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2006 Medicinal Chemistry Division Award Address

From Natural Products to Bioorganic Chemistry. What's Next?†

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Introduction

I am deeply honored to have been the recipient of the 2006 American Chemical Society Medicinal Chemistry Award in Seattle this past June. This award honors not only me but also my many colleagues and friends that I have had the privilege of working with over the years. The recognition is especially satisfying because my contributions over the past 40 years at Lederle and Wyeth have not fallen within the traditional bounds of medicinal chemistry. So when the invitation came to contribute to the *Journal of Medicinal Chemistry* , I thought it a good opportunity to look back and reminisce over a 40-year odyssey that included screening microbial products for pharmacological activity other than antimicrobial, structural studies of new antibiotics, bioorganic studies with the calicheamicins, new tetracyclines, and finally some bioorganic and biophysical chemistry and enzymology for hit and lead characterization.

My initial interest in chemistry came about in a rather roundabout manner. In fact, it is remarkable that I ever took up chemistry at all because I found my introductory high school chemistry class not at all interesting. Actually, at that period of my life, I was not that interested in science in general—to the great disappointment of my father who was my high school physics teacher and, in hindsight, quite a good one. This became the cause of some rather intense "discussions" at home. However, organic chemistry, and natural products in particular, caught my imagination after a chance encounter during a college summer job in a Forest Products Laboratory in Oregon where I observed chemists working on the isolation and characterization of bark phenolics from Douglas fir. I remember being especially intrigued with the siphoning cycle of a Soxhlet extraction apparatus along with the colors of the various solutions. I was majoring in forestry at Oregon State at the time because I enjoyed wilderness experiences and thought a career in forestry would satisfy these interests. Eventually I realized that this was not the way to choose a career. But after the summer job experience I took a course in organic chemistry and then transferred my major to chemistry where I obtained great satisfaction from identifying unknowns in organic qualitative analysis.

In graduate school at UCLA I worked on the structures of proanthocyanidins, sesquiterpene lactones, and quassinoids, and had excellent mentoring from my thesis advisor, Ted Geissman. The structural studies on proanthocyanidins, from avocados no less, did not work out primarily because of my inexperience with amorphous and easily air-oxidized phenolic materials. And the relatively unsophisticated state of separation science at that time (1958−1960), at least compared to the methodologies available today, did not help. The sesquiterpene and quassinoid projects, on the other hand, were successful because nicely crystalline compounds were obtained on which to carry out structural studies. I was also very fortunate at this time in receiving some very good mechanistic tutoring from Jim Hendrickson who taught me how to push electrons, essential in explaining the many fascinating rearrangements that occur in natural products, and most importantly to pass cumulative exams. But as would be expected from the Cram and Winstein environment at that period of UCLA, I also got exposed to a great deal of physical organic chemistry that was to become very useful in the later part of my career in the drug industry.

A postdoctoral position with Basil Whalley at the University of London provided an introduction to microbial products. My research there involved studies on the absolute stereochemistry of sclerotiorin, an azaphilone, and some structural ambiguities of rosenonolactone, a novel fungal diterpenoid which contained the unusual trans−cis−trans ABC ring system. This experience suggested to me that the pharmaceutical industry might be a

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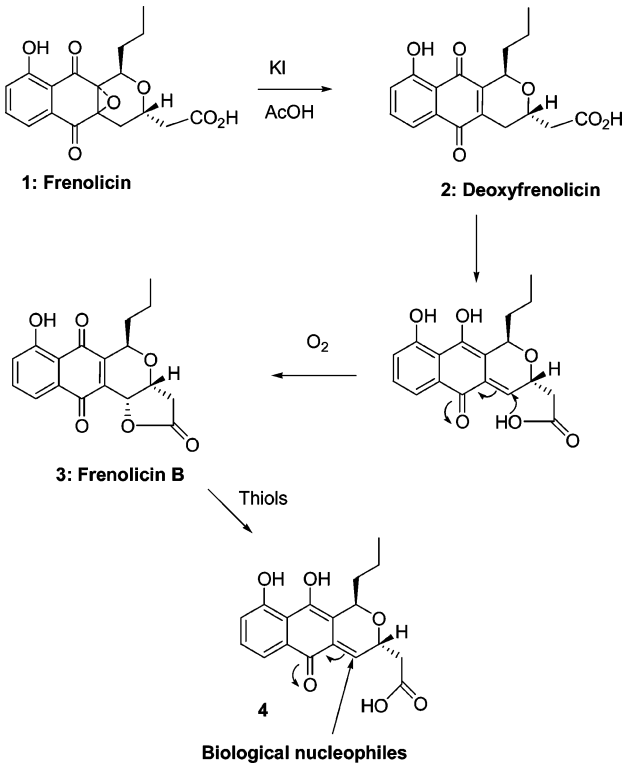

Figure 1. Structure of frenolicin (**1**), deoxyfrenolicin (**2**), the oxidative lactonization of deoxyfrenolicin to frenolicin B (**3**), and the bioreductive alkylation (**4**) of frenolicin B.

good place to work, especially since microbial fermentations seemed to provide a bountiful supply of interesting natural products.

