Synthesis and Cyclization Reactions of 4-[Bis(2-Hydroxyethyl)amino]-9-(3-Dimethylaminopropylamino)-1-Nitroacridines: Approaches to the Synthesis of 'Nitracrine Mustard'

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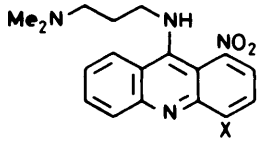
Compounds which can be selectively activated to cytotoxic species in hypoxic mammalian cells have potential for the treatment of solid tumours, which uniquely contain cells in such an environment. One approach to such drugs is exemplified by the title compound (**2**), 'Nitracrine Mustard,' where selective reduction of the nitro group in hypoxic cells (known to occur in the case of Nitracrine itself) would activate the mustard moiety. Synthetic routes to the penultimate dihydroxy derivative (**22**) are reported. Attempts to prepare the mustard derivative for this and a series of analogous compounds were frustrated by rapid intramolecular cyclization reactions to give the dihydroxpyrazinoacridines (**25**).

One of the major reasons for the poor response of many solid tumours to chemotherapy is the existence of hypoxic, drug-resistant cell populations in the interior of these tumours.[1-3] Thus, there has been increasing interest in the design of drugs which will be activated only in such hypoxic regions.[3-5] Much work with nitroaromatic compounds has shown that if the nitro group has a suitable redox potential (E_7^1 in the range approximately -200 to -400 mV), selective reduction will occur in hypoxic cells. In the two most well-documented cases of Misonidazole[6,7] and Nitracrine (**1**),[8] reduction of the nitro group appears to be followed by fragmentation of the molecule to DNA-alkylating species, but these fragmentations are poorly understood. A more direct approach to hypoxia-selective nitroaromatics would be to design compounds with preformed alkylating functions, *e.g.* a nitrogen mustard moiety. The mustard function would be expected to exhibit very low reactivity in the parent compounds owing to the electron-withdrawing properties of the nitro group, but would be activated upon nitro group reduction.

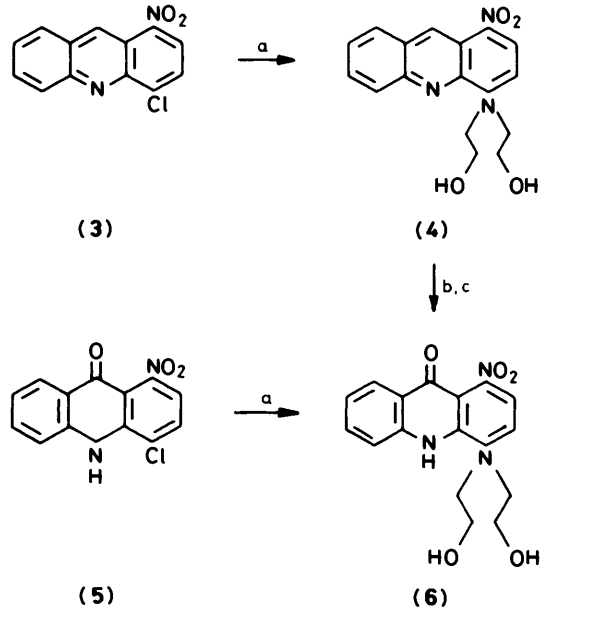


(**1**) X = H
(**2**) X = $N(CH_2CH_2Cl)_2$

For the above reasons, the 4-[bis(2-chloroethyl)amino] mustard derivative of Nitracrine (**2**: 'Nitracrine mustard') seemed an attractive candidate. Nitracrine itself has a redox potential of -250 mV,[9] towards the higher end of the suggested acceptable range, and has been shown to be rapidly and selectively metabolised in hypoxic mammalian cells.[8] Attachment of the mildly electron-donating $-N(CH_2CH_2Cl)_2$ function (σ_p approximately -0.10)[10] *para* to the nitro group would be expected to reduce this reduction potential only slightly. Cellular reduction of the nitro group of (**2**) (σ_p 0.78) to the amine (σ_p -0.66) or even to the hydroxylamine, (σ_p -0.32) would provide a very large electron release to the mustard group, even if the ring did not fragment.

