24953-82-0; **42**, 91971-25-4; **43**, 19672-03-8; **44**, 98527-11-8; **45**, 52959-34-9; **46**, 98527-12-9; **47**, 36680-47-4; **48**, 36674-07-4; **49**, 98527-13-0; **50**, 98527-14-1; **51**, 17715-70-7; **52**, 98527-15-2; **53**, 98527-16-3; **54**, 98527-17-4; **55**, 98527-18-5; **55** (acid chloride), 98527-33-4; **55** (amide), 98527-34-5; **56**, 98527-19-6; **57**, 98527-20-9; **58**, 98527-21-0; **58** (amide), 98527-35-6; **59**, 98527-22-1; **60**, 98527-23-2; **61**, 84225-86-5; **62**, 98527-24-3; **63**, 98527-25-4; **64**, 98527-26-5; **65**, 98527-27-6; **66**, 98527-28-7; II (X^1 = X^2 = Cl), 73219-91-7; II (X^1 = Cl, X^2 = Br), 73219-92-8; II (X^1 = H, X^2 = Br), 96897-96-0; II (X^1 = H, X^2 = Pr), 96897-98-2; II (X^1 = H, X^2 = F), 52189-67-0; (2*S*)-2-(aminomethyl)-1-ethylpyrolidine, 22795-99-9; 2,5-dibromo-2-methoxybenzoic acid, 13130-23-9; 2,6-dimethoxy-3-ethylbenzene, 19672-03-8; methyl 3-bromo-2,6-dimethoxy-5-nitrobenzoate, 98527-36-7; **66**, 98527-28-7; II (x^1 = x^2 = Cl), 73219-91-7; II (x^1 = Cl, x^2 = Br), 73219-92-8; II (x^1 = H, x^2 = Br), 96897-96-0; II (x^1 = H, x^2 = Pr), 96897-98-2; II (x^1 = H, x^2 = F), 52189-67-0; (2*S*)-2-(aminomethyl)-1-ethylpyrolidine, 22795-99-9; 2,5-dibromo-2-methoxybenzoic acid, 13130-23-9; 2,6-dimethoxy-3-ethylbenzoic acid, 96897-97-1; methyl 3-bromo-2,6-dimethoxy-5-nitrobenzoate, 98527-36-7.

Potential Antitumor Agents. 45. Synthesis, DNA-Binding Interaction, and Biological Activity of Triacridine Derivatives

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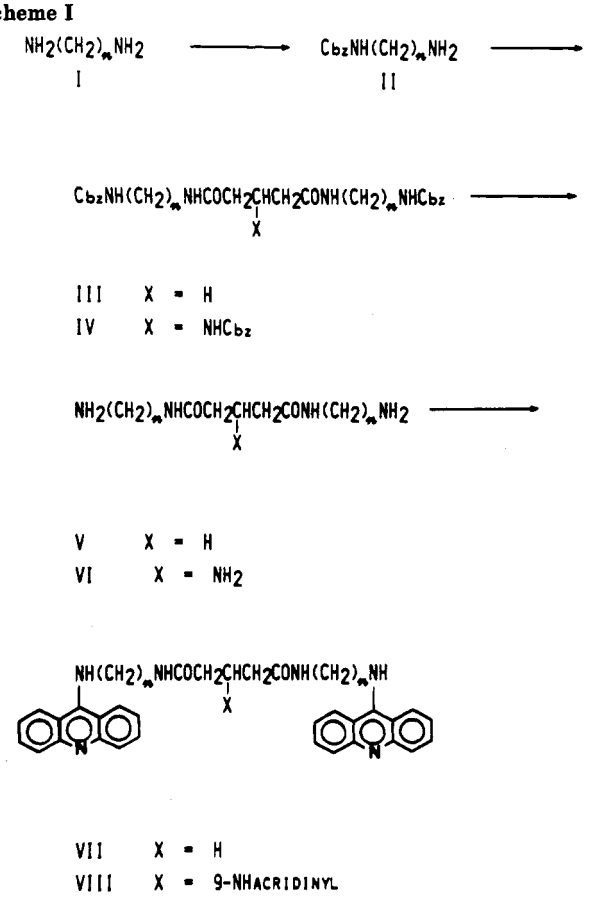
A series of amide-linked triacridines of varying interchromophore separation were synthesized as potential DNA trisintercalating agents. The corresponding diamide diacridines (lacking the central chromophore) were also prepared, and the DNA-binding and biological activities of both series of compounds were evaluated. Although one of the triacridines shows evidence of a trisintercalative binding mode, most of the triacridines (and all the diacridines) bound by bisintercalation. The diacridines showed greater cytotoxicity and higher DNA association constants than the corresponding 9-[[3-(dimethylamino)propyl]amino]acridine monomer, but addition of a third chromophore did not provide corresponding increases in either DNA affinity, inhibition of RNA synthesis, or cytotoxicity. Some members of both series show minimal in vivo antileukemic activity. The results suggest that further development of trimeric molecules as potential antitumor agents should focus on smaller chromophores with lower capacity for nonspecific binding and/or the employment of rigid linker chains to provide true molecular "staples" for DNA.

