

Trimethylene Bridges as Synthetic Spacers for the Detection of Intramolecular Interactions

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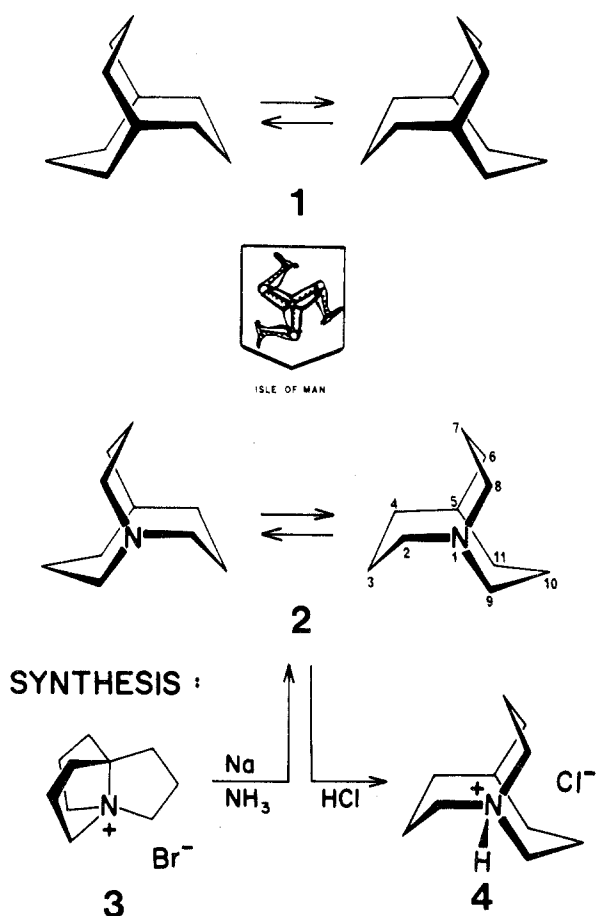
The rational synthesis of organic compounds is being applied to a broad range of problems regarding function, property, stereochemistry, and biological activity. One illustration is our use of polymethylene bridges, in particular the trimethylene bridge, $-(CH_2)_3-$, as synthetic spacers for examining repulsive and attractive intramolecular interactions.¹

Three Trimethylene Bridges. Linking two bridgeheads with three trimethylene bridges gives conjoined arrays of eight-membered rings,^{2,3} which are of special interest because of their geometry, internal strain, and spectroscopic and chemical behavior. Bicyclo[3.3.3]undecane (1), dubbed "manxane" by the late W. Parker, was synthesized by a double ring expansion of the short bridge of bicyclo[3.3.1]non-2-en-9-one.⁴ Manxane (1) has C_{3h} molecular symmetry and at room temperature is in a state of rapid conformational equilibrium between two degenerate forms. In terms of the eight-membered rings, the $BC-BC-BC$ (all boat-chair) conformation was considered to be at least 6 kcal/mol more stable than the alternative conformations.

For the related 1-azabicyclo[3.3.3]undecane (2), or "manxine", which has C_3 symmetry, the two corresponding forms are dissymmetric (chiral). Manxine⁴ was synthesized by sodium and ammonia reduction, probably proceeding via homolytic central bond cleavage,⁵ of 1-azoniatricyclo[3.3.3.0]undecane bromide (3).⁶ A "frozen" ¹H NMR spectrum at 100 MHz was obtained for 2 in $CDCl_3/CD_2Cl_2$ near $-80^\circ C$. The chemical shifts for the methine proton and carbon in manxine and its hydrochloride (4) reflected unusual hybridization, and the ¹³C-H coupling constant indicated increased p character in the C-H bond.

The corresponding flattening of the bridgehead region, with an increase in the s character of the carbon bonds to the bridgehead atom, was indicated clearly by the X-ray crystal study of manxine hydrochloride (4),^{7,8} which showed the C-C-C and C-N-C valency angles

THREE TRIMETHYLENE BRIDGES



at the bridgeheads to be $113.5-114.8^\circ$ and $115.0-115.9^\circ$, respectively. This flattening is accompanied by wid-

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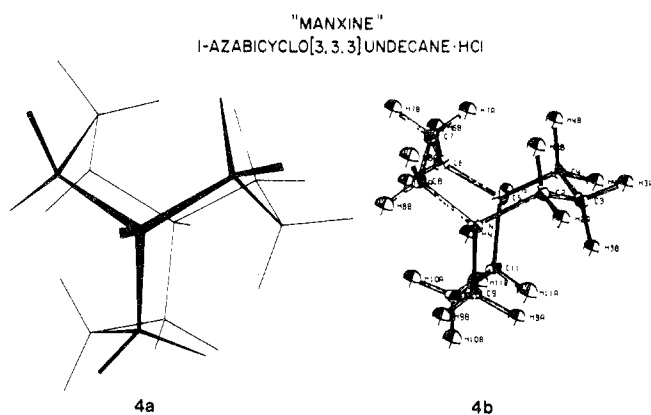
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Nelson J. Leonard was born in Newark, NJ, in 1916. He received his undergraduate education at Lehigh University. After 2 years at Oxford University as a Rhodes Scholar and graduate work at Columbia University, where he received the Ph.D. degree, he went to the University of Illinois for postdoctoral work with Roger Adams. Except for U.S. Government service and for visiting professorships, he has ever since been on the faculty of the University of Illinois, where he is now Professor of Chemistry and Biochemistry and a Member of the Center for Advanced Study.

