DNA-Directed Alkylating Agents. 6. Synthesis and Antitumor Activity of DNA Minor Groove-Targeted Aniline Mustard Analogues of Pibenzimol (Hoechst 33258)

G. Lance Gravatt,† Bruce C. Baguley,† William R. Wilson,‡ and William A. Denny*,†

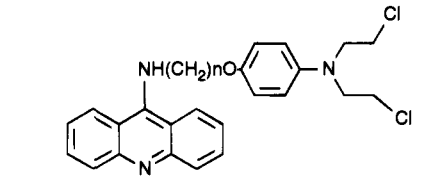
Cancer Research Laboratory and Department of Pathology, University of Auckland School of Medicine, Private Bag 92019, Auckland, New Zealand

Received July 25, 1994®

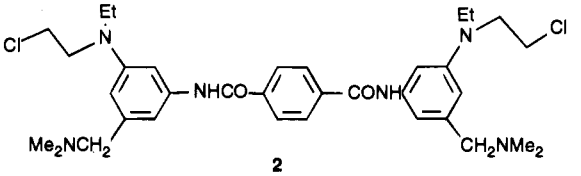
A series of nitrogen mustard analogues of the DNA minor groove binding fluorophore pibenzimol (Hoechst 33258) have been synthesized and evaluated for antitumor activity. Conventional construction of the bisbenzimidazole ring system from the piperazinyl terminus, via two consecutive Pinner-type reactions, gave low yields of products contaminated with the 2-methyl analogue which proved difficult to separate. An alternative synthesis was developed, involving construction of the bisbenzimidazole from the mustard terminus, via Cu^{2+}-promoted oxidative coupling of the mustard aldehydes with 3,4-diaminobenzonitrile to form the monobenzimidazoles, followed by a Pinner-type reaction and condensation with 4-(1-methyl-4-piperazinyl)-*o*-phenylenediamine. This process gives higher yields and pure products. The mustard analogues showed high hypersensitivity factors (IC_{50} AA8/IC_{50} UV4), typical of DNA alkylating agents. There was a large increase in cytotoxicity (85-fold) across the homologous series which cannot be explained entirely by changes in mustard reactivity and may be related to altering orientation of the mustard with respect to the DNA resulting in different patterns of alkylation. Pibenzimol itself (which has been evaluated clinically as an anticancer drug) was inactive against P388 *in vivo* using a single-dose protocol, but the short-chain mustard homologues were highly effective, eliciting a proportion of long-term survivors.

Recent work on the targeting of nitrogen mustard alkylating agents to DNA by the use of both DNA-intercalating[1-3] and DNA minor groove-binding[4,5] ligands has shown that this strategy can greatly enhance both the *in vitro* cytotoxicity and the *in vivo* antitumor activity of the mustard moiety, when compared with untargeted mustards of similar reactivity. Such targeting can also significantly alter the pattern of DNA alkylation by the mustard. Simple nitrogen mustards alkylate almost completely from the major groove at the N7 of guanine, and the lethal interstrand cross-links they form are almost exclusively between guanine N7 positions.[6] However, in a series of DNA-intercalator targeted compounds (e.g., **1**), there was a progressive switch from alkylation at N7 of guanine in 5′-GT sequences to alkylation at N7 of adenine in 5′-AC sequences.[7] Even more drastic alterations in alkylation pattern were observed with the minor groove-targeted "split" mustard **2**, based on the well-known[8] series of synthetic minor groove-binding bisquaternary ammonium heterocycles. Alkylation by **2** occurred almost exclusively at the N3 position of adenine in the minor groove.[9]

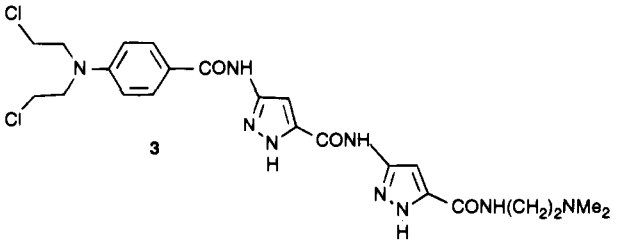
Compounds which alkylate in the DNA minor groove are very potent cytotoxins; typical examples are **2** (IC_{50} in P388 = 7 nM),[9] which compares well with other well-known (albeit non-mustard) compounds such as CC-1065 (IC_{50} in L1210 = 3 nM).[10] The only other example of minor groove-targeted nitrogen mustards are a series of compounds based on the antibiotic distamycin. Recent reports[4,5] indicate that they are also potent cytotoxins, with **3** in particular showing *in vivo* activity against a number of solid tumor cell lines and promising results in clinical trial.[11] This general class of compounds are thus attractive synthetic targets.



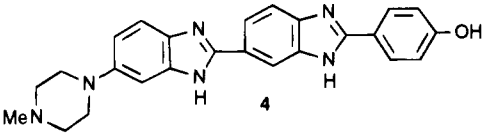
1 : n = 2-6



2



3



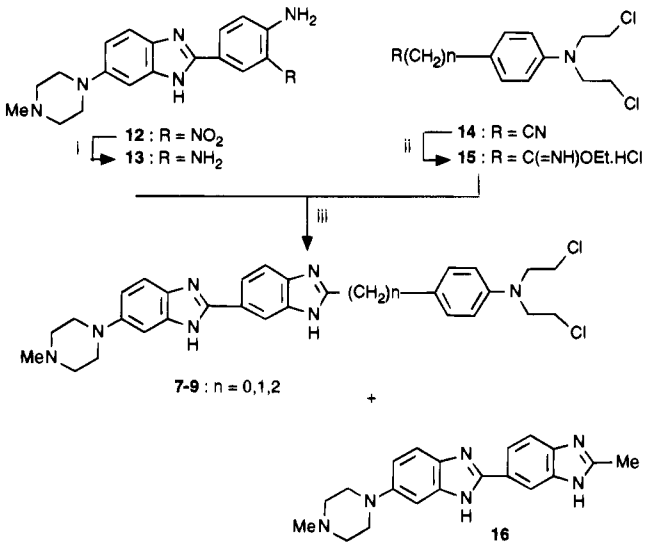
4

Another well-described series of synthetic minor groove-binding agents is the bisbenzimidazoles. In particular, compound **4** (pibenzimol, Hoechst 33258) has

† Cancer Research Laboratory.
‡ Department of Pathology.
® Abstract published in *Advance ACS Abstracts*, November 1, 1994.

0022-2623/94/1837-4338$04.50/0

Scheme 1



[a] (i) H_2/Rh–Al_2O_3/Pt–C/EtOH/glacial AcOH; (ii) EtOH/HCl(g)/(0–20 °C)/2 days; (iii) EtOH/glacial AcOH/3 h.

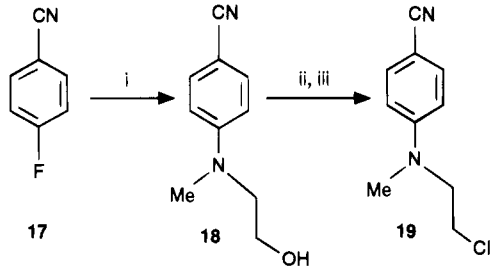
been widely studied for its sequence-specific interaction with DNA[12–14] and has also undergone phase I clinical evaluation as an anticancer agent;[15] an analogue has recently been reported to have topoisomerase I activity.[16] We have been interested in using bisbenzimidazoles as DNA sequence-selective carriers of alkylating functionality and report here the synthesis and biological evaluation of a series of such compounds (**6**–**11**), together with the corresponding reference compounds (**4**, **5**).

Chemistry

The first reported synthesis of the fluorophore Hoechst 33258 (**4**) and a wide variety of congeners (including **5**) was more than 25 years ago.[17] The synthetic strategy employed construction of the bisbenzimidazole ring system from the piperazinyl terminus via two consecutive Pinner-type reactions (cf. Scheme 1). Despite many reported modifications to the preparation of simple monomeric benzimidazoles, the paper of Loewe *et al.*[17] has remained the definitive synthetic route to the dimeric bisbenzimidazoles, and we initially employed this method.

We therefore initially prepared the mustard-bearing bisbenzimidazoles (**6**–**9**) by an analogous method (Scheme 1). Pinner-type reaction of the imine ether hydrochlorides (**15**) derived from the corresponding nitriles (**14**) with phenylenediamine (**13**) (prepared immediately prior to use by catalytic hydrogenation (Pt–C/Rh–Al_2O_3) of nitroaniline **12**) afforded the desired products (**7**–**9**), although in low yield (ca. 20%). The crude products obtained by this method were consistently contaminated with significant amounts of the 2-methylbisbenzimidazole (**16**), presumably arising from condensation of diamine **13** with the acetic acid employed as solvent, and required extensive chromatography to purify. Attempts to condense the butyronitrile mustard (**14**: $n = 3$) directly with **13** produced no detectable (by TLC) quantities of the desired bisbenzimidazole **10**. Synthesis of the benzonitrile monomustard derivative **19** required for preparation of **6** was achieved according to the method shown in Scheme 2. Direct nucleophilic aromatic substitution by methyl-

Scheme 2



[a] (i) HN(Me)(CH_2CH_2OH)/DMSO/120 °C/8 h; (ii) MsCl/Et_3N/CH_2Cl_2/0 °C; (iii) LiCl/DMF/140 °C.

aminoethanol on 4-fluorobenzonitrile gave the anilino ethanol **18** which was converted to the corresponding mustard **19** via mesylation and displacement by LiCl.

