Pergamon

PII: S0040-4020(96)00868-X

Tetrahedron, Vol. 53, No. 7, pp. 2325–2355, 1997

0040-4020/97 $17.00+0.00

TETRAHEDRON PERSPECTIVE NUMBER 5

THE 'CHEMISTRY' OF RESEARCH COLLABORATION

NELSON J. LEONARD
Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125, USA

Abstract—This paper examines the process of research collaboration with other scientists based solely upon the experiences of the author: initiation of cooperation; agreement on purpose and responsibility; development of the joint project; information exchange and writing; and consequences unique to each research project. © 1997, Elsevier Science Ltd. All rights reserved.

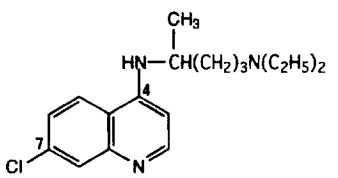
In retrospect, it appears that some of the greatest enjoyment I have had doing chemical research has been in collaboration with other scientists. The joint effort was initiated by a variety of factors: mutual interest, a combination of skills, expansion of initial goals, recognition of applications, or, quite simply, friendship. The joint project was continued in each instance until major goals were reached, and it resulted in advances beyond those envisaged by either research group. Every research collaboration proved to be an educational experience. In no case did the participants have an overweening concern as to credit due. A captious critic might suggest that was because the achievements were not that notable—a judgment that would not devalue, however, the mutual satisfaction and pleasure to be gained from each experience.

The stories of research collaboration in which the author was a participant follow along the lines of individual projects at the University of Illinois during a 50-year period. In my recollection of the events, which may be faulty, there is no standard pattern of research project initiation, development, and conclusion. In the very disparate nature of the experiences, nevertheless, may lie some interest and also a basis for comparison with the collaborative research experiences of others.

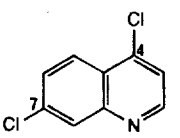
ANTIMALARIALS: CHLOROQUINE

Each research collaboration has a story and each has a trademark consisting of a single organic compound, a series of compounds, a combination of functional groups, or a reaction pathway. The symbol for the collaborative research on antimalarials is Chloroquine (**1**). When I became an Instructor at the University of Illinois in 1943, I was encouraged to direct the research of senior undergraduates majoring in chemistry, and that was a highly satisfactory experience because of their knowledge and training. However, a faculty member was not permitted to direct the research of graduate students until he (only 'he' in those days) was appointed to the Graduate College Faculty, a process that required submission of proof of such ability, including numerous research publications. My colleagues, Professors Charles C. Price, III and Harold R. Snyder, helped me out of the catch in that academic archaism by having me join them on the NDRC-funded Antimalarial Research Program and share in the direction of their excellent graduate students. The wartime duties of the senior staff included considerable travel,

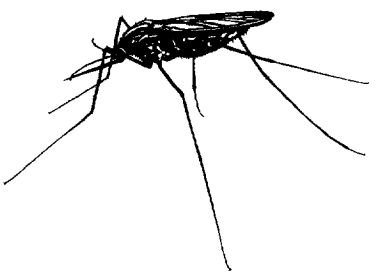
with the result that I was given additional teaching duties along with my practice of consulting with the graduate students each day on the progress of their antimalarial research. I well recall some 72-hour stints in the laboratory when together we prepared the intermediate 4,7-dichloroquinoline (**2**) on a grand scale. Our pilot effort was sufficient for Chloroquine to be produced in time for its use in the Pacific against the assaults of the *Anopheles* mosquitoes. The research publications that resulted from the Antimalarial Program helped me to obtain Graduate Faculty status and with it the authority to direct my own graduate students. The lesson in unselfish collaboration was not lost on this junior faculty member.



1



2

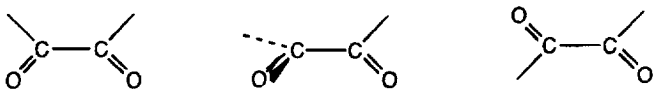


Anopheles sp.

1,2-DICARBONYL COMPOUNDS

My interest in the relationship between the geometry and the visible/ultraviolet spectra of 1,2-dicarbonyl compounds, which had been stimulated by a published lecture by Sir Robert Robinson in 1932,[1] was further developed during my preparation of one of the 'propositions' required for the Ph.D. (1942) at Columbia University. With two senior research students at Illinois, I was finally able to put my ideas to practice. Our synthetic procedures were satisfactory, but the spectroscopic capability at Illinois at that time was deficient. I therefore turned to Elkan R. Blout for help. Elkan, who had been my laboratory partner at Columbia, had become employed in the Chemical Research Laboratory of Polaroid Corporation, where the crucial ultraviolet spectra of our compounds could be determined accurately and quickly. The first paper on representative alkoxy- and hydroxybenzils[2] was written by vigorous exchange of information, including drafts and figures, and the second paper on hindered benzils[3] was written when we met at an American Chemical Society National Meeting in Washington, DC. The latter paper described our finding that the dicarbonyl absorption is shifted to longer wavelength with increasing hindrance at the *ortho* carbons of benzil, corresponding to increasing tendency to coplanarity of the two carbonyl groups, whereas the phenyl–carbonyl absorption is diminished in intensity, corresponding to decreasing tendency to coplanarity of the phenyl and carbonyl groups. This steric relationship, which was novel for the time, was further developed at Illinois in a corresponding study of a series of alicyclic 1,2-diketones of varying ring size (5, 6, 7, 8, 18 and acyclic), α-disubstituted so that enolization could not occur.[4] The position of the long wavelength excitation varies in an orderly way with the average angle between the planes of the carbonyl groups. Thus, the band moves toward shorter wavelengths as the angle increases from 0° (*cis* or *syn*) (**3**) to about 90° (skew) (**4**) and shifts back again toward longer wavelengths as the angle increases beyond 90° (toward *trans* or *anti*) (**5**). Many years later, our results were confirmed and refined in a more thorough investigation at the University of Amsterdam that included determination of the ground state intercarbonyl angles by electrical dipole moment and correlation of

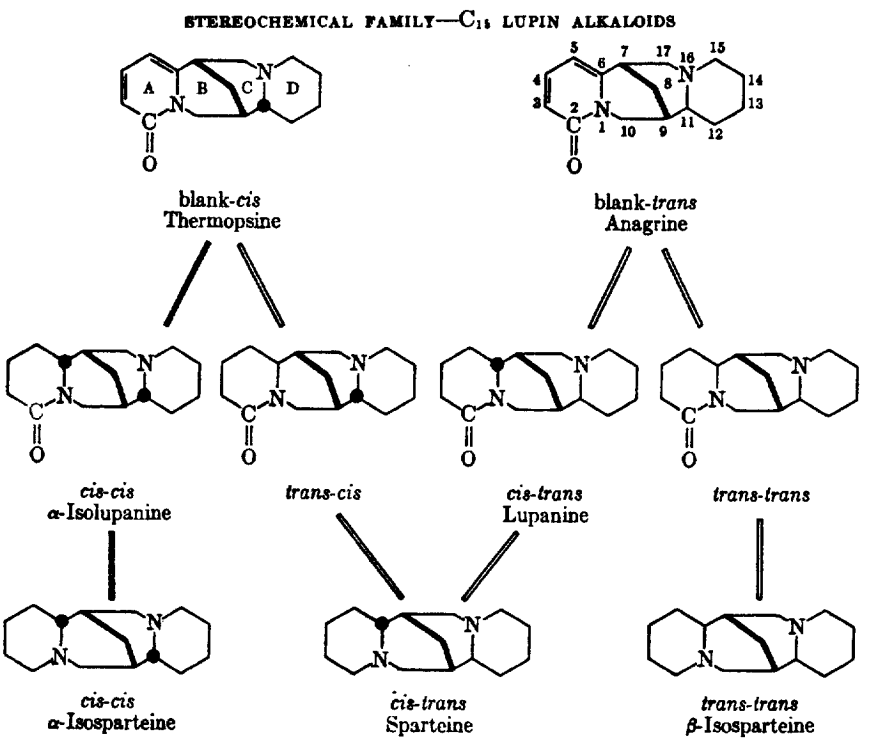
these angles with photoelectron and emission spectra as well as ultraviolet absorption spectra.[5] In addition, a correlation of ^{17}O NMR chemical shifts with the intercarbonyl dihedral angle revealed that the shifts are linearly dependent on both the electron density at the oxygen atoms and the $n \rightarrow \pi^*$ excitation energy of the 1,2-dicarbonyl chromophore.[6]



3 **4** **5**

LUPIN ALKALOIDS

Our total synthesis of the lupin alkaloids sparteine and isosparteine at Illinois, *inter alia*, earned me an invitation to spend the summer of 1950 at the Canadian National Research Council in Ottawa to work with Léo Marion. A fellowship from the Rockefeller Foundation made it possible for our family of four to spend three months there. I did isolation, identification, and partial synthesis of lupin alkaloids in the company of the very talented and compatible coworkers of Dr. Marion. I complained to my wife about my slow progress, probably because I



Scheme 1. Reproduced, with permission, from: Marion, L.; Leonard, N. J. *Can J. Chem.* **1951**, *29*, 355–362.

was doing all the work myself without the benefit of students. Accordingly, it must have come as

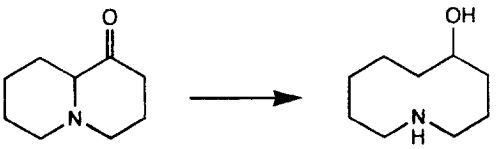
a shock to her when she asked much later whether I had any publications to show for my time at the CNRC and I had replied that there were three. In that moment I lost the privilege of ever complaining about the lack or slowness of research progress. My favorite joint paper was the one in which we assigned stereochemical structures to the members of the C_{15} family of lupin alkaloids (Scheme 1).[7] The assignments (one set of enantiomers is shown) were based upon (a) the structural similarity but configurational difference between rings B and C of sparteine and its derivatives, (b) the study of accurate scale molecular models, and (c) the recognized surface nature of the catalytic hydrogenation process as applied to certain of the alkaloid interconversions. Both stereochemical families related to the thermopsine and anagyrine series are to be found in *Lupinus caudatus* Kellogg. Stereochemistry was not Marion's forte, and he was relieved, I think, when the crystal structure determination of α-isosparteine monohydrate[8] fully confirmed the stereochemical structures that we had proposed that linked all members of the class of C_{15} lupin alkaloids.

Lupinus caudatus

ELECTROLYTIC REDUCTION OF α-AMINOKETONES

In chemistry closely related to that of the lupin alkaloids, we established the general nature of the rearrangement of α-aminoketones during Clemmensen reduction. In our desire to interrupt the cleavage–condensation process in search of intermediates and new methodology, it was natural that we should consult Professor Sherlock Swann, Jr. He was a member of the Chemical Engineering (then) Division at the University of Illinois and the reigning world expert on the literature of electrolytic methodology. He guided us in the selection of conditions and apparatus for the electrolytic reduction of representative α-aminoketones. We were aided by an unsolicited, unrestricted grant from E. I. DuPont de Nemours and Company, Inc. Because Swann was a strong proponent of cathode studies, we first studied the electrolytic reduction of representative

monocyclic α-aminoketones at cathodes of cadmium, copper, lead, and tin at 60 °C and 20 °C.[9]



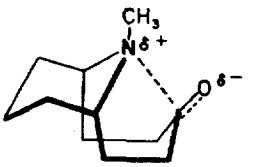
6 **7**

The current density was carefully selected and the quantity of current allowed to pass through the electrolysis cell was set equivalent to six faradays per mole of aminoketone, which is the theoretical current for scission of the C_γ–N bond and reduction of the carbonyl group to methylene. While we could model the Clemmensen reduction–rearrangement using a cadmium cathode in a catholyte of 30% sulfuric acid at 60 °C, we discovered that by the use of a lead cathode we had actually devised a new method of making medium-size rings (9–12) containing nitrogen, e.g., **6**→**7**.[10,11] The method was extended to the synthesis of medium-size rings containing nitrogen and having a benzo grouping fused to the newly formed 11- or 12-membered ring.[12] The general applicability of the method is limited only by the average reluctance of an organic chemist to set up an electrolytic reaction; there are certainly adequate means of synthesizing the precursor α-aminoketones. In my case, reluctance to continue the research stemmed from the students' lack of interest in extended cathode studies.