The acridine-derived DNA-intercalating agents comprise an important class of antitumor drugs.[1] The mode of action of such compounds is surmised to be inhibition of nucleic acid synthesis[2] and/or the induction of irreparable DNA strand breaks.[3,4] The drug physicochemical properties that contribute most to the cytotoxic processes include strong equilibrium binding to DNA,[5,6] long drug residence times at a particular DNA site,[7] and the ability to deliver radical species to the DNA.[8,9] While it is conceptually clear how the latter property might facilitate DNA breakage, the biochemical reasons for the favorable effect of the binding requirements have not been elucidated. Suggestions include the blocking of polymerase progression along the DNA[2,10] and selective reaction of the drug–DNA complex with proteins involved in DNA replication, such as the topoisomerases.[11,12] In efforts to obtain compounds with higher equilibrium binding constants than achievable by the monomers, much work has gone into the synthesis and evaluation of dimeric molecules, where the two chromophores are joined by a linker chain of sufficient length and flexibility to allow both chromophores to intercalate when bound to DNA. Most of this work has involved derivatives of 9-aminoacridine, and compounds of significantly higher binding constants and lower dissociation rates[13,14] than those of the monomer 9-aminoacridine have been obtained. In a logical extension of this argument, several groups[15–18] have now prepared and evaluated the DNA-binding properties of triacridine derivatives that appear to act as DNA trisintercalating agents. Some preliminary reports on the biological activity of these compounds have appeared.[17,19]

Scheme I



In this paper we report the synthesis, physicochemical properties, and biological activity of a series of triacridine

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‡ Institute of Physiology & Biochemistry, Lodz.

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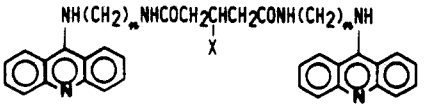
Table I. Physiochemical and Biological Data for Diacridines VII and Triacridines VIII



VII X = H
VIII X = 9-NHACRIDINYL

no.	formula	n	R_m[a]	X[b]	unwinding angle,[c] deg	C_{50}[d] AT	C_{50}[d] GC	RNA inhibn in vitro: IC_{50}[e]		L1210 in vitro: IC_{50}[f]	P388 in vivo OD[g]	P388 in vivo ILS[h]
14			−0.76		16	0.56	0.75	12.1	8.4	530	45	*i*
1	VII	2	−0.31	14.5	36	0.30	0.29	9.2	6.7	3080	30	*i*
2	VII	3	−0.10	17.0	29 (29)			7.5	6.2	350	13.3	*i*
3	VII	4	0.03	19.5	33 (30)	0.28	0.13	4.6	4.1	240	20	30
4	VII	5	0.19	22.0	32	0.28	0.24	5.2	4.2	170	20	33
5	VII	6	0.35	24.5	30	0.22	0.33	4.9	4.7	250	45	*i*
6	VII	7	0.54	27.0	29	0.15	0.23	6.2	4.5	960	30	*i*
7	VIII	2	−0.59	6.0/8.0	33	0.43	0.38	30.3	30.0	5900	3.9	*i*
8	VIII	3	−0.28	7.0/9.0	37 (45)	0.10	0.19	13.2	12.8	800	8.9	*i*
9	VIII	4	−0.19	8.5/10.5	32 (31)	0.08	0.13	5.9	5.4	2300	8.9	*i*
10	VIII	5	0.02	10.0/11.5	32	0.35	0.25	4.1	4.5	580	8.9	21
11	VIII	6	0.25	10.5/13.5	29	0.11	0.11	4.1	4.0	350	20	21
12	VIII	7	0.40	12.0/14.5	22	0.10	0.14	4.6	3.7	590	20	*i*
13	XIII (Scheme II)		−0.75	17.0	(33)	0.09	0.21	4.2	3.1	470		NT[j]

[a] R_m values were determined by liquid–liquid chromatography as in ref 27 and are for drug cations. [b] Distance (in Å) between the chromophores, measured on Cortauld models when the side chains are in fully extended conformations (see text). The asymmetry of the triacridines VIII result in nonidentical distances. [c] Unwinding angles for closed circular supercoiled DNA from *E. coli* plasmid PNZ116, determined at 20 °C and reported in ref 15, relative to ethidium bromide as 26°. Values in parentheses were determined with use of DNA from *E. coli* plasmid PML-21 at 25 °C (ref 16). [d] C_{50}: the micromolar concentration of drug necessary to displace 50% of previously bound ethidium bromide from the DNA; see ref 29. [e] IC_{50}: concentration of drug in micromolar to inhibit RNA synthesis in vitro to 50% of control value; see text and Figures. Values are averages of two to four determinations. [f] IC_{50}: concentration of drug in nanomolar to inhibit growth of L1210 leukemia cells in culture by 50%, following a 70-h continuous exposure; see ref 41. [g] OD: optimal dose of drug in milligrams/kilogram per day, administered intraperitoneally as a solution in 0.1 mL of 30% v/v ethanol–water on days 1, 5, and 9 after intraperitoneal inoculation of 10^6 P388 leukemia cells. [h] ILS: the percentage increase in lifespan of drug-treated tumor-bearing animals compared to tumor-bearing controls when treated at the optimal dose; values above 20% are considered significant. [i] ILS: of 20% not reached at any dose level. [j] NT: drug not tested in vivo.