Improved yields of pure bisbenzimidazoles (**7**–**11**) were finally achieved using the alternative synthetic strategy outlined in Scheme 3, where the bisbenzimidazole unit was built up from the mustard terminus. The mustard-bearing alkyl aldehydes (**21**: $n = 2, 3, 6$) were synthesized from the corresponding mustard acids (**20**: $n = 2, 3, 6$) in good overall yield by a reduction/selective oxidation sequence (Scheme 3). Treatment of the acids (**20**: $n = 2, 3, 6$) with borane–dimethyl sulfide gave the crude alcohols, which were in turn oxidized under Swern conditions[18] to yield the desired aldehydes (**21**: $n = 2, 3, 6$). A variety of alternative oxidation systems, including pyridinium chlorochromate[19] and tetrapropylammonium perruthenate,[20] were attempted without success. All attempts to prepare the acetaldehyde analogue (**21**: $n = 1$) were unsuccessful, possibly due to facile enolization of this compound.

Cu^{2+}-promoted oxidative coupling[21] of the mustard aldehydes **21** with 3,4-diaminobenzonitrile **22**[22] afforded the mustard-bearing benzimidazoles **23** in fair yield. Pinner-type reaction of the imine ether hydrochlorides **24** derived from the nitriles **23** with the freshly-prepared diamine **26** cleanly yielded the target compounds **7**–**11**. This protocol represents a considerable improvement over the classical procedure (Scheme 1), affording bisbenzimidazoles of greater purity (free of **16**) and in higher yield.

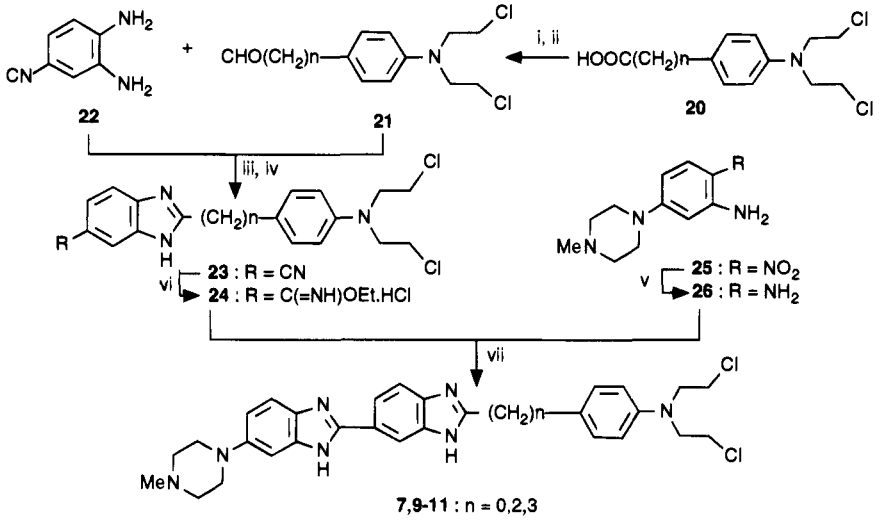
The phenylenediamines **13** and **26** were freshly prepared immediately prior to use via catalytic hydrogenation (Pt–C/Rh–Al_2O_3) of nitroanilines **12** and **25**, respectively. This mixed catalyst system was found to be superior to conventional methods, affording rapid and clean reduction.

Results and Discussion

Growth Inhibition. The structures of the compounds are given in Table 1, and their DNA binding[23] and biological properties in Table 2. Reversible DNA binding constants and binding site sizes to calf thymus DNA were determined spectrophotometrically by Scatchard analysis, using analysis times sufficiently short that covalent alkylation reactions did not interfere.[23] The reversible binding of these compounds is somewhat stronger than that of pibenzimol itself and decreases slightly across the homologous series. The binding site size remains essentially constant, suggesting similar binding contacts across the series.

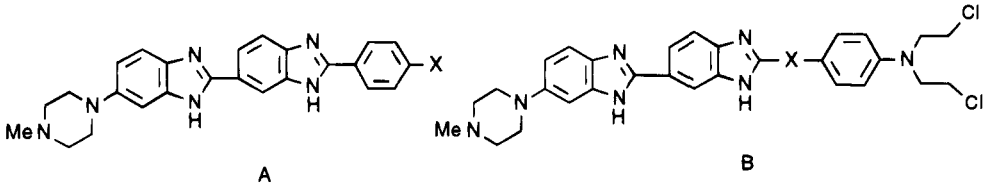
The compounds were evaluated for their growth inhibitory properties against three mammalian tumor

Scheme 3



[a] (i) BH_3·DMS/THF/0 °C; (ii) DMSO/$(COCl)_2$/THF/−78 °C; Et_3N/-78 °C to 20 °C; (iii) $Cu(OAc)_2$/MeOH(aq)/Δ/20 min; (iv) H_2S(-CuS); (v) Pt−C/Rh−Al_2O_3/60 psi H_2/AcOH; (vi) HCl(g)/EtOH/0−20 °C/2 days; (vii) AcOH/100 °C/2 h.

Table 1. Structures and Physicochemical Properties of Pibenzimol Mustards



no.	form	X	mp (°C)	formula	analyses
4	A	OH	*a*		
5	A	NEt_2	>300	$C_{29}H_{33}N_7$·3HCl·4H_2O	C, H, N
6	A	$N(Me)CH_2CH_2Cl$	>300	$C_{28}H_{30}NCl$·3HCl·3H_2O	C, H, N
7	A	$N(CH_2CH_2Cl)_2$	>300	$C_{29}H_{31}N_7Cl_2$·3HCl·1.5H_2O	C, H, N, Cl
8	B	CH_2	>300	$C_{30}H_{33}N_7Cl_2$·3HCl	C, H, N
9	B	$(CH_2)_2$	>300	$C_{31}H_{35}N_7Cl_2$·3HCl·2H_2O	C, H, N
10	B	$(CH_2)_3$	>300	$C_{32}H_{37}N_7Cl_2$·3HCl·H_2O	C, H, N
11	B	$(CH_2)_6$	>300	$C_{35}H_{43}N_7Cl_2$·3HCl·4H_2O	C, H, N

[a] Purchased from Sigma Chemical Co., St. Louis, MO.

cell lines *in vitro*: wild-type P388 leukemia, AA8 Chinese hamster fibroblasts, and the AA8-derived mutant line UV4 (Table 2). The latter cell line is deficient in aspects of the excision repair pathway for removing DNA adducts[24] and is hypersensitive to DNA-alkylating agents, especially interstrand cross-linking agents. Previous work[1,25,26] has shown that the ratio of IC_{50}s determined in the wild-type AA8 and mutant UV4 lines (the hypersensitivity factor HF) is a determinant of the mode of cell killing by alkylating agents. Compounds with HFs of ca. 1 are unlikely to kill cells via DNA alkylation events. Compounds which show high HF values are likely to form bulky adducts or cross-links, with the latter being especially likely for compounds showing the highest HF values (> ca. 25).[26]

In the present series, both of the nonalkylating compounds (pibenzimol itself (**4**) and the $N(Et)_2$ derivative (**5**)) were moderately cytotoxic against wild-type P388 leukemia, with IC_{50} values of ca. 1 μM. The compounds were much less sensitive in the AA8 cell line (although a shorter exposure time was used; see the Experimental Section). In other studies,[27] pibenzimol has shown a similar IC_{50} value against L1210 leukemia, but very low cytotoxicity in solid tumor lines. Both **4** and **5** had AA8/UV4 IC_{50} ratios (HFs) of about unity, as expected for compounds which do not act by DNA alkylation. Some phase I−II clinical studies with pibenzimol have been reported,[15,16] but the mode of action is not known.

The half-mustard analogue **6** showed slightly increased cytotoxicity against the wild-type P388 and AA8 lines but was much more effective against UV4, resulting in an HF of 14. This is strong evidence for the mode of cytotoxicity being by the formation of DNA adducts. The corresponding full mustard **7** had roughly similar cytotoxicities against the wild-type lines but had an increased HF of 24. This compound has the capability of forming DNA cross-links, and the increased HF is consistent with this possibility. Compounds **8**−**11** form a series where the aniline mustard moiety is tethered to the end of an increasingly long polymethylene chain. While IC_{50} values in both wild-type cell lines differ little between **7** and **8**, there is a large increase in cytotoxicity (80-fold in P388, 5-fold in AA8) for the $(CH_2)_2$-linked compound **9**. This is probably not due simply to increasing lipophilicity, since the longer chain compounds **10** and **11** are less cytotoxic.

