

Distinguished Lecturer 2010
The Institute of Cancer Research

Sutton, UK
Thursday, November 4, 2010

Professor K.C. Nicolaou, Ph.D.
The Scripps Research Institute
and
University of California, San Diego

**“Chemistry, Biology and Medicine:
Molecules That Changed the World”**



Synthesis and the Molecule in Perspective



- The advent of *organic synthesis* coupled with the discovery of the *molecule* in the 19th century is one of the most profound scientific developments of all time.
 - These discoveries set in motion a revolution that shaped the landscape of the molecular sciences and changed the world.
 - The atomic-molecular theory of matter and the art and science of synthesis provided the foundation for understanding and shaping nature at the molecular level.
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THE ESSENCE OF TOTAL SYNTHESIS



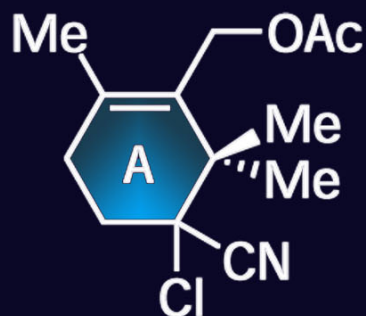
- **Rendering Scarce Natural Products Readily Available for Biological Investigations**
 - **Providing Opportunities for Discovery and Invention of New Synthetic Methods and Strategies**
 - **Opening Access to Otherwise Inaccessible Natural Product Analogs for Biological Evaluation**
 - **Structural Elucidation of Natural Products – Assigning Absolute Configurations and Revising Wrongly Assigned Structures**
 - **For Advancing the Art of Total Synthesis for its Own Sake and for the Excitement it Provides**
-



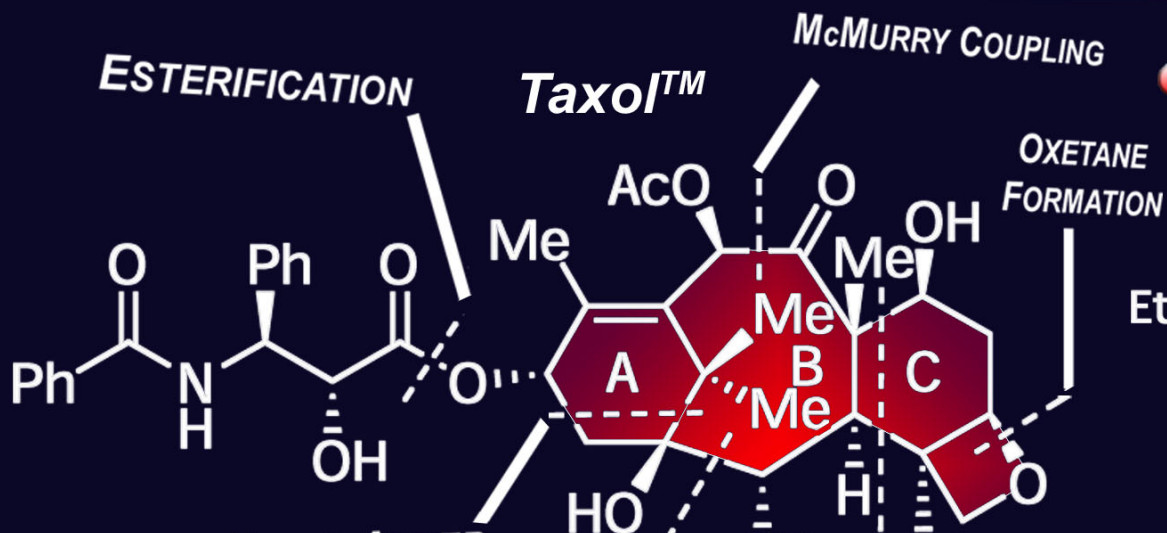
TOTAL SYNTHESIS OF TAXOL™ [1994]



● Water soluble pro-drugs



ESTERIFICATION



● Fluorescent probes



DIELS-ALDER

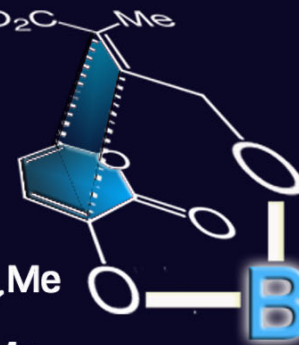
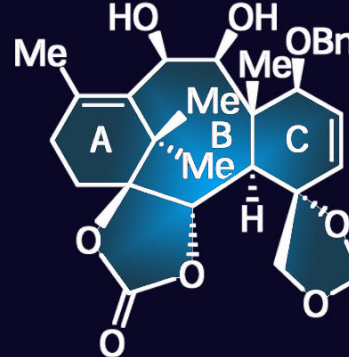
● Isolated from *Taxus brevifolia*

Convergent strategy

SHAPIRO COUPLING

DIELS-ALDER

EtO2C



Ph



CHEMICAL SYNTHESIS AND BIOLOGY OF EPOTHILONES [1996-2003]

● Isolated from myxobacterium *sorangium cellulosum* strain ce90

Epothilones A ($R=H$) and B ($R=Me$)

● Potent anticancer agents

● Tubulin polymerization/microtubule stabilization

● Designed Epothilones

● Subnanomolar potencies

● Olefin metathesis

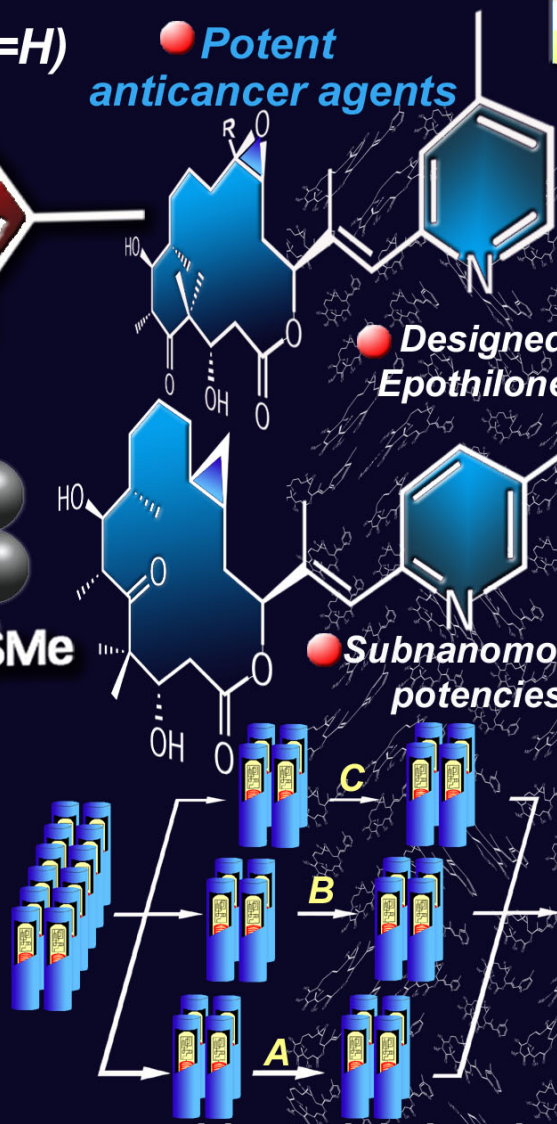
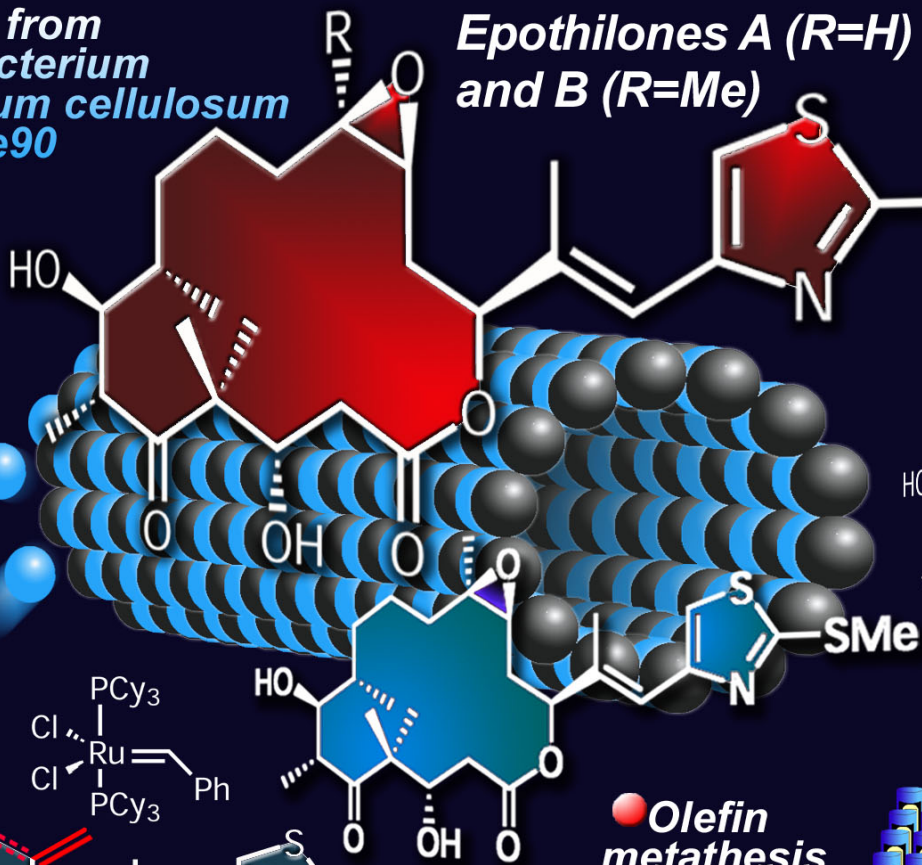
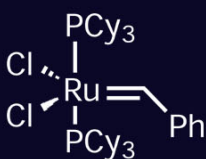
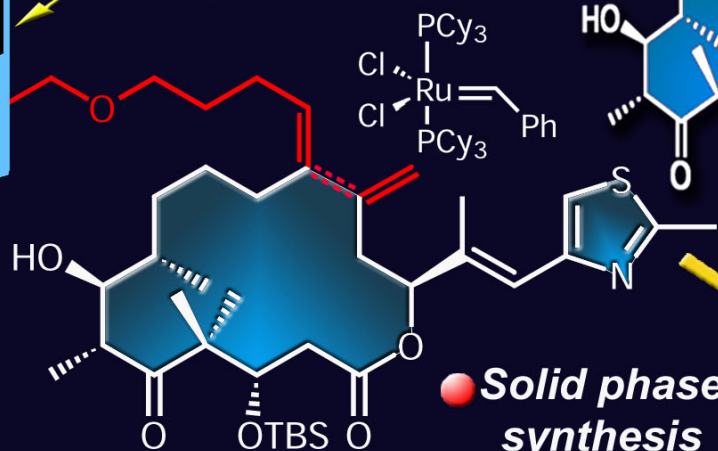
● Cyclorelease strategy

● Epothilones

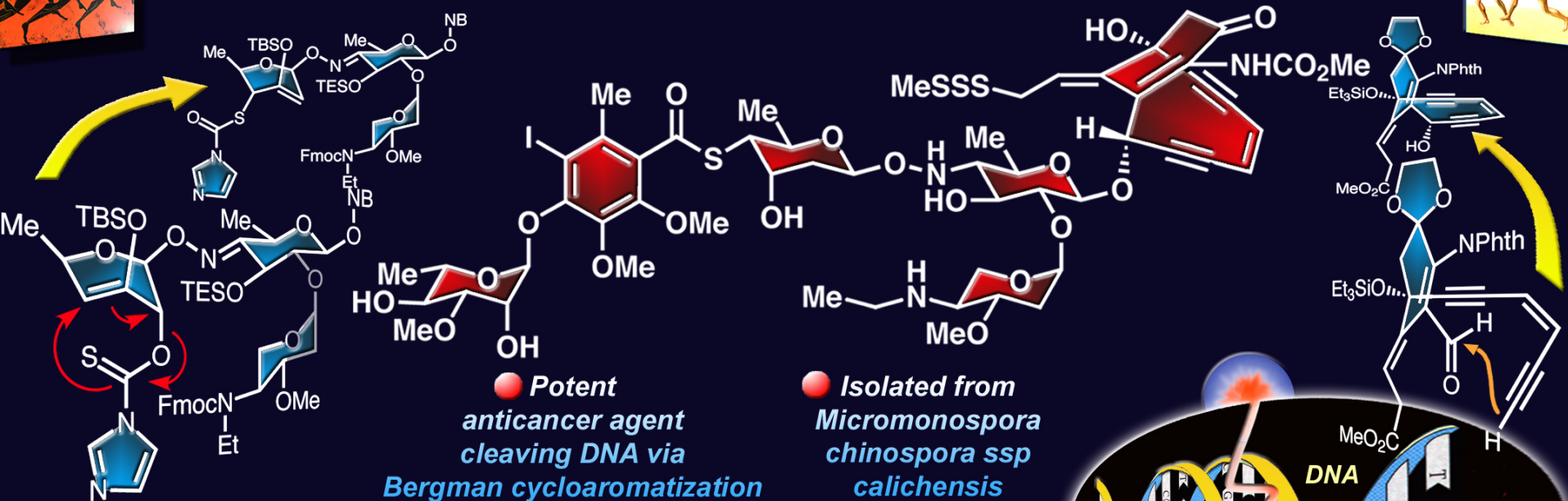
● Split-and-pool combinatorial chemistry

● Solid phase synthesis

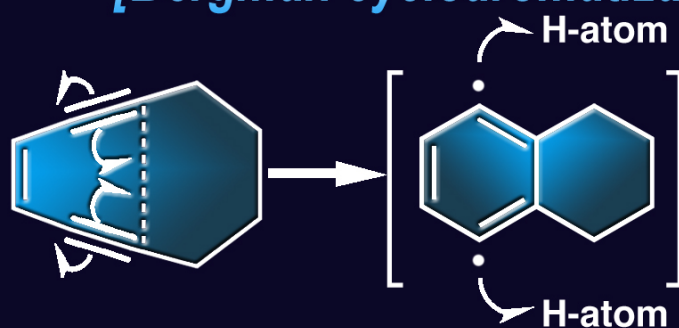
Radiofrequency tag



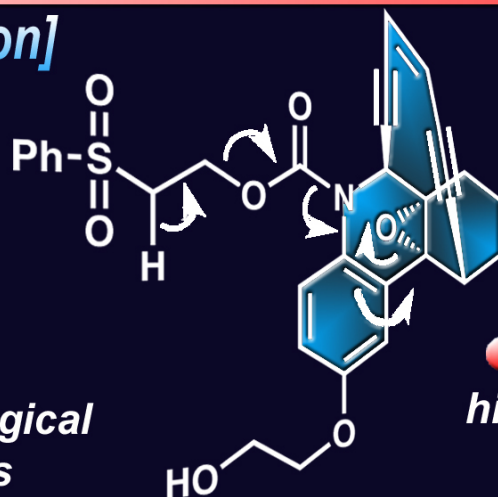
TOTAL SYNTHESIS OF CALICHEAMICIN γ_1' [1992]



[Bergman cycloaromatization]



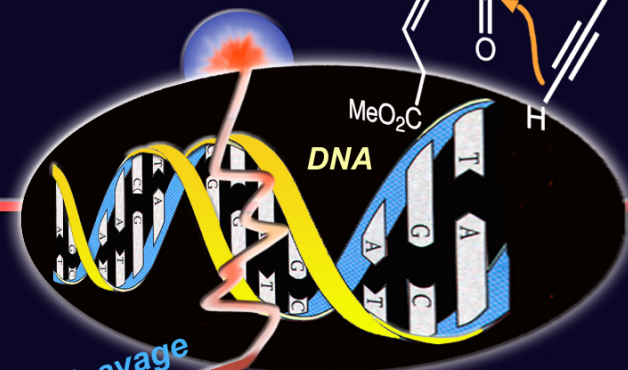
● **Elucidation of chemical and biological properties of cyclic enediynes**



DNA cleavage
Cell death

Double strand cuts

● **Designed enediynes with highly potent DNA cleaving and antitumor properties**







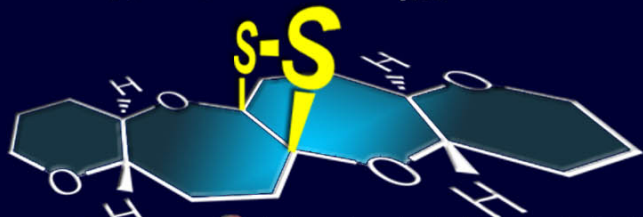
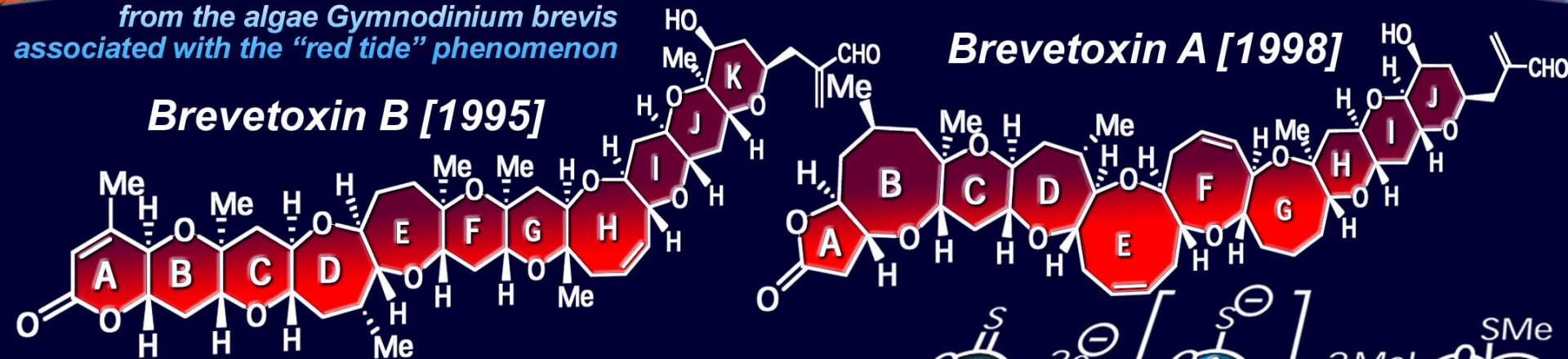
Total Synthesis of Brevetoxins B and A [1982-1998]



● Marine neurotoxins isolated from the algae *Gymnodinium brevis* associated with the "red tide" phenomenon

Brevetoxin B [1995]

Brevetoxin A [1998]

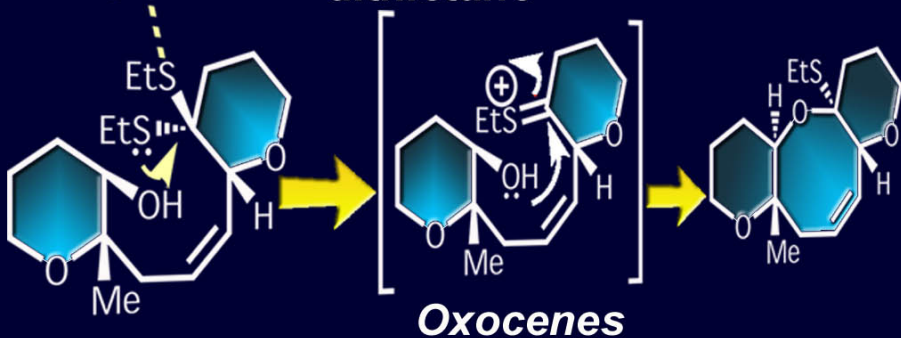


● First stable dithietane

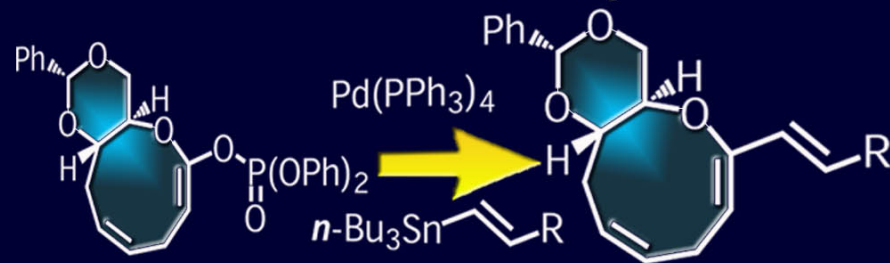
● New synthetic technologies



● Macrocycles to bicycles



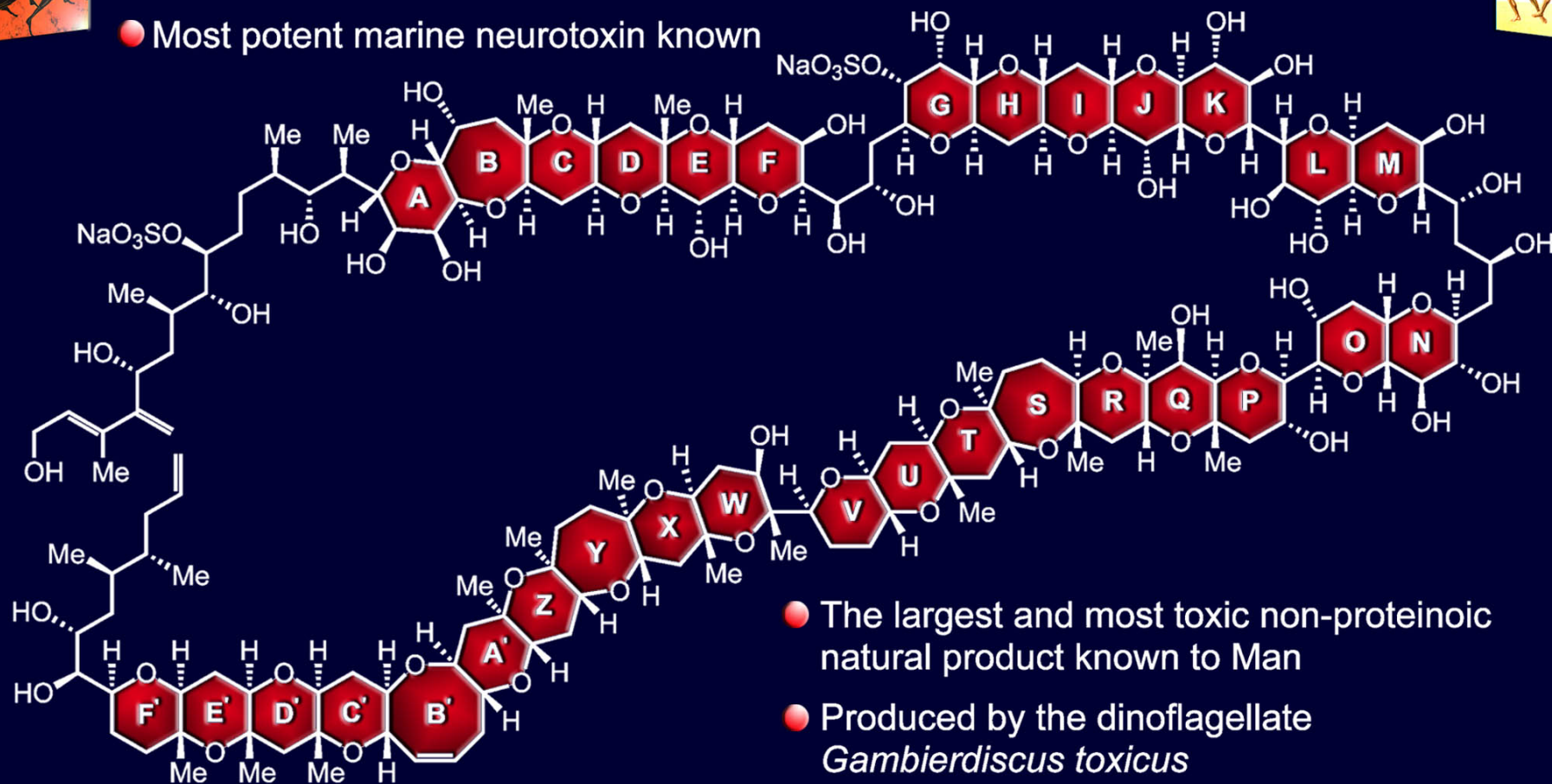
Oxocenes



Palladium-catalyzed couplings



Proposed Structure of Maitotoxin



- Most potent marine neurotoxin known

● $C_{164}H_{256}O_{68}S_2Na_2$ [MW 3422 Da]

● 32 rings, 98 stereogenic centers

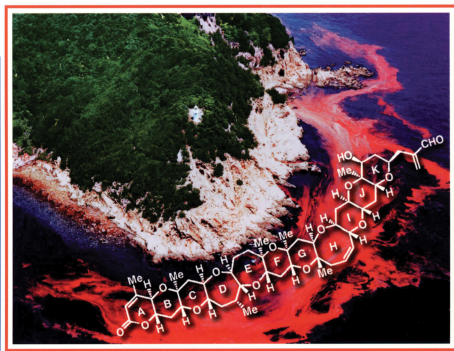
- The largest and most toxic non-proteinogenic natural product known to Man
- Produced by the dinoflagellate *Gambierdiscus toxicus*
- $LD_{50}=50\text{ng/Kg}$, 1 mg can kill a million mice
- Activates voltage-dependent Ca ion channels

● originally proposed maitotoxin structure [Yasumoto, Tachibana, Kishi, 1996]

● JK ring junction challenged [Gallimore and Spencer, 2006]

K. C. NICOLAOU, E. J. SORENSEN

CLASSICS IN TOTAL SYNTHESIS



TARGETS, STRATEGIES, METHODS

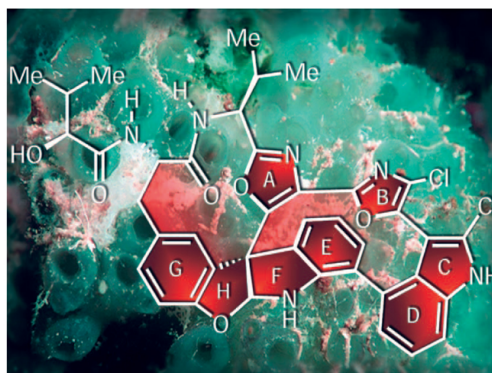


Nicolaou · Snyder

WILEY-VCH

Classics in Total Synthesis II

Targets, Strategies, Methods

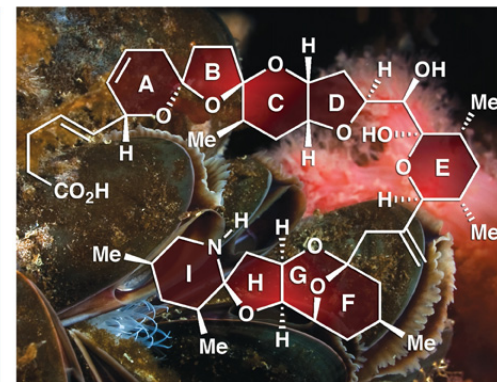


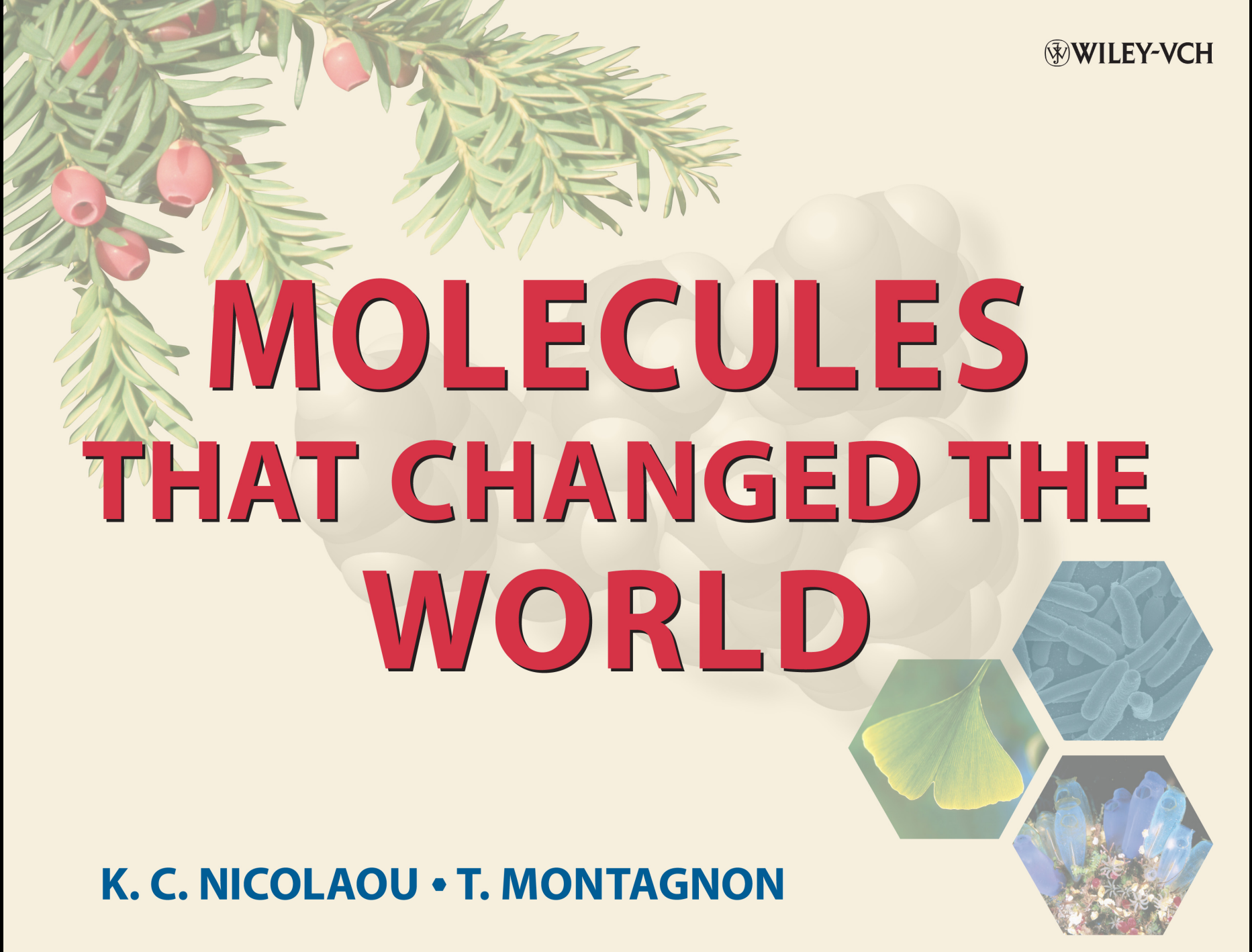
Nicolaou · Chen

WILEY-VCH

Classics in Total Synthesis III

New Targets, Strategies, Methods





MOLECULES THAT CHANGED THE WORLD

K. C. NICOLAOU • T. MONTAGNON

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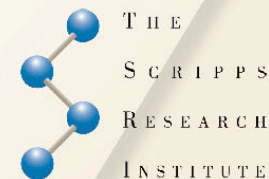
The Skaggs Institute for Chemical Biology

The Scripps Research Institute



Wyeth

GVKBIO





About the author



K. C. Nicolaou holds joint appointments at The Scripps Research Institute, where he is the Chairman of the Department of Chemistry and holds the Darlene Shiley Chair in Chemistry and the Aline W. and L. S. Skaggs Professorship of Chemical Biology, and the University of California, San Diego, where he is Distinguished Professor of Chemistry. He is also the Program Director for the Chemical Synthesis Laboratory@Biopolis, A*Star, Singapore.

His impact on chemistry, biology and medicine flows from his research works in chemical synthesis and chemical biology

described in his numerous publications and patents. For his contributions to science and education, he was elected Fellow of the New York Academy of Sciences, Fellow of the American Association of Arts and Sciences, and Member of the National Academy of Sciences, USA, and a Foreign Member of the Academy of Athens, Greece. He is the recipient of numerous prizes, awards and honors, and a co-author of *Classics in Total Synthesis* (with his student Erik J. Sorensen) and *Classics in Total Synthesis II* (with his student Scott A. Snyder).

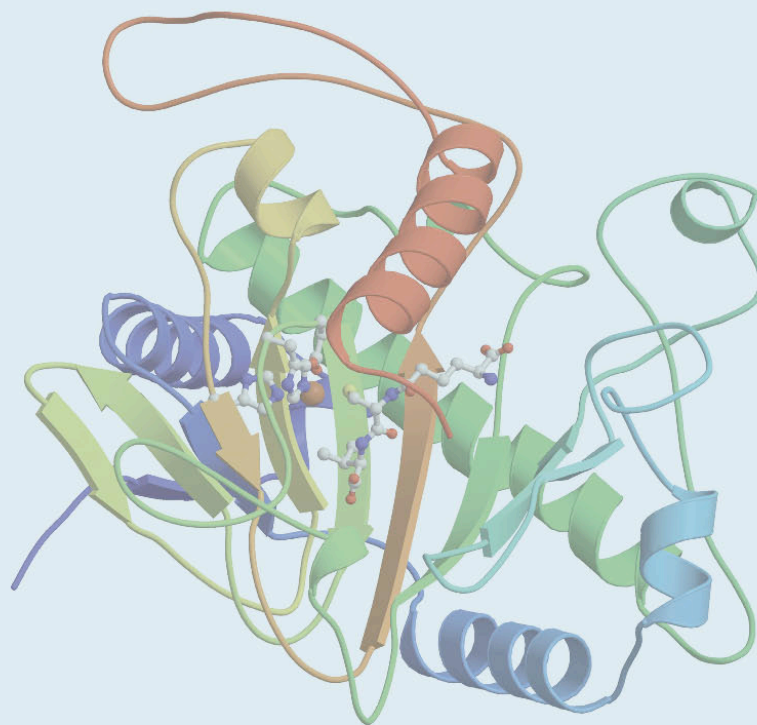


About the co-author



Tamsyn Montagnon received her B.Sc. in Chemistry with Medicinal Chemistry from the University of Leeds which was followed by a move to the University of Sussex, where she obtained a D.Phil in 2000 for research conducted under the supervision of Professor P. J. Parsons. She was awarded a GlaxoWellcome postdoctoral fellowship and joined Professor K. C. Nicolaou's group in January 2001 for three years. She is presently at the University of Crete doing research under the auspices of an EU Marie Curie Fellowship. Her research interests include natural product synthesis, medicinal chemistry, and reaction methods and mechanisms.







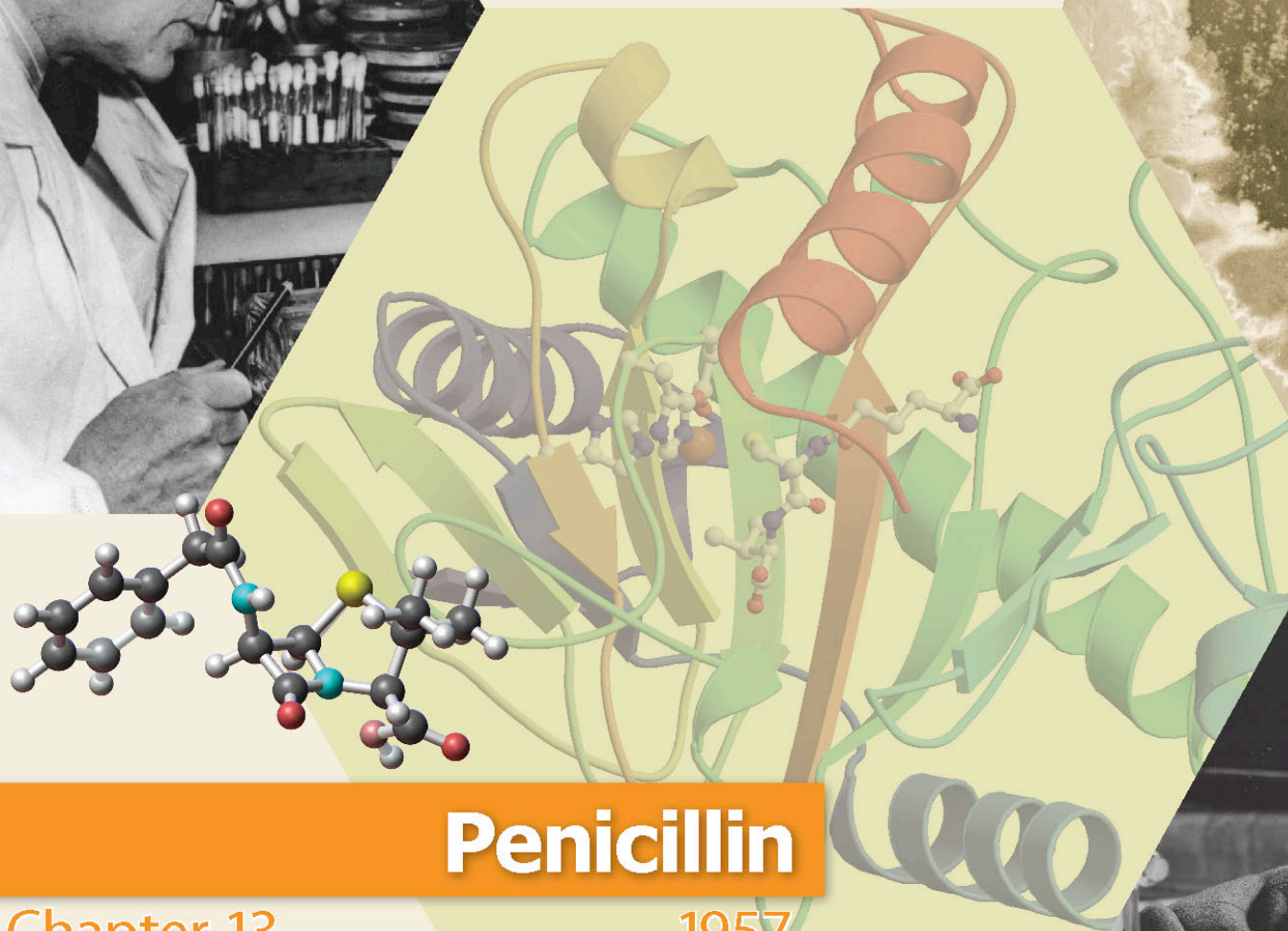
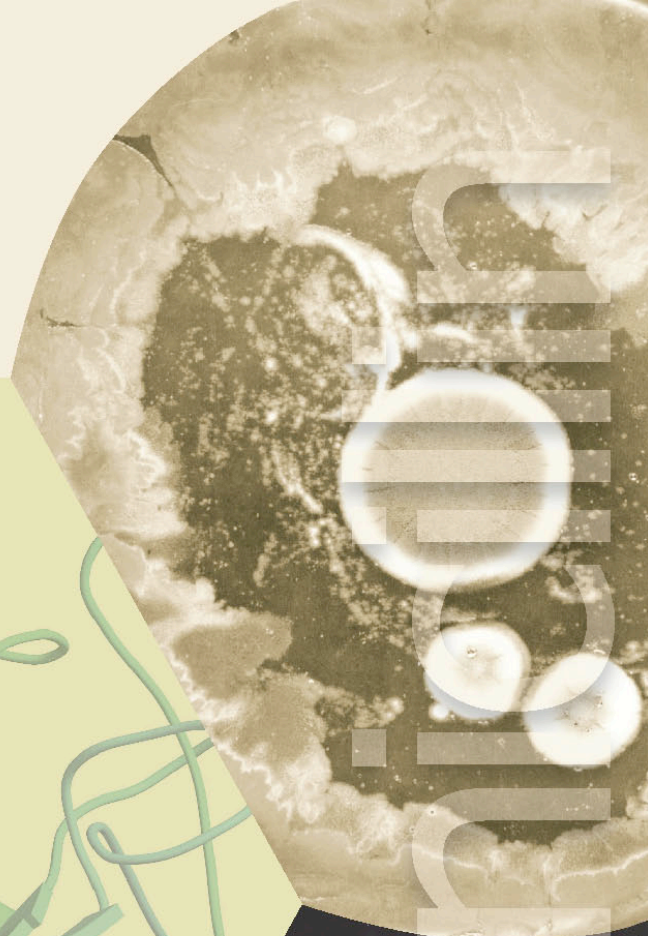
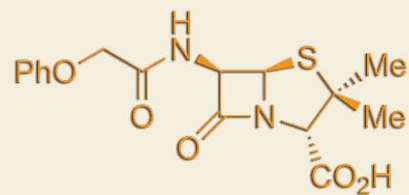
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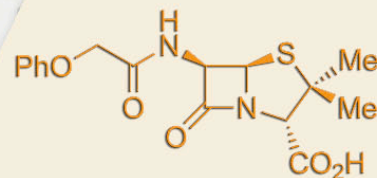
Penicillin

Chapter 13

1957



Alexander Fleming in his laboratory



penicillin V

The narrative surrounding the discovery and development of penicillin is truly remarkable, possessing all the ingredients of a best-selling novel: serendipitous discovery, wartime political intrigue, fierce competition, and an eclectic cast of characters. In addition, the dreadful problem of fatal bacterial infections was solved.