Table II. Physiochemical Properties for the Compounds of Table I

no.	mp, °C	formula	anal.
1	290–291	$C_{35}H_{34}N_6O_2 \cdot 2HCl$	C, H, N, Cl
2	61–64	$C_{37}H_{38}N_6O_2 \cdot 2HCl$	C, H,[a] N, Cl
3	170–173	$C_{39}H_{42}N_6O_2 \cdot 2HCl$	C, H, N, Cl
4	144–146	$C_{41}H_{46}N_6O_2 \cdot 2HCl$	C, H, N, Cl
5	77–79	$C_{43}H_{56}N_6O_2 \cdot 2HCl \cdot {}^1/_2H_2O$	C, H, N, Cl
6	62–64	$C_{45}H_{54}N_6O_2 \cdot 2HCl$	C, H, N
7	223–226	$C_{48}H_{42}N_8O_2 \cdot 3HCl$	C, H, N, Cl
8	216–218	$C_{50}H_{46}N_8O_2 \cdot 3HCl \cdot H_2O$	C, H, N, Cl
9	208–210	$C_{52}H_{50}N_8O_2 \cdot 3HCl$	C, H, N, Cl
10	199–202	$C_{54}H_{54}N_8O_2 \cdot 3HCl$	C, H, N, Cl
11	199–203	$C_{56}H_{58}N_8O_2 \cdot 3HCl$	C, H, N, Cl
12	198–200	$C_{58}H_{62}N_8O_2 \cdot 3HCl \cdot {}^1/_2H_2O$	C, H,[b] N, Cl
13	194–197	$C_{37}H_{39}N_7O_2 \cdot 3HCl \cdot 2H_2O$	C, H, N, Cl

[a] H out by 0.7%. [b] H out by 0.6%.

derivatives, where the interchromophore spacing is systematically varied.

Chemistry. The diacridines of Table I were prepared by the methods outlined in Scheme I. The initial step required suitably monoprotected alkanediamines. The *tert*-butyloxycarbonyl (Boc) moiety has been used as a protecting group for this purpose,[20,21] but in this work we

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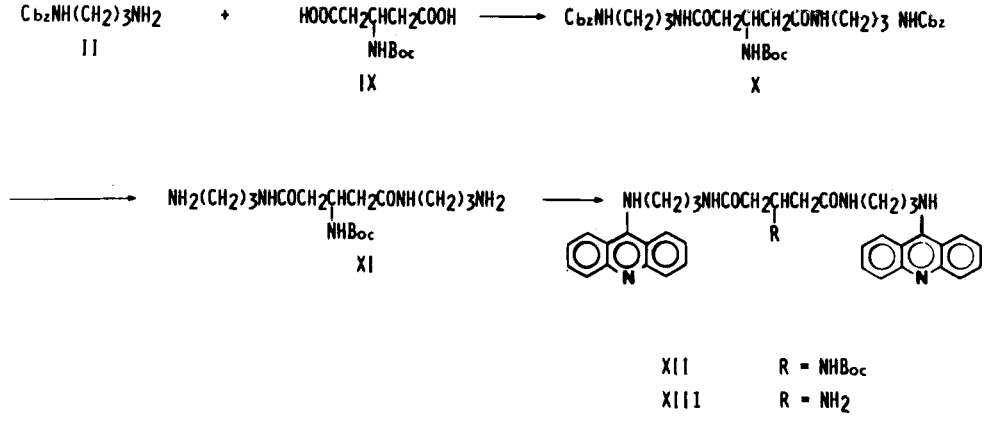
Scheme II



chose to use the more highly-crystalline benzyloxycarbonyl (Cbz) derivatives. The mono-Cbz protected alkanediamines II were prepared by the method previously reported[22] and coupled with glutaric acid with use of diethyl phosphorocyanidate[23] as the amide-forming reagent. Hydrogenolysis of the resulting highly crystalline bis-Cbz compounds III gave the corresponding diamines V, which were coupled with 9-chloroacridine in excess phenol[10] to give the diacridines VII as yellow crystalline, water-soluble dihydrochlorides.

A similar procedure was used for preparation of the triacridines VIII. To avoid the complication of optical isomers in the linker chain of these compounds, the symmetric 3-aminoglutaric acid[24] was chosen. Coupling of the Cbz-protected amino diacid as above with the appropriate mono-Cbz protected diamine gave the highly crystalline tri-Cbz derivatives IV. Hydrogenolysis and reaction of the resulting triamine with 3 mol of 9-chloroacridine provided the triacridines. A minor modification of this route was used to prepare the diacridine trihydrochloride XIII. In this case (Scheme II) the Boc-protected 3-aminoglutaric acid (IX) was used. Coupling provided the heteroprotected triamine X. Removal of the Cbz groups followed by reaction with 2 mol of 9-phenoxyacridine gave the diacridine XII. Use of 9-chloroacridine in this reaction lead to some hydrolysis of the Boc group by the released HCl. Mild acid hydrolysis of XII gave the desired diacridine XIII, which crystallized as a yellow, water-soluble trihydrochloride.