Increasing the polymethylene chain has two effects. One is to increase the σ values of the *para* substituent on the aniline mustard (**7**, Ph, σ = −0.01; **8**, $PhCH_2$, σ = −0.09; **9**−**11**, $Ph(CH_2)_n$, σ = ca. −0.17).[28] Using a previous equation (eq 7 of ref 26) relating the reactivity

Table 2. Biological Data for the Pibenzimol Mustards of Table 1

	DNA binding		growth inhibition: IC_{50} (μM)			P388 in vivo	
no.	M^{-1} (10^{-6}K)[a]	n[b]	P388(W)[c]	AA8[d]	HF[e]	OD[f]	ILS[g]
4	1.82	2.4	1.2			20	NA[h]
5	6.24	2.8	1.2	10	1.5	20	50
6			0.5	5.75	14	30	86
7	7.52	2.6	0.8	1.68	24	30	106 (2)[i]
8	6.73	3.0	0.85	1.50	59	13.3	71
9	6.30	2.8	0.01	0.33	47	3.9	52 (1)
10	4.55	3.0	0.02	1.32	46	5.9	NA
11	3.89	2.4	0.06	0.4	20	8.9	23

[a] Intrinsic association constant for reversible binding of compounds to calf thymus DNA at 20 °C in 0.01 ionic strength at pH 7.00 (data from ref 23). [b] Binding site size expressed in nucleotide residues (data from ref 23). [c,d] IC_{50}: concentration of drug (nM) to inhibit cell growth in culture to 50% of controls, using the protocols detailed in refs 34 and 36). Values are means of three determinations, and vary by $<\pm 10\%$. P388(W): wild-type P388 murine leukemia. AA8: Chinese hamster ovary derived cell line. [e] HF: hypersensitivity factor = IC_{50}(AA8)/IC_{50}(UV4), where UV4 is a mutant AA8 line lacking the ability to perform incision repair (see text). [f] OD: optimal dose of drug, in milligrams/kilogram, administered as a single dose intraperitoneally as a solution in 0.1 mL of 30% v/v EtOH/water on day 1, 24 h after intraperitoneal inoculation of 10^6 tumor cells. [g] ILS: percentage increase in lifespan of drug-treated tumor-bearing animals when treated at the optimal dose. Values of ILS >20% are considered statistically significant. [h] NA: compound inactive in all nontoxic doses. [i] Figure in parentheses is the average number of animals (out of a group of 6) which were long-term survivors.

of substituted aniline mustards (their ability to alkylate 4-nitrobenzylpyridine) to substituent σ values,[25] it can be calculated that, purely on electronic grounds, compound **8** will be ca. 1.5-fold more reactive than **7**, and compounds **9**–**11** will be ca. 2.5-fold more reactive. Such changes in mustard reactivity would be expected (see eq 7 of ref **1**) to provide increases of similar magnitudes in the relative cytotoxicities of the compounds for untargeted mustards, although in aniline mustards targeted to DNA by intercalating chromophores the effects of electronic factors are attenuated.[1]

The other effect of increasing the polymethylene chain is altered positioning of the mustard moiety on the DNA. Several studies of pibenzimol itself[12-14] show that it binds to DNA by lying snugly in the minor groove in AT-rich regions. While the orientation of the drug with respect to the helix direction can alter with DNA sequence,[14] the positioning of the benzene ring with respect to minor groove elements is essentially the same. While the reported crystal structures would place the mustard group of **7** some distance from the susceptible adenine N3 site (although well-placed for phosphate alkylation), later compounds in the series would have a better positioning. The relative cytotoxicities of compounds **7**–**11** do suggest a positive role for the DNA-targeting chromophore. The increased HF of compounds **8**–**10** is consistent with them forming a higher proportion of cross-links than **7**. The lower HF and absolute cytotoxicity of **11** suggest there may be an optimal chain length, beyond which entropic factors become important. We have noted this phenomenon previously in a series of acridine-targeted mustards.[2]

***In Vivo* Activity.** The compounds were also evaluated for their antitumor activity against P388 leukemia (Table 2). Pibenzimol (**4**) is reported[16] to have moderate *in vivo* activity in L1210 leukemia on a repeated dose schedule but was not active against P388 using the single-dose protocol (Table 2). However, the $N(Et)_2$ derivative **5** showed low but significant activity (ILS 50%). The monoalkylating half-mustard **6** was also active, but the highest activity was shown by the corresponding bis-mustard **7**, with a single dose of 30 mg/kg resulting in some long-term survivors (Table 2). The antitumor activity of the higher homologues decreased as their toxicity increased, but the CH_2 and $(CH_2)_2$ compounds (**8** and **9**) retained significant *in vivo* activity.

Conclusions

The concept of targeting reactive moieties to DNA by the use of either intercalating or minor groove-binding carriers has given rise to a novel class of potent cytotoxic agents. The present study has shown that mustard analogues of the well-characterized reversible minor groove-binding ligand pibenzimol have greatly increased cytotoxicity and *in vivo* antitumor activity in antiproliferative assays, compared with pibenzimol itself. However, the very high reversible DNA binding of these compounds (greater than that of pibenzimol) is of concern, since it is likely to limit diffusion of the intact molecule into solid tumors (pibenzimol itself is used as a stain for outlining the vasculature). Further studies of structure–activity relationships in this series, directed toward evaluation of the role of the DNA binding chromophore on the pattern of DNA alkylation of these compounds, will address this issue.

Experimental Section

Chemistry. Elemental analyses were carried out in the Microchemical Laboratory, University of Otago. Melting points were determined on an Electrothermal apparatus using the supplied, stem-corrected thermometer and are as read. NMR spectra were obtained on either Bruker AM-400 or CW-60 spectrometers (Me_4Si). Mass spectra were obtained on an AEI MS-30 spectrometer at nominal 5000 resolution. Fluorescence spectra were obtained on a Hitachi F-2000 spectrofluorimeter.

Preparation of 2-[2-[4-(*N*,*N*-Bis(2-chloroethyl)amino)phenyl]-6-benzimidazolyl]-6-(1-methyl-4-piperazinyl)benzimidazole (7) via the method of Scheme 1: Example of General Procedure. Dry HCl gas was slowly bubbled into a suspension of 4-(*N*,*N*-bis(2-chloroethyl)amino)benzonitrile (**14**: $n = 0$)[29] (0.73 g, 3 mmol) in dry EtOH (30 mL) maintained at 0–5 °C, and addition of HCl was continued until the solution became saturated. The mixture was brought to room temperature and stirred for a further 48 h, and the resulting suspension was then concentrated under reduced pressure to give the crude imine ether hydrochloride (**15**; $n = 0$) which was used without purification.

A solution of 2-(3-nitro-4-aminophenyl)-6-(1-methyl-4-piperazinyl)benzimidazole[17] (**12**) (1.06 g, 3 mmol) in EtOH/glacial AcOH (1:1, 60 mL) was hydrogenated over Rh–Al_2O_3/Pt–C (0.05 g) at 60 psi H_2 for 12 h. After this time the catalysts were removed by filtration and the solution of crude diamine **13** was added immediately to the imine ether hydrochloride (**15**: $n = 0$) prepared above. The slurry was refluxed under N_2 for 3 h and concentrated under reduced pressure, and the crude residue was basified with concentrated ammonia and partitioned between EtOAc and water. The aqueous phase was repeatedly extracted with portions of EtOAc, the combined organic phases were dried (Na_2SO_4), filtered, and concentrated, and the residue was chromatographed on Al_2O_3 (grade III). Elution with EtOAc/MeOH (99:1) afforded **7** (0.30 g, 17% yield) as a pale yellow foam. The trihydrochloride salt crystallized from MeOH/EtOAc: mp >300 °C; ^1H NMR (CD_3OD) δ 8.52 (d, $J_{4,6} = 1.4$ Hz, 1 H, H-4), 8.25 (dd, $J_{6,7} = 8.6$ Hz and $J_{4,6} = 1.4$ Hz, 1 H, H-6), 8.11 (d, $J_{2'',3''} = 9.1$ Hz, 2 H, H-2″,H-6″), 8.03 (d, $J_{6,7} = 8.6$ Hz, 1 H, H-7), 7.77 (d, $J_{6',7'} = 9.1$ Hz, 1 H,

H-7'), 7.44 (dd, $J_{6',7'}$ = 9.1 Hz and $J_{4',6'}$ = 2.0 Hz, 1 H, H-6'), 7.37 (d, $J_{4',6'}$ = 2.0 Hz, 1 H, H-4'), 7.10 (d, $J_{2'',3''}$ = 9.1 Hz, 2 H, H-3'',H-5''), 4.00 (br d, J = 11.5 Hz, 2 H, piperazinyl methylene), 3.96 (br t, J = 6.5 Hz, 4 H, NCH_2CH_2Cl), 3.80 (br t, J = 6.5 Hz, 4 H, NCH_2CH_2Cl), 3.70 (br d, J = 12.0 Hz, 2 H, piperazinyl methylene), 3.30 (broad m, 4 H, piperazinyl methylene), 3.01 (s, 3 H, NCH_3); ^{13}C NMR δ 154.09, 153.42, 151.08, 149.02, 136.20, 134.35, 133.73, 131.44, 127.63, 126.31, 121.36, 119.94, 116.02, 115.72, 114.14, 113.99, 109.92, 100.80, 54.59, 53.85, 48.34, 43.63, 41.46; fluorescence excitation λ_{max} (MeOH) 383 nm, emission λ_{max} (MeOH) 510 nm. Anal. Table 1.