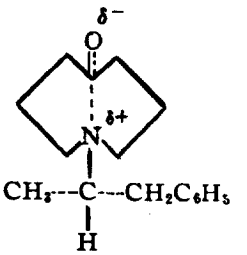
TRANSANNULAR INTERACTIONS

From the making of medium ring compounds containing amine and hydroxyl functions, the research flowed toward the making of medium-to-large rings containing amine and carbonyl functions. The original purpose was not realized, but a wonderful source of research excitement was found: transannular interactions and reactions. Such interactions and reactions between 3°-amine and carbonyl groups were identified first in alkaloid chemistry[13] and were studied in our laboratory in several related series of cyclic aminoketones and aminoacyloins. Using these, we provided evidence for occurrence of transannular nitrogen–carbonyl interaction and for its limitation by ring-size, steric interference, environmental and electronic factors. We had at hand spectroscopic means of observation, namely infrared and ultraviolet absorption spectra, and we relied upon the cooperation of Dr Harold Boaz of Eli Lilly and Company for more detailed infrared spectral analysis and for differential pK_a determinations of the conjugate acids of the aminoketones and aminoacyloins. A visit to Michigan State University gave me the opportunity of interesting Professor Max T. Rogers in determining the dipole moments of representative aminoketones in our series. That of 11-methyl-11-azabicyclo[5.3.1]hendecan-4-one (**8**) in benzene was observed to be 4.87 D, or 1.27 D units higher than the moment calculated for an interactive conformation devoid of partial charge separation. The difference was estimated roughly at 11–12% charge separation in our joint publication.[14] My reluctance to do our own dipole moment determinations was probably occasioned by the fact that I had spent two years of my early research life doing just that, and somehow I did not want to return to the procedure. In any case, working with Max Rogers made the research more enjoyable, and he supplied the definitive numbers for the joint publication. In further investigation of transannular phenomena

involving sulfide or ether with ketone groups, my colleague at Illinois, Professor Theodore L. Brown, was our research collaborator in supplying the crucial electric moments.[15]



8

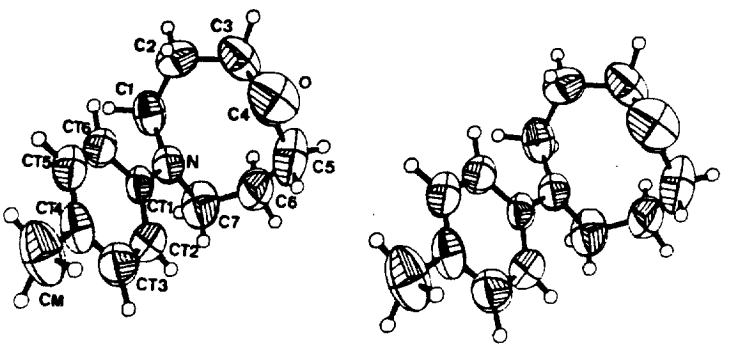


9

On the same trip to Michigan mentioned above, I conferred with Professor Carl Djerassi, then at Wayne State University and about to go to Mexico City on a leave of absence, about the possibility of detection of transannular interaction in an optically active aminoketone of medium ring size. As a result of this discussion, the Illinois contribution was the synthesis of (+)-1-(α-methylphenethyl)-1-azacyclooctan-5-one (**9**) and the Wayne State contribution was the determination of the optical rotatory dispersion. The latter was agreeably abnormal—specifically, a negative Cotton effect superimposed on a positive plain curve.[16] Thus, the asymmetry of the α-carbon on nitrogen exerts its influence on the carbonyl group diametric, but conformationally in close proximity, to the nitrogen across the eight-membered ring. I wrote up the paper and, for a reward (asked for and granted), I was given a pancake breakfast in Carl's hotel room during a San Francisco American Chemical Society Meeting. The amusing part of the action was that during my breakfast Carl paced the room and outlined for me how the paper should be written. Luckily, his verbal version was exactly like the written version I had brought with me, so that very little time elapsed before our joint manuscript was sent to the Journal. My other reward was to receive a T-shirt from Carl many years later on some celebratory occasion when he was at Stanford University. A suitable inscription on it mentioned my one-thousandth contribution to Carl's total number of research papers.

In 1960, I enjoyed a sabbatical leave and spent a fruitful half year at the University of Basel. On the occasion of a lecture visit to the E.T.H. in Zürich, I placed satisfactory crystals of two important compounds in the hands of Professor Jack D. Dunitz: 1-*p*-tolyl-1-azacyclooctane-5-one (**9**, but with *p*-tolyl attached to N(1)) and 11-methyl-11-azabicyclo[5.3.1]undecan-4-one (alternative name of **8**). I was convinced that he would be able to determine the X-ray crystal structures and to confirm thereby the strong transannular N···C=O interaction that we had observed in solution measurements. The issues that X-ray could solve included the conformation of the eight-membered rings, the angle of N approach to C=O, and, of course, the interatomic distances. However, the methodology was not yet in hand in 1960 for facile solution of the X-ray structural problems presented by **8** and **9**. The situation changed with time. In the Dunitz laboratory, the concept was developed that attractive intramolecular interactions can be regarded as representing incipient stages of chemical reactions, including nucleophilic addition to the carbonyl group.[17] By determining the experimental structural parameters for N···C=O interactions for six crystal-structure analyses, Bürgi, Dunitz, and Shefter concluded that the nucleophilic approach trajectory is such that the angle between the developing N—C bond and

the C=O bond is about 107°. In addition, as the nucleophile approaches the carbonyl C, the two alkyl or alkylene substituents bend away and the C—O distance becomes slightly longer.



10

Reproduced, with permission, from: Kraftory, M.; Dunitz, J. D. *Acta Cryst.* **1975**, *B31*, 2912–2914.

What of our two synthetic aminoketones? The words of Kaftory and Dunitz in 1975 concerning 1-*p*-tolyl-1-azacyclooctan-5-one are most descriptive:[18]

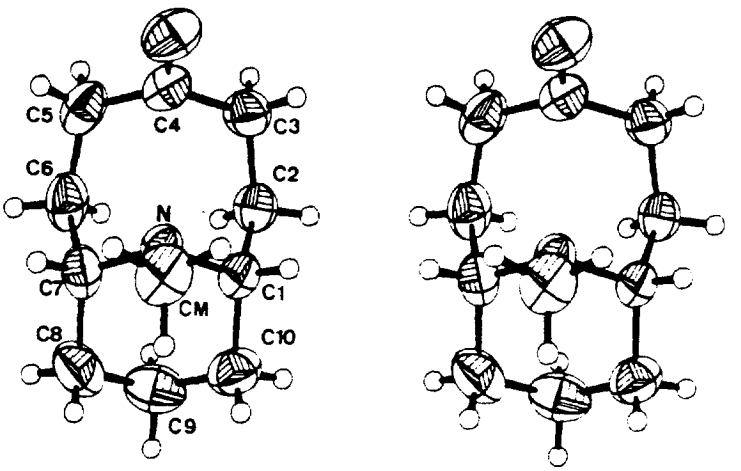
> "A sample of the title compound, sealed under nitrogen, was obtained from Professor N. J. Leonard in 1960, mislaid soon afterwards, eventually forgotten and finally rediscovered in 1973 when our interests had turned towards N···C=O interactions as examples of incipient stages of nucleophilic addition reactions."

A stereoscopic view of the molecule is shown in **10**. The conformation of the eight-membered ring is boat–chair and the N–C_{co} distance of 2.76 Å is 0.5 Å less than the sum of the N and C packing radii. The conclusion that there was weak transannular interaction fortified the earlier decision from our laboratory on the basis of infrared determinations and pK_a comparisons concerning the effect of *N*-aryl substitution.[19]

Here again are the words of Kaftory and Dunitz, this time relating to 11-methyl-11-azabicyclo[5.3.1]undecan-4-one (**8**):[20]

> "A small sample of the title compound shared the same fate as the sample of 1-*p*-tolyl-1-azacyclooctan-5-one described in the preceding paper."

The crystal structure confirmed the existence of strong transannular interaction that had been concluded on the basis of the abnormally large dipole moment and strongly displaced infrared carbonyl absorption in solution.[14] In the crystal, the ten-membered ring adopts the BCB conformation and the eight-membered ring, the BB conformation, with a transannular N–C_{co} distance of 2.46 Å. Along with the deviation of the carbonyl carbon from the plane of its three bonded neighbors, the data for **8** are in good agreement with the empirical correlations of data for the Dunitz compounds examined two years earlier.[17] A stereoscopic view of the molecule is reproduced in **11**. The structural research in Zürich also included examination of the directional preference of nonbonded electrophilic and nucleophilic atom contacts with S···C=O[21] and O···C=O interactions.[22] My interaction with Jack Dunitz might be described as intersectional collaboration, and I have drawn great value from each 'intersection'.



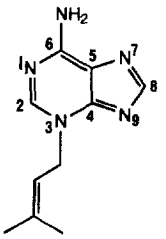
11

Reproduced, with permission, from: Kraftory, M.; Dunitz, J. D. *Acta Cryst.* **1975**, *B31*, 2914–2916.

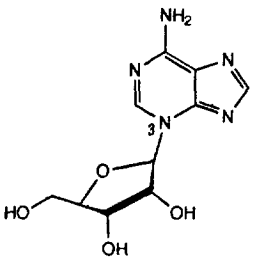
The area of investigation has been reviewed thoroughly by Rademacher[23] under the title 'Transannular Interactions in Difunctional Medium Rings—Modelling Bimolecular Reactions.' An array of methods, including ultraviolet photoelectron (PE) spectroscopy, quantum chemical calculations, nuclear magnetic resonance (NMR), and conformational analysis by molecular mechanics, has provided quantitative data on through-space electronic interactions that relate to nucleophilic and electrophilic addition reactions.

TRIACANTHINE

The sudden origin and just as sudden demise of veterinary interest in the leaves of the honey locust tree are recorded amazingly in two editorials that appeared in the American Journal of Pharmacy in 1887.[24] Two-thirds of a century later, Belikov, Bankowsky, and Tsarev[25] isolated from the new leaves of the honey locust, *Gleditsia triacanthos* L., a compound having an unusually high ratio of nitrogen to carbon (1:2) for an alkaloid. This feature, together with the abundance of honey locust trees in Illinois, made an investigation of triacanthine especially attractive. We repeated the described extraction procedure, and we isolated, purified, and then established the structure of triacanthine as 3-isopentenyladenine (**12**).[26] This was the first natural plant product to be described that had substitution on the 3-position. We were misled initially by the absence of any models and by the ultraviolet spectrum and pK_a value into deciding in favor of 7-substitution,[27] and it was not until later that we assembled a body of UV spectral characteristics that makes it possible to distinguish among all N-substituted[28] and all N,N-disubstituted adenines.[29] With the correct structure of triacanthine in hand, an easy collaboration was arranged with colleagues in France and Belgium. In each of our three laboratories we determined that triacanthine, togholamine from *Holarrhena floribunda* (G. Don) Dur. and Schinz (fam. Apocyanaceae) from Togo, and chidlovine from *Chidlowia sanguinea* Hoyle (fam. Caesalpiniaceae) from the Ivory Coast were identical.[30] These sources in which triacanthine appears are in distantly related families, and triacanthine, in other sources, may be overlooked because its concentration is significant only in the beginning of the growing season. The 3-substitution on adenine sparked the next research collaborations.



12



13

Gleditsia triacanthos

PURINE NUCLEOSIDES INCLUDING 3-ISOADENOSINE

Attendant with developing spectral methods for differentiation between adenine derivatives substituted at various nitrogen atoms was the reinvestigation of a number of compounds related to puromycin that had been regarded as '7'-substituted 6-dimethylaminopurine nucleosides. We joined forces with the nucleoside experts, Roland K. Robins and Leroy B. Townsend, then at Arizona State University in Tempe, and, with the utilization of ultraviolet absorption data supplemented by proton magnetic resonance spectra, we were able to show that previously described 6-dimethylamino-'7'-glycosylpurines should be reassigned as the corresponding 3-glycosyl derivatives.[28] The more important result was that it became clear for the first time that the combination of a halosugar with the mercury salt of a purine, the method of synthesis in these cases, does not guarantee that glycosidation will occur on the imidazole ring only. The possible N-sites of attack on the purine nucleus depend upon the nature and size of the group(s) already attached. This research collaboration was carried out mainly by letters and telephone calls plus one conference at the time of an American Chemical Society meeting. Substitution at N3 on purines gained biological importance with the appearance during 1961–1964 of reports that 3-ribosyluric acid is found in beef blood and can be converted to the 5′-mono- and diphosphates by a pyrimidine ribonucleotide pyrophosphorylase of beef erythrocytes.[31]

The chemistry of triacanthine, including its synthesis by the direct isopentenylation of adenine, lead us to the synthesis of 3-isoadenosine, that is, 3-β-D-ribofuranosyladenine (**13**).[32] The reaction of adenine with protected sugar halides gives an easily separable mixture containing more or less equal amounts of N3 and N9 derivatives. The question as to whether the 3-isoadenosine (**13**) can mimic natural adenosine in enzyme systems was directed first toward its behaviour with adenosine deaminase (from *Aspergillus oryzae*). We were approached by Dr Richard V. Wolfenden, then at Princeton University and fast becoming an authority on deaminases, and together we agreed that his laboratory would examine the possible enzymatic deamination of **13** while ours would carry out the chemical deamination. The results were described in a joint communication[33] that indicated the flexibility of interaction at the binding and catalytic sites of the deaminase.

It was during 1963–1964 through the urging of my student Richard A. Laursen, now a Professor at Boston University, that we ventured into the realm of 5′-mono-, di-, and triphosphates and the NAD^+ analog based on 3-isoadenosine, in the anticipation that these would serve as spatial probes of enzyme–coenzyme binding sites. Indeed they are.[34] We were fortunate to secure the collaboration of Dr A. M. Michelson, Institut de Biologie Physico-chimique, Paris, and Dr Koert Gerzon and Dr Irving S. Johnson, Lilly Research Laboratories, Eli Lilly and

Company, Indianapolis, Indiana. I had made the acquaintance of Dr Michelson when he was a summer visitor at the University of Illinois and gave an outstanding lecture course on nucleosides, nucleotides, and nucleic acids. Koert Gerzon was an old friend. When our families vacationed near each other in Michigan in 1960, he and I spent time tracing chemical structures in the beach sand of Lake Michigan, mainly of nucleosides, as I recall. Our research collaboration was delayed but seemed inevitable due to our common interests and my fortunate relationship as a consultant to Eli Lilly and Company.