Results and Discussion

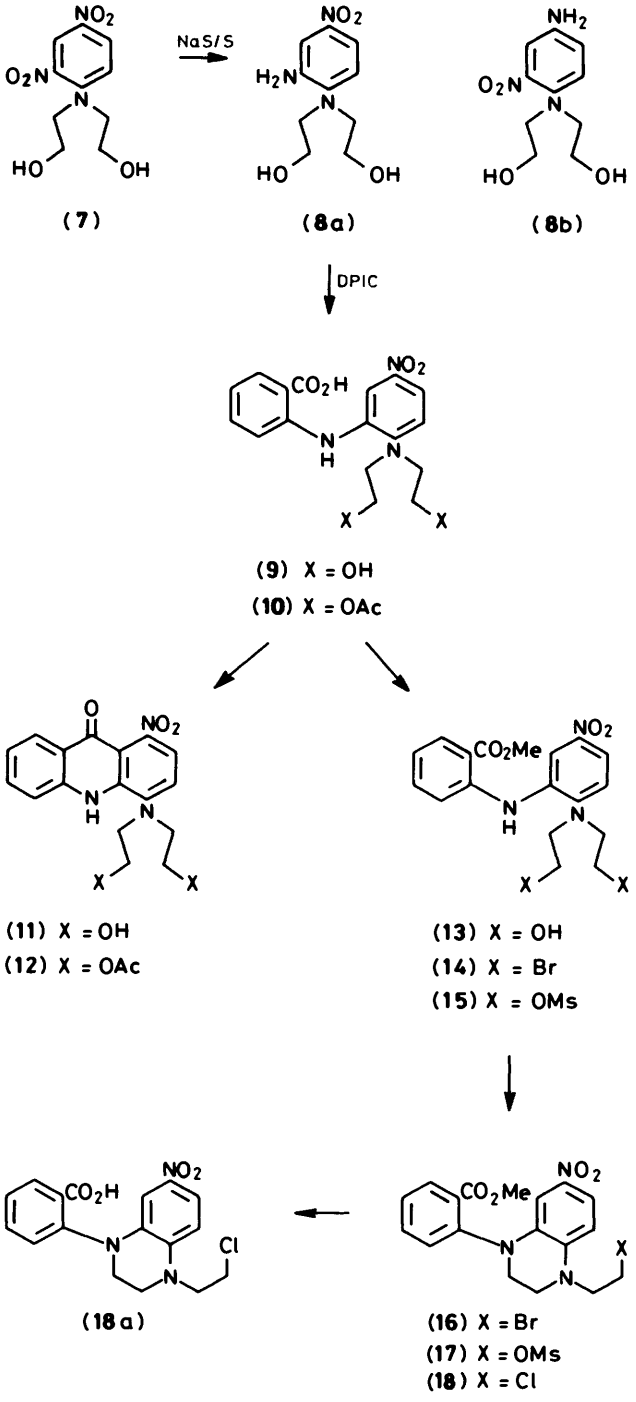
Our initial objective was the synthesis of the suitably functionalized acridone derivative (**6**). Direct replacement of the halogen atom of 4-halogeno-1-nitroacridines (**3**) and 4-halogeno-1-nitroacridones (**5**) with 2,2'-iminodiethanol (Scheme 1) was not successful. Formation of (**6**) *via* a suitably functionalized 2,2'-iminodiethanol acid was then investigated (Scheme 2). Selective reduction of *N,N*-bis(2-hydroxyethyl)-2,4-dinitroaniline (**7**) with sodium sulphide/sulphur in aqueous ethanol gave only one detectable product, 2-[bis(2-hydroxyethyl)amino]-5-nitroaniline (**8a**), in 63% yield, after recrystallization. The initial tentative assignment of structure [(**8a**) rather than (**8b**)] was made by analogy with similar reductions of the *N,N*-dimethylamino analogue,[11,12] since the ^{1}H n.m.r. data were inconclusive. This structure was later confirmed (*vide infra*).



Scheme 1. *Reagents and Conditions:* a, diethanolamine/Heat; *b*, S, Heat, OH^-; *c*, H^+

The normal Jourdan–Ullmann condensation[13] of (**8a**) with 2-halogenobenzoic acids was not successful, but use of diphenyliodonium-2-carboxylate (DPIC)[14] and Cu^{II} acetate under non-basic conditions in DMF at 90—100 °C gave the desired acid (**9**) (93%).

We decided to investigate elaborating the mustard moiety at this point in the synthesis, since it would be stabilized by the



Scheme 2.

para nitro group. The methyl ester (**13**) was prepared, and a number of different methods for functionalizing the hydroxy groups were attempted. However, owing to rapid intramolecular alkylation of the diphenylamine nitrogen, the only isolated products were various 1-substituted 4-(2-methoxycarbonylphenyl)-2,3-dihydroquinoxalines (**16**)—(**18**). Thus, Vilsmeier bromination of (**13**) gave only (**16**) [presumably *via* the dibromide (**14**)]. Mesylation of (**13**) (MsCl/pyridine) gave an unstable product (t.l.c.) tentatively identified as the dimesylate (**15**); this was rapidly converted into the oily monomesylate (**17**), and this crude product gave a good yield of the same bromo derivative (**16**) on treatment with NaBr–acetone. Similarly, treatment of the monomesylate with NaCl–DMF afforded (**18**), which on hydrolysis gave the corresponding acid (**18a**).

Since formation of the mustard at the diphenylamine acid stage was not possible owing to rapid intramolecular alkylation, the next goal was the dihydroxyacridine (**22a**), the direct precursor to (**2**). Two broad methods for elaborating 9-alkylaminoacridines from precursor diphenylamine acids have been described,[13] and both were successfully used. Although the dihydroxy compound (**9**) could not be cyclized with any of the usual dehydrating agents ($SOCl_2$, $POCl_3$, H_2SO_4, PPA, or PPE), the corresponding diacetate (**10**) could be smoothly cyclized to (**12**) with PPE in 88% yield. Activation of the diacetoxyacridone (**12**) with $POCl_3$ gave the crystalline 9-chloro derivative (**19**), which reacted smoothly with 3-dimethylaminopropylamine in hot phenol to give the acridine (**21a**). This compound could also (and more expeditiously) be made from (**10**) by treatment with $SOCl_2$ in benzene (to form the acid chloride without ring closure), followed by amine treatment to give (**20a**). Cyclodehydration with PPE gave (**21a**) in good yield, and deacetylation with methanolic KOH gave the desired compound (**22a**).