Further elution with EtOAc/MeOH (19:1) yielded 2-(2-methyl-6-benzimidazolyl)-6-(1-methyl-4-piperazinyl)benzimidazole (**16**) (0.31 g, 27% yield): mp (MeOH/EtOAc) >260 °C dec; ^1H NMR ($(CD_3)_2SO/D_2O$) δ 8.62 (s, 1 H, H-4), 8.28 (d, $J_{6,7}$ = 8.4 Hz, 1 H, H-6), 8.03 (d, $J_{6,7}$ = 8.4 Hz, 1 H, H-7), 7.74 (d, $J_{6',7'}$ = 9.1 Hz, 1 H, H-7'), 7.38 (dd, $J_{6',7'}$ = 9.1 Hz and $J_{4',6'}$ = 1.7 Hz, 1 H, H-6'), 7.28 (d, $J_{4',6'}$ = 1.7 Hz, 1 H, H-4'), 3.94 (br d, J = 11.9 Hz, 2 H, piperazinyl methylene), 3.61 (br d, J = 10.8 Hz, 2 H, piperazinyl methylene), 3.27 (t, J = 11.9 Hz, 2 H, piperazinyl methylene), 3.20 (t, J = 11.4 Hz, 2 H, piperazinyl methylene), 2.91 (s, 3 H, CH_3), 2.88 (s, 3 H, CH_3); ^{13}C NMR δ 154.25, 148.61, 146.95, 133.70, 133.04, 131.44, 126.09, 124.45, 119.95, 117.22, 114.69, 114.33, 113.55, 98.64, 51.94, 46.01, 41.76, 12.60. Anal. ($C_{20}H_{23}N_6 \cdot 5H_2O$) C,H,N.

2-[2-[[4-(*N*,*N*-Bis(2-chloroethyl)amino)phenyl]methyl]-6-benzimidazolyl]-6-(1-methyl-4-piperazinyl)benzimidazole (8). A similar reaction between **13** and the crude imine ether hydrochloride (**15**: n = 1) (prepared from the nitrile **14**: n = 1)[29] followed by chromatography on Al_2O_3 grade III (elution with CH_2Cl_2 followed by CH_2Cl_2/MeOH (49:1)) afforded **8** (38% yield): trihydrochloride salt; mp (MeOH/EtOAc) >300 °C; ^1H NMR (CD_3OD) δ 8.60 (s, 1 H, H-4), 8.28 (dd, $J_{6,7}$ = 8.7 Hz and $J_{4,6}$ = 1.5 Hz, 1 H, H-6), 8.07 (d, $J_{6,7}$ = 8.7 Hz, 1 H, H-7), 7.78 (d, $J_{6',7'}$ = 9.1 Hz, 1 H, H-7'), 7.47 (dd, $J_{6',7'}$ = 9.1 Hz and $J_{4',6'}$ = 2.0 Hz, 1 H, H-6'), 7.39 (d, $J_{4',6'}$ = 2.0 Hz, 1 H, H-4'), 7.33 (d, $J_{2'',3''}$ = 8.7 Hz, 2 H, H-2'', H-6''), 6.85 (d, $J_{2'',3''}$ = 8.7 Hz, 2 H, H-3'', H-5''), 4.53 (s, 2 H, $ArCH_2Ar$), 4.00 (br d, J = 13.7 Hz, 2 H, piperazinyl methylene), 3.80 (broad t, J = 6.4 Hz, 4 H, NCH_2CH_2Cl), 3.70 (broad t, J = 6.5 Hz, 6 H, NCH_2CH_2Cl and piperazinyl methylene), 3.30 (m, 4 H, piperazinyl methylene), 3.01 (s, 3 H, NCH_3); ^{13}C NMR δ 158.70, 151.18, 149.04, 147.78, 135.49, 134.37, 133.13, 131.80, 127.70, 126.56, 122.07, 121.81, 120.13, 116.81, 115.75, 115.33, 114.32, 100.90, 54.60, 54.38, 48.39, 43.61, 41.52, 32.99; fluorescence excitation λ_{max} (MeOH) 335 nm, emission λ_{max} (MeOH) 462 nm. Anal. Table 1.

2-[2-[2-[4-(*N*,*N*-Bis(2-chloroethyl)amino)phenyl]ethyl]-6-benzimidazolyl]-6-(1-methyl-4-piperazinyl)benzimidazole (9). A similar reaction between **13** and the crude imine ether hydrochloride (**15**: n = 2) (prepared from the nitrile **14**: n = 2)[30] followed by chromatography on Al_2O_3 (grade III) (elution with EtOAc followed by EtOAc/MeOH (33:1)) gave **9** (19% yield): trihydrochloride salt; mp (MeOH/EtOAc) >300 °C; ^1H NMR (CD_3OD) δ 8.60 (d, $J_{4,6}$ = 1.7 Hz, 1 H, H-4), 8.28 (dd, $J_{6,7}$ = 8.7 Hz and $J_{4,6}$ = 1.7 Hz, 1 H, H-6), 8.08 (d, $J_{6,7}$ = 8.7 Hz, 1 H, H-7), 7.79 (d, $J_{6',7'}$ = 9.1 Hz, 1 H, H-7'), 7.48 (dd, $J_{6',7'}$ = 9.1 Hz and $J_{4',6'}$ = 2.1 Hz, 1 H, H-6'), 7.39 (d, $J_{4',6'}$ = 2.1 Hz, 1 H, H-4'), 7.12 (d, $J_{2'',3''}$ = 8.7 Hz, 2 H, H-2'',H-6''), 6.72 (d, $J_{2'',3''}$ = 8.7 Hz, 2 H, H-3'',H 5''), 4.01 (br d, J = 13.8 Hz, 2 H, piperazinyl methylene), 3.73 (br t, J = 7.3 Hz, 4 H, NCH_2CH_2Cl), 3.69 (br d, J = 12.6 Hz, 2 H, piperazinyl methylene), 3.62 (br t, J = 7.3 Hz, 4 H, NCH_2CH_2Cl), 3.52 [t, J = 7.4 Hz, 2 H, $ArCH_2CH_2C_6H_4N(CH_2CH_2Cl)_2$], 3.31 (broad m, 4H, piperazinyl methylene), 3.21 [t, J = 7.3 Hz, 2 H $ArCH_2CH_2C_6H_4N(CH_2CH_2Cl)_2$], 3.01 (s, 3 H, NCH_3); ^{13}C NMR δ 158.44, 150.90, 148.98, 146.60, 135.30, 134.30, 133.00, 130.47, 128.36, 127.76, 126.25, 121.78, 120.02, 116.70, 115.78, 114.98, 113.93, 101.00, 54.54, 54.28, 48.33, 43.77, 41.74, 33.06, 30.01; fluorescence excitation λ_{max} (MeOH) 331 nm, emission λ_{max} (MeOH) 449 nm. Anal. Table 1.

2-[2-[4-*N*-methyl-*N*-(2-chloroethyl)amino)phenyl]-6-benzimidazolyl]-6-(1-methyl-4-piperazinyl)benzimidazole (6). A solution of 4-fluorobenzonitrile (**17**) (5.35 g, 44.2 mmol) in DMSO (30 mL) containing excess methylaminoethanol was heated with stirring to 120 °C for 8 h. The solution was then cooled and concentrated under reduced pressure. The crude residue was partitioned between H_2O/EtOAc, and the organic phase was then washed with water (×2), dried (Na_2SO_4), filtered, concentrated, and filtered through a short column of SiO_2 (CH_2Cl_2 then EtOAc elution) to give 4-(*N*-methyl-*N*-(2-hydroxyethyl)amino)benzonitrile (**18**) (5.6 g, 72% yield) as a colorless liquid, which was used without further purification. A solution of alcohol **18** (5.6 g, 31.8 mmol) and Et_3N (8.8 mL, 63.6 mmol) in dry CH_2Cl_2 (50 mL) was treated at 0 °C with MsCl (3.0 mL, 38.2 mmol). The mixture was stirred for 20 min and partitioned between aqueous $NaHCO_3$/EtOAc. The organic phase was then dried (Na_2SO_4), filtered, and concentrated under reduced pressure to yield the crude mesylate, which was treated with excess LiCl in DMF (25 mL) at 140 °C for 5 min with rapid stirring. Excess DMF was removed under reduced pressure, and the residue was partitioned between H_2O and EtOAc. Workup of the organic layer, chromatography of the residue on SiO_2, and elution with petroleum ether/EtOAc (3:1) afforded pure 4-(*N*-methyl-*N*-(2-chloroethyl)amino)benzonitrile (**19**) (3.6 g, 58% yield): mp (petroleum ether/CH_2Cl_2) 104.5–105.5 °C; ^1H NMR ($CDCl_3$) δ 7.48 (d, $J_{2,3}$ = 9.1 Hz, 2 H, H-2, H-6), 6.67 (d, $J_{2,3}$ = 9.1 Hz, 2 H, H-3, H-5), 3.74 (complex t, J = 7.3 Hz, 2 H, NCH_2CH_2Cl), 3.64 (complex t, J = 7.3 Hz, 2 H, NCH_2CH_2Cl), 3.09 (s, 3 H, NCH_3); ^{13}C NMR δ 150.95, 133.66, 120.30, 111.50, 98.50, 53.77, 40.16, 39.02. Anal ($C_{10}H_{11}ClN_2$) C, H, N.