My career at Lederle Laboratories in Pearl River, New York started in the fall of 1964 after being convinced by Les Mitscher — now at the University of Kansas — that I would have the opportunity to do some interesting natural products chemistry at Lederle. As described below, I was eventually able to work on some extremely challenging compounds with incredibly potent biological activity. So it has indeed been a remarkable 40-year scientific odyssey that I would never have dreamed of back in 1964, let alone back in high school chemistry. I have highlighted several projects that were interesting chemically and biologically and tell something about the progress of drug discovery of primarily new antimicrobials and antitumor agents, from the middle 1960s to the early 2000s at Lederle and finally Wyeth.

Frenolicin

One of my first projects at Lederle was the structure elucidation of frenolicin (Figure 1) on which I collaborated with Howard Whaley. Frenolicn was a nicely crystalline metabolite from fermentation broths of *Streptomyces fradiae* and turned out to be a novel naphthopyranoquinone epoxide (**1** in Figure 1).[1] Crude extracts of the fermentation broths had previously exhibited antitumor activity in animal models, and so Howard and I wanted to see if this molecule accounted for the original activity. It did not. Our structure−activity efforts, however, were cut short after some 6 months after we both developed severe facial edema that seemed to correlate with the quinone epoxide functionality of frenolicin. The corresponding quinone, deoxyfrenolicin (**2**), however, proved to be relatively nontoxic and exhibited significant antifungal activity. The structure elucidation of frenolicin using chemical transformations, NMR, and mass spectroscopy was straightforward, but we missed the most

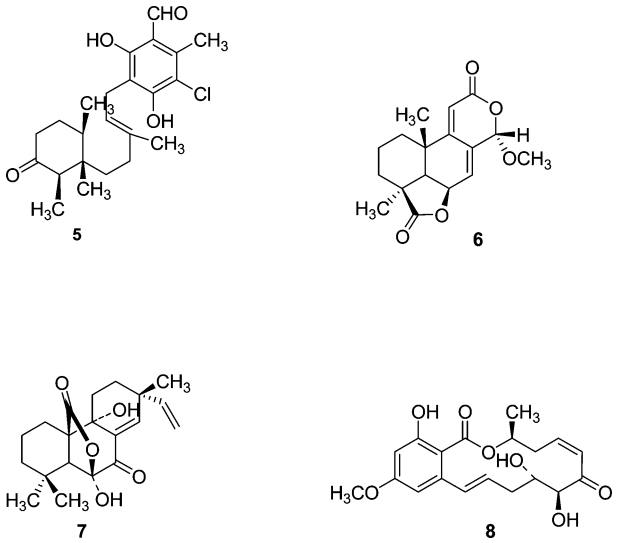

Figure 2. Structures of selected mold metabolites (**5**−**8**) that were active in the *Tetrahymena pyriformis* screen for hypocholesteremic agents.

important features of deoxyfrenolicin both chemically and biologically. That is, upon standing in organic solvents in air for a few days it undergoes oxidative lactonization to frenolicin B (**3**, Figure 1).

We did not realize this until about 10 years later when a Japanese group published their findings on the oxidative lactonization of a related pyranonaphthquinone.[2,3] At about the same time Harold Moore at University of California−Irvine published an article in which he speculated that such compounds upon bioreduction with thiols could provide an intermediate that could act as an alkylating agent with biomolecules, especially DNA.[4] It seems likely that this chemistry (Figure 1, **4**) explains the antitumor activity observed with the early fermentation extracts. Very recently the lactone form of frenolicin and related compounds have been shown to be of interest as inhibitors of farnesylation of viral K-ras protein by bovine farnesyltranferase[5] and as anticoccidial agents.[6]

The Search for Hypocholesteremic Agents

After the frenolicin project, I collaborated with Ralph Evans and Marty Kunstmann on a screening program to identify hypocholesteremic agents from fungal metabolites. The bioassay used at that time was somewhat of a black box in which we screened crude extracts from fermentations of fungi for activity inhibiting the growth and motility of the ciliated protozoan *Tetrahymena pyriformis*. It had previously been shown that certain hypocholesteremic steroidal derivatives were active in this assay, apparently as inhibitors of squalene cyclase.[7,8] Unfortunately we did not come up with any statins, but we did identify several interesting natural products designated by their culture numbers as **5** (LL-1272),[9] **6** (LL-S491),[10] **7** (LL-1271),[11] and **8** (LL-Z1640)[12] (Figure 2) that exhibited antifungal activity but no significant hypocholesteremic activity. However, after about 25 years the zearalenone-related metabolite **8** was shown to be a signal-specific inhibitor of the mitogen-activated protein kinase cascade (MAPK) and, in particular, that of the c-Jun N-terminal kinase (JNK) and to a lesser extent p38 kinase.[13] Clearly, the rather crude assays we had available at that time greatly limited the potential for success.

