Synthetic Models Related to DNA Intercalating Molecules: Preparation of 9-Aminoacridines Linked to the Nucleotide Bases Adenine, Guanine and Thymine

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A series of novel compounds were prepared in which the aromatic ring of the drug quinacrine (2-methoxy-6-chloro-9-alkylaminoacridine) is linked to the nucleotide bases adenine, guanine and thymine by penta- and hexamethylene bridges.

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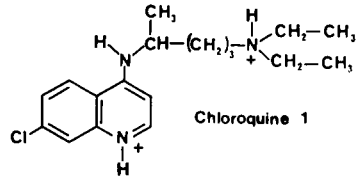
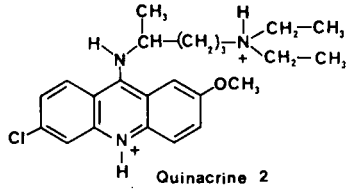
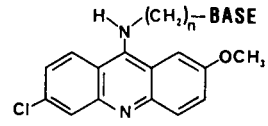
The phenomenon of reversible binding to DNA by planar aromatic molecules has been shown to influence their biological and physico-chemical properties [1,2,3]. Various biophysical techniques have shown that the strongest of the binding modes is that of intercalation [4,5]. In an ongoing investigation into the forces which intervene in the interactions between DNA and intercalating agents in general, we prepared a series of model compounds in which the aromatic moiety of the antimalarial chloroquine (**1**) is linked *via* a trimethylene chain to the nucleotide bases, adenine, thymine and guanine [6,7,8]. Both uv and nmr investigations confirmed that attractive intramolecular interactions do exist in water between the pairs of aromatic nuclei as witnessed by the very high degree of stacking observed. In a subsequent study, comparable models were prepared in which the quinoline ring of chloroquine was replaced by the acridine nucleus of the strong intercalating drug quinacrine (**2**). The additional ring present in the latter led to a dramatic increase in the attractive intramolecular interactions exhibited by the corresponding models. Total ring-ring stacking was observed in water in the whole temperature range from 0° to 80° for the model in which adenine is linked to quinacrine by a trimethylene bridge [9]. Here we present the synthesis of new series of compounds **3a, 3b, 4a, 4b, 5a** and **5b**, in which the aromatic portion of quinacrine is linked respectively to adenine, thymine and guanine by penta and hexamethylene chains. These "hetero-dimers" which possess longer polymethylene bridges facilitate the investigations between the acridine moiety and pairs of complementary nucleotide bases. The results of these studies will be published at a later date.



Chloroquine 1



Quinacrine 2



3 a,b
4 a,b
5 a,b

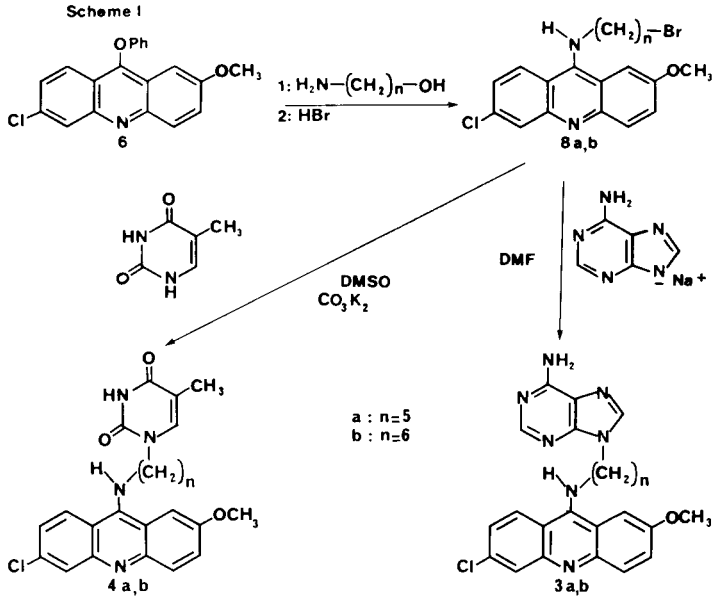
Base = Adenine, Thymine, Guanine

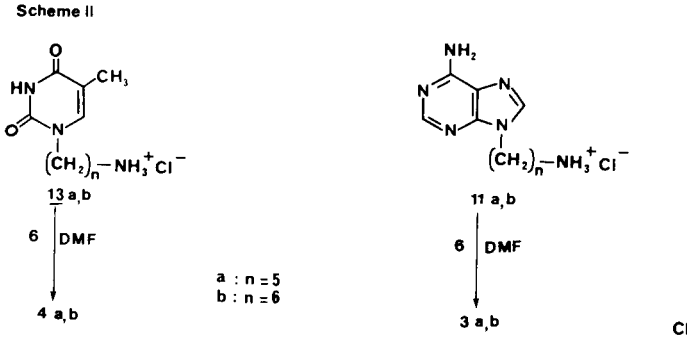
a: n = 5
b: n = 6

Synthesis of the Adenine and Thymine Models **3a, 3b, 4a, 4b**.

Initial considerations suggested that the route in which the ω-bromoalkylaminoacridine is prepared *via* the corresponding alcohol (Scheme I) would lead more rapidly to the model compounds. For example, the reactions of the corresponding ω-aminoalkyl-1-ols with 6-chloro-2-methoxy-9-phenoxyacridine (**6**) in dimethylsulfoxide at 110° for 12 hours gave *ca.* 45% yields of the 9-(ω-hydroxyalkylamino)-acridines **7a, 7b**. Subsequent reaction of compounds **7a, 7b** with 47% hydrobromic acid in sulphuric acid at 70° for several hours followed by filtration, dissolution of the residue in water and extraction with dichloromethane afforded the crude bromo derivatives **8a** and **8b** which happened to decompose during the purification steps. Indeed, column chromatographic separations on silica gel gave only small samples of the unstable desired bromides.

Exploratory reactions with thymine in dimethylsulfoxide in the presence of anhydrous potassium carbonate and with sodium adenylate in dimethylformamide, however, were attempted on the crude mixture of **8b**. Work-up of the resulting reaction mixture and column separation afforded milligram quantities of the desired product. As significant quantities of the model compounds were required for extensive physico-chemical and biological testing, this route was abandoned in favour of that outlined in Scheme II.