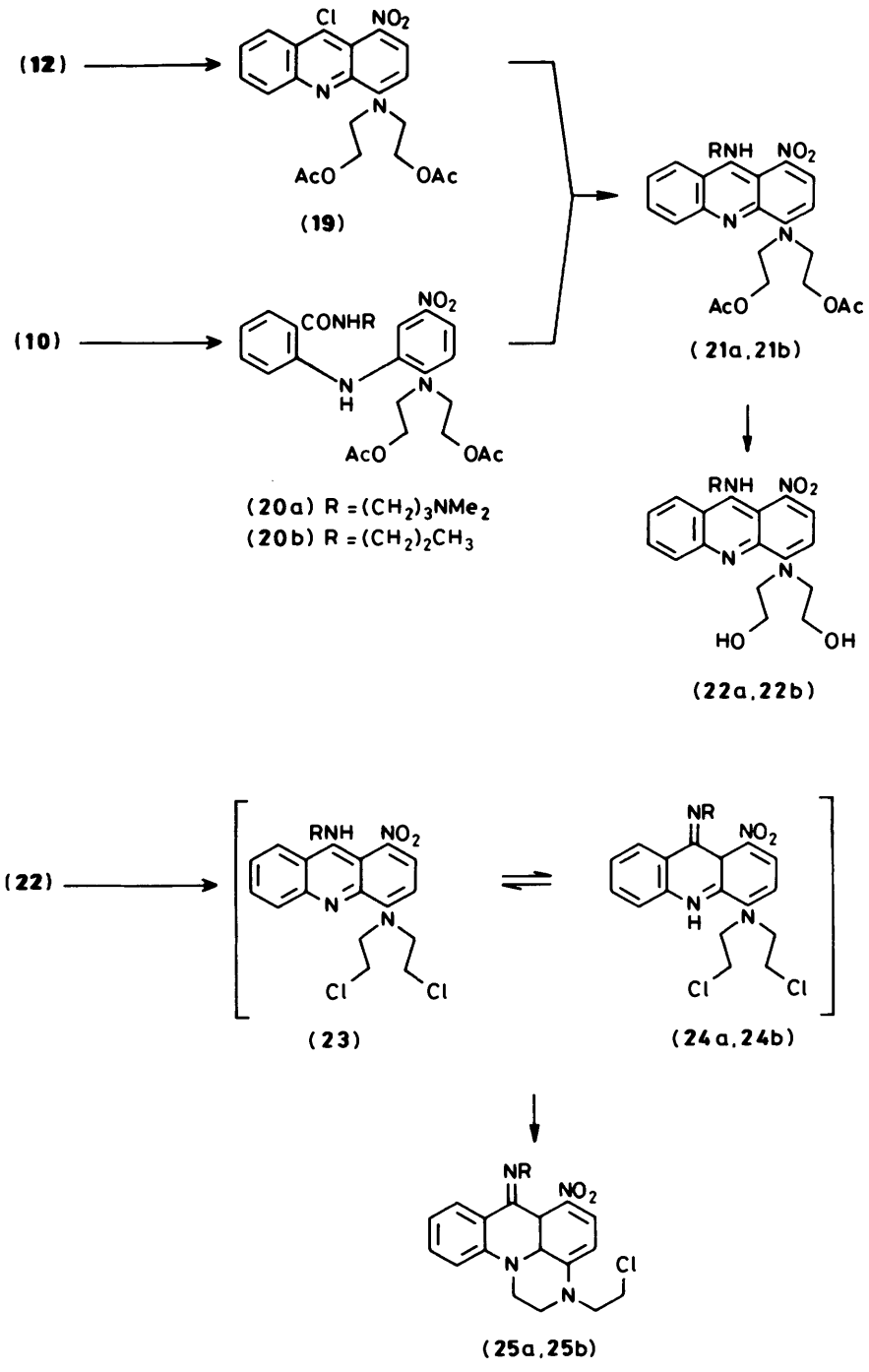
Treatment of (**22a**) with a number of halide-forming reagents [$POCl_3$–DMF: NaH–oxalyl chloride–DMF: CCl_4–PPh_3: MsCl–py: $SOCl_2$–dichloroethane: $(Tf)_2O$–py: SO_2Cl_2–py: I_2–PPh_3–imidazole: LDA–THF–oxalyl chloride: PPE–KI: and Vilsmeier salts under various conditions] gave only polymeric material and/or unchanged starting material (due to salt formation with the highly basic side chain and subsequent precipitation).

A model compound (**22b**), without this highly basic side chain, was thus prepared by a similar route, and this reacted smoothly with $SOCl_2$ and other halide-forming reagents (Vilsmeier salt, MsCl–Py–NaX–DMF) to give a single product in good yield. However, this was shown to be the pyrazinoacridine (**25b**) formed by internal cyclization of the (presumed) dichloride (**24b**).

This internal alkylation reaction provides proof of the structure of (**22b**), and thus shows the product of selective reduction of the dinitro compound (**7**) to have been the presumed regioisomer (**8a**) since the corresponding products (**26**) and (**27**) arising from (**8b**) could not take part in ring-forming reactions.

Spectroscopic and hydrolysis studies of 9-alkylaminoacridines have shown[15] that the presence of electron-withdrawing substituents such as NO_2 in the 1-position displace the prototropic equilibrium (**23**) $\rightleftharpoons$ (**24**) in favour of the iminoacridan form (**24**) and a crystal structure of nitracrine (**1**) clearly shows the compound to exist in this form.[16] The ring nitrogen is then suitably disposed to take part in intramolecular alkylation with a proximal leaving group. The rate of this reaction is governed by the nature of the leaving group. When this is chloro [as in (**2**)], the only isolated product is the ring-closed pyrazinoacridine (**25**), whereas the diacetate (**21**) is stable to refluxing dioxane under both neutral and acid conditions.

