shrinking of the N(2) to N(2) distances may not constitute a major energy difference between the two models, but it does indicate that the d(GpC) adduct requires a larger conformational change, which might occur at a slower rate than the smaller conformational change required for d(CpG) binding. Thus, the d(CpG) bifunctional adduct may be more rapid-forming than the d(GpC) bifunctional adduct. This gives an advantage to the formation of the d(CpG) bifunctional adduct, even if it is similar in energy to the d(GpC) bifunctional adduct.

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Registry No. $C_{16}H_2ON_4O_5$, 801-52-5.

Supplementary Material Available: Tables of phase angles of pseudorotation, C(1')-C(1') distances, bond lengths, angles, anisotropic thermal parameters, and figures showing overlap of drug-bound and drug-free minimized DNA structures (7 pages). Ordering information is given on any current masthead page.

DNA-Directed Alkylating Agents. 2. Synthesis and Biological Activity of Platinum Complexes Linked to 9-Anilinoacridine

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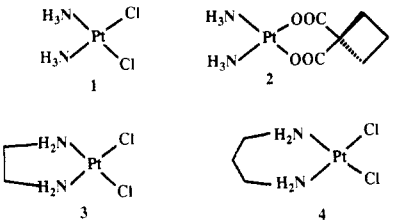
Two different classes of *cis*-diaminedichloroplatinum(II) complexes linked to the DNA-intercalating chromophore 9-anilinoacridine have been synthesized and evaluated as DNA-targeted antitumor agents. Two different Pt chelating ligands were investigated (based on 1,2-ethanediamine and 1,3-propanediamine), designed to deliver the Pt in an orientation likely to respectively enhance either intrastrand or interstrand cross-linking. Although both sets of ligands were somewhat unstable under neutral or basic conditions with respect to disproportionation, the corresponding Pt complexes, once prepared, appeared to be quite stable. All the Pt complexes were monitored for purity by TLC, HPLC, and FAB mass spectra, and the mode of Pt coordination was established by ^{195}Pt NMR spectroscopy. The complexes appeared to cause simultaneous platination and intercalative unwinding of plasmid DNA. In vitro studies were carried out with both wild-type and cisplatin-resistant P388 cell lines. Whereas cisplatin itself and the ethylenediamine and 1,3-propanediamine complexes used as standards were about 10-fold less active against the resistant line, the ethylenediamine-linked Pt complexes showed no differential toxicity between the two lines and the propanediamine-linked complexes showed significant differentials (up to 8-fold) in favor of the cisplatin-resistant line. However, these were no greater than those shown by the unplatinated ligands themselves. The majority of the acridine complexes were inactive in vivo against the wild-type P388 leukemia. They were very insoluble, and although a suitable formulation was found, this may have been a factor. It is also possible that these compounds bind in such a way as to direct the Pt away from the major groove.

Cisplatin (*cis*-diamminedichloroplatinum(II)) (1) is one of the very few drugs with significant clinical activity against a range of solid tumors[1] and is therefore widely used despite its severe side effects[2] and steep dose-response curve.[3] The primary mechanism of cytotoxicity of cisplatin and analogues is by initial cross-linking of cellular DNA (primarily through the N7 of guanine,[4] although bonding to both N1 and N3 of purines has also been reported[5]). However, the relative importance of the more common intrastrand cross-linking compared with the much less common (<1% of total platination)[6] but potentially more lethal interstrand cross-links has yet to be decided. The actual bonding to DNA occurs via the aquo species, with relatively slow kinetics compared to that of the organic alkylators, especially for the second step to form the cross-link, which can take some hours.[7] The development of cellular resistance to cisplatin in mammalian cells is common, via three main mechanisms:[8] (1) increased efficiency of repair of platinum-DNA lesions,[9,10] (2) increased inactivation of drug by elevated levels of cellular low-molecular weight thiols, particularly glutathione,[11] and (3) decreased cellular uptake of drug.[12,13]

Many analogues of cisplatin have been prepared, where the labile chloro ligands have been replaced by other leaving groups (e.g. carboplatin (2)) and/or the stable



amine ligands have been extended by a series of either cyclic or acyclic alkyldiamines [e.g. dichloro(ethylenedi-

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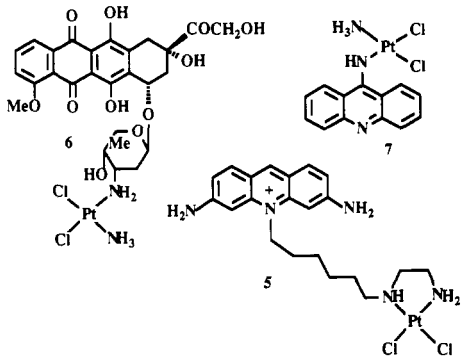
0022-2623/90/1833-3008$02.50/0

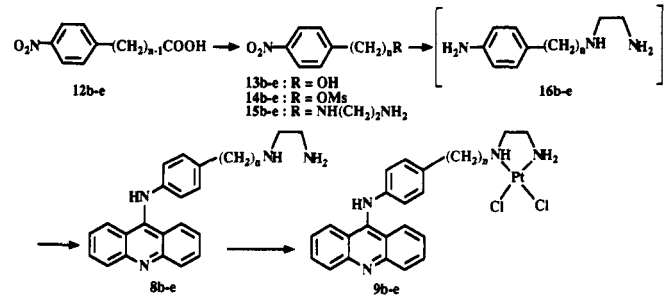
amine)platinum(II) (3)[14] and dichloro(propane-1,3-diamine)platinum(II) (4)]. Although the primary aim in the development of these new analogues was an improved spectrum of anticancer activity and/or reduced side effects, the work has generally been empirical.[15] However, increasing knowledge about the mechanisms of cytotoxicity and cellular resistance to cisplatin has made possible the more deliberate design of new analogues. In particular, the fact that cisplatin acts by cross-linking DNA makes the evaluation of analogues with improved DNA-targeting ability of some interest. Although the actual reactive species is probably a cationic aquo form which has some DNA affinity, targeting might be greatly improved by the use of suitable DNA-affinic "carriers". Such DNA-targeted compounds would be likely to enter cells by passive diffusion and provide lower intracellular levels of unbound drug, which would have interesting implications for overcoming resistance by these mechanisms. Additionally, regiospecific delivery of the platinum to the DNA has the potential to alter the pattern and kinetics of reaction, which also has significant biological implications.[16]

There have been some recent reports of the design and evaluation of cisplatin analogues linked to various DNA-binding ligands, although the rationales for this work have been somewhat different to that proposed above. Acridine orange derivative **5** has been prepared as part of a study[17] of the cooperative interactions of different DNA-binding ligands, and a doxorubicin–Pt complex (**6**) has been studied[18] as a "multifunctional" drug, combining two antitumor agents which are often administered together in combination chemotherapy. The synthesis of a 9-aminoacridine–Pt(II) complex (**7**), with the metal linked directly to the acridine exocyclic amino group, has also been reported.[19]