All this was a consequence of the extraction of a broad-spectrum antibiotic from an ordinary mold, following an observation made by Alexander Fleming in 1928. Despite its importance and the efforts of many chemists, the total synthesis of penicillin would not be accomplished until 1957.

This epic story begins much earlier, however, with a revolution in our understanding of the underlying cause of disease. Before the magnificent accomplishments of Louis Pasteur, disease, death, and illness were frequently ascribed metaphysical causes. In the western world, this idea commonly meant that sickness, especially in the form of epidemics, was deemed to

be the wrath of God, punishing man for his sins. Pasteur's seminal works culminated in the 'germ theory of disease,' which asserted that infectious diseases were caused not by God's vengeance, but by microbes.

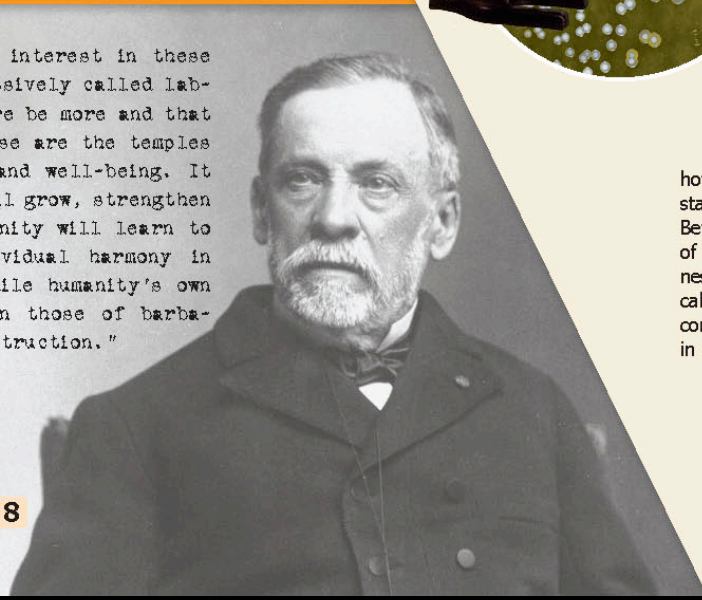
Louis Pasteur studied chemistry in Paris, and in 1848 he made his first profound contribution to the advancement of science, aged just twenty-six. He examined tartaric acid crystals under a microscope and noticed that they existed in two distinct forms that were mirror images of each other. He was able to separate these forms and discovered that, in solution, they rotated the plane of polarized light in opposite directions. This investigation represents the inception of the pivotal and fundamental field of stereochemistry. The tartaric acid Pasteur studied came from wine sediments, and his interest in the science of fermentation would lead to more important discoveries. In 1856, Pasteur came to the aid of a student's family, who were experiencing production problems at their fermentation plant; sometimes alcohol was produced as expected, but other batches gave lactic acid instead. Pasteur examined the fermentation mixtures by microscopy and noticed that during normal production the yeast cells were plump and budding, but when lactic acid was being produced, the yeast cells were smaller and accompanied by rod-like microbes. He found that briefly heating the liquid medium before fermentation began would kill the undesirable microbes and lead to reproducible fermentations. This sterilization procedure, known as *pasteurization*, is still in common use today, particularly for dairy products. Pasteur was subsequently able to show, using an ingeniously designed swan-necked flask containing a fermentable solution, that microbes such as these were airborne particles. Next, he tackled a serious disease in silk-producing



Box 1 Pasteur's philosophy on nature, research, and humanity

"I beseech you to take interest in these sacred domains so expressively called laboratories. Ask that there be more and that they be adorned for these are the temples of the future, wealth and well-being. It is here that humanity will grow, strengthen and improve. Here, humanity will learn to read progress and individual harmony in the works of Nature, while humanity's own works are all too often those of barbarism, fanaticism and destruction."

Louis Pasteur



worms whose malaise was having a devastating economic impact on the buoyant European silk trade. He showed that healthy worms could become infected by nesting on leaves previously occupied by diseased specimens. These early influential studies led Pasteur to develop his idea that germs spread contagious diseases, and that these foreign particles were living micro-organisms. Pasteur went on to do more groundbreaking work. Building on Edward Jenner's discovery of a vaccine for smallpox, Pasteur developed vaccination as a prophylactic strategy to outwit other viral contagions, such as rabies, as well as the bacterial infections of anthrax and cholera. Having sensed the widespread recognition of his work, Pasteur died with the knowledge that he had succeeded in bequeathing France a powerful and vibrant establishment for research, The Pasteur Institute, in Paris. He had pursued this project with passion for he strongly believed in applying experimental science to eradicate humanity's plagues and often spoke emphatically on this subject (Box 1). His words ring true and are surprisingly relevant even in today's much altered world.

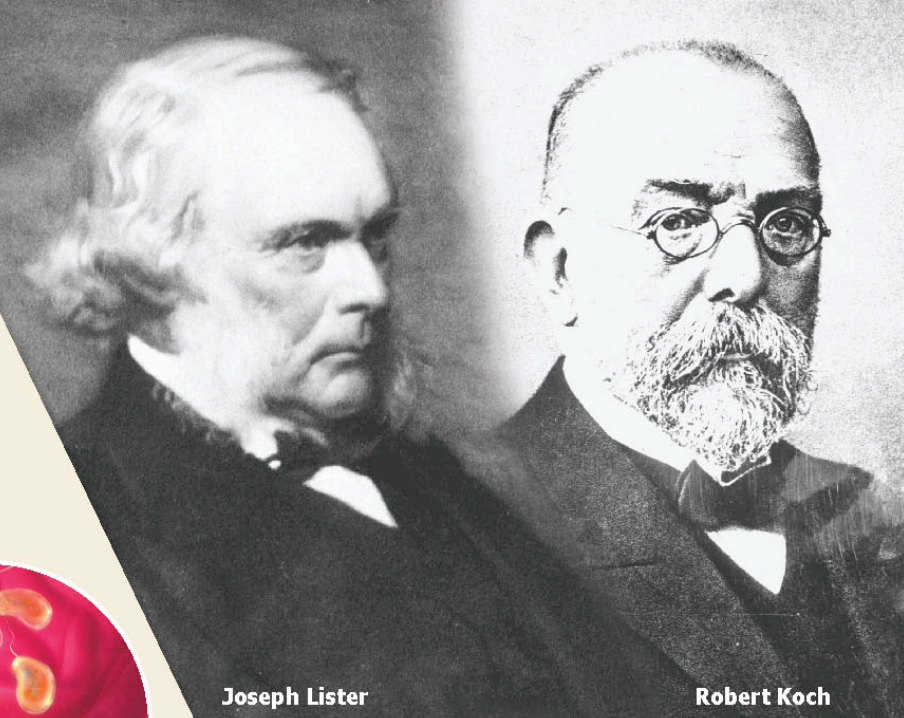
Meanwhile, in the United Kingdom, a young Quaker surgeon by the name of Joseph Lister was enthralled by Pasteur's work, prompting him to propose a connection between wound sepsis and microbes from the air. Lister's search for the cause of sepsis had begun because he was troubled by the assertion made earlier by the famous chemist Justus von Liebig that sepsis was a kind of combustion occurring when expanding moist body tissue met with oxygen from the air. In Lister's surgical ward at the Glasgow Royal Infirmary, wound sepsis killed fifty percent of his patients. Lister was further inspired to do something about this dreadful situation when he read that carbolic acid (Box 2)

was being used to treat sewage in Carlisle, and that fields treated with the resulting slurry were freed of a contagion that would normally lead to infection in cattle grazing on the same site. Lister began to clean wounds and dressings with a carbolic acid solution, and in 1867 he was in a position to announce to the British Medical Association that his ward had been sepsis-free for an astonishing nine month period due to the implementation of this protocol. Lister had pioneered the use of antiseptic solutions and broadcast the importance of hygiene in operating theatres, thereby saving innumerable patients from a painful gangrenous death.

The German doctor Robert Koch, working alone as the District Medical Officer for Wollstein during the Franco-Prussian War in the 1870s, was finally able to show definitively that the anthrax bacillus directly causes disease in test animals. He also developed techniques for culturing bacteria, thereby illustrating the phenomenal resilience of these specific microbes, while also proving that, in general, a host animal was not necessary for these germs to thrive. He photographed cultures, studied the conditions required for bacterial growth, and developed stains to improve their visibility. It was by employing this knowledge that he was able to identify both the *Vibrio cholerae* and the *Mycobacterium tuberculosis* that cause the deadly diseases of cholera and tuberculosis, respectively, work which earned him the 1905 Nobel Prize in Physiology or Medicine.



Vibrio cholerae

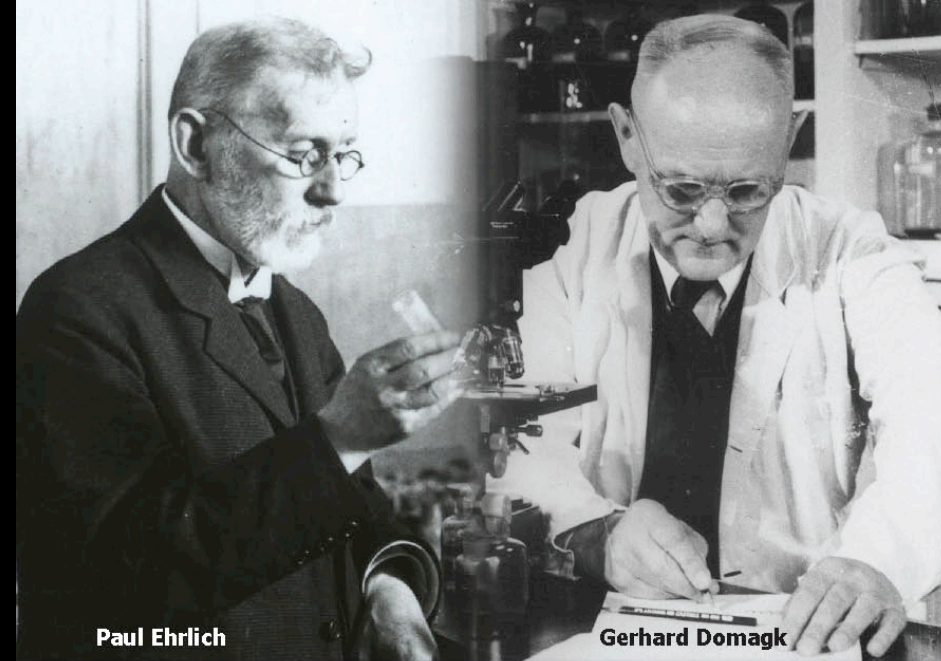


Joseph Lister

Robert Koch

Joseph Lister, pioneer of antiseptics, attending to a patient

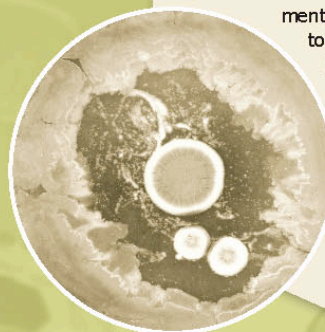
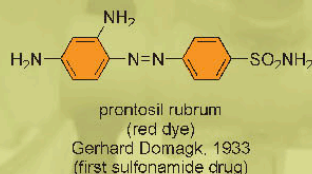
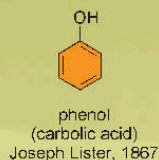
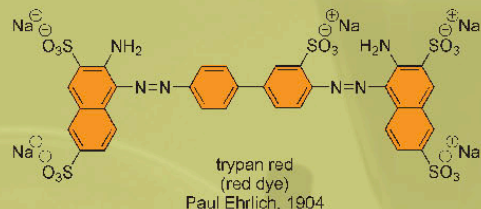




Paul Ehrlich

Gerhard Domagk

Box 2 Pre-penicillin antibacterial agents



Petri dish

Identifying these disease-causing agents had been a long and arduous battle requiring all the energy of those distinguished scientists whose ingenious work had already revolutionized medical practice, as we have just seen. Each of these men left his own indelible mark on history, immediately contributing to saving lives, yet our story is just in its beginning. At the start of the twentieth century life expectancy in England was just forty-five years, and infant mortality was at a rate of one hundred and fifty per thousand live births. Many of the premature deaths that contributed to these appalling statistics could be attributed to fatal bacterial infections. Bacteria had been identified, routes of transmission had been elucidated, and sterilization and surgical hygiene to prevent infection had been proven, yet still no solution to the problem of successfully treating an already-infected patient was available. People had good reason to dread bacterial infections since they frequently ended with the death of their victim.

Paul Ehrlich, a friend and one-time colleague of Koch, suggested that bacterial infection might be curable by treatment with a drug that was toxic towards the bacteria, whilst being harmless to the patient, thus introducing the so-called 'magic bullet' concept. This idea had been inspired by the selective uptake of dyes into bacterial cells, a technique that had been developed for enhancing microscopy, and Ehrlich was

fascinated by what influenced the disparity between various cell types. The large number of dyes produced by the German dye industry at the time afforded Ehrlich with a vast array of chemical candidates for testing. He and his Japanese colleague, Kiyoshi Shiga, eventually found that trypan red (an azo dye, Box 2) could effectively kill the bacteria *Trypanosoma gambiense*, the causative agent of the fatal sleeping sickness transmitted by the tsetse fly in Africa. However, in tests on humans in Uganda, its use resulted in unacceptable side effects ranging from blindness to death. Ehrlich is also famous for introducing the arsenic salts salvarsan (arsphenamine) and neosalvarsan (neoarsphenamine) as drugs to treat syphilis (caused by *Treponema pallidum* infection). Pain at the injection site, side effects, and frequent relapses rapidly consigned these agents to history. Nevertheless, Ehrlich's studies launched the age of chemotherapy. Ehrlich was also a pioneer in the fields of hematology and immunology, with his contributions being recognized by the awarding of the 1908 Nobel Prize in Physiology or Medicine, which he shared with the Russian Ilya Ilyich Mechnikov, "in recognition of their work on immunity."

In 1927, Gerhard Domagk, while continuing investigations in the same vein at the laboratories of I. G. Farbenindustrie, concentrated mainly on finding agents effective against haemolytic *Streptococci* (the bacteria associated with throat infections). He recommended prontosil rubrum (Box 2), a red azo dye, for clinical trials – a fortunate choice, for this compound contains the critical sulfonamide functionality and proved to be very effective against these bacteria. So it was that the first clinically used sulfonamide antibacterial agent was born, to be followed soon by many other members of this same class. The dis-

covery of the antibacterial effects of prontosil won Gerhard Domagk the Nobel Prize in Physiology or Medicine in 1939. His real triumph, however, was much more personal, as it emerged later that Domagk had, in desperation, used prontosil rubrum successfully to treat his infant daughter, who was dying of *Staphylococcal* septicemia, long before the drug became available to the public. After an initial period of euphoria over the discovery of the sulfonamides the febrile activity calmed and their shortcomings began to become all too apparent. Although still used in rare cases today, these antibacterials are very narrow in spectrum and essentially obsolete.

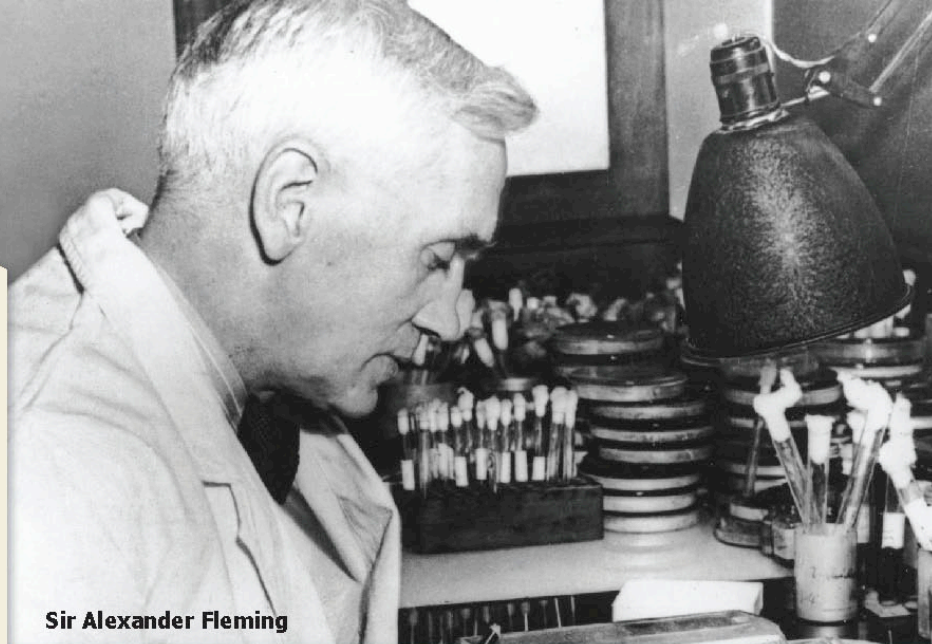
Enter Sir Alexander Fleming, the son of a farmer from rural Scotland, whose entire career could be said to have been based on a series of serendipitous happenings. In 1900, the young Alec, as he was called, joined a Scottish regiment in order to fight in the Boer War with two of his brothers. This experience honed his swimming and shooting skills, but never actually took him as far as the war-torn Transvaal region of South Africa (a province lying between the Vaal and Limpopo rivers). On returning to London, and in need of a profession, Fleming decided to follow his older brother into medicine. He obtained top scores in the qualifying examinations, giving him a free hand over which school he might choose for his studies. He elected to enroll at St. Mary's, one reason being that he had played water polo against them in a previous sporting fixture. In 1905 Fleming found himself specializing in surgery, a career choice which would require him to leave St. Mary's in order to take up a posi-



Fleming's famous petri dish

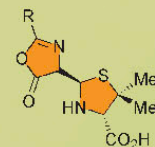
tion elsewhere. The captain of St. Mary's rifle club, who relied on Fleming's flawless shooting skills, heard of his impending departure and did his best to prevent it by winning him over to his own discipline, bacteriology, thereby maintaining the integrity of his champion team. Alexander Fleming never left St. Mary's, becoming instead the world's most famous bacteriologist.

In the 1920s, Fleming identified lysozyme, an enzyme found in tears, which exhibited a natural and mild antibacterial action. This protein was the first antibiotic to be isolated from the human body, but as it was not powerful enough to attack the most prevalent and aggressive infections, Fleming continued his search. One day, so the legend goes, when he was clearing out petri dishes containing bacterial cultures that had begun to accumulate in one of his sinks, he noticed that one of the containers had a mold growing on the nutritional agar. This rather common occurrence was made fascinating by the fact that Fleming's habit of careful observation also revealed that no bacterial colonies were growing around the periphery of the fungus. Fleming went on to show that not only was bacterial growth inhibited, but that healthy bacteria underwent cell lysis and death when exposed to the mold (*Penicillium notatum*). It should be noted at this point that the full detailed tale of the discovery of penicillin has its roots earlier than Fleming's investigations.

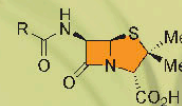


Sir Alexander Fleming

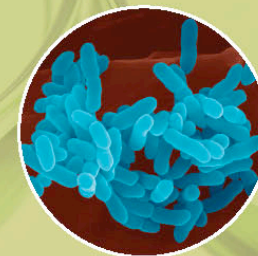
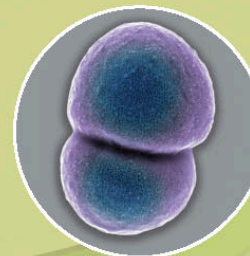
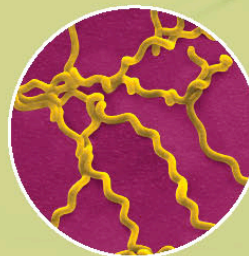
Box 3 Highly contested structures of penicillin



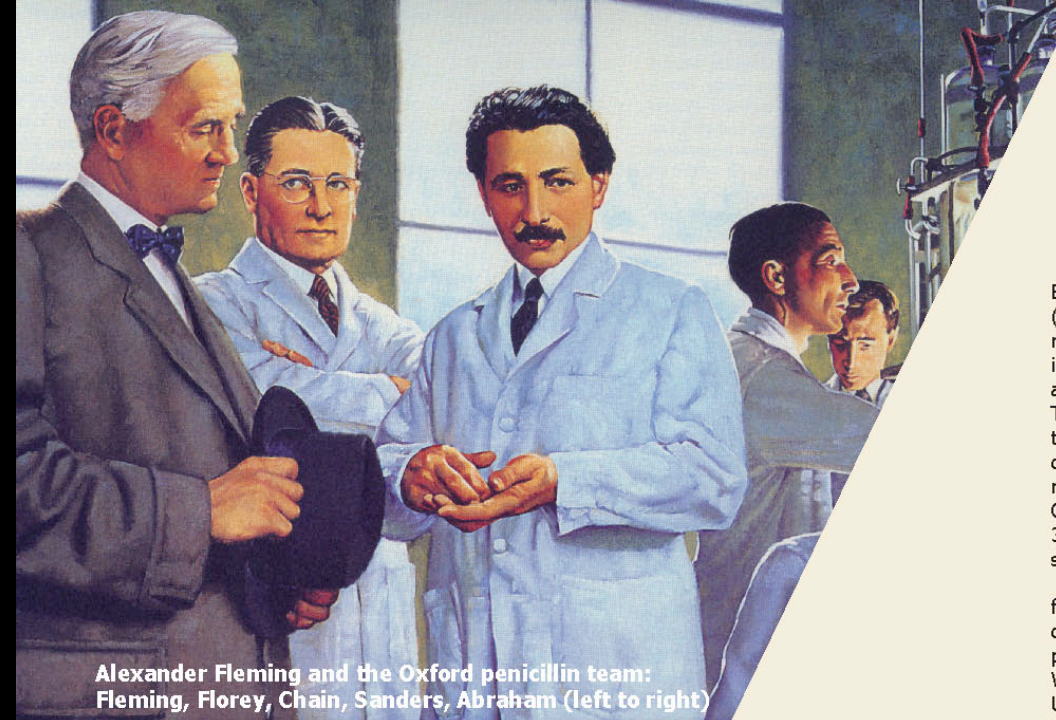
oxazoline thiazolidine structure (incorrect)



β -lactam thiazolidine structure (correct)

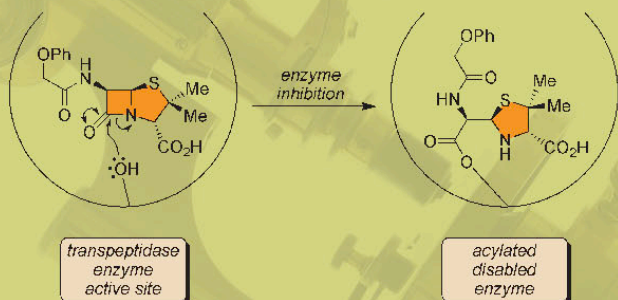


Bacterial strains



Alexander Fleming and the Oxford penicillin team: Fleming, Florey, Chain, Sanders, Abraham (left to right)

Box 4 The mechanism of action of penicillin



Both Joseph Lister and Ernest Duchesne (a French medical student) independently reported the use of *Penicillium* molds in bandages to treat infected patients at the end of the nineteenth century. These compresses were ignored by the scientific and medical communities due to their low potency until Fleming rekindled interest in them. Furthermore, Chinese medical texts dating back some 3000 years advocated the use of moldy soybean curd to treat skin infections.

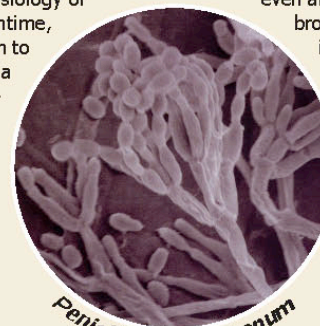
At the beginning of the 1930s, frustrated by progress in advancing his discovery to the next stage, Fleming passed on some of his culture to Howard W. Florey and Ernst B. Chain at Oxford University. The Oxford group, which also included Norman G. Heatley and Edward P. Abraham, refined the growth and isolation of the penicillin extracts just enough to facilitate the instigation of clinical trials, which immediately began to deliver very promising results. However, pure penicillin was still in such short supply that it had to be recovered from the urine of patients for reuse. The spectacular success of penicillin as an antibiotic would later earn Fleming, Chain, and Florey the 1945 Nobel Prize in Physiology or Medicine. In the meantime, the elevation of penicillin to its legendary status as a world-changing antibiotic would require the launching and successful execution of one of history's most

captivating international scientific adventures, the so-called Penicillin Project.

With the outbreak of World War II, interest in penicillin had intensified. Numerous scientists strove to produce the antibiotic on a large scale in response to an urgent new need for the drug to treat wounded soldiers and civilians who had subsequently contracted infections. The proximity of the battle frontline, the frequent aerial bombardments of the UK, and the need for a rapid solution to the problem led to a huge Anglo-American collaborative project on penicillin. The Rockefeller Foundation in New York arranged for Florey and Heatley to come to America in 1941 to meet with Charles Thom, chief mycologist at the US Department of Agriculture. A two-pronged strategy for the procurement and development of penicillin was immediately formulated. The first approach was directed towards the elucidation of the structure of penicillin, which would make the ultimate goal of its chemical synthesis at least conceivable. Upwards of forty independent laboratories and hundreds of chemists became involved in this labyrinthine task. The second line of attack was directed towards further improving the fermentation process for production of the drug. The latter of these pivotal works was relocated to the US Agriculture Department Laboratories in Peoria, Illinois. Here an intense research program drew on a myriad of sources to find extra momentum; in this eclectic project, progress was

even aided by local residents who brought moldy household items to the laboratories for investigation in the search for more productive penicillin strains.

It was the second of these two approaches that paid



Penicillium chrysogenum

the earliest dividends. Thus, a much-improved yield of penicillin was secured in the 1940s from *Penicillium chrysogenum*, a discovery made courtesy of a moldy cantaloupe melon brought into the laboratory by Mary Hunt, an employee at the Peoria laboratories. Within three



Ripening cantaloupe on a vine

years, twenty-nine plants were fermenting this high-yielding fungal strain to produce penicillin using a corn-steep liquid medium (a by-product of the massive Mid-West corn production) in an amazing effort organized by an extraordinary conglomeration of scientific establishments. The pharmaceutical companies of Merck, Pfizer, Squibb, and Abbott were all involved, along with leading British and American academic and governmental institutions.

General Dwight D. Eisenhower began the invasion of Europe from the southern shores of England on D-Day supported by some three million doses of penicillin (300 billion units or approximately 180 tonnes), the product of one of the most exciting and lucrative joint ventures in history. Only the notorious Manhattan Project directed towards the development of the atom bomb exceeded it in magnitude during that period. Thus, in less than four years, scientists had gone from recovering penicillin from patients' urine, due to its short supply, to the phenomenal level of production whereby 1,633 billion units of penicillin were produced in 1944 alone. The tremendous effort extended towards the development of penicillin may be less familiar to us than the Manhattan Project, but it is easy to argue that the former collaboration yielded far greater benefits to

mankind. Overall, this venture succeeded admirably in optimizing fermentation and production protocols, allowing for a successful and practical supply of the new miracle drug, ultimately saving countless lives. The process, however, still relied on natural biosynthesis of the intact penicillin molecule, a fact that limited investigations into producing analogs with enhanced activity, especially to combat the new demon of bacterial resistance that was just beginning to rear its ugly head. These imperatives stipulated urgent attention be paid to finding a chemical synthesis.

Before a chemical synthesis could be attempted the non-trivial task of deconvoluting the molecular structure of penicillin had to be accomplished. From the ardent debate amongst many of the most renowned chemists of the time, two possible structures emerged in the early 1940s as leading contenders for the honor of representing the magical molecule of penicillin (see Box 3). The so-called oxazolone-thiazolidine formula was proposed by Sir Robert Robinson (Nobel Prize in Chemistry, 1947, see also Chapter 7) and fiercely defended by him, as well as by a number of other notable chemists such as Sir John Cornforth (Nobel Prize in Chemistry, 1975). Its β -lactam rival was advocated by Merck scientists and by the Oxford axis of Abraham and Chain. Despite

X-Ray crystallography



Dorothy Crowfoot Hodgkin and husband

General Dwight D. Eisenhower (left) conferring with General Bernard Montgomery

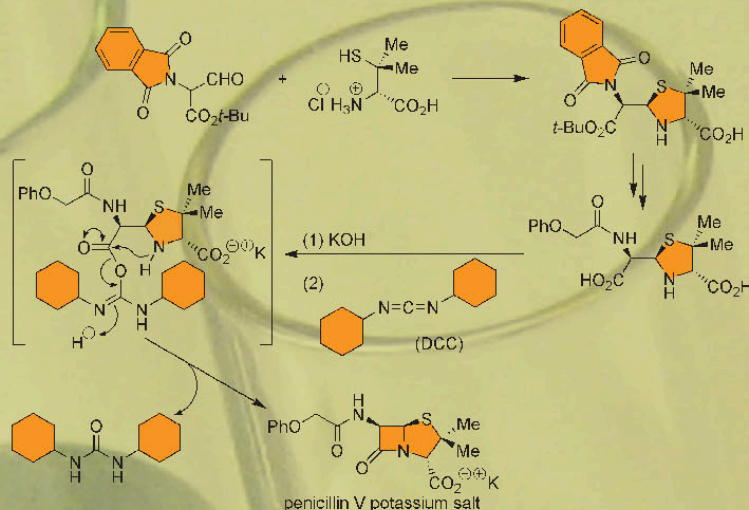


Box 5 Sheehan's thoughts on the total synthesis of penicillin V

"At the time of my successful synthesis of penicillin V in 1957, I compared the problem of trying to synthesize penicillin by classical methods to that of attempting to repair the mainspring of a fine watch with a blacksmith's anvil, hammer and tongs."

John C. Sheehan

Box 6 Sheehan's total synthesis of penicillin V



experimental evidence for the existence of a β -lactam structural motif provided by the Merck scientists, conventional wisdom could not accept the presence of such a strained and reactive feature within a naturally occurring substance. It was only after the brilliant crystallographic work of Dorothy Crowfoot Hodgkin that the dispute was finally settled in favor of the β -lactam structure in 1945, pleasing its backers and winning fame for the unobtrusive crystallographer from Oxford.

The striking four-membered β -lactam ring of penicillin, which was so decisively revealed by Hodgkin's crystallographic analysis, also turned out to be the motif that was responsible for the lethal action of the drug against bacteria. This activity was found to be related to the conformation adopted by penicillin, wherein the fused 4,5-ring system enforces an orthogonal alignment of the nitrogen lone pair and the carbonyl π -bond such that the resonance stabilization exhibited by traditional amides cannot be attained in this case. This feature, in combination with the intrinsic strain of the four-membered ring, creates a situation where the carbonyl functionality of the β -lactam ring acts as a highly effective acylating agent due to its particularly strong electrophilic reactivity. Thus, it is now known that penicillin irreversibly acylates the bacterial transpeptidase enzyme responsible for the cross-linking reaction which unites the terminal glycine residue of a pentaglycine strand with the D-alanine residue of a neighboring pentapeptide, in

an indispensable step during the construction of bacterial cell walls (Box 4). The acylation process deactivates this cross-linking enzyme, thereby compromising the integrity of the bacterial cell wall, resulting in rapid cell death. Unlike bacteria, only a phospholipid membrane surrounds mammalian cells, so transpeptidase inhibition is completely selective for bacterial cells. It has been shown that the penicillin molecule adopts an overall conformation that is very similar to the D-alanine-D-alanine residue of the substrate involved in this chain elongation process, thus it gains ready access to the active site of the enzyme where it reacts to disable its host.

The biosynthesis of penicillin, including the unprecedented β -lactam ring, was elucidated through a series of brilliant chemical and biological studies, many of which were carried out by Sir Jack E. Baldwin and his group at Oxford University. In addition to this seminal work, Baldwin is also known for his contributions to biomimetic synthesis, as well as for a set of rules he devised to predict the outcome of certain ring-closing reactions.

Ironically, while the biological activity of penicillin relies on the characteristic lability of the β -lactam ring, it is this same feature that led most synthetic organic chemists of the wartime period to consider penicillin an impossible target to conquer by chemical synthesis. Indeed, despite a huge effort directed towards its synthesis, both during and after World War II, no success was reported until much later. Indeed, it was 1957 before John C. Sheehan at the Massachusetts Institute of Technology (USA) was able to announce triumphantly the total synthesis of penicillin V, the result of a relentless ten-year campaign (Boxes 5 and 6).

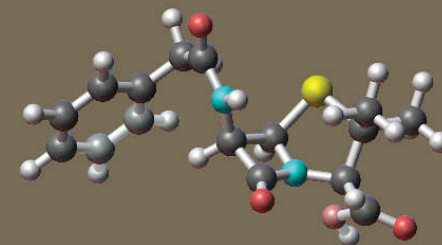
Sheehan's celebrated success was due to his innovative and daring approach. He

had recognized very early on that the main hurdle to be overcome prior to any total synthesis of penicillin was the construction of the highly strained and sensitive β -lactam ring. In addition, it was clear to him that a suitable method for accomplishing this challenging ring formation had to be developed since none of the existing technologies could be expected to rise to the challenge. It is here that Sheehan's insight and brilliance ensured his team's triumph. He conceived of and developed the *N,N'*-dicyclohexylcarbodiimide (DCC)-mediated coupling of carboxylic acids with amines to afford amides. Applied in its intramolecular version (wherein the amine and acid both belong to the same molecule) on an appropriately functionalized precursor, this method would solve one of the most recalcitrant problems in chemical synthesis of the 1940s and 1950s – the construction of the β -lactam ring of penicillin. The DCC coupling reaction, later extended to provide a solution for ester bond formation, remains a powerful synthetic tool in contemporary organic synthesis, and has been the inspiration for many similar reactions. This innovation was not the only one made by Sheehan during the penicillin synthesis; the phthaloyl protecting group for primary amines was another important and enduring contribution emanating from his group.

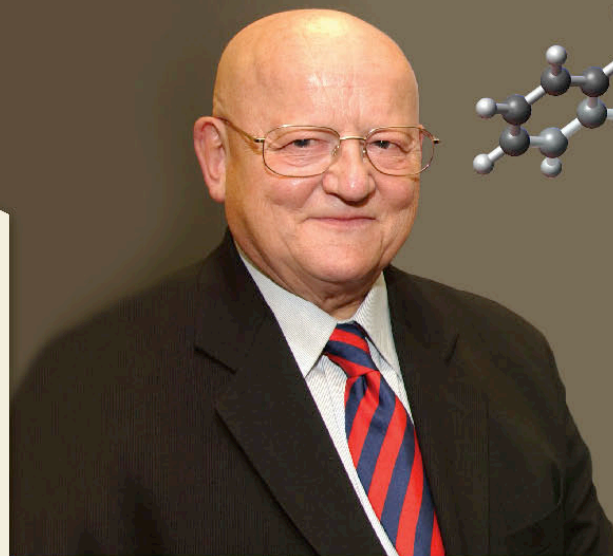
With these two innovations at their disposal, the Sheehan group was able to complete the synthesis of this previously impossible target through the sequence briefly outlined in Box 6. Thus, coupling of a phthaloyl-protected amino aldehyde with a suitable amino thiol led to the construction of the thiazolidine ring of penicillin. Further elaboration furnished an advanced amino diacid intermediate which served as the precursor for the β -lactam ring of penicillin V. Indeed, exposure of this precursor

to Sheehan's DCC coupling conditions provided penicillin V as its potassium salt. So it was that the first total synthesis of penicillin was accomplished, a milestone event in the history of the art of total synthesis. This feat marked not only the beginning of a highly productive era in the synthesis of β -lactam derivatives, many of which were subsequently synthesized, but also represented the addition of a new dimension to total synthesis endeavors, that of seeking to invent new synthetic technologies along the way. The task of developing this paradigm further, taking it to impressive new heights, would be assumed by one of Sheehan's students, the now highly celebrated master of this science E. J. Corey, about whom we will learn much more later in subsequent chapters.

Penicillin ushered in a new epoch in antibiotic research, one that grew by leaps and bounds over the ensuing decades. A series of new naturally occurring β -lactams such as cephalosporin C, clavulanic acid, and thienamycin were delivered in quick succession for use as drugs (Box 7). As soon as these discoveries had been made in microbiology laboratories, synthetic chemists busily focused on synthesizing the newly discovered natural products and modifying their structures in an effort to discover new antibacterial agents with improved pharmacological profiles. Their work led to an equally impressive collection of synthetic or semisynthetic β -lactam antibiotics including ampicillin, amoxycillin, and methicillin (Box 7). During this golden



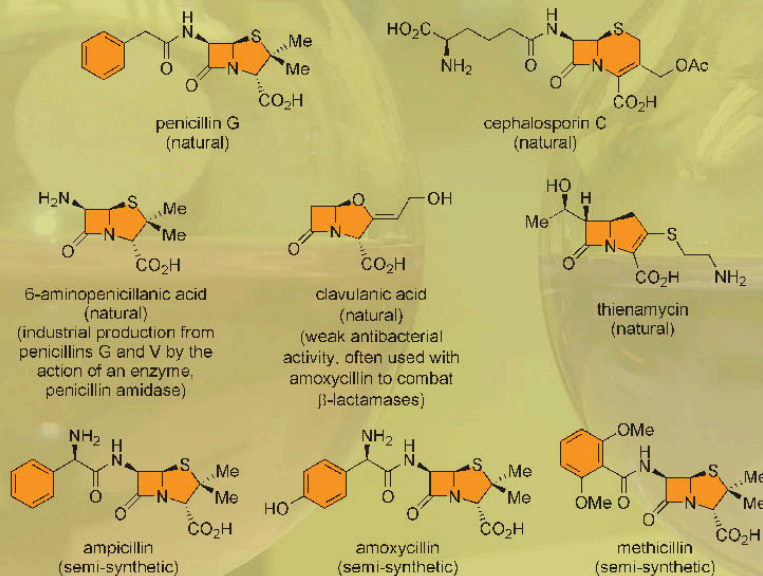
Ball and stick model of the molecule of penicillin G



Sir Jack E. Baldwin

Ribbon model of isopenicillin N-synthase





and of the antibiotics used to defend us against them, will be revisited in the chapter introducing vancomycin (Chapter 31), a natural product that is today the lifesaving drug of last resort in cases of severe infection.

era for antibiotics, however, came some disturbing news. Their widespread use, and sometimes misuse, led to the rapid evolution and spread of antibiotic-resistant bacterial strains. A new menace for humanity was now looming on the horizon!