RNA Polymerase Assays. *Escherichia coli* DNA-dependent RNA polymerase was prepared as described previously.[25] Phage T7 DNA was isolated according to Kiss et al.[26] Calf thymus DNA (Worthington, USA), [^{14}C]ATP (Chemapol, Czechoslovakia), and BSA fraction V (BDH, England) were used. RNA synthesis in vitro was assayed according to Richardson,[27] except that samples were incubated for 10 min and [^{14}C]ATP was used. The reaction was initiated by the addition of *E. coli* RNA polymerase (0.65 μg in 20 μL) to mixtures of template (2.5 μg of DNA) and drug in 80 μL of buffer (resulting in final concentrations: 0.1 M KCl, 10 mM $MgCl_2$, 40 mM Tris-HCl, pH 8.0; 0.2 mM GTP, UTP, CTP, and [^{14}C]ATP, 0.1 mM dithiothreitol, 0.1 mM EDTA, 0.4 mg/mL BSA).[27,28] Reaction was terminated by addition of trichloroacetic acid–pyrophosphate,[27,28] the precipitates were collected on GF/C filters, and the radioactivity was determined by scintillation counting.[25,27] DNA and drug concentrations were determined by UV absorption in 0.01 SHE buffer, pH 7.0, using published[15] values for the drugs. All determinations were carried out in triplicate.

Results and Discussion

Table I records physicochemical and biological data for the homologous series of triacridine diamides VIII, where the linker chain between the chromophores is varied to provide minimum interchromophore distance from ca. 6 Å (compound 7) to 12 Å (compound 12). Also given in Table I are data for the corresponding diacridine diamides VII, which retain the same relative distance between the terminal chromophores but lack the central acridine.

For the diacridines, the interchromophore distances along the linker chain were measured with use of Courtauld space-filling models with the amides planar, the methylene chains fully staggered, and the chromophores pointing to the same side. For odd numbers of methylene units (compounds 2, 4, and 6), the amide carbonyls were on the same side as the chromophores and for the compounds with even-numbered chains, on the opposite side. This alternation might help account for the corresponding small alternations in unwinding angle values found (at least for the shorter members of the series).

Addition of the central acridinyl group to give triacridines introduces some distortion and asymmetry into the central unit. Interchromophore distances, measured so that the amides are again planar with all three chromophores on the same side of the molecules and approximately coplanar, are thus not identical (Table I). The values are sensitive to slight alterations in methylene staggering but are useful for relative comparison.

The ethidium displacement assay[29] was used to evaluate relative binding and to determine any selectivity of binding to different sites (represented by the extremes of poly(dA–dT) and poly(dG-dC). The micromolar concentration of the ligand required to displace 50% of previously bound ethidium from the DNA (the C_{50} value) can be shown to be inversely proportional to the ligand–DNA association constant for monointercalating ligands,[30] and for such compounds K values can be derived by allowance for

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drug-induced quenching of ethidium fluorescence.[29,30] For compounds such as minor groove binders[31] and the current polyintercalating ligands, determination of K values by this method is less certain, but comparison of determined C_{50} values does provide a valuable comparative measure of DNA binding by the triacridines VIII and the corresponding diacridines VII. The diacridines VII show little selectivity for binding to the two different polymers. Although they bind much more tightly than the monomer 9-aminoacridine (which has a C_{50} value for binding to poly(dA-dT) of 9.6 μM[10]), the binding levels are similar to those previously observed[10] for bisintercalating polymethylenediacridines and are not greatly above those for the more closely related dicationic monomer 9-[[3-(dimethylamino)propyl]amino]acridine (**14**; Table I). Introduction of a third point charge into the center of the molecule does increase DNA binding somewhat (compound **13**), but replacement of this with a charged acridine (compound **8**) provides no further increase in binding as determined by C_{50} values. Generally, the increase in binding in going from the diacridines **1–6** to the triacridines **7–12** is small, in agreement with data from other workers.[17]

All the diacridine diamides bind bisintercalatively to DNA, as shown by the unwinding angles of 29–36°,[15] compared to that (16°) of 9-[[3-(dimethylamino)propyl]-amino]acridine monomer (**14**). The triacridine derivative **7** has spacings between the chromophores of 6 and 8 Å (Table I), incapable of spanning even a single base pair, and clearly binds by bisintercalation (unwinding angle 33°), probably with the central chromophore outside the helix. When the linker chain is extended by a methylene unit to provide **8**, the interchromophore distance (7 Å) becomes large enough to span a single base pair, and all three chromophores have the ability to intercalate. Although the unwinding angles determined under different conditions are somewhat variable for this compound (Table I), its behavior is sufficiently different from that of its homologues to support the contention[16] of trisintercalative binding. However, unwinding and helix extension data for the remaining triacridines with longer chains suggest only bisintercalative binding.