ening of the C(N)-C-C angles in the bridges and by torsional twisting of the bridges. A comparison of the Dreiding model (4a) and the X-ray structure (4b) of



1-azabicyclo[3.3.3]undecane hydrochloride shows that the endo hydrogen on C-3 is pushed away from the intraannular hydrogens on C-9 and C-11 and the same action takes place in the other bridges. By this action, the torsion angles in the trimethylene bridges, which appear slightly greater than 90° in the model (4a), are reduced to the observed 66.5 – 68.7° in 4b, thus providing some energy compensation for the internal strain.

The unusual ultraviolet spectrum of 1-azabicyclo[3.3.3]undecane, or manxine (2), is compatible with this strained geometry. The absorption maximum for 2 is at exceptionally long wavelength, 240 nm with ϵ 2935 in ether, possibly a record for a saturated tertiary amine.^{4,7} The shift to longer wavelength (lower energy) for the $n \rightarrow p$ transition is compatible with the flattening of the nitrogen pyramid in the ground state since, by analogy with quinuclidine and ammonia, the excited state would be expected to approach coplanar bonding.⁹ For manxine in the vapor state, the 0–0 band of the lowest transition lies at 278.3 nm. Four distinct electronic Rydberg transitions are observed above 200 nm, each characterized by a prominent 0–0 band, which is the most intense vibronic member within similar Franck–Condon envelopes. From the shape of the envelopes, Halpern¹⁰ concluded that the upper state geometry of the amine is similar to that of the ground state. In the vapor, manxine is fluorescent, with a fluorescence lifetime of 91 ns, a quantum yield near unity at 0.10 torr, and emission extending above 300 nm upon excitation at 258 nm.

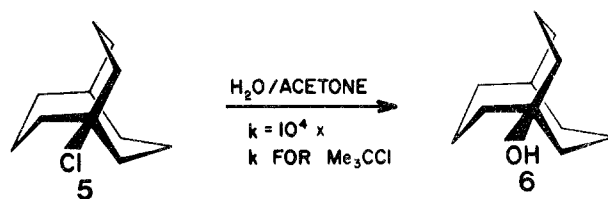
The photoelectron spectrum for the bridgehead lone pair in manxine is very different from that of other tertiary amines.¹¹ The sharp onset and narrow band width indicate vertical ionization from a preferred planar geometry in manxine to a planar radical cation. The vertical ionization potential of 7.01 eV is very low, especially when set against the gradual onset of ioni-

zation realized at 7.04 eV for tri-*n*-propylamine, 7.20 for triethylamine, and 7.55 for quinuclidine. The proton affinity of manxine is 1.6 kcal/mol lower than that of tri-*n*-propylamine as determined by equilibrium ion cyclotron resonance techniques in the gas phase.¹¹ In aqueous solution, manxine is correspondingly 1.5 kcal/mol less basic than quinuclidine in terms of $\Delta G^\circ_{\text{prot}}$.⁴

In addition to the lowered basicity of manxine, the bicyclo[3.3.3]undecane system exhibits several other unusual chemical properties. Both manxane and manxine are extremely reactive and are unstable in the presence of air. Manxane, even stored in a brown screw-capped vial in the absence of light, absorbs oxygen rapidly. Parker described the extreme reactivity of the bridgehead position of manxane (1) to radical reactions,¹² which depends upon the lowered energy of activation for the formation of a flattened radical intermediate.

There is also lowered energy of activation for the formation of a bridgehead cation in the manxane series, as shown by the finding that 1-chlorobicyclo[3.3.3]undecane, or 1-manxyl chloride (5), undergoes solvolysis

ENHANCEMENT OF REACTIVITY AT BRIDGEHEAD



to the corresponding alcohol (6) in aqueous acetone about 10^4 times faster than *tert*-butyl chloride in the same solvent.¹³ Empirical force-field calculations including allowance for $sp^3 \rightarrow sp^2$ rehybridization at the bridgehead indicate that among the components: bond stretching, angle strain, torsional strain, and nonbonded strain, the relief of angle strain during ionization ($\Delta H = -4.31$ kcal/mol) accounts for the major portion of the enthalpy change associated with strain ($\Delta H = -6.77$ kcal/mol).