To determine whether the existence of the iminoacridan tautomer facilitated the reaction, the unsubstituted derivative (**4**) (which cannot undergo such prototropic equilibria) was synthesized. Previous attempts to prepare this compound by halogen displacement on 4-chloro-1-nitroacridine were not successful (see above and Scheme 1), and an alternative route was developed from (**10**) (Scheme 4). Reaction of the acid chloride of (**10**) with bis(triphenylphosphine)copper[I] tetrahydroborate[17] gave the aldehyde (**28**), which was subjected to a variety of cyclodehydration conditions. The best of these proved to be polyphosphoric acid at 90 °C for 1 h, but the yield of isolated product (**29**) was only 4%. Deacetylation of this gave the desired diol (**4**). Treatment of this diol with $SOCl_2$ in dichloroethane gave an immediate purple colouration, followed after several minutes by precipitation of a dark purple solid,



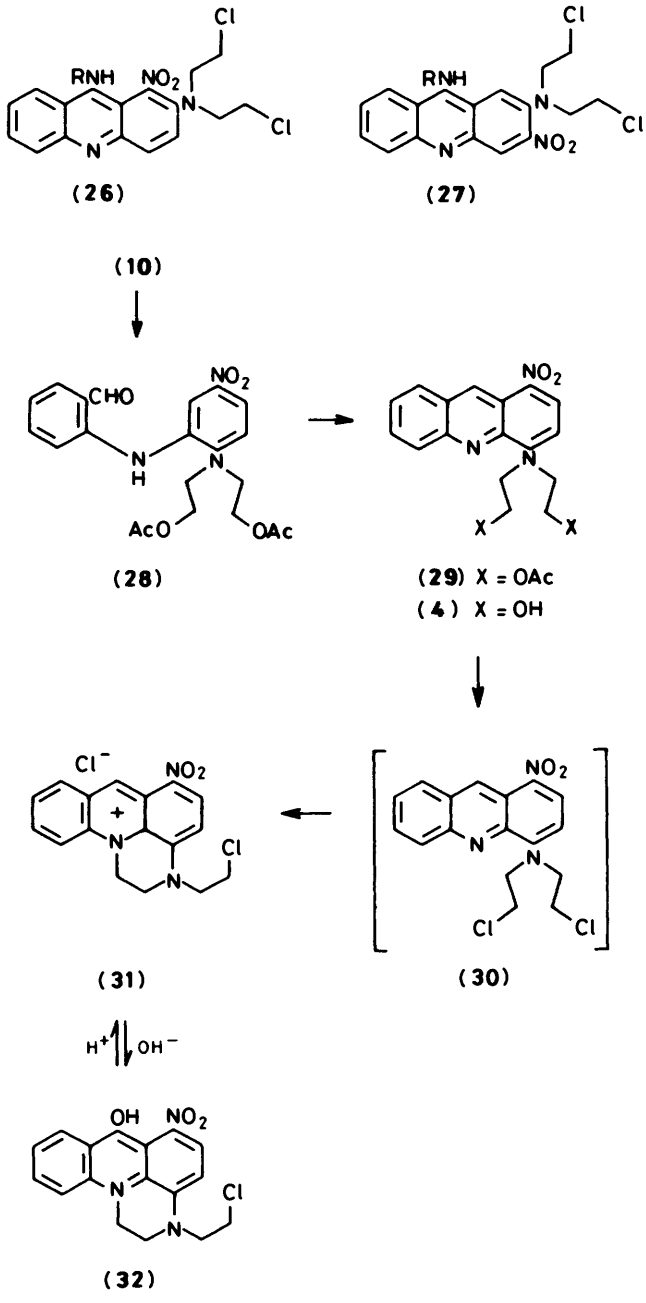
Scheme 3.

which was assigned the structure (**31**) on the basis of its physicochemical properties. Treatment with base gave an orange, organic-soluble material assigned the leuco base form (**32**), which upon mild acid treatment immediately regenerated the purple water-soluble quaternary salt (**31**).

Thus, rapid internal cyclization of these derivatives is not restricted to compounds such as (**24**), which exist in the iminoacridan form, but occurs also for compounds such as (**30**), where attack is on a fully aromatic nitrogen. These results show that the original target compounds 'nitracrine mustard,' formulated as compound (**2**), is unattainable.

Experimental

Analyses were carried out in the Microchemical Laboratory, University of Otago, Dunedin, N.Z., under the direction of Professor A. D. Campbell. M.p.s were determined on an Electrothermal apparatus using the supplied stem-corrected thermometer, and are as read. N.m.r. spectra were obtained on a Varian 360-L spectrometer (Me_4Si). To monitor the progress of reactions and the purity of products, t.l.c. on SiO_2 (Merck SiO_2, F_{254}) was used. The most convenient solvent systems were $CHCl_3$–MeOH (9:1) or the top phase of BuOH–HOAc–H_2 (5:1:4 v/v), and visualization was by u.v. spectroscopy.



Scheme 4.

4-*Chloro*-1-*nitro*-9-*acridone* (**5**).—2-Chloro-5-nitroaniline (5 g, 29.06 mmol), DPIC (14 g, 43.20 mmol, 1.48 equiv.), and copper(II) acetate (500 mg) in DMF (50 ml) was heated to 90 °C overnight. The mixture was poured into water and extracted with ethyl acetate, and the extract was filtered, washed with 2M HCl, and the product back extracted into 2M NH_4OH. The residual crust was also dissolved in 2M NH_4OH and the combined base extracts were charcoaled, filtered, and then acidified to give a yellow precipitate (1 spot t.l.c.) of *N*-(2-chloro-5-nitrophenyl)anthranilic acid (7.04 g, 82%). This acid (4.8 g) in polyphosphate ester solution (PPE) (20 ml) was heated to 100 °C for 2 h. After cooling, the mixture was diluted with methanol and then refluxed for several minutes. The precipitated crystalline acridone (**5**) (4.0 g, 90%) was filtered off, washed with MeOH and dried (Na_2SO_4), m.p. 320—323 °C (lit.,[18] m.p. 320 °C).