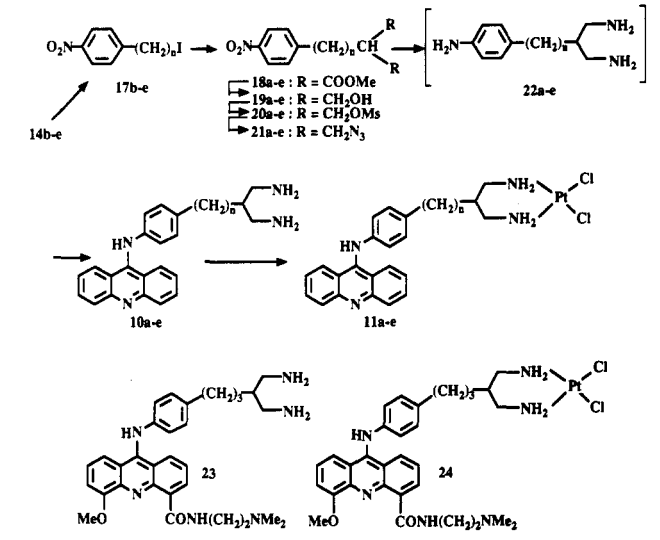


We have been interested in the general concept of using DNA-binding ligands to "target" reactive functionality to DNA.[16,20,21] We report here the synthesis and preliminary biological activity of two classes of platinum-chelating ligands (**8b–8e** and **10a–10e**) linked to the DNA-affinic chromophore 9-anilinoacridine and the corresponding *cis*-diaminedichloroplatinum(II) complexes (**9b–9e** and **11a–11e**). (Ethylenediamine complex **9a**, where $n = 1$, was not prepared, since CPK models suggested this linker chain was too short to correctly position the platinum). 9-Anilinoacridine is a lipophilic, cationic, DNA-intercalating chromophore with no intrinsic antitumor activity of its own,[22] and 9-anilinoacridine species such as amsacrine have been shown to enter cells very rapidly by passive diffusion.[23] Two different Pt chelating ligands were investigated. Assuming that the anilino group lies in the DNA minor groove on binding, CPK models showed that these two different chelating units, 1,2-ethylenediamine, with the linker chain attached via one of the coordinated nitrogens (compounds **9b–9e**), and a 1,3-propanediamine chelator, linked symmetrically via the 2-position (compounds **11a–11e**), can position the Pt in quite different ways with respect to the available nucleophilic sites on DNA. Intercomparison of the biological properties of the classes, both *within* series as the chain is lengthened and *between* series at each chain length, might thus provide interesting structure–activity relationships.

Scheme I



Scheme II



Chemistry

Ethanediamine ligands **8b–8e** ($n = 2$–5) were synthesized from the known ω-(4-nitrophenyl)alkanoic acids (**12**) by the route outlined in Scheme I. Reduction of the acids with borane–methyl sulfide complex afforded alcohols **13**, which were converted into mesylates **14** and then reacted

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Table I. Analytical and Biological Properties of Anilinoacridine–Platinum(II) Complexes and Ligands

no.	n	mp	formula	analyses	IC_{50},[a] cytotoxicity P388/W	IC_{50},[a] cytotoxicity P388/P	in vivo P388/W OD[b]	in vivo P388/W ILS[c]
1	cisplatin				1.2	5.5	5.3	120
3	dichloro(1,2-ethanediamine)platinum(II)				0.3	1.03	13.3	70
			Dichloro(1,2-ethanediamine)platinum(II) Analogues					
8b	2	215 dec	$C_{23}H_{24}H_4 \cdot 3HCl \cdot 0.5H_2O$	C,H,N,Cl	12.5	1.5	45	NA[d]
8c	3	246–248	$C_{24}H_{26}N_4 \cdot 3HCl \cdot 1.5H_2O$	C,H,N	3.7	0.7	20	NA
8d	4	250–251	$C_{25}H_{28}N_4 \cdot 3HCl \cdot 2H_2O$	C,H,N,Cl	7.8	1.3	65	NA
8e	5	276–277	$C_{26}H_{30}N_4 \cdot 3HCl$	C,H,N,Cl	7.3	3.0	20	NA
9b	2	290 dec	$C_{23}H_{24}Cl_2N_4Pt$	C,H,N	2.9	2.4	13.3	NA
9c	3	295 dec	$C_{24}H_{26}Cl_2N_4Pt \cdot 3H_2O$	C,H,N	1.8	2.4	20	NA
9d	4	280 dec	$C_{25}H_{28}Cl_2N_4Pt$	C,H,N	1.2	1.4	20	NA
9e	5	270 dec	$C_{26}H_{30}Cl_2N_4Pt$	C,H,N	1.5	1.6	20	NA
4	dichloro(1,3-propanediamine)platinum(II)				0.12	0.38	8.9	31
			Dichloro(1,3-propanediamine)platinum(II) Analogues					
10a	1	250–260 dec	$C_{23}H_{24}N_4 \cdot 3HCl \cdot 0.5H_2O$	C,H,N	7.2	0.7	45	NA
10b	2	245–250 dec	$C_{24}H_{26}N_4 \cdot 3HCl$	C,H,N,Cl	0.31	0.63	30	NA
10c	3	280–290 dec	$C_{25}H_{28}N_4 \cdot 3HCl \cdot H_2O$	C,H,N	0.12	0.12	30	NA
10d	4	310–325 dec	$C_{26}H_{30}N_4 \cdot 3HCl \cdot 0.5H_2O$	C,H,N	0.72	0.68	20	NA
10e	5	250 dec	$C_{27}H_{32}N_4 \cdot 3HCl$	C,H,N,Cl	0.72	0.69	13.3	NA
11a	1	250–260 dec	$C_{23}H_{24}Cl_2N_4Pt \cdot HCl$	FAB MS[e]	2.9	1.9	20	NA
11b	2	250–275 dec	$C_{24}H_{26}Cl_2N_4Pt \cdot HCl$	FAB MS	3.7	0.93	20	NA
11c	3	235–250 dec	$C_{25}H_{28}Cl_2N_4Pt \cdot HCl$	FAB MS	6.2	1.5	30	NA
11d	4	220–225 dec	$C_{26}H_{30}Cl_2N_4Pt \cdot HCl$	FAB MS	7.2	0.9	30	NA
11e	5	210–220 dec	$C_{27}H_{32}Cl_2N_4Pt \cdot HCl$	FAB MS	20	3.4	30	NA
23		240–245 dec	$C_{31}H_{40}N_6O_2 \cdot 4HCl \cdot 5H_2O$	C,H,N,Cl			30	NA
24		270–280 dec	$C_{31}H_{40}Cl_2N_6Pt \cdot 2HCl$	FAB MS	1.6	1.0	45	28