Poly(3-isoadenylic acid) was obtained by the action of polynucleotide phosphorylase on 3-isoadenosine 5′-diphosphate.[35] In Michelson's laboratory, similarity to poly(A) was observed in that poly(3-iso-A) was not degraded by pancreatic ribonuclease, was hydrolyzed to the free nucleoside by crude rattlesnake venom, and to the 5′-phosphate by purified venom diesterase. Strong interaction between 3-isoadenosine and uridine was indicated in the 1:1 complex of poly(3-iso-A) and poly(U), T_M 88 °C, versus T_M 44 °C for poly(A)·2 poly(U). Confirmation of the strong interaction and indication of the nature of the base-pairing followed much later and was dependent upon the development of new methodology (see below).

Comparison of the observed effects of 3-isoadenosine (**13**) on bacterial and mammalian cell growth with the effects of adenosine and several adenosine analogs indicated that the pattern of activities of 3-iso-A is unique. Such were the findings of Koert Gerzon and Irving Johnson at the Lilly Research Laboratories.[36] While 3-iso-A supported the growth of an *Escherichia coli* mutant (strain B 97) that is dependent upon an exogenous supply of adenine or adenosine, it inhibited the growth of various mammalian cell lines *in vitro*. Moreover, the isomer was cytotoxic to the lymphoblastic leukemia cell line L 5178Y and also inhibited the growth of Adeno III virus in tissue culture. Appreciable mammalian toxicity *in vivo*, combined with modest activity against some ascites tumours and a narrow antitumour spectrum, prevented 3-iso-A from becoming a candidate chemotherapeutic agent. Apparently I was not able to abandon the idea of a chemotherapeutic agent based upon 3-iso-A, because twenty-five years later I engaged the cooperation of Professor Vasu Nair of the University of Iowa, who had been earlier a postdoctorate at the University of Illinois, for the synthesis of 2′,3′-dideoxy-3-isoadenosine as a regioisomer of the anti-HIV drug ddA. Our friend Professor Erik De Clercq of the Rega Institute of Medical Research at the Katholieke Universiteit in Leuven, Belgium found that our 3-iso-ddA exhibited relatively low activity against HIV-1 and HIV-2 replication in MT-4 cells,[37] insufficient for further development.

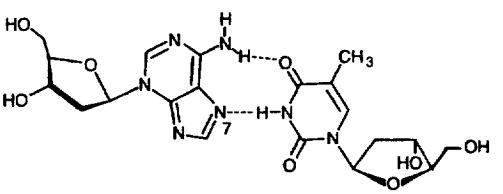
Prebiotic chemistry

A lectureship at the University of California, San Diego in 1981 brought me in contact with Professor Leslie Orgel, also of the Salk Institute for Biological Studies, and made me aware especially of his fundamental research on the oligomerization of activated mononucleotides on polynucleotide templates.[38] Together we published on the poly(U) template-directed oligomerization of 3-isoadenosine 5′-phosphate as the activated imidazolide.[39] The reaction of the imidazolide of 3-iso-AMP is more efficient than the reaction of the corresponding derivative of AMP and produces 3′-5′-linked oligomers to the extent of an estimated 90%. It seemed probable that the base-pairing between oligo(3-iso-A) and oligo(U) is of the Hoogsteen type (involving 6-NH_2 and N7 of the 3-isoadenosine), as in the earlier case of the high-melting (T_M) 1:1 complex of poly(3-iso-A) and poly(U), but this was not yet established. When I returned to UCSD as a Visiting Professor in 1990, we completed a sequel to the first oligomerization paper.[40] The amusing feature of this paper was the way in which it was written—at a series of four lunches in a delightful restaurant in La Jolla overlooking the Pacific. Each week I would produce a draft, and each week Leslie Orgel would provide me with revisions he considered necessary to incorporate in the next draft. I had the distinct impression of being a graduate

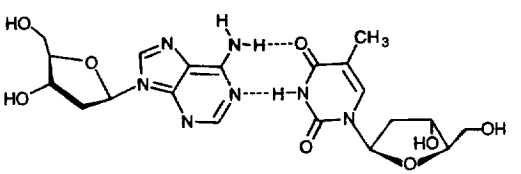
student again and of being under the tutelage of Dr Leslie Sutton of the University of Oxford, who had been a mentor for both Leslie (Orgel) and me in earlier years.

Interstrand hydrogen bonding

Currently we have addressed this question in a collaborative examination by NMR of the 3D structure of a DNA hexamer, d(CGATCG), as a control, and its 3-iso-A-substituted analog, d(CG[iA]TCG).[41] Dr Howard Robinson and Professor Andrew Wang of the Department of Cell and Structural Biology at the University of Illinois performed the rigorous NMR structure determination on hexanucleotide that Dr Balkrishen and Dr Neelima Bhat made in my laboratory using a unique combination of protection/deprotection methodology. Andrew Wang and I had published together twenty-five years ago when he was a graduate student at Illinois, so that the current collaboration represents an attack on another problem of mutual interest. The water-exchangeable spectrum of $d(CG[iA]TCG)_2$ displayed two clear imino proton resonances at 12.87 (G2), 14.96 (T4) ppm plus a rapidly exchanging peak at 13.23 (G6). The T4H3 imino resonance is further downfield than that in the normal Watson–Crick A:T, as in $d(CGATCG)_2$, and a strong NOE crosspeak between the T4H3 imino proton and the iA3H8, but not the iA3H2 proton, is consistent with a Hoogsteen-type base pair. The 2D-NOESY and TOCSY spectra in D_2O were used to assign the resonances of all non-exchangeable protons. The uninterrupted sequential crosspeak connectivity of the aromatic protons to the H1′ protons suggested a



14



15

Figure 1. 3-iso-dA hydrogen bonding with T (**14**); dA hydrogen bonding with T (**15**).

helical structure, and their intensities indicated that all nucleotides are in the *anti* conformation. The *anti* (to the purine ring) conformation of the iA3 residue defines how iA pairs with T4 of the opposite strand (Fig. 1, **14**), with Hoogsteen-type hydrogen bonding, in comparison with Watson–Crick A:T pairing (**15**). The C1′–C1′ distance in both base pairs is ~10.5 Å. The

refined, energy-minimized model of the iA-modified duplex shows that the Hoogsteen-like iA:T base pairs can be incorporated into a B-DNA duplex with minimum conformational perturba-

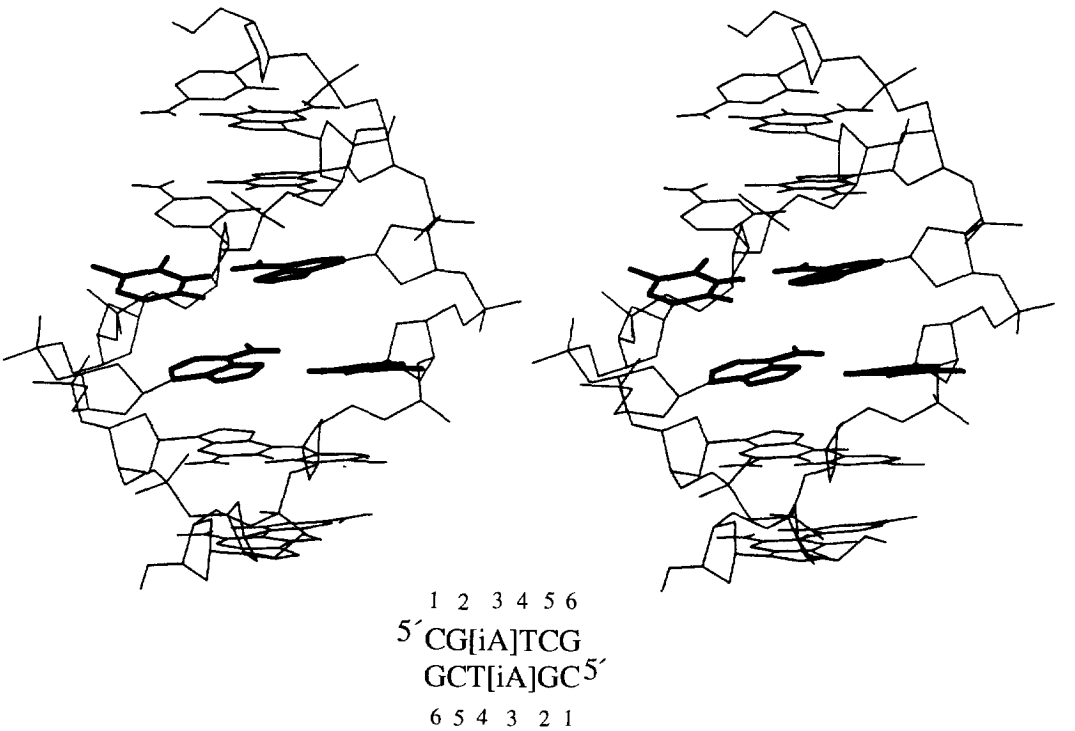


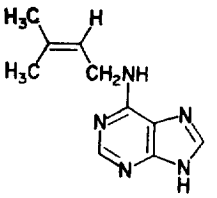
Figure 2. Reproduced, with permission, from: Bhat, B.; Neelima; Leonard, N. J.; Robinson, H.; Wang, A. H.-J. *J. Am. Chem. Soc.* **1996**, *118*, 3065–3066. Copyright 1996 (American Chemical Society).

tion (Fig. 2) and flanked by Watson–Crick pairs. These results support the Hoogsteen-type base pairing predicted much earlier for the oligo(iA)·oligo(U) complexes.[35,39] We also deduced that nature might have discarded the N3 attachment site for purines due to chemical instability rather than because of structural incompatibility.

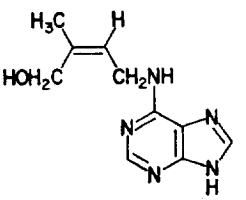
CYTOKININS: PLANT CELL GROWTH AND DIFFERENTIATION FACTORS

In the course of establishing the structure of triacanthine (see above), we synthesized all of the isomeric N-substituted isopentenyladenines, actually *N*-(Δ^2-isopentenyl)adenines, including those with substitution on N1 and N^6. I interested Professor Folke Skoog of the Department of Botany, University of Wisconsin, whom I had not yet met, in the testing of these compounds for biological activity, namely, the growth and differentiation of plant callus tissue and in the prevention of leaf senescence. My stimulation of his interest was based on the structural similarity of the compounds to kinetin, an unnatural product of the autoclaving of DNA.[42,43] We

were pleased to learn that N^6-(Δ^2-isopentenyl)adenine (**16**) was ten times as active as kinetin and



16



17

that the N1-substituted isomer had fractional activity as a result of its partial conversion to **16** during the autoclaving process.[44,45] Moreover, the N3-isomer, triacanthine (**12**), was found to be inactive when the test solutions were filter sterilized and added to the culture medium after it had been autoclaved, whereas when the triacanthine was added before autoclaving or was autoclaved prior to incorporation in the medium, it promoted the growth of tobacco callus tissue. We realized that there were many questions to be answered. After Folke Skoog and I finally met—as I recall, it was on the front steps of the National Academy of Sciences building in Washington, DC—we decided on several directions for research and on intended joint publication, to be preferred over separate or adjoining articles, our initial method of communication. (a) We considered that nature would not have missed such a good bet as **16** and that we should therefore look for this isoprenoid derivative and possible congeners in natural sources. (b) The rearrangements to give biologically active products were of interest chemically and therefore should be investigated. (c) A study of structure–activity relationships was also in order. As we found the answers to these propositions, other problems surfaced that were worth pursuing. Our particular combination of chemistry and plant physiology was celebrated all through a close research collaboration between Illinois and Wisconsin that lasted eighteen years and resulted in about fifty publications. It involved many visits back and forth, exchange of collaborators from one laboratory to the other for the effective exchange of ideas and techniques, and eventual involvement and collaboration with other scientists, including Professor Jack L. Strominger, University of Wisconsin, now at Harvard University; Dr John Occolowitz, Eli Lilly and Company; Professors Ian Gillam and Gordon M. Tener, University of British Columbia; Professor Muttaiya Sundaralingam, University of Wisconsin, now at Ohio State University; and Dr Barbara S. Vold, then at the University of New Mexico.

(a) N^6-*Isopentenyladenine (16) in nature*

The compound was indeed found and identified in nature in the plant pathogen *Corynebacterium fascians*,[46,47] and as the riboside, N^6-isopentenyladenosine (iA), synthesized earlier,[30,48] in the two main serine tRNAs of brewer's yeast.[49] The *trans*-methyl hydroxylated derivative of **16**, which is zeatin, was isolated from sweet corn kernels, and its structure was identified by spectroscopy[50] and synthesis.[51] The Wisconsin–Illinois team isolated, identified, and synthesized cytokinins that were present in *E. coli* tRNA, *Staphylococcus epidermidis* tRNA, wheat germ tRNA, tRNA of 7-day-old green pea shoots, yeast cysteine and phenylalanine tRNAs, and the tRNA of tobacco callus that had been grown in the presence of N^6-benzyladenine (6-benzyl-

aminopurine).[52–54] The new structures that were identified motivated the stereoselective synthesis of *cis*-zeatin (**17**)[55] and ribosyl-*cis*-zeatin.[56]

(b) *Rearrangements*

The development of cytokinin activity during the autoclaving of N1- and N3-substituted adenines alone or in the test medium and the identification of the products as N^6-substituted adenines prompted a study of the pathways of these rearrangements.[57,58] The solution to the problem of the route of rearrangement of 3-benzyladenine to N^6-benzyladenine, as an illustration, was based on the synthesis of specifically ^{15}N-labeled 3-benzyladenines and comparison of the products of their autoclaving in water at 120 °C and pH 5.8 under pressure, by mass spectrometry and NMR ^{15}N–^1H coupling, with N^6-benzyladenines specifically labeled with ^{15}N. We determined that the rearrangement was not due to a trivial sequence of N3-αC solvolysis followed by N^6-αC alkylation. Rather, the rearrangement follows a contortional route involving ring opening and ring closing, during which the side chain does not leave its original nitrogen. In the process both rings open, the pyrimidine ring more readily than the imidazole ring. The findings relate to the mutability of the adenine nucleus in a steam atmosphere and suggest a chemical route for possible natural conversion to cytokinin-active substances.