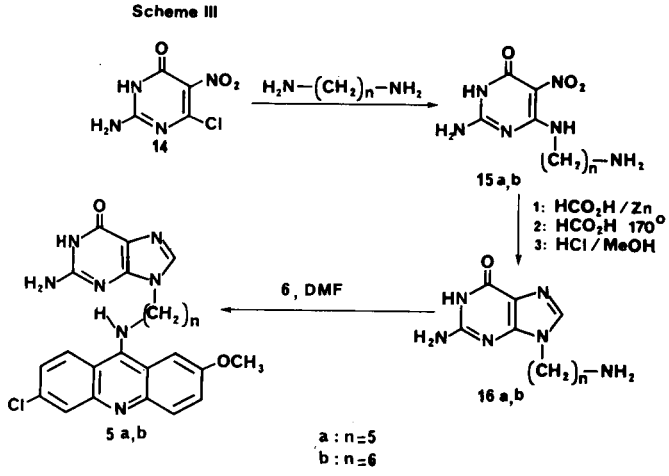


Reaction of sodium adenylate with ω-bromopentylphthalimide and ω-bromohexylphthalimide respectively, in dimethylformamide at room temperature, followed by acidic hydrolysis in 1:1:1 mixtures of concentrated hydrochloric acid, acetic acid and water furnished 85 and 75% yields of the corresponding amine hydrochlorides **11a** and **11b**. These aminoalkyladenines were caused to react with equimolar 6-chloro-2-methoxy-9-phenoxyacridine (**6**) in different solvents (phenol, dimethylformamide, dimethylsulfoxide) with or without added non-nucleophilic amines. The best results were obtained in neat dimethylformamide. Precipitation in water (*p*H 13) afforded crude products which turned out to be highly polar when chromatographed on silica gel. Repetitive chromatographic procedures and crystallization furnished the pure adenine-acridine hetero-dimers **3a** and **3b**.

The corresponding thymine-acridine models **4a** and **4b** were obtained by comparable routes. Silylation of thymine with hexamethyl disilazane yielded the protected base which was quantitatively alkylated with ω-bromopentyl- and ω-bromohexylphthalimides, respectively, in dimethylsulfoxide at 80°, to give the corresponding phthalimidoalkylthymines **12a** and **12b**. Acid hydrolysis furnished 70 and 75% of the resulting amine hydrochlorides **13a** and **13b**. Nucleophilic displacement of the phenoxy group of the acridine **6** by the terminal amino function of **13a** and **13b** in dimethylformamide at 110° afforded crude mixtures from which the thymine-acridine compounds **4a** and **4b** could be isolated by repeated chromatography on silicagel.

Synthesis of the Guanine Models **5a** and **5b**.

Due to the presence of two acidic protons in the guanine residue (N_1H p*K*a 12.4; N_9 p*K*a 9.6) [11], the direct base catalysed alkylation procedure is not feasible. The synthetic pathway described by Leonard *et al.* [12] was therefore employed in which a substituted guanine moiety was constructed from a conveniently substituted pyrimidine ring system (Scheme III).



Chlorination of 2-amino-4,6-dihydroxypyrimidine in phosphoryl chloride/dimethylaniline followed by precipitation in ice and recrystallization in benzene with activated charcoal gave 45% of 2-amino-4,6-dichloropyrimidine. Treatment with refluxing 1*N* sodium hydroxide solution for four hours and precipitation with acetic acid furnished the desired 2-amino-6-chloro-4(3*H*)-pyrimidinone. This compound was added to a cooled 1:1 mixture of fuming nitric acid and concentrated sulphuric acid under nitrogen while limiting the reaction temperature to less than 5° and excellent yields of the nitro compound **14** were prepared. If the procedure is carried out at higher temperatures an unidentified bright yellow compound is isolated. Overnight reaction with the appropriate diamines in dimethylformamide and in the presence of triethylamine afforded, after work-up and crystallization, 80 and 70% respectively of the corresponding ω-aminoalkylpyrimidinones, **15a** and **15b**.

Preliminary reduction-cyclization sequences as described by Leonard *et al.*, for the preparation of substituted

guanines [12] were attempted. For example the product isolated from the zinc-formic acid treatment of **15b** was cyclized both in formic acid heated to 175-180° and in dimethylformamide refluxed under nitrogen in the presence of anhydrous potassium carbonate. Analysis (hplc and nmr) indicated that side reactions including formylation of the aliphatic primary amine had occurred besides cyclization. The most satisfactory method for the preparation of **16a** and **16b** turned out to be that in which the formic acid solution, after the zinc reduction step, is concentrated and heated to 175-180° for three hours. Simple evaporation and treatment of the residue with methanolic hydrochloric acid completely hydrolysed all formylated products to form *ca.* 95% of the desired guanine substituted amines **16a** and **16b**. The characteristic new signal at 8.4 ppm in the nmr spectrum (deuteriotrifluoroacetic acid) due to the H-8 of the guanine ring definitely proves that cyclization following reduction has occurred. Reaction of **16a, 16b** with 6-chloro-2-methoxy-9-phenoxyacridine (**6**) gave the guanine-acridine model compounds **5a** and **5b** which were isolated and purified by repetitive chromatographies on silicagel.

For the three couples of heterodimers thus obtained **3a, 3b, 4a, 4b** and **5a, 5b**, all the analytical and spectroscopic data are in accordance with the given structures. Particularly informative are the ^1H nmr spectra measured at 270 MHz in dimethylsulfoxide which correspond quite closely for each heterodimer to the addition spectrum of the constitutive "half molecules", respectively the base and the acridine nucleus. The same is observed for the uv absorptions as measured in organic solvents. All the compounds are very polar in some cases highly hygroscopic. The purity which is quite critical for the physicochemical studies was checked by a number of techniques including hplc, tlc, elemental analysis. The physicochemical and biological properties of these substances are now under study, such as their binding characteristics to DNA and to synthetic polynucleotides.