[a] IC_{50}: the concentration of drug in μM to inhibit cell growth by 50%, measured in 96-well cultures as described in ref 34. [b] OD: optimal dose of drug in mg/kg, administered intraperitoneally as a solution in 0.1 mL of 30% v/v ethanol/water (ligands) or 0.1 mL of 1:1:2 DMA/glycerol/water (Pt complexes) on days 1, 5, and 9 after intraperitoneal inoculation of 10^6 P388/W leukemia cells. [c] ILS: percentage increase in lifespan of treated animals (at the optimal dose) compared with tumor-bearing control animals. Figures are the mean of at least two determinations. Average lifespan of control animals was 11 days. [d] Compound inactive (KS < 20%) at all dose levels up to toxic ones. [e] Analyses by FAB mass spectra (see the Experimental Section).

with an excess of 1,2-diaminoethane to give 4-nitrophenyldiamines **15**. Catalytic reduction of these then gave anilines **16**. These were reacted with 1 equiv of 9-chloroacridine under mild acid conditions, where coupling occurs selectively to the aromatic amine,[22] to give the desired ligands (**8b–8e**) as the trihydrochloride salts.

Propanediamine ligands **10a–10e** (n = 1–5) were also prepared from the same starting materials (Scheme II). Mesylates **14** were converted into the more reactive iodides **17**, and these were condensed with the sodium salt of dimethyl malonate to give diesters **18** [for the case of **18a** (n = 1), the known 4-nitrobenzyl bromide was used directly]. The diesters were reduced slowly but cleanly by borane–methyl sulfide complex to diols **19**, and these were converted successively into dimesylates **20** and diazides **21**, which were purified by silica gel chromatography. Hydrogenation of the diazides over Pd/C gave triamines **22**, which were isolated as crystalline trihydrochloride salts. These were then coupled selectively with 1 equiv of 9-chloroacridine to give the required ligands (**10a–10e**) as their trihydrochloride salts.

Both sets of ligands (**8b–8e** and **10a–10e**) were found to be unstable under neutral or basic conditions with respect to disproportionation, a property described before for 9-[ω-(alkylamino)alkyl]acridines[24] but not previously reported for 9-[ω-(alkylamino)anilino]acridines. Since the neutral ligands are the coordinating species, this presented problems during platination of the ligands. Initial attempts therefore used the more reactive K_2PtI_4 platinum reagent, following the procedure of Dhara,[25] but the products were found to be contaminated with varying amounts of inorganic platinum species, which appeared by mass spectral and combustion analyses to be mainly PtI_4. Cleaner platination reactions were achieved by using the less reactive K_2PtCl_4 in aqueous MeOH solution, although extensive purification was still necessary to separate propanediamine complexes **11a–11e** from decomposition products (see the Experimental Section).

Once prepared, the Pt complexes appeared quite stable to the disproportionation reactions seen for the ligands. Ethylenediamine complexes **9b–9e** were homogeneous by both TLC and HPLC, and satisfactory combustion analyses were obtained for these compounds (Table I). 1,3-Propanediamine complexes **11a–11e** were shown by TLC and HPLC to be persistently contaminated with small quantities of other Pt-containing material, but FAB mass spectroscopy and ^{195}Pt NMR spectroscopy indicated that they were >90% pure. They were converted into their hydrochloride salts in order to improve aqueous solubility and to stabilize their solutions toward platinum aquation reactions, a process which appears to be more facile in the cases of these six-membered ring chelates than for ethylenediamine complexes **9b–9e**. The two series of platinated complexes were quite insoluble in water, both as the free bases and the monohydrochloride salts, and were therefore made up in an aqueous dimethylacetamide/glycerol formulation for biological testing.

NMR Spectra

^{195}Pt NMR spectra were obtained for all of the complexes (**9b–9e** and **11a–11e**) in both series in order to confirm the mode of coordination of the platinum (Table II). Ethylenediamine-linked complexes **9b–9e** all exhibited ^{195}Pt resonances at δ values of ca. −2340 ppm (from $PtCl_6^{2-}$), compared with a shift of −2312 ppm for $Pt(en)Cl_2$ (**3**) itself (the literature shift for cisplatin (**1**) is −2088 ppm[26]). Similarly, propane-1,3-diamine-linked compounds

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Table II. Physical Properties of 9-Anilinoacridine–Platinum(II) Complexes

no.	R_f[a]	t_R,[b] min	^{195}Pt resonance,[c] δ
1			−2088[d]
Dichloro(1,2-ethanediamine)platinum(II) Analogues			
3			−2312
9b	0.64	12.7	−2345
9c	0.55	18.0	−2344
9d	0.49	31.1	−2338
9e	0.43	52.2	−2339
Dichloro(propane-1,3-diamine)platinum(II) Analogues			
4			−2250
11a	0.73 (0.44)[e]	12.7 (8)[f]	−2276
11b	0.65 (0.38)	15.2 (9.6)	−2274.5
11c	0.56 (0.27)	24.8 (15.6)	−2274
11d	0.47 (0.16)	13.4	−2275
11e	0.41 (0.11)	16.4 (20)	−2275
24			−2272

[a] R_f: chromatographic mobility; values for the compounds on glass reverse-phase silica-coated plates (Merck RP-18 $F_{254}S$), pretreated by immersion in 95% MeOH and fixed in an oven at 100 °C for 30 min. The compounds were applied to the prepared plates as dilute solutions in DMF and run with saturated aqueous NaCl/glycerol/MeOH (1:1:2) as mobile phase. [b] t_R: HPLC retention values on Waters Novapak C_{18} Radialpak cartridge, with a maximum flow rate of 0.675 mL/min^{-1} and a maximum backflow pressure of 2500 psi, using UV detection at 254 nm. The mobile phase was saturated aqueous NaCl/glycerol/H_2O/MeOH (1:1:1:2), adjusted to pH 4.00 with 1 N HCl. [c] Values for ^{195}Pt resonance, in ppm upfield from $PtCl_6^{2-}$ as internal standard, with the instrument parameters described in the Experimental Section. [d] Reference 26. [e] Values in parentheses are those of a minor yellow component. [f] Values in parentheses are those of a minor component containing both Pt and 9-anilinoacridine moieties by UV and AA analyses (see the Experimental Section).