Two Trimethylene Bridges. Not all transannular interactions are unfavorable. In rings of eight, nine, and ten members (mesocyclic rings) containing electron-donor and electron-acceptor groups on opposite sides of the ring, the negative enthalpy and the restriction of the degrees of freedom lead to favorable interaction between such groups.^{14–16} In general, the zest for research on transannular interactions and reactions^{14–22} was derived from discoveries of phenomena that were

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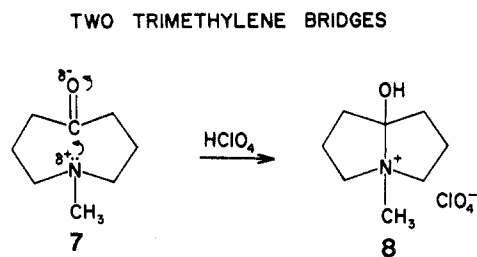
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observed neither in smaller or larger rings nor intermolecularly. Transannular *interaction* between such groups as tertiary amine and ketone carbonyl (7), for



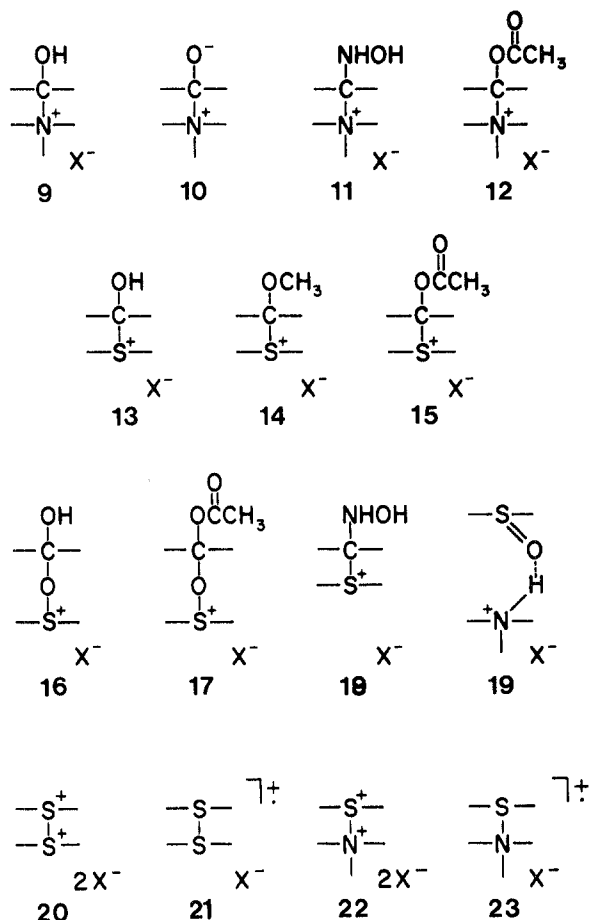
TRANSANNULAR INTERACTION TRANSANNULAR REACTION

example, in which the lone-pair electrons on N are modifying the sp^2 $C=O$ toward sp^3 , has been detected by IR and UV spectra, dipole moments, optical rotatory dispersion,²³ ^{17}O NMR,²⁴ and ^{13}C NMR²⁵ spectra. Transannular *reaction* between the proximate amine and carbonyl groups, with development of a σ bond between nitrogen and carbon (8), which becomes a possibility when the conjugate acid is formed, is readily detectable by IR and NMR spectra and by the disappearance of any UV absorption detected in the amino ketone.

Eight-membered rings allow spatial control of organic functionality, as illustrated in the accompanying collection of partial structures where the new functionality has been dissected from the original eight-membered ring and where X^- is generally a nonnucleophilic anion. The functional group combinations thus created would not exist without the benefit of ring constraint. For example, the α -hydroxy quaternary ammonium functionality 9 is uniquely generated intramolecularly. There is multiple evidence for the existence of the (hydrated) zwitterionic form 10 (of 7) in aqueous solution. The oxime of 1-benzyl-1-azacyclooctan-5-one (7, with $PhCH_2$ in place of CH_3) was converted to a perchlorate salt that lacked $C=N$ infrared absorption, was readily hydrogenolyzed to pyrrolizidine, and therefore could be recognized as having the α -hydroxylamino quaternary ammonium functionality of 11.²⁶ Excess $HClO_4$ produced a diperchlorate that was also a transannular-bonded salt. The α -acetoxy quaternary ammonium functionality 12 was created by acetylation of 9 with acetic anhydride²⁷ or ketene,²⁸ and transannular salts of types 9 and 12 proved useful in assigning the positions of microbiological oxygenation of azacycloalkanes.²⁷ A substituted transannular salt of type 9 was also used for the stereospecific synthesis of the alkaloid derivative (\pm)-isoretroecanone.²⁹ The behavior of the salt 12 as a powerful acetylating agent was verified in two rather extreme examples: the acetylation of piperidine and of potassium acetate.²⁸