4-*Chloro*-1-*nitroacridine* (**3**).—The acid chloride (100 mg) of *N*-(2-chloro-5-nitrophenyl)anthranilic acid [$SOCl_2$ (slight excess), 1,2-dichloroethane, DMF (1 drop), reflux 30 min)] was treated with bis(triphenylphosphine)copper(I) tetrahydroborate[17] (193 mg, 1 equiv.) and triphenylphosphine (170 mg, 2 equiv.) in acetone (10 ml) for 5 min at 20 °C. The white solid of tris(triphenylphosphine)copper(I) chloride was filtered off and the crude product was concentrated; further precipitated material was then removed. This procedure was repeated several times. The solvent was then evaporated off from the filtrate and the residue treated immediately with aqueous PPE at 90 °C for 15 min. After work-up, the oily non-polar yellow product (20 mg) was purified on p.l.c. and then crystallized from ether–hexane to give yellow plates of the chloronitroacridine (**3**), m.p. 69—71 °C (Found: C, 60.4; H, 2.7; N, 10.5. $C_{13}H_7ClN_2O_2$ requires C, 60.36; H, 2.73; N, 10.83%).

Reaction of either (**3**) or (**5**) with 2,2′-iminodiethanol at 110—150 °C for several hours afforded only unchanged starting material and/or baseline polymer (due to decomposition).

N,N-*Bis*(2-*hydroxyethyl*)-2,4-*dinitroaniline* (**7**).—A mixture of 1-chloro-2,4-dinitrobenzene (50 g, 241.5 mmol), 2,2′-iminodiethanol (54.5 g, 518.3 mmol, 2.15 equiv.), and ethyl acetate (200 ml) was heated to reflux for 1 h. The heterogeneous mixture was poured into water, the organic layer was removed, and the aqueous layer was extracted twice with ethyl acetate. The combined organic fractions were washed with brine and dried (Na_2SO_4). Solvent removal afforded a quantitative yield of (**7**) as a yellow solid, m.p. 106—107 °C (lit.,[19] m.p. 90 °C).

2-*Bis*(2-*hydroxyethyl*)*amino*]-5-*nitroaniline* (**8a**).—A well-shaken mixture of sodium sulphide (19.48 g, 80 mmol, 1.1 equiv.) and sulphur (2.6 g, 80 mmol, 1.1 equiv.) in water (100 ml) was added (over 5 min) to a refluxing solution of (**7**) (20 g, 74 mmol) in EtOH (100 ml). After 30 min the solvents were removed and the dark red slurry was partitioned in EtOAc–H_2O. The organic layer was dried (Na_2SO_4), concentrated, and the precipitate was recrystallized (EtOAc–Et_2O) to give orange crystals of (**8a**) (11.25 g, 63%), m.p. 92—94 °C (Found: C, 49.3; H, 6.4; N, 17.4. $C_{10}H_{15}N_3O_4$ requires C, 49.78; H, 6.27; N, 17.42); δ_H[$CDCl_3$–$(CD_3)_2SO$] 3.21 (t, 4 H, CH_2N), 3.62 (t, 4 H, CH_2O), 4.40 (br s, 4 H, exch. with D_2O), and 6.90—7.60 (m, 3 H, ArH).

N-{2-[*Bis*(2-*hydroxyethyl*)*amino*]-5-*nitrophenyl*}*anthranilic Acid* (**9**).—A mixture of (**8a**) (10g, 36.9 mmol), diphenyliodonium carboxylate (DPIC) (20 g, 61.7 g, 1.67 equiv.), and copper(II) acetate (*ca.* 500 mg) in DMF (100 ml) was heated to 100 °C (initial rapid heating on a hot plate with stirring) for 30 min. The mixture was quenched in water (500 ml) and extracted thrice with ethyl acetate. The organic phase was then extracted thrice with ammonium hydroxide to give a deep red solution. After charcoal treatment the aqueous layer was acidified with 2M-HCl and the precipitated oil extracted with ethyl acetate. The extract was dried (Na_2SO_4) and evaporated and the residual oil was triturated with dichloromethane to yield the acid (**9**) (13.9 g, 93%) as a yellow-orange powder, m.p. 185—187 °C (Found: C, 55.15; H, 5.4; N, 11.1. $C_{17}H_{19}N_3O_6 \cdot 0.5\ H_2O$ requires C, 55.12; H, 5.44; N, 11.34); δ_H[$CDCl_3$–$(CD_3)_2SO$] 3.20—3.73 (m, 8 H, CH_2N, CH_2O), 6.00 (br s, 4 H, exch. with D_2O), and 6.65—8.30 (m, 7 H, ArH).

Methyl N-{2-[*Bis*(2-*hydroxyethyl*)*amino*]-5-*nitrophenyl*}*anthranilate* (**13**).—A stirred slurry of (**9**) (6.46 g, 17.78 mmol), dimethyl sulphate (1.86 ml, 1.1 equiv.), potassium carbonate (2.7 g, 1.1 equiv.), and acetone (50 ml) was refluxed for 30 min. The mixture was filtered and the solvent removed. The product was taken up in ethyl acetate and washed with dilute acid followed

by dilute base. Concentration afforded the oily methyl ester (**13**) in quantitative yield; $\delta_H(CDCl_3)$ 3.35 (t, 4 H, CH_2N), 3.68 (t, 4 H, CH_2OH), 3.90 (s, 3 H, OMe), and 6.75—8.36 (m, 7 H, ArH).

Methyl 2-[4-(2-*Bromoethyl*)-2,3-*dihydro*-6-*nitroquinoxalin*-1-*yl*]*benzoate* (**16**).—To a solution of Vilsmeier's salt [PPh_3 (810 mg), DMF (238 μl), Br_2 (159 μl), 3.09 mmol, 2.2 equiv.] in benzene (20 ml) was added (**13**) (530 mg, 1.41 mmol) dissolved in THF (5 ml). The mixture was refluxed for 5 min, when an oil separated from the solution. The solvent was removed and the crude material was flash chromatographed on silica gel (hexane–ethyl acetate, 2:1) to yield the benzoate (**16**) (270 mg, 45%), m.p. 79—80 °C (EtOAc–hexane) (Found: C, 51.4; H, 4.3; Br, 18.95; N, 9.9. $C_{18}H_{18}BrN_3O_4$ requires C, 51.43; H, 4.31; Br, 19.01; N, 10.00); δ_H ($CDCl_3$) 3.25—4.00 (m, 8 H, CH_2N and CH_2Br), 3.52 (s, 3 H, OMe), 6.50 (d, *J* 7 Hz, 1 H, ArH), and 7.00—7.97 (m, 6 H, ArH). Starting material was also recovered.