Bacterial strains have evolved the capability to evade the action of β -lactam antibiotics by producing an enzyme, called β -lactamase, which can cleave the β -lactam ring, thus deactivating the molecules before they reach their site of action. To combat these newly acquired enemies, scientists have since sought out and developed various novel antibacterial agents; some of these powerful new antibiotics came from nature and some emerged from totally manmade designs. The story of drug-resistant bacteria,

We must conclude this account by underscoring once again the profound significance of the discovery of penicillin. Besides constituting a landmark medical breakthrough that saved lives and alleviated human suffering, this fortunate event also revealed to scientists a new treasure trove of biologically active molecules ripe for exploration. Thus, to the forest, which held the key to the development of Aspirin®, we now add the kingdom of microbes as a rich hunting ground for molecules endowed with healing powers. Indeed, many such molecules have since been isolated from the soil and other habitats where bacteria and fungi hold sway and, from this bountiful harvest, scientists have derived a host of 'magic bullets' and billion dollar drugs, as we shall see in forthcoming chapters.

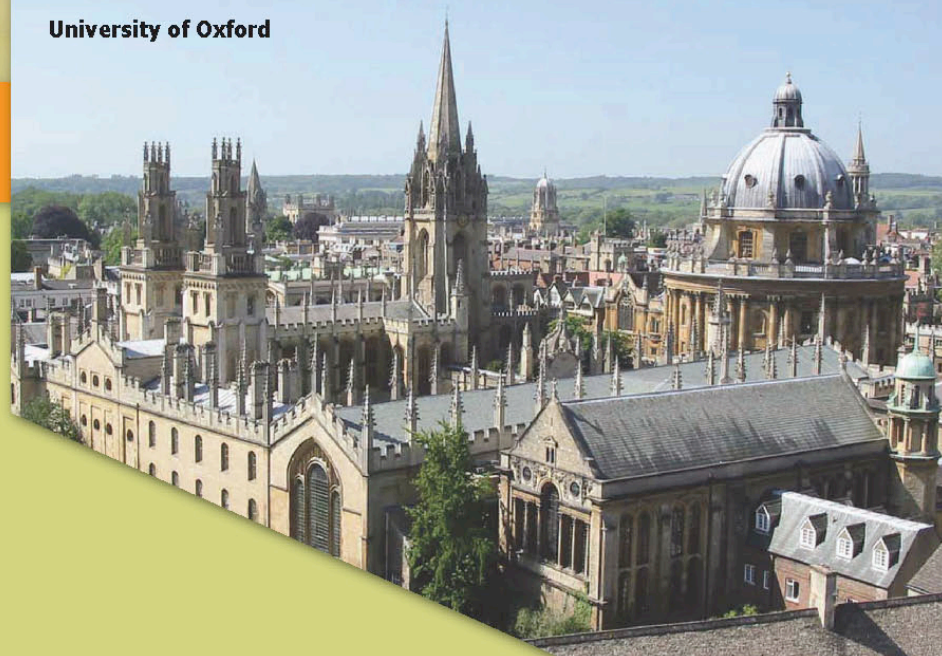
Further Reading

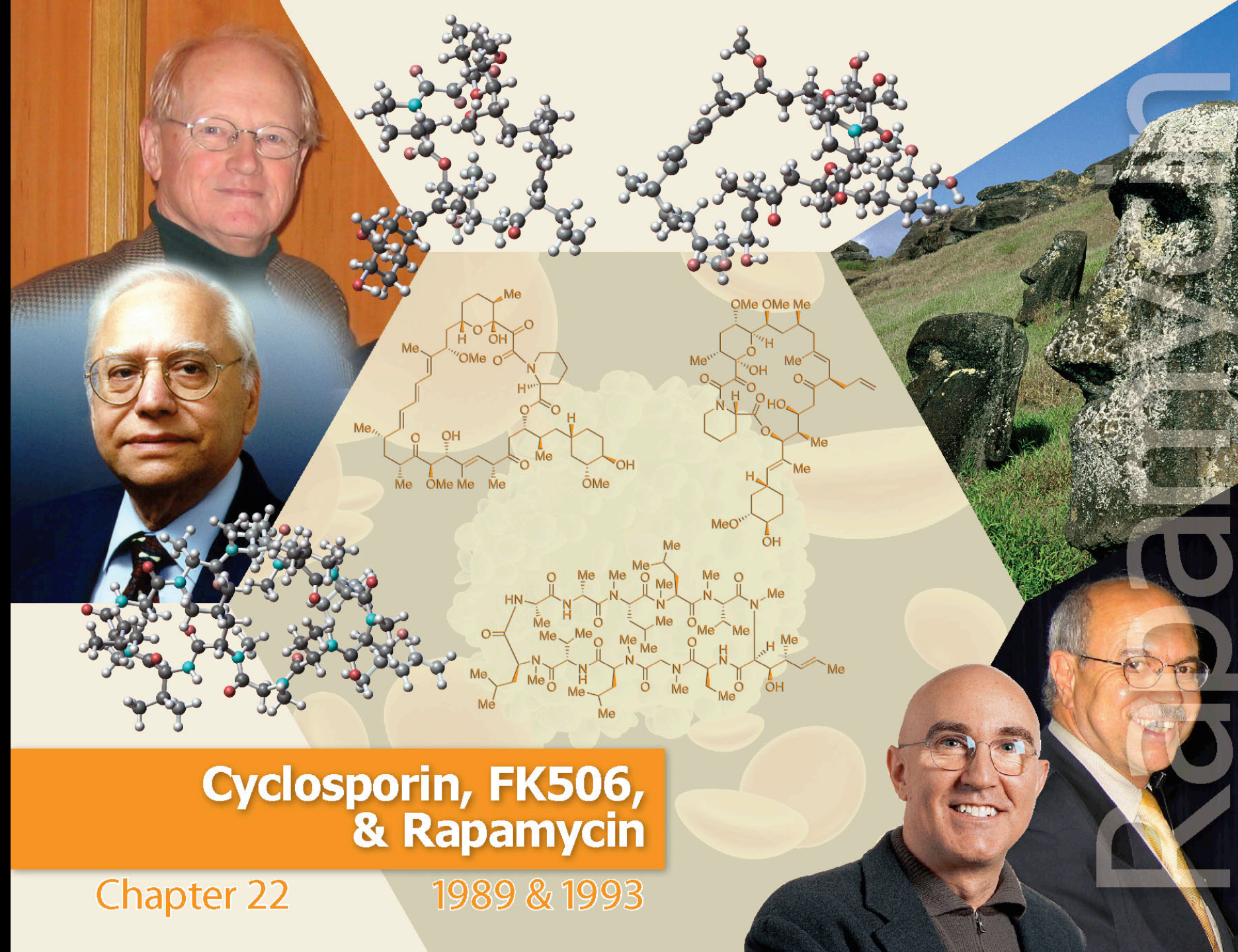
J. C. Sheehan, *The Enchanted Ring: The Untold Story of Penicillin*, The MIT Press, Cambridge, Massachusetts, 1982.

J. Mann, M. J. C. Crabbe, *Bacteria and Antibacterial Agents*, Oxford University Press, Oxford, 1996.

K. C. Nicolaou, E. J. Sorensen, *Classics in Total Synthesis*, Wiley-VCH, Weinheim, 1996, pp. 41–53.

University of Oxford





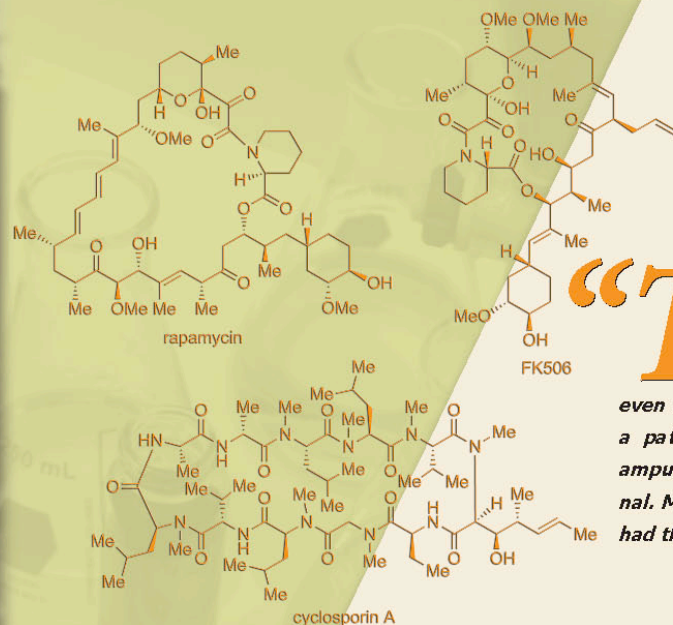
Cyclosporin, FK506, & Rapamycin

Chapter 22

1989 & 1993



Alexis Carrel



The idea of replacing diseased organs by sound ones, of putting back an amputated limb or even of grafting a new limb on to a patient who has undergone an amputation, is far from being original. Many surgeons before me have had this idea...."

Alexis Carrel (Surgeon and Nobel Laureate, 1912)

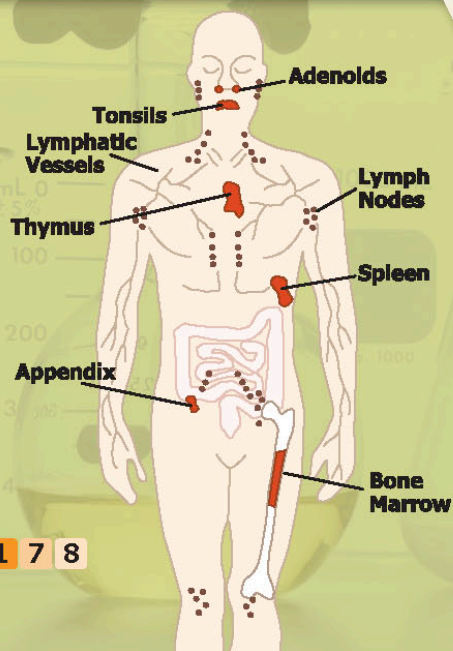
Despite the optimism of the above quotation, it would be a further forty-two years before the first successful organ transplant took place. As significant as this feat was, this first success lay in the donation of a kidney between identical twins. Identical twins have exactly the same genetic makeup, so the surfaces of their cells express indistinguishable recognition proteins. The cells of any other donor, even relatives as close as mother and child, carry subtle differences in these proteins, which allow the alien organ to be recognized by the immune system, provoking a reaction against what it sees as the invading tissue. The free transplantation of organs between unrelated patients would have to wait many more years to become a practical reality, even though surgeons had already developed the necessary tissue reconstruction skills. Success would only come when organic chemists embraced

the problem, and developed new drugs to counter tissue rejection effectively. In this chapter, we will explore a number of such molecules. Prominent amongst these so-called immunosuppressors is a natural product, FK506, with a most unremarkable name which belies its fascinating chemistry and biology. We will also discuss cyclosporin A and rapamycin, two other naturally occurring molecules that have had a major impact on medicine, particularly in the field of organ transplantation.

The 1912 Nobel Prize in Physiology or Medicine was awarded to the surgeon Alexis Carrel for his advancement of vascular surgery, and the quote introducing this chapter is an excerpt from his acceptance speech. His speech indicated that tissue transplantation had been occupying the minds of surgeons for some time. Carrel was accomplished in the practice of removing limbs from laboratory animals and reattaching them, either onto their original host or onto another individual. After describing all the procedures, precautions, and precision engineering that was required to undertake these delicate operations, he noted, "...vascular surgery can today be considered as completed from the standpoints both of technical and experimental results." While caution is generally prudent before announcing that an advance in any given field marks the summit, with no further progress possible, Carrel perhaps understood the limiting issues better than many of his successors. He knew his dogs, with their transplanted limbs, would die soon after surgery, not because of any mechanical dysfunction of the limb or its blood vessels, but because of some biological factor communicating between the dog's tissue and the new appendage. Thus, he was correct in saying that there was little to be gained from refining surgical techniques until, "...the

Box 1 The human immune system

Stress causes physiological changes that tend to weaken our immune system, and may negatively affect our health.



biological relationships existing between living tissues..." could be understood and the problems highlighted by his investigations fully addressed.

Despite Carrel's awareness and his accurate assessment of the challenges, many people continued to attempt hopeless transplants. The donor species was even ignored; sheep, goats, and pigs were all having their organs fruitlessly interchanged. Then, with the onset of World War II, this research gained a new impetus as many casualties, both civilian and military, suffered terrible burns. There was no hope for these patients unless new skin could be grafted onto their ravaged bodies. Encouraged by the Medical Research Council in Great Britain, a talented professor of zoology at Oxford University, Peter B. Medawar, began to investigate the problem of achieving a successful permanent skin graft with skin donated by a healthy per-



Sir Peter B. Medawar

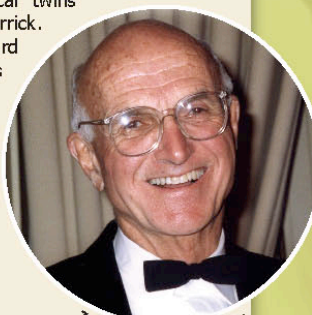
son to a burn victim. From this research, Medawar collected data and developed theories for achieving transplantation immunity, work for which he was awarded the 1960 Nobel Prize in Physiology or Medicine. He showed that if fetal or new-

born mice were injected with cells from an incompatible mouse strain early enough, not only would the cells not be rejected immediately, but when the juvenile mouse grew to reach maturity it could accept grafts from the donor mouse whose cells it had received early in life. Medawar and his colleagues went on to show that a combination of lymphocytes and antibodies circulating in the blood destroyed foreign cells in test animals that had not been given the opportunity to develop this tolerance. Thus, Medawar had identified the two distinct components of the body's immune defense system (see Box 1), humoral (antigen-antibody responses) and cellular (lymphocytes), and began to untangle their roles in the rejection of grafts. Of course, in so doing, he had also finally discovered Carrel's mysterious biological factor.

Following Medawar's work, early investigators in the field of human genetics had noticed that identical twins did not have this immunological barrier. For this reason, the first organ transplant was between the identical twins Richard and Ronald Herrick. In December 1954, Richard received one of Ronald's kidneys under the direction of surgeon Joseph E. Murray at the Brigham and Women's Hospital in



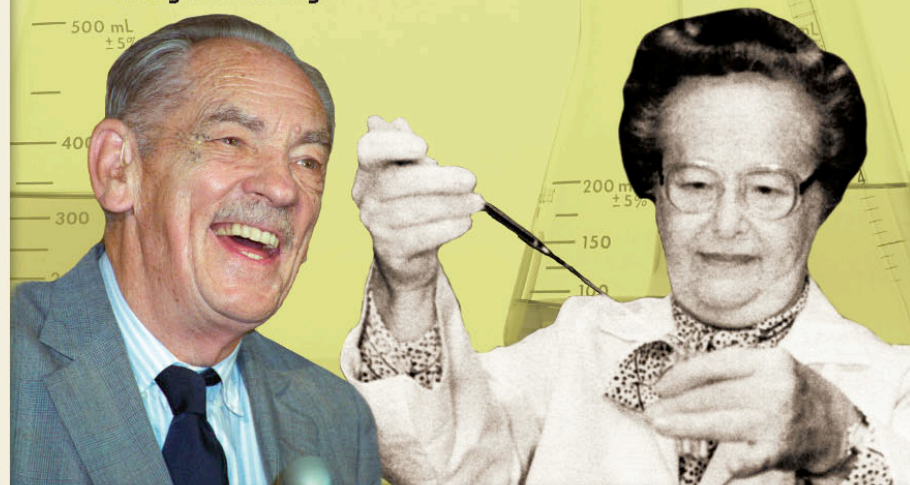
Richard and Ronald Herrick



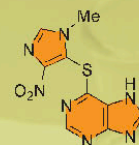
Joseph E. Murray

George H. Hitchings

Gertrude B. Elion

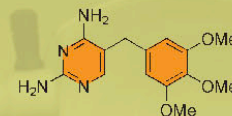


Box 2 Selected antimetabolite drugs developed by Hitchings and Elion



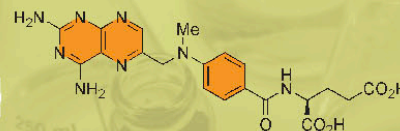
azathioprine [Imuran®]

(still used as part of a cocktail of drugs administered to prevent graft rejection, certain leukemias and Crohn's disease)



trimethoprim [Proloprim® Monotrim®]

(originally an anti-malarial drug, now used to combat urinary and respiratory tract infections)



methotrexate [Trexall®, Rheumatrex®]

(still used in cancer chemotherapy and to treat rheumatoid arthritis and psoriasis)

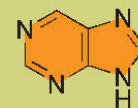


6-mercaptopurine [Purinethol®]

(still used as part of a cocktail of drugs administered to prevent certain leukemias and Crohn's disease)

Jean-François Borel

Hartmann F. Stähelin



purine

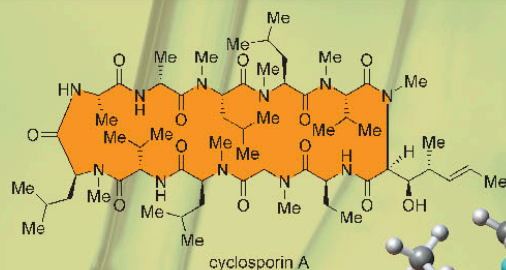


pyrimidine

Boston (Massachusetts, USA). Murray had first taken Richard and Ronald to the police in order to analyze their fingerprints to ascertain whether they were indeed identical twins. The success of the transplant was sealed soon afterwards, when a thriving Richard Herrick married the recovery room nurse who had tended to him. They had two children before disaster struck; Richard Herrick died eight years later, when the original kidney disease recurred in his newly transplanted organ. Murray and another pioneering transplant clinician, E. Donnall Thomas, from the Fred Hutchinson Cancer Research Center in Seattle (Washington, USA), received the 1990 Nobel Prize in Physiology or Medicine for "their discoveries concerning organ and cell transplantation in the treatment of human disease." In their writings, preceding the award of the Nobel Prize, both of these doctors reiterated that the great turning point in the long term success of transplant surgery came with the discovery and use of immunosuppressant drugs, beginning with antimetabolites such as azathioprine (Box 2).

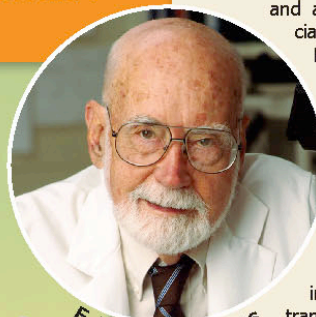
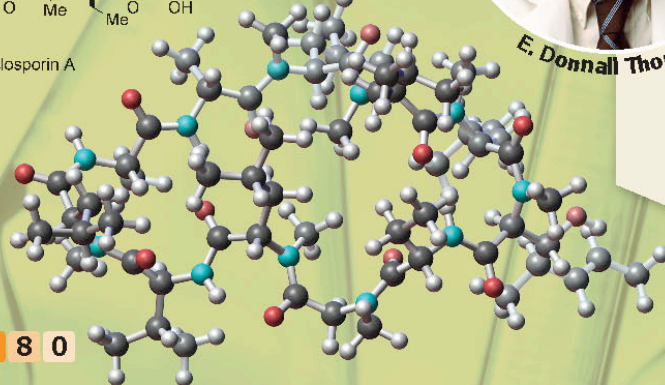
Laboratories in Tuckahoe (New York, USA), became interested in *The Antimetabolite Theory*. This concept had first been discussed in relation to the action of the sulfonamide antibiotics (see Chapter 13), which were proposed to act by mimicking a component (*para*-aminobenzoic acid, PABA) of the dihydrofolate metabolic pathway. This reaction sequence is responsible for the biosynthesis of nucleotide units for incorporation into DNA and the supply of cellular energy in the form of ATP (adenosine triphosphate). The sulfonamide substitute is incorporated up to a certain point, after which it blocks the dihydrofolate cycle. When the bacteria cannot make DNA they are unable to reproduce, and eventually die out. Hitchings theorized that, since the latter stages of this pathway are essentially the same in mammalian cells, small molecule inhibitors could also be used to attack other rapidly dividing cells, such as those that make up tumors. The naturally occurring purine and pyrimidines, which form the foundation of the genetic code and are components of nucleotides, were obvious templates on which to base such inhibitors. Elion undertook the synthesis of molecular mimics of these natural bases, analogs that would later be tested for potential biological activities. Elion and

Box 3 The molecular structure of cyclosporin A, the first discovered natural immunosuppressant



cyclosporin A

Ball and stick model of the molecule of cyclosporin A



E. Donnall Thomas

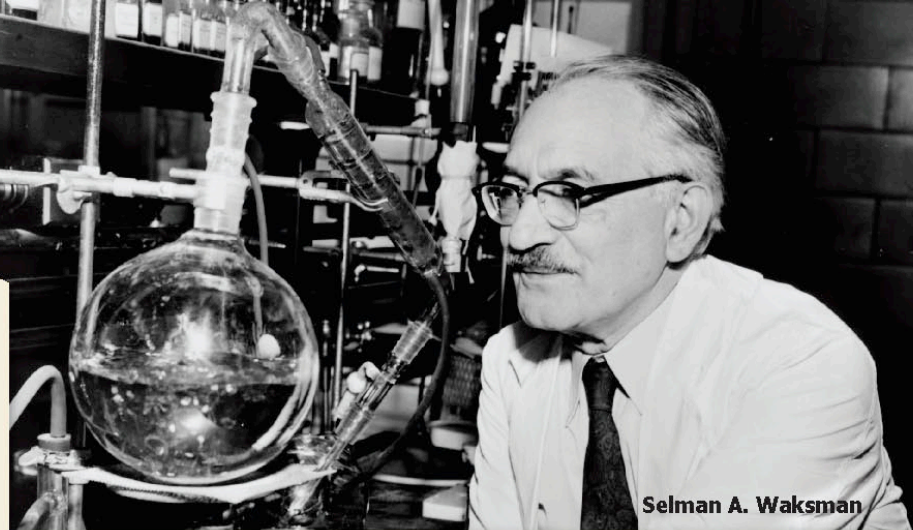
In the early 1950s, medicinal chemists George H. Hitchings and Gertrude B. Elion, working at the Wellcome Research

Hitchings made hundreds of purines and pyrimidines as part of their drug discovery program. Such programs typically involve the design, synthesis, and biological testing of many molecules in an iterative process that aims to fine-tune the structure of a compound in order to optimize its pharmacological properties. Early success came with the discovery of 6-mercaptopurine (6-MP), used to treat leukemia, and then azathioprine (Box 2). In 1958, two Boston doctors, Robert Schwartz and William Dameshek, rationalized that the body's immune response leads to an overproduction of lymphocytes in a manner not dissimilar to that involved in leukemia. Schwartz showed that Elion and Hitchings' 6-MP, when administered to rabbits for several days, prevented the production of antigens by the animal's immune system when blood from an alien species was injected into the test subject. A British surgeon, Sir Roy Y. Calne (Royal Free Hospital, London, UK), stimulated by the promise these results held for kidney transplantations, investigated such grafts in laboratory animals. He had significant success with 6-MP, but eventually the performance of this drug was overshadowed by that of azathioprine. By 1962, kidney transplants between unrelated human patients had become a therapeutic reality when accompanied by immunosuppression induced by azathioprine. E. Donnall Thomas subsequently managed to diminish organ rejection further with a treatment employing another of Hitchings and Elion's compounds, the cytotoxic anticancer compound methotrexate (Box 2). Over the ensuing fifteen years, some 25,000 kidney transplants were carried out, with the first liver (1963), lung (1963), and heart (1967) transplants also recorded in rapid succession. Later investigations showed that the success of this approach

is predicated on the fact that T-cell (T-lymphocyte) proliferation, which is key to the immune system response, is critically dependent on *de novo* synthesis (synthesis from scratch) of purines because these cells have a far reduced capacity to salvage and reuse nucleotides when compared with other cell types. Hitchings and Elion were awarded the 1988 Nobel Prize in Physiology or Medicine "for their discoveries of important principles for drug treatment." This prize was also shared with Sir James W. Black, another pioneer of medicinal chemistry. More about these biomedical researchers can be found in Chapter 33 (Small Molecule Drugs).

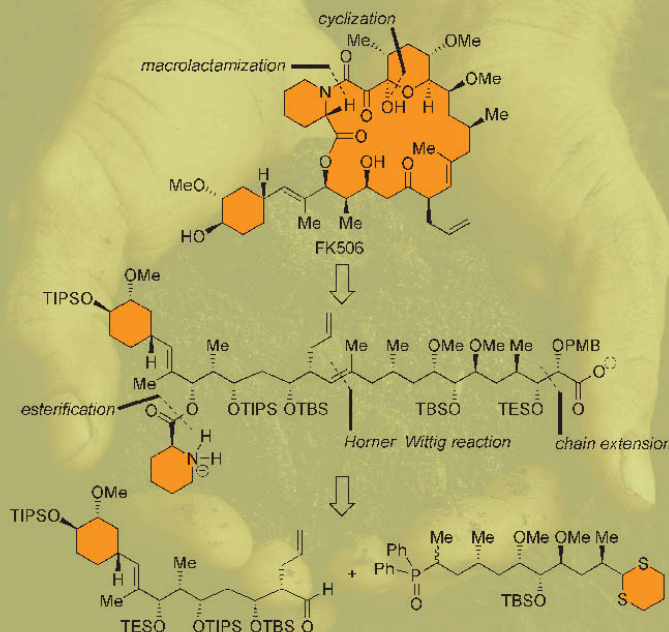
Azathioprine, methotrexate, and certain other related compounds are still in use today in cancer chemotherapy, and to counter certain autoimmune diseases in which the body's own immune system malfunctions and attacks itself, such as rheumatoid arthritis, multiple sclerosis, and systemic lupus. However, their use in post-transplant therapy has, for the most part, been superseded by natural products discovered in the 1970s and 1980s, beginning with cyclosporin (Box 3).

Selman A. Waksman's discovery of the natural antibiotic streptomycin from a soil sample in 1943, and its subsequent huge success in combating the scourge of epidemic tuberculosis, sparked the scientific fashion of examining soil samples in order to find potential drugs. The large number of medicinal leads discovered by this method has been ascribed to the abundance of defense chemicals produced by



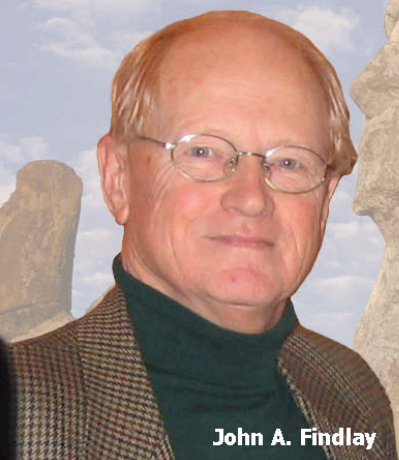
Selman A. Waksman

Box 4 Highlights from the Merck team's retrosynthetic analysis of FK506





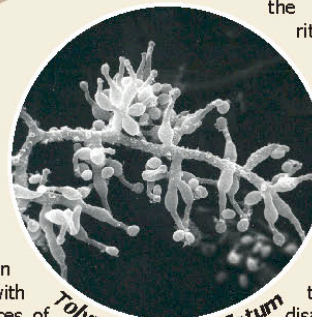
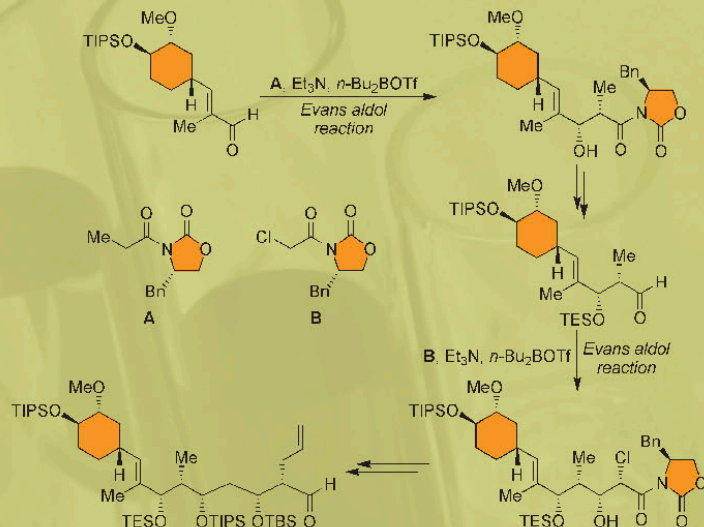
David A. Evans



John A. Findlay

Box 5

The use of the Evans auxiliary in the Merck total synthesis of FK506



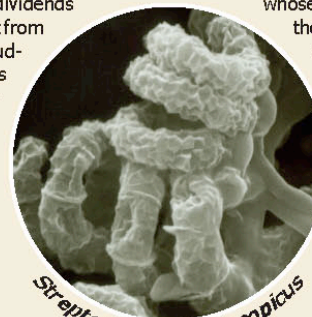
Tolypocladium inflatum

the millions of organisms living in the soil as they compete with each other for nutrients. This chemical warfare between germs has afforded us with one of the richest sources of bioactive molecules, and throughout this book you will encounter many examples, including erythromycin, avermectin, amphotericin, the epothilones, and thiostrepton (Chapters 17, 19, 20, 29, and 32, respectively). Cyclosporin was isolated in 1971 from a Norwegian soil fungus, *Tolypocladium inflatum* Gams, by a team under the leadership of two scientists from the Sandoz Pharmaceutical Company (now part of Novartis) in Switzerland. Antibacterial chemotherapy was their main investigative focus, and Jean-François Borel and Hartmann F. Stähelin initially found no practical therapeutic use for their new and complex discovery, since it did not exhibit useful levels of activity in any of their bioassays. Fortunately, they were sufficiently intrigued by its chemical properties to continue their research. Their persistence paid substantial dividends when it became evident from immune modulation studies that cyclosporin was a selective suppressor of T-cells. Sandoz then rushed to develop cyclosporin for immunosuppression chemotherapy, thus rescuing

the compound from obscurity. They started with full structural elucidation and synthesis of the peptidic fungal metabolite (comprising eleven amino acid units, including one previously unknown amino acid). It is interesting to note that the progress of cyclosporin to the clinic nearly met with disaster on one more occasion, because there was some

debate within the Sandoz management group as to whether organ transplantation would ever become a practical operation with broad applicability. In 1978, Sir Roy Calne, the British surgeon who had pioneered the use of antimetabolites in kidney transplant surgery, tested cyclosporin, first in pigs (which are good animal models for many drugs as they have much in common with humans) and then in human subjects, with excellent results. In the human patients, rejection was inhibited in five out of the seven cases where a new kidney had been received from mismatched deceased donors. By 1983, cyclosporin A had been approved by the regulatory authorities and entered the market as Sandimmun®. Throughout the 1980s, Sandimmun® (also sold as

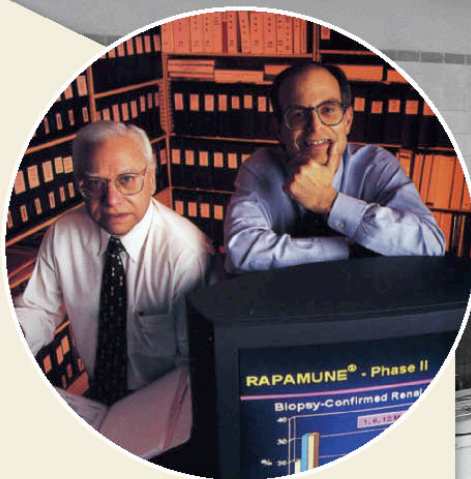
Neoral®) provided a lifeline for people whose failing organs had led them to depend on transplant therapy. Though still used today in more extreme cases, it quickly emerged that cyclosporin had a serious drawback; not only is it highly toxic, thus requiring cautious administration and dos-



Streptomyces hygroscopicus

age control, but it also increases patients' risk of developing cancer. It directly affects tumor growth, encouraging cancerous cells to divide and spread by inducing the synthesis of transforming growth factor- β (TGF- β). Furthermore, patients must also receive a high dose of steroids along with cyclosporin, to ward off the damaging side effects of this powerful drug. Even under these circumstances, however, the side effects meant that some patients experienced only a marginal improvement in their health after their transplant operation. Fortunately, new immunosuppressive agents that would improve the prognosis of transplant patients considerably were soon to appear on the horizon.

In 1975, Suren Sehgal and co-workers from the pharmaceutical company Ayerst Laboratories (now part of Wyeth) reported the identification of a new antibiotic produced by a streptomyces culture (*Streptomyces hygroscopicus*) contained in soil samples collected from Easter Island. Named rapamycin, after the native name for Easter Island (Rapa Nui), it had weak antifungal properties and marginal activity against a limited number of Gram-positive bacteria. As with cyclosporin, rapamycin could easily have become lost in the vast annals of unused natural products. It had simply been tested in the 'wrong' bioassay when isolated, although this could not have been recognized at the time. Three years later, John A. Findlay and co-workers (University of New Brunswick, Canada) completed the structural elucidation of the rapamycin molecule, based on spectroscopic analysis and x-ray



Above circle: Suren Sehgal (left), discoverer of rapamycin, and Dr. Joe Camardo (right), who was responsible for development of Rapamune® in the clinic and its current use in transplantation.

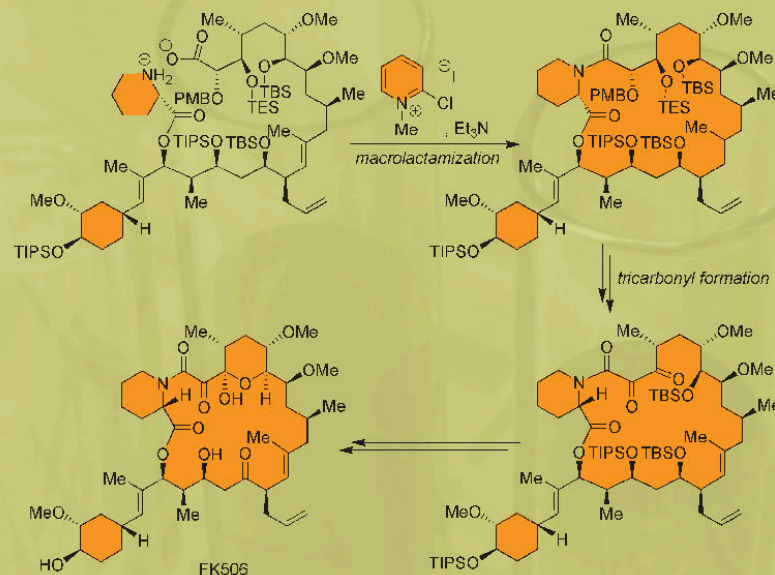


Thomas E. Starzl

crystallography. Their painstaking work unveiled an ornate macrocycle incorporating a highly sensitive triene system. Despite the new information regarding its beautiful structure, the development of rapamycin still stalled because a medicinal niche had yet to be found. A change in its fortune would only occur when it was reconsidered in the light of the discovery of FK506, another macrocyclic natural product with exciting biological properties.

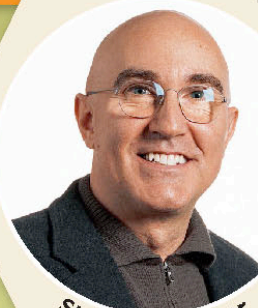
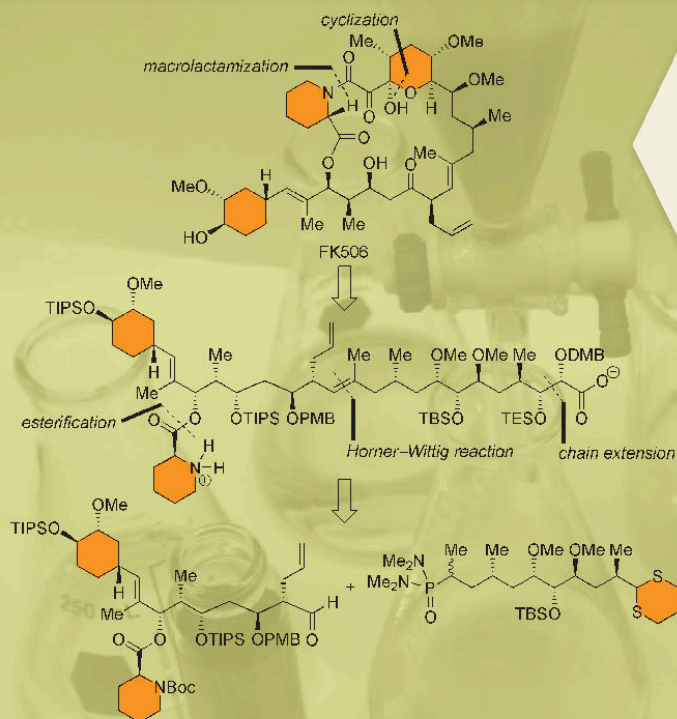


Box 6 The final stages of the Merck total synthesis of FK506



Box 7

Highlights of Schreiber's retrosynthetic analysis of FK506



Stuart L. Schreiber

FK506 was first reported in the scientific literature in 1987 by a group of scientists from the Fujisawa Pharmaceutical Company (Japan), who had been looking directly for immunosuppressor molecules in their bioassay-driven program to identify and isolate novel natural products. By now, you will not be surprised to learn that it too was isolated from a streptomyces culture (*Streptomyces tsukubaensis*). Its molecular structure features remarkable overlaps with that of rapamycin. Initial reports revealed that FK506 was more potent *in vitro* and *in vivo* (in the test tube and in live test animals, respectively) than cyclosporin. FK506 (sometimes also called tacrolimus), a smaller molecule than cyclosporin, although perhaps more structurally complex, was championed by Thomas Earl Starzl, a charismatic surgeon who ran the world's most successful and busiest, at the time, transplantation clinic at the University of Pittsburgh (Pennsylvania, USA). Despite early fears stemming from fatalities with laboratory dogs, Starzl had directed the first clinical trials for this drug by 1989 and had urged its continuing development in his well-known assertive and driven style. The findings from his studies were breathtaking, defying belief amongst those familiar with the field. The initial application of FK506 had been in patients whose new replacement organs were being rejected despite cyclosporin therapy. FK506 turned the prognosis of these people upside

down; some, who had been so ill that they were facing a second, and usually hopeless, transplant to replace failing and rejected organs, were rescued. In almost every case where FK506 was administered, dramatic improvements were seen, and in many of these patients the episodes of rejection petered out completely. FK506 was estimated to be between 50 and 100 times more powerful than cyclosporin on a gram per gram basis, a figure reflected in the dramatic increase in one-year survival rates seen once FK506 (marketed as Prograf®) became established as the preferred therapy to accompany transplant surgery. FK506 proved to be a resounding success, and it continues to be a wonderful drug today. In 2001, it recorded annual sales of \$416 million, and this figure looks set to grow as the use of cyclosporin declines.