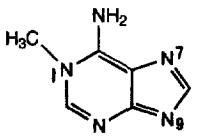
(c) *Structure–activity relationships*

A thorough study established the relative importance of the side-chain parameters: length; planarity; stereochemistry; saturation or unsaturation; substitution (degree, position, type—also on the ring system, replacement of NH by O, S, or CH_2), etc.[53,54,59–62] Sidney M. Hecht moved from the Illinois laboratory to Wisconsin to originate and coordinate research activities on two important questions. One was whether the addition of a ribosyl moiety at the 9 position of exogenously supplied cytokinins is or is not a prerequisite for their promotion of cell division and growth of plant tissue. It is *not* a prerequisite.[63] The other was whether a class of compounds, cytokinin antimetabolites, could be devised.[54] He developed a protocol for the detection of cytokinin antagonism and synthesized a very active antagonist, one that required alteration of **16** at three locations and implied a competition between the cytokinin and antagonist for the receptor sites associated with the promotion of cell division and growth.[63,64]

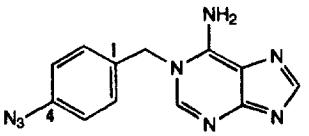
Further collaborative research included the synthesis of highly active cytokinins that are Westheimer-type photoaffinity labeling agents for cytokinin-binding protein,[65,66] and the production and characterization of an antibody for ribosylzeatin.[67] In the latter case, a radioimmunoassay was established that was capable of detection of pmol quantities of ribosyl-*trans*-zeatin. The joint research was a logical development from the work of Barbara Vold on antibodies to N^6-isopentenyladenosine and its nucleotide that could be employed for the detection of the isopentenyladenosine unit in tRNAs.[68,69]

An unexpected hazard for the research, at least in Madison, was the bomb blast in 1970 at the computer facility that was adjacent to the laboratory where the tRNA cytokinin fractionation was being done. All the windows in the plant physiology laboratory were smashed, which required massive clean-up and repair, but the large chromatographic columns luckily remained intact.

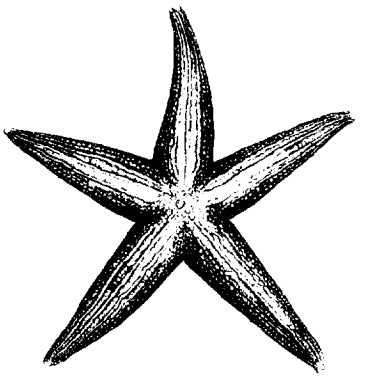
An unexpected bonus of our cytokinin research originated in a request for samples sent to me by Marcel Dorée and Pierre Guerrier, scientists at the Biological Station of Roscoff in France. In **starfish**, full-grown **oocytes** are arrested at the prophase stage of meiosis, and **meiosis** is reinstated by a relay hormone identified as 1-methyladenine (**18**). Dorée and Guerrier[70] were studying the specificity of the 1-methyladenine receptors which are localized on the cell



18



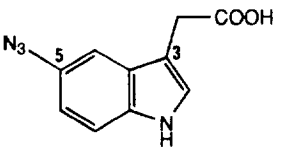
19



Asterias rubens

membrane of starfish oocytes, so that our collection of 1-substituted adenines[57] was of interest to them. We supplied them with series of variously monosubstituted and disubstituted adenines in sufficient variety to define the structural limitations for the expression of activity. I did not hear from Roscoff for some time, until suddenly the first draft of a 'joint' manuscript arrived in Illinois on the day in 1975 my wife and I were about to start driving to Colorado for our Christmas vacation. I could not approve the manuscript because our chemistry and the conventional theory at that time for the triggering of the release of meiosis inhibition were incompatible. With apology to my wife and the family whom we were to meet in Colorado, I disappeared into the library to read the literature on meiosis of *Marthasterias glacialis* and *Asterias rubens* oocytes. Everyone thought I had left Illinois, so I was undisturbed. My revised draft of the manuscript, produced after two days of intensive reading on starfish, accommodated the structure–activity results and put forth an improved concept of hormonal binding in the control of meiosis. My French colleagues approved the second draft with alacrity when it was submitted to them in January 1976.[71] Our family developed from this experience a new habit of publicly stating our date of departure from home to be one or two days before the actual intended event. This sneaky practice tends to confer privacy and to remove stress.

In due course we were able to provide a photoaffinity-labeling hormone-equivalent, 1-(4-azidobenzyl)adenine (**19**), which, because of its high level of biological activity, is a logical candidate for photo-attachment to the receptor site.[72] I have never met Dr Dorée or Dr Guerrier, but I did enjoy our joint adventure in research and publication. From the development of cytokinin photoaffinity labeling agents mentioned earlier, it was a short step to the synthesis of **auxin photoaffinity labeling agents**, the first of these in collaboration with Professor Skoog.[73] He remained more interested in cytokinins than in auxin; accordingly, my postdoctoral research associate Dr L. Lee Melhado and I sought the collaboration of our University of Illinois colleagues, Professor Larry N. Vanderhoef, Department of Botany, and Professor T.-H. David Ho, Department of Plant Physiology (change of department name). They were the successor mentors of Alan M. Jones, whom I inherited after both left the University of Illinois and who wanted to have synthetic organic chemical experience in addition to the auxin (indole-3-acetic acid) studies. We synthesized azido auxins and settled upon 5-azidoindole-3-acetic acid (**20**) as the most effective analog of auxin itself.[74–76] Together we laid the fundamental groundwork concerning physical behaviour, biological activity, products of photolysis, radiochemical labeling, fluorescence upon photolysis, and covalent attachment upon photolysis at auxin-specific sites in

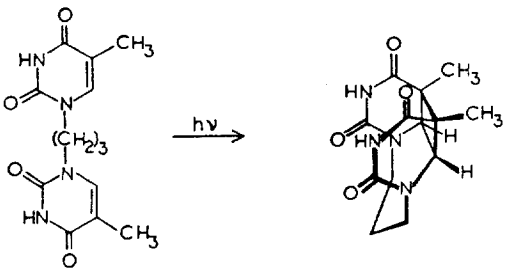


20

maize and soybean. However, we did not continue the work at the University of Illinois after Alan Jones received his Ph.D. Now, as an Associate Professor in the Department of Biology at the University of North Carolina, Alan has used radiolabeled, photolabeling **20** to identify several different auxin-binding proteins in maize and squash:[77] two on the plasma membrane, two in the cytosol, and a family in the membrane system. He has accomplished receptor identification, receptor characterization, determination of auxin transport pathways, and has tested hypotheses concerning the cellular site of auxin perception. These are some of the scientific gains that have resulted from the original collaboration and substitute mentoring.

SYNTHETIC SPECTROSCOPIC MODELS RELATED TO COENZYMES AND BASE PAIRS

In order to study the interactions between adjacent bases in a nucleic acid strand in the absence of complicating factors associated with hydrogen bonding or the usual carbohydrate and phosphate units, we synthesized a series of simplified dinucleotide analogs in which the bases (9-substituted adenine or guanine or 1-substituted cytosine, thymine or uracil residues) were connected by a polymethylene chain: B-$(CH_2)_n$-B′ or, more simply, B-C_n-B′ (**21**). In those models in which the bases are connected by a trimethylene chain, the chain is of sufficient length to allow (but not dictate) vertical ring stacking in aqueous solution similar to that found in nucleic acids. Shorter chains would not allow the rings to lie in parallel planes, while longer chains would presumably result in decreased interaction due to an entropy effect. We wanted to study these compounds optically at concentrations low enough ($3–8 \times 10^{-5}$ M) to preclude formation of intermolecular complexes while examining the perturbations associated with the 1:1 intramolecular interaction of a pair of bases, the same or different, within compounds of type **21**. Because Dr J. (Terry) Eisinger whom I had met at a tRNA conference, had been involved in optical studies of dinucleotides, polynucleotides and DNA,[78,79] I invited myself to Bell Telephone Laboratories to obtain his agreement that a related study of our simplified models would be worthwhile. The characterization was accomplished by ultraviolet spectroscopy in aqueous solution at room temperature and by emission spectroscopy in 1:1 ethylene glycol:water glass at ca 77 K.[80] We were satisfied on the basis of earlier work that our analogs (**21**) would probably have similar conformations in water at ambient temperature and in the mixed solvent used for emission studies. The simplified models behaved similarly to the natural dinucleoside phosphates and provided additional information concerning hypochromism, fluorescence emission characteristic of eximer formation, the stacking of adenine with the anti-codon-adjacent base in tRNAs,[81] the reason for the 'bend' at N^2-dimethylguanosine in tRNAs (ninth nucleoside component from the first letter of the anticodon),[82] and the photodimerization of 1,1′-trimethylenebisthymine (Thy-C_3-Thy) (**22**), 3.5 times faster than TpT. Somehow this research collaboration is associated in my memory with a Chinese restaurant in Greenwich Village and music in a loft in SoHo, New York City. Anyone who knows Terry Eisinger will recognize the stimulating venues.

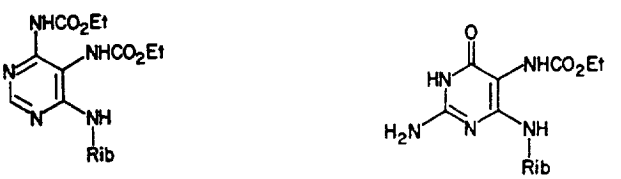


22 23

In a research collaboration with my Illinois colleague Professor Iain Paul, the structure of the photodimer from **22** was established by chemical and X-ray analysis as having *cis–syn* stereochemistry (**23**).[83] The four membered ring in **23** is very close to planar (dihedral angle = 178°). The employment of bases linked at specific positions by a trimethylene bridge[84] has found many other applications in our laboratory and elsewhere.

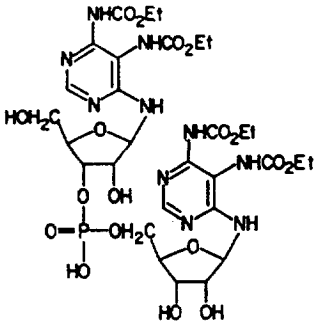
DIETHYL PYROCARBONATE AND NUCLEIC ACIDS (CHEMICAL PROBES)

Professor M. E. Reichmann, Department of Microbiology, University of Illinois, brought me the next problem in nucleic acid chemistry. He had been using diethyl pyrocarbonate (DEP) for ribonuclease inhibition in the preparation of tobacco mosaic virus (TMV) and was finding that the infectivity of the virus was destroyed completely by DEP at 37 or 23 °C and reduced to near zero at 0 °C. Our first chemical experiments assessed the reaction of DEP with the natural purines and pyrimidines, e.g. adenine,[85] and nucleic acid components, e.g. adenosine.[86] While DEP opens the pyrimidine ring of adenine and N^6-substituted adenines, this reagent opens the imidazole ring of 9-substituted adenines. Adenosine reacts with excess DEP in water to give the

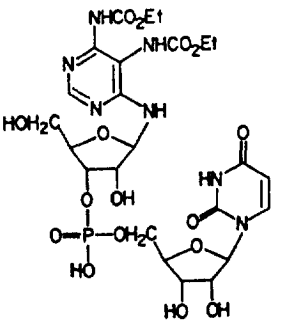


24 25

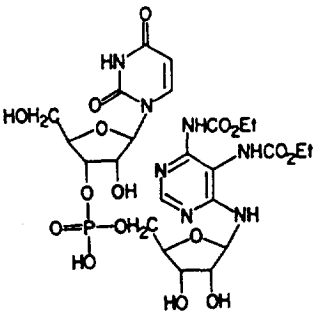
ring-opened dicarbethoxylated derivative **24** as the major product.[86,87] Guanosine undergoes imidazole ring opening to yield **25**, and cytidine reacts with DEP in unbuffered aqueous medium with carbethoxylation of the exocyclic amino group.[87] There is usefulness in the structure determination of these nucleoside reaction products for understanding and assessing the behaviour of DEP with polynucleotides, RNA, and tRNA, but careful consideration must be given to the use of this reagent as a nuclease inhibitor for the isolation of unaltered nucleic