The compounds of Table I span a considerable range of lipophilicity, as determined by R_m values for the cations at pH 1–2 (Table I). The range within each series (ca. 0.9 R_m unit, corresponding to ca. 2.3 log P units[32]) is almost exactly that expected for the addition of 10 methylene units, after allowing for the appropriate H-polar proximity effects.[33] Addition of a point charge to compound **2** resulting in compounds **13** gives an expected large increase in hydrophilicity (0.65 R_m unit, almost 2 log P units), and replacement of this with a charged acridine provides a more lipophilic compound (**8**).

It is interesting to compare the in vitro cytotoxicity of the diacridine diamides **1–6** against L1210 leukemia cells with related 9-linked diacridines.[10] While the simple polymethylene-linked diacridines showed high potency (IC_{50} values of 50–100 nM), the corresponding diacridine monoamides showed much poorer activity (IC_{50} > 4000 nM).[10] The possibility of such dialkylamides being hydrolyzed intracellularly could not be ruled out, but the relatively high cytotoxicities of the diacridine diamides (Table I) suggest this is not the reason for the low cytotoxicity of the previous series. The longest offrate of the diamide **1** from calf thymus DNA has been measured by stopped-flow UV spectrophotometry as 0.61×10^{-3} s^{-1}, compared to a value of 15.8×10^{-3} s^{-1} for a similar diacridine monoamide derivative,[34] suggesting increased interactions between the diamide linker chain and the DNA, but it is not possible to decide if this is the reason for the improved cytotoxicity of such diacridine diamides.

However, the most interesting conclusion to be drawn from the data of Table I is that addition of a third acridine chromophore to the molecule does not provide any increase in cytotoxicity. Pairwise comparison of compounds **1–6** and **7–12** suggests in fact that the triacridines are less active in vitro than their diacridine counterparts.

Since the primary site of action of diacridines linked by polymethylene chains is thought to be inhibition of RNA synthesis,[35,36] the effects of compounds **1–13** against *E. coli* DNA-dependent RNA polymerase was measured, using both calf thymus and phage T7 DNA templates.[27,28] Curves for the inhibition of ^{14}C-labeled ATP incorporation into RNA by the drugs were plotted and used to determine IC_{50} values (the drug concentration to inhibit incorporation by 50% compared to controls). These values are recorded in Table I.

Previous work on polymethylene-linked diacridines showed[36] that the efficiency of inhibition of the T7 DNA-dependent T7 RNA polymerase increased with increasing chain length up to C_8, with the C_8 diacridine being more than 7-fold more effective than 9-aminoacridine (IC_{50} of 10 μM compared to 75 μM). Further extension of the linker chain had no effect on the IC_{50} value. The drugs have been suggested[36] to work by preventing unwinding of the DNA to allow polymerase binding, since they mainly inhibit chain initiation. The fact that the inhibitory effect reaches a maximum with the C_8 compound, which is also the shortest polymethylene-linked diacridine to show completely relaxed bisintercalative binding, suggests that the more strained drug/DNA complexes formed by shorter diacridines are sensitive to distortion of the DNA by binding or translocation of the enzyme.

For the diamide-linked diacridines of Table I, a similar (but less dramatic) increase in inhibitory potency is seen as the interchromophore separation is increased, with the IC_{50} values for calf thymus DNA falling from 9.2 (**1**) to 4.6 μM (**3**), thereafter remaining essentially constant. Even less variation is seen with T7 DNA, but in each case the compounds showing the strongest inhibition (**3** and **4**) also are the most cytotoxic in vitro and are the only diacridines to show confirmed in vivo activity (Table I). The limited variation in RNA synthesis inhibition is not unexpected on the basis of the polymethylene diacridine results,[36] since the shortest compound (**1**) has a chromophore separation of 14.5 Å, and all show completely relaxed bisintercalative binding as judged by unwinding angles (Table I) and helix extension data.[15]

Larger differences in inhibition of RNA synthesis are seen with the triacridine derivatives, with a 10-fold difference across the series. Compound **7**, where the chains are too short to allow the central acridine to intercalate, is by far the least active, and potency steadily increases to reach a maximum for compound **11**. Although the chromophore separations of this compound (10.5 and 13.5 Å) are clearly enough to permit all three acridines to intercalate, physical measurements (Table I and ref 15)

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suggest only bisintercalative binding. Again, the most active compounds against RNA synthesis in vitro (**10** and **11**) also are the most cytotoxic and have low but reproducible in vivo antileukemic activity.

However, comparison of the RNA synthesis inhibition of corresponding pairs of diacridines and triacridines show that addition of the third chromophore has had no effect on potency. In fact, comparison of compounds **2** and **13** shows that while addition of a point charge to the side chain of diacridine **2** to give **13** doubled the potency (as shown earlier for polymethylene diacridines[36]), replacement of this by a thrid chromophore provided a compound (**8**) less active than either.