EXPERIMENTAL

Melting points were determined on a Tottoli apparatus and are not corrected. Routine nmr spectra were recorded at 60 MHz and 80 MHz on a Bruker WP60 and WP80 spectrometers, respectively. The nmr spectra at 270 MHz were recorded on a Bruker WP 270 spectrometer. Infrared spectra were registered on a Perkin-Elmer 237 Model. A Beckman DB-GT grating spectrophotometer was employed to record uv spectra. Mass spectra were recorded on Riber-Mag 10-10 spectrometer. Reverse phase hplc was performed using a μ-Bondapak RP 18 analytical column (Waters Associates) with a Model 660 solvent programmer and two M-6000 pumps (Waters Associates). The effluent was analysed by the Model 440 dual wave-length detector: 254 and 265 nm (Waters Associates) and a linear gradient of solvents was used from 10 to 100% methanol in water (*p*H 2.5, phosphoric acid) during 10 minutes with a flow rate of 2 ml/minute. Sample microanalysis was performed by the "Service Central de Microanalyse du CNRS" (France). Analyses (tlc) were performed on "Merck 5 × 10 cm Kieselgel $60F_{254}$" plates. Liquid chromatography was performed on silica "Merck Kieselgel 60".

The 2-amino-4,6-dihydropyrimidine was prepared using the procedure adopted by Rembold and Schramm [14]. Chlorination of this product to give the 2-amino-4,6-dichloropyridine [15] was carried out in the presence of dimethylamine [16], subsequent basic hydrolysis afforded the 2-amino-6-chloro-4(3*H*)-pyrimidinone [17].

6-Chloro-2-methoxy-9-[(5-hydroxypentyl)amino]acridine (**7a**).

A mixture of 6-chloro-2-methoxy-9-phenoxyacridine (**6**) [13] (11.7 g, 0.035 mole) and 5-aminopentanol (11.1 g, 0.108 mole) in DMSO (250 ml) was heated at 110° under a nitrogen atmosphere for 12 hours. The reaction mixture was then cooled to room temperature and added to cold water (1000 ml). The resulting solution was left overnight at 0° after which the precipitated product was filtered off, washed with water and then dried in a dessicator to give 9.04 g (75%) of crude material. Crystallization from ethanol afforded samples which were homogeneous on tlc, mp 154-156°; ir (Nujol): 3320, 1625, 1600, 1560, 1520, 1500, 1350, 1255, 1235, 1165, 1070, 1030, 950, 920, 825, 780, 765 cm^{-1}; nmr (90 MHz, dimethylsulfoxide-d_6): δ 8.55 (d, $AcrC_8H$, 1H), 8.10-7.50 (m, $AcrC_1HC_3H$-C_5HC_7H, 5H), 7.05 (s, AcrN*H*, 1H), 4.55 (s, O*H*, 1H), 4.15 (s, $AcrOCH_3$, 3H), 3.95 (m, $AcrNHCH_2$- or $HOCH_2$-, 2H), 3.55 (m, $AcrNHCH_2$- or $HOCH_2$, 2H), 1.80 (m, $(CH_2)_3$, 6H); uv (ethanol): λ max 267 (ϵ = 58200), 278 (ϵ = 51400), 410 (ϵ = 8600) nm; ms: 344 (M^+, 100), 271 (M-73, 24), 257 (M-87, 12).

Anal. Calcd. for $C_{19}H_{21}ClN_2O_2$: C, 66.1; H, 6.1; N, 8.1. Found: C, 65.7; H, 6.0; N, 8.1.

6-Chloro-2-methoxy-9-[(6-hydroxyhexyl)amino]acridine (**7b**).

Compound **7b** was prepared as described for **7a**, using 12.6 g (0.108 mole) of 6-aminohexanol to give 10.0 g (80%) of **7b**, which was crystallized from chloroform, mp 148-149°; ir (Nujol): 3315, 1625, 1605, 1500, 1365, 1345, 1255, 1230, 1075, 1030, 925, 860, 825, 817, 792, 755 cm^{-1}; nmr (90 MHz, dimethylsulfoxide-d_6): δ 8.50 (d, $AcrC_8H$, 1H), 8.10-7.45 (m, $AcrC_1HC_3HC_4HC_5HC_7H$, 5H), 7.50 (s, AcrN*H*, 1H), 4.50 (s, O*H*, 1H), 4.15 (s, $AcrOCH_3$, 3H), 3.95 (t, $AcrNHCH_2$- or $HOCH_2$-, 2H), 3.55 (t, $AcrNHCH_2$, or $HOCH_2$, 2H), 2.1-1.5 (m, $(CH_2)_4$, 8H); uv (ethanol): λ max 269 (ϵ = 55500), 280 (ϵ = 48700), 412 (ϵ = 7300) nm, ms: 358 (M^+, 100), 285 (M-73, 13), 271 (M-87, 44).

Anal. Calcd. for $C_{20}H_{23}ClN_2O_2$: C, 66.9; H, 6.5; N, 7.8. Found: C, 66.9; H, 6.5; N, 7.7.

6-Chloro-2-methoxy-9-[(6-bromohexyl)amino]acridine (**8b**).

Compound **7b** (3.5 g, 0.102 mole) was dissolved in a mixture of bromohydric acid (47% in water, 200 ml) and concentrated sulfuric acid (50 ml). The solution was heated at 70° under nitrogen for 7 hours. The reaction mixture was then cooled to room temperature and added dropwise to a potassium hydroxide solution (5*N*, 1000 ml) at 4°. The resulting suspension was filtered, washed with water and dried to give 1.8 g (45%) of crude material. Because of the instability, it was impossible to obtain analytically pure samples of the product. The purification method was a dissolution in chloroform followed by precipitation with petroleum ether and rapid chromatography on silica, mp 90° dec; ir (Nujol): 3500, 3000, 1625, 1590, 1560, 1500, 1270, 1245, 1090, 840, 760 cm^{-1}; nmr (90 MHz, dimethylsulfoxide-d_6): δ 8.55 (d, $AcrC_8H$ 1H), 8.10-7.45 (m, $AcrC_1HC_3HC_4HC_5HC_7H$, 5H), 7.25 (broad signal, AcrN*H*, 1H), 4.15 (s, $AcrOCH_3$), 3.65-3.95 (m, $(CH_2)_2$, 4H), 1.6-2.2 (m, $(CH_2)_4$, 8H); uv (ethanol): λ max 268 (ϵ = 53500), 280 (ϵ = 46000), 412 (ϵ = 7700) nm; ms: 422 ($(M+1)^+Br^{81}$, 44), 420 ($(M-1)^+Br^{79}$, 34), 271 (M-151, 35).