11a–11e exhibited resonances at positions (ca. −2275 ppm) similar to that for the parent compound dichloro(propane-1,3-diamine)platinum(II) (**4**) (−2250 ppm). In particular, the ^{195}Pt NMR spectra contained no resonances attributable to $[PtCl_4]^{2-}$ salts of the ligands (expected[27] at ca. δ −1610 ppm) or to platinum coordinated via aromatic ring nitrogens.[28]

Results and Discussion

As expected, the ligands **8b–8e** and **10a–10e** showed bathochromic and hypsochromic shifts in their UV spectra when added to excess DNA (data not shown), indicative of intercalative binding. The Pt complexes showed similar UV properties. More detailed unwinding measurements using gel electrophoresis and supercoiled plasmid DNA showed that compound **9e** caused a DNA unwinding per bound ligand molecule of about twice that observed for dichloro(ethylenediamine)platinum(II) (**3**). This is strong evidence for simultaneous platination and intercalative binding.[29]

Table I gives the in vitro cytotoxicities of the ligands **8b–8e**, **10a–10e**, and **23** and the corresponding platinated compounds **9b–9e**, **11a–11e**, and **24** against both wild-type P388 leukemia and a cisplatin-resistant mutant P388 cell line (obtained from Mason Research, Worcester, MA). Cisplatin itself (**1**) and the ethylenediamine and 1,3-propanediamine complexes (**3** and **4**) were used as standards and were about 5-fold less active against the resistant line. As expected, the ligands themselves generally showed relatively poor in vitro cytotoxicity (IC_{50} in the 1–10 μM range) against the wild-type P388 leukemia and were inactive against it in vivo. It was felt that selection of inactive carrier moieties would provide more clear-cut answers about the utility of DNA-targeting the cisplatin unit. In previous studies[18] where carrier units such as doxorubicin were used, it was difficult to assess the individual contributions of the carrier and the cisplatin moieties to the biological activity of the compounds. However, the ligands were significantly more cytotoxic (3–5-fold) toward the cisplatin-resistant P388.

The ethylenediamine-linked Pt complexes **9b–9e** were generally slightly more cytotoxic (3–5-fold) than the corresponding ligands toward the wild-type line but, unlike the standards (**1**, **3**, **4**), exhibited no differential toxicity between the wild-type and cisplatin-resistant lines. Propanediamine-linked Pt complexes **11a–11e** did in general show significant differentials (up to 8-fold) in favor of the cisplatin-resistant line, although these were no greater than those shown by the unplatinated ligands themselves.

While cisplatin (**1**) and the two standards (**3** and **4**) showed in vivo activity against the wild-type P388 leukemia, none of the acridine-linked cisplatin complexes **9b–9e** or **11a–11e** were active. However, carboxamide derivative **24** showed low but significant in vivo activity.

Conclusions

Although a very large number of platinum complexes have been tested for antitumor activity, there have been few studies on the consequences of DNA-targeting. In this paper we show that such targeting has relatively little effect on the intrinsic cytotoxicity of two types of cisplatin complexes. This is in contrast to the DNA-targeting of aniline mustards, where very large increases in in vitro cytotoxicity have been observed.[16]

However, the primary requirement for new platinum analogues is demonstration of activity in platinum-resistant lines,[30] and 1,3-propanediamine complexes **11a–11e** did show preferential cytotoxicity toward the cisplatin-resistant P388. Nevertheless, while this is encouraging, it is not possible to decide the role of the carrier moiety in this effect. Although a noncytotoxic carrier (9-anilinoacridine) was deliberately chosen, the ligands themselves showed some selective toxicity toward the resistant line.

However, none of the acridine complexes proved active in vivo even against the wild-type P388 leukemia, so that further testing against the resistant line in vivo was unwarranted. The complexes were very insoluble, and although a suitable formulation was found, this may have been a factor. The more soluble carboxamide derivative **24** did show significant (albeit low) in vivo antileukemic activity in the wild-type line.

Finally, it should be noted that the preferred binding orientation of 9-anilinoacridines on DNA is not settled,[31] and the possibility that these compounds bind in such a way as to direct the Pt away from the major groove cannot be ruled out. Thus future work in this area will be directed toward more soluble DNA-targeted Pt complexes of varying topologies.

Experimental Section

Where elemental analyses are indicated by the symbols of the elements, results were within ±0.4% of theoretical. Melting-points were determined on an Electrothermal apparatus using the supplied stem-corrected thermometer and are as read. ^1H NMR

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spectra were measured on Bruker WP-60 or AM-400 spectrometers. ^{195}Pt NMR spectra were determined at 86.0 MHz for solutions (ca. 1.5×10^{-2} M) in DMF, with an external D_2O lock. Spectra were collected by using a 10-μs pulse width and acquisition times from 0.123 to 0.4 s, with spectral widths from 11 to 33 kHz. Shifts are referenced to Na_2PtCl_6 in H_2O as 0 ppm. Mass spectra were determined for propane-1,3-diamine complexes **11a–11e** in the FAB mode from a DMF/glycerol/0.5N HCl matrix, on a Varian VG70-SE mass spectrometer. The complexes all displayed molecular ions corresponding to M + 1, with an isotopic cluster pattern characteristic of the $PtCl_2$ unit.

Synthesis of 1,2-Diaminoethane Ligands. Preparation of 8e: General Example. 5-(4′-Nitrophenyl)pentyl Methanesulfonate (14e). Borane–methyl sulfide complex (Aldrich; 10 M solution, 3.0 mL, 30 mmol) was added at 0 °C under N_2 to a stirred solution of 5-(4′-nitrophenyl)pentanoic acid (**12e**, 5.01 g, 22.5 mmol) in dry THF (60 mL). After stirring for a further 1 h at 20 °C, MeOH (30 mL) was added, and after completion of gas evolution, the mixture was evaporated under reduced pressure. The residue was partitioned between CH_2Cl_2 and 0.5% NaOH, and the organic layer was washed with water, dried, and evaporated to give crude alcohol **13e** as a colorless oil (4.58 g, 97% yield): ^1H NMR ($CDCl_3$) δ 8.15 (d, J = 8.7 Hz, 2 H, H-3′,5′), 7.33 (d, J = 8.7 Hz, 2 H, H-2′,6′), 3.65 (t, J = 6.5 Hz, 2 H, H-1), 2.77 (t, J = 7.7 Hz, 2 H, H-5), 1.73–1.40 (m, 6 H, H-2,3,4), 1.35 (br s, 1 H, exch with D_2O, OH).

Treatment of this alcohol (4.5 g) with methanesulfonyl chloride (7 mL) in pyridine (20 mL) at 20 °C for 3 h, followed by partitioning between CH_2Cl_2 and aqueous NaCl, gave the crude methanesulfonate. Chromatography on silica and elution with CH_2Cl_2 gave pure **14e** (6.1 g, 91% yield), which crystallized from EtOAc as colorless needles: mp 56–57 °C; ^1H NMR ($CDCl_3$) δ 8.15 (d, J = 8.5 Hz, 2 H, H-3′,5′), 7.35 (d, J = 8.5 Hz, 2 H, H-2′,6′), 4.25 (t, J = 6.4 Hz, 2 H, H-1), 3.02 (s, 3 H, SO_2Me), 2.77 (t, J = 7.6 Hz, 2 H, H-5), 1.90–1.40 (m, 6 H, H-2,3,4). Anal. ($C_{12}H_{17}NO_5S$) C, H, N.