Transannular interaction was detected between sulfide and carbonyl carbon separated by two trimethylene bridges in 1-thiacyclooctan-5-one.³⁰⁻³² Salt formation

SPATIAL CONTROL OF ORGANIC FUNCTIONALITY



led to the α -hydroxysulfonium functionality 13, convertible to the corresponding α -methoxy- (14) and α -acetoxy-sulfonium (15) species by simple recrystallization from methanol and by treatment with acetic anhydride, respectively.³² Structures analogous to 13-15 were not formed from the open-chain analogue 2-thiaheptan-6-one. The unusual functionality in 16 was obtained by transannular conversion of 1-thiacyclooctan-5-one 1-oxide to the bicyclic perchlorate protonated on carbonyl oxygen.³³ The structures of the hydroxy (16) and corresponding methoxy and acetoxy (17) perchlorates were assignable by kinetics and ^{18}O exchange data as well as by IR and NMR spectra. The additional oxygen bridge between the sulfur and the carbonyl carbon permitted similar, but less stable, salt formation across a seven-membered ring.³⁴ In the eight-membered-ring series, the conjugate acid of the sulfide ketoxime provided the functionality 18, corresponding to the tertiary amine-ketoxime combination (11),³⁵ while the compound having two trimethylene bridges between tertiary amine and sulfoxide moieties, upon acidification, led to the first example of $N^+-H-O=S$ bonding across a ring to form a bicyclic system (19), comprising an eight-membered ring of BC con-

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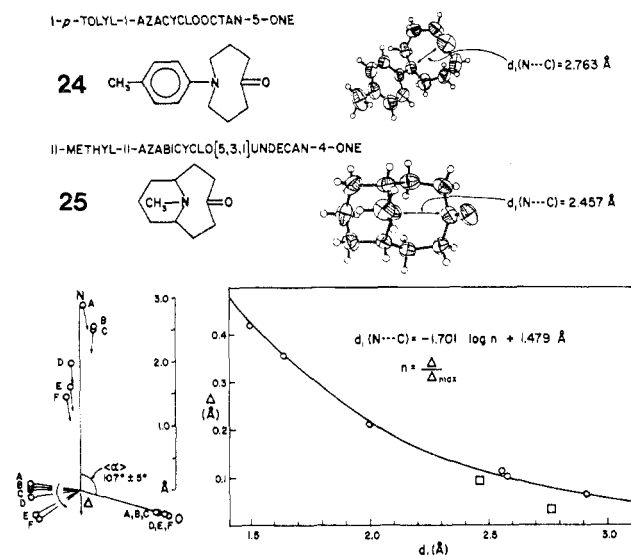


Figure 1. Nucleophilic addition to a carbonyl group (taken in part from ref 47 and 48 and reprinted with permission; copyright 1975 International Union of Crystallography).

formation in the crystal.³⁶⁻³⁸

Dicationic dithioethers containing a sulfur-sulfur bond (20) were obtained for the first time by a two-electron oxidative coupling of mesocyclic dithioethers (1,5-dithiacyclooctane and 1,6-dithiacyclodecane) with NOBF_4 or NOPF_6 in acetonitrile.³⁹ The functionality in 20 is unique in that it can behave as either an electrophile or a two-electron oxidizing agent. The ^1H and ^{13}C NMR spectra confirmed the structure and, in the case of the eight-membered ring, the ESR spectrum confirmed the intermediacy of the cation radical species 21. The latter could also be obtained from 1,5-dithiacyclooctane by mixing with an equimolar portion of 20 or by oxidation with $\text{Cu}(\text{CH}_3\text{CN})_4(\text{BF}_4)_2$ in nitromethane.^{40,41} The ESR spectrum of the yellow solution, λ_{max} 412 nm, containing 21 was observable for at least 72 h at room temperature. Formed from the analogous 5-methyl-1-thia-5-azacyclooctane were the dication 22 and the short-lived N-S bonded cation radical 23, the first examples of aliphatic dication and cation radical in which two different heteroatoms are bonded together.⁴² It may be assumed that additional new organic functionality will result from similar spatial control.