As with any molecule that exhibits important medicinal properties, investigations relating to FK506 hastily became an intense cauldron of activity, heated by anticipation, competition, and rivalry. The very best minds from a range of scientific disciplines focused their energies on determining its mechanism of action and biochemistry, and devising plausible strategies for its total synthesis. At the center of the efforts, originating from both industrial and academic settings, emerged a young and talented chemistry professor, Stuart L. Schreiber, from Harvard University (Massachusetts, USA). He not only exhibited a strong passion for chemical synthesis, but was also a major proponent of research at the interface of chemistry and biology. Simultaneously, the Schreiber group, in collaboration with Matthew Harding (a rising star in immunology from Yale Medical School, Connecticut, USA), and a team from the pharmaceutical giant Merck (Rahway,

Prograf® capsules

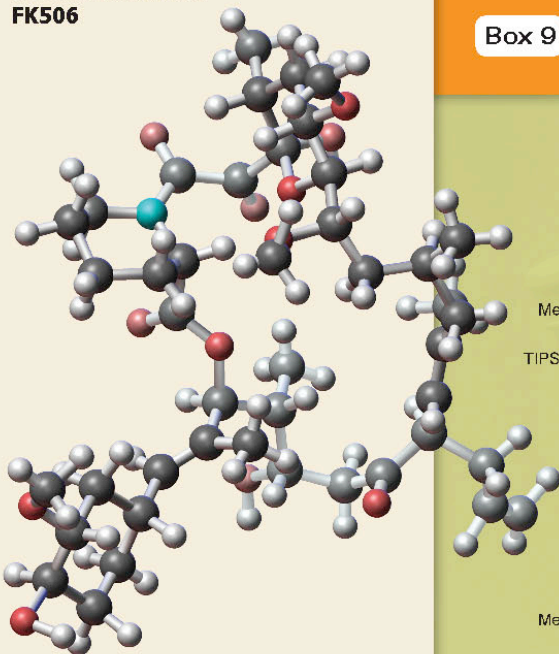


New Jersey, USA) independently identified and expressed quantities of the cellular target protein for FK506. The protein, named FKBP-12, was found to bind FK506, along with calcium and another cellular protein, calmodulin, inside the T-cell, thus disrupting the function of an enzyme called calcineurin. Details of the resultant cellular reverberations are beyond the scope of this text, but the effects are felt throughout a complex sequence of signal transduction events that govern the machinery of T-cells. Ultimately, the pathways that are blocked are those signaling and activating other T-cells (following the secretion of chemical messengers, such as interleukin-2). In addition, the FKBP-FK506 complex blocks the entry of resting immune cells into the reproductive section of a normal cell cycle (the transition from the G_0 to the G_1 phase of the cell cycle is prevented). Hindering T-cell activity in this manner prevents their proliferation with the net result being the shutdown of the immune system (Box 1). Studies by Robert E. Handschumacher and his colleagues at Yale University demonstrated that cyclosporin binds to a different protein, called cyclophilin. However, the resultant complex also inhibits the enzyme calcineurin, so, in essence, the same mechanism of action is in play for both of these agents. The proteins that bind these two immunosuppressors were given a common classification and collectively named the immunophilins. Alongside these intriguing biological investigations, Schreiber had also been working on the total synthesis of FK506. The task was completed within a year, along with the synthesis of a collection of analogues, which were to be used to probe the binding characteristics of the FKBP-FK506 complex. As is often the case in such circumstances,

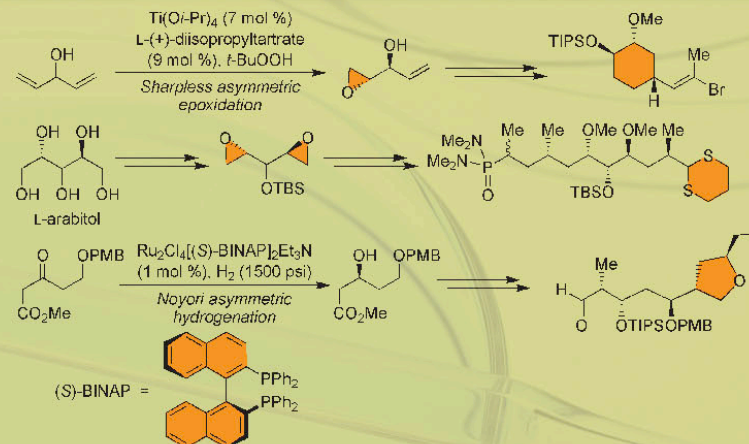
the synthesis had been a two horse race, and in the end the winners were a team of chemists from Merck led by Todd K. Jones, who published their FK506 total synthesis less than five weeks ahead of that of the Schreiber group (Boxes 4–9).

The FK506 molecule includes a rich array of stereochemistry, a striking 21-membered macrolactam ring, a pyran ring and a sensitive pipercolic acid motif. Of the numerous possible disconnections that could be envisaged for FK506, a number were selected by both the Merck team and the Schreiber group independently. Furthermore, in several key instances the two teams relied on the same powerful reactions to forge pivotal FK506 bonds in the synthetic direction. For example, an

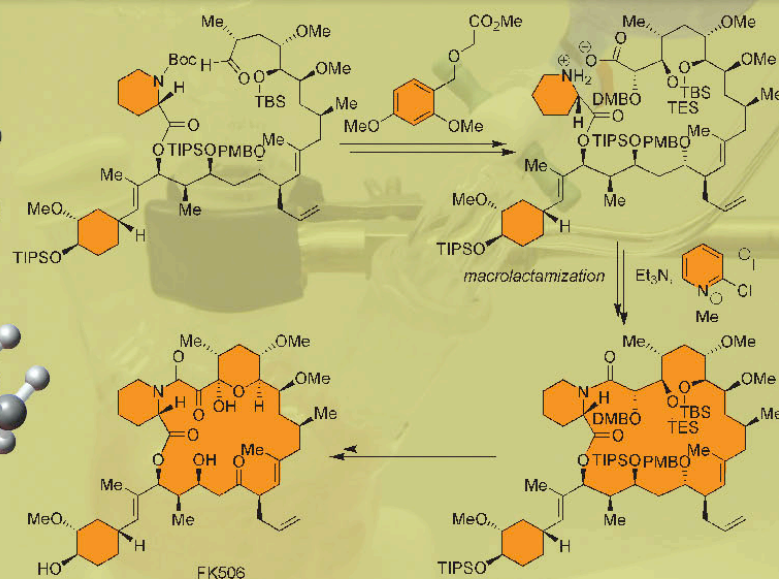
Ball and stick model of the molecule of FK506

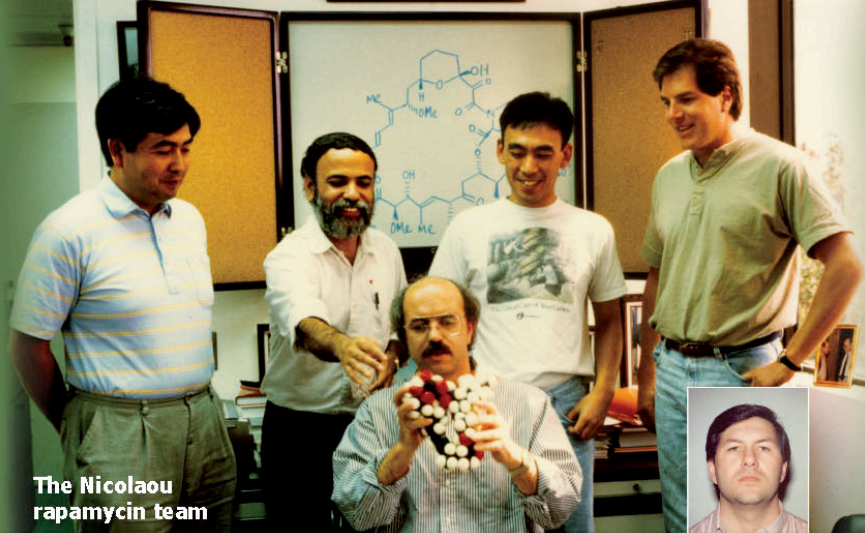


Box 8 Highlights from Schreiber's synthesis of FK506



Box 9 The completion of Schreiber's total synthesis of FK506

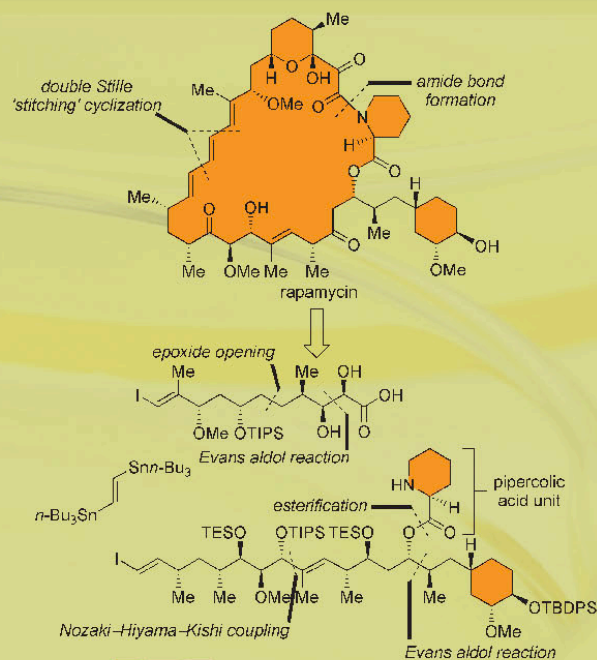




The Nicolaou rapamycin team

Box 10

Highlights from the Nicolaou team's retrosynthetic analysis of rapamycin



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Cyclosporin, FK506,
& Rapamycin

olefination was chosen by both groups to construct the endocyclic double bond, and, likewise, macrolactamization was used to join the pipercolic ring to an appropriate terminal carboxylic acid, thus accomplishing the final fusion of the macrocycle (Boxes 4 and 7). The two groups, however, employed different conditions in these pivotal reactions and used distinctly different strategies to build up the stereochemically complex linear precursors. Merck relied heavily on the stalwart chemistry developed by David A. Evans (Harvard University, USA) wherein oxazolidinone chiral auxiliaries are employed to direct aldol reactions (see Box 5 and Chapter 31). Schreiber's group relied on a more eclectic series of reactions to obtain the desired stereochemical arrays (Box 8). A Sharpless asymmetric epoxidation (see Chapter 26) forged two new stereocenters, an inexpensive sugar (arabitol) provided a series of asymmetric centers in another fragment, and finally the asymmetric reduction of a ketone provided the correct stereochemistry



Amos B. Smith, III



Steven V. Ley

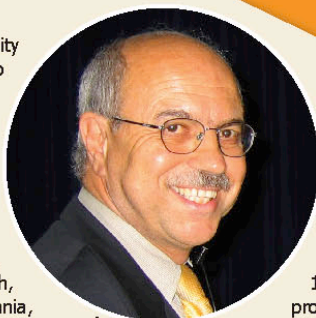


Samuel J. Danishefsky

at yet another key site. The ruthenium catalyst used in this last reaction, which employs a chiral phosphine ligand known as BINAP, was developed by Ryoji Noyori (see Chapter 28). The final delicate orchestrations of these approaches are summarized in Boxes 6 and 9. Since the reports of these syntheses in 1989 and 1990, a number of other groups have made worthy contributions in the form of formal syntheses of FK506 (i.e. novel syntheses that converge on a late intermediate from a previously known route).

When we introduced FK506 we commented on its structural overlaps with rapamycin. These similarities did not escape the attention of scientists working in the frenzied world of FK506 development. Suddenly, rapamycin was drawn into the limelight as its own immunosuppressive activity was confirmed. The race then began in earnest to tap into the potential of this natural product, and the chemical literature came to be filled with synthetic and biological studies focused on rapamycin. In 1993, three groups accomplished total syntheses of this challenging molecular architecture; first was that of K. C. Nicolaou of The Scripps Research

Institute and the University of California, San Diego (USA), followed in close succession by the groups of Schreiber and Samuel J. Danishefsky (then at Yale University, now at the Sloan-Kettering Institute and Columbia University, New York, USA). Later, Amos B. Smith, III (University of Pennsylvania, USA), Charles J. Sih (University of Wisconsin, USA), Steven V. Ley (University of Cambridge, UK), and Robert E. Ireland (University of Virginia, USA) added their own innovative variations. With these syntheses of the molecule accomplished, it quickly became apparent that rapamycin effects its immunosuppressive activity through a slightly different mechanism than FK506. This mechanistic dichotomy occurs despite the structural similarities between the two, a reminder of the functional intricacy and complexity of biological systems. Before we discuss its biological activity, we will highlight some details of how the first synthesis of rapamycin was accomplished by the Nicolaou group.



K. C. Nicolaou

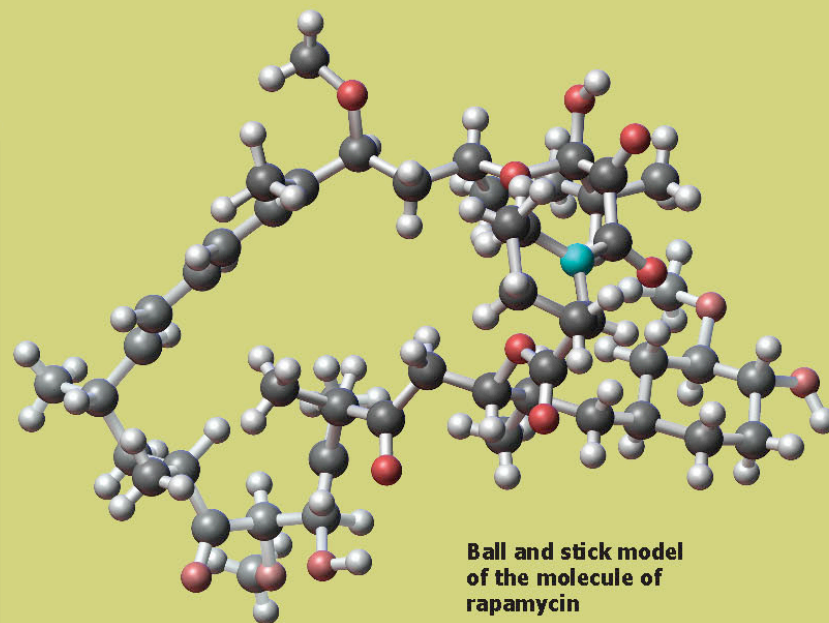
that complications arising from its sensitivity might be avoided (Boxes 10 and 11). They daringly proposed that this unit could be introduced via a double inter- then intramolecular, Stille reaction in a cascade sequence, simultaneously forming the triene moiety and closing the macrocyclic ring. Other key retrosynthetic disconnections included operations to remove the pipercolic ring motif by cleavage of the amide and ester bonds, thus revealing two major fragments for synthesis. The macrocyclic system of rapamycin is even more stereochemically rich than that of FK506, and the Nicolaou team opted to employ a broad range of techniques to access the desired stereoisomers. Certain stereocenters were derived from readily available naturally occurring substances; examples include a mannitol derivative (a sugar), citronellene (a naturally occurring terpene), and a derivative of vitamin C (ascorbic acid). A number of powerful asymmetric reactions were also used to synthesize pivotal building blocks with a high degree of convergency, such as several Evans auxiliary-mediated aldol reactions (see

Rapamycin possesses the same delicate pipercolic system as FK506, united to a similar pyran ring by the same 1,2-ketoamide unit. Rapamycin, however, contains the additional synthetic obstacle of a sensitive triene array as part of its intimidating 29-membered macrocyclic structure. The Nicolaou team chose this latter unstable motif as the first that should be excised in their retrosynthetic analysis. This proposal ensures that its inclusion would be left until a late stage of the synthesis, so



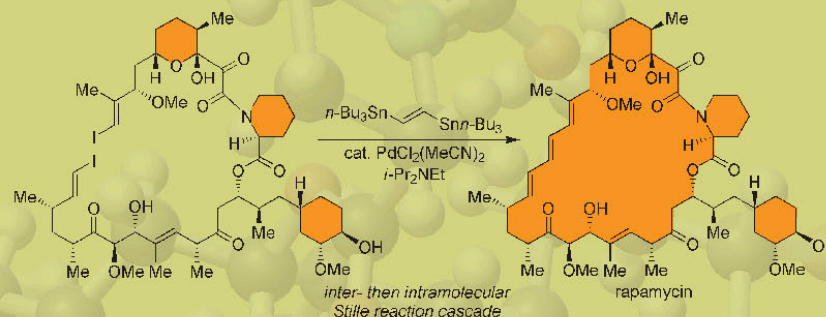
Irving Weissman

Chapter 31) and an asymmetric boron-mediated allylation, developed by Herbert C. Brown (see Chapter 18). After the many hurdles were overcome, the stage was set at last for the audacious macrocyclization event.



Ball and stick model of the molecule of rapamycin

Box 11 The double-Stille reaction to complete the Nicolaou team's total synthesis of rapamycin



The striking double-Stillé coupling used by the Nicolaou group to complete the synthesis of rapamycin (Box 11) belongs to a class of related reactions known collectively as palladium-catalyzed cross-couplings. These reactions constitute an important group of carbon-carbon bond-forming processes that has come of age only in the last thirty years. Although initially investigated using stoichiometric palladium (i.e. one part metal for each part substrate), these processes have now been refined to use catalytic quantities of this rather expensive metal.

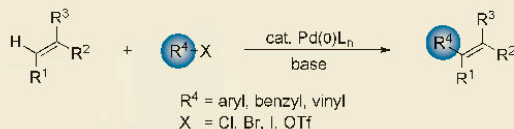
The earliest example of this class involves the coupling of an alkenyl or aryl halide or triflate with an alkene, which has come to be known as the Heck reaction, in honor of one of its pioneers, Richard F. Heck. The first examples of this coupling were reported by Mizoroki and Heck in 1971 and 1972, respectively. Since then, a number of other cou-

pling processes have been developed which differ primarily in the nature of the nucleophilic component. In each case, the reactions have been named after their primary developer, although it is important to remember that such developments are often influenced by the results of several groups. The Stille reaction, pioneered by John K. Stille and David Milstein, involves the coupling of an aryl or alkenyl halide with an alkyl tin reagent. This coupling reaction has found widespread use in total synthesis due to the mild conditions and convenient preparation of the organotin coupling partners. A related process involves the coupling of aryl and alkenyl boronic acids and esters, which has become known as the Suzuki reaction. The Sonogashira reaction replaces the organometallic component with a terminal alkyne. The Negishi coupling uses organozinc reagents, which can be prepared directly from organohalides, or from other organometallics. This reaction

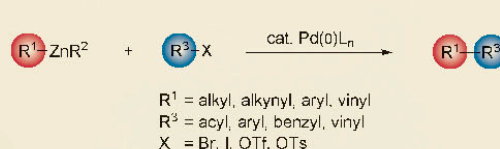
predates the Stille and Suzuki procedures, but has gained popularity in total synthesis only recently.

The Tsuji-Trost reaction, another palladium-catalyzed coupling process, involves nucleophilic substitution at an allylic position. Allylic esters and carbonates are the most commonly employed electrophiles, and they couple with a range of carbon nucleophiles. This process is particularly useful for the coupling of two alkyl units, and the asymmetric variant is commonly used in total synthesis. The scope of palladium-catalyzed coupling reactions continues to expand and, in addition to the reactions described here, a range of other processes to form carbon-carbon and carbon-heteroatom bonds are gaining popularity. The latter reactions have been developed recently primarily by Stephen L. Buchwald (Massachusetts Institute of Technology, USA) and John F. Hartwig (University of Illinois at Urbana-Champaign, USA).

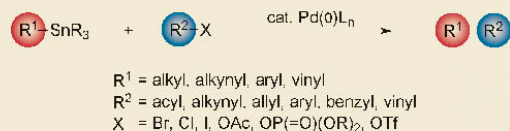
The Heck Reaction (pioneer - R. F. Heck, 1972)



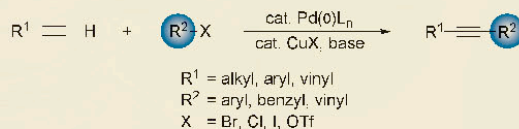
The Negishi Reaction (pioneer - E. Negishi, 1977)



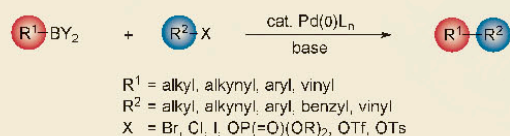
The Stille Reaction (pioneers - J. K. Stille and D. Milstein, 1978)



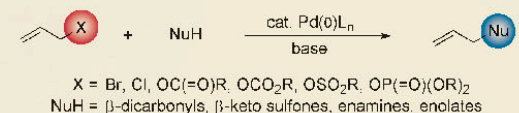
The Sonogashira Reaction (pioneers - K. Sonogashira, R. F. Heck and L. Cassar, 1975)



The Suzuki Reaction (pioneers - A. Suzuki and N. Miyaura, 1979)

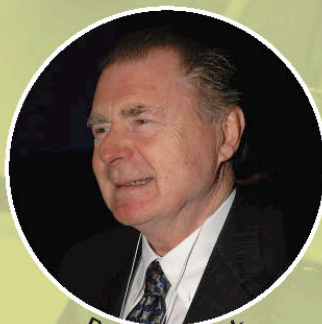


The Tsuji-Trost Reaction (pioneers - J. Tsuji, 1965 and B. M. Trost, 1976)





Barry M. Trost



Richard F. Heck



John F. Hartwig



Akira Suzuki



John K. Stille



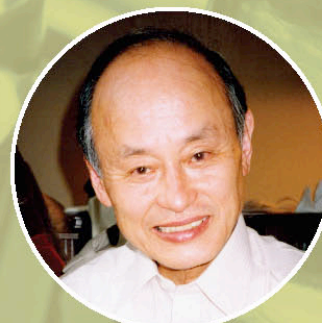
Stephen L. Buchwald



Ei-ichi Negishi



David Milstein



Jiro Tsuji

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B. Werth, *Billion-Dollar Molecule: The Quest for the Perfect Drug*, Simon and Schuster, New York, **1994**.

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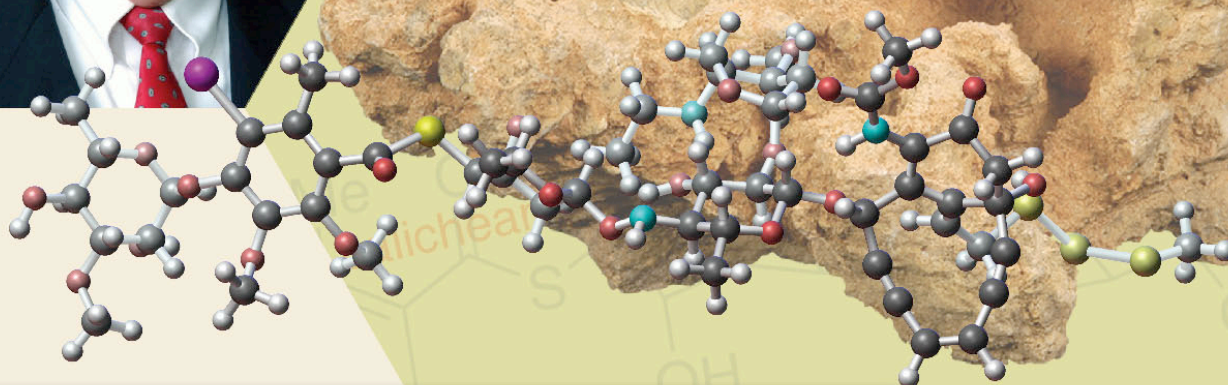
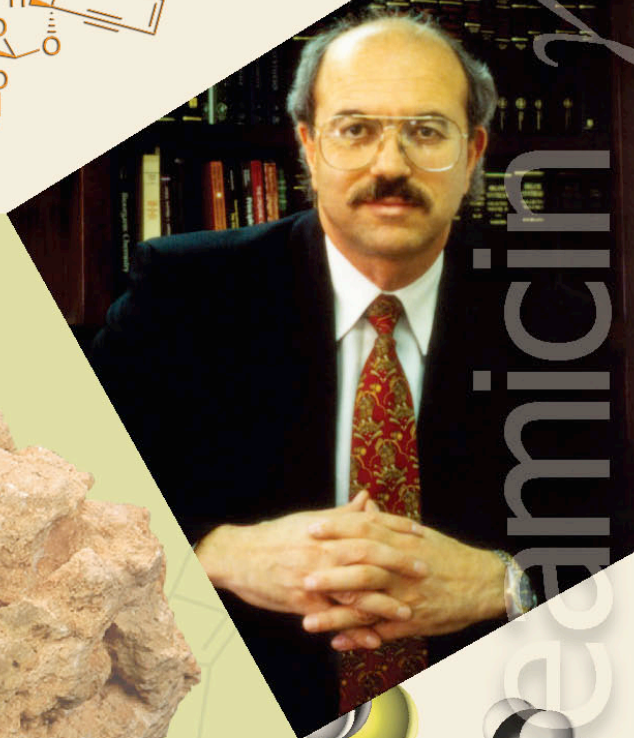
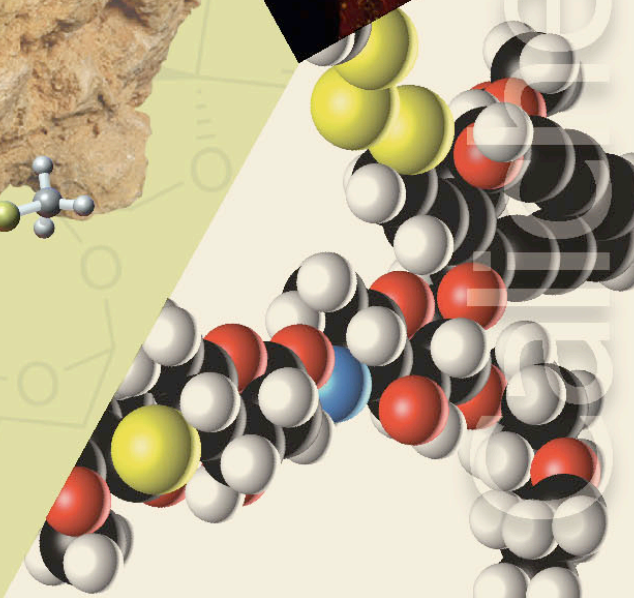
Fortunately, their daring strategy paid off and rapamycin, triene intact, was isolated from this cascade Stille reaction (Box 12), thereby completing the total synthesis (Box 11).

The elucidation of the mechanism of action of rapamycin was once again spearheaded by Schreiber, but with several other scientists playing prominent roles, most notably Irving Weissman at Stanford University (California, USA). Their discoveries were both fascinating and highly relevant to the future practice of immunotherapy. In addition, the new knowledge that was obtained explained much about cytoplasmic signal transduction, an area of cellular biology that had long been shrouded in mystery. Early studies had shown that rapamycin binds to the same protein, FKBP-12, as FK506 in the cytoplasm of the cell. It came as no surprise to the investigators that the most important binding sites on the two natural products were located in their common structural domain. Both of the natural products were shown to inhibit a rotamase activity of the enzyme, but then their effects diverge. Rapamycin does not inhibit calcineurin, and therefore does not act on the same signaling pathway as FK506 and cyclosporin; rather, it blocks a regulatory kinase involved in a later pathway associated with T-cell activation. The FKBP-rapamycin complex blocks the progression of many cell types through an early phase of the cell cycle (at the G₁ to S phase transition), thereby causing cell cycle arrest.

Ultimately, this difference has presented benefits to patients with respect to other proliferative disorders such as cancer. As mentioned earlier, cyclosporin, and to some extent FK506, has a propensity to promote tumor growth. With cancer as one of the main causes of death in post-operative transplant patients, rapamycin, fortunately, have been found to have the opposite effect in that it inhibits primary and metastatic tumor growth. Relatively small doses of rapamycin can cause regression in tumors, but only after angiogenesis. In simple terms, rapamycin stops, or at least slows down, the growth of the blood supply that the tumor develops to nourish itself after it has grown too big to rely on hijacking the blood supply of proximal tissue. Furthermore, this remarkable molecule also moderates antibody production, another major constituent of the immune response. The study of the biochemistry of rapamycin is still relatively youthful, and as it is further unraveled it promises to teach us much more in coming years. It is quite possible that a host of other medical benefits will emerge from this intrepid journey of discovery.

Rapamycin, also sometimes known as sirolimus, was approved by regulatory authorities (Food and Drug Administration, USA) in September 1999 and is marketed by Wyeth as Rapamune®. It looks increasingly likely that Rapamune® will become the immunosuppressant of choice for transplant patients, given its advantages over FK506 and cyclosporin. As we herald the dawning of these miracle drugs, we should keep in mind that transplants have only become as routine as they are today due to the heroic efforts of the chemists, biologists, and clinicians who discovered these natural products and developed them into the useful medications.



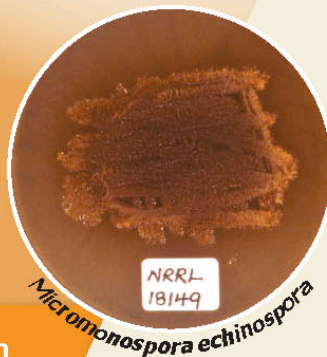
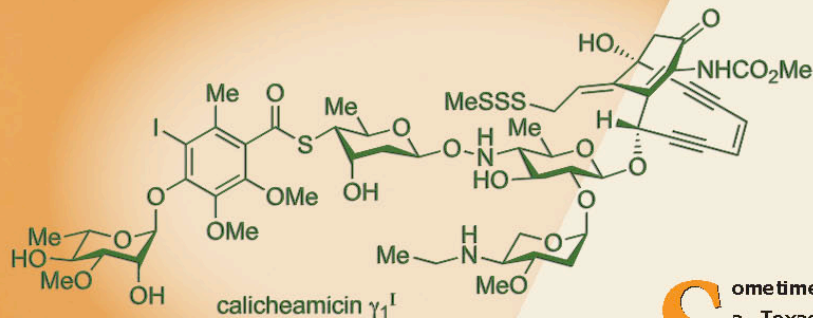


Chapter 23

1992



Robert G. Bergman



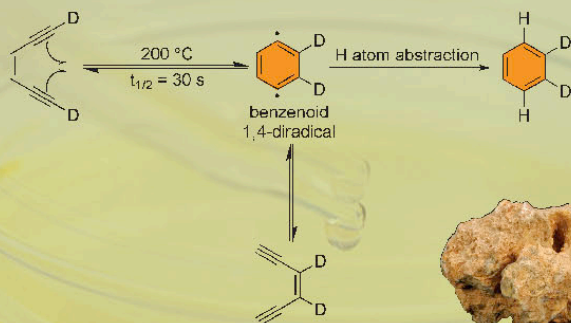
Sometime in the 1980s, beside a Texas highway, a touring scientist from Lederle Laboratories picked up a pebble. Living inside this chalky rock were bacteria, subsequently identified as *Micromonospora echinospora*, subspecies *calichensis*, the name having been derived from the Greek word for a small lime stone pebble, *caliche*. These bacteria were cultured in the laboratory and their metabolites screened for useful biological activity. This led to the discovery of a phenomenally cytotoxic agent, called calicheamicin γ_1^I , which was destined to become the most prominent member of the then newly emerging enediyne family of antitumor antibiotics. Apparently, these bacteria had evolved over the eons with a super-weapon for waging chemical warfare against their competitors and potential predators.

Harnessing calicheamicin γ_1^I and using the valuable knowledge derived from this beautiful molecule, scientists have been able to develop a new weapon for use against cancer, in the form of

Mylotarg®. This drug represents a significant new advance in the treatment of cancer wherein the cytotoxic molecule is delivered, like a 'guided missile', directly to cancer cells. To achieve this feat, calicheamicin γ_1^I was chemically linked to a monoclonal antibody, forming a unique molecular assembly that constitutes an exciting new approach to cancer chemotherapy (for a more detailed discussion of cancer and its treatment, see Chapter 25). To fully appreciate the development and efficacy of this approach, we must first consider the chemistry of calicheamicin γ_1^I and, in particular, the properties of the enediyne structural motif.

Annulenes and aromaticity make seemingly esoteric subjects for study within organic chemistry. In spite of this, these topics captivated a generation of pioneering organic chemists during the second half of the twentieth century. In 1971, Robert G. Bergman, then at the California Institute of Technology (CalTech, USA), made an important observation. Following the works of Franz Sondheimer (University College London, UK) and Satoru Masamune (University of Alberta, Canada), he designed experiments that demonstrated the cycloaromatization reaction of conjugated enediyne compounds through a hypothetical benzenoid diradical intermediate (Box 1). This process came to be known as the Bergman cyclization reaction. Bergman's work would later become central to the study of the calicheamincins and related naturally occurring enediynes. A Bergman cyclization is the key event in the mechanism by which these compounds exert their cytotoxic activity. Cell death is caused when the calicheamicin γ_1^I molecule undergoes a series of transformations within the target cell that alter its conformation, triggering a Bergman cycloaromatization to

Box 1 The Bergman cycloaromatization reaction



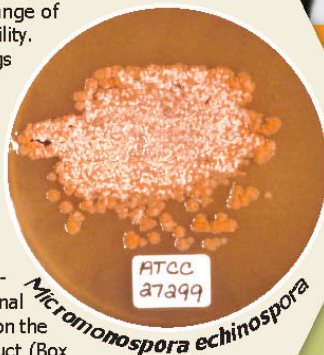
The pebble (*caliche*) from which the calicheamicin γ_1^I producing bacteria were isolated

give a benzenoid diradical that severs both strands of any nearby DNA molecule.

An early indication of the small victory over cancer that was to follow came in 1985 when the antitumor antibiotic neocarzinostatin was discovered in Japan (Box 2). This material consists of a highly unstable chromophore molecule, which includes an epoxy-diyne moiety shielded within a protective protein molecule. Further investigations into this seminal finding were, however, overshadowed by a spate of papers published in 1987 by scientists from the Lederle Laboratories and Bristol-Myers companies in which the structures of the calicheamicins and their cousins, the esperamicins, were disclosed. The biological assay-driven identification of these extraordinarily potent anticancer antibiotics had provided the impetus for their isolation and structural elucidation, which revealed that this new class of molecules boasted magnificent molecular architectures, including a particularly intriguing and unprecedented, conjugated, enediyne structural motif. Since these early reports, a number of other enediyne natural products have been discovered. Their mesmerizing chemical structures, combined with their spectacular biological properties, generated great interest and excitement and prompted many chemists to initiate programs directed towards their total synthesis. In order to meet this formidable synthetic challenge, chemists first had to develop an understanding of how these beautiful molecules exert their lethal action in nature.

Even more than the other naturally occurring enediynes, calicheamicin γ_1^1 is a masterpiece of molecular design. The carefully engineered central core incorporates the deadly enediyne motif along with a number of finely balanced architectural features, ensuring that the cytotoxic

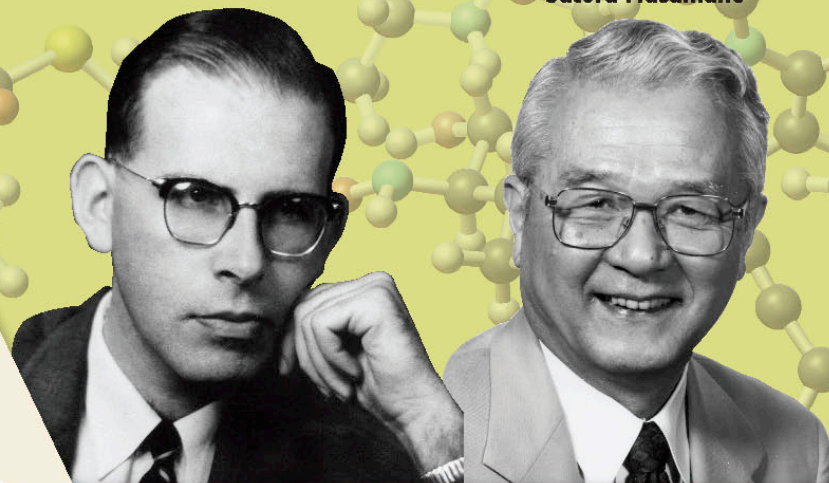
effects of the molecule are manifested only in the right circumstances. Thus, the producing bacteria can manufacture this powerful cytotoxin and yet avoid falling victim to it themselves. The first key element of the design is the incorporation of the enediyne group within a ten-membered ring. The acyclic enediynes originally investigated by Bergman (Box 1) require excessively high temperatures to effect the cyclization. Chemists have since constructed enediynes within a range of ring sizes and studied their stability. Those within ten-membered rings were found to undergo Bergman cyclization at body temperature. Within calicheamicin γ_1^1 , the ends of the enediyne motif are held at a distance by the rigid shape of the bridged bicyclic skeleton, preventing cyclization. The first step in the cascade that brings about the Bergman cyclization is the attack of an external nucleophile (such as glutathione) on the trisulfide unit of the natural product (Box 3). This generates a thiolate anion, which is perfectly positioned to attack the enone group in an intramolecular conjugate addition. The conversion of the bridgehead enone into a tricyclic ketone causes a structural change, bringing the ends of the enediyne system closer together and triggering the Bergman cyclization reaction. The resulting diradical abstracts hydrogen atoms from the sugar-phosphate backbone of the DNA of the target cell, initiating a further sequence leading



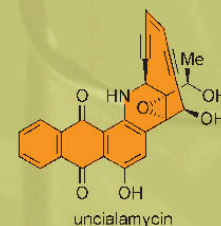
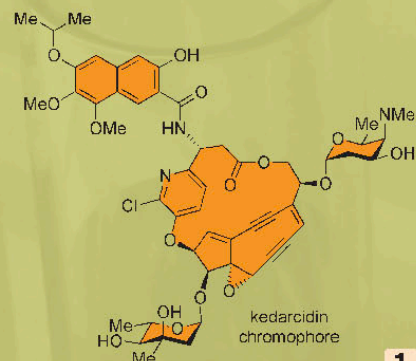
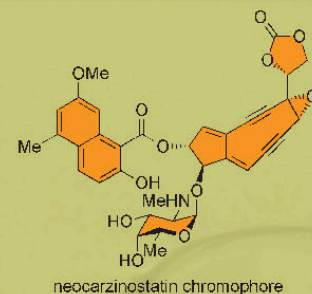
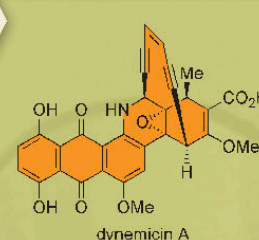
Micromonospora echinospora
ATCC 27399

Franz Sondheimer

Satoru Masamune

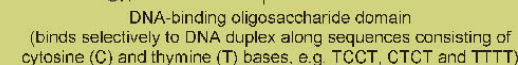


Box 2 Selected natural enediynes





Proposed mechanism of action of calicheamicin γ_1 ¹

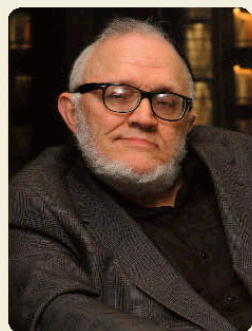
Calicheamicin γ_1^I

194

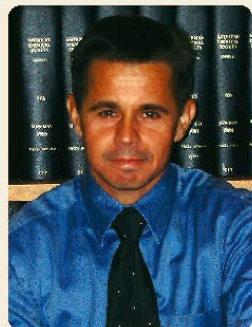
Inspired by the stunning architecture of calicheamicin γ_1^I , chemists began to consider how to achieve its total synthesis. Despite the daunting challenge posed by such a target, some dared to embark on a journey that was sure to be full of adventure and risk, reasoning that the opportunities it held were too valuable to ignore. The first to complete this task were K. C. Nicolaou and his group, who published their total synthesis of calicheamicin γ_1^I in 1992, after a five-year campaign. Their work on the enediyne was multifaceted from the start and involved concurrent investigations on several fronts, including molecular design, chemical synthesis, and chemical biology studies. The crowning achievement was the total synthesis



The first, a highly complex oligosaccharide domain, confers the sequence specificity to the binding of this molecule within the minor groove of the DNA duplex, while the second domain, the aglycon, consists of the enediyne warhead. The oligosaccharide fragment is far from being just a simple carbohydrate anchor for the impressive enediyne moiety. Its own intimidating structure includes a fragile N—O bond that bridges two of the carbohydrate units through a challenging β -glycoside bond, and a thioester linkage connecting a rare hexasubstituted aromatic ring to one of the sugar units. Each of the building blocks identified in the retrosynthetic analysis (Box 4) of this part of the molecule would require its own careful assembly. For the aglycon, the retrosynthetic analysis suggested the strategy shown in which



Samuel J. Danishefsky



Andrew G. Myers



Masahiro Hirama

key bonds would be formed by an intramolecular acetylide addition to an aldehyde, an intramolecular nitrile oxide/olefin cycloaddition, and a Sonogashira coupling. Having decided upon a synthetic strategy, the Nicolaou group proceeded to construct the key building blocks required for the final stages of the synthesis. Needless to say, the roads to these goals were both arduous and demanding, for each of these intermediates presented unique synthetic challenges, but the endeavor also proved rewarding and, in a relatively short period of time, the two main fragments of calicheamicin γ_1^I had been synthesized and were ready for unification. A successful glycosidation reaction, facilitated by a Lewis acid at low temperature, locked the entire carbon skeleton of the target molecule in place (Box 5). Following the introduction of the trisulfide unit (the molecule's triggering device) and the unmasking of several protected functional groups, the total synthesis of calicheamicin γ_1^I was completed on the September 29, 1992, at the Scripps laboratories of the Nicolaou team. With this extraordinary structure reached by total synthesis, the field was entering a golden age of unprecedented accomplishments, as the subsequent decade was to witness.