Similar reactions starting with 2-(4′-nitrophenyl)acetic acid (**12b**), 3-(4′-nitrophenyl)propanoic acid (**12c**), and 4-(4′-nitrophenyl)butyric acid (**12d**) gave, respectively, 2-(4′-nitrophenyl)-ethyl methanesulfonate (**14b**) as an oil, 3-(4′-nitrophenyl)propyl methanesulfonate (**14c**) as an oil, and 4-(4′-nitrophenyl)butyl methanesulfonate (**14d**): mp ($EtOAc/CH_2Cl_2$) 52.5–53.5 °C; ^1H NMR ($CDCl_3$) δ 8.15 (d, J = 8.8 Hz, 2 H, H-3′,5′), 7.35 (d, J = 8.8 Hz, 2 H, H-2′,6′), 4.27 (m, 2 H, H-1), 3.05 (s, 3 H, SO_2CH_3), 2.78 (m, 2 H, H-4), 1.8 (m, 4 H, H-2,3).

***N*-[5-[4′-[9″-Acridinylamino]phenyl]pentyl]ethanediamine Trihydrochloride (8e).** Ethane-1,2-diamine (25 mL, large excess) was added slowly to a solution of methanesulfonate **14e** (2.2 g, 7.67 mmol) in MeOH (50 mL) kept at 0 °C, and the reaction was stirred for a further 22 h at 20 °C. Most of the solvent was removed under reduced pressure, and the residue was diluted with CH_2Cl_2 (200 mL) and washed with dilute aqueous $NaHCO_3$ and water to give crude amine **15e** as an orange oil (1.8 g, 93% yield): ^1H NMR ($CDCl_3$) δ 8.15 (d, J = 8.8 Hz, 2 H, H-3′,5′), 7.35 (d, J = 8.8 Hz, 2 H, H-2′,6′), 2.82 (br t, J = 6.0 Hz, 2 H, $CH_2NHCH_2CH_2NH_2$), 2.73 (t, J = 7.5 Hz, 2 H, $CH_2NHCH_2CH_2NH_2$), 2.68 (br t, J = 6.0 Hz, 2 H, $CH_2NHCH_2CH_2NH_2$), 2.62 (t, J = 7.3 Hz, 2 H, $PhCH_2$), 1.78 (s,3 H, exch with D_2O, NH), 1.72–1.35 (m, 6 H, H-2,3,4).

Crude amine **15e** (1.8 g) was dissolved in MeOH and hydrogenated over Pd/C. The resulting solution was filtered directly into a flask containing 2 equiv of HCl in MeOH, and the solution was evaporated to dryness to give the crude dihydrochloride of triamine **16e** (2.1 g). This was dissolved in a mixture of MeOH (130 mL) and DMF (40 mL) and treated with 9-chloroacridine (1.62 g, 7.57 mmol) and 0.2 mL of concentrated HCl at 20 °C for 21 h. Dilution with EtOAc gave an orange solid which was collected and washed with EtOAc. The solid was dissolved in 0.5 N HCl and the aqueous layer was extracted with CH_2Cl_2 and then evaporated to dryness at low temperature (<40 °C) under reduced pressure. The resulting solid was crystallized from aqueous MeOH to give pure **8e** as the trihydrochloride salt (3.09 g, 80% yield): mp 276–277 °C; ^1H NMR (CD_3SOCD_3) δ 8.6–7.3 (m, 12 H, aromatic protons), 3.20 (br s, 4 H, CH_2NHCH_2), 2.95 (br s, 2 H, CH_2NH_2), 2.68 (t, J = 7.5 Hz, 2 H, H-5), 1.8–1.4 (m, 6 H, H-2,3,4). Anal. in Table I.

Similar treatment of methanesulfonates **14b–14d** gave, respectively, ***N*-[2-[4′-[9″-acridinylamino]phenyl]ethyl]-ethane-1,2-diamine trihydrochloride (8b)** [mp 215 °C dec; ^1H NMR (CD_3SOCD_3) δ 8.4–7.4 (m, 12 H, aromatic protons), 3.30 (br s, 6 H, $CH_2NHCH_2CH_2NH_2$), 3.10 (t, J = 7.9 Hz, 2 H, H-2). Anal. in Table I.], ***N*-[3-[4′-[9″-acridinylamino]phenyl]-propyl]ethane-1,2-diamine trihydrochloride (8c)** [mp 246–248 °C dec; ^1H NMR (CD_3SOCD_3) δ 8.5–7.3 (m, 12 H, aromatic protons), 3.23 (m, 4 H, $NHCH_2CH_2NH_2$), 2.98 (br t, J = 6.9 Hz, 2 H, H-1), 2.80 (br t, J = 7.7 Hz, H-3), 2.03 (m, 2 H, H-2). Anal. in Table I.], and ***N*-[4-[4′-[9″-acridinylamino]phenyl]butyl]ethane-1,2-diamine trihydrochloride (8d)** [mp 250–251 °C dec; ^1H NMR (CD_3SOCD_3) δ 9.00–7.25 (m, 12 H, aromatic protons), 3.25 (br s, 4 H, CH_2NHCH_2), 3.05 (br s, 2 H, CH_2NH_2), 2.75 (br s, 2 H, H-4), 1.75 (br s, 4 H, H-2,3). Anal. in Table I.].

Synthesis of 1,3-Diaminopropane Ligands. Preparation of 10a: General Example. Dimethyl 2-(4′-Nitrobenzyl)-malonate (18a). A suspension of hexane-washed NaH (2.91 g of a 60% dispersion in mineral oil, 73 mmol) in a mixture of THF (80 mL) and DMF (30 mL) was cooled to 0 °C under N_2 and treated dropwise with dimethyl malonate (7.9 mL, 69 mmol). After a further 10 min at 20 °C, a solution of 4-nitrobenzyl bromide (15.0 g, 69 mmol) in a mixture of THF (20 mL) and DMF (20 mL) was added, and the mixture was heated under gentle reflux for 1 h. The reaction was then poured into brine and extracted with EtOAc to give an oil, which was subjected to Kugelrohr distillation at 130 °C (20 mmHg) to remove unreacted dimethyl malonate. The residue was chromatographed on silica gel, with EtOAc/petroleum ether (1:4) eluting diester **18a** (11.3 g, 61% yield). Crystallization from $CHCl_3$/petroleum ether gave cubes: mp 82–84 °C; ^1H NMR ($CDCl_3$) δ 8.13 (d, J = 9 Hz, 2 H, ArH), 7.38 (d, J = 9 Hz, 2 H, ArH), 3.71 (br s, 7 H, $CH(COOCH_3)_2$), 3.31 (d, J = 7 Hz, 2 H, $ArCH_2$). Anal. ($C_{12}H_{13}NO_6$) C, H, N.

