these conditions they are less persistent than dimesitylcarbene.[14]

The steric interactions of the ortho-methyl groups must have a profound effect on the structure of I. They force it to adopt a structure that is close to linear and presumably overhang the reactive site so that attack at solvent molecules is disfavored and intramolecular abstraction at a methyl group becomes a viable decay pathway.[2] Perhaps the most intriguing aspect of its chemistry is that unlike other diaryl carbenes I does not react to form an azine, even though this molecule is thermally and photochemically stable, but preferentially forms a sterically congested dimer (reactions 1 and 2). As was previously suggested,[2] azine formation should in principle proceed from the singlet state while dimer formation can take place in a triplet–triplet reaction.

Dimesitylcarbene has a geometry that effectively maximizes the triplet–singlet energy gap.[15] The steric interactions of the methyl groups will also disfavor ring rotations and reduction of the central C–C–C bond angle, which could provide a pathway for intersystem crossing from the triplet to singlet state, thus discriminating against azine formation. However, another intriguing possibility is that the steric influence of the methyl groups is so important that carbene molecule reactions require substantial activation energy, and as a consequence carbene–carbene combination becomes a significant reaction pathway. The kinetics for these reactions are currently under investigation.

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Triacridine Derivative: First DNA Tris-Intercalating Ligand

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In 1961 it was recognized[1] that the acridine derivative proflavine binds to double-stranded DNA primarily by intercalation of the aromatic chromophore between the base pairs.

In efforts to obtain compounds of higher DNA affinity for use both as possible antitumor agents[2,3] and as probes of ligand–DNA interactions,[4,5] several groups have investigated compounds containing two chromophores joined by various linker groups. These compounds generally show much greater DNA affinity than the corresponding single chromophores, and many examples have been shown by a variety of techniques[6] to be true DNA bis-intercalators.

We now report the synthesis of the triacridine derivative **1c** (Scheme I) together with the two model compounds **1a** and **1b**. A comparison of the DNA-binding behavior of the three compounds strongly indicates that **1c** is the first DNA tris-intercalating ligand. Acridine was chosen as the chromophore because of its proven[7] propensity to bind to DNA in the intercalative mode. To keep unfavorable entropic effects to a minimum, the spacing distance between the chromophore needed to be the shortest that would permit them all to intercalate.

From the above considerations, the linker chain **4b** was chosen. The two amide linkages provide excellent water solubility for the resulting ligand (**1c**), and Courtauld models of the side chain in the fully staggered conformation indicate a fairly constrained structure with the three amine groups positioned on the same side of the molecule, providing interchromophore distances of about 7 Å. Reaction of 3-(*N*-benzyloxycarbonyl)aminoglutaric acid (**2d**[8]) with *N*-(benzyloxycarbonyl)propane-1,3-diamine and diethyl phosphorocyanidate[9] gave the tris(carbobenzyloxy) derivative **3d**. Hydrogenolysis of the protective groups and usual coupling of the resulting triamine **4b** with 9-chloroacridine gave the triacridine trihydrochloride **1c** as a yellow, crystalline water-soluble solid. The synthetic scheme offers considerable scope for variation; thus use of glutaric acid provided the diacridine **1a**, while employment of the Boc-protected aminoglutaric acid **2e** gave the triply charged diacridine **1b**. Reaction of the last compound with 1 equiv of 9-chloroacridine converted it to the triacridine **1a**.[10]

Scheme I

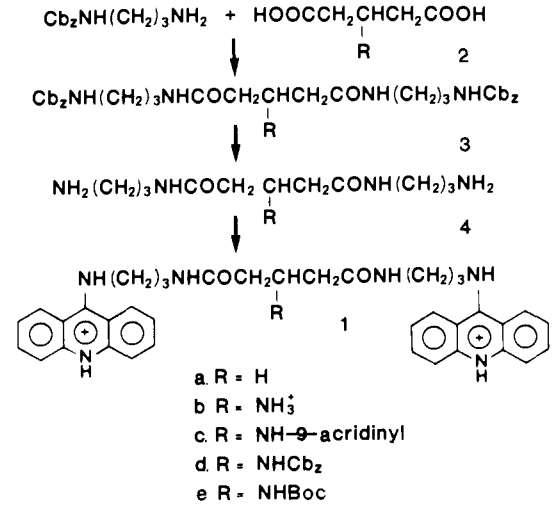

Table I. Interaction of Compounds 1a-c with DNA

	UV-vis absorption[a]				
	free ligand		bound ligand		
compd	λ, nm	ε, M^{-1} cm^{-1}	λ, nm	ε, M^{-1} cm^{-1}	r^b
1a	437	11100	440	8600	0.0365
	413	13600	417	9800	
	267	60300			
1b	437	11700	440	9300	0.0409
	413	15000	417	10500	
	259	63400			
1c	435	15600	440	13700	0.0265
	412	21300	417	16600	
	257	108000			

[a] In 0.01 M SHE buffer, pH 7.0, T = 25 °C; [ligand] = 10^{-5} M; D/P ratio = 1/100; calf thymus DNA. [b] Ligand per phosphate ratio in 0.01 M SHE buffer at the equivalence point of the viscometric titration of PML-21 DNA; T = 25.3 °C; [DNA] = 1.59 × 10^{-4} M (P). Under the same condition, r values were determined for ethidium bromide (0.0464), 9-aminoacridine (0.0812), and 9-(ethylamino)-acridine (0.0770). These values are not corrected for free ligand, but the error thus introduced is less than 10%.