The X-ray analysis of the two amino ketones (24, 25)^{43,44} featured in the solution to problems relating to the approach of a nucleophile to a carbonyl group, namely, the favored angle of approach and the change in multiplicity of bonding with proximity. A survey of crystal-structure analyses of a series of constrained compounds: A, methadone; B, cryptopine; C, protopine; D, clivorine; E, retusamine; F, *N*-brosylmitomycin A,

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ONE TRIMETHYLENE BRIDGE

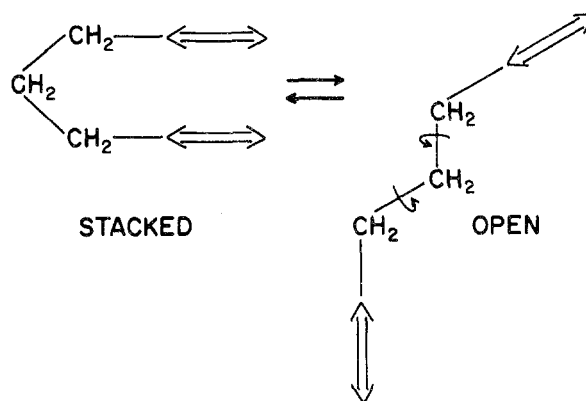


Figure 2. Nucleic acid bases.

having progressively shorter N-C distances, permitted the mapping of the reaction coordinate for the addition reaction (Figure 1).⁴⁵ The minimum energy pathway, expressed as the favored NCO angle, was virtually constant for the series, $\langle \alpha \rangle 107 \pm 5^\circ$, over an N-C distance (d_1) decreasing from 2.9 to 1.5 Å as the deviation (Δ) of the C atom from the plane defined by its O and the two groups (R, R') attached to carbon increased from 0.06 to 0.42 Å. The corresponding values for eight-membered-ring amino ketone alkaloid senkirkine⁴⁶ led to a point ca. 4% off the curve shown in Figure 1, and the points for compounds 24 and 25, shown as squares,^{47,48} are also off the curve. This⁴⁵ and alternative⁴⁶ relationships probably need further refining as further examples are accumulated. Nevertheless, each example can be regarded as a case in which the addition reaction has been frozen at a certain stage by the constraints imposed by the molecular and crystal environment.

As the $\text{RR}'\text{C}=\text{O}$ system becomes nonplanar, the π and π^* orbitals of the carbonyl group develop into a lone-pair orbital on oxygen and an empty sp^n -type orbital on carbon. The closer the approach of the nucleophile, the greater the development of nonplanarity of the $\text{RR}'\text{C}=\text{O}$ system, the higher the s character of the empty orbital, the lower its orbital energy, and the greater its interaction with the nitrogen lone pair.⁴⁵

One Trimethylene Bridge. In water solution, *interactions* are observable between nucleic acid bases connected by polymethylene chains, particularly a trimethylene chain, $\text{B}-(\text{CH}_2)_3-\text{B}'$, where B and B' are 9-substituted adenine or guanine or 1-substituted cytosine, thymine, or uracil residues⁴⁹ These analogues of dinucleotides or dinucleoside phosphates, which are free from the complicating factors associated with hydrogen bonding and carbohydrate and phosphodiester conformations, were studied at concentrations low enough to preclude formation of intermolecular complexes. Thereby, perturbations associated with the 1:1 interaction of a pair of bases could be characterized by UV spectra in aqueous solution at room temperature

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and by emission spectra in 1:1 ethylene glycol–water glass at 77 K.

Conclusions from UV spectroscopic determinations were based on Tinoco's demonstration that Coulombic interaction between dipoles induced in the chromophores by light could result in either a decrease (hypochromism) or increase (hyperchromism) in light absorption, depending upon the relative orientation of the transition moments in the ordered array.⁵⁰ Hypochromism, within limitations, corresponds to an ordered or stacked structure (Figure 2).

Conclusions from fluorescence emission spectra were based upon Eisinger and Shulman's demonstration that interaction between nucleic acid bases, as in DNA, in the excited state leads to fluorescence emission spectra that are broad and red-shifted compared with those of the monomeric bases.⁵¹ A complex between a molecule in the singlet excited-state and a neighboring ground-state molecule is then implicated. The high % *H* (percent hypochromism) values observed for the analogues B–(CH₂)₃–B' suggested a short time-average base–base separation in the analogues. Triplet energy transfer is also an efficient mechanism in these molecules, since the phosphorescence emission from all the neutral dinucleotide analogues was characteristic of the base with the lower lying triplet state.

In the model compound Ade⁹-C₃-i⁶Ade⁹ (IUPAC–IUB abbreviations), UV absorption spectra, absorption coefficients, and fluorescence and phosphorescence spectra showed the adenine and N⁶-(Δ²-isopentenyl)-adenine moieties to be in close proximity, with stacked conformations present in aqueous solution.⁵² From this it could be concluded that the i⁶A anticodon-adjacent base in tRNA can stabilize the stacked conformation of the anticodon loop. For m₂²Gua⁹-C₃-Ade⁹ and m₂²Gua⁹-C₃-Cyt¹, the spectroscopic evidence points to strong stacking interaction of the dimethylguanine unit with either adenine or cytosine, its normal tRNA neighbors.⁵³ Accordingly, while normal base-pair hydrogen bonding is ruled out for dimethylguanine, it would not be a lack of stacking ability that would encourage a "bend" at m₂²G in a tRNA.