26



27



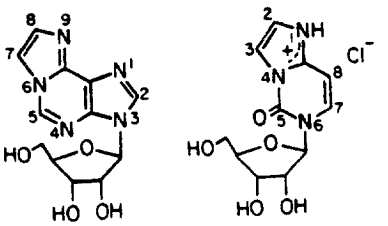
28

acids. As organic chemists, we were able to turn DEP to good structural purpose. We determined that the dinucleoside phosphates ApA, ApU, and UpA were converted by excess DEP in high yields to products **26**, **27**, and **28**, respectively.[88] Of further interest was the finding that the ApA product **26** is not hydrolyzed by venom phosphodiesterase, ribonuclease T_2, bovine pancreatic ribonuclease A, or bovine spleen phosohodiesterase, and the modified ApU (**27**) and UpA (**28**) are hydrolyzed very slowly, if at all. Thus, in 1973 we suggested[88] that the reagent would serve the purpose of detecting adenosine or deoxyadenosine, for example, at exposed sites in an RNA or DNA and of initiating sequential analysis at a modified A at a spot in the molecule where enzymatic cleavage would not occur readily. In 1976 Ehrenberg, Fedorcsák, and Solymosy[89] reviewed the chemistry of DEP, its reactions with proteins, and, with emphasis on the kinetics of competing reactions, its applications in nucleic acid research. Selected applications since that time will be further illustrative but are not intended to be complete. For example, it was found in a study with terminally ^{32}P-labeled **tRNA^{Phe}**—native, semi-denatured, and denatured—that DEP reacts at the N7 of an adenosine only if the site is not involved in structural interactions, i.e., is unstacked.[90] Purine residues are modified preferentially within regions that have the potential to form left-handed **Z-helical structures** and is accordingly diagnostic.[91,92] The N7 carbethoxylation creates piperidine-sensitive sites. Hyperreactivity is dependent on the degree of negative superhelicity of the circular DNA. DEP carbethoxylates N7 of A's (and G's) in presumptive single-stranded loops of **DNA cruciform structures**, and the backbone is then cleavable with piperidine.[93,94] Similar methodology has been used for the footprinting of **binding of quinoxaline antibiotics** to DNA[95] and of **intercalation-induced helix unwinding** in DNA that leads to hypersensitivity of adenines upon exposure of N7s.[96]

ETHENO-SUBSTITUTED NUCLEOTIDES AND COENZYMES (FLUORESCENT PROBES)

3-Isoadenosine and its phosphate derivatives served as **spatial probes** of adenosine and its corresponding derivatives (see above) and were distinguishable by such properties as NMR, chromatographic behaviour, and ultraviolet spectra. An analog that possesses fluorescence would have greater advantage in terms of detection and studies of binding, and we were in the process of making fluorescent derivatives of as many nucleosides as possible. This enterprise was inspired by my auditing a course on fluorescence given by my Illinois colleague, Professor Gregorio Weber, and by the realization that spectroscopic investigation of coenzyme–enzyme interactions and of nucleic acid–protein interactions is facilitated when one of the members is rendered fluorescent. Stimulated by the report of Kochetkov, Shibaev, and Kost,[97] my young

colleague Dr Jorge Barrio, joined by Jack Secrist, predicted enthusiastically that the product of the chemical modification of adenosine with chloroacetaldehyde in aqueous solution at room temperature, namely ϵ-adenosine, **29**, should be fluorescent. Indeed, it is fluorescent, as is the protonated form of ϵ-cytidine (**30**).[98]

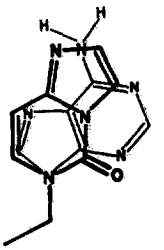


29 **30**

There followed a flurry of activity in our Laboratory on reactions of chloroacetaldehyde, e.g. as a spray reagent for the detection of adenine-containing residues by fluorescence on thin-layer or paper chromatograms; conversion of ATP to fluorescent ϵATP and following its activity in representative enzyme systems; conversion of NAD^+ to ϵNAD^+ for investigation by fluorescence yield and lifetime of intramolecular association and intermolecular interaction and activity in dehydrogenase systems; and modification of dinucleoside phosphates for the determination of dynamic fluorescence quenching and quenching due to intramolecular complexation. We teamed up with Gregorio Weber for a thorough study of the fluorescent modification of adenosine-containing coenzymes and their biological activities and spectroscopic properties,[99] including a determination that it is the unprotonated form of the ϵ-adenine fluorophore that is responsible for the fluorescence emission.[100] Agbaria, Parola, and Gill[101] have since shown that the photophysics of ϵ-adenosine relies on the properties of $n\pi^*$ and $\pi\pi^*$ excited states and that protonation quenches the $n \rightarrow \pi^*$ absorption. With Professor Weber, we also determined that the modified coenzyme ϵFAD exists mainly (90%) as an internally complexed or stacked form by an analysis of the dynamic and static quenching of fluorescence.[102]

In order to investigate the activity of ϵ-adenosine 3′,5′-monophosphate, cyclic ϵAMP, in enzyme systems, we collaborated with Professor Alfred G. Gilman, at the time in the Department of Pharmacology of the University of Virginia School of Medicine. He very kindly invited my student Jack Secrist to come to Charlottesville and work in his laboratory. When the intended study was completed in two weeks' time, I congratulated Jack; however, he informed me modestly that during a major portion of the time he had been watching over the shoulder of Professor Gilman while Gilman eagerly demonstrated how the experiments should be done. The conclusion was that cyclic ϵAMP is a highly acceptable substitute for cyclic AMP in protein kinase systems, with utility based upon its long fluorescent lifetime, detectability at low concentration, and relatively long wavelength of excitation.[103]

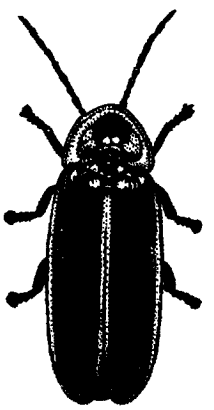
Back at Illinois, we collaborated with Professor Robert L. Switzer, Department of Biochemistry, in the Υ-^{32}P labeling of ϵATP and ϵCTP.[104] In the enzymatic phosphorylation of 3-phosphoglyceric acid, ϵCTP is essentially equivalent to ATP and is a significantly better coenzyme than ϵATP. The ability to replace ATP permitted the enzymatic synthesis of [Υ-^{32}P]ϵCTP and [Υ-^{32}P]ϵATP by phosphate exchange. With Professor Richard I. Gumport, we compared the activity of nicotinamide 3,N^4-ethenocytosine dinucleotide, ϵNCD^+, with the natural coenzyme, NAD^+, in selected enzyme systems: yeast alcohol, horse liver alcohol, pig heart malate, beef liver glutamate, rabbit muscle lactate, and glyceraldehyde-3-phosphate



31

dehydrogenases.[105] An inspection of the spatial relationship (**31**) between the three-dimensional models of NAD^+ and ϵNCD^+ and the coenzyme binding domains (Kendrew models) of dogfish lactate dehydrogenase and lobster muscle glyceraldehyde-3-phosphate dehydrogenase allowed a rationalization of the binding behaviour of the unnatural analog. With Professor James L. Robinson, we studied the kinetic and fluorescence behaviour of ϵADP and ϵATP in terms of reaction rates. Moreover, the binding of ϵADP and ϵATP was observable by their fluorescence polarization,[106] which indicated that the fluorophor portion can rotate somewhat freely in the enzyme solutions. It was concluded thereby that the base part of the ligand is not strongly associated with the protein through multiple points of attachment.

Although ϵATP will substitute for ATP in phosphoryl, pyrophosphoryl, and adenylyl transfer reactions,[107] in a collaboration with Dr Marlene DeLuca and Professor W. D. McElroy, of the University of California, San Diego, we found that ϵATP was inactive as a substrate for firefly luciferase (from *Photinus pyralis*).[108] However, chemically synthesized luciferyl-ϵAMP is oxidized by luciferase and light is emitted. Because the color of the light is red in contrast to the normal yellow-green observed with luciferyladenylate, we could conclude that more of the excited state monoanion (red) is present and that the analog is bound in the active site in such a way that the normal proton-accepting group on the enzyme is no longer adjacent to the C5-hydrogens of the



Photinus pyralis

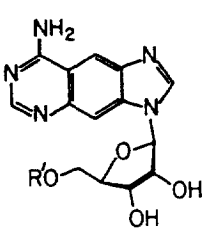
luciferyl unit, i.e. to produce the dianion (yellow-green). It is interesting that 3-iso-ATP, the triphosphate of **13**, which is active with luciferase, also produces red light at pH 7.5.[109]

A second collaboration with my Columbia laboratory partner, Elkan R. Blout, now in the Department of Biological Chemistry, Harvard Medical School, developed from the idea of **fluorescent photoaffinity labeling**.[110] With the urging of Dr Gideon Dreyfuss, also at Harvard, our Illinois team synthesized a fluorescent photoaffinity label for cyclic AMP receptor sites of cyclic AMP-dependent protein kinases in both unlabeled and radioactive forms. The probe, 8-azido-1,N^6-ethenoadenosine 3′,5′-cyclic monophosphate, closely mimics cyclic AMP in its ability to stimulate the phosphotransferase activity of the protein kinases and competes strongly with cyclic AMP for its binding sites on rabbit muscle protein kinase and beef heart protein kinase, as examples. After equilibration of the protein kinase and the probe in the dark, photolysis brings about irreversible binding of the intermediate nitrene specifically to the cyclic AMP sites. An extrinsic fluorescent probe is thereby attached to a particular protein that may be part of a complex mixture.

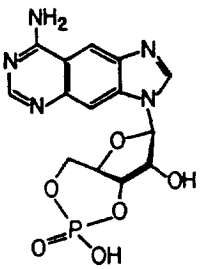
After a dozen papers and two patents[111,112] on etheno-substituted nucleotides and coenzymes, we decided to turn our attention elsewhere because as the etheno-bridged compounds became available from a number of commercial sources, many scientists were finding utility in the chloroacetaldehyde/bromoacetaldehyde-modified compounds. I adopted a new, less active role, i.e. reviewing the field about every ten years[113–115] in order to keep up with developments. The exercise was gratifying, for it revealed the continuing use of the etheno-bridged compounds in many applications, including **DNA and tRNA structural diagnosis, carcinogen activation, coenzyme–enzyme interactions, and coenzyme binding to proteins**.

FLUORESCENT DIMENSIONAL PROBES

In fluorescent ∈ATP and its congeners, the N1 hydrogen acceptor and N^6 hydrogen donor positions of ATP are blocked. Because these positions may be involved in enzyme or protein interactions in some systems, I felt I had to devise an improved type of probe in which analogous loci would remain free while the property of fluorescence would be retained by the inclusion of a third ring in the adenine system. Why not try a central 'benzo' ring between the terminal pyrimidine and imidazole rings? The answer was found in the *lin*-benzoadenosine (**32a**) series



32



32e

32a R′= H

32b R′= O=P(OH)–OH

32c O=P(OH)–O–P(=O)(OH)–OH

32d O=P(OH)–O–P(=O)(OH)–O–P(=O)(OH)–OH

(**32**).[116] The fact that the new, nitrogen-containing ring system had to be synthesized *de novo* gave us some respite so that we could do ample experimentation before the compounds would

have any off-the-shelf availability. We shortened the synthetic methodology[117,118] for the *lin*-benzopurines in general, we know that other laboratories have been able to repeat our published syntheses, and we offered samples selectively as gifts for research collaboration where the synthetic capability did not exist. The naming of the compounds in this stretched-out (by 2.4 Å) series (e.g. **32**) was anticipatory of biological activity and seemed justifiable chemically since the basic heterocyclic system is a benzolog of adenine. Experiments with an initial group of kinases showed that the formal insertion of a benzene ring in the center of the adenine nucleotides, *lin*-benzo-ADP (**32c**) and *lin*-benzo-ATP (**32d**), does not greatly diminish their binding properties with respect to those of the normal nucleotides but tends to decrease the rate of reaction.[119] Armed with the knowledge of the similarity in behavior of *lin*-benzo-ATP and ATP in a number of systems and convinced of the desirability of relating the nucleotide names, I had the temerity to approach Dr Waldo E. Cohn for approval. We had collaborated earlier on nomenclature abbreviations for pyrimidine photo-products.[120] It took some argumentation, however, and the conviviality of a Gordon Research Conference in New Hampshire for me to obtain his imprimatur on the nomenclature abbreviations of the *lin*-benzo series (also applicable to *lin*-naphtho, etc.). I call it a 'two Scotches' name.

Research on the fluorescent dimensional probes moved forward at a brisk pace at the University of Illinois when Dr Jorge R. Barrio returned to us from an interim teaching position in his native Argentina where dangerous political fracturing was in progress. We found usefulness in the fluorescence properties of the *lin*-benzoadenine nucleotides **32b–e** and in their enhanced π interactions.[120–124]

These properties could be directed to many studies of static and dynamic behavior with different moieties; complexation; the dimensional probing of enzyme binding sites; and conformational changes induced by surrogate coenzyme–enzyme binding. Together with my colleague Olke Uhlenbeck at the University of Illinois in the Department of Biochemistry, we found that *lin*-benzoadenosine, modified by conversion by pyrophosphoryl chloride to the 3′(2′),5′-bisphosphate, could be incorporated onto the 3′ end of $(Ap)_3C$ using T4 RNA ligase.[125] Together with Dr M. J. Schmidt of the Lilly Research Laboratories, Eli Lilly and Company, we published on the maximal activation of brain protein kinase and protein kinase from skeletal muscle by *lin*-benzoadenosine 3′,5′-monophosphate (**32e**).[126] We also reported the inhibition of kinase activity by *lin*-benzoadenosine (**2a**). In both cases the active sites can accommodate the lateral extension of the purine ring by 2.4 Å.

Professor Henry A. Lardy, of the Institute for Enzyme Research and the Department of Biochemistry at the University of Wisconsin, and I were consulting together for Eli Lilly and Company at the time, and during the frequent meetings we exchanged accounts of our current research. One outcome of these discussions was the decision to collaborate on the interaction of *lin*-benzo-ADP (**32c**) and *lin*-benzo-ATP (**32d**) with the enzymes of the mitochondrion.[127] The major conclusions of the collaborative research were that **32c** and **32d** substituted well for ADP and ATP as substrates for the enzymes of oxidative phosphorylation in the submitochondrial particles and for hydrolysis by purified mitochondrial ATPase. With intact mitochondria, *lin*-benzo-ADP was a poor acceptor for oxidative phosphorylation, which demonstrated that it had little, if any, activity as a substrate for the adenine nucleotide carrier.