2-(4′-Nitrobenzyl)propane-1,3-diol (19a). A solution of borane–methyl sulfide complex (12.7 mL of a 10.5 N complex, 0.13 mol) was added to a solution of the above diester **18a** (11.3 g, 40 mmol) in THF (100 mL) under N_2, and the resulting solution was heated under reflux for 40 h. MeOH was added slowly to the cooled solution to destroy excess reagent, and the mixture was diluted with brine and extracted with EtOAc. The residue from workup of the organic layer was chromatographed on silica gel. After foreruns, EtOAc/petroleum ether (4:1) eluted diol **19a** (8.36 g, 94% yield) as a viscous yellow oil: ^1H NMR ($CDCl_3$) δ 8.15 (d, J = 9 Hz, 2 H, ArH), 7.35 (d, J = 9 Hz, 2 H, ArH), 3.77 (m, 4 H, CH_2OH), 2.80 (d, J = 7 Hz, 2 H, $ArCH_2$), 2.50 (br s, 2 H, OH), 2.12 (m, 1 H, CH); mass spectrum, m/e 193 (M – H_2O, 18), 174 (M – $2H_2O$, 62), 163 (48), 152 (47), 150 (63), 149 (55), 146 (21), 145 (42), 144 (37), 137 (26), 133 (19), 117 (39), 116 (100), 115 (95), 91 (68), 77 (47), 63 (32).

2-(4′-Aminobenzyl)propane-1,3-diamine (22a). A solution of the above diol **19a** (8.0 g, 37.8 mmol) in CH_2Cl_2 (150 mL) and Et_3N (15.8 mL, 113 mmol) was treated dropwise at 0 °C with methanesulfonyl chloride (6.5 mL, 83.3 mmol). After an additional 15 min the solution was washed with water, aqueous $NaHCO_3$, and brine, and worked up to give crude dimesylate **20a**, which was homogeneous on TLC. This was immediately dissolved in DMF (30 mL), NaN_3 (15 g, 0.23 mol) was added, and the suspension was stirred at 120 °C for 1 h and then poured into brine. Extraction with EtOAc, thorough washing of the organic layer with brine, and workup gave an oil, which was chromatographed on silica gel. Elution with EtOAc/petroleum ether (1:9) gave diazide **21a** as a viscous yellow oil (3.16 g, 32% yield from the diol). The compound was pure by TLC, and was used directly. Diazide **21a** (3.16 g, 12 mmol) was dissolved in MeOH (100 mL) and hydrogenated over 10% Pd/C (0.5 g) at 60 psi for 20 h. The catalyst was removed by filtration and washed well with MeOH, and the combined filtrates were immediately saturated with HCl gas and concentrated to dryness under reduced pressure. The resulting solid was dried under vacuum and recrystallized from $MeOH/Et_2O$ to give the trihydrochloride of **22a** as a hygroscopic white solid (3.04 g, 87% yield): mp 220–235 °C (solvent loss at 110 °C); ^1H NMR (D_2O) δ 7.50 (d, J = 8.6 Hz, 2 H, ArH), 7.45 (d, J = 8.6 Hz, 2 H, ArH), 3.21 (dd, J = 13.5, 6.6 Hz, 2 H, $CHHNH_3$), 3.10 (dd, J = 13.5, 6.8 Hz, 2 H, $CHHNH_3$), 2.93 (d, J = 7.5 Hz, 2 H, $ArCH_2$), 2.55 (sp, J = 6.8 Hz, 1 H, CH). Anal. ($C_{10}H_{17}N_3 \cdot 3HCl$) C, H, N, Cl.

2-[4'-(9''-Acridinylamino)benzyl]propane-1,3-diamine (10a). 9-Chloroacridine (1.13 g, 5.30 mmol) was added to a solution of triamine trihydrochloride **22a** (1.53 g, 5.30 mmol) in MeOH (40 mL) containing 3 drops of concentrated HCl. The mixture was stirred at 20 °C for 18 h and then diluted with EtOAc. The precipitate was collected and dried under vacuum, and the resulting hygroscopic orange solid was dissolved in MeOH (40 mL). Et_2O (200 mL) was then added and the mixture was cooled to give a granular orange solid. Final recrystallization from MeOH/iPr_2O gave the trihydrochloride of **10a** as a hygroscopic orange powder (2.11 g, 85% yield): mp 270 °C dec; ^1H NMR (CD_3SOCD_3) δ 15.0 (br, 1 H, NH), 11.7 (br, 1 H, NH), 8.44 (br, NH), 8.27 (d, J = 8.8 Hz, 2 H, acridine-H), 8.15 (d, J = 8.5 Hz, 2 H, acridine-H), 7.99 (dd, J = 8.8, 8.2 Hz, 2 H, acridine-H), 7.48 (dd, J = 8.5, 8.2 Hz, 2 H, acridine-H), 7.47 (d, J = 6.8 Hz, 2 H, ArH), 7.43 (d, J = 6.8 Hz, 2 H, ArH), 3.01 (dd, J = 12.8, 5.6 Hz, 2 H, C*H*HNH$_3$), 2.87 (d, J = 6.36 Hz, 2 H, ArCH_2), 2.80 (dd, J = 12.8, 5.6 Hz, 2 H, CH*H*NH$_3$), 2.47 (sp, J = 6.3 Hz, 1 H, CH). Anal. in Table I.

Synthesis of 10b. A mixture of mesylate **14b** (26.3 g, 0.11 mol) and anhydrous NaI (50 g, 0.33 mmol) in Me_2CO (200 mL) was stirred vigorously at 20 °C in the dark for 18 h. The mixture was evaporated to dryness and the residue was partitioned between EtOAc and water. Workup of the organic layer gave a yellow solid which was chromatographed on silica gel. Elution with EtOAc/petroleum ether (1:9) gave 1-iodo-2-(4'-nitrophenyl)ethane (**17b**; 21.4 g, 70% yield). Crystallization from benzene/petroleum ether gave white plates: mp 96–98 °C (lit.[32] mp 97 °C); ^1H NMR ($CDCl_3$) δ 8.17 (d, J = 8 Hz, 2 H, ArH), 7.34 (d, J = 8 Hz, 2 H, ArH), 3.31 (br s, 4 H, CH_2I, ArCH_2).