The influence of ethanol, an effective denaturing agent for DNA and single-stranded poly(A), was determined for Ade⁹-C₃-Ade⁹ and Thy¹-C₃-Thy¹. Virtually no interaction was detectable in 95% ethanol in either case.

The effect of temperature was determined by quantitative comparison of the UV absorption curves for Ade⁹-C₃-Ade⁹ vs. Ade⁹-C₃ in aqueous solution at 5° intervals from 5 to 60 °C.⁵⁴ The % *H* increased with decreasing temperature, corresponding to an increased degree of base stacking and possibly approaching a maximum value. The value calculated for Δ*H*^o of stacking was –8.9 kcal/mol on the basis of hypochromism (Δ*S*^o = –30 eu), in the high range of the many values reported for ApA.

The effect of synthetic spacer length of % *H* at 25 °C for the series Ade⁹-C_{*n*}-Ade⁹ is: *n* = 2, 11.3%; 3,

14.0–14.8%;⁵⁴ 4, 14.4%; 5, 10.1%; 6, 6.1%. Qualitative observations of MCD spectra confirm this order of interaction.⁵⁵

Variation in interaction between two adenine rings with position of attachment of a trimethylene bridge was also determined.⁵⁶ The % *H* values calculated for the 9,9'-, N⁶,N⁶'-, N⁶,9'-, and 7,9'-trimethylene bis-adenines in aqueous solution are roughly equal, 15 ± 1%, and indicate strong interaction between the trimethylene-separated adenines in each case, corresponding to folded or stacked conformations. For the isomers Ade⁸-C₃-Ade⁸ and Ade⁸-C₃-Ade⁹, the % *H* values, 21 and 19%, respectively, were significantly above the range for the other four isomers and suggest conformations with opposite-facing adenines stacked so that the NH₂ group of one adenine lies over the pyrimidine ring of the other.⁵⁶ For trimethylene-bridged compounds 8,8'-trimethylenebistheophylline⁵⁷ and 1,1'-trimethylenebisthymine⁵⁸ in the crystal, the X-ray structure analyses show opposite facings of the stacked rings making up each molecule.

The thymine rings in the crystal of Thy¹-C₃-Thy¹ are arranged so that both intra- and intermolecular photoreaction could occur, although for either event, trans-syn geometry in the product would be anticipated. The intramolecular is shorter than the intermolecular interplanar distance, but the intermolecular overlap provides more favorable geometric interaction for the π-electron orbitals of the two 5,6 double bonds.⁵⁸ Solid-state ultraviolet irradiation of 1,1'-trimethylenebisthymine at 300 nm yielded the intermolecular photoproduct, a polymer of trans-syn thymine cyclobutane dimer units, each joined to the next by a trimethylene chain.⁵⁹

The photosensitized irradiation of Thy¹-C₃-Thy¹ in acetone (10%)–water solution and also of the di- and tetramethylene homologues yielded almost exclusively the cis-syn cyclobutane dimers, corresponding to the major thymine photoproduct from native DNA. The relative rate of internal photodimerization depends on the chain length (C₃ > C₄ > C₂ ≫ C₆) and is maximal for the trimethylene chain (→ 26).^{59,60} The relative rates were faster than those for the unsensitized internal photodimerizations in aqueous solution, reflecting the longer lifetime of the triplet state.

Only in the case of the trimethylene-bridged compound was the unsensitized photoreaction practical. Irradiation of 1,1'-trimethylenebisuracil, acetone-photosensitized to suppress photohydration, also gave a cis-syn internal dimer (26, H in place of CH₃). The acetone-photosensitized photolysis at 300 nm in aqueous solution of synthetic bisthymine analogues linked by trimethylene bridges between the 3,3', 1,5', and 1,3' positions yielded cis-syn (27), trans-anti (28), and cis-anti (29) cyclobutane products, thus establishing the stereochemical control of intramolecular dimerization.⁶¹ This series of internal dimeric photoproducts allowed

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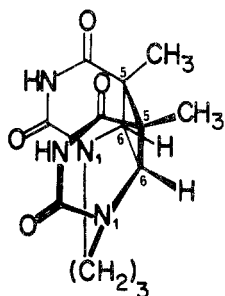
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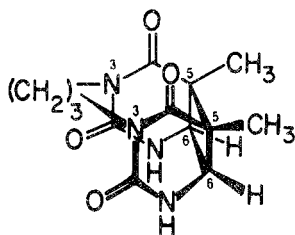
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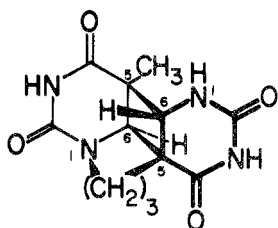
TRIMETHYLENE-LINKED THYMINES