The crude imino ether was prepared from **19** as above and reacted with **13** as before. Chromatography on Al_2O_3 (grade III) (elution with EtOAc then EtOAc/1–3% MeOH) afforded **6** (0.57 g, 13% yield): trihydrochloride; mp (MeOH/EtOAc) >300 °C; ^1H NMR (CD_3OD/D_2O) δ 8.16 (d, $J_{4,6}$ = 1.6 Hz, 1 H, H-4), 8.00 (dd, $J_{6,7}$ = 8.6 Hz and $J_{4,6}$ = 1.6 Hz, 1 H, H-6), 7.87 (d, $J_{6,7}$ = 8.6 Hz, 1 H, H-7), 7.82 (d, $J_{2'',3''}$ = 9.0 Hz, 2 H, H-2'', H-6''), 7.68 (d, $J_{6',7'}$ = 8.9 Hz, 1 H, H-7'), 7.32 (dd, $J_{6',7'}$ = 8.9 Hz and $J_{4',6'}$ = 2.1 Hz, 1 H, H-6'), 7.29 (d, $J_{4',6'}$ = 2.1 Hz, 1 H, H-4'), 6.80 (d, $J_{2'',3''}$ = 9.0 Hz, 2 H, H-3'', H-5''), 3.95 (broad d, J = 12.1 Hz, 2 H, piperazinyl methylene), 3.72–3.68 (m, 6 H, NCH_2CH_2Cl and piperazinyl methylene), 3.31 (t, J = 12.4 Hz, 2 H, piperazinyl methylene), 3.24 (t, J = 11.8 Hz, 2 H, piperazinyl methylene), 3.04 (s, 3 H, NCH_3), 2.99 (s, 3 H, NCH_3); ^{13}C NMR δ 154.15, 153.42, 150.44, 148.16, 136.00, 134.03, 133.53, 130.62, 127.41, 125.41, 120.26, 119.31, 115.88, 115.68, 113.17, 112.95, 108.54, 100.50, 54.45, 54.20, 48.06, 43.81, 42.00, 39.30; fluorescence excitation λ_{max} (MeOH) 397 nm, emission λ_{max} (MeOH) 511 nm. Anal. Table 1.

2-[2-[4-(Dimethylamino)phenyl]-6-benzimidazolyl]-6-(1-methyl-4-piperazinyl)benzimidazole (5). A similar reaction between **13** and the crude imine ether hydrochloride prepared from 4-(diethylamino)benzonitrile,[31] followed by chromatography on Al_2O_3 (grade III) (EtOAc then EtOAc/1–3% MeOH), yielded **5** (0.33 g, 12% yield): trihydrochloride salt; mp (MeOH/EtOAc) >300 °C; ^1H NMR (CD_3OD/D_2O) δ 8.16 (s, 1 H, H-4), 7.98 (d, $J_{6,7}$ = 8.5 Hz, 1 H, H-6), 7.86 (d, $J_{6,7}$ = 8.5 Hz, 1 H, H-7), 7.81 (d, $J_{2'',3''}$ = 8.8 Hz, 2 H, H-2'',H-6''), 7.65 (d, $J_{6',7'}$ = 9.5 Hz, 1 H, H-7'), 7.26 (br envelope, 2 H, H-4',H-6'), 6.78 (d, $J_{2'',3''}$ = 8.8 Hz, 2 H, H-3'',H-5''), 3.93 (br d, J = 11.6 Hz, 2 H, piperazinyl methylene), 3.70 (br d, J = 11.9 Hz, 2 H, piperazinyl methylene), 3.36 (q, J = 7.1 Hz, 4 H, NCH_2CH_3), 3.28 (t, J = 12.7 Hz, 2 H, piperazinyl methylene), 3.22 (t, J = 13.3 Hz, 2 H, piperazinyl methylene), 3.03 (s, 3 H, NCH_3), 1.16 (t, J = 7.1 Hz, 6 H, NCH_2CH_3); ^{13}C NMR δ 153.26, 152.76, 150.46, 147.90, 135.82, 133.86, 133.32, 130.89 (2 signals superimposed), 127.10, 125.38, 119.94, 119.27, 115.65, 113.40, 112.79, 100.36, 54.41, 47.95, 46.19, 43.80, 12.75; fluorescence excitation λ_{max} (MeOH) 405 nm, emission λ_{max} (MeOH) 519 nm. Anal. Table 1.

Alternative Preparation of 7 via the Method of Scheme 3: Example of a General Coupling Procedure. The procedure followed was essentially that described by Weiderhagen and Weedon.[21] A solution of 4-(bis(2-chloroethyl)amino)-benzaldehyde (**21**: n = 0)[32] (1.32 g, 5.4 mmol) in MeOH (25 mL) was added to a stirred solution of 2,3-diaminobenzonitrile[22] (**22**) (0.72 g, 5.4 mmol) in aqueous MeOH (25 mL), immediately followed by a 0.05 M aqueous solution of cupric acetate (1.1 equiv).[21] The suspension was brought briefly to the boil and filtered hot, and the precipitate was washed with

aqueous MeOH and redissolved in concentrated HCl. This acid solution was treated with an aqueous 5% solution of Na_2S (1.5 equiv) and the precipitated CuS removed by filtration. The filtrate was then basified with concentrated ammonia, diluted with water, and repeatedly extracted with EtOAc. The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated, and the residue was chromatographed on SiO_2. Elution with CH_2Cl_2/EtOAc (3:1) yielded 2-[4-(*N*,*N*-bis(2-chloroethyl)amino)phenyl]-5-cyanobenzimidazole (**23**: $n = 0$) (0.80 g, 40% yield): mp (EtOAc/petroleum ether) 80 °C dec; ^{1}H NMR ($(CD_3)_2CO$) δ 8.15 (d, $J_{2',3'} = 9.0$ Hz, 2 H, H-2′, H-6′), 7.94 (d, $J_{4,6} = 1.5$ Hz, 1 H, H-4), 7.69 (d, $J_{6,7} = 8.5$ Hz, 1 H, H-7), 7.50 (dd, $J_{6,7} = 8.5$ Hz and $J_{4,6} = 1.5$ Hz, 1 H, H-6), 6.99 (d, $J_{2',3'} = 9.0$ Hz, 2 H, H-3′, H-5′), 3.94 (br t, $J = 7.5$ Hz, 4 H, NCH_2CH_2Cl), 3.83 (br t, $J = 7.5$ Hz, 4 H, NCH_2CH_2Cl); ^{13}C NMR δ 155.98, 149.75, 143.08, 140.50, 129.62, 126.31, 120.58, 120.13, 118.40, 116.15, 112.93, 105.47, 53.52, 41.43. Anal. ($C_{18}H_{16}Cl_2N_4$) C, H, N, Cl.

Dry HCl gas was slowly bubbled into a suspension of the nitrile (**23**: $n = 0$) in dry EtOH (10 mL/mmol of nitrile) maintained at 0–5 °C, with addition of HCl being continued until the solution became saturated. The mixture was brought to room temperature, stirred for 48 h, and then concentrated under reduced pressure to give the crude imine ether hydrochloride (**24**: $n = 0$) which was employed without further purification.

A solution of 4-(1-methyl-4-piperazinyl)-1-nitroaniline[17] (**25**) (0.40 g, 1.7 mmol) in glacial AcOH (10 mL/mmol of nitroaniline) was hydrogenated over Rh–Al_2O_3/Pt–C (0.02 g) at 60 psi of H_2 for 12 h. After this time the catalysts were removed by filtration and the resulting crude triamine **26** was added immediately to the imine ether hydrochloride (**24**: $n = 0$) (0.75 g, 1.7 mmol) prepared above, and the slurry was refluxed under N_2 for 3 h. The reaction mixture was then concentrated under reduced pressure and the crude residue was basified with concentrated ammonia and then partitioned between EtOAc and water. The aqueous phase was repeatedly extracted with portions of EtOAc, and the combined organic phases were dried (Na_2SO_4), filtered, and concentrated. Chromatography of the residue as before afforded **7** (0.75 g, 74% yield) (identical chromatographic behavior and NMR spectrum to an authentic sample).

Alternative Preparation of 9 via the method of Scheme 3: Example of the General Procedure for the Synthesis of Aldehydes (21). A solution of borane–dimethyl sulfide (23 mmol of a 10 M solution in THF) was added slowly to a stirred solution of 3-(4-(*N*,*N*-bis(2-chloroethyl)amino)phenyl)-propanoic acid[33] (**20**: $n = 2$) (5.5 g, 19 mmol) in THF (190 mL) maintained at 0 °C under a N_2 atmosphere. After the initial effervescence the solution was stirred for a further 4 h before being quenched with MeOH and concentrated under reduced pressure. The product was partitioned between aqueous $NaHCO_3$ and EtOAc, and the residue from the organic phase was filtered through a short column of SiO_2 (EtOAc elution) to give essentially pure 4-(4-(*N*,*N*-bis(2-chloroethyl)amino)-phenyl)butanol, which was used without further purification.