Glycocinnamoylspermidine Antibiotics: Cinodine

In the mid 1970s the antimicrobial program at Lederle began a search for new aminoglycosides with the hope of obtaining

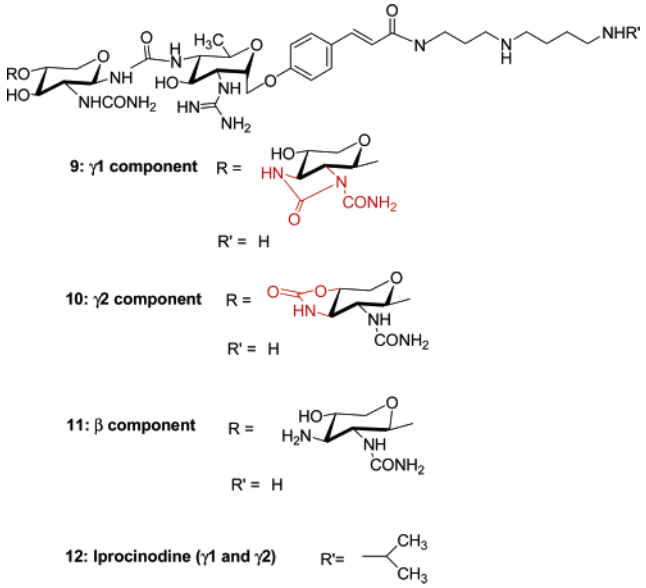

Figure 3. Structures of cinodine antimicrobial agents (**9**−**12**).

agents with better safety profiles than those identified at that time. This program led to the very basic, water soluble glycocinnamoylspermidine antibiotics designated cinodine $\gamma 1$ (**9**), $\gamma 2$ (**10**), and β (**11**) (Figure 3).[14]

The structural chemistry carried out by myself, Donna Cosulich, John Martin, Marty Kunstmann, George Morton, and Morris Lovell involved a combination of degradative chemistry and X-ray, ^{1}H NMR, and ^{13}C NMR analyses. These bacteriocidal agents were extremely potent, broad spectrum agents, especially against Gram-negative organisms. At the same time they were quite toxic with a very limited therapeutic index. It turned out that they were not inhibitors of bacterial protein synthesis, as are the aminoglycosides, but rather DNA gyrase inhibitors.[15] Not surprisingly they bind to DNA but even more tightly to gyrase B, one of the two subunits of DNA gyrase important for ATP binding. The carbonyl groupings in the strained oxazolidone and imidazolidone rings (due to the 5/6 trans ring fusion in the terminal sugars) in **9** and **10** (Figure 3) are quite labile chemically, which could be important for explaining the ~20 fold greater activity of **9** and **10** over that of the **11**. Thus, they could serve as a target for a nucleophile on the gyrase or perhaps provide additional hydrogen-bonding opportunities for binding with the gyrase.

The alkylated derivative iprocinodine (**12**) was developed for veterinary purposes because of its better toxicity profile and usefulness for the treatment of shipping fever in cattle where in feed lots about 20% of the animals are lost to respiratory infections. But chronic toxicology studies revealed the development of squamous cell carcinomas around the oral cavity, and so the project was terminated.

Glycopeptide Antibiotics: Avoparcin

Toward the end of the cinodine project in 1977 our agricultural division became interested in marketing the avoparcin complex of antimicrobial agents for use in feed lots. The α and β avoparcins (**12**, **13**, Figure 4) are complex glycopeptide antibiotics related to vancomycin. Avoparcin had been isolated at Lederle in the mid-1960s, and some very preliminary structural studies were carried out at that time. However, no further structural work had been done since then. A great deal of effort had been put in by Dudley Williams at Cambridge University on vancomycin, and his group had managed to

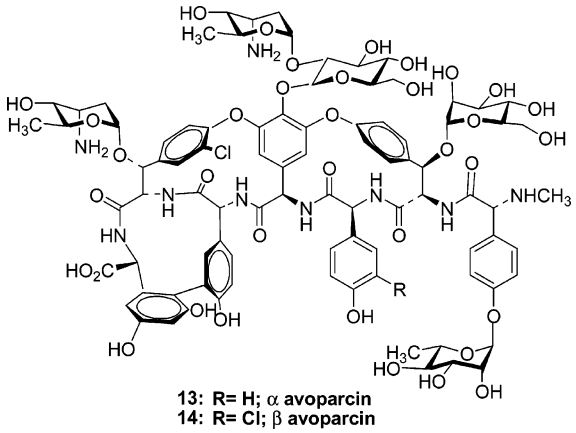

Figure 4. Structures of avoparcin α (**13**) and β (**14**).

identify its building blocks but not the complete structure, although they were close. Thus, in order to help register avoparcin for commercialization, our group was given a year to determine its structure, which I thought was somewhat unrealistic given the complexity of these agents. I said that we could probably identify the structural components but whether we could piece them together into a complete structure within a year's time frame was another matter. No crystals were available, and so an X-ray analysis was not possible. By taking advantage of Williams' prior studies on vancomycin and some help by Stan Holker's group at the University of Liverpool, Jim McGahren, John Martin, George Morton, and I were able to identify the avoparcin building blocks using degradative chemistry and a great deal of ^{1}H and ^{13}C NMR spectroscopy. Fortunately, Sheldrick and Williams' X-ray structure of a vancomycin degradation product[16] became available toward the end of our alloted time, and we were able to establish the complete structure of avoparcin.[17]

Enediynes: Calicheamicin

The highlight of my career, though, was the calicheamicin project where my group contributed to the isolation, structural elucidation, and bioorganic chemistry of these remarkable molecules. For me, calicheamcin was the crème de la crème of natural products. The unusual structural features and bioreductive DNA cleavage chemistry of these agents made for a truly exciting period of research. The calicheamicin story began in the early 1980s when a bacterium, *Micromonospora echinospora* ssp. *calichensis*, was isolated from a sample of caliche clay collected near Kerrville, TX, in August of 1981. This effort was part of a screening program at Lederle aimed at identifying new classes of fermentation-derived antitumor antibiotics using a very sensitive biochemical induction assay. Indeed, the calicheamicins turned out to be some 1000 times more potent than adriamycin (a widely used clinical antitumor agent) against tumor models in mice.