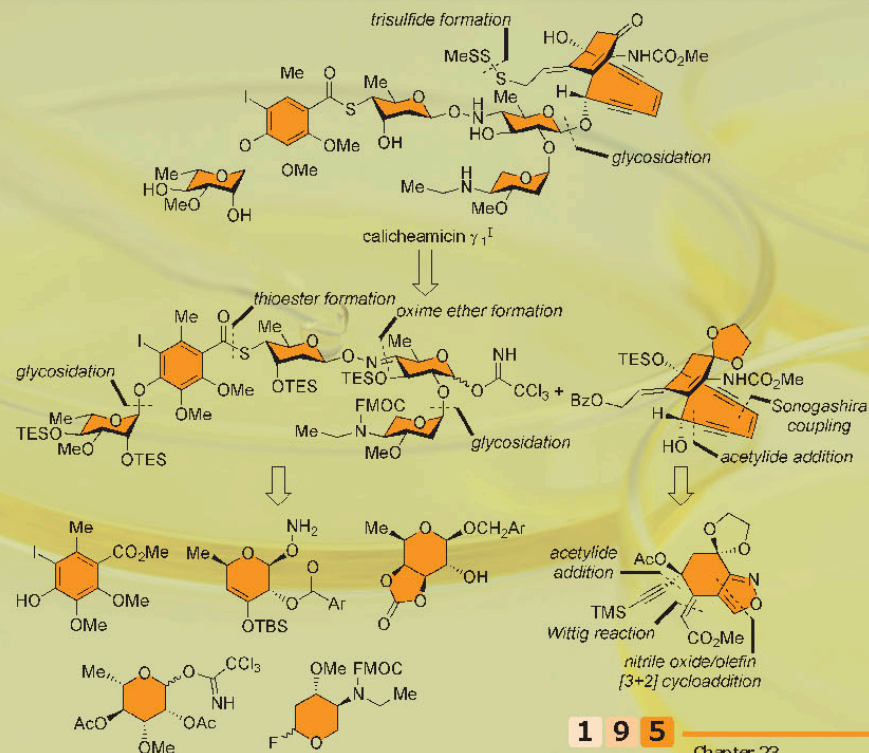
Samuel J. Danishefsky and his group, then at Yale University (USA) accomplished a second total synthesis of calicheamicin γ_1^I , in 1994, adding a host of further discoveries and inventions to the collection of those made during the Nicolaou synthesis. The challenges posed by the enediyne class led many groups to attempt syntheses of the various members, however, only a few such adventures have ended in success. Amongst these successes are two independent syntheses of dynemicin A (Box 2), published in 1995, by the groups of Andrew G. Myers (then at CalTech) and Danishefsky (now



K. C. Nicolaou

Box 4

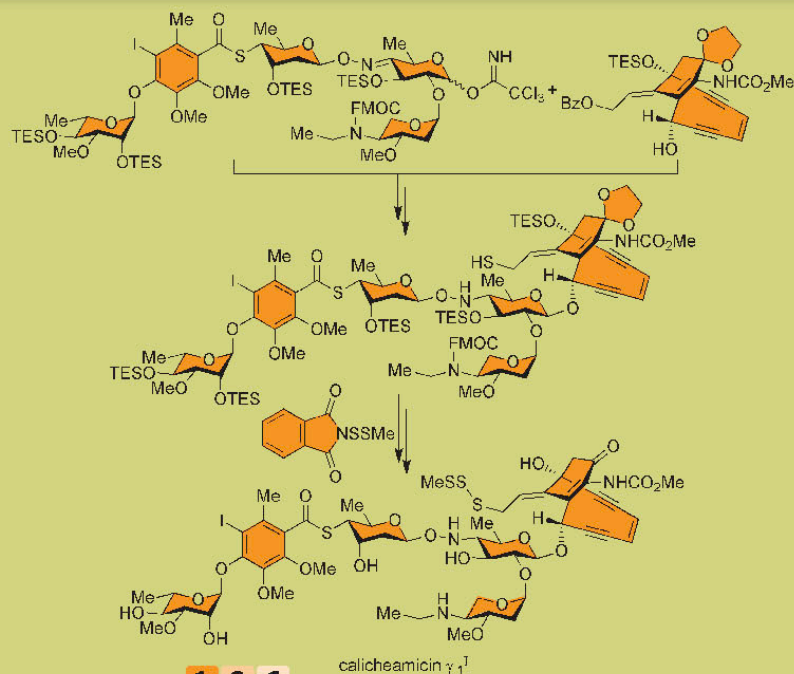
The Nicolaou group's retrosynthetic analysis of calicheamicin γ_1^I





The Nicolaou calicheamicin γ_1^1 team

Box 5 The final stages of the Nicolaou group's total synthesis of calicheamicin γ_1^1



Calicheamicin γ_1^1

1 9 6



César Milstein

at the Sloan-Kettering Institute and Columbia University, USA), and two of the neocarzinostatin chromophore, by the Myers group (now at Harvard University, USA) in 1998, and the Masahiro Hiram group (Tohoku University, Japan) in 2006.

Despite falling to the advances of total synthesis in the early 1990s, it would be a further eight years before calicheamicin γ_1^1 could be tamed to afford an approved drug for the treatment of cancer. The development of Mylotarg[®] was made possible not only by advancements in chemistry, but also by the increased understanding of our immune system, which had developed over the preceding thirty years. In 1984, the Nobel Prize in Physiology or Medicine was awarded to Niels K. Jerne, Georges J. F. Köhler, and César Milstein, in part for their "discovery of the principle for production of monoclonal antibodies." To generate a monoclonal antibody (which has been chosen because it will attack disease ridden cells), the antibody-synthesizing cells (B cells) are fused to specially treated tumor cells, which proliferate rapidly. The result is a hybrid cell line that can produce large numbers of monoclonal antibodies with absolute integ-

rity (i.e. clones). Early attempts to use this approach to treat disease in humans were complicated by the fact that the patient's own immune system often attacked the foreign antibodies, causing a worsening of the illness.

The problem of rejection has been overcome in recent years by transplanting the engineered antigen recognition domain of the cloned antibody onto human antibodies to furnish chimeric (~66 % human) or humanized (~90 % human) antibodies. This new technique has now been applied to the production of antibodies designed to attack specific tumor cells with admirable success. The anticancer drugs Rituxan[®] (developed by Idex, now Biogen Idec) and Herceptin[®] (developed by Genentech) have recently been introduced to treat non-Hodgkin's lymphoma and breast cancer, respectively, and are already significantly enhancing the prospects for patients. The monoclonal



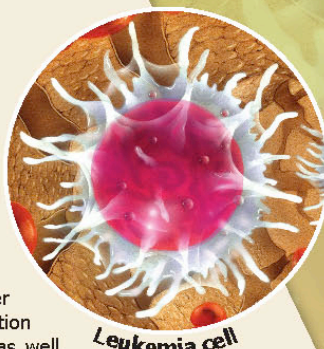
Georges J. F. Köhler and Niels K. Jerne

antibodies are frequently administered in combination with a chemotherapeutic agent, such as Taxol® (see Chapter 25), in order to double the force of the attack on the tumor. The application of monoclonal antibodies has expanded considerably as a result of phage display. This technique was pioneered by George P. Smith (University of Missouri, Columbia, USA), and further developed by the groups of Richard A. Lerner (The Scripps Research Institute, La Jolla, USA) and Sir Gregory Winter (Medical Research Council Laboratories, University of Cambridge, UK), for the production and screening of very large combinatorial libraries of antibodies. This application of phage display was exploited by scientists at Abbott Laboratories to discover Humira®, a monoclonal antibody for the treatment of rheumatoid and psoriatic arthritis. Further discussion on antibody therapies can be found in Chapter 34.

Mylotarg® represents the natural evolution of the antibody/chemotherapy combination strategy, as it consists of a chemotherapeutic agent, calicheamicin γ_1^I , chemically bound to a delivery system targeting certain leukemia cells, a monoclonal antibody. The antibody domain was designed to bind specifically to an adhesion protein found on the surface of leukemia cells. The pioneering research that culminated in the approval of Mylotarg® for the treatment of relapsed acute myeloid leukemia was led by George Ellestad, Philip Hamann, and Janis Upeslacis, working at Lederle Laboratories (later American Home

Products and now Wyeth). Their team modified calicheamicin γ_1^I and designed a linker that maintained the function of the cytotoxic agent as well as that of the antibody. The overall result was a powerful molecular weapon capable of delivering a very effective and carefully directed two-fold blow against leukemia cells, which utilized the very best of what the biological and chemical sciences had to offer at the end of the twentieth century. This advance, which comes closer than any previous attempts to reaching the magic bullet ideal of chemotherapy, is continuing to impress clinicians, who are now able to treat certain types of cancer with unprecedented rates of success. During the twenty-first century, we can expect the development of more targeted chemotherapeutic agents in order to help in the fight against cancer.

The design of calicheamicin γ_1^I has been honed by nature to form a molecular machine that is ideally suited to perform its given function. The evolutionary refinement of this molecule has now been combined with the ingenuity of scientists to furnish a formidable weapon against



Leukemia cell



Herceptin®

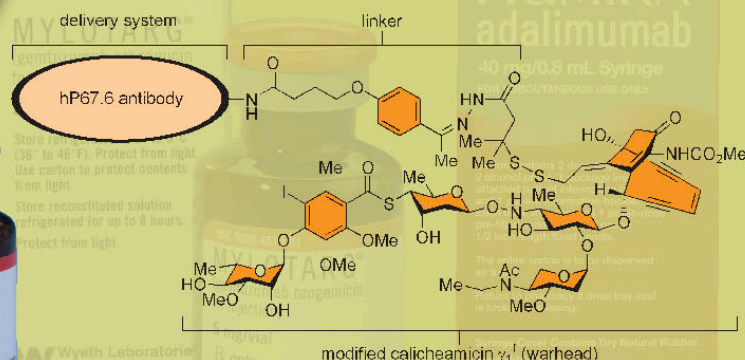


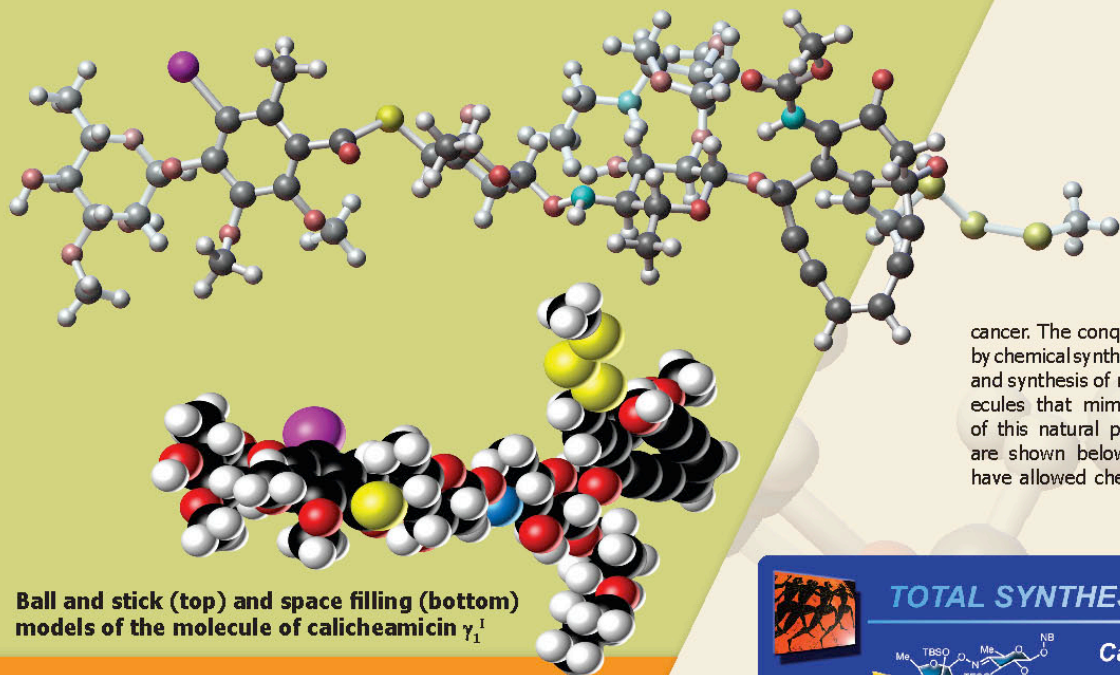
George Ellestad

Philip Hamann

Janis Upeslacis

Box 6 Mylotarg®, a molecular weapon targeting cancer cells





Ball and stick (top) and space filling (bottom) models of the molecule of calicheamicin γ_1^I

Further Reading

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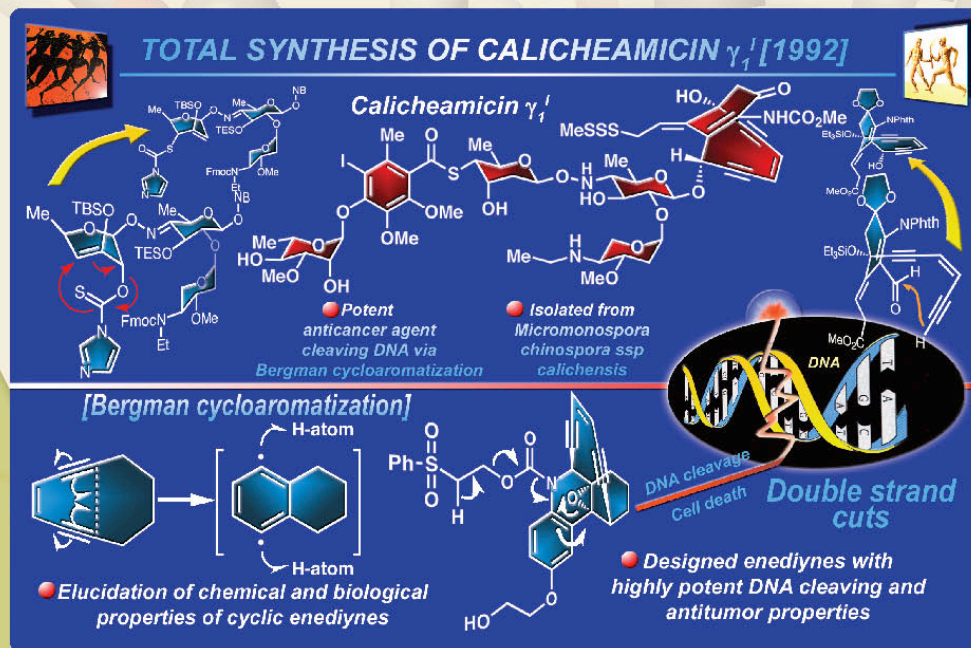
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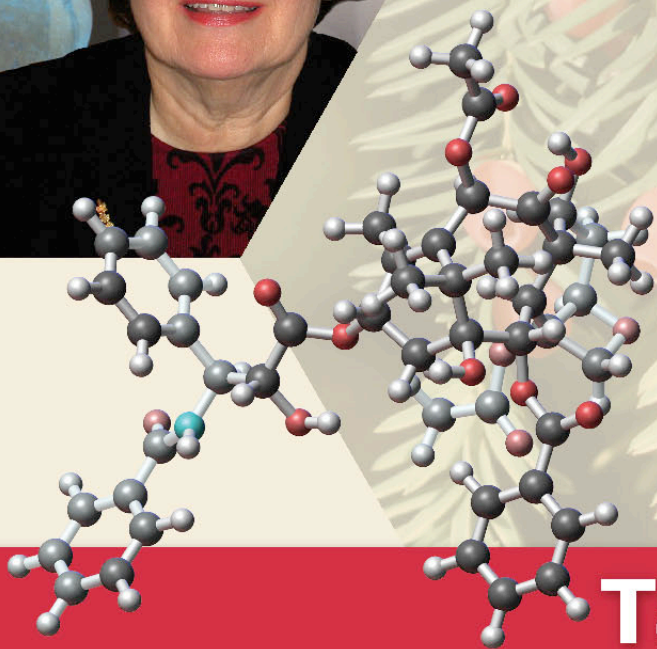
E. L. Sievers, M. Linenberger, Mylotarg: Antibody-Targeted Chemotherapy Comes of Age, *Curr. Opin. Oncol.* **2001**, *13*, 522–527.

C. Ezzell, Magic Bullets Fly Again, *Sci. Am.* **2001**, *285*, 34–41.

cancer. The conquest of calicheamicin γ_1^I by chemical synthesis facilitated the design and synthesis of numerous eneidyne molecules that mimic the biological action of this natural product (some examples are shown below). Synthetic eneidyne have allowed chemists to probe multiple

chemical and biological issues relevant to biomedical research. Synthetic organic chemistry has, once again, played a pivotal role in organizing and driving forward the frontiers of the combined biological and chemical sciences for the betterment of mankind.

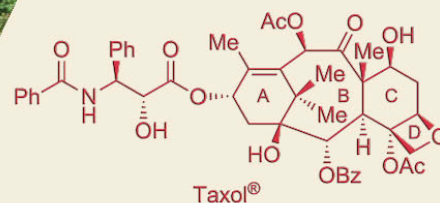




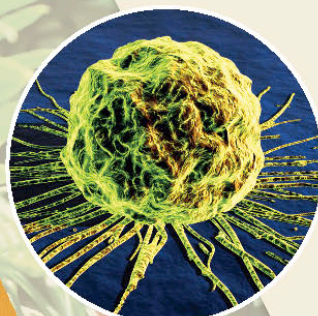
1994



Taxus brevifolia



The legend of Taxol® reveals a prime example of how natural products and chemical synthesis influence things at the very heart of our lives. It is a tale of hope for humanity which also brims with scientific drama and intrigue. Taxol® is one of the best-selling anti-cancer drugs, saving and improving the quality of countless lives around the world. It is also a scarce natural product with a beautiful and complex molecular architecture that chemists were able to isolate and construct in the laboratory, thus learning about its intricate properties and facilitating its advancement to the clinic as an effective cancer-fighting drug.



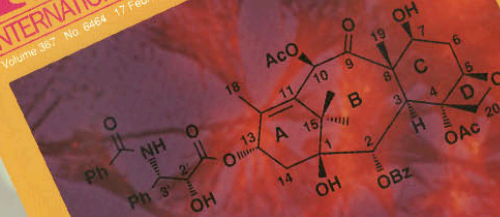
Cancer cell

Cancer is a word that instills a deep-seated fear because we immediately associate it with grave illness and a high mortality rate. Almost all of

us know someone whose life has been blighted by a cancer diagnosis, and who has suffered the prolonged pain of the illness. Cancer patients are forced to tolerate a tough treatment regime with all the accompanying side effects and subsequent problems. Few people are fortunate enough to escape the distress of cancer over their lifetime, since the frightening statistics would suggest that the vast majority of us will either experience it first hand, or have a loved one afflicted. However, as we advance our understanding of the mechanisms involved in causing and propagating cancer, we are gradually uncovering a host of new leads and hopes for cures. Scientists and clinicians diligently and continuously harness such intelligence and powerful resources, laboring to convert them into practical strides forward, giving us hope for a future where cancer is not a death sentence, but a curable disease. The story of the development of Taxol® provides a striking example of how advances in several fields of science and technology can be combined to provide new lifesaving therapies.

Cancer is a collection of a number of rather disparate diseases, all characterized by the uncontrolled proliferation of abnormal cells which invade and disrupt tissues, beginning locally and then spreading through the body to extend the reach of their destructive behavior. Both external causes (e.g. chemicals, radiation, viruses) and internal factors (e.g. hormones, immune conditions, inherited genes), acting either alone or in combination, may be responsible for the initiation and promotion of carcinogenesis. Furthermore, many years can pass between cause and detection, and, with some types of cancer, there exists the obstinate problem of detecting malig-

nature
INTERNATIONAL WEEKLY JOURNAL OF SCIENCE
Volume 387 No. 6664 17 February 1994 \$8.50



Taxol®

208

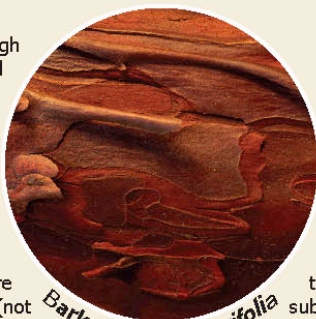
Total synthesis of taxol
... of light-harvesting complex II
... general anaesthesia

Separation technology
PRODUCT REVIEW

nant growths early enough for intervention to stand any chance of success.

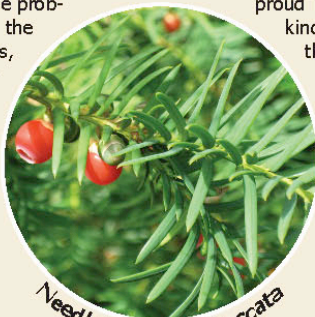
The global impact of cancer cannot be overstated. It constitutes a major public health problem with an ever growing worldwide occurrence. In 2003, approximately 1.34 million people were diagnosed with cancer (not including basal and squamous cell skin cancers) and more than half a million patients died from the disease in the United States alone. The only condition attributed with more deaths per annum is cardiovascular disease, which is responsible for one in every four deaths in the industrialized world. The American Cancer Society estimates that men have slightly less than a one in two lifetime risk of developing cancer; for women the risk is slightly more than one in three. Quite apart from the personal suffering caused by this high incidence, there is an immense financial cost to both the individual and society as a whole in the form of direct medical expenses and lost productivity. As a consequence, each small step scientists make in advancing our ability to treat this heinous group of diseases translates into a major unburdening for society, not to mention the lives saved.

The seriousness of the problem was recognized in the United States in the 1950s, prompting the rapid passage of new legislation over the ensuing two decades as part of a campaign against the disease. This legislation included the 1971



Bark of *Taxus brevifolia*

National Cancer Act which aimed to invigorate cancer research through a directed and substantial increase in funding. As a result of this act proclaiming "the conquest of cancer a national crusade," several new cancer centers were established around the country, and research in the field was accelerated considerably. However, while the long and arduous journey of Taxol® to the clinic would certainly benefit from this infusion of funds, the story begins much earlier. A specific project directed by the National Cancer Institute (NCI), already underway during the 1960s, aimed to conduct a widespread screening of substances and extracts obtained from a variety of natural sources for anti-neoplastic activity. Thus, United States Department of Agriculture (USDA) botanist Arthur S. Barclay collected samples of bark from the relatively rare Pacific yew, *Taxus brevifolia*, while on a field trip to an Oregon forest in the summer of 1962. Barclay's choice of the yew was an astute one, since these trees had a long and proud history of use by mankind, and buried within this ancient knowledge were the clues that they might contain cytotoxic compounds. For example, Julius Caesar recorded that, after defeat at the hands of the Roman



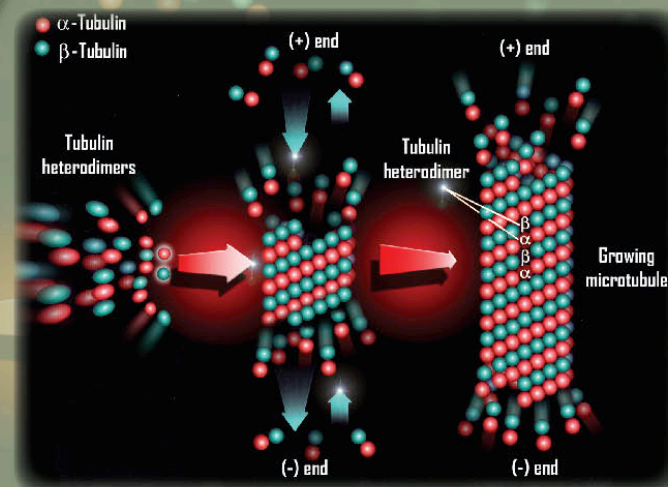
Needles of *Taxus baccata*



Julius Caesar

Box 1

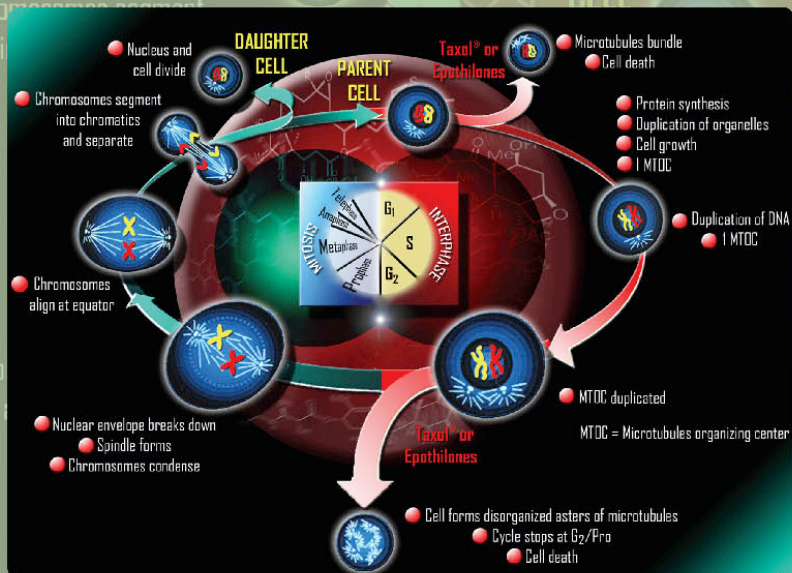
Tubulin polymerization and microtubules



Monroe E. Wall and Mansukh C. Wani

Susan B. Horwitz

Box 2 The cell cycle and the mechanism of action of Taxol®

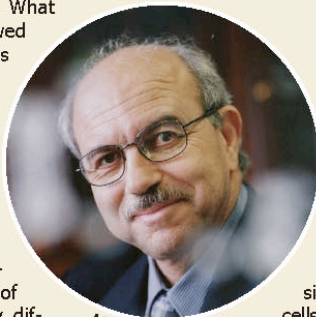


legions, the Gallic chieftain Cativolcus committed suicide by drinking tea made from yew bark. Barclay's samples were eventually packed up and sent to the Research Triangle Institute (North Carolina, USA) in 1964, where two chemists, Monroe E. Wall and Mansukh C. Wani, were charged with their investigation. The crude extracts of the yew bark were shown to exhibit an unusually broad spectrum of cytotoxic activity against leukemia cells, as well as against a variety of other cancer cells. This activity spurred on Wall and his colleagues to isolate the active constituent of the extracts, the complete structure of which was definitively established, in collaboration with researchers at Duke University (North Carolina, USA), by x-ray crystallographic analysis in 1971. Of the more than 110,000 compounds from 35,000 plant species tested by the NCI between 1960 and 1981, this active ingredient, named Taxol®, proved to be the most promising. Despite its promise, however, Taxol® was set to languish on laboratory shelves for almost another full decade before the next advance was made. The reluctance to pursue investigations into the potential therapeutic benefits of Taxol® was largely due to difficulties associated with its isolation and low solubility, as well as the belief that it was simply another microtubule-destabilizing agent akin to the other available natural products, colchicine and the vinca alkaloids, already in use as anti-cancer agents. However, in 1979, Susan B. Horwitz (Yeshiva University, New York,

USA) and coworkers reported in the journal *Nature* that Taxol® actually possessed the unique characteristics of stabilizing and promoting the formation of microtubules. This exciting disclosure led to new research momentum, since this mode of action constituted an entirely new mechanism of intervention against the aberrant replication of cancerous cells (Boxes 1 and 2).

Microtubules are involved in many aspects of cellular biology; they give shape and structure to the cell, assist in reorganizing organelles, and, most importantly for cancer, play an essential role in mitosis, the process of cell division. Their pivotal role in the growth, function, and division of cells led to their description in the 1990s as "the most strategic subcellular targets of anticancer chemotherapeutics." Microtubules are predominantly composed of two similar protein subunits, α - and β -tubulin, which combine to form a heterodimer (Box 1). The tubulin dimer binds two molecules of guanosine 5'-triphosphate (GTP), and in the presence of magnesium these dumbbell-shaped dimers begin to unite in a head-to-tail fashion forming protofilaments. These, in turn, assemble in a staggered manner, leading to the left-handed helices that make up the microtubule. Typically, exchanges are set up at both ends of the microtubule with regular loss and gain of tubulin subunits at relative rates that are often different, thus endowing the tubule with a growing directionality. In general, microtubules are not static structures and, after a certain period of growth, the microtubule and free tubulin will reach a constant equilibrium concentration. At this point, termed the critical concentration, disassembly and growth are finely balanced in a situation regulated by the

bound GTP molecules. What Horwitz's group showed was that Taxol® affects the tubulin-microtubule equilibrium. Taxol® decreases both the concentration of free tubulin (to almost zero) and the induction time for polymerization, with the result that microtubules forming in the presence of Taxol® have a distinctly different morphology from the control variants. They have a shorter average length and are resistant to change under conditions that would depolymerize normal microtubules. In addition, the Taxol® promoted polymerization of tubulin does not require GTP.

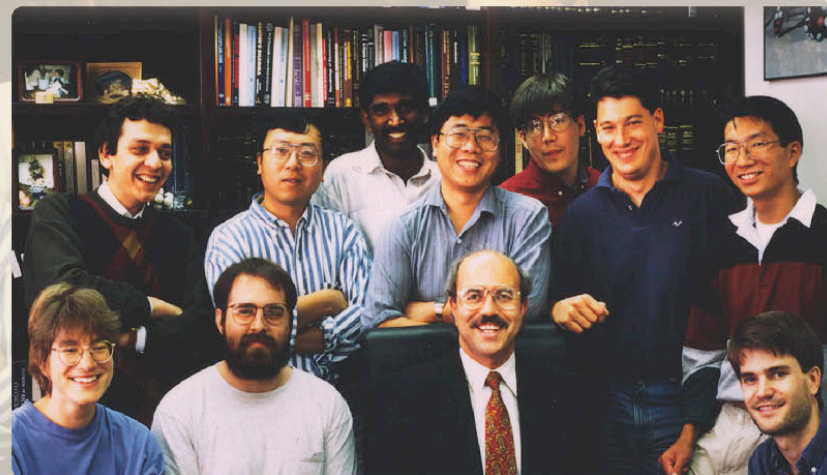


K. C. Nicolaou

From the brief discussion above, we can now understand the interactions of Taxol® with tubulin and the subsequent effects on the gross architecture of microtubules, but how does this translate into the powerful cytotoxicity of the drug? Most cells, excluding a few that cannot replicate, spend the majority of their time in a nonproliferative state called quiescence, yet they are able to switch rapidly into a reproductive cycle (mitosis) when their populations are low and in need of a boost. The mitotic cycle itself has a number of defined stages (Box 2). Cancer cells, unlike their normal counterparts, do not enter the quiescent state when the population density is high, and furthermore, during the mitotic cycle, can-



cer cells suffer from a deficiency in the checkpoints which regulate the rate of division so that the problem of excessive expression of these cells is grossly exacerbated. Horwitz showed that tumor cells treated *in vitro* with Taxol® were arrested at the transition from metaphase to anaphase. Morphologically these cells exhibited unnatural bundles of microtubules and no mitotic spindle. Other research groups quickly extended these findings, proving that Taxol® causes the general and irreversible formation of bundles of microtubules at several cell lifecycle stages, visually reminiscent of a log jam. The net effect of this phenomenon is the sequestering of tubulin in the form of stable structures, ultimately preventing the formation of a mitotic spindle, halting cell division, and leading to rapid cell death by apoptosis (suicidal death). Furthermore, the disturbance to microtubule mobility was also shown to have dramatic effects on other processes within the cell, such as inhibiting the secretion of certain proteins, leading to the general conclusion that the full nature of the attack of Taxol® on cancerous cells is likely to be a complex conglomeration of cell function shutdowns.

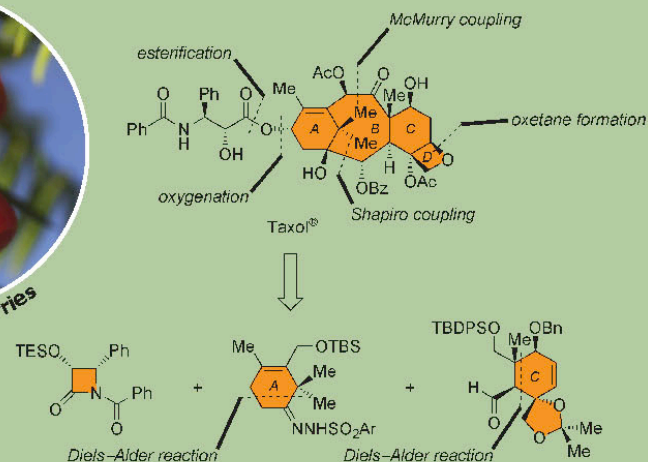


The Nicolaou Taxol® team

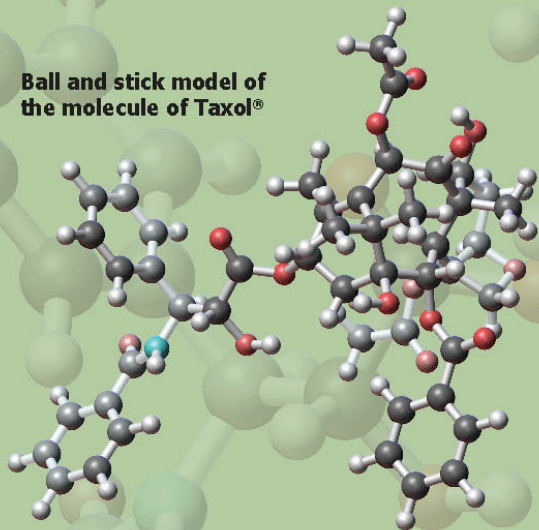
Box 3 The Nicolaou retrosynthetic analysis of Taxol®



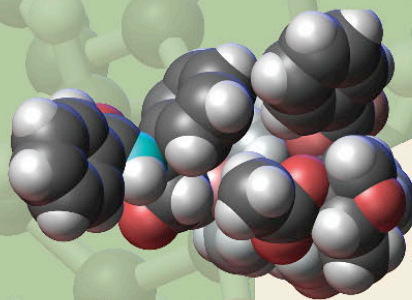
Taxus baccata berries



Ball and stick model of the molecule of Taxol®



Space filling model of the molecule of Taxol®



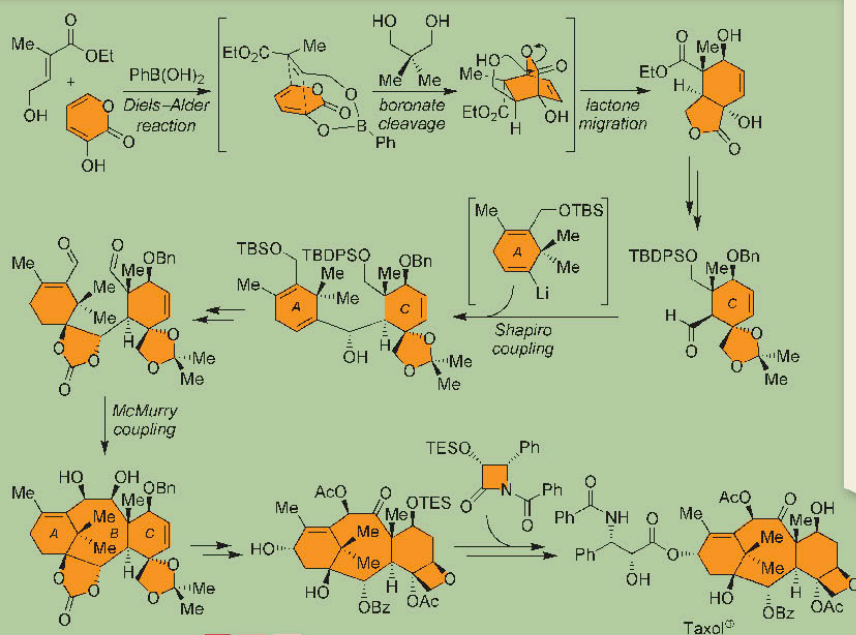
As these groundbreaking investigations were gradually revealed, scientists became increasingly excited about Taxol® because of its emerging portrayal as a long sought-after ally in the fight against cancer. In the 1980s and 1990s, the media was buzzing with stories of a miraculous cure and a drug that could change the face of cancer treatment forever. However, before these dreams could be realized, passage around a number of difficult logistical obstacles still had to be navigated. The most pressing of these issues was that of supply. The Pacific yew is a slow growing tree and has a tendency to grow in dispersed microsites hidden in ecosystems containing mostly older growth and larger tree species. Prior to the discovery of Taxol®, these trees were regarded as scrub and were, for the most part, ignored in primary logging operations, instead being destroyed in the slash and burn disposal fires that followed. Large numbers of mature specimens concentrated in sizeable and harvestable regions were therefore not available. The extraction of Taxol® itself was also a tedious process requiring large quantities of tree bark to yield relatively small amounts of the compound. For example, 38,000 *Taxus brevifolia* trees were sacrificed to obtain just 25 kg of Taxol®, enough to treat 12,000 patients (that is more than three trees per person) in one of the initial clinical studies of this new medication. Such short supplies were hampering the progress of these

early clinical trials, and yet the problem was complicated even further by environmental concerns. Indeed, there was a major controversy and a lively debate surrounding the harvest of so many trees from the delicate forest environments. The concern was voiced that the success of Taxol® would drive the Pacific yew to the verge of extinction and cause irreparable damage to other members of these fragile ecosystems, including the endangered Northern Spotted owl.