Reaction of (**13**) (590 mg, 1.57 mmol) with MsCl (0.266 ml, 2.2 equiv.) and triethylamine (0.5 ml) in dichloromethane at 0 °C for 30 min gave an unstable product tentatively identified as the dimesylate (**15**). With time the material converted (t.l.c. analysis) into the monomesylate (**17**). Treatment of the mixture of (**15**) and (**17**) with NaBr in refluxing acetone overnight gave a 69% overall yield of the same benzoate (**16**).

Methyl 2-[4-(2-*Chloroethyl*)-2,3-*dihydro*-6-*nitroquinoxalin*-1-*yl*]*benzoate* (**18**).—Treatment of the above mesylate (**17**) with an excess of powdered NaCl in hot DMF for 15 min afforded, after work-up, the benzoate (**18**), m.p. 105—106 °C (EtOAc–hexane) (Found: C, 56.85; H, 4.45; Cl, 9.45; N, 11.6. $C_{17}H_{16}ClN_3O_4$ requires C, 56.43; H, 4.45; Cl, 9.80; N, 11.61); $\delta_H(CDCl_3)$ 3.65 and 3.80 (2s, 8 H, CH_2N and CH_2Cl), 3.69 (s, 3 H, OMe), 6.50 (d, *J* 9 Hz, 1 H, ArH), and 7.06—7.98 (m, 6 H, ArH).

2-[4-(2-*Chloroethyl*)-2,3-*dihydro*-6-*nitroquinoxalin*-1-*yl*]-*benzoic Acid* (**18a**).—A mixture of (**18**) (270 mg, 0.66 mmol) and KOH (0.72 ml of a 1M KOH solution) in EtOH (2 ml) was refluxed for 10 min. The solvent was removed and the residue was extracted with ethyl acetate–1M HCl. The organic layer was extracted with dilute NH_4OH, leaving behind changed starting material, and then acidified with HCl. The acid was then extracted into ethyl acetate, dried (Na_2SO_4) and concentrated to yield the acid (**18a**) (150 mg) which was crystallized from acetone, m.p. 212—214 °C (Found: C, 56.85; H, 4.45; Cl, 9.45; N, 11.69. $C_{17}H_{16}ClN_3O_4$ requires C, 56.43; H, 4.45; Cl, 9.80; N, 11.61); δ_H[$CDCl_3$–$(CD_3)_2SO$] 3.65 and 3.80 (2s, 8 H, CH_2N and CH_2Cl), 6.00 (br s, 1 H, CO_2H), 6.55 (d, *J* 9 Hz, 1 H, ArH), and 6.90—8.00 (m, 6 H, ArH).

N-{2-[*Bis*(2-*acetoxyethyl*)*amino*]-5-*nitrophenyl*}*anthranilic Acid* (**10**).—A mixture of (**9**) (4.7 g, 12.02 mmol), acetic anhydride (3.6 ml, 3 equiv.), and pyridine (15 ml) was heated under gentle reflux for 30 min. The solvents were removed and the residue was diluted with ethyl acetate and extracted with 2M-NH_4OH. The aqueous layer was acidified with 2M-HCl and extracted with ethyl acetate to yield, after concentration, the diacetate (**10**) (5.11 g, 89%), m.p. 128—130 °C (ether) (Found: C, 56.8; H, 5.35; N, 9.2. $C_{21}H_{23}N_3O_8$ requires C, 56.62; H, 5.20; N, 9.43); $\delta_H(CDCl_3)$ 1.99 (s, 6 H, OAc), 3.43 (t, 4 H, CH_2N), 4.20 (t, 4 H, CH_2OAc), and 6.73—8.32 (m, 7 H, ArH).

4-[*Bis*(2-*acetoxyethyl*)*amino*]-1-*nitro*-9-*acridone* (**12**).—Polyphosphate ester solution (PPE) (5 ml) and (**10**) (1.3 g) were heated together to 100 °C for 1.5 h. The mixture was quenched in water, extracted with ethyl acetate, and the extract washed with dilute NH_4OH, dried (Na_2SO_4), charcoaled, and concentrated to yield the acridone diacetate (**12**) (1.10 g, 88%), m.p. 101—103 °C (EtOAc) (Found: C, 59.0; H, 4.95; N, 9.65. $C_{21}H_{21}N_3O_7$ requires C, 59.00; H, 4.95; N, 9.83); $\delta_H(CDCl_3)$ 2.00 (s, 6 H, OAc), 3.39 (t, 4 H, CH_2N), 4.18 (t, 4 H, CH_2OAc), and 7.00—8.37 (m, 6 H, ArH).

9-*Alkylaminoacridine Synthesis*

Method A: via *the* 9-*Chloroacridine Intermediate*.—4-[*Bis*(2-*acetoxyethyl*)*amino*]-9-*chloro*-1-*nitroacridine* (**19**).—A mixture of (**12**) (1.23 g) and $POCl_3$ (10 ml) was gently heated to reflux for 1 h. The $POCl_3$ was removed and the residue was shaken quickly with a mixture of ethyl acetate and dilute NH_4OH. The organic layer was dried (Na_2SO_4) and concentrated and the residue eluted through several short silica gel pads with acetone. The red material was crystallized from ether to afford (**19**) (674 mg, 52%) as red needles, pure by t.l.c.; $\delta_H(CDCl_3)$ 2.00 (s, 6 H, OAc), 4.10 (t, 4 H, CH_2N), 4.58 (t, 4 H, CH_2OAc), 7.90 (d, *J* 8 Hz, 1 H, ArH), and 7.60—8.54 (m, 5 H, ArH). This compound was unstable and was used without further characterization.