N-(5-Bromopentyl)phthalimide (**9a**).

A mixture of phthalimide (15.0 g, 0.10 mole), 1,5-dibromopentane (70 ml, 5-fold-excess) and anhydrous potassium carbonate (30 g, 0.21 mole) were efficiently stirred with dry dimethylformamide (250 ml) under a nitrogen atmosphere at ambiant temperature for three days. At the end of this period the mixture was filtered and the dimethylformamide evaporated *in vacuo*. The resulting viscous liquid was dissolved in a mixture of petroleum and diethyl ether (3/2) and left overnight at −10° to crystal-

lize slowly. The resulting colourless crystals were filtered off, washed with cold petroleum ether and crystallized another time to give colourless needles (21.7 g, 80%) of **9a**, mp 59-61°; ir (Nujol): 1770, 1710, 1265, 1245, 1210, 1040, 1025, 940, 880, 715 cm^{-1}; nmr (60 MHz, trifluoroacetic acid-d): δ 7.80 (m, Phth*H*, 4H), 3.65 (t, PhthN-CH_2, 2H), 3.40 (t, Br-CH_2, 2H), 1.60 (m, (CH_2)$_3$, 6H); uv (ethanol): λ max 295 (ε = 2400), 242 (ε 12200), 233 (ε = 19200), 221 (ε = 39400) nm; ms: 297 ((M + 1)$^+$Br^{81}, 7), 295 ((M-1)$^+$Br^{79}, 6), 216 (M-80, 14), 188 (M-109, 23), 160 (M-136, 100).

Anal. Calcd. for $C_{13}H_{14}BrNO_2$: C, 52.7; H, 4.7; N, 4.7. Found: C, 52.7; H, 4.8; N, 4.7.

N-(6-Bromopentyl)phthalimide (**9b**).

This compound was prepared according to Leonard and Lambert, mp 171-173° lit [18] 177-180°.

9-(5-Phthalimidopentyl)adenine (**10a**).

A mixture of adenine (3.00 g, 0.022 mole), anhydrous potassium carbonate (6.00 g, 0.043 mole) and **9a** (9.20 g, 0.031 mole) were stirred vigourously under a nitrogen atmosphere in dimethylformamide (60 ml). After 48 hours the mixture was filtered *in vacuo* and the residue washed with dimethylformamide (3 × 10 ml). The combined organic fractions were then evaporated *in vacuo* and the resulting semi-solid residue triturated with ether (3 × 15 ml) to leave a pale yellow solid (3.12 g). Two subsequent crystallizations yielded a pale yellow powder (2.81 g, 40%). Two further crystallizations of a small sample and treatment with animal charcoal gave colourless crystals of **10a**, mp 203-205°; ir (Nujol): 3250, 3100, 1770, 1720, 1665, 1600, 1455, 1395, 1350, 1240, 1210, 1050, 1035, 975, 710 cm^{-1}; nmr (60 MHz, trifluoroacetic acid-d): δ 9.40, 8.80 (2s, AdeC$_2$*H*C$_8$*H*, 2H), 7.90 (s, Phth*H*, 4H), 4.85 (m, AdeN$_9$-CH_2, 2H), 3.90 (m, PhthN-CH_2, 2H), 1.80 (m, (CH_2)$_3$, 6H); uv (methanol): λ max 283 (ε = 2700), 263 (ε = 14200), 242 (ε = 15500), 234 (ε = 17900), 219 (sh, ε = 56600), 216 (ε = 53000) nm; ms: 350 (M$^+$, 28), 204 (M-146, 3), 190 (M-160, 100), 176 (M-174, 19), 135 (M-215, 30).

Anal. Calcd. for $C_{18}H_{18}N_6O_2$: C, 61.7; H, 5.2; N, 24.0. Found: C, 61.8; H, 5.2; N, 24.0.

9-(5-Aminopentyl)adenine Dihydrochloride (**11a**).

A sample of **10a** (7.0 g, 0.02 mole) was heated at 100° in a 1:1:1 mixture of glacial acetic acid, water and concentrated hydrochloric acid for 10 hours under a nitrogen atmosphere. At the end of the reaction the solution was evaporated to half-volume *in vacuo* and then filtered. The filtrate was then evaporated to dryness *in vacuo* and crystallized from absolute alcohol to give colorless crystals (4.45 g, 85%) of the desired product **11a**, mp 240-258° dec; ir (Nujol): 3250, 3100, 2640, 1030, 1690, 1630, 1600, 1520, 1415, 1220 cm^{-1}; nmr (60 MHz, trifluoroacetic acid): δ 9.55, 8.80 (2s, AdeC$_2$*H*C$_8$*H*, 2H), 6.90 (broad signal, Ade-NH_2, 2H), 4.70 (m, AdeN$_9$-CH_2, 2H), 3.25 (m, NH_3^+-CH_2, 2H), 1.85 (m, (CH_2)$_3$, 6H); uv (water): λ max 261 (ε = 14300), 205 (ε = 21300) nm; ms: 220 (M-2 HCl, 43), 204 (M-16, 13), 190 (M-30, 43), 176 (M-44, 25), 135 (M-85, 80).

Anal. Calcd. for $C_{10}H_{16}N_6 \cdot 2HCl$: C, 41.0; H, 6.2; N, 28.6; Cl, 24.2. Found: C, 40.8; H, 5.7; N, 28.2; Cl, 24.0.

9-(6-Aminohexyl)adenine Dihydrochloride (**11b**).

This compound was prepared according to Leonard and Lambert, mp 224-226° hydrochloride lit [18] 164-165° free base.

1-(5-Phthalimidopentyl)thymine (**12a**).