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Compounds **1a-c** bind strongly to DNA, as indicated by changes in their fluorescence quantum yields and by hyperchromism and bathochromic shifts in their absorption spectra (Table I) on DNA addition.

Evidence that the DNA binding of **1a-c** is intercalative comes from consideration of their molar extinction coefficient $\epsilon(\lambda_{max})$ at the maximum of their first absorption band. In the free state, ϵ(412 nm) of **1c** is only about twice as large as ϵ(409 nm) for the monomeric 9-(ethylamino)acridine, due to intramolecular stacking, a phenomenon observed for many polychromophores.[5] However, ϵ(417 nm) for bound **1c** is just 3 times as large as ϵ(417 nm) for bound 9-(ethylamino)acridine, suggesting that all three chromophores of **1c** are in environments identical with that of 9-(ethylamino)acridine (i.e., intercalated).

Definitive evidence concerning the mode of DNA intercalation of **1a-c** comes from their ability to unwind and rewind closed circular supercoiled DNA. The D/P ratios (r) for the compounds to completely relax the supercoils of PML-21 closed circular DNA as determined by viscometry are recorded in Table I,[11] together with the r values for ethidium bromide, 9-aminoacridine, and 9-(ethylamino)acridine measured under the same conditions. Assuming an unwinding angle of 26° for ethidium[12] results in an unwinding angle of 15° for the monomeric 9-aminoacridines, in agreement with other determinations.[6,13] This indicates that the chromophore determines the geometry of the ligand-DNA complex. The diacridines **1a** and **1b** have comparable unwinding angles (29 and 33°, respectively), of about twice that of the monomer, indicating bis-intercalation. A similar value for the triply charged compound **1b** suggests that the point charge in the chain has little effect on ligand-DNA geometry.

The triacridine **1c**, where the point charge of **1b** has been replaced by a third acridine chromophore, has an unwinding angle of 45°, 3 times that of the monomer, indicating strongly that all three chromophores bind by intercalation. The value of 45° is a minimum, for the compound binds avidly to all surfaces, including glass. The binding constant K of **1c** to PML-21 DNA was estimated to be $>10^6$ M^{-1} from a Vinograd plot[14] by using a site exclusion model.[15]

The spacing of 7 Å between the chromophores demands that **1c** forms single base pair sandwiches on intercalation as suggested for the $(CH_2)_6$- and $(CH_2)_7$-linked diacridines.[6] However, the present data do not allow determination of the exact nature of the triacridine-DNA complex. NMR studies of triacridine-oligonucleotide complexes provide more information and are underway. The possibility that **1c** tris-intercalates only in presence of certain DNA sequences is under study using several analogous compounds. This work is facilitated by the synthetic scheme outlined (Scheme I), which permits easy variation of both linker chain and chromophores.

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Registry No. 1a·2HCl, 85185-21-3; **1b**·3HCl, 85185-22-4; **1c**·3HCl, 85185-23-5; **2a**, 110-94-1; **2d**, 17336-01-5; **2e**, 85185-24-6; **3d**, 85185-25-7; **4b**, 85185-26-8; 9-chloroacridine, 1207-69-8; *N*-(benzyloxycarbonyl)propane-1,3-diamine, 46460-73-5.

(10) All compounds analyzed correctly for the assigned structure. The end products **1** had NMR spectra consistent with the assigned structures. The compounds showed one spot on TLC (Merck silica gel 60, developed in the top phase of *n*-BuOH/water/AcOH (5:4:1) and eluted for 20 cm).

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Sequential Resonance Assignments in DNA ^{1}H NMR Spectra by Two-Dimensional NOE Spectroscopy

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The utility of ^{1}H NMR in structural studies of biological macromolecules has grown enormously since the advent of two-dimensional (2-D) techniques.[1-7] Systematic methods have been developed to obtain resonance assignments in ^{1}H NMR spectra of small proteins[8,9] by a combination of 2-D NOE (NOESY)[10,11] and 2-D J-correlated spectroscopy (COSY, SECSY).[6,12] These assignments are a prerequisite for a detailed analysis of 2-D NOE data, which yields a large amount of proton-proton "contacts" (proton-proton distances within about 0.4 nm) from which the solution structure of the protein may be deduced.[13]

In this communication we show that a similar strategy can be used in nucleic acid conformational studies. A sequential assignment procedure is described for most of the base and deoxyribose protons in DNA duplexes employing 2-D NOE spectroscopy. As in the case of proteins the individual resonance assignments thus found may form the basis for a quantitative analysis of 2-D NOE spectra in terms of proton-proton distances and, eventually, the DNA structure in solution.

A 360-MHz 2-D NOE spectrum was recorded of a mixture of two synthetic[14,15] complementary heptamers d(TGAGCGG) and d(CCGCTCA), which form a duplex under the experimental conditions employed (see legends to Figure 1). This DNA fragment is homologous to part of the *lac* operator of *Escherichia coli*, which is the subject of current research on specific protein-nucleic acid interactions in our laboratory. In the 2-D NOE experiment dipole-dipole cross relaxation between a pair of closely spaced protons results in the appearance of an off-diagonal peak linking the two corresponding diagonal resonances, thereby establishing the promixity of the two protons.[10]

Three parts of the 2-D spectrum will be discussed in detail here. Figure 1A shows the region of cross peaks that link the base proton (AH8, GH8, CH6, TH6) resonances with those of the CH5 and H1′ protons. In Figure 1B the contacts are shown between the same base protons and the TCH_3 and sugar H2′, H2″ protons.

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