4-[*Bis*(2-*acetoxyethyl*)*amino*]-9-[3-*dimethylaminopropylamino*]-1-*nitroacridine* (**21a**). A mixture of dry (benzene azeotrope) phenol (2.5 g) and (**19**) (614 mg, 1.34 mmol) was heated to 100 °C for 10 min then cooled to *ca.* 50 °C. 3-Dimethylaminopropylamine (274 mg, 2 equiv.) was added and the solution was heated to 80 °C for 45 min after which a further 1 equiv. of amine was added; heating was continued for a further 30 min, and the mixture was then poured into water and extracted with ethyl acetate. The organic phase was extracted with dilute acid and residual phenol was removed with ethyl acetate.

The aqueous layer was basified with dilute NH_4OH and the product was taken up in ethyl acetate. Evaporation of the solvent and flash chromatography on silica gel, eluting with 2% Et_3N–EtOAc afforded pure (**21a**) (335 mg, 49%), as a red solid, m.p. 101 °C (Found: C, 61.1; H, 6.5; N, 13.48. $C_{26}H_{33}N_5O_6$ requires C, 61.04; H, 6.50; N, 13.69); $\delta_H(CDCl_3)$ 1.38—2.62 (m, 4 H, CH_2N), 1.95 (s, 6 H, OAc), 2.17 (s, 6 H, NMe_2), 3.00—4.52 (m, 10 H, CH_2N and CH_2OAc), and 6.55—8.75 (m, 6 H, ArH).

4-[*Bis*(2-*hydroxyethyl*)*amino*]-9-(3-*dimethylaminopropylamino*-1-*nitroacridine* (**22a**). A mixture of (**21a**) (240 mg) and methanolic KOH (1 ml of a 2.197M-solution) in methanol (5 ml) was refluxed for 30 s. The solvent was removed and the residue was extracted with dichloromethane and washed with water. T.l.c. showed one polar product. Removal of solvent gave the diol (**22a**) (170 mg, 85%) as a gum; $\delta_H(CDCl_3)$ 1.50 (m, 2 H, CH_2), 2.15 (s, 6 H, NMe_2), 2.42 (t, 2 H, CH_2N), 3.25—3.95 (m, 10 H, CH_2N and CH_2OH), and 6.70—7.93 (m, 6 H, ArH).

Method B: via *the Amide* (**20a**).—A mixture of (**10**) (500 mg), thionyl chloride (1 ml), and DMF (1 drop) (10 ml) were refluxed together in 1,2-dichloroethane for 15 min. The solvents were removed and dichloromethane (10 ml) was added, followed by 3-dimethylaminopropylamine (1 ml). The mixture was refluxed for 2 min and then quenched with water. Additional dichloromethane (20 ml) was added and the organic layer was washed with water. Removal of the solvent gave a quantitative yield of the oily amide (**20a**).

The crude product was taken up in PPE (10 ml) and heated at 100 °C for 1.5 h. Similar work-up to that detailed above, followed by chromatography on silica gel gave (**21a**), in a similar yield to that of Method A.

The use of propylamine in place of 3-dimethylaminopropylamine in Methods A and B gave comparable yields of 4-[bis(2-acetoxyethyl)amino]-9-propylamino-1-nitroacridine (**21b**) as a yellow oil; $\delta_H(CDCl_3)$ 0.95 (t, 3 H, Me), 1.60 (m, 2 H, CH_2), 2.00 (s, 6 H, OAc), 3.47 (t, 6 H, CH_2N), 4.15 (t, 4 H, CH_2OAc), and 7.00—7.95 (m, 6 H, ArH).

Hydrolysis of the diacetate (**21b**) in refluxing methanolic KOH (5 min) afforded the oily diol (**22b**); $\delta_H(CDCl_3)$ 0.75 (t, 3 H, Me_3), 1.50 (q, 2 H, CH_2), 3.05—3.90 (m, 10 H, CH_2N and CH_2O), 5.70 (br, 2 H, OH), and 6.75—7.95 (m, 6 H, ArH).

Cyclization of 4-[*Bis*(2-*hydroxyethyl*)*amino*]*acridines* (**22**).—Treatment of (**22a**) with a wide variety of halide-forming reagents under many different conditions gave only starting material or polymeric products (or sometimes mixtures of the above: see Discussion).

Treatment of (**22b**) with a slight excess of $SOCl_2$ in refluxing 1,2-dichloroethane for 30 min gave a quantitative yield of the dihydropyrazinoacridine derivative (**25b**), m.p. 174—175 °C (EtOAc–hexane) (Found: C, 62.55; H, 5.35; Cl, 9.35; N, 14.1. $C_{20}H_{21}ClN_4O_2$ requires C, 62.41; H, 5.50; Cl, 9.21; N, 14.56). This compound could also be formed by treatment of the mesylate derived from (**22b**) (MsCl, Et_3N, acetone, room temp.) with powdered NaCl in hot DMF, or by direct ring closure of the propylamide derivative of the acid (**18a**) in refluxing $POCl_3$.

Similar treatment of the above mesylate with NaBr in hot DMF for 2 min gave the analogous bromopyrazinoacridine, characterized by its similar chromatograph behaviour and n.m.r. spectrum; $\delta_H(CDCl_3)$ 0.92 (t, 3 H, Me), 1.57 (m, 2 H, CH_2), 3.37—4.17 (m, 10 H, CH_2N and CH_2Br), 6.45 (dd, *J* 9, 4 Hz), and 6.90—8.13 (m, 5 H, ArH).