A sample of thymine (3.0 g, 0.024 mole) was heated at reflux with hexamethyldisilazane (HMDS, 30 ml) and a catalytic amount of ammonium sulphate (0.1 g) under a nitrogen atmosphere. Although after 2 hours all of the thymine had dissolved, the silylation reaction was continued overnight (18 hours). At the end of this period the HMDS was evaporated *in vacuo* leaving a colourless opaque oil. To this was added dry dimethylformamide (30 ml) and then a sample of **9a** (8.15 g, 0.028 mole) and the mixture stirred under a nitrogen atmosphere at 80° for 18 hours. The resulting brown solution was evaporated *in vacuo* to leave a red brown oil which was dissolved in a minimum volume of an ethanol/water (80/20) mixture with warming on a steam bath. The solution was filtered while hot. Cooling and scratching induced a slow crystallization of the product. This sample was left overnight at 4° to effect complete crystallization and filtered. The residue was then washed with absolute alcohol, ether and weighed, gave 5.80 g (70%) of a crude product. Further crystallizations afforded colourless crystals of **12a** mp 138-140°; ir (Nujol): 3160, 1767, 1710, 1660, 1390, 1345, 1215, 1040, 965, 925, 895, 845, 760 cm^{-1}; nmr (60 MHz, dimethylsulfoxide-d$_6$): δ 7.80 (s, Phth*H*, 4H), 7.50 (s, ThyC$_6$*H*, 1H), 3.50 (m, ThyN$_1$-CH_2, and PhthN-CH_2, 4H), 1.80 (s, ThyCH_3, 3H), 1.50 (m, (CH_2)$_3$, 6H); uv (methanol): δ max 275 (ε = 15600), 220 (ε = 77200), 205 (ε = 86700) nm; ms: 341 (M$^+$, 95), 216 (M-125, 8), 160 (M-181, 100).

Anal. Calcd. for $C_{18}H_{19}N_3O_4$: C, 63.3; H, 5.6; N, 12.3. Found: C, 63.0; H, 5.7; N, 12.0.

1-(6-Phthalimidohexyl)thymine (**12b**).

Compound **12b** was prepared as described previously for **12a** to give colourless crystals, mp 165-167°; ir (Nujol): 3160, 1765, 1710, 1660, 1390, 1345, 1215, 1040, 965, 925, 895, 845, 760 cm^{-1}; nmr (60 MHz, dimethylsulfoxide-d$_6$): δ 7.85 (s, Phth-*H*, 4H), 7.55 (broad signal, ThyC$_6$*H*, 1H), 3.55 (m, Thy-CH_2 and Phth-CH_2, 4H), 1.75 (s, Thy-CH_3, 3H), 1.40 (m, (CH_2)$_4$, 8H); uv (absolute ethanol): λ max 273 (ε = 11000), 241 (ε = 14800), 234 (ε = 19600), 221 (ε = 49200) nm; ms: 355 (M$^+$, 34), 230 (M-125, 7), 195 (M-260, 27).

Anal. Calcd. for $C_{19}H_{21}N_3O_4$: C, 64.2; H, 6.0; N, 11.8. Found: C, 64.1; H, 6.1; N, 11.9.

1-(5-Aminopentyl)thymine Hydrochloride (**13a**).

A sample of **12a** (5.42 g, 0.018 mole) was refluxed in 100 ml of 1:1:1 mixture of water, glacial acetic acid and concentrated hydrochloric acid for 36 hours. The mixture was then evaporated to half the original volume and the crystallized benzoic acid filtered off. The filtrate was evaporated *in vacuo* to near dryness and dissolved in a minimum volume of absolute alcohol with heating on a steam bath. The resulting solution was left overnight at 5° to crystallize and gave colourless crystals (3.07 g, 70%) which were homogeneous on tlc, mp 192-195°; ir (Nujol): 3175, 2760, 2075, 1695, 1585, 1415, 1335, 1050, 865 cm^{-1}; nmr (60 MHz, dimethylsulfoxide-d$_6$): δ 8.50 (broad signal, -N*H*3$^+$, 3H), 7.55 (s, ThyC$_6$*H*, 1H), 3.60 (t, ThyN$_1$-CH_2, 2H), 2.70 (t, NH_3^+-CH_2, 2H), 1.70 (s, Thy-CH_3, 3H), 1.35 (m, (CH_2)$_3$, 6H); uv (water): λ max 261 (ε = 14300), 205 (ε = 21300) nm; ms: 221 (M$^+$-HCl, 44), 181 (M-65, 100).

Anal. Calcd. for $C_{10}H_{16}N_3O_2 \cdot HCl$: C, 48.7; H, 6.9; N, 17.0. Found: C, 48.6; H, 7.2; N, 16.6.

1-(6-Aminohexyl)thymine Hydrochloride (**13b**).

Compound **13b** was prepared as described for **13a**, mp 253°; ir (Nujol): 3115, 1765, 1255, 1210, 1100, 950, 910, 800, 730 cm^{-1}; nmr (60 MHz, trifluoroacetic acid-d): δ 7.45 (broad signal, ThyC$_6$*H*, 1H), 3.90 (t, ThyN$_1$-CH_2, 2H), 3.40 (t, NH_3^+-CH_2, 2H), 2.10 (s, Thy-CH_3, 3H), 1.60 (m, (CH_2)$_4$, 8H); uv (water): λ max 271 (ε = 8475), 203 (ε = 9300) nm; ms: 225 (M-HCl, 23).

Anal. Calcd. for $C_{11}H_{18}N_3O_2 \cdot HCl$: C, 50.5; H, 7.7; N, 16.1; Cl, 13.5. Found: C, 50.4; H, 7.8; N, 15.7; Cl, 13.5.

6-Chloro-2-methoxy-9-[(5-(thym-1-yl)pentyl)amino]acridine (**4a**).