DMSO (1.15 equiv) was added to a stirred solution of oxalyl chloride (1.1 equiv) in THF (5 mL/mmol of alcohol) maintained at −78 °C under a N_2 atmosphere. Stirring was continued for 10 min, after which a solution of the above crude alcohol (1 equiv) in THF (5 mL/mmol of alcohol) was added. Stirring was continued at −78 °C for a further 15 min before Et_3N (5.0 equiv) was added and the mixture brought slowly to room temperature. The reaction mixture was partitioned between H_2O and EtOAc, and the residue from the organic layer was chromatographed on SiO_2 and eluted with petroleum ether/EtOAc (3:1) to give pure 3-(4-(*N*,*N*-bis(2-chloroethyl)amino)-phenyl)propanal (**21**: $n = 2$) as an unstable oil (3.4 g, 68% overall yield): ^{1}H NMR ($CDCl_3$) δ 9.80 (t, $J_{1,2} = 1.6$ Hz, 1 H, CHO), 7.08 (d, $J_{2',3'} = 8.8$ Hz, 2 H, H-2′, H-6′), 6.62 (d, $J_{2',3'} = 8.8$ Hz, 2 H, H-3′, H-5′), 3.69 (complex t, $J = 6.5$ Hz, 4 H, NCH_2CH_2Cl), 3.68 (complex t, $J = 6.5$ Hz, 4 H, NCH_2CH_2Cl), 2.87 (t, $J_{2,3} = 7.4$ Hz, 2 H, H-3), 2.72 (broad dt, $J_{2,3} = 7.4$ Hz, and $J_{1,2} = 1.6$ Hz, 2 H, H-2), 2.12 (complex m, 4 H, H-2); ^{13}C NMR δ 201.88, 144.56, 129.53, 129.36, 112.28, 53.54, 45.50, 40.48, 27.04; HRMS (EI, 70 eV) Calcd for $C_{13}H_{17}NO_3Cl_2$ 273.068 720; found 273.068 81.

Reaction between diamine **22** and aldehyde (**21**: $n = 2$) as above, followed by chromatography on SiO_2 and elution with CH_2Cl_2/EtOAc (9:1), gave the nitrile (**23**: $n = 2$) (1.6 g, 32% yield), mp (EtOAc/petroleum ether) 50 °C dec; ^{1}H NMR ($(CD_3)_2CO$) δ 7.95 (d, $J_{4,6} = 1.5$ Hz, 1 H, H-4), 7.66 (d, $J_{6,7} = 8.4$ Hz, 1 H, H-7), 7.50 (dd, $J_{6,7} = 8.4$ Hz and $J_{4,6} = 1.5$ Hz, 1 H, H-6), 7.12 (d, $J_{2',3'} = 8.7$ Hz, 2 H, H-2′, H-6′), 6.70 (d, $J_{2',3'} = 8.7$ Hz, 2 H, H-3′, H-5′), 3.73 (m, 8 H, NCH_2CH_2Cl), 3.22 (br t, $J = 7.9$ Hz, 2 H, benzylic methylene), 3.10 (br t, $J = 7.9$ Hz, 2 H, benzylic methylene); ^{13}C NMR δ 158.95, 145.86, 142.30, 140.28, 130.44, 130.26, 125.97, 120.69, 120.57, 116.08, 113.08, 105.27, 53.82, 41.58, 33.42, 31.96. Anal. ($C_{20}H_{20}Cl_2N_4$) C, H, N. Conversion of **23** ($n = 2$) to the imino ether **24** ($n = 2$), and condensation of this with the crude triamine **26** was carried out as described above to give **9** (1.4 g, 65% yield) (identical chromatographic behavior and NMR spectrum to an authentic sample).

2-[2-[3-[4-(*N*,*N*-Bis(2-chloroethyl)amino)phenyl]propyl]-6-benzimidazolyl]-6-(1-methyl-4-piperazinyl)benzimidazole (10). A similar reaction of 4-(4-(*N*,*N*-bis(2-chloroethyl)-amino)phenyl)butanoic acid[33] (**20**: $n = 3$) (4.75 g, 15.6 mmol) according to the above protocol gave 4-(4-(*N*,*N*-bis(2-chloroethyl)amino)phenyl)butanal (**21**: $n = 3$) as a pale yellow oil (3.3 g, 73% overall yield): ^{1}H NMR ($CDCl_3$) δ 9.75 (t, $J_{1,2} = 1.6$ Hz, 1 H, CHO), 7.06 (d, $J_{2',3'} = 8.6$ H z, 2 H, H-2′,H-6′), 6.63 (d, $J_{2',3'} = 8.6$ Hz, 2 H, H-3′,H-5′), 3.71 (t, $J = 7.4$ Hz, 4 H, NCH_2CH_2Cl), 3.63 (t, $J = 7.4$ Hz, 4 H, NCH_2CH_2Cl), 2.56 (t, $J_{3,4} = 7.4$ Hz, 2 H, H-4), 2.45 (dt, $J_{2,3} = 7.3$ Hz, and $J_{1,2} = 1.6$ Hz, 2 H, H-2), 1.90 (quintet, $J = 7.4$ Hz, 2 H, H-3); ^{13}C NMR δ 202.52, 144.41, 130.28, 129.62, 112.21, 53.51, 43.10, 40.59, 33.82, 23.85; HRMS (EI, 70 eV) calcd for $C_{14}H_{19}NO^{35}Cl_2$ 287.084 37, found 287.084 27.

Reaction of **21** ($n = 3$) and diamine **22** as above, followed by chromatography on SiO_2 and elution with CH_2Cl_2/EtOAc (5:1), gave the nitrile **23** ($n = 3$) as an oil (2.1 g, 46% yield): ^{1}H NMR ($(CD_3)_2CO$) δ 7.94 (dd, $J = 0.8$, 0.6 Hz, 1 H, H-4), 7.66 (dd, $J_{6,7} = 8.3$, 0.6 Hz, 1 H, H-7), 7.49 (dd, $J_{6,7} = 8.3$ Hz and $J_{4,6} = 1.6$ Hz, 1 H, H-6), 7.10 (d, $J_{2',3'} = 8.8$ Hz, 2 H, H-2′, H-6′), 6.71 (d, $J_{2',3'} = 8.8$ Hz, 2 H, H-3′, H-5′), 3.79–3.69 (m, 8 H, NCH_2CH_2Cl), 2.97 (t, $J = 7.4$ Hz, 2 H, $ArCH_2CH_2CH_2C_6H_4NR_2$), 2.64 (t, $J = 7.4$ Hz, 2 H, $ArCH_2CH_2CH_2C_6H_4NR_2$), 2.15 (quintet, $J = 7.4$ Hz, 2 H, $ArCH_2CH_2CH_2C_6H_4NR_2$); ^{13}C NMR δ 159.39, 145.63, 142.28, 140.41, 131.12, 130.23, 125.91, 120.63, 120.58, 116.05, 113.11, 105.20, 53.90, 41.63, 34.77, 30.40, 29.06. Anal. ($C_{21}H_{22}Cl_2N_4 \cdot H_2O$) C, H, N. Conversion of **23** ($n = 3$) to the imino ether **24** ($n = 3$) and condensation of this with **26** was carried out as above. Chromatography of the product on Al_2O_3 (grade V) and elution with EtOAc followed by EtOAc/MeOH (66:1) gave **10** as an orange oil (1.2 g, 41% yield): trihydrochloride salt; mp (MeOH/EtOAc) >300 °C; ^{1}H NMR (CD_3OD) δ 8.50 (s, 1 H, H 4), 8.26 (dd, $J_{6,7} = 8.7$ Hz and $J_{4,6} = 1.7$ Hz, 1 H, H-6), 7.97 (d, $J_{6,7} = 8.7$ Hz, 1 H, H-7), 7.83 (d, $J_{6',7'} = 9.1$ Hz, 1 H, H-7′), 7.48 (dd, $J_{6',7'} = 9.1$ Hz and $J_{4',6'} = 2.2$ Hz, 1 H, H-6′), 7.44 (d, $J_{4',6'} = 2.2$ Hz, 1 H, H-4′), 7.09 (d, $J_{2'',3''} = 8.6$ Hz, 2 H, H-2″, H-6″), 6.52 (d, $J_{2'',3''} = 8.6$ Hz, 2 H, H-3″, H-5″), 4.02 (broad d, $J = 12.9$ Hz, 2 H, piperazinyl methylene), 3.72 (br d, $J = 11.4$ Hz, 2 H, piperazinyl methylene), 3.54 (t, $J = 6.0$ Hz, 4 H, NCH_2CH_2Cl), 3.46 (t, $J = 6.0$ Hz, 4 H, NCH_2CH_2Cl), 3.40–3.27 (m, 6 H, piperazinyl methylenes and $ArCH_2CH_2CH_2C_6H_4NR_2$), 3.03 (s, 3 H, NCH_3), 2.79 (t, $J = 6.7$ Hz, 2 H, $ArCH_2CH_2CH_2C_6H_4NR_2$), 2.41 (quintet, $J = 6.3$ Hz, 2 H, $ArCH_2CH_2CH_2C_6H_4NR_2$); ^{13}C NMR δ 159.12, 150.93, 148.70, 145.18, 135.06, 134.19, 132.70, 130.87, 130.67, 127.59, 126.06, 121.48, 120.06, 116.46, 114.52, 113.80, 100.88, 54.59, 54.51, 48.27, 43.77, 41.73, 34.97, 28.91, 27.53; fluorescence excitation λ_{max} (MeOH) 332 nm, emission λ_{max} (MeOH) 453 nm. Anal. Table 1.