The biochemical induction assay developed by Mike Greenstein and co-workers was critical for the discovery of the calicheamicins.[18] Crude extracts showed activities down to the femtogram level if the assay was done carefully. This assay uses a genetically engineered strain of *E. coli*. (λ) lysogen containing the lacZ reporter gene, which encodes the enzyme β-galactosidase. DNA damaging agents result in the induction of the β-galactosidase gene and, in the presence of a suitable chromogenic substrate, produces a color that can be quantitated in a spectrophotometer. The assay was used to assay bacterial colonies on agar plugs and to follow the isolation and purification of the various calicheamicin metabolites by TLC.

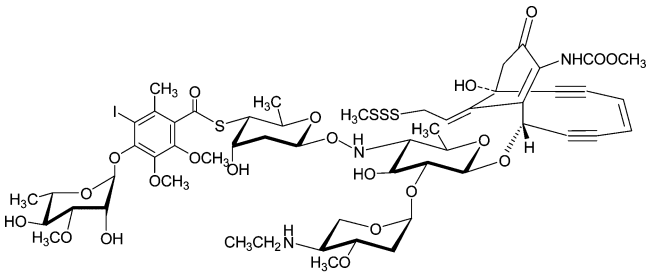

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Figure 5. Structure of calicheamicin γ_1^I (**15**).

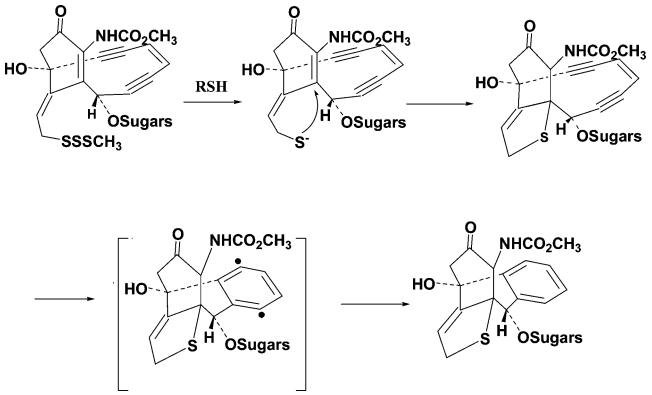

Figure 6. Bioreductive aromatization of calicheamicin via the carbon-centered diradical *p*-benzyne intermediate.

Early fermentation titers were extremely low, but with successful strain improvement and fermentation development efforts led by Al Fantini and Ray Testa, sufficient material was eventually available for structural studies. Specifically, the addition of potassium iodide to fermentations enhanced yields and provided a new analogue in the form of the iodinated γ_1^I component.[19]

The structure determination of calicheamicin γ_1^I (**15**, Figure 5) was a monumental task and required the combined efforts of a number of natural product chemists in addition to NMR and IR spectroscopists. This effort, under the leadership of Don Borders, was carried out by May Lee, Conway Chang et al.[20] May Lee and Conway Chang performed some very careful degradation experiments on the very small amounts of compound that were available at that time and were responsible for most of the structural characterization. Calicheamicin γ_1^I (**15**, Figure 5) is the metabolite to which a targeting antibody was eventually conjugated by a group led by Janis Upeslacis, Philip Hamann, Lois Hinman, et al. for the treatment of acute myologenous leukemia in elderly patients who are refractory to traditional chemotherapy.[21] This calicheamicin-antibody conjugate is now commercialized by Wyeth.

My group's contribution to the calicheamicn effort began in 1985 as participants in the isolation and structure determination. We initially developed improved isolation procedures to increase the amount of calicheamicin γ_1^I from fermentation broths and then started to study a very mystifying aromatization reaction initiated by phosphines and reducing thiols in an attempt to define the nature of the sulfur atoms in the molecule.[22] Lee and Chang finally discerned the presence of a cyclic enediyne moiety that then explained the aromatization by a room temperature Bergman rearrangement (Figure 6).[23] It was not clear at this time, however, how calicheamicin exerted its potent cytotoxicity. On the basis of our group's subsequent DNA cleavage studies, we were able to relate directly the calicheamicin-induced DNA

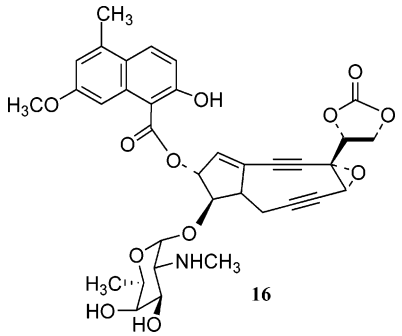

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Figure 7. Neocarzinostatin chromophore (**16**).

double-stranded cleavage to the transient intermediacy of the *p*-benzyne that initiated oxidative strand cleavage by abstracting proximal hydrogen atoms from the DNA deoxyribose backbone.[24,25] Thus, these experiments provided critical insight into the biochemical basis of the potent antitumor activity of these agents.