As mentioned, some members of both series showed low but reproducible activity against the P388 leukemia in vivo, when given using the rather stringent d1,5,9 protocol (drug dosing on days 1, 5, and 9 after tumor inoculation). With use of the same protocol the polymethylenediacridines, including the preclinical candidate *N,N'*-bis(9-acridinyl)-hexanediamine (NSC 219733),[37] proved completely inactive.[10] The active compounds were not ones of the same chain length in each series but instead were those with similar R_m values (0.0–0.2). This suggests that penetration into cells (governed among other things by drug lipophilicity) may be the important determinant for in vivo activity rather than intrinsic cytotoxicity.

Conclusions

Previous work has shown that dimeric DNA-intercalating molecules can have DNA association constants,[13] dissociation time constants,[13,29] and chromophore residence times[10,38] much greater than those achieved by the corresponding monomers. In some cases such dimeric molecules have also been found to have higher levels of biological activity than the monomers,[10,39] especially when connected by a neutral, rigid linker chain that reduces ligand movement on the DNA site.[10,38] Recent work has thus focused on trimeric molecules, in the hope of achieving further increases in binding and activity.[16-18] However, at least for the compounds evaluated to date, this has not proved to be the case. The DNA binding affinity of a triacridine tetraamide increased by only a small factor over that of a related diacridine diamide,[17] and the results of the present study show a similar trend. C_{50} values drop over 30-fold on going from the monomeric 9-aminoacridine to the dimeric compounds **1–6**, but only a further 2.5-fold for the trimeric derivatives **7–12**, and all of this binding increase could be accounted for by the additional cationic charge (compound **13**). Studies on the activity of amine-linked triacridines and diacridines found similar levels of cellular uptake and inhibition of macromolecular synthesis for both classes.[18] For the larger series of compounds discussed here, no biological advantage is again seen for the triacridines **7–12** over similar dimeric molecules (**1–6**), with only minimal levels of antitumor activity in vivo for both series. Further development of trimeric molecules as potential antitumor agents will have to focus on smaller chromophores with less nonspecific binding capacity and/or the employment of more rigid linker chains to provide true molecular "staples".[1] The example of echinomycin[40] (NSC 526417) is salutory. Although the compound possesses only uncharged, bicyclic quinoxaline chromophores which are not by themselves intercalating agents, they are rigidly held by the peptide backbone in a coplanar geometry about 10 Å apart. The molecule is a classical bisintercalating ligand currently in phase II clinical trials as an antitumor drug.

Table III. *N,N'*-Bis[[(benzyloxycarbonyl)amino]alkyl]pentanediamides III

n	mp, °C	formula	yield, %
2	209–212	$C_{25}H_{32}N_4O_6$	78
3	171–172	$C_{27}H_{36}N_4O_6$	72
4	191–192	$C_{29}H_{40}N_4O_6$	84
5	161–162	$C_{31}H_{44}N_4O_6$	71
6	180–181	$C_{33}H_{48}N_4O_6$	65
7	155–156	$C_{35}H_{52}N_4O_6$	71

[a] All compounds analyzed satisfactorily for C, H, and N.

Experimental Section

Where analyses are indicated only by the symbols of the elements, analytical results for those elements were obtained within ±0.4% of the theoretical values. Analyses were performed under the direction of Professor A. D. Campbell, Microchemical Laboratory, University of Otago, New Zealand. Melting points were determined on an Electrothermal apparatus using the manufacturer's stem-corrected thermometer and are as read. To monitor the progress of reactions and purity of products, TLC on SiO_2,F_{254} was used. Compounds that could not be seen by UV were visualized by heat treatment after impregnation with polyphosphomolybdic acid. For the very polar polycationic end products, TLC on cellulose using the top phase of a mixture of *i*-BuOH, HOAc, H_2O, and DMF (30:6:24:2.25) was used.

3-[*N*-(*tert*-Butoxycarbonyl)amino]pentanedioic Acid (IX). 3-Aminoglutaric acid hydrochloride[24] (1.47 g, 8 mmol) dissolved in a mixture of water (20 mL) and dioxane (25 mL) containing KOH (1.8 g) was treated with di-*tert*-butyl dicarbonate (2.18 g, 10 mmol). The reaction mixture was stirred at 20 °C for 12 h and at 60 °C for 30 min and then evaporated in vacuo. The residue was dissolved in water (25 mL), washed with $CHCl_3$ (2 × 25 mL), and acidified with citric acid and then solid NaCl was added to saturation. Following extraction with EtOAc (3 × 25 mL), the combined organic phases were washed with saturated NaCl, dried (Na_2SO_4), and evaporated to provide the crude Boc derivative (0.99 g). Two recrystallizations from EtOAc–light petroleum gave pure product as colorless prisms (0.84 g, 42%), mp 149–150 °C. Anal. ($C_{10}H_{17}NO_6$) C, H, N.