The frustrated medicinal promise that Taxol® held in these heady days created unprecedented interest among synthetic organic chemists, who were motivated by the recognition that a chemical synthesis could potentially provide a solution to the supply problem. This urgency spurred additional teams of researchers to follow the many groups, from a host of countries, who had already embarked on the quest for a total synthesis of Taxol®. The lure was not only the issue of the limited stocks, but also the intrigue and challenge presented by the highly complex and unique architecture of this molecule. The structure of Taxol® consists of a diterpene core that is distinguished by its ring arrangement, a periphery densely populated with oxygen functionalities, and nine asymmetric centers. Appended to this core, at a relatively hindered site, is an ester side chain bearing two additional asymmetric carbon atoms. Taxol® became the Holy Grail of total synthesis, and the race to reach it took on many twists and turns until it finally ended in success in 1994.

The first total synthesis of Taxol® to be published was that emanating from the Nicolaou laboratories at The Scripps Research Institute in La Jolla, California (USA), reported in *Nature* early in 1994. This achievement, together with another

Box 4 Highlights of the Nicolaou group's total synthesis of Taxol®



total synthesis which appeared at almost the same time and was accomplished in the laboratories of Robert A. Holton at Florida State University in Tallahassee (USA), ended nearly two decades of recalcitrance exhibited by Taxol®. The Nicolaou group's success relied on the implementation of a highly convergent strategy devised by retrosynthetic analysis (Box 3). Thus, five strategic bonds had been identified and retrosynthetically disconnected, disassembling the molecule of Taxol® into three key building blocks: a β -lactam corresponding to the side chain, a hydrazone encoding the A-ring, and an aldehyde containing the necessary carbon framework and functionality to complete rings C and D. It was anticipated that ring B, which perhaps posed the thorniest problem of the synthesis, might be cast through an intermolecular Shapiro reaction followed by an intramolecular McMurry coupling process. Finally, the venerable Diels–Alder reaction was to be called upon twice, to assemble each of the two requisite cyclohexene rings.

This strategy was successfully implemented, more or less the way it was designed, after a relentless campaign that included many twists and turns and dramatic moments, as summarized in Box 4. Thus, the six-membered C-ring was assembled through the application of an innovative boron-tethered Diels–Alder reaction, developed by Koichi Narasaka (University of Tokyo, Japan), which caused a switch of regioselectivity from the unwanted natural bias of the reactants towards the desired isomer. The A-ring was prepared through a different Diels–Alder



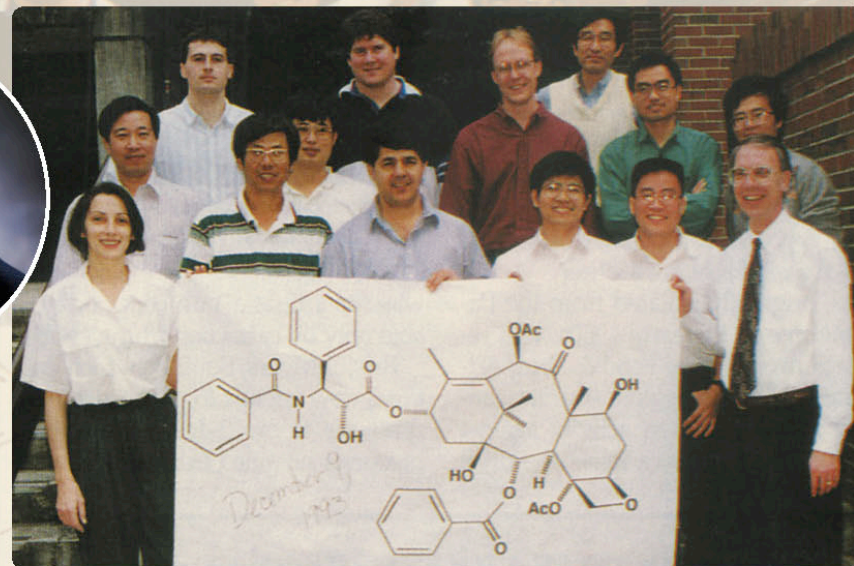
Taxus baccata

reaction, and was then coupled to the C-ring in a subsequent Shapiro reaction. Between each of these key stages adjustment of oxidation levels and functional group manipulations steered the growing molecule towards its final destination. Pleasingly, the challenge of cyclization to furnish the highly strained and crowded 8-membered B-ring was met, as projected, by the McMurry reaction. This remarkable process proved capable of uniting the two aldehyde groups to close the highly strained ring, yielding the tricyclic framework of Taxol® molecule, despite the significant opposing forces. A more advanced intermediate exhibiting all of the structural motifs of the Taxol® was then reached after a number of sensitive and frequently treacherous chemical modifications. Included among these stringent tests were the generation of the highly strained oxetane ring and the regioselective cleavage of the cyclic carbonate to form the desired

hydroxy benzoate moiety. To complete the Herculean task of Taxol®'s total synthesis, the hindered ester side chain was finally installed by the reaction of the completed tetracyclic core with an appropriate electrophilic β -lactam ring, according to the

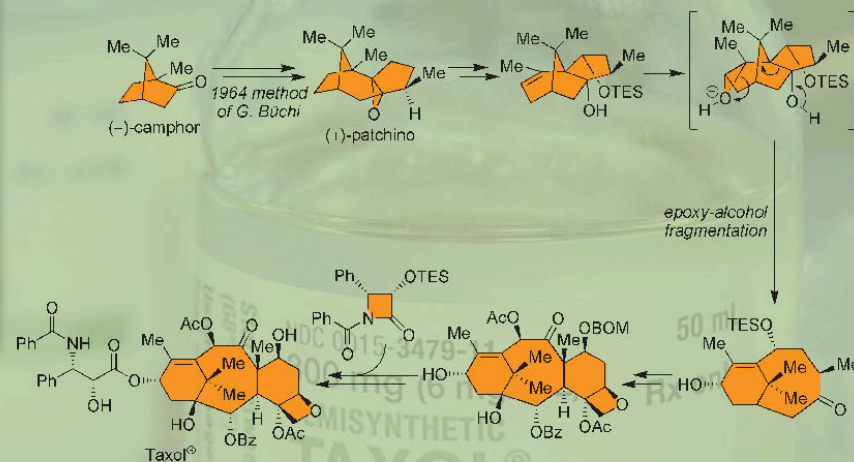


Robert A. Holton



The Holton Taxol® team

Box 5 Highlights of the Holton group's total synthesis of Taxol®

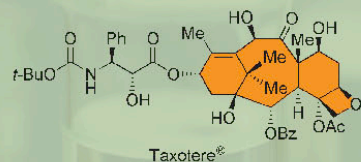
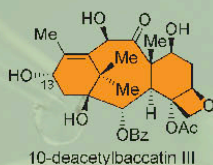




Pierre Potier and Françoise Gueritte-Voegelein

Box 6

Structures of 10-deacetylbaicatin III and Taxotere®



Holton–Ojima procedure. The final protecting groups were then removed to reveal the trophy - laboratory synthesized Taxol®.

Within days of the *Nature* report describing the Nicolaou synthesis, Robert Holton and his group at Florida State University published their elegant route to Taxol®. Holton had chosen an altogether different strategy to access the taxane skeleton in which he employed a rather unusual and novel epoxy-alcohol fragmentation that he had previously developed especially for this application (Box

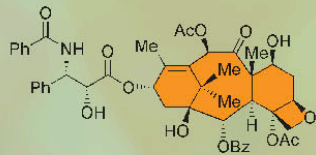
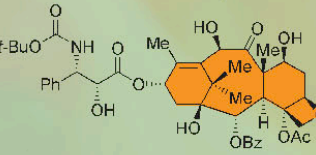
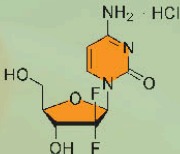
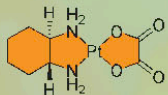
5). His choice of camphor as the starting point for his synthesis of Taxol® provides a telling reminder of how far organic synthesis had advanced over the preceding century, for as you will recall from one of our stories (Chapter 5) camphor itself was once an ardously sought-after synthetic target. In Holton's synthesis of Taxol®, camphor served as a cheap and readily available source of chirality, and the start of the synthesis, not its end. These two essentially simultaneous total syntheses from the Nicolaou and Holton camps were followed over the next few years by completed syntheses from the groups of Samuel J. Danishefsky (1995, Sloan–Kettering Institute and Columbia University, USA), Paul A. Wender (1996, Stanford University, USA), Teruaki Mukaiyama (1997, Tokyo University of Science, Japan), and Isao Kuwajima (1998, Tokyo Institute of Technology, Japan). All of these disparate syntheses can be characterized by one unifying feature; they all exhibit the use of bold strategies and novel tactics, which have contributed considerably to the advancement of total synthesis and exemplified both its awesome power and its enabling nature for biology and medicine.

The conquest of Taxol® by chemical synthesis was not the only victory of science and medicine, nor did it signal the end of the story. In 1992, the US Food and Drug Administration (FDA) approved the use of Taxol® for the treatment of ovarian cancer, and, in 1994, the drug was approved for the treatment of recurrent breast cancer. At its peak in 2001, Taxol®, distributed by Bristol-Myers Squibb (BMS), was the world's top-selling anti-cancer drug with sales approaching \$2 billion. Furthermore, the clinical applications of Taxol® are constantly expanding, opening up a plethora of new opportuni-



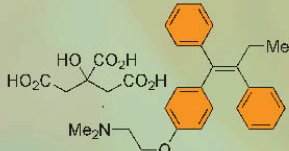
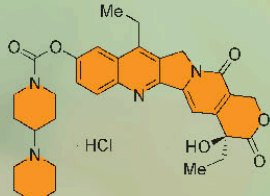
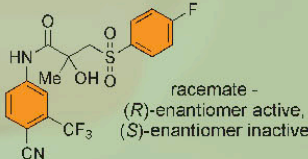
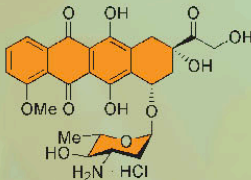
Box 7 Selected anticancer drugs

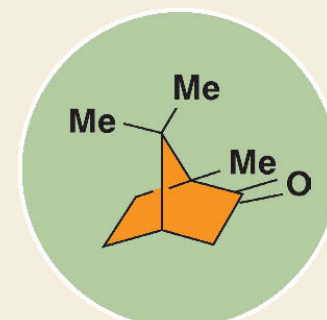
ties for the treatment of a wider variety of cancers, especially with new formulations, including exciting and omnipotent new combination therapies in which Taxol® is combined with other anticancer drugs. It should be noted that BMS secured the rights to the name Taxol® for use as a trademark, thereby causing the renaming of the natural product as 'paclitaxel', despite the fact that scientists had been using the former name since 1971. To reach a position from which Taxol® could dominate the therapy of breast and ovarian cancer, BMS had to find a synthesis capable of meeting the large demand for such a drug. This task was facilitated by the findings of Françoise Guéritte-Voegelein, Pierre Potier, Andrew E. Greene, and coworkers in France, who discovered that the foliage and other renewable biomass of the more common European yew, *Taxus baccata*, contained significant quantities of a related compound, 10-deacetylbaccatin III (Box 6), that could be converted into Taxol® through a short synthetic sequence. Although three thousand kilograms of needles from *Taxus baccata* were needed to obtain one kilogram of the drug precursor, unlike the isolation of Taxol® from *Taxus brevifolia* bark, the harvesting did not signal the death of the tree. Thus a sustainable supply of Taxol® could be guaranteed. Today, Taxol® and related compounds are produced industrially from 10-deacetylbaccatin III through the attachment of the side chain to the hindered C-13 hydroxyl group in a semi-synthesis which is related to the protocols originally developed by Holton and Iwao Ojima. As we shall see next, the discovery of 10-deacetylbaccatin III led to yet another chapter in the tale of Taxol® and a new advancement in cancer chemotherapy.

Generic name [trade name] (source)	Structure or description	Mode of action	Marketer (worldwide sales 2005, US \$, in millions)	Indication
paclitaxel [Taxol®] (natural product)		microtubule stabilization	Bristol-Myers Squibb (747)	ovarian and breast cancers
docetaxel [Taxotere®] (semi-synthetic)		microtubule stabilization	Sanofi-Aventis (1,609)	breast and lung cancers
bevacizumab [Avastin®] (biotechnology)	anti-VEGF (vascular endothelial growth factor) antibody	angiogenesis inhibitor	Genentech and Roche (1,333)	metastatic colorectal cancer (in combination with 5-fluorouracil)
rituximab [Rituxan®] (biotechnology)	first monoclonal antibody approved for use in treatment of malignant disease in the USA (chimeric murine/human antibody)	targets CD20 antigen on surface of normal and malignant B lymphocytes	Genentech and Biogen Idec (1,831)	non-Hodgkin's lymphoma
goserelin [Zoladex®] (synthetic analogue)	decapeptide mimicking natural hormone gonadorelin	causes androgen hormone depletion	AstraZeneca (1,004)	palliative treatment of prostate cancer
leuprolide acetate [Leuplin®] (synthetic analogue)	decapeptide mimicking natural hormone gonadorelin	causes androgen hormone depletion	Takeda and TAP Pharmaceuticals (1,782)	palliative treatment of prostate cancer
gemcitabine hydrochloride [Gemzar®] (synthetic cytosine nucleotide analogue)		inhibits enzyme producing nucleotides for DNA, also competes with cytosine for incorporation into DNA. Leads to cell death in S-phase and blocks to progression from G1/S-phase	Eli Lilly (1,335)	lung cancer
oxaliplatin [Eloxatin®] (synthetic compound)		prodrug - hydrolysis gives active compound that produces interstrand DNA crosslinks, cell-cycle non-specific	Sanofi-Aventis (1,947)	colorectal cancer (in combination with 5-fluorouracil and leucovorin)

(continues on next page)

Box 7 Selected anticancer drugs (continued)

Generic name [trade name] (source)	Structure or description	Mode of action	Marketer (worldwide sales 2005, US \$, in millions)	Indication
tamoxifen citrate [Nolvadex [®]] (synthetic compound)		potent anti-estrogen, binds competitively to estrogen receptors in breast tissue	AstraZeneca (114)	breast cancer
irinotecan hydrochloride [Camptosar [®]] (semi-synthetic analogue)		inhibits topoisomerase I	Pfizer (910)	metastatic colorectal cancer
bicalutamide [Casodex [®]] (synthetic compound)		non-steroidal anti-androgen, binds to cytosol androgen receptors	AstraZeneca (1,123)	palliative treatment of prostate cancer
trastuzumab [Herceptin [®]] (biotechnology)	recombinant DNA-derived monoclonal antibody	antibody binds to human epidermal growth factor 2 (HER2) protein, whose overexpression is common in primary breast cancers	Genentech and Roche (1,722)	breast cancer
epirubicin hydrochloride [Ellence [®]] (natural product)		forms a complex with DNA by intercalation, thus inhibiting DNA and RNA synthesis	Pfizer (367)	solid tumors, particularly in the lung and breast



Camphor

The poor solubility of Taxol[®] in water was recognized as a serious impediment in early clinical studies. The drug originally had to be formulated with ethanol and Cremophor EL (a castor oil derivative) to alleviate this problem; however, the latter component induced major hypersensitivity reactions in some patients. Some claim that this obstacle delayed the clinical trials by as much as five years. Whatever the true timescale, this problem undoubtedly invigorated the search for Taxol[®] analogues (variations of the drug) with improved pharmacological profiles. One of the earliest and most successful analogues of Taxol[®] was originally discovered by Guéritte-Voegelein and Potier at Gif-sur-Yvette in France. This analogue was later developed and distributed by Rhône-Poulenc, now Sanofi-Aventis, under the trademark Taxotere[®]. This anticancer agent possesses a *tert*-butoxycarbonyl group instead of a benzoyl group on the nitrogen atom and a free hydroxyl moiety at C10 rather than an acetoxy group as in Taxol[®] (Box 6). Taxotere[®] is today a highly successful anticancer drug in its own right, with sales figures that have well surpassed those of its elder sibling. In early 2005, the FDA approved

Abraxane® for use in the treatment of metastatic breast cancer. This new treatment consists of nanoparticles of albumin-bound paclitaxel, thus overcoming the insolubility problem and offering an improved administration profile with fewer side effects than Taxol® itself.

Chemotherapy using Taxol® and Taxotere® proved to be a decisive advance in the struggle against cancer, but there are other drugs with their own long and rich chemical and clinical histories (Box 7). As the taxoid drugs show continuing success alongside the many other cancer chemotherapies available, we can view their development as an instructive illustration of what mankind can achieve when academia, industry, and government join forces

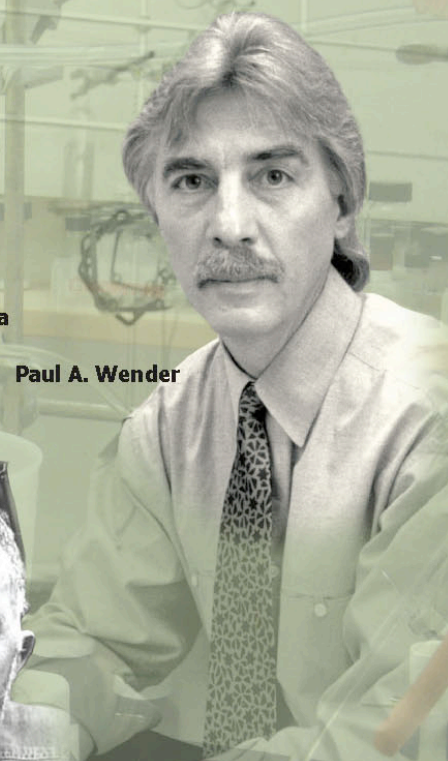
to solve seemingly intractable and pressing problems that seriously affect society. To be sure, there were skirmishes, competitions, and controversies, but the number of lives saved and improved by Taxol® and Taxotere® make these incidents pale in comparison to the overall accomplishment. Chemists should be particularly proud for the crucial contributions that their science has made to the successful outcome of this legendary project.



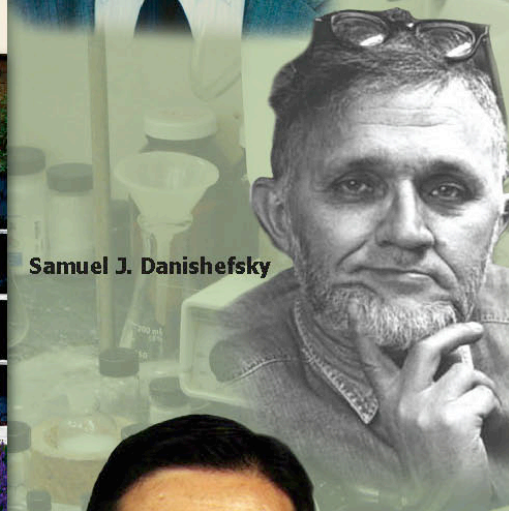
Taxus baccata, 'Standishii', flank the entrance to a house



Teruaki Mukaiyama



Paul A. Wender



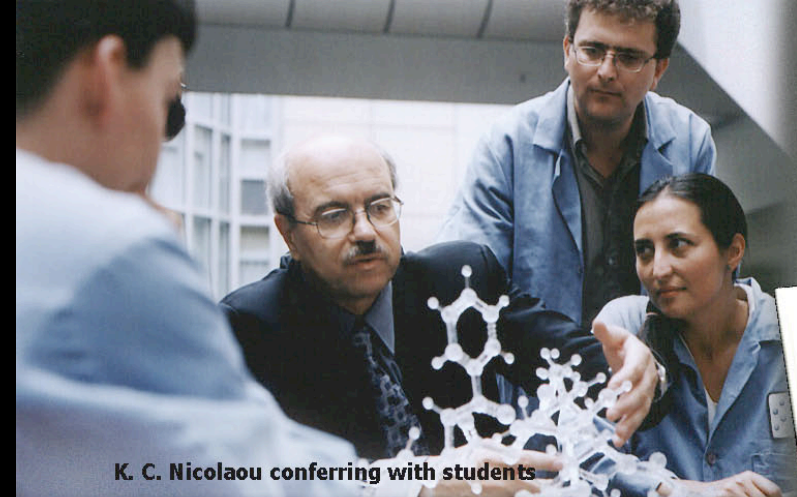
Samuel J. Danishefsky



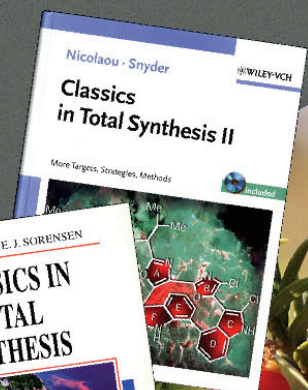
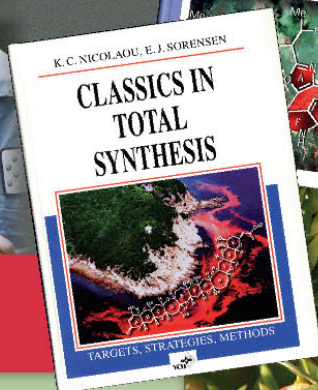
Iwao Ojima



Isao Kuwajima



K. C. Nicolaou conferring with students



Taxus baccata

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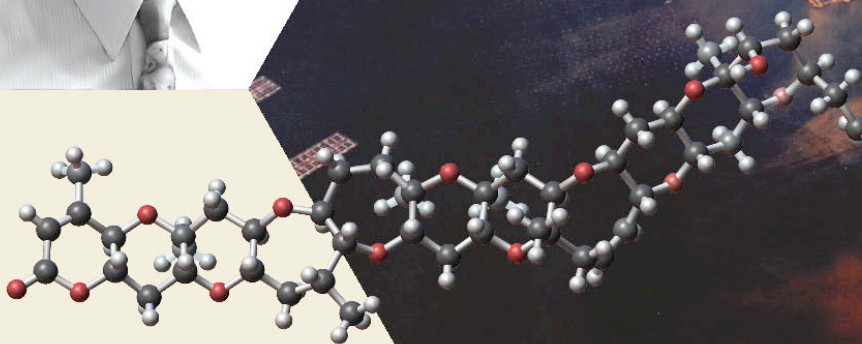
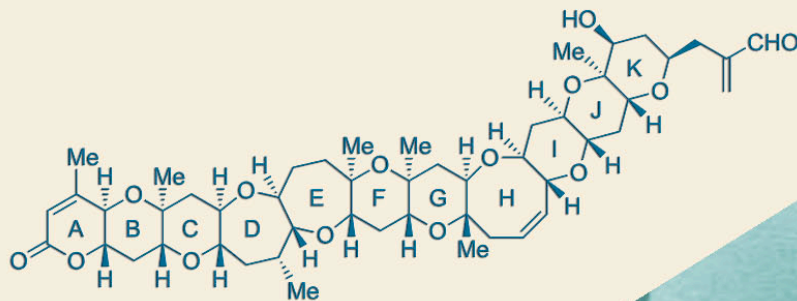
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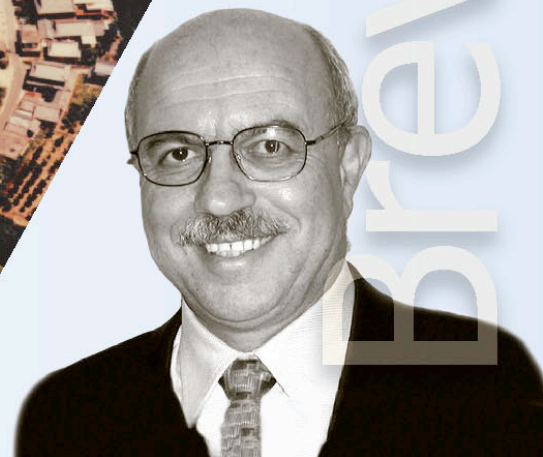
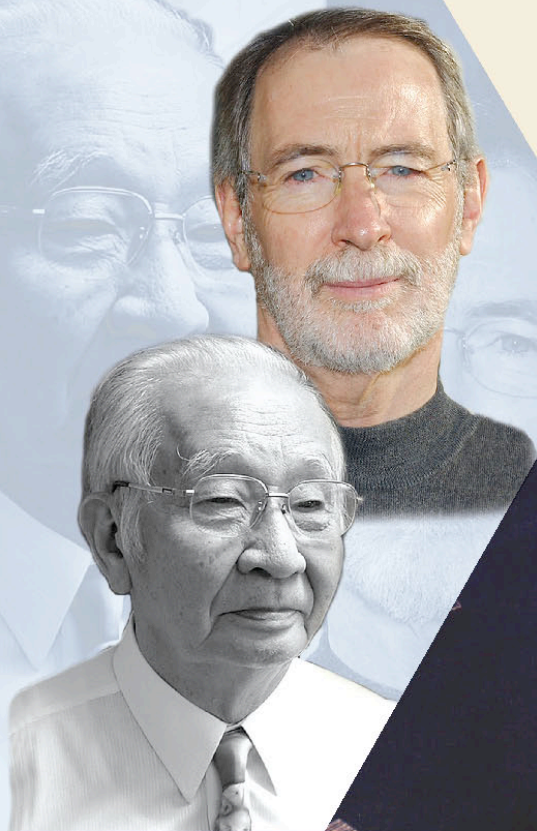




Brevetoxin B

Chapter 27

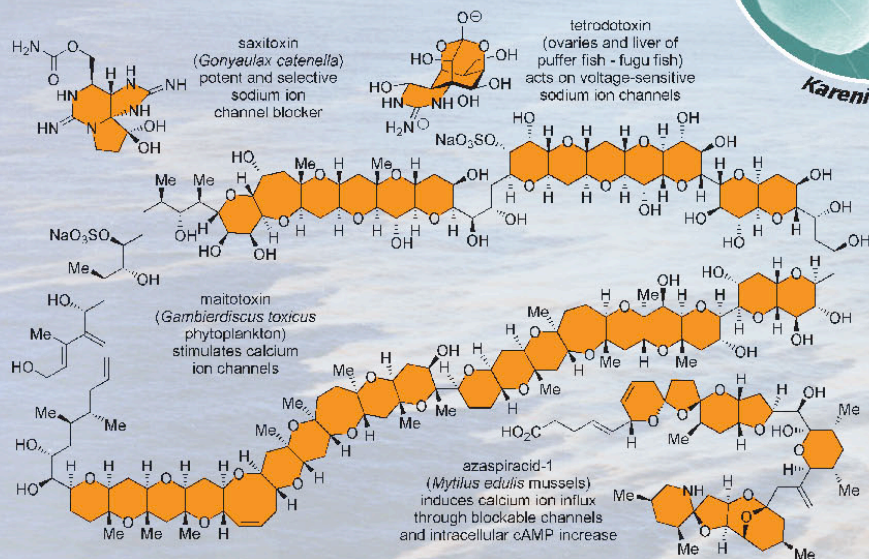
1995



Prevent toxin B

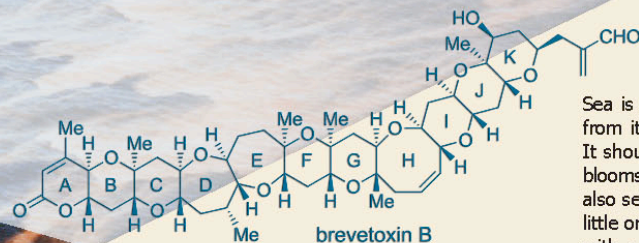
Red tide

Box 1 Selected marine toxins



Brevetoxin B

2 3 4



Karenia brevis

“**b**ehold, I will smite
with the rod that
is in mine hand
upon the waters
which are in the river, and they shall
be turned to blood. And the fish that
is in the river shall die, and the river
shall stink; and the Egyptians
shall loathe to drink of the
water of the river.”
Exodus 7:17-18

Above is a short excerpt from what some believe is one of the first descriptions of an occurrence known around the world today as a red tide phenomenon. The quote comes from the Bible, where the story of the opening plague executed at the command of God against the Egyptians who had enslaved Moses and the Israelites is recounted. ‘Red tide’ is the modern name commonly given to the vast blooms of single-celled phytoplankton occurring sporadically in coastal waters, which derive a red hue from the dense growth of algae containing the carotenoid pigment peridinin. The Red

Sea is thought to have acquired its name from its susceptibility to such epidemics. It should be noted that not all toxic algal blooms are red; various other colors are also seen, and outbreaks even occur with little or no associated color. Oceans tinged with a bright crimson stain present spectacular opportunities for photography, but, as the biblical quote ominously implies, the red tide also carries a sinister threat. A small number of the algae causing these outbreaks produce deadly defense toxins that can bring catastrophe to marine life within the bloom. These toxins persist and can reach dangerous concentrations throughout the food chain, so humans are also at risk if they consume contaminated seafood. There are no geographical restrictions to these algal blooms; they occur frequently throughout the world. The members of one class of powerful neurotoxic molecules isolated from the waters associated with such events were named the brevetoxins after the organism responsible for producing the deadly compounds, a dinoflagellate called *Karenia brevis* (formerly *Gymnodinium breve*). The first structure to be determined from this class, that of brevetoxin B, was reported in 1981. The story of the conquest of brevetoxin B by total synthesis constitutes the main theme of this chapter, but before

we hear of the incredible twelve-year odyssey that it took to construct brevetoxin B in the laboratory, let us delve further into the extraordinary history and effects of red tides.

In 1793, the English seafaring captain George Vancouver became the first European to navigate and explore the coastal waters around British Columbia

K. C. NICOLAOU, E. J. SORESENSEN

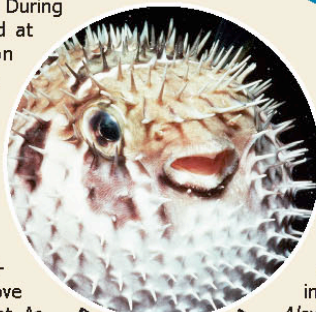
CLASSICS IN TOTAL SYNTHESIS



TARGETS, STRATEGIES, METHODS

WILEY

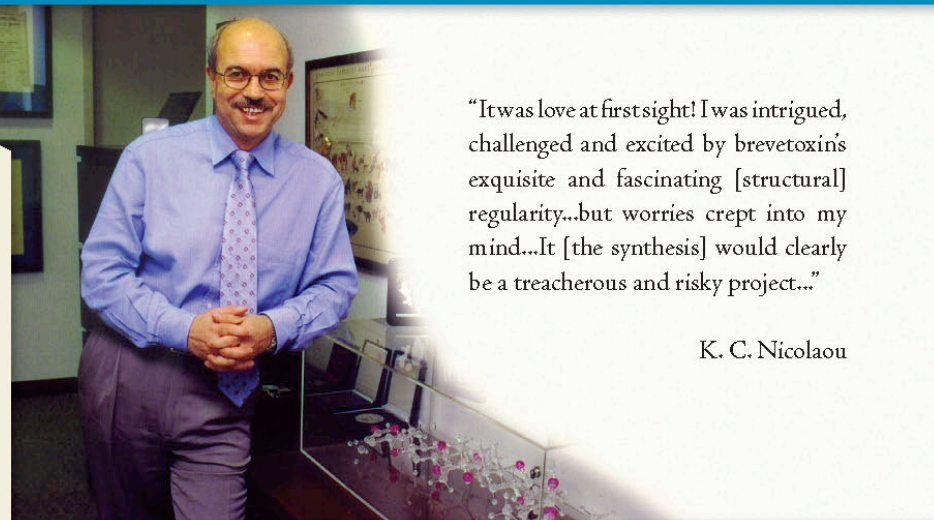
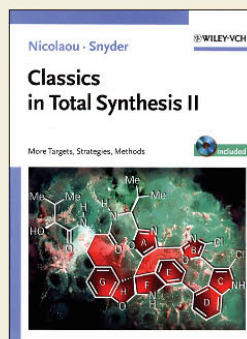
(present-day Canada). During this expedition, he landed at a place now called Poison Cove. Vancouver noted in his diary that the native Indians would not eat the shellfish from the colored waters of the bay because they were considered too dangerous. It now seems reasonable to attribute this historical episode, which gave Poison Cove its name, to a red tide event. As we move forward in time, we can be more certain of the incidences of this phenomenon. For example, in 1972, a massive bloom stretching along the northeast coast of the United States from Maine all the way to Massachusetts was definitively identified as a red tide. The incident followed a hurricane that had originated further south in the warm waters of the Gulf of Mexico and which had presumably been responsible for relocating the toxic algae to new waters as the storm moved northwards. 1972 was also a disastrous year for the Japanese fishery industry due to a severe red tide outbreak in the Seto Inland Sea killing caged yellowtail tuna worth an estimated \$500 million. The tiny unicellular algae responsible for the blooms are



Puffer fish (fugu)

to algal bloom toxins. In the case of the whales the poisoning was traced to mackerel contaminated by feeding on toxic algae called *Alexandrium tamarense*, and with the dolphins to fish containing lethal brevetoxins from *Karenia brevis*. In 1991, a red tide incident was blamed for hundreds of sick and dying pelicans found on the beaches of Monterey, California (USA). Humans have also become ill as a result of such blooms. In 1987, a mass poisoning was reported in Canada with patients complaining of vomiting, diarrhea, disorientation, and abdominal cramps. In this instance, the culprits were identified as mussels from Prince Edward Island that were apparently contaminated by marine toxins from the red tide bloom of a *Pseudonitzschia* species. For this very reason, folklore in Nova Scotia warns people not to eat shellfish in the months whose name contains a letter "r".

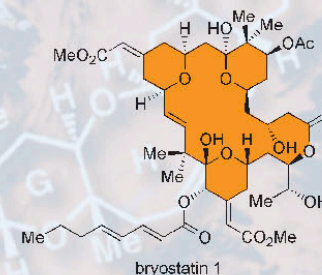
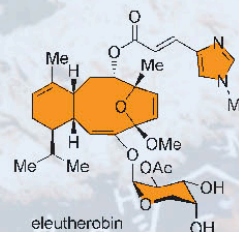
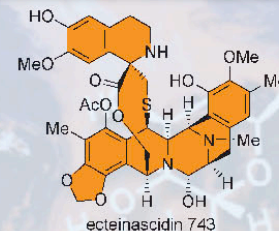
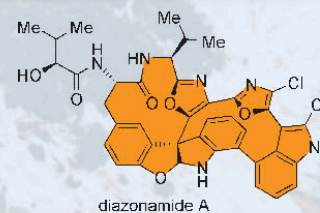
The prevalence of red tide blooms has increased in recent times as population, pollution, and maritime transport have all swelled to previously unimagined levels. During the period between 1976 and 1986, when the population of Hong Kong grew six-fold, this startling expansion was matched in nearby

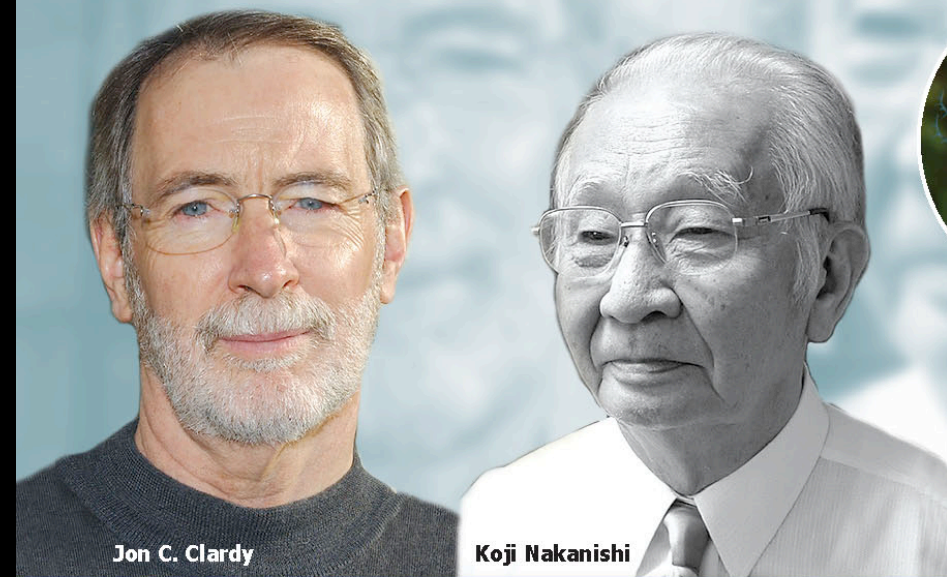


"It was love at first sight! I was intrigued, challenged and excited by brevetoxin's exquisite and fascinating [structural] regularity...but worries crept into my mind...It [the synthesis] would clearly be a treacherous and risky project..."

K. C. Nicolaou

Box 3 Selected cytotoxic marine natural products



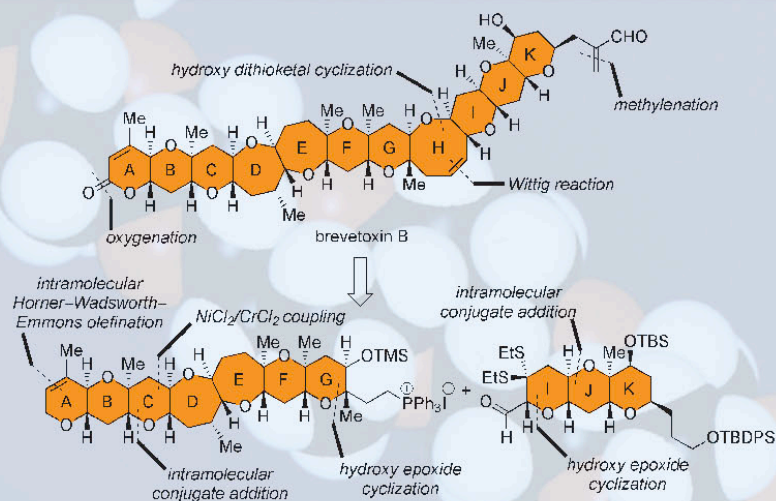


Jon C. Clardy

Koji Nakanishi

Box 4

Nicolaou's strategic bond disconnections and retrosynthetic analysis of brevetoxin B



Eleutherobia grayi coral

Tolo Harbor by an eight-fold rise in the occurrence of red tides. Pollutants from the land in the form of agricultural run-offs and sewage provide algae with the requisite nutrients to grow, sometimes out of control, triggering a bloom. In Florida (USA), high levels of chelated iron compounds in the Peace River effluent have been demonstrated to cause outbreaks in the immediate vicinity. Gibberellic acid, a plant hormone often washed into our waterways, has also been implicated as a red tide promoter. Long distance shipping by oil tankers, cargo vessels, and naval fleets has also facilitated the uncontrolled distribution of algal colonies. A number of these species have the ability to form hardened cysts that travel the oceans in a dormant state only to be reawakened when they encounter conditions that are conducive to their growth and reproduction (temperature range 10–33 °C, salinity levels 27–37 ‰, and appropriate duration and intensity of light). It is unfortunate that certain human activities are somewhat responsible for this growing hazard. The damage to marine ecology and human health, and the considerable worldwide economic losses due to red tides, all suggest that we ought to take this issue more seriously and look for prophylactic solutions.