2-[2-[6-[4-(*N*,*N*-Bis(2-chloroethyl)amino)phenyl]hexyl]-6-benzimidazolyl]-6-(1-methyl-4-piperazinyl)benzimidazole (11). A similar reaction of 7-(4-(*N*,*N*-bis(2-chloroethyl)-amino)phenyl)heptanoic acid **20** ($n = 6$) (1.50 g, 4.34 mmol) gave 7-(4-(*N*,*N*-bis(2-chloroethyl)amino)phenyl)heptanal (**21**: $n = 6$) as an unstable colorless gum (1.34 g, 96% overall yield): ^{1}H NMR (60 MHz) ($CDCl_3$) δ 9.8 (t, $J_{1,2} = 1.7$ Hz, 1 H,

CHO), 6.9 (d, $J_{2',3'}$ = 8.9 Hz, 2 H, H-2', H-6'), 6.4 (d, $J_{2',3'}$ = 8.9 Hz, 2 H, H-3', H-5'), 3.6 (broad s, 8 H, NCH_2CH_2Cl), 2.7–2.2 (broad m, 4 H, H-2, H-7), 1.8–0.9 (broad m, 8 H, H-3, H-4, H-5, H-6). HRMS (EI, 70 eV) calcd for $C_{1H25}NO^{35}Cl_2$ 329.131 32; found 329.131 76. Reaction of **21** (n = 6) and diamine **22** as above, followed by chromatography on SiO_2 and elution with CH_2Cl_2/EtOAc (5:1), afforded the nitrile **23** (n = 6) (0.65 g, 40% yield) as an oil: ^1H NMR ($(CD_3)_2CO$) δ 7.94 (d, $J_{4,6}$ = 1.5 Hz, 1 H, H-4), 7.67 (d, $J_{6,7}$ = 8.3 Hz, 1 H, H-7), 7.50 (dd, $J_{6,7}$ = 8.3 Hz and $J_{4,6}$ = 1.5 Hz, 1 H, H-6), 7.05 (d, $J_{2',3'}$ = 8.7 Hz, 2 H, H-2', H-6'), 6.70 (d, $J_{2',3'}$ = 8.7 Hz, 2 H, H-3', H-5'), 3.78–3.68 (m, 8 H, NCH_2CH_2Cl), 2.97 (t, J = 7.5 Hz, 2 H, $ArCH_2(CH_2)_5C_6H_4NR_2$), 2.49 (t, J = 7.4 Hz, 2 H, $Ar(CH_2)_5CH_2C_6H_4NR_2$), 1.88 (quintet, J = 7.5 Hz, 2 H, $ArCH_2CH_2(CH_2)_4C_6H_4NR_2$), 1.57 (quintet, J = 7.5 Hz, 2 H, $Ar(CH_2)_4CH_2CH_2C_6H_4NR_2$), 1.49–1.34 (m, 4 H, $ArCH_2CH_2CH_2CH_2CH_2CH_2C_6H_4NR_2$); ^{13}C NMR δ 159.41, 145.46, 142.12, 140.12, 132.21, 130.24, 126.02, 120.59, 120.54, 116.04, 113.12, 105.35, 53.96, 41.65, 35.33, 32.37, 30.41, 29.76, 29.58, 29.57, 28.44; HRMS (EI, 70 eV) calcd for $C_{24}H_{28}N_435Cl_2$ 442.169 103; found 442.167 64.

Conversion of **23** (n = 6) to the imino ether **24** (n = 6) and condensation of this with **26** was carried out as above. Chromatography of the product on Al_2O_3 (grade V) and elution with EtOAc followed by EtOAc/MeOH (66:1) gave **11** (0.20 g, 23% yield): trihydrochloride salt; mp (MeOH/EtOAc) >300 °C; ^1H NMR (CD_3OD) δ 8.48 (d, $J_{4,6}$ = 1.6 Hz, 1 H, H-4), 8.17 (dd, $J_{6,7}$ = 8.7 Hz and $J_{4,6}$ = 1.6 Hz, 1 H, H-6), 8.00 (d, $J_{6,7}$ = 8.7 Hz, 1 H, H-7), 7.71 (d, $J_{6',7'}$ = 9.0 Hz, 1 H, H-7'), 7.36 (dd, $J_{6',7'}$ = 9.0 Hz and $J_{4',6'}$ = 1.9 Hz, 1 H, H-6'), 7.32 (d, $J_{4',6'}$ = 1.9 Hz, 1 H, H-4'), 7.03 (d, $J_{2'',3''}$ = 8.5 Hz, 2 H, H-2'', H-6''), 6.75 (d, $J_{2'',3''}$ = 8.5 Hz, 2 H, H-3'', H-5''), 3.89 (d, J = 12.4 Hz, 2 H, piperazinyl methylene), 3.66 (t, J = 6.5 Hz, 4 H, NCH_2CH_2Cl), 3.59 (d, J = 11.4 Hz, 2H, piperazinyl methylene), 3.52 (t, J = 6.5 Hz, 4 H, NCH_2CH_2Cl), 3.28–3.10 (complex m, 6 H, piperazinyl methylenes and $ArCH_2(CH_2)_5C_6H_4NR_2$), 2.90 (s, 3 H, NCH_3), 2.45 (t, J = 7.5 Hz, 2 H, $Ar(CH_2)_5CH_2C_6H_4NR_2$), 1.89 (quintet, J = 7.5 Hz, 2 H, $ArCH_2CH_2(CH_2)_4C_6H_4NR_2$), 1.51 (quintet, J = 7.1 Hz, 2 H, $Ar(CH_2)_4CH_2CH_2C_6H_4NR_2$), 1.39–1.30 (m, 4 H, $ArCH_2CH_2CH_2CH_2CH_2CH_2C_6H_4NR_2$); ^{13}C NMR δ 159.16, 150.86, 148.90, 143.13, 136.04, 135.20, 134.18, 132.84, 130.73, 127.63, 126.31, 121.71, 120.02, 116.75, 115.78 (two signals superimposed), 114.99, 100.97, 55.71, 54.51, 48.31, 43.79, 41.05, 35.54, 32.17, 29.71, 29.39, 27.91, 27.70; fluorescence excitation λ_{max} (MeOH) 328 nm, emission λ_{max} (MeOH) 467 nm. Anal. Table 1.

Measurement of Selective Cytotoxicity by Growth Inhibition Assay. 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.[25,34] IC_{50} values were determined 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, using eight control cultures on each microplate. For P388 cultures, drug was present throughout the growth period (72h), and final cell densities were determined using a minor modification of the MTT method of Mossman.[35] For AA8 and UV4 cultures, drug exposure was terminated after 18h by washing three times with fresh medium. Cultures were then grown for a further 72 h before determining cell density by staining with methylene blue.[36]

Acknowledgment. This work was supported by the NZ Lotteries Board, the Auckland Division of the Cancer Society of New Zealand, and the Health Research Council of New Zealand. The authors thank Susan Pullen for the cytotoxicity assays.

References

(1) Gourdie, T. A.; Valu, K. K.; Gravatt, G. L.; Boritzki, T. J.; Baguley, B. C.; Wilson, W. R.; Woodgate, P. D.; Denny, W. A. DNA-directed alkylating agents. 1. Structure-activity relationships for acridine-linked aniline mustards: consequences of varying the reactivity of the mustard. *J. Med. Chem.* **1990**, *33*, 1177–1186.

(2) Valu, K. K.; Gourdie, T. A.; Gravatt, G. L.; Boritzki, T. J.; Woodgate, P. D.; Baguley, B. C.; Denny, W. A. DNA-directed alkylating agents. 3. Structure-activity relationships for acridine-linked aniline mustards : consequences of varying the length of the linker chain. *J. Med. Chem.* **1990**, *33*, 3014–3019.

(3) Koyama, M.; Takahashi, K.; Chou, T.-C.; Darzynkiewicz, Z.; Kapuscinski, J.; Kelly, T. T.; Wanatabe, K. A. Intercalating agents with covalent bond forming capability. A novel type of potential anticancer agents. 2. Derivatives of chrysophanol and emodin. *J. Med. Chem.* **1989**, *32*, 1594–1599.

(4) Krowicki, K.; Balzarini, J.; De Clercq, E.; Newman, R. A.; Lown, J. W. Novel DNA groove binding alkylators : design, synthesis and biological activity. *J. Med. Chem.* **1988** *31*, 341–345.