I vividly recall the first cleavage specificity experiment we ran with the aid of Akyat Sinha, a molecular biologist who was an expert on how to run the gels and, most conveniently, had available some 5′ ^{32}P end-labeled restriction fragments. I made up a cocktail with the DNA fragment and calicheamicin and then added some β-mercaptoethanol to trigger the cleavage chemistry. We compared the electrophoretic mobilities of the drug-induced cleavage products with Maxam−Gilbert chemically produced markers on a high-resolution polyacrylamide sequencing gel. To my astonishment, we observed very pronounced cleavage specificity with a non-Gaussian cleavage profile supporting a nondiffusible, carbon-centered radical as opposed to a diffusible species such as hydroxyl radicals. There was skepticism from some of my colleagues but repeat experiments confirmed the initial results. Nada Zein, a very creative postdoctoral scientist, joined our group a few months later and carried out many experiments that indicated specificity for duplex DNA containing T**C**CT/C**C**CAGGA and similar pyrimidine/purine-containing sequences. These initial DNA cleavage experiments provided evidence for abstraction of one of the 5′ hydrogens from the preferred cytidine in the TCCT sequence and the 4′ hydrogen abstraction from the preferred cytidine on the complementery C**C**CAGGA-containing strand.[24,25]

These gel-shift experiments were helped immensely by some work on neocarzinostatin (**16**, Figure 7), a nine-membered ring enediyne, carried out by Irving Goldberg's group at Harvard Medical School a few years earlier and before the structure and chemistry of that molecule was known. He had speculated that neocarzinostatin's DNA cleavage chemistry might be related to a carbon-centered radical as opposed to a more diffusible small molecule based on the non-Gaussian cleavage profile on sequencing gels.[26] However, at the time, the nature of the reactive neocarzinostatin species was unknown. But once we had seen the results of the first gel experiments with calicheamicin and the dependency of the cleavage on bioreductive activation, we were pretty sure that the *p*-benzyne was the reactive species involved in the oxidative DNA cleavage initiated by calicheamcin.

At Nada's urging, a cleavage experiment with sonicated calf thymus DNA in the presence of deuterated buffer and solvent indicated abstraction of hydrogen atoms from the calf thymus DNA and not deuterium from the deuterated buffer.[27] After hearing these results presented by Nada at an American Chemical Society meeting in Dallas, Stuart Schreiber (Harvard) and Craig Townsend (Johns Hopkins) suggested the preparation

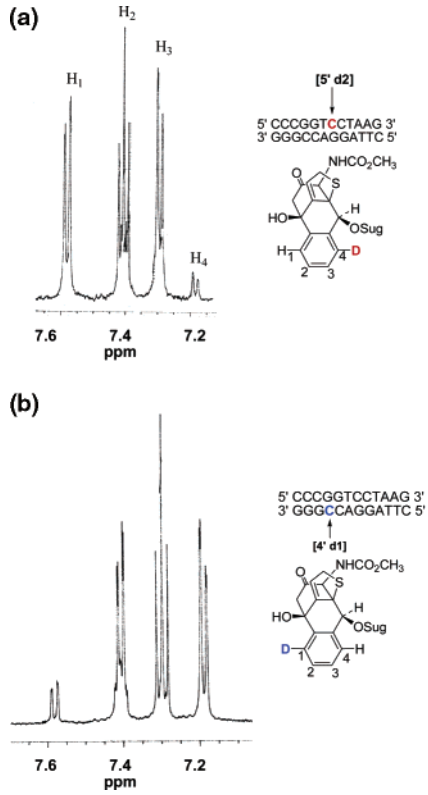

Figure 8. NMR aromatic region of aromatized calicheamicin from deuterium transfer experiments with specifically deuterium-labeled 12-mers. Deuterium labeled nucleotides are colored red and blue.

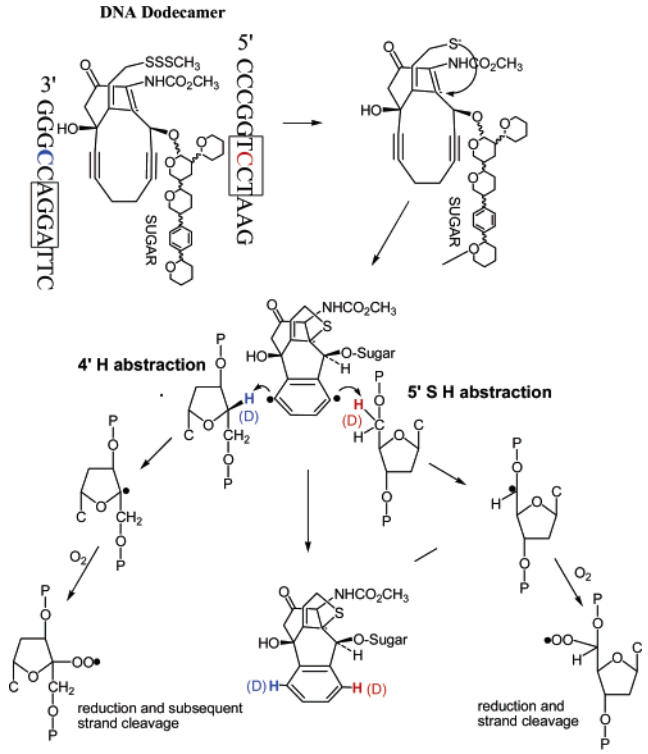

Figure 9. Summary of calicheamicin DNA cleavage.