The search for understanding of these catastrophic phenomena began sometime ago and it led to the identi-

cation of several biotoxins from poisoned waters. Amongst the most notorious marine toxins identified thus far are tetrodotoxin (associated with the fugu or puffer fish, a delicacy in the Far East), saxitoxin, palytoxin, maitotoxin, ciguatoxin, azaspiracid-1, and brevetoxins A and B (Boxes 1 and 6). One should not, however, associate the oceans only with harmful chemical compounds, for certain marine natural products can be tamed and exploited to provide powerful leads for treating disease, particularly cancer. Indeed, static or slow-moving aquatic creatures, such as sponges, algae, and anemones, have survived evolutionary pressures by producing powerful toxins to fend off potential predators. While several of these molecules have already been found and are providing clues towards new chemotherapeutic approaches to cancer treatment, it is certain that many more remain to be discovered. Included among the stunning known molecular architectures from the marine environment are those of the antitumor agents ecteinascidin 743, eleutherobin, diazonamide A, and bryostatin 1 (Box 3). Exploring, in environmentally benign ways, the largely untapped molecular diversity stored in the oceans is bound to lead to new beginnings in science and medicine, and therefore will ultimately be of great benefit to mankind.

Brevetoxin B, the potent and highly complex neurotoxin associated with red tides, was the first structure of its kind to be unraveled and as such it holds a special place within the annals of natural products chemistry. The exact structure was elucidated by Koji Nakanishi (Columbia University, USA), in collaboration with Yong-Yeng Lin (University of Texas, USA) and Jon C. Clardy (Cornell University, USA), who carried out the x-ray crystallographic analysis of brevetoxin B. The mode of



oxocene
ring



oxapane
ring



tetrahydropyran
ring



K. C. Nicolaou

action of the brevetoxins involves activation of voltage-dependent sodium channels embedded within neurons. When these channels are activated by brevetoxin A or B they open, allowing a continuous influx of sodium ions, rather than the normal regulated flow, thus disabling the neuron. Symptoms in humans include a tingling sensation in the mouth and digits, disrupted coordination, hot/cold reversal of temperature sensitivity, dilated pupils, brachycardia, and diarrhea.

The amazing molecular architecture of brevetoxin B is breathtaking to organic chemists (see Box 2). Its fifty carbon, fourteen oxygen, and seventy hydrogen atoms are woven together in a stunning array of eleven contiguous rings locked into a rigid ladder-like structure. Despite its complex nature, this magnificent structure shows remarkable regularity. Thus, all eleven rings are *trans*-fused and each one contains a single oxygen atom.



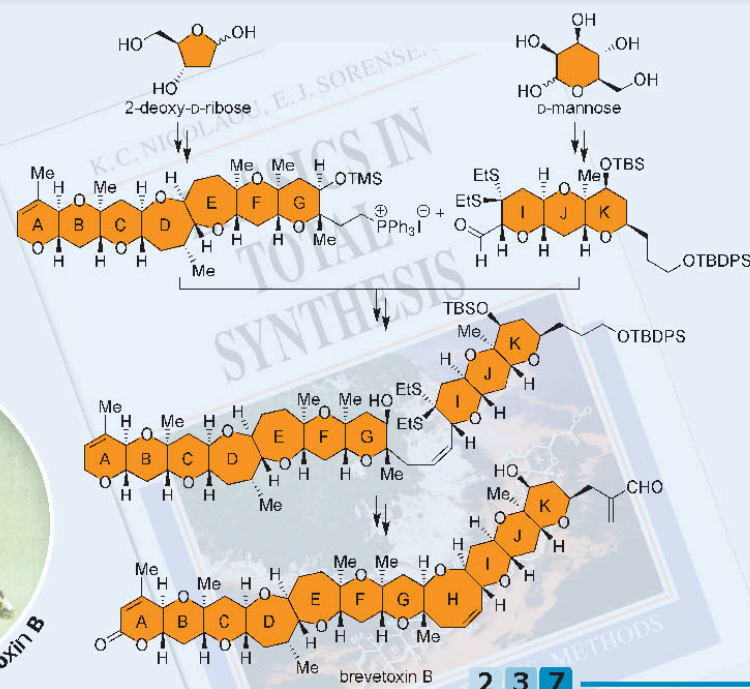
Crystals of synthetic brevetoxin B



The final
brevetoxin B team

Box 5

Completing the odyssey: highlights of the Nicolaou brevetoxin B total synthesis



Paul J. Scheuer

Yuzuru Shimizu

Masahiro Hirama



All the ring oxygen atoms are separated from their closest neighboring ring oxygens by two carbons and each is flanked by two *syn*-disposed hydrogen (or methyl) substituents, except for the first (ring A) which carries a carbonyl group to its 'left', and the last (ring K) which is flanked by two *anti*-orientated hydrogen atoms.

The first total synthesis of brevetoxin B was accomplished by the group of K. C. Nicolaou in 1995, following an arduous campaign. This twelve-year "synthetic odyssey" required tremendous stamina and skill, not to mention ingenuity and imagination on the behalf of those working at the front line. After several abortive attempts based on a myriad of diverse strategies, brevetoxin B finally fell, with the details of the conquest reported in the *Journal of the American Chemical Society*. This celebrated total synthesis was accompanied by the discovery and invention of much new chemistry. Included among the methods endowed by this program were new means to construct certain types of oxygen-containing rings, such as tetrahydropyrans, oxepanes, and oxocenes, methods that today remain as widely used tools in organic synthesis.

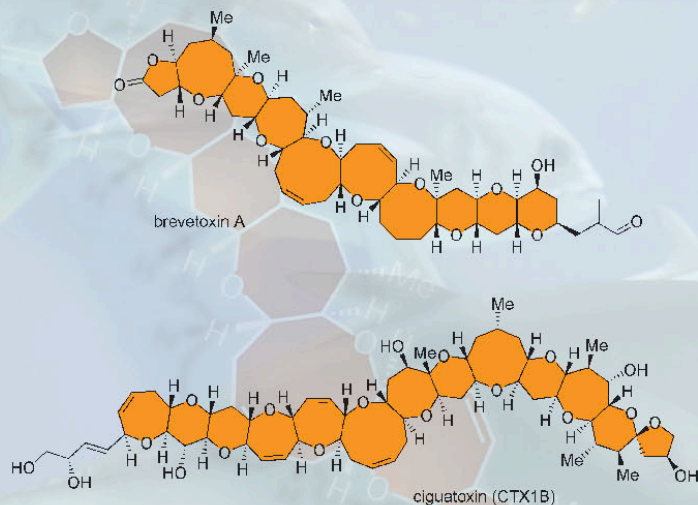
The final and successful strategy employed in the Nicolaou total synthesis of brevetoxin B is shown retrosynthetically in Box 4. While experts will not be surprised, many read-

ers may be interested to know that this strategy toward brevetoxin B was not the one originally designed (it very rarely is in complex molecule construction), but rather it was one that had evolved over the duration of the campaign. It involved eighty-six steps in its longest

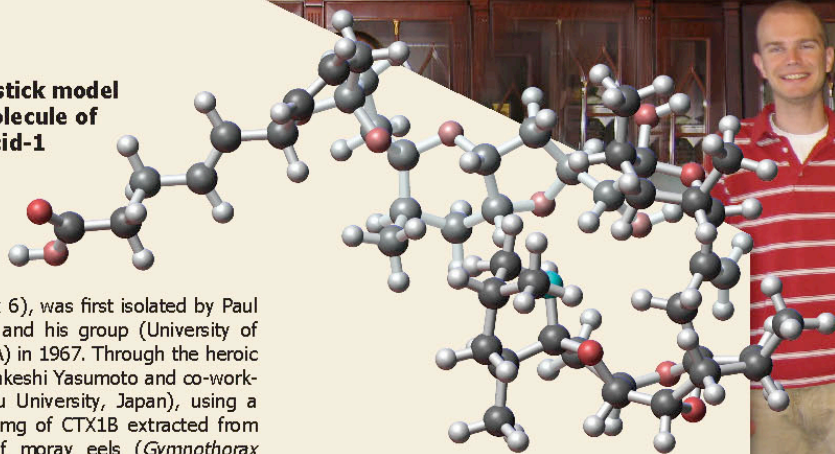
linear sequence (from 2-deoxy-D-ribose) and traversed a terrain full of surprises, puzzles, and fascinating sites of exploration. The convergent synthesis began with 2-deoxy-D-ribose and D-mannose, two readily available carbohydrate building blocks which were separately elaborated towards two advanced intermediates (Box 5). These key fragments were then united using a Wittig reaction, and the product advanced to brevetoxin B through a carefully orchestrated synthetic sequence. Brevetoxin A (Box 6), which presents its own unique synthetic hurdles, was also synthesized by the Nicolaou group, in 1998. Brevetoxin A is the most potent neurotoxin isolated from *Karenia brevis*, and its structure had been elucidated as the result of a collaboration, undertaken during the early 1980s, between Yuzuru Shimizu (University of Rhode Island, USA) and Jon C. Clardy.

The ciguatoxins are a class of polyether marine toxins produced by another dinoflagellate, *Gambierdiscus toxicus*, which lives on macro-algae. The ciguatoxins are also potent neurotoxins and, through accumulation up the aquatic food chain, are thought to be responsible for the poisoning of over 20,000 people annually in subtropical and tropical regions of the world. The parent member of this natural product class,

Box 6 Molecular structures of brevetoxin A and ciguatoxin CTX1B

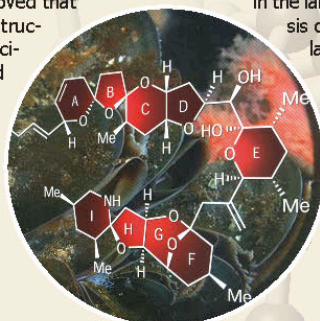


Ball and stick model of the molecule of azaspiracid-1



CTX1B (Box 6), was first isolated by Paul J. Scheuer and his group (University of Hawaii, USA) in 1967. Through the heroic efforts of Takeshi Yasumoto and co-workers (Tohoku University, Japan), using a mere 0.35 mg of CTX1B extracted from 4000 kg of moray eels (*Gymnothorax javanicus*), the structure of this molecule was finally elucidated in 1989. In a similar feat of skill and endurance to that accomplished earlier by the Nicolaou brevetoxin teams, ciguatoxin (CTX1B, Box 6) was synthesized by Masahiro Hirma (Tohoku University, Japan) and his group in 2006. The Hirma group has also completed the total syntheses of other members of this challenging class.

Another accomplishment in this area is the total synthesis, by the Nicolaou group, of azaspiracid-1 (Box 1), a neurotoxin which contaminates mussels causing human poisoning in Europe. Originally isolated by the Yasumoto team from poisoned mussels (*Mytilus edulis*) collected from Killary Harbor (Ireland), this molecule inspired a campaign towards its synthesis that became a high-drama detective story once the first synthesis proved that the originally proposed structure was incorrect. The scientists then re-designed their strategy so as to decipher the true structure of azaspiracid-1, a formidable task that was finally accomplished



Azaspiracid-1

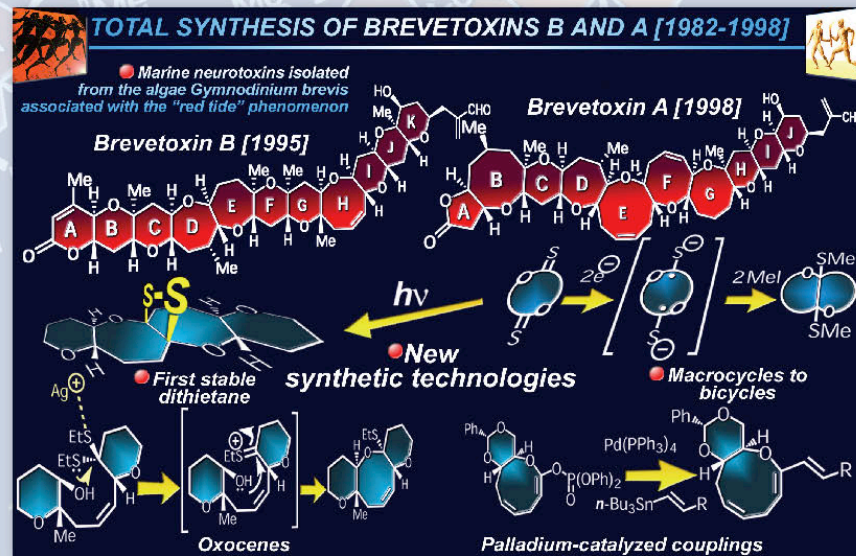
by opportunistic intelligence gathering and total synthesis. As with the story of strychnine (Chapter 12), this tale exemplifies another value of chemical synthesis, that of providing the ultimate proof of a natural product structure.

Completion of all these syntheses may be hailed as a testament to the awesome power of modern chemical synthesis. Yet, if we compare the overall efficiency of the processes involved in these syntheses to the facile biosyntheses of these marine toxins, we must admit our inferiority and conclude that we still have a long way to go before we can claim equal status with nature. Incidentally, while both saxitoxin and tetrodotoxin (Box 1) have also been synthesized in the laboratory, the total synthesis of maitotoxin (Box 1), the largest secondary metabolite yet isolated, remains a challenge – perhaps to be met one day by one or more of the young students reading this book!



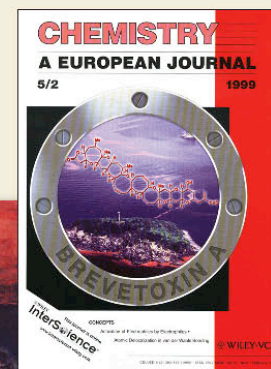
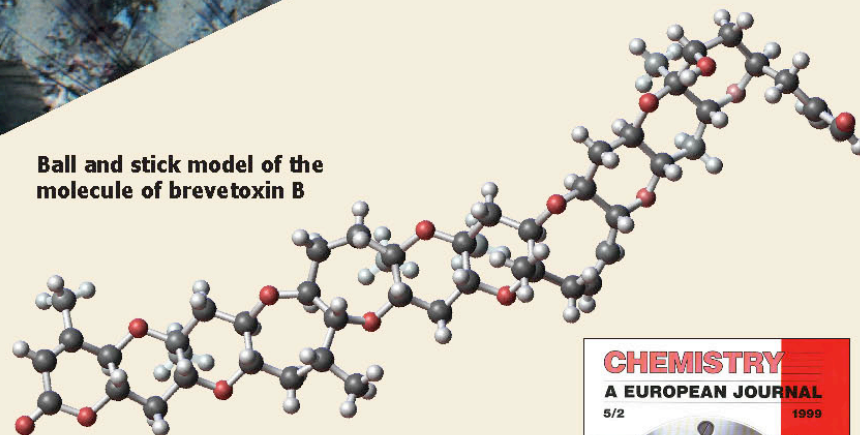
The Nicolaou azaspiracid-1 team

Box 7 Highlights of the Nicolaou group's endeavor in the brevetoxin area





Ball and stick model of the molecule of brevetoxin B



Further Reading

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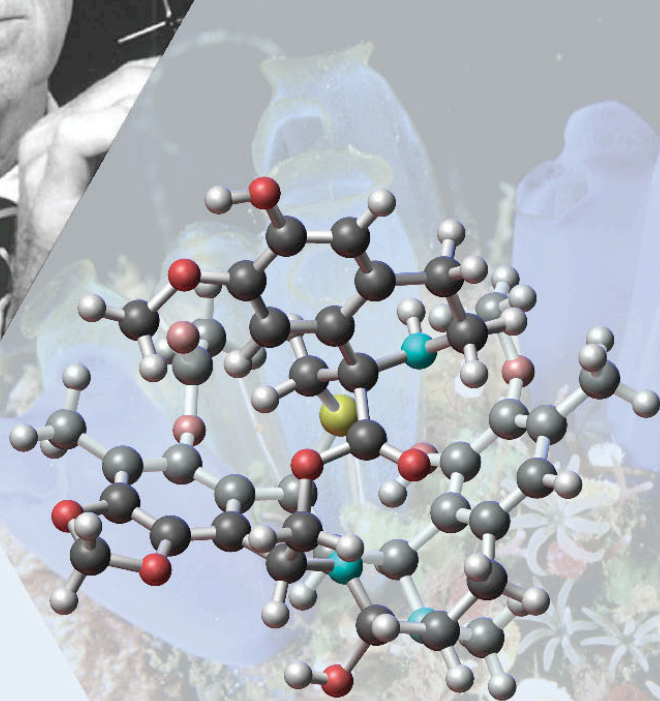
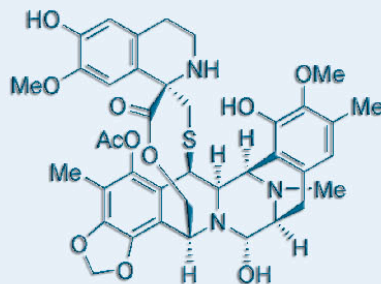
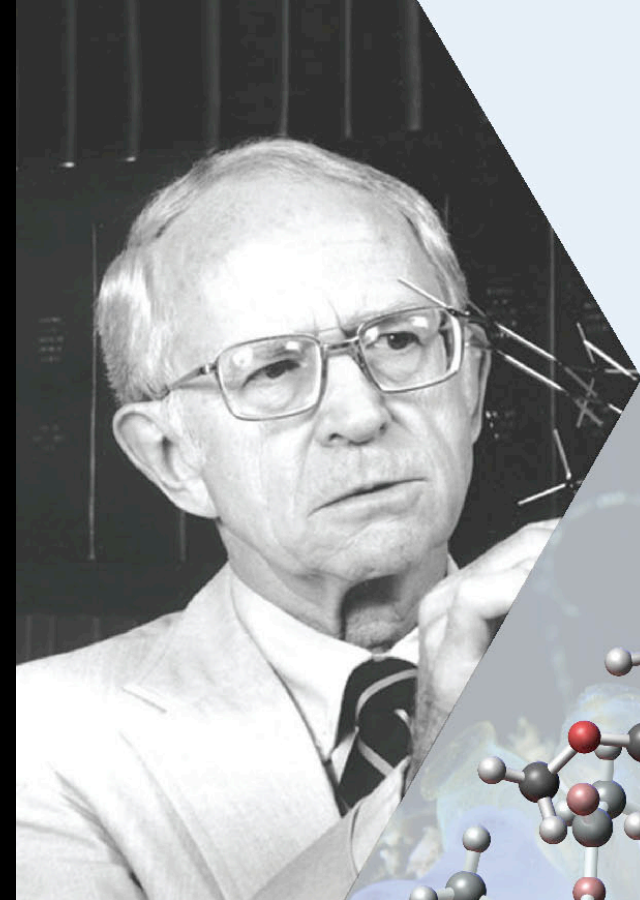
K. C. Nicolaou, The Total Synthesis of Brevetoxin B: A Twelve-Year Odyssey in Organic Synthesis, *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 589–607.

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M. Inoue, K. Miyazaki, Y. Ishihara, A. Tatami, Y. Ohnuma, Y. Kawada, K. Komano, S. Yamashita, N. Lee, M. Hiram, Total Synthesis of Ciguatoxin and 51-HydroxyCTX3C, *J. Am. Chem. Soc.* **2006**, *128*, 9352–9354.

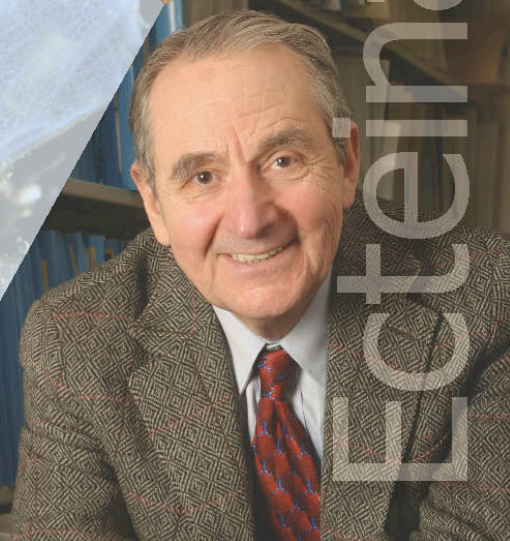
K. C. Nicolaou, T. V. Koftis, S. Vyskocil, G. Petrovic, T. Ling, Y. M. A. Yamada, W. Tang, M. O. Frederick, Structural Revision and Total Synthesis of Azaspiracid-1. Part 2: Definition of the ABCD Domain and Total Synthesis, *Angew. Chem. Int. Ed.* **2004**, *43*, 4318–4324.



Ecteinascidin 743

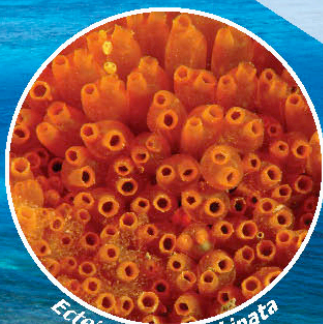
Chapter 28

1996



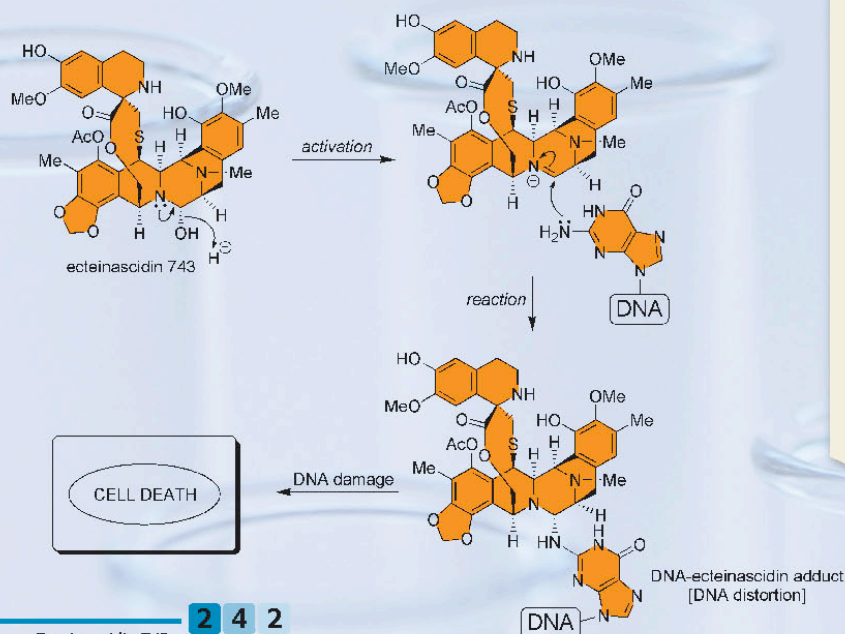
Ecteinascidin 743

Caribbean Sea



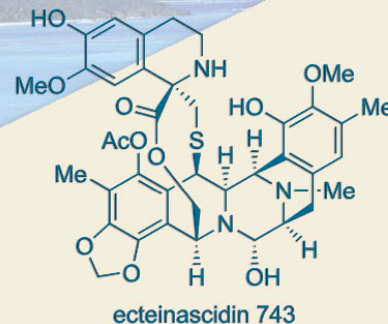
Ecteinascidia turbinata

Box 1 The mechanism of action of ecteinascidin 743



Ecteinascidin 743

2 4 2



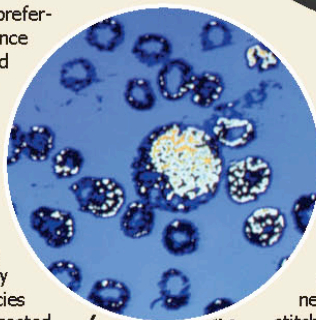
The oceans and seas, especially the shallow warm waters of the tropics, are home to many soft-bodied invertebrates. These strange and resplendent creatures include soft corals, sponges, starfish, and sea anemones. The story of ecteinascidin 743 begins in 1969 when a preparation containing a mixture of compounds was extracted from one such marine creature, a sea squirt, collected during a scientific diving expedition in the Caribbean Sea.

Sea squirts (or ascidians) have sack-like bodies into which they draw water so that nutritional microorganisms can be absorbed; the water is then squirted out, hence their rather comical name. Sea squirts contain an array of organs within their body wall, including a circulatory system driven by a heart which, curiously, can change the direction of its pumping. The mixture extracted following maceration of the entire body of this particular ascidian,

Ecteinascidia turbinata, was propelled into the scientific limelight because it showed an extraordinary level of lethal activity against P388 murine leukemia cells. This cancer cell line has been specially engineered and can be cultivated in the laboratory to test for anticancer properties in new compounds. Usually, the next steps in such a process would have been separation of the mixture, analysis and identification of its individual components, and their respective activities, followed by a full-scale investigation pursuing the constituents that had been identified as the most biologically promising. In this instance, however, such work was stymied for more than fifteen years due to the instability of the components. Irrespective of the care employed, almost any attempt to purify the extracted mixture led to the decomposition of its constituent compounds. During this frustrating period this medley of compounds continued to tantalize scientists, owing to the fact that it was the most potent marine natural product tested by the National Cancer Institute (USA) in the eight years to 1980. The development of a number of new chromatographic techniques finally allowed the composition of the mixture to be deconvoluted in 1986 by Kenneth L. Rinehart and his colleagues at the University of Illinois (USA). With pure materials now available, the full structural elucidation was also completed shortly thereafter (1990), unveiling the intricate molecular architectures of several ecteinascidins. The most abundant member of this new class of natural products was ecteinascidin 743, and experts were anxious to understand the origins of its impressive biological activity, having become convinced that a novel mode of action was at work.

It was not long before investigators found that ecteinascidin 743 (Et743) binds tightly in the minor groove of the DNA

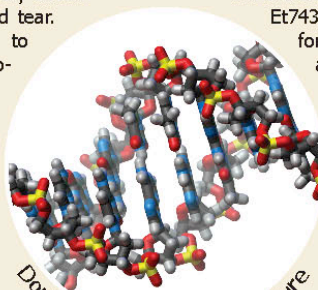
double helix, showing a preference for a specific sequence of bases (guanine followed by cytosine). Upon docking in the minor groove, the ecteinascidin 743 molecule reacts chemically with the DNA, forming a covalent bond with a nearby DNA functional group and converting the Et743 molecule into a highly reactive chemical species (Box 1). It has been suggested



Leukemia cells

that the DNA–Et743 adduct absorbs oxygen from its environment, generating a reactive hydroxyl radical. This radical then slices the backbone of the DNA strands in the locality. This mechanism is thought to be the manner by which the ecteinascidins (and another family of structurally related molecules, the saframycins, see Box 5) destroy the genetic material that is essential to living cells. However, our cells, cancerous or normal, are not so weak as to submit to this violent attack without resistance; enzymes exist within cells whose sole purpose is repairing lesions in DNA. They shuttle up and down DNA duplexes in search of stretches requiring maintenance, like methodical train track engineers fixing railroads. These enzymes evolved because DNA is constantly being damaged under the ordinary circumstances of daily wear and tear.

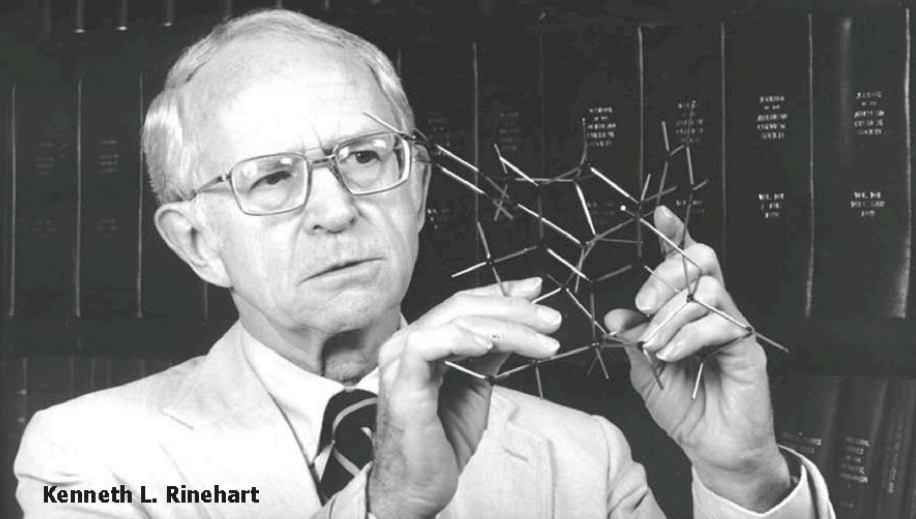
For example, exposure to ultraviolet light can promote dimerization reactions within DNA to form cyclobutane rings that distort the helix. One of these enzymes, called nucleotide excision repair (NER) enzyme, removes lesions in dam-



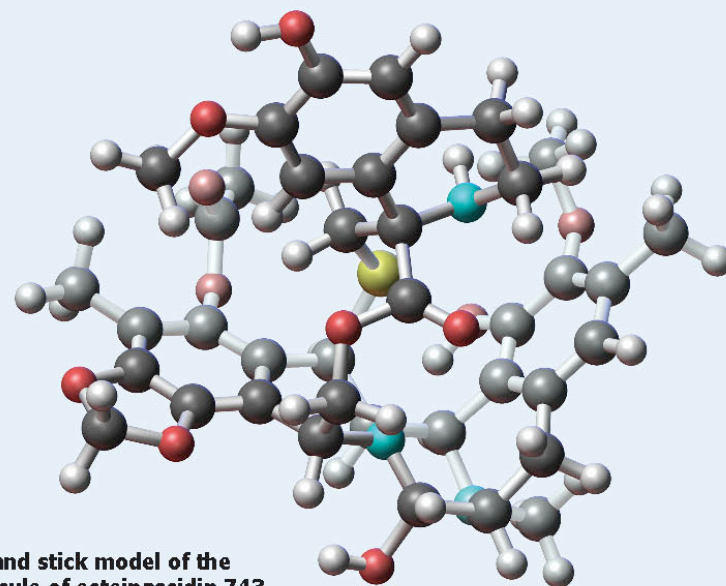
Double-stranded DNA structure

aged helices by excising them out and then, in conjunction with other cellular machinery, incorporates new nucleotides and stitches the DNA back up to restore a perfect helix. A recent investigation offered an interesting new insight into the mode of DNA cleavage by Et743, which accounts for the correlation between the cytotoxicity of Et743 and the presence of intact NER enzyme in a cell. In this hypothesis, the kink made in DNA by its reaction with Et743 is recognized by the NER enzyme, which then proceeds to snip out the damaged region. However, Et743 has the last word, since it disables the enzyme afterwards, such that the gaping hole in the helix is not mended following this surgery. With damage this severe, the cell dies rapidly. Since it has been established beyond doubt that Et743 is capable of cleaving DNA, it may be that both the mechanistic hypotheses mentioned above are valid and that there

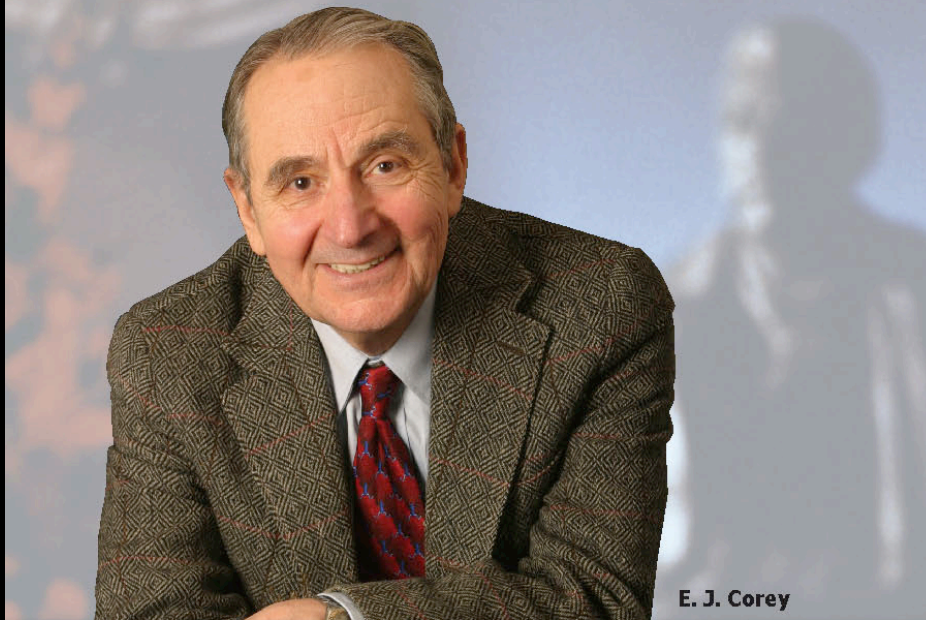
is more than one cellular target for Et743, with multiple modes for DNA interference operating simultaneously. At a molecular level, fine details such as these are always difficult to tease apart. What is beyond dispute is that ecteinascidin 743 exerts its effects through a new mode of



Kenneth L. Rinehart



Ball and stick model of the molecule of ecteinascidin 743

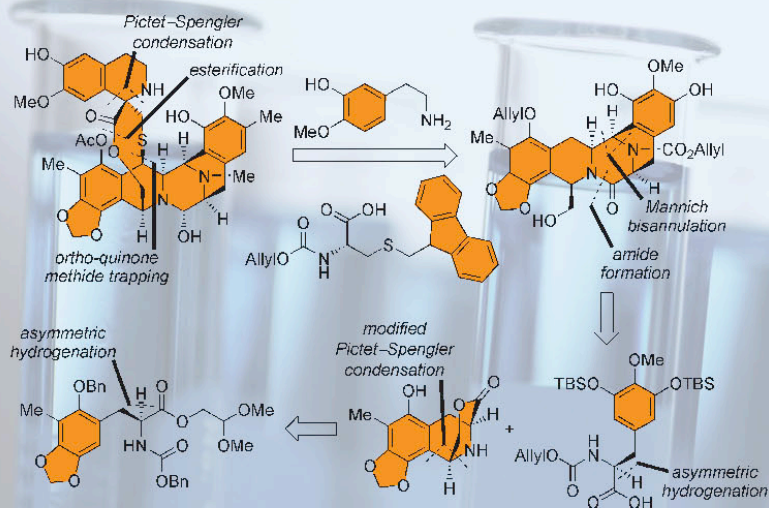


E. J. Corey



Tunicates (*Rhopalaea crassa*)

Box 2 Corey's retrosynthetic analysis of ecteinascidin 743



Ecteinascidin 743

2 4 4

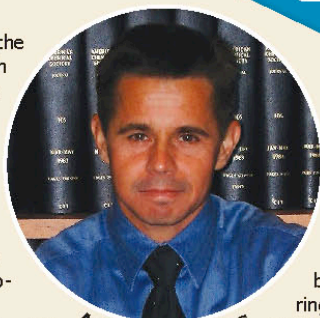
action. Thus, it is hoped that Et743 may become a powerful new weapon in our fight against cancer, provided of course that it makes it through the final stages of clinical trials. It should be emphasized that approval is not automatic, once a drug candidate has entered clinical trials. This period is one of the most arduous in the whole process of drug discovery and development, and may not ultimately end with the launch of a new medicine. All clinical drug candidates are required to pass certain safety and efficacy criteria in humans, and also to demonstrate advantages over existing therapies before they receive the official stamp of ratification from the appropriate regulatory authorities.

Before the ecteinascidin 743 clinical trials could commence, a pressing ques-

tion had to be answered, that of supply. A single dose of Et743 would require the harvesting and processing of such vast numbers of the rare and inaccessible sea squirts that this approach is not remotely practical. A chemical synthesis, carried out by E. J. Corey and his group at Harvard University in 1996, was to provide the solution, and allow the exploration of this potential drug in the clinic. The first clues towards solving this synthetic challenge were suggested in the hypothesis regarding the biosynthetic origins of the ecteinascidins proposed by Rinehart and his colleagues, the same group of scientists that had succeeded in isolating and structurally characterizing the individual ecteinascidins. They proposed a sequence of transformations beginning with the dimerization of the amino acid tyrosine,

that eventually led to the ecteinascidin skeleton. In nature, of course, this construction is a trivial operation when compared to a laboratory synthesis because of the enzymatic machinery that helps to organize and accelerate the reactions within the producing organism.