3-[*N*-(Benzyloxycarbonyl)amino]pentanedioic Acid. A vigorously stirred solution of 3-aminoglutaric acid hydrochloride (2.76 g, 15 mmol) in water (40 mL) containing $KHCO_3$ (6.8 g) was diluted with diglyme (40 mL) and treated dropwise at 5 °C with benzyl chloroformate (3.1 g, 18 mmol). The reaction mixture was stirred for a further 4 h at 20 °C and evaporated under vacuum, and the residue was dissolved in 2 N KOH (25 mL). Following filtration, the solution was washed with benzene and then acidified in the cold with 12 N HCl. The crystalline solid which slowly separated on prolonged cooling was collected, washed with ice-cold water, and then crystallized from a small volume of hot water. Recrystallization from EtOAC–light petroleum gave pure product as colorless needles (1.20 g, 39%), mp 162–163 °C. Anal. ($C_{13}H_{15}NO_6$) C, H, N.

Preparation of Diacridines VII. As an example, the preparation of compound VII (n = 3) is given in detail.

***N,N'*-Bis[3-[(benzyloxycarbonyl)amino]propyl]pentanediamide (III, *n* = 3).** A solution of glutaric acid (0.4 g, 3 mmol) and *N*-(benzyloxycarbonyl)-1,3-propanediamine (1.29 g, 6.2 mmol) in dry DMF (8 mL) was treated at 0 °C with diethyl phosphorocyanidate (1.14 g, 7.0 mmol), followed by NEt_3 (0.71 g, 7.0 mmol). The mixture was stirred at 20 °C for 1 h and then at 100 °C for

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Table IV. *N,N'*-Bis[[(benzyloxycarbonyl)amino]alkyl]-3-[*N*-(benzyloxycarbonyl)amino]pentanediamides IV

n	mp, °C	formula[a]	yield, %
2	215–217	$C_{33}H_{39}N_5O_8$	84
3	199–200	$C_{35}H_{43}N_5O_8$	72
4	174–175	$C_{37}H_{47}N_5O_8$	82
5	166–167	$C_{39}H_{51}N_5O_8$	71
6	161–162	$C_{41}H_{55}N_5O_8$	68
7	157–159	$C_{43}H_{59}N_5O_8$	70

[a] All compounds analyzed satisfactorily for C, H, and N.

30 min. Concentration to half volume under vacuum followed by addition of excess 10% aqueous $KHCO_3$ provided the diamide. Crystallization from aqueous EtOH gave a white solid (72% yield), mp 171–172 °C (Table III). The other homologues were prepared in similar fashion (Table III).

The above diamide (III, n =3; 3 mmol) in MeOH (100 mL) was hydrogenated (5% Pd/C, 60 psi, 12 h). Removal of the catalyst and all volatiles gave a quantitative yield of the corresponding diamine (V, n = 3), homogeneous to TLC. The compound was used directly in the next step.

***N,N'*-Bis[3-(9-acridinylamino)propyl]pentanediamide (VII, *n* = 3).** The above diamine (V, n = 3; 0.36 g, 1.5 mmol) and freshly crystallized 9-chloroacridine (0.66 g, 3.1 mmol) in dry phenol (2 g) were heated together at 115–120 °C for 3 h. Addition of acetone to the cooled mixture precipitated a solid, which was collected, washed well with acetone, dissolved in water, and basified with excess NH_4OH. The free base was purified by column chromatography on alumina (activity II–III), eluting with MeOH–CH_2Cl_2. Appropriate fractions were pooled and crystallized from MeOH–EtOAc to give the pure free base. Conversion to the dihydrochloride salt (MeOH–EtOAc–HCl) followed by crystallization from MeOH–EtOAc gave pure diacridine VII (n = 3) as yellow hygroscopic crystals (60% yield), mp 61–64 °C (Table II).

Use of the homologous diamines similarly gave the homologous diacridines VII (n = 2–7), in yields ranging from 48% to 69%.

Preparation of Triacridines VIII. As an example the preparation of compound VIII (n = 3) is given.

***N,N'*-Bis[3-[(benzyloxycarbonyl)amino]propyl]-3-[*N*-(benzyloxycarbonyl)amino]pentanediamide (IV, *n* = 3).** A mixture of 3-[*N*-(benzyloxycarbonyl)amino]glutaric acid (3.0 mmol) and *N*-(benzyloxycarbonyl)-1,3-diaminopropane (6.2 mmol) was treated as detailed above for preparation of compounds III. Initial workup gave a gelatinous product, which was purified by slow crystallization first from aqueous DMF followed by aqueous EtOH to give a bulky white solid (72% yield), mp 199–200 °C (Table IV). Other homologues were prepared similarly, and the crude products were purified by repeated crystallization from aqueous DMF or aqueous EtOH until homogeneous on TLC; to avoid gelatinous products, crystallizing solutions were allowed to cool very slowly.

The above diamide (IV, n = 3) was hydrogenated as detailed above to yield the crude triamine VI (n = 3), which was used directly.

***N,N'*-Bis[3-(9-acridinylamino)propyl]-3-(9-acridinylamino)pentanediamide (VIII, *n* = 3).** The above triamine (VII, n = 3; 0.39 g, 1.5 mmol) and 9-chloroacridine (1.0 g, 4.7 mmol) were reacted together as described above for the preparation of the bis compound. The crude free base was extracted twice with hot EtOAc. Evaporation left a solid, which was crystallized from a small amount of MeOH to give pure free base. Conversion to the trihydrochloride salt (MeOH–EtOAc–HCl) followed by crystallization from MeOH–EtOAc gave pure product (51% yield), mp 216–218 °C (Table II).