We turned again to collaboration with Dr Marlene DeLuca, Department of Chemistry, University of California, San Diego, in this case for the dimensional probing of the ATP binding site on firefly luciferase.[128] *lin*-Benzo-ATP (**32d**) was shown to be an acceptable substrate for light production in the firefly luciferase–luciferin system. This is significant because firefly luciferase has long been known for its fastidious choice of nucleotide substrate and has been used for detection of the specific presence of ATP on the basis of its light-emitting response. *Lin*-benzo-ATP (**32d**) and ATP have similar binding affinities. The spectral variation in the

bioluminescence for *lin*-benzo-ATP (relative quantum yield 0.6) versus ATP suggested that the lateral extension in the purine ring system induces a change in the conformation of the luciferase, affecting the immediate environment of the excited light emitter.

What would be the activity of *lin*-benzo-ADP and *lin*-benzo-ATP in association with the cyclic nucleotide dependent protein kinases? Through my one-time neighbor and colleague at the University of Illinois, Professor Carl S. Vestling, I learned of the work of Robert Roskoski, Jr, and we engaged in some science together. Professor Roskoski and Dr F. Thomas Hartl in the Department of Biochemistry of the Louisiana State Medical Center, New Orleans examined the interaction of *lin*-benzoadenosine di- and triphosphates with type II holoenzyme and the catalytic subunit of adenosine cyclic 3′,5′-monophosphate dependent protein kinase by steady-state kinetics and fluorescence spectroscopy.[129] The relevant utility of the fluorescent analogs lay in their similar behaviour to the natural nucleotides, and **fluorescence polarization** results taught us the degree of rotation within the binding site.[129,130] While I had earlier experience with fluorescence polarization, it was Professor Roskoski and Dr Deepak Bhatnagar who devised the rapid, nondestructive, and sensitive technique that we call **fluorescence polarization titration** for characterizing the ATP binding site of the catalytic subunit of the type II cAMP-dependent protein kinase.[131] This was done by determination of the dissociation constants (K_d) of a series of nucleotide analogs for the catalytic subunit by displacing *lin*-benzo-ADP with increasing concentrations of competing nucleotide. The K_d of each nucleotide was calculated from the decreases in the fluorescence polarization of *lin*-benzo-ADP that accompany its displacement from the catalytic subunit. By appropriate choice of nucleotide analogs it was possible to map the exact requirements for the most tenacious binding of the nucleotide moiety. Application of the same method made it possible to determine which metal ions promote binding and to what extent and to correlate these findings with the phosphotransferase activity observed in their presence,[132] and the method was extended to the examination of guanosine cyclic 3′,5′-monophosphate-dependent enzyme.[133] During the period of our research collaboration, we kept abreast of developments by telephone, frequent mailings, and one or two visits to New Orleans (not a hardship).

NITROGEN-15 NUCLEAR MAGNETIC RESONANCE

Research collaboration with Professor John D. Roberts of the California Institute of Technology was initiated and continued on the ski slopes of Aspen/Snowmass, Colorado. We were part of an intense group of chemists and biochemists who met together in an annual series referred to as the Aspenyl Chemistry and Biochemistry Meetings. While the 'Aspen' part of the name referred to the location and inferred one of the purposes, the ending 'yl' was supposed to denote the radical nature of the enterprise. We celebrated après-ski with seminars on our current research! The small audience was such that discussion of purpose, experiment, and conclusions was incisive, so much so that the speaker of the day usually heaved a sigh of relief when he or she had passed the first slide or overhead. Jack Roberts' description of his pathfinding work on ^{15}N NMR and my contributions to nucleic acid chemistry first found a meeting place in an analysis of 5-azacytidine ^{15}N resonances in neutral and protonated form.[134] A number of downhill runs and ski-lift rides later, we collaborated in a study of the tautomers and of the protonation of adenine and its derivatives by ^{15}N NMR spectroscopy.[135] Specific ^{15}N labeling at N1, N3, N6′, N7, and N9 was accomplished by chemical synthesis at Illinois; uniform labeling was accomplished by conversions of *S*-adenosylmethionine isolated from yeast grown with $^{15}NH_4Cl$ as the principal nitrogen source at Caltech. The results included unequivocal assignments of the chemical shifts, elucidation of the position of tautomeric equilibria, and

determination of the sites of proton addition and proton removal for adenine, adenosine, and substituted derivatives of these. Great care had to be taken in making the assignments across the pH scale 1 to 12 since there was much crossing of the curves corresponding to the individual ^{15}N shifts versus pH (Fig. 3).

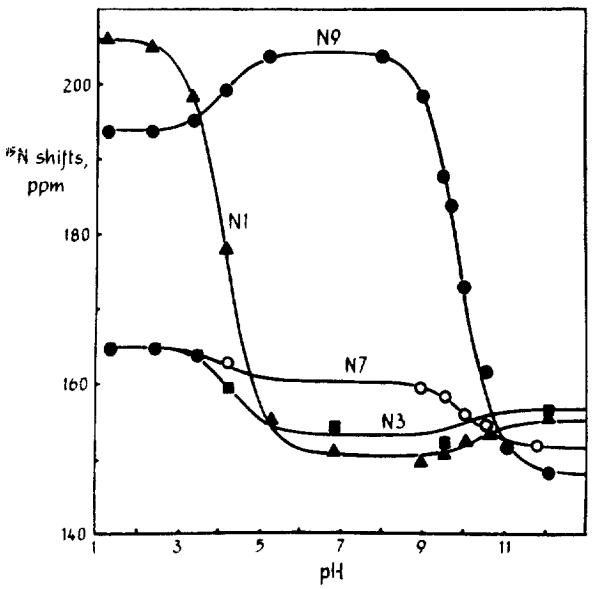


Figure 3. Changes in the ^{15}N resonance shifts of adenine in aqueous solution with pH. Reproduced, with permission, from: Gonella, N. C.; Nakanishi, H.; Holtwick, J. B.; Horowitz, D. S.; Kanamori, K.; Leonard, N. J.; Roberts J. D. *J. Am. Chem. Soc.* **1982**, *105*, 2050–2055. Copyright 1983 (American Chemical Society).

The unequivocal synthesis of (+)-[1-^{15}N]biotin was linked to the assignments of the ^{15}N NMR resonances of biotin.[136] The intent was to provide another probe for following the biological carboxylation and transcarboxylation of biotin and for investigating the phenomenal interaction between biotin and avidin.The final research collaboration with Jack Roberts required a fruitful visit to Pasadena in addition to the contacts generated in Snowmass Village. The paper that resulted was concerned with the ^{15}N NMR assignments in a systematic series of azacycl[3.3.3]-azines of varying nitrogen content,[137] and, as such, presented data that were closely related to the other spectroscopic results assembled at Illinois for the series (see below). At a dinner in Pasadena to celebrate Jack Roberts' seventieth birthday, I had the pleasure of describing his contributions to chemistry, universities, foundations, and friends. When I mentioned that I could probably pinpoint his four major research contributions as references 134–137, the audience groaned, appreciating the hyperbole. My most meaningful collaboration with Jack and Edith Roberts had its roots in their introducing me to one Peggy Phelps that resulted in our marriage in 1992.

TRI-*S*-TRIAZINES AND TETRAAZAPENTALENES

In our Laboratory at the University of Illinois at Urbana-Champaign, we discovered methods for the synthesis of nitrogen heterocycles that were of particular theoretical interest, namely,

tri-*s*-triazines and tetraazapentalenes. Pauling and Sturdivant[138] devised the correct formulation of a ring system consisting of a coplanar arrangement of three fused *s*-triazine rings as the common nucleus present in some heat-stable materials that had been made by Liebig and Gmelin more than a century earlier. Our synthesis of the unsubstituted nucleus, tri-*s*-triazine (cyamelurine) (**33**), for the first time was inspired by the behaviour of the intermediate **35**, formed in the reaction of 2,4-diamino-1,3,5-triazine (**34**) with methyl *N*-cyanomethanimidate, in the mass spectrometer.[139] It showed a major m/e peak corresponding to the loss of cyanamide. I suggested that we try a vacuum pyrolysis method that duplicated, in bulk, the inlet conditions in the mass spectrometer, i.e. short residence time, high temperature, and very low pressure. My young colleagues Mitchell Rossman and Dr. Ram Hosmane enjoyed some teasing when they said something to the effect, "We tried your idea, *but* it worked." The conversion ($\geq 60\%$) of **35** to **33** probably proceeds through the intermediacy of **36**, resulting from a single ring closure and elimination. The physical and spectroscopic properties and the structure, established by X-ray crystallography, satisfied most of the theoretical predictions related to the 12-π electron periphery and the question of the involvement of the n electrons of the central N. The compound is yellow, with a lowest energy transition close to that predicted by Leupin and Wirz,[140] and it is also weakly fluorescent. Our Communication elicited a letter from Linus Pauling, reproduced here, which had a very stimulating effect on my young coworkers:

LINUS PAULING INSTITUTE of SCIENCE and MEDICINE

440 Page Mill Road, Palo Alto, California 94306
Telephone: (415) 327-4064

5 November 1982

Professor N. J. Leonard
School of Chemical Sciences
University of Illinois
470B Roger Adams Laboratory
1209 West California Street
Urbana, IL 61801

Dear Professor Leonard:

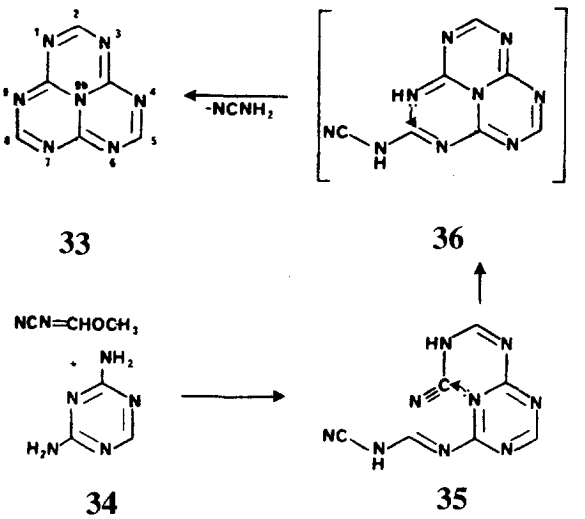
I am surely pleased that you have succeeded in synthesizing the compound cyamelurine.

I must say that I was also pleased, nearly 50 years ago, when I realized that I had thought of a sensible structure for cyameluric acid and hydromelonic acid. Professor Franklin at Stanford had given me nicely crystallized samples of some of the compounds, and I had been trying to think of a sensible structure - the ones that he had written for the compounds and that other early chemists had written did not seem to me to be sensible.

Sincerely,

Linus Pauling

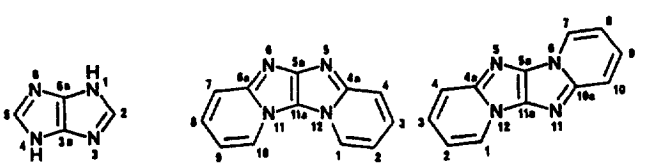
My colleague Professor Pierre LeBreton at the University of Illinois at Chicago was intrigued with the structure **33**. When we talked together at a National Meeting of the American



33 36

34 35

Chemical Society, he agreed to add UV photoelectron spectroscopic data and quantum mechanical calculations to our chemical findings for a better definition of the valence orbital structure of tri-*s*-triazine (**33**), accounting for its low basicity and overall high chemical stability.[141] When we completed the abbreviated two-step syntheses (modeled after **34**→**35**→**36**→**33**) of the azacycl[3.3.3]azines in a systematic series with six, five, four, and three peripheral nitrogens, Pierre LeBreton and his colleague Shigeyuki Urano established the influence of sequential removal of Ns on the π and lone-pair electronic structures,[142] bolstered in interpretation by quantum mechanical calculations. The Illinois contribution to the latter resided only in the preparation of appropriate figures. Further collaboration with Pierre LeBreton resulted in another publication,[143] but then we both ran out of manpower. Professor Arthur M. Halpern,



37 38 39

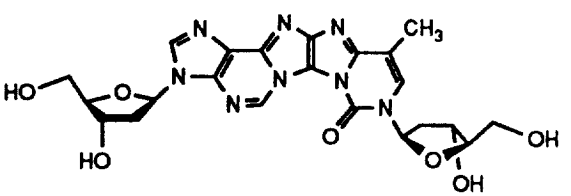
then at Northeastern University, was another collaborator on the tri-*s*-triazine problem, with a spectroscopic and photophysical study.[144]

1,3,4,6-Tetraazapentalene (**37**)[145] represents another nitrogen-containing ring system of theoretical interest. It has a 10 π-electron system, isoelectronic with the pentalene dianion. Art Halpern and his coworkers determined the spectroscopic, photophysical, and photochemical properties of the *syn* (**38**) and *anti* (**39**) isomers of the dipyrido-substituted 1,3,4,6-tetraazapentalene that we had synthesized in connection with covalently-linked double-helical cross sections (see below).[146] Both isomers and their *N*-ethyl bromide derivatives have the ability to illuminate

fragments of DNA in a manner similar to that observed with ethidium bromide.[147] The four nitrogen atoms in both isomers occupy sites of high charge density which gives them stability in accord with the tenets of topological charge stabilization theory.[148] Differences lie in the phosphorescence observed for the *syn* isomer (**38**) in EPA glass at 77 K and in its photolability in aerated solution, corresponding to the addition of singlet oxygen to the ground state of **38**. These properties are not shown by the *anti* isomer (**39**).