of specifically labeled deuterated nucleotides for incorporation into a short DNA fragment for deuterium transfer experiments with calicheamicin. Depending on where the label ended up, either the 1 or 4 position of the aromatic ring of the aromatized end-product (Figure 8), we would be able to discern the binding orientation of the drug in the DNA minor groove. On the basis of our DNA cleavage experiments, we had originally proposed a binding orientation with the sugar moieties aligned toward the 5′ side of the TCCT-containing strand. On the other hand, on the basis of modeling studies, Schreiber had proposed a binding orientation with the sugars toward the 3′ side of the TCCT sequence. In addition, these proposed deuterium transfer experiments would also confirm or disprove our original evidence from the cleavage experiments on which hydrogen atoms were abstracted during oxidative strand cleavage.

Thus, the Schreiber and Townsend groups prepared the appropriately labeled nucleotides, which Vladimir Axelrod in our laboratory then incorporated into a synthetic DNA 12-mer containing the preferred TCCT/AGGA-containing sequence. Wei-dong Ding carried out the transfer experiments and isolated the deuterium labeled aromatized derivative. The NMR spectra, shown in Figure 8, provided amazingly conclusive evidence for the binding orientation of calicheamicin as predicted by Schreiber—sugar binding to the 3′ side of the TCCT sequence instead of the 5′ side. And it confirmed the remarkable regio- and stereospecificity in the abstraction of the 5′S hydrogen from the T**C**CT-containing strand and the 4′ hydrogen from the C**C**CAGGA-containing strand as suggested by our initial cleavage studies.[28,29] The calicheamicin cleavage chemistry is summarized in Figure 9.

These studies stimulated a great deal of interest in the calicheamcin binding and DNA cleavage chemistry, and a number of academic laboratories subsequently made significant contributions. However, the biological relevance of the TCCT/AGGA and related cleavage sites remains uncertain. But from a purely biophysical point of view, NMR studies by several academic laboratories, notably by the Kahne,[30] Nicolau,[31] and Patel[32] groups, along with gel-shift experiments by Dedon et al.,[33] suggested that these sites are amenable to drug-induced bending to ensure a strong and complementary fit between the DNA minor groove site and the drug. The circular dichroism (CD) titration experiments with calicheamicin carried out by Krishnamurthy et al.[34] showed a decrease in the normal CD of B-form DNA (12-mer) at both the positive extremum at 265 nm and negative extremum at 240 nm with saturable binding and are consistent with drug-induced conformational changes in the DNA. This CD titration experiment permitted the determination of binding affinities in the micromolar region, in good agreement with Chatterjee et al.[35] who used microcalorimetry.

An interesting feature of the optical activity of the calicheamicin−esperamicin class of 10-membered ring-containing enediynes is the strong, negative, exciton couplet at 310 ($\Delta\epsilon = -40$) and 270 nm ($\Delta\epsilon = +38$) in the circular dichorism (CD) spectrum. In the initial structural studies this exciton couplet was used to predict the absolute stereochemical relationship between the dienone and enediyne chromophores in the aglycon portion of the molecule as indicated.[36] A theoretical simulation of the circular dichroism spectrum of calicheamicin carried out by Giorgio et al. at Columbia using density functional theory and DeVoe coupled oscillator quantum chemical calculations showed that the CD of calicheamicin in the 240−400 nm region is indeed a combination of exciton coupling between the dienonecarbamate and enediyne chromophores plus the intrinsic CD of the twisted dienonecarbamate chromophore itself (Figure 10).[37] Thus our initial assignment of the stereochemical relationship between the enediyne and dienone chromophores based on CD proved to be valid.

One of the key findings linking calicheamicin to a targeting antibody was the discovery by Jim McGahren that in acetonitrile, relatively smooth disulfide exchange chemistry could be ac-

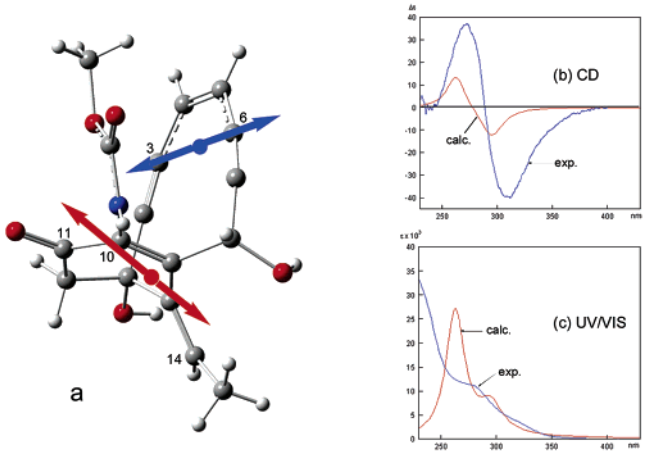

Figure 10. (a) Orientation of electric transition moments of the diencarbamate (red) and enediyne (blue) chromophores, (b) experimental and calculated CD spectra of calicheamicin, and (c) experimental and calculated UV/vis spectra of calicheamicn. Adapted from *Bioorganic and Medicinal Chemistry* **2005**, *13*, 5072−5079 (Giorgio et al. Theoretical simulation of the electronic circular dichroism spectrum of calicheamcin).[37] Copyright 2005, with permission from Elsevier.