(5) Arcamone, F. M.; Animati, F.; Barbieri, B.; Configliacchi, E.; D'Alessio, R.; Geroni, C.; Giuliani, F. C.; Lazzari, E.; Menozzi, M.; Mongelli, N.; Penco, S.; Verini, M. A. Synthesis, DNA-binding properties and antitumor activity of novel distamycin derivatives. *J. Med. Chem.* **1989**, *32*, 774–778.

(6) Millard, J. T.; Raucher, S.; Hopkins, P. B. Mechlorethamine crosslinks deoxyguanosine residues at 5'-GNC sequences in duplex DNA fragments. *J. Am. Chem. Soc.* **1990**, *112*, 2459–2460.

(7) Prakash, A. S.; Denny, W. A.; Gourdie, T. A.; Valu, K. K.; Woodgate, P. D.; Wakelin, L. P. G. DNA-directed alkylating ligands as potential antitumor agents: sequence specificity of alkylation by DNA-intercalating acridine-linked aniline mustards. *Biochemistry* **1990**, *29*, 9799–9807.

(8) Denny, W. A.; Atwell, G. J.; Baguley, B. C.; Cain, B. F. Potential Antitumour Agents. 29. QSAR for the antileukemic bisquaternary ammonium heterocycles. *J. Med. Chem.* **1979**, *22*, 134–151.

(9) Prakash, A. S.; Valu, K. K.; Wakelin, L. P. G.; Denny, W. A. Synthesis and antitumour activity of the spatially-separated mustard bis-N,N'-[3-(N-(2-chloroethyl)-N-ethyl)amino-5-((N,N-dimethylamino)methyl)aminophenyl]-1,4-benzenedicarboxamide, which alkylates DNA exclusively at adenines in the minor groove. *Anti-Cancer Drug Design* **1991**, *6*, 195–206.

(10) Hurley, L. H.; Lee, C.-S.; McGovren, J. P.; Warpehoski, M. A.; Mitchell, M. A.; Kelly, R. C.; Aristoff, P. A. Molecular basis for sequence-specific DNA alkylation by CC-1065. *Biochemistry* **1988**, *27*, 3886–3892.

(11) Pezzoni, G.; Grandi, M.; Biasoli, G.; Capolongo, L.; Ballinari, D.; Giuliani, F. C.; Barbieri, B.; Pastori, A.; Pesenti, E.; Mongelli, N.; Spreafico, F. Biological profile of FCE-24517, a novel benzoyl mustard analogue of distamycin-A. *Br. J. Cancer* **1991**, *64*, 1047–1050.

(12) Pjura, P. E.; Grzeskowiak, K.; Dickerson, R. E. Binding of Hoechst 33258 to the minor groove of B-DNA. *J. Mol. Biol.* **1987**, *197*, 257–271.

(13) Teng, M.; Usman, N.; Frederick, C. A.; Wang, A. H.-J. The molecular structure of the complex of Hoechst 33258 and the DNA dodecamer d(CGCGAATTCGCG). *Nucleic Acids Res.* **1988**, *16*, 2671–2690.

(14) Carrondo, M.; Coll, M.; Ayami, J.; Wang, A. H.-J.; van der Marel, G. A.; van Boom, J. H.; Rich, A. Binding of a Hoechst dye to d(CGCGATATCGCG) and its influence on the conformation of the DNA fragment. *Biochemistry* **1989**, *28*, 7849–7859.

(15) Patel, S. K.; Kvols, L. K.; Rubin, J.; O'Connell, M. J.; Edmonson, J. H.; Ames, M. M.; Kovach, J. S. Phase I-II study of pibenzimol hydrochloride (NSC 322921) in advanced pancreatic carcinoma. *Invest. New Drugs* **1991**, *9*, 53–57.

(16) Chen, A. Y.; Yu, C.; Bodley, A.; Peng, L. F.; Liu, L. F. A new mammalian DNA topoisomerase-I poison Hoechst-33342 - cytotoxicity and drug resistance in human cell cultures. *Cancer Res.* **1993**, *53*, 1332–1337.

(17) Loewe, H.; Urbanietz, J. Basisch substituerte 2,6-bisbenzimidazolderivat, eine neue chemotherapeutisch aktive korperclasse. (2,6-Bisbenzimidazoles with basic substituents; a new class of chemotherapeutically active compounds.) *Arzneim-Forsch. (Drug Design)* **1974**, *24*, 1927–1933.

(18) Omura, K.; Swern, D. Oxidation of alcohols by "activated" dimethyl sulfoxide. A preparative steric and mechanistic study. *Tetrahedron* **1978**, *34*, 1651–1660.

(19) Corey, E. J.; Suggs, J. W. Pyridinium chlorochromate. An efficient reagent for oxidation of primary and secondary alcohols to carbonyl compounds. *Tetrahedron Lett.* **1975**, 2647–2550.

(20) Griffith, W. P.; Ley, S. V.; Whitcombe, G. P.; White, A. D. Preparation and use of tetra-n-butylammonium perruthenate (TBAB reagent) and tetra-n-propylammonium perruthenate (TPAB reagent) as new catalytic oxidants for alcohols. *J. Chem. Soc., Chem. Commun.* **1987**, 1625–1627.

(21) Weidenhagen, R.; Weeden, U. Eine neue synthese von benzimidazol-derivaten. (A new synthesis of benzimidazole derivatives.) *Chem. Ber.* **1936**, *69B*, 2263–2272.

(22) Stephens, F. F.; Bower, J. D. The preparation of benziminazoles and benzoxazoles from Schiff's bases. Part II. *J. Chem. Soc.* **1950**, 1722–1726.

(23) O'Connor, C. J.; Denny, W. A.; Jan, J.-Y. Physicochemical properties and DNA interactions of DNA-directed alkylating agents based on pibenzimol, a bisbenzimidazole which binds to the DNA minor groove. Manuscript in preparation.
(24) Hoy, C. A.; Thompson, L. H.; Mooney, C. L.; Salazar, E. P. Defective DNA crosslink removal in Chinese hamster cell mutants sensitive to bifunctional alkylating agents. *Cancer Res.* **1985**, *45*, 1737–1747.
(25) Palmer, B. D.; Wilson, W. R.; Pullen, S. M.; Denny, W. A. Hypoxia-selective antitumor agents. 3. Relationships between structure and cytotoxicity against cultured tumor cells for substituted N,N-bis(2-chloroethyl)anilines. *J. Med. Chem.* **1990**, *33*, 112–121.
(26) Wilson, W. R.; Thompson, L. H.; Anderson, R. F.; Denny, W. A. Hypoxia-selective antitumor agents. 2. Electronic effects of 4-substituents on the mechanisms of cytotoxicity and metabolic stability of nitracrine analogues. *J. Med. Chem.* **1989**, *32*, 31–38.
(27) Finlay, G. J.; Baguley, B. C. Potentiation of the cytotoxicity of topoisomerase-II directed anticancer drugs by DNA minor groove binders. *Eur. J. Cancer* **1990**, *26*, 585–589.
(28) Hansch, C.; Leo, A. J. *Substituent Constants for Correlation Analysis in Chemistry and Biology;* Wiley: New York, 1979.
(29) Degutis, J.; Sukelienne, D. Synthesis and some reactions of nitriles of N,N-bis(2-chloroethyl)aminophenylalkanoic acids. *Zh. Obshch. Khim.* **1961**, *31*, 3326–3330; *Chem. Abstr.* **1962**, *57*, 3354e.
(30) Degutis, J.; Jodelyte. A.; Sukelienne, D. New synthesis of p-[bis-(2-chloroethyl)amino]-β-phenylpropionic acid and its derivatives. *Lietuvos TSR Aukstuju Mokyklu Mokslo Darbai, Chem. ir Chem. Tech.* **1962**, *2*, 39–46; *Chem. Abstr.* **1963**, *59*, 8651a.
(31) Lohaus, G. Nitrilsynthesen mit chlorosulfonylisocyanat. (Synthesis of nitriles using chlorosulfonyl isocyanate.) *Chem. Berichte* **1967**, *100*, 2719–2729.
(32) Anker, R. M.; Cook, A. H. Quinoxaline cyanines. Part IV. Some halogenated styryl derivatives. *J. Chem. Soc.* **1944**, 489–492.
(33) Everett, J. L.; Roberts, J. J.; Ross W. C. J. Aryl-2-halogenoalkylamines. Part XII. Some carboxylic acid derivatives of N,N-di-2-chloroethylaniline. *J. Chem. Soc.* **1953**, 2386–2392.
(34) Baguley, B. C.; Wilson, W. R. Comparison of in vivo and in vitro drug sensitivities of Lewis lung carcinoma and P388 leukemia to analogues of amsacrine. *Eur. J. Cancer Clin. Oncol.* **1987**, *23*, 607–613.
(35) Mossman, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55–63.
(36) Finlay, G. J.; Baguley, B. C.; Wilson, W. R. A semiautomated microculture method for investigating growth inhibitory effects of cytotoxic compounds on exponentially growing carcinoma cells. *Anal. Biochem.* **1984**, *139*, 272–277.