A mixture of **13a** (1.20 g, 4.9 mmoles), DABCO (2.75 g) and dimethylsulfoxide (35 ml) was stirred at room temperature for 15 minutes. A sample of **6** (1.77 g, 5.3 mmoles) was then added and the reaction stirred under a nitrogen atmosphere for 18 hours at 70°. At the end of this period the mixture was added in a dropwise manner to rapidly stirred and cooled water (100 ml, *p*H 13). The resulting semi-solid was recuperated and dried *in vacuo* to give 2.12 g (90%) of a mixture containing starting material and final product. Repeated column chromatographic separations (silica, ethanol, methanol) furnished pure product, mp 209-211° dec; ir (Nujol): 3440, 3160, 1695, 1650, 1525, 1255, 1235, 1135, 1075, 1035, 925, 860, 820, 770, 755, 720 cm^{-1}; nmr (270 MHz, dimethylsulfoxide-d$_6$): δ 11.10 (s, ThyN$_1$*H*, 1H), 8.25 (d, AcrC$_8$*H*, 1H), 7.78 (m, AcrC$_4$*H* C$_5$*H*, 2H), 7.35 (s, ThyC$_6$*H*, 1H), 7.31 (m, AcrC$_1$*H* C$_3$*H*, 2H), 7.24 (d, AcrC$_7$*H*, 1H), 6.81 (broad signal, AcrN*H*, 1H), 3.86 (s, AcrOCH_3, 3H), 3.67 (m, ThyNCH_2, 2H), 3.49 (t, AcrNHCH_2, 2H), 1.67 (m, Thy-CH_3 and CH_2,

5H), 1.50 (m, CH_2, 2H), 1.27 (m, CH_2, 2H); uv (water *p*H 5.5, 5% of ethanol); λ max 442.5 (ε = 8210), 420 (ε = 8750), 342.5 (ε = 4390) nm; ms: 453 (M^+, 8), 417 (M-36, 23), 327 (M-126, 11).

Anal. Calcd. for $C_{24}H_{25}ClN_4O_3$: C, 63.6; H, 5.6; N, 12.4. Found: C, 63.4; H, 5.6; N, 12.2.

6-Chloro-2-methoxy-9-[(6-(thym-1-yl)hexyl)amino]acridine (**4b**).

A mixture of **13b** (1.42 g, 6.7 mmoles) DABCO (3.00 g) and dimethylsulfoxide (30 ml) was stirred for 15 minutes. A sample of **6** (2.34 g, 7 mmoles) was added and the reaction mixture stirred under nitrogen for 18 hours at 70°. The mixture was added to ice-water (100 ml, *p*H 13). The resulting semi-solid precipitated and dried *in vacuo* to give 2.74 g (85%) of mixture containing the desired compound. Several column chromatographic separations afforded pure samples of the model compound, mp 209-211° dec; ir (Nujol): 3370, 1675, 1635, 1605, 1560, 1520, 1500, 1335, 1255, 1235, 1120, 1075, 1040, 940, 920, 820, 800, 760, 725 cm^{-1}; nmr (270 MHz, dimethylsulfoxide-d_6): δ 11.10 (s, ThyN$_1$*H*, 1H), 8.25 (d, AcrC$_8$*H*, 1H), 7.76 (m, AcrC$_4$*H*C$_5$*H*, 2H), 7.56 (s, ThyC$_6$*H*, 1H), 7.40 (s, AcrC$_1$*H*, 1H), 7.34 (d, AcrC$_3$*H*, 1H), 7.24 (d, AcrC$_7$*H*, 1H), 6.96 (broad signal, AcrN*H*, 1H), 3.84 (s, AcrOCH_3, 3H), 3.67 (m, ThyN-CH_2, 2H), 3.49 (m, AcrNCH_2, 2H), 1.67 (m, ThyCH_3 and CH_2, 5H), 1.46 (m, CH_2, 2H), 1.24 (m, (CH_2)$_2$, 4H); uv (water *p*H 5.5, 5% of ethanol): λ max 445 (ε = 8250), 422.5 (ε = 8770), 340 (ε = 4020) nm; ms: 466 (M^+, 0.2), 431 (M-35, 0.2).

Anal. Calcd. for $C_{25}H_{27}ClN_4O_3$: C, 64.3; H, 5.8; N, 12.0. Found: C, 64.4; H, 5.8; N, 12.0.

6-Chloro-2-methoxy-9-[(5-(aden-9-yl)pentyl)amino]acridine (**3a**).

A mixture of **11a** (0.20 g, 7 mmoles) and compound **6** (0.19 g, 0.56 mmole) in dimethylformamide (2.5 ml) were heated at 110° for 10 hours under a nitrogen atmosphere. The resulting mixture was added slowly to water (30 ml, *p*H 13) and the resulting hygroscopic yellow-brown solid filtered off. Immediate drying *in vacuo* gave 0.179 g (70%) of a product whose tlc indicated a complex mixture. Repeated chromatographic separations (silica, methanol/ethanol (1:1)/ammonium hydroxide 1%) furnished small quantities of final product, mp 164-168°; ir (Nujol): 3400, 3250, 3150, 1625, 1560, 1520, 1300, 1255, 1230, 1160, 1145, 1060, 1035, 920 cm^{-1}; nmr (270 MHz, dimethylsulfoxide-d_6): δ 8.29 (d, AcrC$_8$*H*, 1H), 8.06 (2s, AdeC$_2$*H*C$_8$*H*, 2H), 7.78-7.29 (m, AcrC$_1$*H*C$_3$*H*C$_5$*H*C$_7$*H*, 2H), 7.13 (s, AdeNH_2, 1H), 3.86 (s, AcrOCH_3, 3H), 3.75 (t, AcrNHCH_2, 5H), 1.75 (m, (CH_2)$_2$, 4H), 1.23 (m, CH_2, 2H); uv (water *p*H 5.5, 5% of ethanol): λ max 445 (ε = 7650), 422.5 (ε = 8140), 342.5 (ε = 4230) nm; ms: 461 (M^+, 16), 426 (M-35, 32), 271 (M-190, 47).

Anal. Calcd. for $C_{24}H_{24}ClN_7O\cdot H_2O$: C, 60.1; H, 5.5; N, 20.4; Cl, 7.4. Found: C, 60.5; H, 5.9; N, 20.0; Cl, 7.5.

6-Chloro-2-methoxy-9-[(6-aden-9-yl)hexyl)amino]acridine (**3b**).