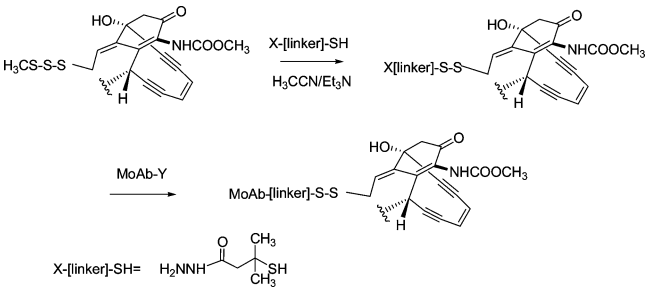

Figure 11. Disulfide exchange chemistry for attaching linkers to the aglycon of calicheamicin for use in conjugating monoclonal antibodies through a lysine amino group (MoAb-Y).

complished on the allylic trisulfide to give a disulfide analogue (Figure 11).[38] This proved to be a critical finding for conjugation of the drug to the antibody and provided a bioreductive, susceptible disulfide for release of calicheamicin once the conjugate was internalized within the cytoplasm of the target cell.

Tetracyclines

In 1988, at the same time we were studying the DNA cleavage properties of calicheamicin, Frank Tally, the newly hired head of Infectious Diseases, commandeered me to help him start and coordinate a tetracycline project to synthesize and evaluate a new tetracycline that would overcome resistant organisms. This project was my first exposure to a molecular biology understanding of antimicrobial resistance, and Tally and Yakov Gluzman spent many hours tutoring me so that I had some introductory knowledge of, what was for me, a new scientific discipline. Tetracyclines (**17** is the parent compound; Figure 12) were one of the first truly broad spectrum classes of antimicrobial agents to be discovered and were isolated at Lederle and Pfizer in the late 1940s. However, by the early 1960s resistance to these agents became evident, and structure modification efforts to overcome the resistance were initiated. One of the more clinically successful semisynthetic tetracyclines to be developed was minocycline, (**18**), which was introduced in the clinic in the late 1960s. But after 15 or more years of clinical use, resistance began to be observed with this agent.

About 2 years into our renewed effort and when the project was about to be terminated, P. E. Sum synthesized a 9-glycylamido minocycline analogue that showed good activity against resistant organisms.[39] Resistance in Gram-negative organisms is predominantly the result of expression of an efflux pump, a membrane-bound protein (TetA) that removes the drug from the cytoplasm before it binds to the ribosome and inhibits bacterial protein synthesis. Wolfgang Hillen's group in Erlangen, Germany, had shown with some elegant molecular biological studies that the expression of the tetracycline efflux pump in Gram-negative organisms is under control of the Tet repressor protein (TetR).[40] Binding of the tetracycline−Mg complex to the repressor protein removes it from blocking the operator sequences that control expression of both the TetR and TetA genes. Although at the time it was not clear how this new tetracycline overcame the resistance, studies by Hillen's group and subsequent X-ray crystallographic studies by Winfried Hinrichs, Carolina Kisker, and Wolfrom Saenger in Berlin provided a molecular basis to explain the fact that attachment of a bulky *tert*-butyl-glycylamido grouping at C9 of minocycline prevented binding to TetR.[41] This project eventually led to the development of tigecycline (**19**), the first in a new class of semisynthetic tetracyclines called the glycylcyclines that has been approved for use against bacterial infections resistant to previously useful agents.

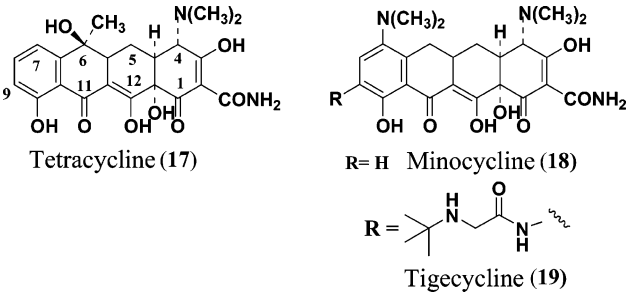

Figure 12. Structures of tetracycline (**17**), minocycline (**18**), and tigecycline (**19**).

Serine Cytomegalovirus Protease Inhibitors: Pyrimidotetrazinones

Toward the end of the calicheamicin and tetracycline projects, I had become interested in biophysical chemistry, a complete turn-about considering my incomprehension of physical chemistry during graduate school years. Actually this was at the urging of Wolfgang Hillen, our consultant on tetracycline resistance, who had encouraged us to become more quantitative in our experimentation. Thus, we had attempted to use recently available microcalorimetry and surface plasmon resonance (BIAcore) instruments to measure the affinity of calicheamicin for different DNA sequences. Although these experiments were only marginally successful, they piqued our interest in the use of these techniques for the more general biophysical characterization of hits and leads. I believed that the routine measurement of binding affinities with fluorescence spectroscopy combined with BIAcore and perhaps binding thermodynamics from microcalorimetry might provide useful information for both medicinal chemists and biologists and would help move projects along. Of course, this idea turned out to be somewhat naive, but eventually our bioorganic and biophysical characterization efforts became useful for a number of different therapeutic areas throughout the company.