Treatment of this as above for 4-nitrobenzyl bromide gave malonate **18b** in 93% yield as an oil: ^1H NMR ($CDCl_3$) δ 8.14 (d, J = 8 Hz, 2 H, ArH), 7.35 (d, J = 8 Hz, 2 H, ArH), 3.75 (s, 6 H, OMe), 3.41 (t, J = 6 Hz, 1 H, C*H*(COOMe)$_2$), 2.75 (m, 2 H, ArCH_2), 2.25 (m, 2 H, CH_2CH_2CH). This in turn gave a 69% yield of diol **19b** as an oil: ^1H NMR ($CDCl_3$) δ 8.13 (d, J = 9 Hz, 2 H, ArH), 7.37 (m, J = 9 Hz, 2 H, ArH), 3.72 (d, J = 6 Hz, 4 H, CH_2O), 2.85 (t, J = 8 Hz, 2 H, ArCH_2), 1.75 (m, 3 H, CH_2C*H*). Reaction of the diol via intermediates **20b** and **21b** gave the trihydrochloride of 2-[2'-(4''-aminophenyl)ethyl]propane-1,3-diamine (**22b**) as a hygroscopic solid: mp 230–240 °C; ^1H NMR (D_2O) δ 7.46 (d, J = 8.5 Hz, 2 H, ArH), 7.40 (d, J = 8.5 Hz, 2 H, ArH), 3.16 (d × q, J = 12.8, 6.5 Hz, 4 H, CH_2NH$_3$), 2.76 (t, J = 7.4 Hz, 2 H, ArCH_2), 2.25 (sp, J = 6.5 Hz, 1 H, CH), 1.74 (m, 2 H, CH_2CH$_2$CH), 1.59 (m, 2 H, CH_2CH_2CH). Reaction of this with 9-chloroacridine gave the trihydrochloride of **10b** as a hygroscopic orange powder: mp 260 °C dec; ^1H NMR (CD_3SOCD_3) δ 15.00 (br, 1 H, NH), 11.75 (br, 1 H, NH), 8.33 (br, NH), 8.30 (d, J = 9.0 Hz, 2 H, acridine-H), 8.16 (d, J = 8.4 Hz, 2 H, acridine-H), 7.99 (dd, J = 9.0, 8.5 Hz, 2 H, acridine-H), 7.44 (dd, J = 8.5, 8.4 Hz, 2 H, acridine-H), 7.43 (d, J = 8.5 Hz, 2 H, ArH), 7.39 (d, J = 8.5 Hz, 2 H, ArH), 3.01 (m, 4 H, CH_2NH$_3$), 2.71 (t, J = 8.1 Hz, 2 H, ArCH_2), 2.17 (sp, J = 6.5 Hz, 1 H, CH), 1.79 (m, 2 H, CH_2). Anal. in Table I. The analogous triamines **10c–10e** were prepared similarly, and all had consistent NMR spectra and analyses (Table I).

Similar reaction of *N*-[(*N*,*N*-dimethylamino)ethyl]-5-methoxy-9-chloroacridine-4-carboxamide[33] with triamine **22c** gave ligand tetrahydrochloride **23** as an orange powder: mp 240–245 °C; ^1H NMR (CD_3SOCD_3) δ 14.72, 12.12, 10.81, 10.00 (each 1 H, exch with D_2O, NH and CONH) 8.89 (d, J = 7.3 Hz, 1 H, acridine-H), 8.58 (d, J = 8.4 Hz, 1 H, acridine-H), 8.34 (br s, 4 H, ArH), 7.83 (d, J = 8.7 Hz, 1 H, acridine-H), 7.52 (br t, J = 8.5 Hz, 2 H, acridine-H), 7.32 (d, J = 9.1 Hz, 1 H, acridine-H), 4.13 (s, 3 H, OMe), 3.80 (t, J = 7.1 Hz, 1 H, CONHCH_2), 3.43 (t, J = 7.1 Hz, 2 H, CH_2NMe$_2$), 2.98 (m, 2 H, CH_2NH$_2$), 2.87 (br s, 8 H, CH_2NH$_2$ and NMe$_2$), 2.67 (t, J = 6.8 Hz, 2 H, ArCH_2), 2.16 (m, 1 H, CH), 1.65 and 1.50 (2 m, 4 H, CH_2CH_2). Anal. ($C_{31}H_{40}N_6O_2$·4HCl).

Platinations. Synthesis of [*N*-[2-[4'-[9''-acridinylamino]phenyl]ethyl]ethane-1,2-diamine]dichloroplatinum(II) (9b). Trihydrochloride **8b** (141 mg, 0.30 mmol) was dissolved in water (1 mL). Dilute (1–2 N) aqueous Na_2CO_3 was added dropwise until a pH of 8–9 was achieved, with the coaddition of the minimum amount of MeOH to maintain a homogeneous solution. The basic, homogeneous mixture was immediately added dropwise to a solution of K_2PtCl_4 (126 mg, 0.30 mmol) in water (1.5 mL) at 20 °C, giving an immediate bright orange precipitate. The reaction was stirred for a further 2.5 h at 20 °C, then 5% aqueous KCl (30 mL) was added and the mixture was stirred for an additional 30 min. The precipitate was collected, washed well with deionized water, and dried to give **9b** as a reddish orange solid, pure by TLC (150 mg, 80% yield): mp 293–296 °C dec; ^{195}Pt NMR δ −2345. An analytical sample was obtained by chromatography on silica gel and elution with CH_2Cl_2/MeOH (10:1), mp 290 °C dec. Anal. in Table I.

Similar reactions gave **[*N*-[3-[4'-[9''-acridinylamino]phenyl]propyl]ethane-1,2-diamine]dichloroplatinum(II) (9c)** [Red-orange solid (71% yield); mp 292 °C dec; ^{195}Pt NMR δ −2344; analytical sample, mp 295 °C dec. Anal. ($C_{24}H_{26}N_4Cl_2Pt$·3H_2O) C, H, N.], **[*N*-[4-[4'-[9''-acridinylamino]phenyl]butyl]ethane-1,2-diamine]dichloroplatinum(II) (9d)** [Red-orange solid (88% yield); mp 279–282 °C dec; ^{195}Pt NMR δ −2338; analytical sample, mp 281–284 °C dec. Anal. ($C_{25}H_{28}Cl_2N_4Pt$) C, H, N.], and **[*N*-[5-[4'-[9''-acridinylamino]phenyl]pentyl]ethane-1,2-diamine]dichloroplatinum(II) (9e)** [Red-orange solid (83% yield); mp 263 °C dec; ^{195}Pt NMR δ −2339; analytical sample, mp 270 °C dec. Anal. ($C_{26}H_{30}Cl_2N_4Pt$) C, H, N.].

Synthesis of [2-[3-[4'-[9''-Acridinylamino]phenyl]propyl]propane-1,3-diamine]dichloroplatinum(II) (11c). A solution of K_2PtCl_4 (0.45 g, 1.10 mmol) and $NaHCO_3$ (0.27 g, 3.28 mmol) in water (3 mL) was added over 30 s to a solution of ligand trihydrochloride **10c** (0.54 g, 1.10 mmol) in MeOH (20 mL) and water (3 mL). After stirring for 2 h in the dark, the supernatant liquid was decanted off, and the residue was washed with 1:1 MeOH/water (2×). Saturated aqueous KCl (30 mL) was then added, and the mixture was stirred vigorously in the dark for 24 h to give a fine orange precipitate which was filtered off and dried under reduced pressure over silica gel. This crude complex was dissolved in DMF, filtered, and precipitated out by addition of iPr_2O. The precipitate was collected and washed well with MeOH and Et_2O and crystallized again from DMF/iPr_2O. These crystals were washed well with Et_2O to give **11c** as an orange powder (0.41 g, 57%): mp 230–240 °C dec; ^{195}Pt NMR δ −2274; FAB mass spectrum, m/z 649, 650, 651, 652, 653, 654, 655 (M + 1). The hydrochloride salt was precipitated as an orange powder by addition of dilute HCl to a DMF solution of the complex, mp 235–250 °C dec.