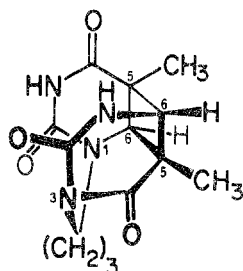
CIS-SYN
1,1
26



CIS-SYN
3,3
27



TRANS-ANTI
1,5
28



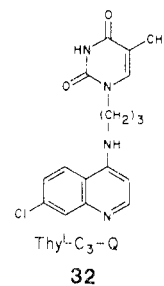
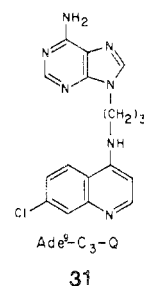
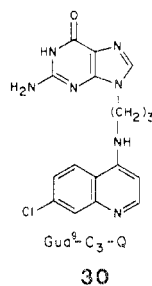
CIS-ANTI
1,3
29

correlation with respect to positions of linkage and relative orientations of the 5,6 double bonds, relative rates of formation within a series, and relative stability of the cyclobutane products (26–29) with respect to acid, base, and response to 254-nm irradiation.

An imposing array of substituted bispyrimidines linked by single and double polymethylene bridges has now been synthesized and subjected to NMR spectroscopic analysis and photochemical study.^{62–64} The irradiation of Thy¹-C₃-Ade⁹, as one example of a series of trimethylene-linked pyrimidine-purine combinations, led to a photostationary state which contained a thermally unstable internal photoadduct.⁶⁵

The interaction of the antimalarial alkaloid chloroquine with nucleic acid bases has been examined by the synthesis of compounds in which the N⁴ of the quinoline moiety is covalently attached to guanine, adenine, and thymine, for example, by a trimethylene bridge.^{66–68} Water is again the crucial solvent in which intramolecular stacking interaction can be observed by hypochromism. The models Gua⁹-C₃-Q (30) and Ade⁹-C₃-Q

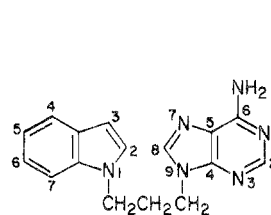
CHLOROQUINE BINDING



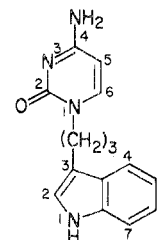
(31) induced practically the same perturbation in the UV spectrum relative to the components. The % *H* value rose to a maximum of about 25% with decreasing temperature, and both models were practically entirely in stacked conformation at 20 °C. At higher temperatures, quinoline seemed to have a slightly greater affinity for guanine than for adenine. The calculated changes in enthalpy and entropy of stacking are $\Delta H^\circ = -9$ kcal/mol, $\Delta S^\circ = -24$ eu. There was a substantially lower degree of stacking interaction in the representative pyrimidine-quinoline model, Thy¹-C₃-Q (32).

The results, which are of obvious biological interest, suggest that the action of chloroquine is related to its binding to DNA, particularly by stacking interaction with the purine deoxyribonucleotides. The polarizable chloro substituent, which aids in the association, is a prerequisite for the expressed antimalarial activity.

Among the specific stacking interactions that may contribute to the positioning of protein with respect to nucleic acid, interaction between tryptophan and nucleic acid bases has been strongly implicated. We assessed the stacking interactions between indole, as a neutral representative of tryptophan, and the nucleic acid bases in aqueous solution at 25 °C by means of UV hypochromism and fluorescence emission. To this end, we synthesized 15 compounds in which indole and the nucleic acid bases adenine, cytosine, guanine, and thymine were connected by a C₃ or C₄ bridge.⁶⁹ Ring attachments were at the 1 or 3 position of indole and at the 9 position of adenine and guanine, the 1 position of cytosine and thymine, and the N⁶ position of adenine. Formulas 33 and 34, with their descriptive abbreviations, are representative of the series.



Ind¹-C₃-Ade⁹
33



Ind³-C₃-Cyt¹
34

The relative quantum efficiencies ($q = F/F_0$) were determined by comparing the fluorescence intensity of the Ind-C₃-base with a solution containing equivalent concentrations of the half-molecules, Ind-C₃ and base-C₃. The reduced quantum efficiency of indole

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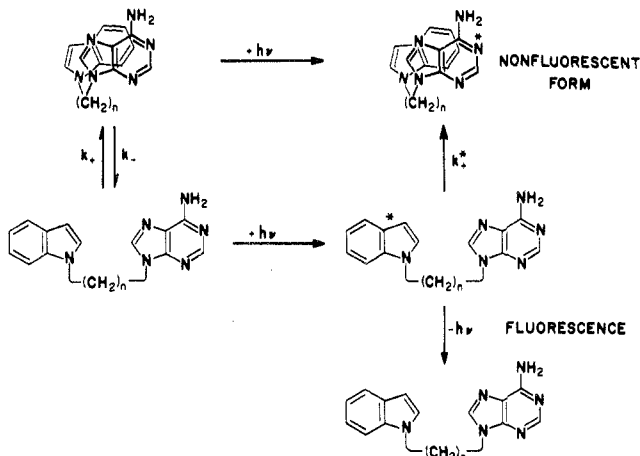