So when Wyeth took over Lederle in the summer of 1994, I was given the charge by Magid Abou Gharbia, head of chemical research at Wyeth, of setting up a group to biophysically characterize small-molecule hits and leads for purity, binding affinities, and specificity with targeted macromolecules. In

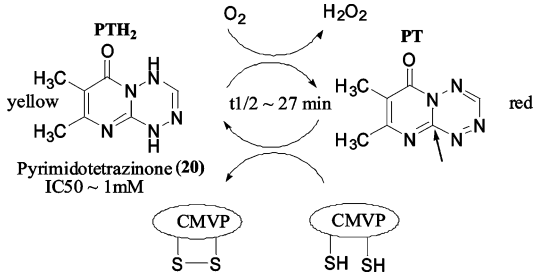

Figure 13. Redox chemistry of pyrimidotetrazinone (**20**) and CMPV thiols.

connection with our antiviral program we had occasion to examine the purity of a hit against a serine cytomegalovirus protease (CMVP), sUL80. It had been noticed previously that many compounds from the company library were mislabeled or had undergone some transformation or degradation during storage. The pyrimidotetrazinones **20** (Figure 13) proved to be an interesting example where the labeled compound, the dihydrotetrazinone, was not the active agent. HPLC analysis showed the presence of two compounds, a yellow and a red one. The yellow dihydro form, PTH_2, was originally deposited in the library but over the years had undergone slow oxidation by air to a small amount of the oxidized form, PT, and it was this form that actually inhibited the serine protease in question. Inhibition of sUL89 thus prevented cleavage of the viral assembly precursor protein necessary for encapsidation of the DNA in the virion. The mechanism of inhibition of the oxidized pyrimidotetrazinone was shown by Baum et al., by site-specific mutagenesis, to be the oxidation of cys138−cys161 to the corresponding disulfide.[42] There are three other cysteines on the surface of this protease, but disulfide formation from these thiols is not essential to inhibit the virus. Reduction of the pyrimidotetrazinone is dependent on the presence of the protease because no reaction was observed with serum albumin. Substoichiometric amounts of PT are sufficient for the inhibitory activity and are consistent with redox recycling of the inhibitor because PTH_2 is rapidly converted back to PT by dissolved oxygen in the buffer. Riboflavin also exhibited activity against this viral protease but was not as active as PT. Thus, our bioorganic interests spawned by the calicheamicin studies became useful in other projects.

What's Next

In 1964 cloning and overexpression of macromolecular targets were unknown. Today milligram quantities of targets are available to test new hits and leads for affinity, specificity, and X-ray analysis and computer simulations to visualize binding pockets. Although it is relatively easy to come up with a potent enzyme inhibitor, to convert it into a drug is something else. The discovery of new antimicrobial agents with activities against resistant bacteria has proven to be especially problematic. Indeed so much so that many drug companies have dramatically cut back or completely stopped screening programs for new agents from soil screening programs and plant sources. Although it has proved to be more and more difficult to identify new chemical classes, new agents are being discovered, albeit slowly.

Since the development of HPLC in the middle 1970s, the isolation, purification, and identification of natural products have become significantly more efficient and less labor-intensive. So to disregard the source of incredibly useful agents—β-lactams, aminoglycosides, tetracyclines, and the statins, all derived from millions of years of genetic evolution—seems shortsighted at best. This is especially true at a time when there is a critical need for new antimicrobial agents against resistant organisms, particularly in hospital settings. But screening against new bacterial targets as opposed to the traditional biosynthetic steps of the bacterial cell wall, protein synthesis, and topoisomerases may turn up new structural classes heretofore not observed.

Although recent efforts to identify unnatural and natural products with specificity against other less traditional bacterial targets have not been successful, it is only the past few years that programs have been initiated to screen for such inhibitors compared with about 60 years of screening against the old targets. Attempts at Wyeth to identify potentially useful sortase inhibitors[43] that prevent the anchoring of bacterial surface proteins to the cell wall peptidoglycan to inhibit host−pathogen interactions and small molecules that disrupt protein−protein interactions such as the FtzZ/ZipA binding that is involved in bacterial cell division[44] were not successful. However, new targets will undoubtedly be identified from the sequences of microbial genomes that are now becoming available. But this will take time. And with the ever-increasing demand on the part of company CEOs for faster and faster results, it remains to be seen if the large pharmaceutical companies will provide the necessary resources it will take to keep up with the ever-increasing incidence of bacterial resistance. Let us hope that we are successful.[45]

I have one final comment on the present-day research environment in the drug industry. I have always maintained that most really significant discoveries arise out of exploring a chance observation or hunch.[46] In this time of more and more focused drug discovery involving large compound libraries, which are clearly important, there seems to be less opportunity to explore the chance observation while pursuing the end result. This is largely due to the severe monetary pressures under which pharmaceutical research is carried out. Drug discovery remains an extremely high-risk venture, something the public does not appreciate, despite the incredible scientific advances over the past 40 years. Still, I hope that the more serendipitous part of scientific research can be permitted and respected, at least to some extent, because without this it will be very difficult to come up with significant new discoveries.

Acknowledgment. I have had the privilege of working with some 55 very capable scientists during my career at Lederle and Wyeth, and those directly connected with the topics included in this Award Address have already been mentioned. But I express my appreciation to many others who worked with me on projects not presented here for their hard work and dedication in moving their projects forward.

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