A mixture of **11b** (0.10 g, 0.68 mmole) and compound **6** (0.19 g, 0.56 mmole) in dimethylformamide (2.5 ml) was heated at 110° for 12 hours under a nitrogen atmosphere with stirring. The resulting mixture was added slowly to water (30 ml, *p*H 13) and the resulting semi-solid recovered. Immediate drying *in vacuo* gave 0.23 g (85%) of a product indicating a complex mixture on tlc. Several chromatographic separations (silica, methanol/ethanol (1:1)/ammonium hydroxide 1%) afforded small quantities of **3b**, mp 177-178°; ir (Nujol): 3300, 3100, 1735, 1635, 1600, 1555, 1525, 1410, 1365, 1300, 1255, 1230, 1165, 1070, 1040, 920, 825, 795, 760, 720 cm^{-1}; nmr (270 MHz, dimethylsulfoxide-d_6): δ 8.33 (d, AcrC$_8$*H*, 1H), 8.00 (s, AdeC$_2$*H*C$_8$*H*, 2H), 7.74-7.23 (m, AcrC$_1$*H*C$_3$*H*C$_4$*H*C$_5$*H*C$_7$*H*, 5H), 7.09 (s, AdeNH_2, 2H), 6.79 (broad signal, AcrN*H*, 1H), 4.00 (t, AdeN$_9$-CH_2, 2H), 3.82 (s, AcrOCH_3, 3H), 3.63 (t, AcrNH-CH_2, 2H), 1.64 (m, (CH_2)$_2$, 4H), 1.23 (m, (CH_2)$_2$, 4H); uv (water *p*H 5.5, 5% of ethanol): λ max 445 (ε = 8340), 422 (ε = 8820), 342.5 (ε = 4080) nm; ms: 475 (M^+, 18), 440 (M-35, 43), 285 (M-190, 41).

Anal. Calcd. for $C_{25}H_{26}ClN_7O\cdot H_2O$: C, 63.1; H, 5.5. Found: C, 62.8; H, 5.6.

2-Amino-6-chloro-5-nitro-4(3*H*)-pyrimidinone (**14**).

To a sample of concentrated sulphuric acid (17.5 ml) cooled in an ice bath was added fuming nitric acid (17.5 ml). The mixture was stirred until the temperature of the nitration mixture was below 5°. A sample of 2-amino-6-chloro-4(3*H*)-pyrimidinone (3.25 g, 0.025 mole) was then added to the solution over such a period that the nitration mixture did not exceed 10°. The resulting suspension was left to stir under nitrogen for 2 to 3 hours ensuring that the temperature of the reaction mixture did not exceed 10°. At the end of this period the resulting yellow solution was slowly added with stirring to an ice mixture (130 g) and then left at 5° for a further hour. The pale yellow precipitate was filtered, washed with cold water until neutral, washed successively with absolute alcohol (10 ml), ethyl ether (10 ml) and dried in a dessicator to give 4.00 g (85%) which was used without further purification mp 278° lit [19] 275-276°.

2-Amino-6-(5-aminopentylamino)-5-nitro-4(3*H*)-pyrimidine (**15a**).

To a mixture of dry dimethylformamide (80 ml), triethylamine (25 ml) and 1,5-diaminopentane (8.0 g, 0.078 mole) was added a finely ground sample of **14** (2.0 g, 0.106 mole). The mixture was stirred efficiently under a nitrogen atmosphere at room temperature for 18 hours. The resulting mixture was evaporated *in vacuo* to leave a viscous yellow oil which was dissolved (with warming on a steam bath) in water (70 ml). Standing at 5° for 5 hours furnished a yellow powder (2.14 g, 80%) which exhibited only one major product on hplc. Crystallization from water gave a yellow powder, mp 218-225°; ir (Nujol): 3455, 3390, 3300, 1675, 1640, 1620, 1570, 1530, 1330, 1255, 950, 795, 780 cm^{-1}; nmr (60 MHz, trifluoroacetic acid-d): δ 3.70 (m, PyrCH_2, 2H), 3.30 (m, NH_3^+-CH_2, 2H), 1.75 (m, (CH_2)$_2$, 6H); uv (water, *p*H 7, phosphate buffer): λ max 335 (ε = 9200), 290 (ε = 3480), 210 (ε = 15300) nm; ms: 256 (M^+, 1.2).

Anal. Calcd. for $C_9H_{16}N_6O_3$: C, 40.8; H, 6.5; N, 31.7. Found: C, 40.8; H, 6.4; N, 32.0.

2-Amino-6-(6-aminohexylamino)-5-nitro-4(3†H)-pyrimidinone (**15b**).

A mixture of dimethylformamide (200 ml), triethylamine (60 ml), 1,6-diaminohexane (20 g, 0.172 mole) and compound **14** (4.9 g, 0.026 mole) was stirred under nitrogen at room temperature for 18 hours. The resulting mixture was evaporated to leave a viscous yellow oil which was dissolved (with warming on a steam bath) in water (200 ml). On slow cooling, pale yellow platelets were formed which were filtered off (0.65 mg, mp 213-215°). Further cooling afforded a yellow powder (4.22 g, mp 211-215°). Both of these fractions exhibited similar ir spectra and hplc retention times giving a total of 4.87 g (70%). Crystallization from water furnished analytically pure sample, mp 213-215°; ir (Nujol): 3490, 3320, 3185, 1685, 1630, 1560, 1525, 1325, 1280, 1245, 795, 780 cm^{-1}; nmr (60 MHz, trifluoroacetic acid-d): δ 3.75 (m, Pyr-CH_2, 2H), 3.30 (m, NH^+_3-CH_2, 2H), 1.65 (m, (CH_2)$_4$, 8H); uv (water *p*H 7, phosphate buffer): λ max 335 (ε = 11350), 290 (ε = 6750), 215 (ε = 21300) nm; ms: 270 (M^+, 4.4).

Anal. Calcd. for $C_{10}H_{18}N_6O_3\cdot H_2O$: C, 41.7; H, 7.0; N, 29.1. Found: C, 41.9; H, 6.8; N, 29.4.

6-Chloro-2-methoxy-9-[(5-(guan-9-yl)pentyl)amino]acridine (**5a**).