Figure 3. Quenching processes.

fluorescence attached to a nucleic acid base results from two processes, exemplified in Figure 3, for Ind¹-C_n-Ade⁹: (1) static quenching, a time-independent process resulting from the proximity of the quencher prior to excitation, and (2) dynamic quenching, a time-dependent process that occurs during the excited-state lifetime.⁷⁰⁻⁷³

For the Ind-C₃-base models, the decreasing order of internal association, or stacking, with indole was adenine \approx guanine > thymine \gg cytosine. The equilibrium between open and stacked conformations for the first three types, based on relative fluorescence lifetimes and quantum yields, indicated ΔG values near zero (for Ind¹-C₃-Ade⁹ (33), $\Delta H^\circ = -8.1 \pm 0.9$ kcal/mol; $\Delta S^\circ = -26.6 \pm 3.0$ eu).⁷⁴ The degree of internal association observed for Ind³-C₃-Cyt¹ (34) was 24%. Thus, total fluorescence quenching of the indole of tryptophan in a polypeptide or protein is to be expected if it comes into proximity with a base moiety of a nucleic acid or if intercalation occurs.

There are other applications of the analysis of stacked vs. open forms by measurement of fluorescence yields and lifetimes. The coenzyme flavin adenine dinucleotide (FAD) is 82% stacked in pH 7.0 buffer at 20 °C.^{70,71} Flavin 1,N⁶-ethenoadenine dinucleotide (ϵ FAD), the reaction product of chloroacetaldehyde with FAD, is 90% in stacked form,⁷² and energy transfer from the 1,N⁶-ethenoadenine moiety to the isoalloxazine moiety is nearly 100% efficient. The coenzyme NAD⁺ is not fluorescent, but the fluorescent nicotinamide 1,N⁶-ethenoadenine dinucleotide (ϵ NAD⁺) (35) was found to be 45 \pm 5% stacked.⁷⁴ The fluorescence of the total molecule, with its rings connected by a ribose-

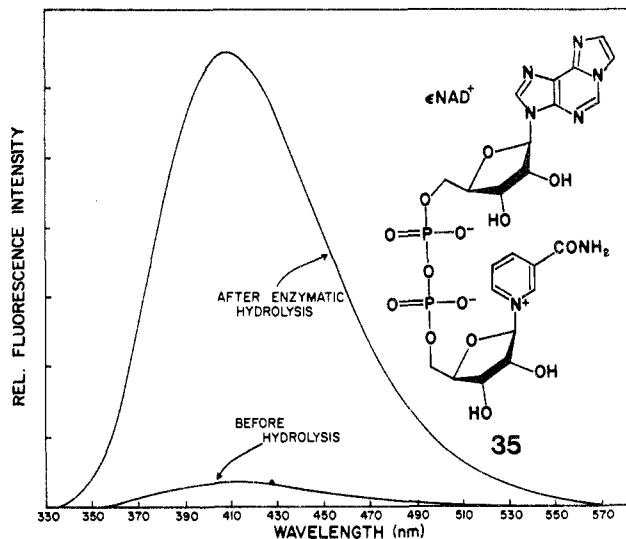


Figure 4. Fluorescence of nicotinamide 1,N⁶-ethenoadenine dinucleotide (ϵ NAD⁺).

phosphate-phosphate-ribose bridge, could be related to that of the half-molecules after snake venom phosphodiesterase hydrolysis of the pyrophosphate linkage. The increase in fluorescence emission on cleavage of the ϵ NAD⁺ (Figure 4) is a dramatic indication of the effect of removing the condition for intramolecular stacking. On the basis of a comparison of stacking in ϵ FAD vs. FAD, it can be reasoned that the stacking in NAD⁺ will be <45%. Details of the dehydrogenase activities observed using the modified coenzymes ϵ NAD⁺ and ϵ NCD⁺, which are of interest in connection with other applications of structural and fluorescent modification, are to be found elsewhere.^{74,75}

Finally, model compounds, Biot-C₃-Ind and Biot-C₄-Ind, having tri- and tetramethylene bridges between the biotin ring system and indole were synthesized to help uncover the basis of the strong binding of biotin to avidin, one of the tightest biological complexes known ($K_D = \text{ca. } 10^{-15}$ M), and to identify any contributory biotin-tryptophan interaction. Fluorescence emission, UV absorption, chemical oxidation, and X-ray single crystal analysis provided useful information that has set some limits on the contribution of avidin's tryptophans to interaction in the complex.^{76,77}

Although this "trimethylene-bridge" theme is not exhaustive, it illustrates how rational organic synthesis can lead to applications far beyond the original purpose, into the realms of structural chemistry, spectroscopy, biochemistry, and molecular biology.

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