N-{2-[*Bis*(2-*acetoxyethyl*)*amino*]-5-*nitrophenyl*}*aminobenzaldehyde* (**28**).—The acid chloride derived from (**10**) (3.38 g, 7.12 mmol) [$SOCl_2$ (3 equiv.), 1,2-dichloroethane (20 ml), DMF (1 drop), gentle reflux 30 min] was treated with bis(triphenylphosphine)copper(I) tetrahydroborate[17] (4.7 g, 1.1 equiv.) and triphenylphosphine (3.72 g, 2 equiv.) in acetone (50 ml) for 30 min. The white solid of tris(triphenylphosphine)copper(I) chloride was removed; the crude product was concentrated and flash chromatographed on silica gel (hexane–EtOAc) to yield the aldehyde (**28**) (3.17 g, 97%) which crystallized with time at 0 °C, m.p. 65—67 °C (Found: C, 58.7; H, 5.55; N, 9.9. $C_{21}H_{23}N_3O_7$ requires C, 58.73; H, 5.39; N, 9.78); $\delta_H(CDCl_3)$ 2.00 (s, 6 H, OAc), 3.48 (t, 2H, CH_2N), 4.18 (s, t, CH_2O), 6.80—7.75 (m, 4 H, Ar), 7.20 (d, *J* 9 Hz, 1 H, Ar), 7.90 (d × d, *J* 9, 3 Hz, 1 H, Ar), 8.36 (d, *J* 3 Hz, 1 H, Ar), 10.01 (s, 1 H, CHO), and 10.14 (br, s, 1 H, NH).

4-[*Bis*(2-*acetoxyethyl*)*amino*]-1-*nitroacridine* (**29**).—Ring closure of the aldehyde (**28**) was effected by heating in polyphosphoric acid (PPA) at 90 °C for 1 h followed by work-up and retreatment of the partially cyclised material (t.l.c.) with polyphosphoric ester (PPE) at 90 °C for 20 min. Extensive decomposition of starting material took place, affording yields of cyclised product (**29**) of *ca.* 4% after silica gel chromatography. ^1H N.m.r. analysis of chromatographed samples of (**29**) showed contamination of up to 24% with starting material; $\delta_H(CDCl_3)$ 2.05 (s, 6 H, OAc), 4.10 (t, 4 H, CH_2N), 4.55 (t, 2H, CH_2O), 6.78 (d, *J* 9 Hz, 1 H, Ar), 6.90—8.05 (m, 5 H, Ar), and 8.35 (d, *J* 9 Hz, 1 H, Ar).

Treatment of (**28**) with PPE at 120 °C for 5 min afforded a mixture of (**28**) and (**29**) (almost identical R_F on t.l.c.—best solvent 2:1 hexane–acetone), while the same treatment for 1 h resulted in total decomposition.

Refluxing (**28**) in PPE–$CHCl_3$ resulted in little or no reaction whilst treatment in refluxing trifluoroacetic acid for 4 h gave mainly starting material, a very small amount of (**29**), and two products of lower but similar R_F, tentatively identified as transesterification adducts of (**28**) and (**29**). Treatment of (**28**) in refluxing conc. HCl–EtOH (10 drops/10 ml) resulted in hydrolysis of the acetate groups with no cyclization whilst gently heating (**28**) in 5% H_2SO_4–HOAc overnight gave a red coloured polymeric material.

4-[*Bis*(2-*hydroxethyl*)*amino*]-1-*nitroacridine* (**4**).—A mixture of (**29**) (130 mg) and 2.5M methanolic KOH (2 ml) in methanol (10 ml) was heated to reflux for 3 min. The solvent was removed and the residue was partitioned between ethyl acetate and M-HCl. The organic layer was concentrated and passed through several silica gel plugs (EtOAc), and afforded, after recrystallization from $CHCl_3$–hexane, the diol (**4**) (50 mg), m.p. 158 °C (Found: C, 61.75; H, 5.25; N, 12.5. $C_{17}H_{17}N_3O_4$ requires C, 62.37; H, 5.23; N, 12.84); $\delta_H[CDCl_3$–$(CD_3)_2SO]$ 3.08 (m, 4 H, CH_2N), 4.05 (m, 4 H, CH_2O), 6.96 (d, *J* 9 Hz, 1 H, Ar), 7.30—8.20 (m, 5 H, Ar), and 8.50 (d, *J* 9 Hz, 1 H, Ar).

Reaction of the Acridine (**4**) *with Thionyl Chloride*.—Thionyl chloride (1 drop) was added to a warmed orange solution of (**4**) (8.3 mg) in 1,2-dichloroethane (3 ml), to give an immediate purple coloration. The mixture was refluxed for several minutes and then kept at 0 °C overnight, resulting in a dark purple precipitate which was then separated by microtube centrifugation, washed several times with fresh solvent, re-centrifuged, and finally dried to afford 6.4 mg of product, homogeneous on t.l.c. (Bu^iOH–HOAc–H_2O–DMF/15:3:12:1.125, top layer). The material instantly dissolved in water to give a purple solution; addition of several drops of concentrated NH_4OH gave an oily orange precipitate which was extracted into ethyl acetate. The process could be reversed by the addition of 1M HCl to the ethyl acetate layer, affording the same purple aqueous phase. Similarly, t.l.c. analysis of the ethyl acetate extract using the solvent system above resulted in reversion to the purple material, but the compound ran as an orange spot using 10% Et_3N–EtOAc as eluant. Based on the above observations the purple compound was assigned structure (**31**) and the compound arising from base treatment of (**31**) was assigned the leuco structure form (**32**). Owing to lack of sufficient material no further investigations were carried out.

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