A sample of **15a** (1.00 g, 3.9 mmoles) was added to desaerated formic acid (100 ml) with warming. To the resulting suspension was added zinc powder (5 mg) and the mixture stirred under a nitrogen atmosphere for 2 hours. The mixture was filtered under nitrogen and the filtrate concentrated to *ca.* 5 ml *in vacuo*. The concentrated solution was then transferred to a bomb for heating 175-180° for 3 hours. On cooling the formic acid solution was evaporated to dryness and the residue dissolved in a mixture of methanol (10 ml) and concentrated hydrochloric acid (10 ml). After boiling for 4 hours on a steam bath this hydrolysis mixture was evaporated to dryness and the residue was added to diethylamine (30 ml). The suspension was stirred for 30 minutes and then filtered and washed with chloroform to give 0.85 g (90%) of 9-(5-aminopentyl)guanine (**15a**) *ca.* 95% purity on hplc, mp 227-230°; ir (Nujol): 3200, 1710, 1625, 1300, 1180, 1010, 915, 775 cm^{-1}; nmr (80 MHz, trifluoroacetic acid-d): δ 8.45 (s, GuaC$_8$*H*, 1H), 3.95 (m, GuaN$_9$-CH_2, 2H), 2.85 (m, NH_3^+-CH_2, 2H), 2.0-1.0 (m, (CH_2)$_3$, 6H); uv (water *p*H 5.5, acetate buffer): λ max 270 (ε = 7460), 253.3 (ε = 9330), 225 (ε = 2950), 212 (ε = 10800) nm; ms: 236 (M^+, 31), 206 (M-30, 90), 151 (M-85, 10). The compound is extremely polar and hygroscopic and was used without further purification.

A solution of **15a** (0.75 g, 3.3 mmoles) in dry dimethylformamide (40 ml) was stirred with molecular sieves (4 Å) for 30 minutes at 80° under a nitrogen atmosphere. At the end of this period and after filtration, **6** (0.88 g, 3.3 mmoles) was added and the mixture was stirred for 4 hours under a nitrogen atmosphere at 80°. The resulting dark brown solution was evaporated to dryness *in vacuo* and trituration with diethyl ether to give 1.05 g of a crude material whose tlc indicated a complex mixture of products including starting materials. The extremely low solubility in all solvents resulted in an extremely long purification procedure including column chromatographic separations on silica using ethanol-ethyl acetate mixtures. Final purification by crystallization from large volumes of methanol-water afforded pure **5a**, mp 258-258.5°; ir (potassium bromide): 3470, 3200, 1715, 1680, 1630, 1565, 1460, 1225, 1180, 1020, 920, 835, 760 cm^{-1}; nmr (270 MHz, dimethylsulfoxide-d_6): δ 8.20 (d $AcrC_8H$, 1H), 7.80 (s, $AcrC_5H$, 1H), 7.75 (d, $AcrC_4H$, 1H), 7.50 (s, $GuaC_8H$, 1H), 7.50 (s, $AcrC_1H$, 1H), 7.30 (d, $AcrC_3H$, 1H), 7.20 (d, $AcrC_7H$, 1H), 6.75 (broad signal, $AcrNH$, 1H), 6.30 (s, $GuaNH_2$, 2H), 3.80 (s, $AcrOCH_3$, 3H), 3.80 (m, $GuaN_9$-CH_2, 2H), 3.60 (m, AcrNH-CH_2, 2H), 1.60 (m, $(CH_2)_2$, 4H), 1.20 (m, CH_2, 2H); uv (water *p*H 5.5, 5.5% of ethanol): λ max 445 (ε = 7750), 422.5 (ε = 8120), 342.5 (ε = 4070) nm; ms 477 (M^+, 0.5), 243 (M-234, 100); ms: FAB (glycerol): 478 ($(M+H)^+$, 40).

Anal. Calcd. for $C_{24}H_{24}ClN_7O_2$: C, 60.3; H, 5.1. Found: C, 60.3; H, 4.9.

6-Chloro-2-methoxy-9-[(6-(guan-9-yl)amino]acridine (**5b**).

The procedure as described above for 9-(5-aminopentyl)guanine (**16a**), afforded 9-(6-aminohexyl)guanine (**16b**), mp 198-201°; ir (Nujol): 3230, 1690, 1625, 1565, 1340, 1280, 950, 780, 730, 715 cm^{-1}; nmr (80 MHz, trifluoroacetic acid-d): δ 8.50 (s, $GuaC_8H$, 1H), 4.00 (m, $GuaN_9$-CH_2, 2H), 2.85 (m, NH_3^+-CH_2, 2H), 2.0-1.0 (m, $(CH_2)_4$, 8H); uv (water, *p*H 5.5, acetate buffer): λ max 270 (ε = 6400), 253.3 (ε = 8240), 225 (ε = 2380), 212 (ε = 8650) nm; ms: 250 (M^+, 33), 234 (M-16, 9), 220 (M-30, 41), 178 (86.4), 165 (100). The compound is extremely polar and hygroscopic and was used without further purification.

Compound **5b** was obtained by reacting **16b** and **6** as described above for **5a** to give the pure compound, mp 249-250°; ir (potassium bromide): 3490, 1675, 1635, 1595, 1470, 1350, 1240, 1175, 1080, 930, 840, 760 cm^{-1}; nmr (270 MHz, dimethylsulfoxide-d_6): δ 8.20 (d, $AcrC_8H$, 1H), 7.75 (s, $AcrC_5H$, 1H), 7.70 (d, $AcrC_4H$, 1H), 7.50 (s, $AcrC_1H$ and $GuaC_8H$, 2H), 7.30 (d, $AcrC_3H$, 1H), 7.20 (d, $AcrC_7H$, 1H), 6.75 (broad signal, $AcrNH$, 1H), 6.60 (s, $GuaNH_2$, 2H), 3.80 (s, $AcrOCH_3$, 3H), 3.80 (m, $GuaN_9$-CH_2, 2H), 3.60 (m, AcrNH-CH_2, 2H), 1.60 (m, $(CH_2)_2$, 4H), 1.20 (m, $(CH_2)_2$, 4H); uv (water *p*H 5.5, 5.5% of ethanol): λ max 445 (ε = 7890), 422.5 (ε = 8290), 342.5 (ε = 3860) nm; ms: 491 (M^+, 2), 285 (M-206, 3), 143 (M-248, 80); ms: FAB (glycerol): 492 ($(M+H)^+$, 25).

Anal. Calcd. for $C_{25}H_{26}ClN_7O_2 \cdot \frac{1}{2}CH_3OH$: C, 60.3; H, 5.6; N, 19.3. Found: C, 60.3; H, 5.2; N, 19.2.

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