COVALENTLY-LINKED DNA/RNA CROSS SECTIONS

The synthesis of 1,3,4,6-tetraazapentalenes, e.g. **38** and **39**, with a core nucleus of 10 π-electrons was crucial to the construction of covalently-linked cross sections with molecular architecture similar to hydrogen-bonded DNA/RNA base pairs for both antiparallel[149] and parallel[150] helical duplexes. An X-ray structure analysis (of the diacetate) showed that compound **40**, dA⟩dT, can be called a spatial mimic of a Watson–Crick double-helical dA·dT cross section.[151] The distance between the top two nitrogens of the core tetraazapentalene is 2.56 Å, which may be compared with the N—H···O distance of 2.85 Å in a dA·dT Watson–Crick base pair. When we had made covalently-linked analogs of nucleic acid base pairs of normal, long, and short C1′ to C1′ dimensions, we again entered into a research collaboration with my Biochemistry colleague Richard Gumport. Together we found that the analogs representing



40

purine–pyrimidine, purine–purine (as observed in a triple helix), and pyrimidine–pyrimidine (hypothetical) base pairs (tetraphosphorylated) could be ligated to $d(A)_6$ with bacteriophage T4 RNA ligase.[152] The analog is joined to only a single oligonucleotide unit in each case. Because the second and third examples are symmetrical, there is no question as to the point of attachment. By the selective blocking of the purine or pyrimidine unit, in a later study, we determined that $d(A)_6$ becomes attached preferentially to the purine side in the first example. The tetraphosphorylated, covalently-linked cross sections offered an extreme test for the T4 RNA ligase which, up to that time, had been recognized as accepting pNp's at its donor site. There are other ways to incorporate such a cross section, i.e. by chemical synthesis, and research collaboration has continued, but now with Dr Balkrishen Bhat, Dr Howard Robinson and Professor Andrew Wang at the University of Illinois and the writer displaced to the California Institute of Technology in a Faculty Associate (no duties) position. In what may be our finale, we have examined intramolecular versus intermolecular dA–dT hydrogen bonding by means of a covalently-linked dimensional analog of dA·dT, namely **40**, that holds added deoxyadenosine and thymidine bases either proximal or distal to each other.

IN CONCLUSION

I am conscious of judgments that have been made over the course of time by sociologists of science to the effect that scientific papers do not necessarily record the actual course of scientific investigations. In this review of some of the collaborative research investigations that I have enjoyed, I have done my best to adhere to a description of events as I believe they took place. I suggest that the combined results amounted to more than the sum of the contributions of the partners. I hope that the article will at least be a contribution to the ethology of a selected group of scientists.

Acknowledgment—During the course of my career at the University of Illinois and up until my retirement, I benefitted from grants from the National Science Foundation, the National Institutes of Health, E.I. Du Pont de Nemours & Co., Eli Lilly and Company, Hoffmann-La Roche Foundation, and Marion-Merrill-Dow. For their assistance in the preparation of this manuscript, I give special thanks to Patricia Silver, Balkrishen Bhat, Michael P. Groziak, and Jennifer Clark. I am most appreciative of the science and friendship of all of my research colleagues.

REFERENCES

1. Robinson, R. *Outline of an Electrochemical Theory of the Course of Organic Reactions*; Institute of Chemistry of Great Britain and Ireland: London, 1932; p. 30.
2. Leonard, N. J.; Rapala, R. T.; Herzog, H. L.; Blout, E. R. *J. Am. Chem. Soc.* **1949**, *71*, 2997–3002.
3. Leonard, N. J.; Blout, E. R. *J. Am. Chem. Soc.* **1950**, *72*, 484–487.
4. Leonard, N. J.; Mader, P. M. *J. Am. Chem. Soc.* **1950**, *72*, 5388–5397.
5. Verheijdt, P. L.; Cerfontain, H. *J. Chem. Soc., Perkin Trans. 2* , **1982**, *12*, 1541–1547.
6. Cerfontain, H.; Kruk, C.; Rexwinkel, R.; Stunnenberg, F. *Can. J. Chem.* **1987**, *65*, 2234–2237.
7. Marion, L.; Leonard, N. J. *Can. J. Chem.* **1951**, *29*, 355–362.
8. Przybylska, M.; Barnes, W. H. *Acta Cryst.* **1953**, *6*, 377–384.
9. Leonard, N. J.; Swann, Jr S.; Dryden, Jr H. *J. Am. Chem. Soc.* **1952**, *74*, 2871–2875.
10. Leonard, N. J.; Swann, Jr S.; Figueras, Jr J. *J. Am. Chem. Soc.* **1952**, *74*, 4620–4624.
11. Leonard, N. J.; Swann, Jr S.; Mottus, E. H. *J. Am. Chem. Soc.* **1952**, *74*, 6251–6253.
12. Leonard, N. J.; Swann, Jr S.; Fuller, G. *J. Am. Chem. Soc.* **1954**, *76*, 3193–3197.
13. See: Leonard, N. J. *Record Chem. Progress* **1956**, *17*, 243–257 for review of the origin and development.
14. Leonard, N. J.; Morrow, D. F.; Rogers, M. T. *J. Am. Chem. Soc.* **1957**, *79*, 5476–5479.
15. Leonard, N. J.; Milligan, T. W.; Brown, T. L. *J. Am. Chem. Soc.* **1960**, *82*, 4075–4084, 6431.
16. Leonard, N. J.; Adamcik, J. A.; Djerassi, C.; Halpern, O. *J. Am. Chem. Soc.* **1958**, *80*, 4858–4862.
17. Bürgi, H. B.; Dunitz, J. D.; Shefter, E. *J. Am. Chem. Soc.* **1973**, *95*, 5065–5067.
18. Kaftory, M.; Dunitz, J. D. *Acta Cryst.* **1975**, *B31*, 2912–2914.
19. Leonard, N. J.; Oki, M. *J. Am. Chem. Soc.* **1955**, *77*, 6241–6244.
20. Kaftory, M.; Dunitz, J. D. *Acta Cryst.* **1975**, *B31*, 2914–2916.
21. Rosenfeld, Jr R. E.; Parthasarathy, R.; Dunitz, J. D. *J. Am. Chem. Soc.* **1977**, *99*, 4860–4862.
22. Bürgi, H. B.; Dunitz, H. B.; Shefter, A. *Acta Cryst.* **1974**, *B30*, 1517–1527.
23. Rademacher, P. *Chem. Soc. Reviews* **1995**, 143–150.
24. *Am. J. Pharm.* **1887**, *59*, 541, 589.
25. Belikov, A. S.; Bankowsky, A. I.; Tsarev, M. V. *Zhur. Obshch. Khim.* **1954**, *24*, 919–922.
26. Leonard, N. J.; Deyrup, J. A. *J. Am. Chem. Soc.* **1962**, *84*, 2148–2160.
27. Leonard, N. J.; Deyrup, J. A. *J. Am. Chem. Soc.* **1960**, *82*, 6202–6203.
28. Townsend, L. B.; Robins, R. K.; Leoppky, R. N.; Leonard, N. J. *J. Am. Chem. Soc.* **1964**, *86*, 5320–5325.
29. Leonard, N. J.; Carraway, K. L.; Helgeson, J. P. *J. Heterocyclic Chem.* **1965**, *2*, 291–297.
30. Cavé, A.; Deyrup, J. A.; Goutarel, R.; Leonard, N. J.; Monseur, X. G. *Annales pharm. franç.* **1962**, *20*, 285–292.
31. Hatfield, D.; Wyngaarden, J. B. *J. Biol. Chem.* **1964**, *239*, 2580–2586 and references therein.
32. Leonard, N. J.; Laursen, R. A. *Biochemistry* **1965**, *4*, 354–365.
33. Wolfenden, R.; Sharpless, T. K.; Ragade, I. S.; Leonard, N. J. *J. Am. Chem. Soc.* **1966**, *88*, 185–186.

34. Leonard, N. J.; Laursen, R. A. *Biochemistry* **1965**, *4*, 365–376.
35. Michelson, A. M.; Monny, C.; Laursen, R. A.; Leonard, N. J. *Biochim. Biophys. Acta* **1966**, *119*, 258–267.
36. Gerzon, K.; Johnson, I. S.; Boder, G. B.; Cline, J. C.; Simpson, P. J.; Speth, C.; Leonard, N. J.; Laursen, R. A. *Biochim. Biophys. Acta* **1966**, *119*, 445–461.
37. Nair, V.; Buenger, G. S.; Leonard, N. J.; Balzarini, J.; De Clercq, E. *J. Chem. Soc. Chem. Commun.* **1991**, 1650–1651.
38. Orgel, L. E.; Lohrmann, R. *Accounts Chem. Res.* **1974**, *7*, 368–377.
39. Hill, Jr A. R.; Kumar, S.; Leonard, N. J.; Orgel, L. E. *J. Mol. Evol.* **1988**, *27*, 91–95.
40. Hill, Jr A. R.; Kumar, S.; Patil, V. D.; Leonard, N. J.; Orgel, L. E. *J. Mol. Evol.* **1991**, *32*, 447–453.
41. Bhat, B.; Neelima; Leonard, N. J.; Robinson, H.; Wang, A. H.-J. *J. Am. Chem. Soc.* **1996**, *118*, 3065–3066.
42. Miller, C. O.; Skoog, F.; Von Saltza, M. H.; Strong, F. M. *J. Am. Chem. Soc.* **1955**, *77*, 1392.
43. Miller, C. O.; Skoog, F.; Okumura, F. S.; Von Saltza, M. H.; Strong, F. M. *J. Am. Chem. Soc.* **1955**, *77*, 2662–2663.
44. Fujii, T.; Leonard, N. J. *Proc. Natl. Acad. Sci. USA* **1964**, *51*, 73–75.
45. Hamzi, H. Q.; Skoog, F. *Proc. Natl. Acad. Sci. USA* **1964**, *51*, 76–83.
46. Klämbt, D.; Thies, G.; Skoog, F. *Proc. Natl. Acad. Sci. USA* **1966**, *56*, 52–59.
47. Helgeson, J. P.; Leonard, N. J. *Proc. Natl Acad. Sci. USA* **1966**, *56*, 60–63.
48. Leonard, N. J. Presented at Fairleigh-Dickenson University, Madison, NJ, 21 Oct., 1963; see *Trans. of Morris County Research Council* **1965**, *1*, 11–41.
49. Zachau, H. G.; Dütting, D.; Feldmann, H. *Angew. Chem.* **1966**, *78*, 392.
50. Letham, D. S.; Shannon, J. S.; McDonald, I. R. *Proc. Chem. Soc.* **1964**, 230–231.
51. Shaw, G.; Wilson, D. V. *Proc. Chem. Soc.* **1964**, 231.
52. Review: Skoog, F.; Armstrong, D. J. *Annu. Rev. Plant Physiol.* **1970**, 21, 359–384.
53. Review: Skoog, F.; Leonard, N. J. *Proc. 6th Inter. Conf. on Plant Growth Subst.*; Ottawa, 1969, pp. 1–18.
54. Review: Leonard, N. J. In *The Chemistry and Biochemistry of Plant Hormones*; *Recent Advances in Phytochemistry* **1974**, *7*, 21–56.
55. Leonard, N. J.; Playtis, A. J.; Skoog, F.; Schmitz, R. Y. *J. Am. Chem. Soc.* **1971**, *93*, 3056–3058.
56. Playtis, A. J.; Leonard, N. J. *Biochem. Biophys. Res. Commun.* **1971**, *45*, 1–5.
57. Leonard, N. J.; Achmatowicz, S.; Loeppky, R. N.; Carraway, K. L.; Grimm, W. A. H.; Szweykowska, A.; Hamzi, H. Q.; Skoog, F. *Proc. Natl. Acad. Sci. USA* **1966**, *56*, 709–716.
58. Leonard, N. J.; Henderson, T. R. *J. Am. Chem. Soc.* **1975**, *97*, 4990–4999.
59. Skoog, F.; Hamzi, H. Q.; Szweykowska, A. M.; Leonard, N. J.; Carraway, K. L.; Fujii, T.; Helgeson, J. P.; Loeppky, R. N. *Phytochemistry* **1967**, *6*, 1169–1192.
60. Henderson, T. R.; Frihart, C. R.; Leonard, N. J.; Schmitz, R. Y.; Skoog, F. *Phytochemistry* **1975**, *14*, 1687–1690.
61. Chwang, A. K.; Sundaralingam, M.; Leonard, N. J. *Acta Cryst.* **1981**, *B37*, 294–296.
62. Schmitz, R. Y.; Skoog, F.; Hecht, S. M.; Leonard, N. J. *Phytochemistry* **1971**, *10*, 275–280.
63. Hecht, S. M.; Bock, R. M.; Schmitz, R. Y.; Skoog, F.; Leonard, N. J.; Occolowitz, J. L. *Biochemistry* **1971**, *10*, 4224–4228.
64. Hecht, S. M.; Bock, R. M.; Schmitz, R. Y.; Skoog, F.; Leonard, N. J. *Proc. Natl. Acad. Sci. USA* **1971**, *68*, 2608–2610.
65. Theiler, J. B.; Leonard, N. J.; Schmitz, R. Y.; Skoog, F. *Plant Physiol.* **1976**, *58*, 803–805.
66. Mornet, R.; Theiler, J. B.; Leonard, N. J.; Schmitz, R. Y.; Moore, III F. H.; Skoog, F. *Plant Physiol.* **1979**, *64*, 600–610.
67. Vold, B. S.; Leonard, N. J. *Plant Physiol.* **1981**, *67*, 401–403.
68. Milstone, D. S.; Vold, B. S.; Glitz, D. G.; Schutt, N. *Nucleic Acids Res.* **1978**, *5*, 3439–3455.
69. Vold, B. S.; Lazar, J. M.; Gray, A. M. *J. Biol. Chem.* **1979**, *254*, 7362–7367.
70. Dorée, M.; Guerrier, P. *Exp. Cell Res.* **1975**, *91*, 297–300.
71. Dorée, M.; Guerrier, P.; Leonard, N. J. *Proc. Natl. Acad. Sci. USA* **1976**, *73*, 1669–1673.
72. Mornet, R.; Leonard, N. J.; Theiler, J. B.; Dorée, M. *J. Chem. Soc., Perkin Trans. I* **1984**, 879–885.
73. Leonard, N. J.; Greenfield, J. C.; Schmitz, R. Y.; Skoog, F. *Plant Physiol.* **1975**, *55*, 1057–1061.
74. Melhado, L. L.; Jones, A. M.; Leonard, N. J.; Vanderhoef, L. N. *Plant Physiol.* **1981**, *68*, 469–475.
75. Melhado, L. L.; Jones, A. M.; Ho, T.-H. D.; Leonard, N. J. *Plant Physiol.* **1984**, *74*, 289–294, 295–301.
76. Jones, A. M.; Melhado, L. L.; Ho, T.-H. D.; Pearce, C. J.; Leonard, N. J. *Plant Physiol.* **1984**, *75*, 1111–1116.
77. Jones, A. M. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1994**, *45*, 393–420.
78. Eisinger, J.; Guéron, M.; Schulman, R. G.; Yamane, Y. *Proc. Natl. Acad. Sci. USA* **1966**, *55*, 1015–1020.
79. Eisinger, J.; Shulman, R. G. *J. Mol. Biol.* **1967**, *28*, 445–449 and references therein.
80. Browne, D. T.; Eisinger, J.; Leonard, N. J. *J. Am. Chem. Soc.* **1968**, *90*, 7302–7323 and references therein.
81. Leonard, N. J.; Iwamura, H.; Eisinger, J. *Proc. Natl. Acad. Sci. USA* **1969**, *64*, 352–359.
82. Iwamura, H.; Leonard, N. J.; Eisinger, J. *Proc. Natl. Acad. Sci. USA* **1970**, *65*, 1025–1032.