Similar reactions gave the other triacridine homologues as water-soluble, hygroscopic, semigelatinous trihydrochloride salts, in yields ranging from 40% to 74%.

***N,N'*-Bis[3-[(benzyloxycarbonyl)amino]propyl]-3-[*N*-(*tert*-butoxycarbonyl)amino]pentanediamide (X).** A mixture of 3-[*N*-(*tert*-butoxycarbonyl)amino]pentanedioic acid (0.80 g, 3.2 mmol) and *N*-(benzyloxycarbonyl)-1,3-diaminopropane (1.41 g, 6.8 mmol) in dry DMF (5 mL) was cooled to 0 °C and treated with diethyl phosphorocyanidate (1.25 g, 7.7 mmol), followed by NEt_3 (0.78 g, 7.7 mmol). The reaction mixture was stirred at 20 °C for 30 min and then at 100 °C for 30 min, cooled, and shaken with excess 10% aqueous $KHCO_3$. The resulting precipitate was washed with 10% aqueous $KHCO_3$ and water and dried. Crystallization from aqueous DMF and then from aqueous MeOH gave pure material as a white solid (1.46 g, 72%), mp 160–162 °C. Anal. ($C_{32}H_{45}N_5O_8$) C, H, N.

A solution of the above compound in MeOH was hydrogenated as described previously to give a quantitative yield of the corresponding diamine as a white solid homogeneous on TLC. This was used directly.

***N,N'*-Bis(9-acridinylamino)-3-[*N*-(*tert*-butoxycarbonyl)amino]pentanediamide (XI).** A mixture of 9-phenoxyacridine (0.81 g, 3.0 mmol) and the above diamine (X; 0.50 g, 1.4 mmol) in dry dimethylacetamide (3 mL) was heated at 115 °C for 3 h. The cooled mixture was treated with HOAc (1 mL) and diluted with EtOAc. The resulting semisolid precipitate was dissolved in water, and basified with NH_4OH, and the precipitated free base was extracted with hot EtOAc. Evaporation and crystallization of the residue twice from small volumes of MeOH gave yellow prisms of the pure free base (XI; 0.62 g, 62%), mp 130–132 °C).

Compound 13 of Table I. The above compound (0.25 g) was dissolved in EtOAc (150 mL). Dry HCl gas was passed into the solution at 0 °C, rapidly precipitating a solid that was collected and washed with EtOAc. Crystallization from MeOH–EtOAc gave a yellow, extremely hygroscopic trihydrochloride (77% yield), mp 194–197 °C (Table II).

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Registry No. I (n = 2), 107-15-3; I (n = 3), 109-76-2; I (n = 4), 110-60-1; I (n = 5), 462-94-2; I (n = 6), 124-09-4; I (n = 7), 646-19-5; II (n = 2), 72080-83-2; II (n = 3), 46460-73-5; II (n = 4), 62146-62-7; II (n = 5), 69747-36-0; II (n = 6), 66095-18-9; II (n = 7), 62146-64-9; III (n = 2), 98577-65-2; III (n = 3), 98577-66-3; III (n = 4), 98577-67-4; III (n = 5), 98577-68-5; III (n = 6), 98577-69-6; III (n = 7), 98577-70-9; IV (n = 2), 98577-71-0; IV (n = 3), 85185-25-7; IV (n = 4), 98577-72-1; IV (n = 5), 98577-73-2; IV (n = 6), 98577-74-3; IV (n = 7), 98577-75-4; V (n = 2), 98577-77-6; V (n = 3), 98577-78-7; V (n = 4), 98577-79-8; V (n = 5), 98577-80-1; V (n = 6), 98577-81-2; V (n = 7), 98577-82-3; VI (n = 2), 98577-83-4; VI (n = 3), 85185-26-8; VI (n = 4), 98577-84-5; VI (n = 5), 98577-85-6; VI (n = 6), 98577-86-7; VI (n = 7), 98611-52-0; VII (n = 2), 98502-80-8; VII (n = 3), 98502-81-9; VII (n = 4), 98502-82-0; VII (n = 5), 98502-83-1; VII (n =6), 98502-84-2; VII (n = 7), 98502-85-3; VIII (n = 2), 98512-16-4; VIII (n = 3), 98502-86-4; VIII (n = 4), 98502-87-5; VIII (n = 5), 98502-88-6; VIII (n = 6), 98502-89-7; VIII (n = 7), 98502-90-0; IX, 85185-24-6; X, 98577-76-5; XI, 98577-87-8; XII, 98577-88-9; XIII, 98577-89-0; 3-aminoglutaric acid, 1948-48-7; 3-[*N*-(benzyloxycarbonyl)amino]pentanedioic acid, 17336-01-5; glutaric acid, 110-94-1; 9-chloroacridine, 1207-69-8.