Similar reactions gave **[2-[4'-[9''-acridinylamino]benzyl]propane-1,3-diamine]dichloroplatinum(II) (11a) hydrochloride** [Mp 250–260 °C dec; ^{195}Pt NMR δ −2276, FAB mass spectrum, m/z 620, 622, 623, 624, 625, 626, 627 (M + 1).], **[2-[2-[4'-[9''-acridinylamino]phenyl]ethyl]propane-1,3-diamine]dichloroplatinum(II) (11b) hydrochloride** [Mp 250–275 °C dec; ^{195}Pt NMR δ −2274.5; FAB mass spectrum, m/z 635, 636, 637, 638, 639, 640, 641 (M + 1).], **[2-[4-[4'-[9''-acridinylamino]phenyl]butyl]propane-1,3-diamine]dichloroplatinum(II) (11d) hydrochloride** [Mp 220–225 °C dec; ^{195}Pt NMR δ −2275.5; FAB mass spectrum, m/z 663, 664, 665, 666, 667 (M + 1).], **[2-[5-[4'-[9''-acridinylamino]phenyl]pentyl]propane-1,3-diamine]dichloroplatinum(II) (11e) hydrochloride** [Mp 210–220 °C dec; ^{195}Pt NMR δ −2275; FAB mass spectrum, m/z 677, 678, 679, 680, 681 (M + 1)], and the dihydrochloride of **23** [Mp 270–280 °C dec; ^{195}Pt NMR δ −2275; FAB mass spectrum, m/z 793, 795, 796, 797 (M + 1)].

Characterization of Platinum Complexes by TLC and HPLC. Ethylenediamine complexes **9b–9e** gave single spots when run on C_{18} reverse-phase bonded silica TLC plates, with saturated MeOH/saline/glycerol (40:40:20 %v/v, 0.1 M in NaCl) as mobile phase, and single peaks in the HPLC (using UV detection at 254 nm). In each case, the values varied as expected with chain length (Table II). Propanediamine compounds **11a–11e** showed an additional minor yellow spot on TLC, suggesting the presence of a second anilinoacridine species which was not the free ligand

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(all of which had R_f values >0.9, compared with R_f values for the complexes of 0.4–0.7).

HPLC studies were performed with a Waters Associates 600 multisolvent delivery system and 712 WISP automatic sample injector, with a Hewlett-Packard 1040A diode-array detector (wavelength range 190–600 nm) directly connected in line. The column was a Waters Associates Novapak C_{18} reverse-phase bonded silica cartridge, and the detector response was monitored with Hewlett-Packard Chemstation software. The mobile phase was saturated aqueous NaCl/glycerol/H_2O/MeOH (1:1:1:2) adjusted to pH 4.00 with 1 N HCl.

The platinum content of collected column fractions was determined by flameless atomic absorption spectroscopy, using a Varian SpectrAA 20 spectrometer fitted with graphite furnace and autosampler.

Formation of Compounds for Biological Testing: Example. The free base of **9b** (23 mg) was suspended in dimethylacetamide (0.5 mL), and 0.5 mL of glycerol was added to give a homogeneous orange solution. Water (1–2 mL) was added last to make up the required concentration.

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DNA-Directed Alkylating Agents. 3. Structure–Activity Relationships for Acridine-Linked Aniline Mustards: Consequences of Varying the Length of the Linker Chain

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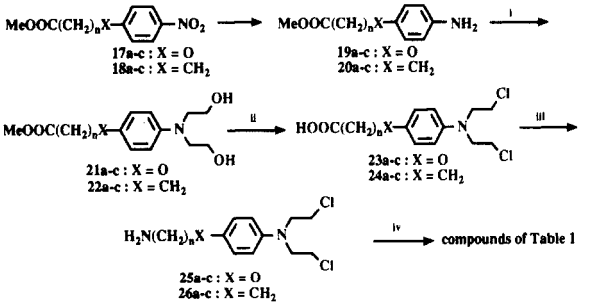
Four series of acridine-linked aniline mustards have been prepared and evaluated for in vitro cytotoxicity, in vivo antitumor activity, and DNA cross-linking ability. The anilines were attached to the DNA-intercalating acridine chromophores by link groups (–O–, $-CH_2-$, –S–, and $-SO_2-$) of widely varying electronic properties, providing four series of widely differing mustard reactivity where the alkyl chain linking the acridine and mustard moieties was varied from two to five carbons. Relationships were sought between chain length and biological properties. Within each series, increasing the chain length did not alter the reactivity of the alkylating moiety but did appear to position it differently on the DNA, since cross-linking ability (measured by agarose gel assay) altered with chain length, being maximal with the C_4 analogue. The in vivo antitumor activities of the compounds depended to some extent on the reactivity of the mustard, with the least reactive SO_2 compounds being inactive. However, DNA-targeting did appear to allow the use of less reactive mustards, since the S-linked acridine mustards showed significant activity whereas the parent S-mustard did not. Within each active series, the most active compound was the C_4 homologue, suggesting some relationship between activity and extent of DNA alkylation.

Several recent papers[1-3] have focused on the concept[4,5] of targeting alkylating agents to DNA by attaching them to DNA-intercalating ligands as DNA-affinic carriers. The aims of such an approach include increasing intrinsic drug potency,[1,6] avoiding some of the common mechanisms of cellular resistance to alkylating agents,[7] and altering the pattern of DNA lesions formed[8,9] and their repair.

We have recently shown[1] that the intrinsic cytotoxicities of simple aniline mustards can be drastically increased (up to 100-fold) by attaching them to the classic DNA-affinic intercalator 9-aminoacridine. The resulting compounds varied primarily in the reactivity of the mustard group (controlled by varying the electronic nature of the link group X), and the results showed that DNA-targeting decreased the usual tight dependence of cytotoxicity on mustard reactivity. While the untargeted aniline mustards showed a variation in cytotoxicity of about 50-fold between the most reactive compound (**1a**) and the least reactive one (**13a**), those of the corresponding targeted mustards **4** and **16** varied by less than 3-fold.[1] Most of the DNA-targeted mustards showed in vivo antitumor activity, being both more dose potent and more active than the clinically used

Scheme I[a]



[a] (i) oxirane/H^+; (ii) MsCl, LiCl/DMF/Δ H^+; (iii) EtOCOCl, NaN_3, H^+; (iv) 9-methoxyacridine.

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