83. Leonard, N. J.; Golankiewicz, K.; McCredie, R. S.; Johnson, S. M.; Paul, I. C. *J. Am. Chem. Soc.* **1969**, *91*, 5855–5862.
84. Hirayama, F. *J. Chem. Phys.* **1965**, *42*, 3163–3171, refers to an "$n=3$ rule."
85. Leonard, N. J.; McDonald, J. J.; Reichmann, M. E. *Proc. Natl. Acad. USA* **1970**, *67*, 93–98.
86. Leonard, N. J.; McDonald, J. J.; Henderson, R. E. L.; Reichmann, M. E. *Biochemistry* **1971**, *10*, 3335–3342.
87. Vincze, A.; Henderson, R. E. L.; McDonald, J. J.; Leonard, N. J. *J. Am. Chem. Soc.* **1973**, *95*, 2677–2682 and references therein.
88. Henderson, R. E. L.; Kirkegaard, L. H.; Leonard, N. J. *Biochim. Biophys. Acta* **1973**, *294*, 356–364.
89. Ehrenberg, L.; Fedorcsák, I.; Solymosy, F. *Prog. Nucleic Acid Res. Mol. Biol.* **1976**, *16*, 189–262.
90. Peattie, D. A.; Gilbert, W. *Proc. Natl. Acad. Sci. USA* **1980**, *77*, 4679–4682.
91. Herr, W. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 8009–8013.
92. Johnston, B. H.; Ohara, W.; Rich, A. *J. Biol. Chem.* **1988**, *263*, 4512–4515 and references therein.
93. Scholten, P. M.; Nordheim, A. *Nucleic Acids Res.* **1986**, *14*, 3981–3993.
94. Furlong, J. C.; Lilley, D. M. *Nucleic Acids Res.* **1986**, *14*, 3995–4007.
95. Portugal, J.; Fox, K. R.; McLean, M. J.; Richenberg, J. L.; Waring, M. J. *Nucleic Acids Res.* **1988**, *16*, 3655–3670.
96. Jeppesen, C.; Nielsen, P. E. *FEBS Lett.* **1988**, *231*, 172–176.
97. Kochetkov, N. K.; Shibaev, V. N.; Kost, A. A. *Tetrahedron Lett.* **1971**, 1993–1996.
98. Barrio, J. R.; Secrist, III J. A.; Leonard, N. J. *Biochem. Biophys. Res. Commun.* **1972**, *46*, 597–604.
99. Secrist, III J. A.; Barrio, J. R.; Leonard, N. J.; Weber, G. *Biochemistry*, **1972**, *11*, 3499–3506.
100. Spencer, R. D.; Weber, G.; Tolman, G. L.; Barrio, J. R.; Leonard, N. J. *Eur. J. Biochem.* **1974**, *45*, 425–429.
101. Agbaria, R. E.; Parola, A. H.; Gill, D. *J. Phys. Chem.* **1994**, *98*, 13280–13285.
102. Barrio, J. R.; Tolman, G. L.; Leonard, N. J.; Spencer, R. D.; Weber, G. *Proc. Natl. Acad. Sci. USA* **1973**, *70*, 941–943.
103. Secrist, III J. A.; Barrio, J. R.; Leonard, N. J.; Villar-Palasi, C.; Gilman, A. G. *Science* **1972**, *177*, 279–280.
104. Barrio, J. R.; Dammann, L. G.; Kirkegaard, L. H.; Switzer, R. L.; Leonard, N. J. *J. Am. Chem. Soc.* **1973**, *95*, 961–962.
105. Greenfield, J. C.; Leonard, N. J.; Gumport, R. I. *Biochemistry* **1975**, *14*, 698–706.
106. Barrio, J. R.; Secrist, III J. A.; Chien, Y.; Taylor, P. J.; Robinson, J. L.; Leonard, N. J. *FEBS Lett.* **1973**, *29*, 215–218.
107. Secrist, III J. A.; Barrio, J. R.; Leonard, N. J. *Science* **1972**, *175*, 646–647.
108. DeLuca, M.; Leonard, N. J.; Gates, B. J.; McElroy, W. D. *Proc. Natl. Acad. Sci. USA* 1973, *70*, 1664–1666.
109. McElroy, W. D.; Seliger, H. H. In *Molecular Architecture in Cell Physiology*; Hayashi, O.; Szent-Gyorgi, A., Eds. Prentice-Hall: Englewood Cliffs, NJ, 1966; pp. 63–79.
110. Dreyfuss, G.; Schwartz, K.; Blout, E. R.; Barrio, J. R.; Liu, F.-T.; Leonard, N. J. *Proc. Natl. Acad. Sci. USA* **1978**, *75*, 1199–1203.
111. Leonard, N. J.; Secrist, III J. A.; Barrio, J. R.; Damman, L. US Patent 3,983,998, July 8, 1975.
112. Leonard, N. J.; Secrist, III J. A.; Barrio, J. R.; Weber, G. US Patent 3, 960,840, June 1, 1976.
113. Leonard, N. J. *CRC Critical Reviews in Biochemistry* **1984**, *15*, 125–199.
114. Leonard, N. J. *Chemtracts: Biochemistry and Molecular Biology* **1992**, *3*, 273–297.
115. Leonard, N. J. *Chemtracts: Biochemistry and Molecular Biology* **1993**, *4*, 251–284.
116. Leonard, N. J.; Sprecker, M. A.; Morrice, A. G. *J. Am. Chem. Soc.* **1976**, *98*, 3987–3994.
117. Leonard, N. J.; Kazmierczak, F.; Rykowski, A. J. *Org. Chem.* **1987**, *52*, 2933–2935.
118. Leonard, N. J.; Petrič, A.; Rykowski, A. *J. Org. Chem.* **1988**, *53*, 3873–3875.
119. Scopes, D. I. C.; Barrio, J. R.; Leonard, N. J. *Science* **1977**, *195*, 296–298.
120. Cohn, W. E.; Leonard, N. J.; Wang, S. Y. *Photochem. Photobiol.* **1974**, *19*, 89–94.
121. Leonard, N. J. *Acc. Chem. Res.* **1982**, *15*, 128–135.
122. Leonard, N. J. In *Biologically Active Principles of Natural Products*, Voelter, W.; Daves, D. G., Eds; Georg Thieme Verlag: Stuttgart, 1984; pp. 237–249.
123. Leonard, N. J. *Pure & Appl. Chem.* **1984**, *56*, 1025–1030.
124. Leonard, N. J.; Hiremath, S. P. *Tetrahedron* **1986**, *42*, 1917–1961.
125. Barrio, J. R.; Barrio, M. del C. G.; Leonard, N. J.; England, T. E.; Uhlenbeck, O. C. *Biochemistry* **1978**, *17*, 2077–2081.
126. Schmidt, M. J.; Truex, L. L.; Leonard, N. J.; Scopes, D. I. C.; Barrio, J. R. *J. Cycl. Nucl. Res.* **1978**, *4*, 201–207.
127. Kauffman, R. F.; Lardy, H. A.; Barrio, J. R.; Barrio, M. del C. G.; Leonard, N. J. *Biochemistry* **1978**, *17*, 3686–3692.
128. Rosendahl, M.; Leonard, N. J.; DeLuca, M. *Photochem. Photobiol.* **1982**, *35*, 857–861.
129. Hartl, F. T.; Roskoski, Jr R.; Rosendahl, M. S.; Leonard, N. J. *Biochemistry* **1983**, *22*, 2347–2352.
130. Bhatnagar, D.; Hartl, F. T.; Roskoski, Jr R.; Lessor, R. A.; Leonard, N. J. *Biochemistry* **1984**, *23*, 4350–4357.
131. Bhatnagar, D.; Roskoski, Jr R.; Rosendahl, M. S.; Leonard, N. J. *Biochemistry* **1983**, *22*, 6310–6317.

132. Bhatnagar, D.; Glass, D. B.; Roskoski, Jr R.; Lessor, R. A.; Leonard, N. J. *Biochemistry* **1985**, *24*, 1122–1129.
133. Bhatnagar, D.; Glass, D. B.; Roskoski, Jr R.; Lessor, R. A.; Leonard, N. J. *Biochemistry* **1988**, *27*, 1988–1994.
134. Roberts, J. D.; Sullivan, G. R.; Pang, P. P.; Leonard, N. J. *J. Org. Chem.* **1981**, *46*, 1014–1016.
135. Gonnella, N. C.; Nakanishi, H.; Holtwick, J. B.; Horowitz, D. S.; Kanamori, K.; Leonard, N. J.; Roberts, J. D. *J. Am. Chem. Soc.* **1983**, *105*, 2050–2055.
136. Abe, A.; Nee, M.; Roberts, J. D.; Wittenberg, W. L.; Leonard, N. J. *J. Am. Chem. Soc.* **1981**, *103*, 4954–4956.
137. Kanamori, K.; Roberts, J. D.; Rossman, M. A.; Leonard, N. J. *Heteroatom Chemistry* **1992**, *3*, 19–23.
138. Pauling, L.; Sturdivant, J. H. *Proc. Natl Acad. Sci. USA* **1937**, *23*, 615–620.
139. Hosmane, R. S.; Rossman, M. A.; Leonard, N. J. *J. Am. Chem. Soc.* **1982**, *104*, 5497–5499.
140. Leupin, W.; Wirz, J. J. *Am. Chem. Soc.* **1980**, *102*, 6068–6075.
141. Shahbaz, M.; Urano, S.; LeBreton, P. R.; Rossman, M. A.; Hosmane, R. S.; Leonard, N. J. *J. Am. Chem. Soc.* **1984**, *106*, 2805–2811.
142. Rossman, M. A.; Leonard, N. J.; Urano, S.; LeBreton, P. R. *J. Am. Chem. Soc.* **1985**, *107*, 3884–3890.
143. LeBreton, P. R.; Yang, Xu; Urano, S.; Fetzer, S.; Yu, Min; Leonard, N. J.; Kumar, S. *J. Am. Chem. Soc.* **1990**, *112*, 2138–2147.
144. Halpern, A. M.; Rossman, M. A.; Hosmane, R. S.; Leonard, N. J. *J. Phys. Chem.* **1984**, *88*, 4324–4326.
145. Ferris, J. P.; Antonucci, F. R. *J. Am. Chem. Soc.* **1974**, *96*, 2014–2019.
146. Halpern, A. M.; Ruggles, C. J.; Zhang, X.; Groziak, M. P.; Leonard, N. J. *J. Phys. Chem.* **1987**, *91*, 1366–1370.
147. Groziak, M. P.; Wilson, S. R.; Clauson, G. L.; Leonard, N. J. *J. Am. Chem. Soc.* **1986**, *108*, 8002–8006.
148. Gimarc, B. M. *J. Am. Chem. Soc.* **1983**, *105*, 1979–1984.
149. Devadas, B.; Leonard, N. J. *J. Am. Chem. Soc.* **1986**, *108*, 5012–5014.
150. Bhat, B.; Wilson, S. R.; Cruickshank, K. A. *J. Am. Chem. Soc.* **1991**, *113*, 1398–1406.
151. Bhat, B.; Leonard, N. J. *J. Am. Chem. Soc.* **1992**, *114*, 7407–7410.
152. Petrič, A.; Bhat, B.; Leonard, N. J.; Gumport, R. I. *Nucleic Acids Res.* **1991**, *19*, 585–590.