Ecteinascidin 743 has three tetrahydroisoquinoline units tightly bound together by a series of complex ring systems, both bridged and fused, including one unusual ten-membered ring bearing an intrinsic sulfide moiety. Corey's enantioselective total synthesis incorporated a number of elegant biomimetic features and impressive 'one-pot' sequences. In addition, and in the tradition of the Corey school, the employed strategy (Box 2) cleverly overcame the hurdles thrown up by the molecule along the way. Highlights of this synthesis include two asymmetric hydrogenations (see Box 6) employing state-of-the-art technologies to furnish the desired stereochemistry, intra- and intermolecular Pictet-Spengler reactions, and an intramolecular Mannich bisannulation process (Box 3). This elegant sequence allowed the researchers to construct an advanced hexacyclic intermediate quickly and efficiently, setting



Andrew G. Myers



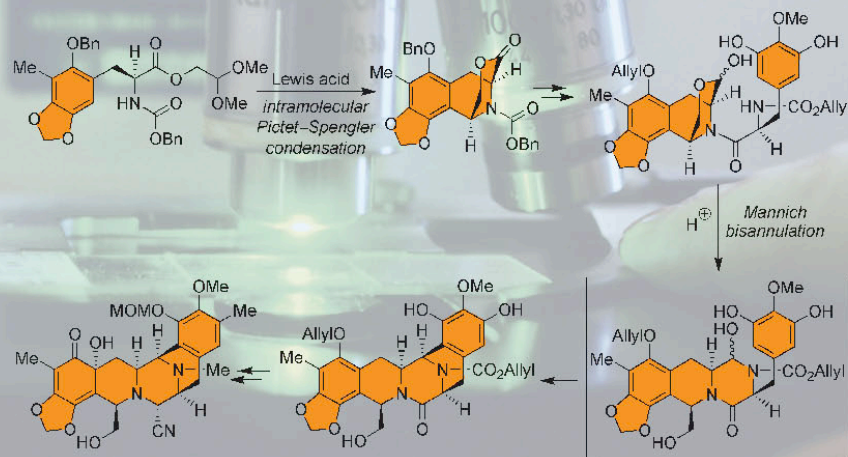
tetrahydroisoquinoline



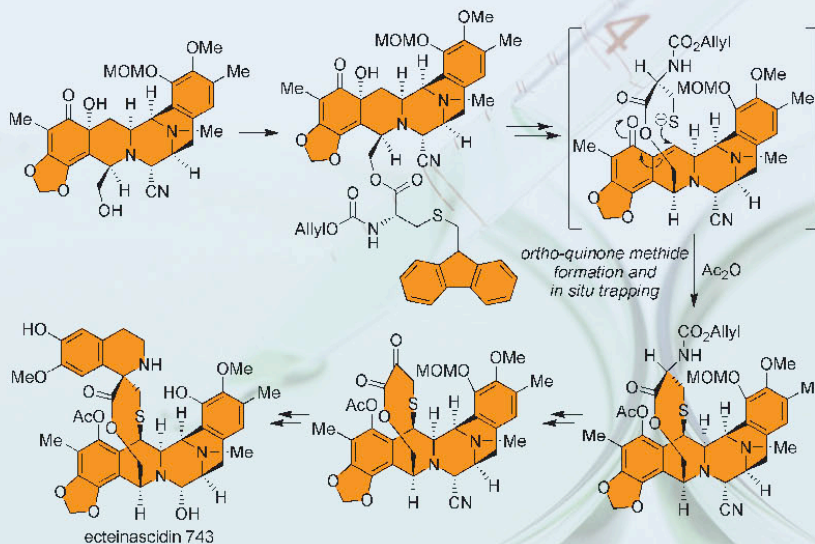
Tohru Fukuyama

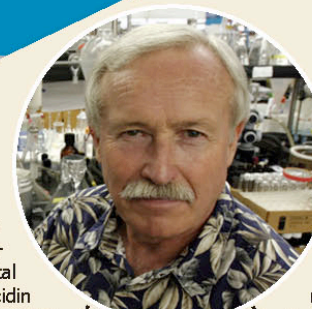
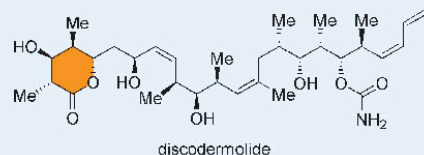
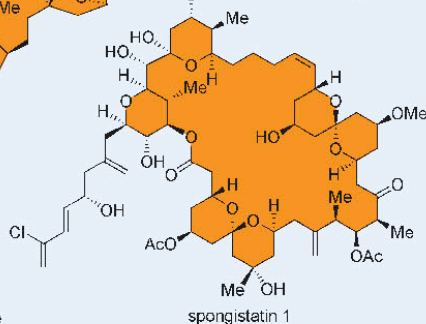
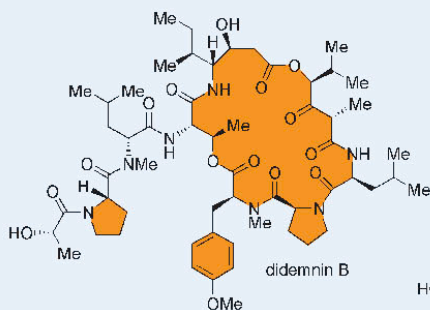
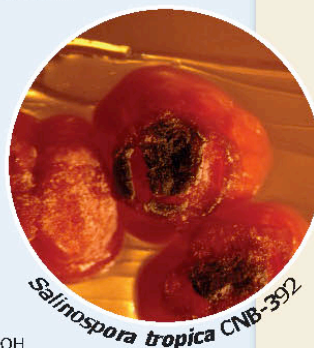
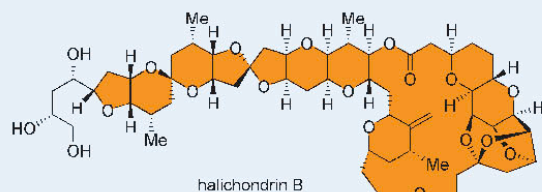
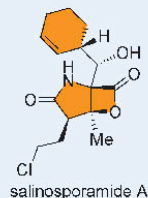
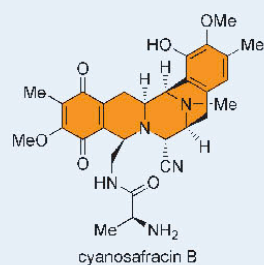
the scene for a dramatic cascade reaction to install the ten-membered sulfide-containing ring (Box 4). This daring reaction involved the generation of an *ortho*-quinone methide, the unmasking of a pendant thiol group, and the intramolecular union of these two highly reactive groups to form the large ring. A further cascade sequence to forge the final ring system, and two deprotection operations completed the total synthesis. The Corey synthesis proved suitable for large-scale production, and was later improved further in a second-generation route from the Corey group. The Spanish pharmaceutical firm PharmaMar has since reported a semi-synthesis of Et743 from cyanosafriin B (Box 5), a simpler natural product available in large quantities from bacterial culture. These triumphs of synthetic organic chemistry have overcome the supply problem, facilitating the clinical trials that are now underway around the world to evaluate ecteinascidin 743 (also known in this regard by the names trabectedin and Yondelis®).

In the year 2002, Tohru Fukuyama and his



Box 4 The late stages of Corey's total synthesis of ecteinascidin 743





William H. Fenical

group at the University of Tokyo (Japan) published their elegant total synthesis of ecteinascidin 743, which was joined in 2006 by a total synthesis from the laboratory of Jieping Zhu (CNRS, Cedex, France) and a formal total synthesis from Samuel J. Danishefsky and co-workers (Columbia University and the Sloan-Kettering Institute for Cancer Research, USA).

The saframycin family of natural products, which includes saframycin A (Box 5) and cyanosafraicin B, has also been the subject of many synthetic studies, including the recent synthesis of large numbers of analogues using solid phase techniques (see Chapter 29) by the group of Andrew G. Myers (Harvard University).

In 2004, Corey and co-workers reported the efficient total synthesis of another potential anticancer marine natural product, salinosporamide A (Box 5).

This highly potent protease inhibitor had been isolated only a year earlier by the group of William H. Fenical at the Scripps Institution of Oceanography (La Jolla, USA). Fenical has been a leading investigator of marine natural products, isolating an impressive number of fascinating chemical structures with a range of important biological activities. D. John Faulkner, also at the Scripps Institution of Oceanography, was another pioneer in the field of marine natural products chemistry, with many important discoveries to his credit.

A great many cytotoxic secondary metabolites have been isolated from marine organisms and ascribed the loose collective title of cytotoxic marine natural products. While they share a common

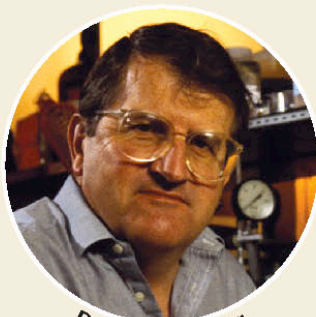




Trididemnum solidum

environmental origin and broadly defined biological activity, the compounds within this group have little else in common. Marine natural products display a wealth of structural diversity and often include intricate arrangements of heteroatoms not seen in terrestrial compounds. These features, combined with the constant need to develop new anticancer drugs, both inspire and entice synthetic organic chemists. Some of the examples shown in

by the group of Madeleine M. Joullié (University of Pennsylvania, USA), with other total syntheses reported by Ulrich Schmidt (Stuttgart University, Germany) and Takayuki Shioiri (Nagoya City University, Japan). The spongistatins are a family of potent anticancer polyketides isolated from marine sponges. The scarcity of these compounds from natural sources has prompted a number of synthetic programs, culminating in seven total syntheses to date. These emanated from the groups of Yoshito Kishi, David A. Evans (Harvard University), Amos B. Smith, III (University of Pennsylvania), Ian Paterson (University of Cambridge, UK), Michael T. Crimmins (University of North Carolina at Chapel Hill, USA), Clayton H. Heathcock (University of California, Berkeley, USA), and Steven V. Ley (University of Cambridge). Finally,



D. John Faulkner

Michael T. Crimmins

Steven V. Ley

Madeleine M. Joullié

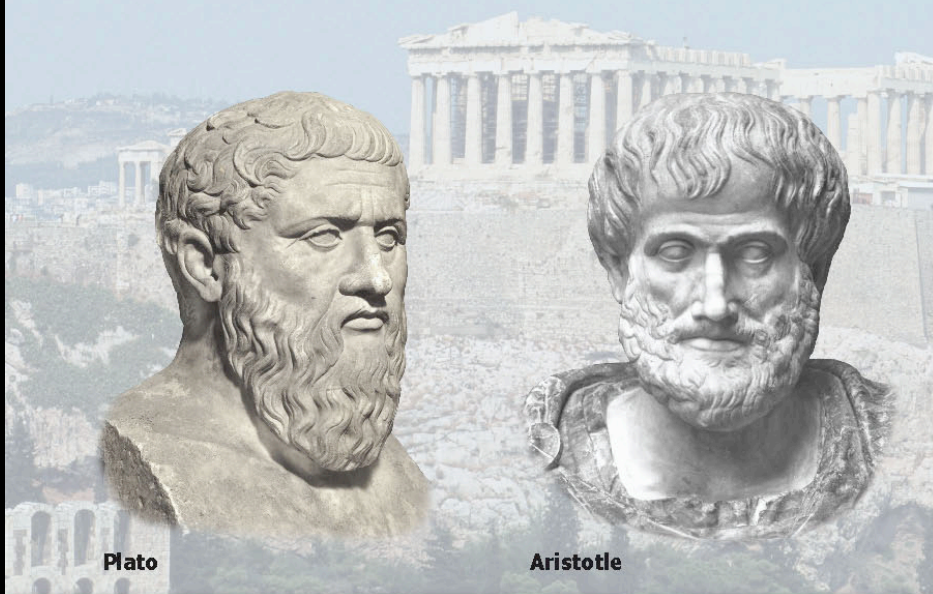
Ian Paterson

Amos B. Smith, III

Clayton H. Heathcock

David A. Evans

Yoshito Kishi



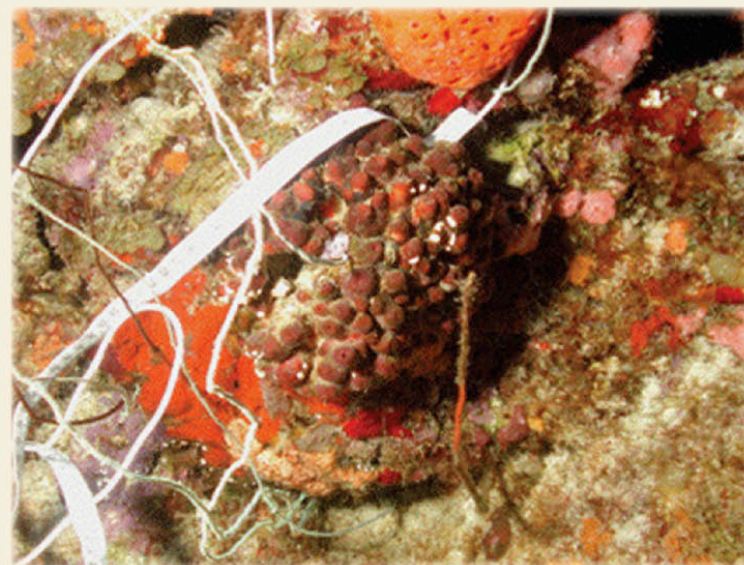
Plato

Aristotle

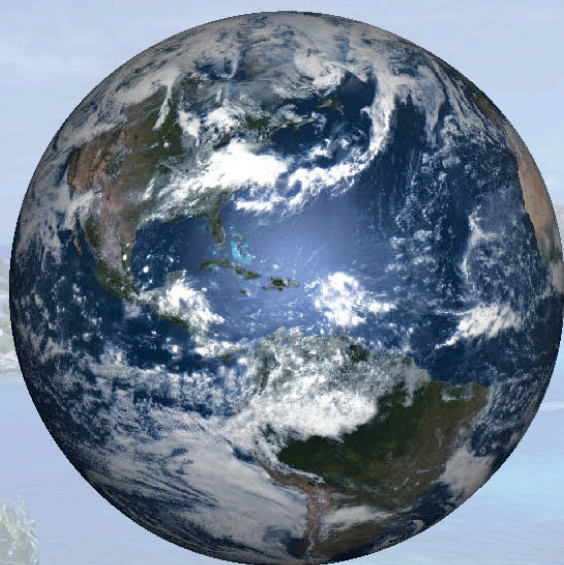
the potent anticancer activity of discodermolide has also inspired numerous total syntheses, including the recent preparation of large quantities by the Novartis pharmaceutical firm, whose hybrid synthesis borrows from the work of both Smith and Paterson.

In closing this chapter, describing some of the many medicinal leads and potential drugs discovered among marine natural products, we must reflect on the modern

tendency to neglect our oceans and seas. In past times, the ocean was revered; the ancient Greek philosopher-scientists were awed by its powerful currents and tides, and the medieval European explorers of its vast and mysterious domains were heralded as heroes, brave pioneers of this frightening and majestic environment. In the Vedic writings from India (3,000 B.C.), the *sagara* (sea) is regarded as a vault of riches, including *Amrita*, the tonic of immortality. These spiritual writers may have been closer to the truth than we care to acknowledge, for if the oceans contain a myriad of new anticancer medicines, antibiotics, and other much needed drugs, then they may indeed have a profound influence on the mortality of Man.



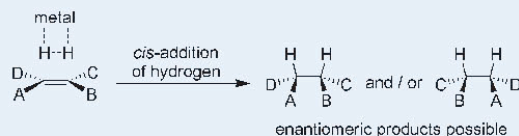
Discodermia dissoluta



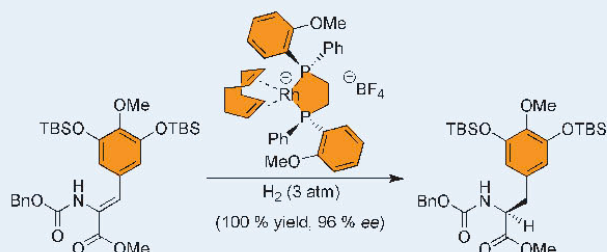
Hydrogenation is a chemical reaction wherein a molecule of hydrogen is added to a substrate, commonly across an alkene double bond to give the corresponding alkane, via a process often mediated by a metal catalyst. This process is an extremely useful transformation in chemical synthesis, for it provides an entry to many intermediates and new compounds required for further investigations. If the alkene is unsymmetrically substituted, then during the hydro-

genation one, or even two, new chiral centers can be generated. If the stereochemistry of these centers is appropriately controlled by employing a suitable chiral catalyst, then the reaction, now elevated to the status of catalytic asymmetric hydrogenation, becomes even more powerful; its usefulness being due to the fact that most naturally occurring substances and active pharmaceutical components are enantiomerically pure, that is to say of one handedness.

Catalytic hydrogenation



Catalytic asymmetric hydrogenation



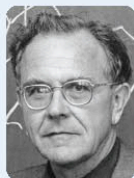
The following milestone events trace the evolution of this important chemical process:



■ **1912** – Paul Sabatier (University of Toulouse, France) is awarded the Nobel Prize in Chemistry “for his method of hydrogenating organic compounds in the presence of finely disintegrated metals.”



■ **1956** – S. Akabori (Osaka University, Japan) reports the use of palladium on silk in asymmetric hydrogenation reactions. Interestingly, the silk had to be wild, not the cultivated type, and the enantiomeric excesses (ee) were low with poor reproducibility.



■ **1965** – The 1973 Nobel Laureate in Chemistry, Geoffrey Wilkinson (Imperial College, London, UK), discovers the rhodium catalyst system [RhCl(PPh₃)₃] which bears his name, and which could be employed in homogeneous hydrogenation reactions, unlike the heterogeneous metal catalysts employed previously.



■ **1968** – William S. Knowles, from the Monsanto Company (USA), publishes a reliable enantioselective catalyst system that favors one enantiomeric product over the other in hydrogenation reactions. Knowles used a variant of Wilkinson’s catalyst, in which he had replaced a non-chiral triphenylphosphine ligand with a chiral analogue. He later refined his chiral catalyst to achieve practical levels of enantiomeric enrichment. This process found applications in the industrial production of both L-DOPA, a drug used to treat Parkinson’s disease, and NutraSweet®, the non-caloric sugar substitute.

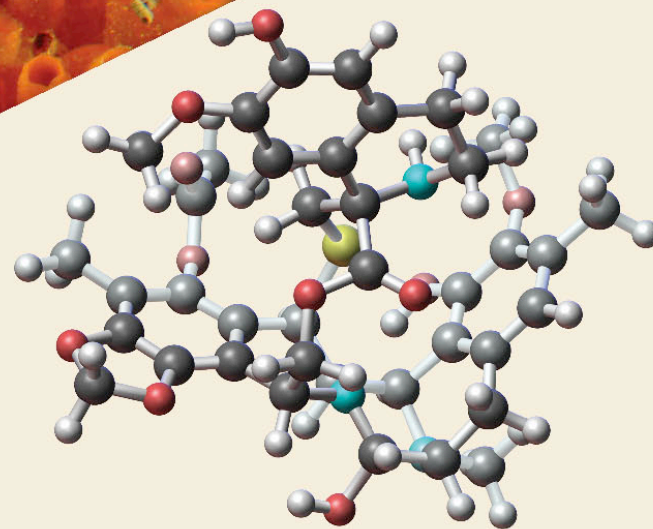


■ **1971** – Henri B. Kagan (Orsay University, France) develops a rhodium catalyst with a DIOP ligand (a diphosphine derived from tartaric acid) that induced impressive asymmetric induction in hydrogenation reactions of dehydro amino acid precursors. This C₂ symmetric ligand provided impetus for further developments in chiral catalyst design, with major implications for asymmetric synthesis.



■ **1980** – Ryoji Noyori and his associates at Nagoya University (Japan) publish the first asymmetric reactions induced by BINAP, a chiral phosphine ligand. This remarkable ligand and its variants have since assumed a prominent role in asymmetric catalysis with important applications in the production of enantiomerically pure compounds, through a wide range of reactions.

■ **2001** – The groundbreaking works of Knowles and Noyori are recognized by the Nobel Foundation with the award of the Nobel Prize in Chemistry “for their work on chirally catalyzed hydrogenation reactions,” which they shared with a third leader in the field of asymmetric catalysis, K. Barry Sharpless (see Chapter 26).



Further Reading

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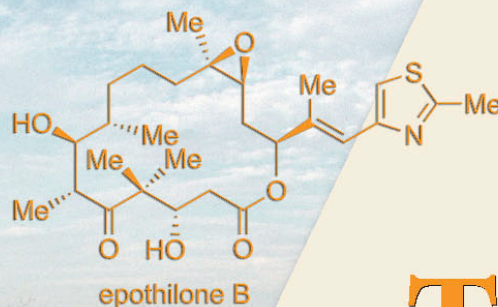
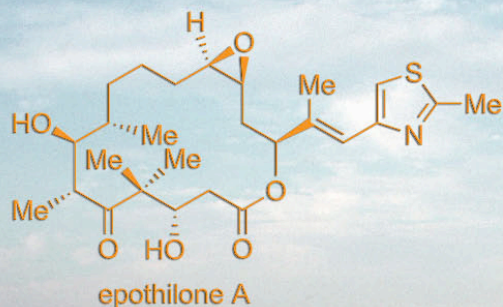




Chapter 29

1996, 1997





The search for anticancer drugs is a major driving force for the isolation and screening of new natural products. Just as the frenzied research on Taxol® (Chapter 25) was reaching its peak in the 1990s, a new class of potential anticancer drugs was, quite literally, being unearthed. The story of these cytotoxic agents, the epothilones, began slowly, but developed rapidly after biological testing indicated the potential of these compounds as medicines to fight drug-resistant cancers.

Epothilones A and B were first isolated in the early 1990s from the culture extracts of the myxobacterium *Sorangium cellulosum* (strain So ce90) which had been found in soil samples taken from the banks of the Zambezi River in southern Africa. The discoverers, Gerhard Höfle, Hans Reichenbach, and their collaborators at the Gesellschaft für Biotechnologische Forschung (Braunschweig, Germany), initially sought to probe the antifungal activity of these compounds with a view to employing them as agricultural fungicides. However, field trials proved that these compounds were too toxic against plants for this application, and their full medicinal potential remained undiscovered for a while. Meanwhile, scientists at the pharmaceutical company Merck (USA) independently isolated both epothilones A and B and, by 1995, had discovered that these polyketide secondary metabolites were highly effective antitumor agents. Indeed, in many of the cell lines used in typical first round tests for cytotoxicity, the epothilones outperformed Taxol®. Perhaps most importantly, further studies revealed that the new substances were

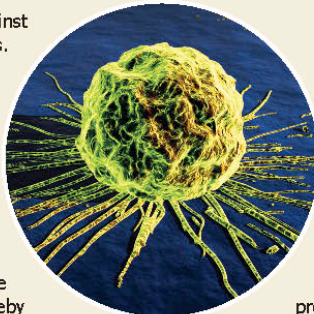


The epothilone-producing myxobacterium *Sorangium cellulosum*: growing cells (left) and spore capsules (right)

Zambezi River

Hans Reichenbach and Gerhard Höfle

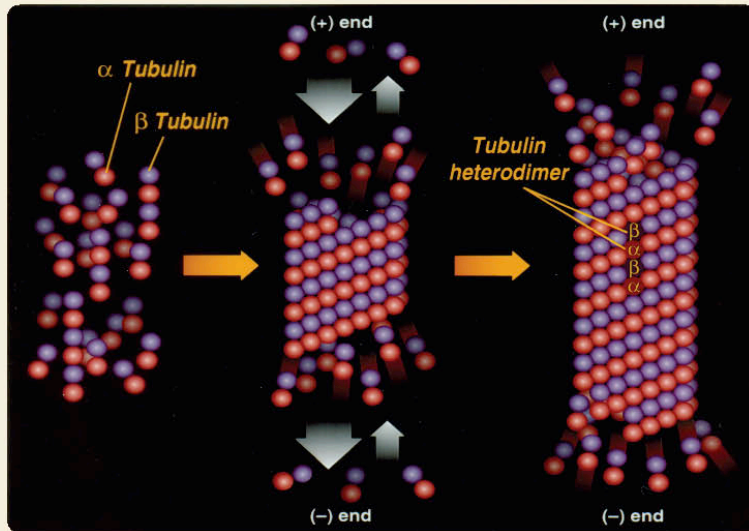
remarkably potent against Taxol®-resistant tumor cells. Intriguingly, they were also found to operate by a mechanism identical to that of Taxol®, even binding to the same site on the same protein target. Specifically, the epothilones were found to induce tubulin polymerization and to stabilize microtubules, and thereby irreversibly damaging replicating cells, driving them to their death (see diagram below and pictures on the right). As a result of this highly significant observation, a great deal of excitement and interest spread amongst the scientific community as hopes were raised about the possibility of developing new and superior agents for use in cancer chemotherapy. In a curious twist, a series of mis-



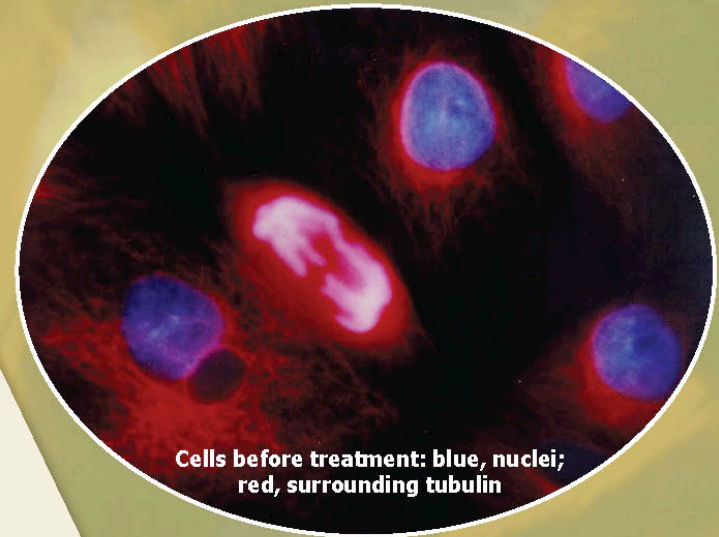
Cancer cell

calculations by the major pharmaceutical players meant that the epothilones were left without patent protection for their potential anticancer application. Fortunately, this feature did not curb their march to the clinic for long, and soon a new wave of investigators opted to pursue them as promising leads.

Despite the fact that the original patent describing the epothilones was filed in early 1990, it was not until July 1996 that the German scientists published the full details of their molecular structures.

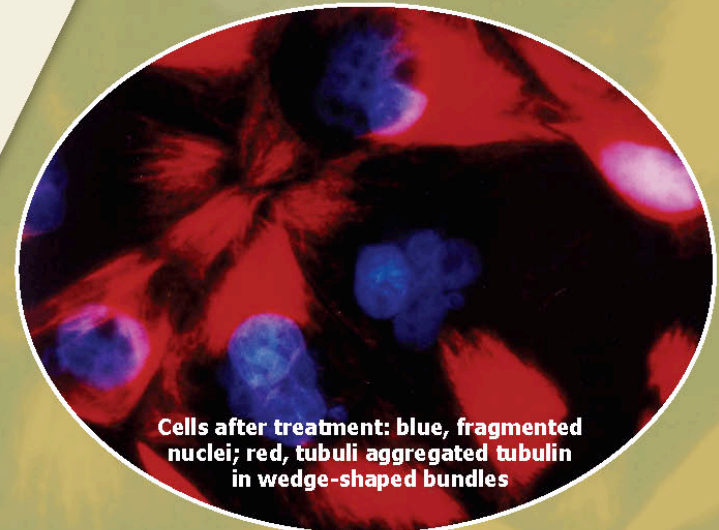


Polymerization of tubulin to microtubules



Cells before treatment: blue, nuclei; red, surrounding tubulin

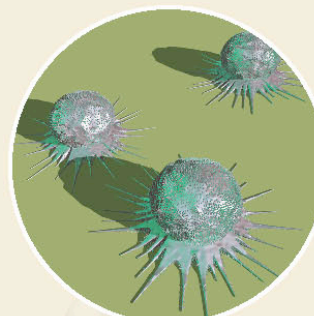
epothilone B



Cells after treatment: blue, fragmented nuclei; red, tubuli aggregated tubulin in wedge-shaped bundles

Samuel J. Danishefsky

Dieter Schinzer

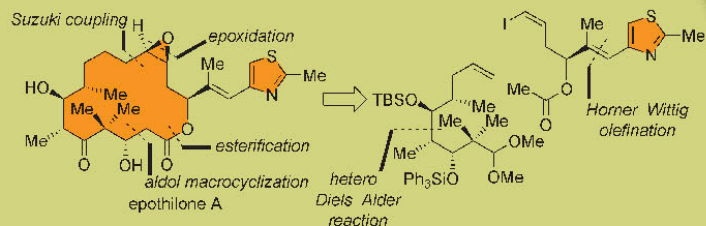


Cancer cells

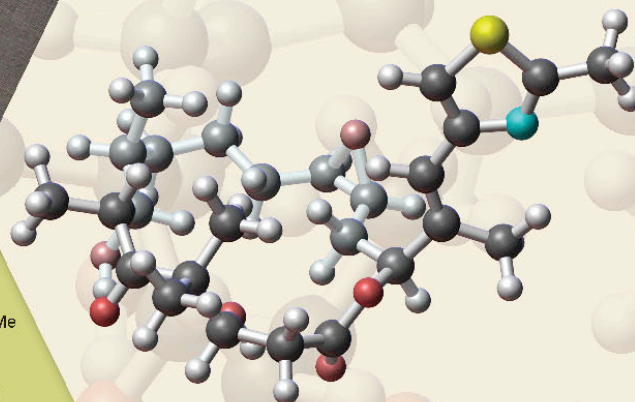
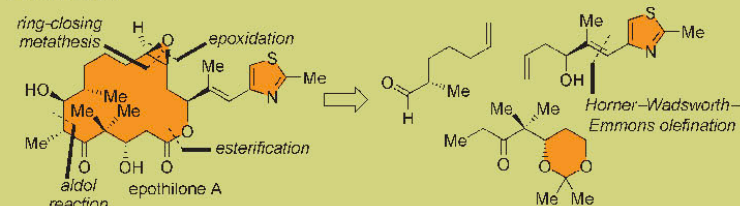
Box 1

Danishefsky and Schinzer's retrosynthetic analyses of epothilone A

Danishefsky *et al.*



Schinzer *et al.*



Ball and stick model of the molecule of epothilone A

Following this revelation, it took less than one year for the first laboratory syntheses of these compounds to appear in the literature, providing testament to the skill of the synthetic chemists involved and the urgency of the need to find new anti-cancer agents. Since then, over twenty additional synthetic routes toward these compounds have joined the original four that were published over a period of only four months by the groups of Samuel J. Danishefsky (Sloan-Kettering Institute and Columbia University, New York, USA), K. C. Nicolaou (The Scripps Research Institute and University of California, San Diego, USA, two strategies), and Dieter Schinzer (then at Braunschweig University, now at the University of Magdeburg, Germany). The key retrosynthetic disconnections of the first four successful approaches to epothilone A are shown in Boxes 1 and 2. All these syntheses were carried out essentially simultaneously, and some common features are evident. All three groups chose related olefination procedures to attach the heterocyclic side chain, although the exact conditions and coupling partners varied. However, significant differences are found in the overall strategies employed, both for forming the macrocyclic ring and for constructing the acyclic precursor. All of these sequences were the result of careful scientific planning, but the individual creativity of the scientists involved is reflected in the different paths followed to the same target. The epothilones also served as an excellent template for chemists to test and exploit a broad range of new techniques. For example, the problem of how to construct the epothilone macrocycle fueled investigations into the scope of olefin metathesis (see Box 6), accelerating its development and significantly expanding its scope and utility. Today, this powerful

reaction has become an indispensable tool to the organic chemist, often providing novel solutions to synthetic puzzles of considerable complexity.

The first total synthesis of epothilone A to use an olefin metathesis approach came from the Nicolaou group and was published early in 1997. Although the molecular architectures of the epothilones are considerably less complex than that of Taxol®, the construction of a sixteen-membered macrolide ring, the installation of seven stereocenters around its periphery, and the attachment of a thiazole side chain still presented serious obstacles. The chemists knew that a better understanding of the biological interactions and structure-activity relationships of the epothilones could potentially lead to the discovery of clinically useful therapeutic agents. Driven by this goal, the Nicolaou group sought to develop a flexible and convergent synthetic strategy that would deliver not only the naturally occurring substances, but also a diverse collection of designed analogues. Thus, contrary to the objective of most other synthetic plans where the controlled installation of stereochemical elements is a major concern, the goal in this instance was to gain access to a range of isomers and related compounds in order to explore which structural motifs were crucial for biological activity. The result of this plan was the olefin metathesis-based approach shown in Box 2. The acyclic precursor was to be formed from three building blocks of roughly equal size via aldol and esterification reactions.



K. C. Nicolaou

This plan was successfully executed to furnish epothilone A. The key olefin metathesis reaction was accomplished using a ruthenium catalyst developed by Robert H. Grubbs at the California Institute of Technology (USA). This remarkable macrocyclization reaction gave rise to a mixture of double bond isomers, slightly favoring the *cis*-olefin, thereby supplying the precursor to the desired final product. Following completion of this synthesis, which was undertaken using conventional solution-phase reactions, the Nicolaou team adapted it for use with solid-phase synthesis technology. This facilitated the synthesis of several hundred epothilone analogues for biological screening.

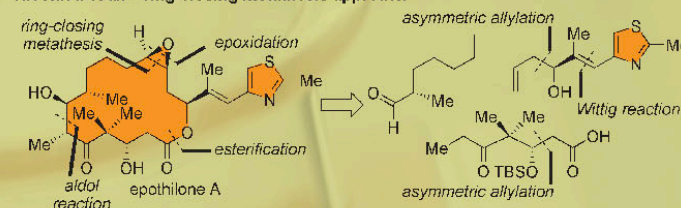
The solid-phase total synthesis of epothilone A (Box 4) and its application to the construction of a collection of designed epothilone analogues (described as an epothilone library) was based on principles pioneered by R. Bruce Merrifield at The Rockefeller University (New York, USA) for the synthesis of peptides, for which he received the 1984 Nobel Prize in Chemistry. In this approach, the growing molecule is chemically attached to a polymer support, or resin, which comes in the form of small beads, usually made of polystyrene. During the synthesis, reactions are driven to completion (with consequently higher yields) by the addition



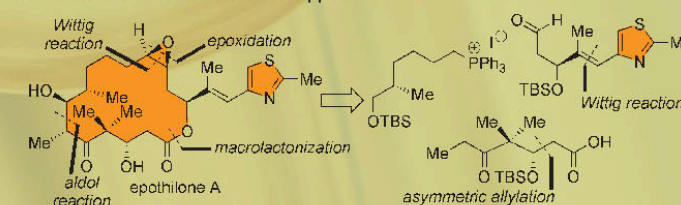
The Nicolaou epothilone team

Box 2 Nicolaou's retrosynthetic analyses of epothilone A

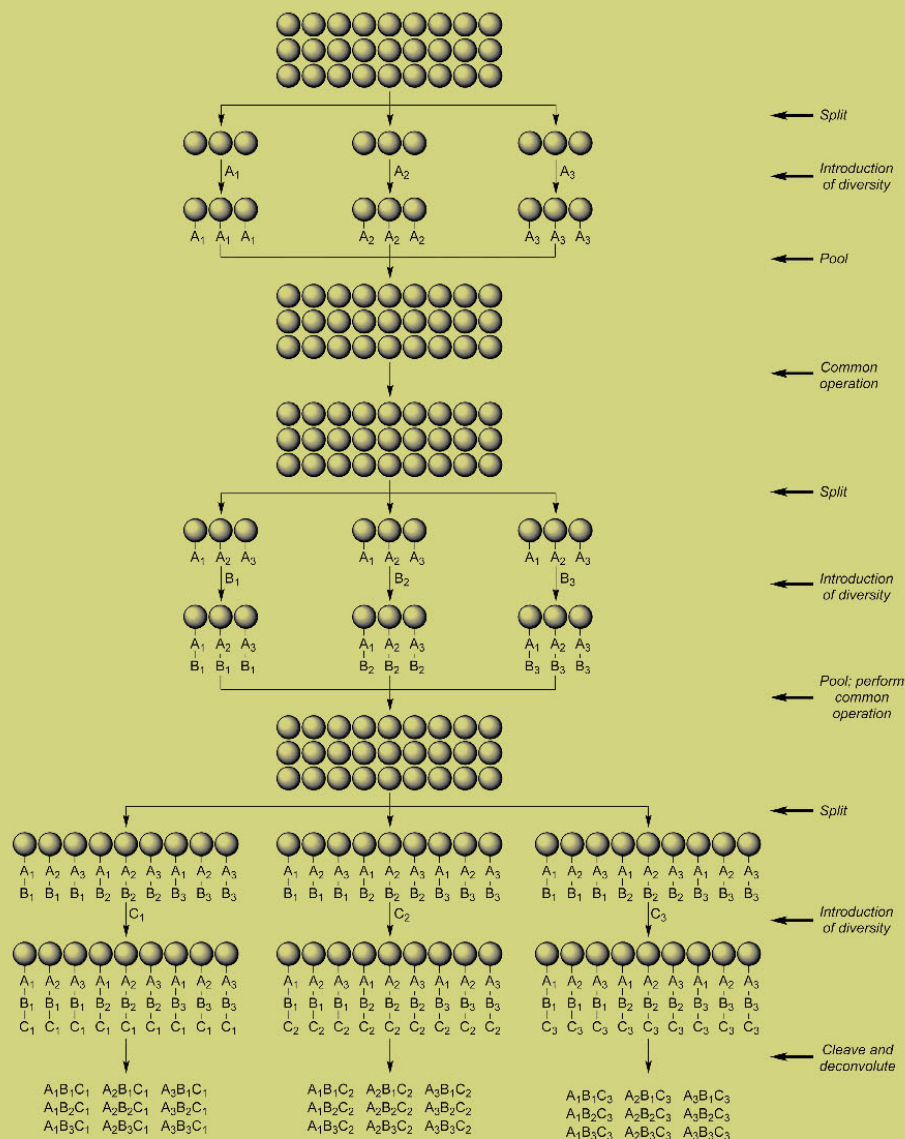
Nicolaou *et al.* – ring-closing metathesis approach



Nicolaou *et al.* – macrolactonization approach



Box 3 The split-and-pool strategy for combinatorial synthesis



R. Bruce Merrifield

of reagents in large excess. The leftover reagent can easily be removed after each step by washing the product-containing beads with appropriate solvents. This method has the advantage of avoiding costly and time-consuming purification procedures at the end of each operation. Furthermore, the characteristics of solid-phase chemistry mean that it is particularly well suited for use in combinatorial chemistry. The purpose of combinatorial chemistry is the assembly of collections (libraries) of many compounds using combinations of building blocks that come in sets with specified variations. The objective is met by forging such fragments into compounds using all possible or logical combinations, thereby building up the maximum number of analogous compounds. The development of solid-phase techniques also allowed for the implementation of the so-called split-and-pool combinatorial strategy, which allows the construction of large compound libraries very rapidly (Box 3). To achieve this goal, the resin is divided into lots and a different variant of the first building block is introduced to each lot. The resin is then pooled so that common operations can be undertaken on the entire batch (saving time and money). These operations range from simple washings to

reactions such as deprotections and functional group manipulations. The resin is subsequently split into lots again and the synthesis is continued by the addition of the next building block, with a different variant employed for each newly divided lot of resin. This iterative process is repeated until the library construction is complete, at which time the products

are cleaved from the resin and purified to provide the desired library of compounds. Even with relatively small numbers of each unit, this approach allows for the construction of very large libraries and requires less time and effort than the conventional synthesis of individual compounds. The structural information regarding the growing molecules that could potentially be lost at the pooling stages may be saved by using encoding techniques, such as bar coding or radio-frequency tagging of the resin cartridges. The epothilone library was synthesized using MicroKan® resin micro-reactors, developed by the IRORI company, bearing barcodes that could be read by a scanner in order to track the synthetic history of each compound.



Microkan® reactors

The elegance of the solid-phase combinatorial synthesis of the epothilones (Box 4) lies in the use of the olefin metathesis not only to bring about macrocyclization, but also as a tactic to cleave the product from the resin in a single process. This so-called cyclorelease strategy has the added advantage of leaving no trace of the resin attachment site in the final products. The final two synthetic steps were carried out in solution and furnished a diverse library of epothilone analogues as discrete and pure compounds ready for biological evaluation.

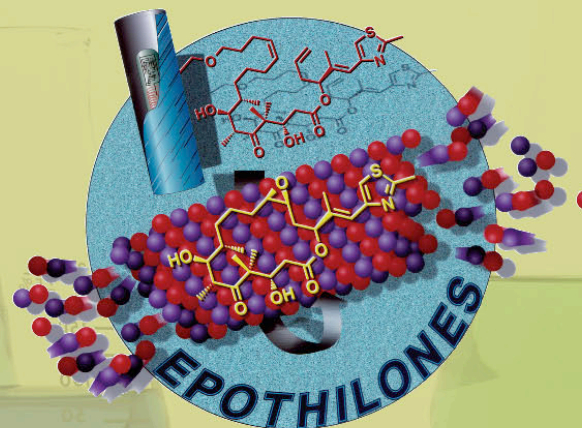
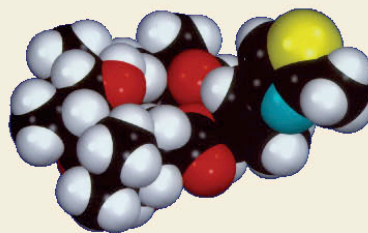
Today, solid-phase chemistry can be applied to the synthesis of both natural and designed molecules, either in a one-at-a-time fashion or as relatively small compound libraries (focused or designed libraries) for screening in various biological assays. Notable developments in the field include the use of solid-supported reagents, and the use of scavenger resins equipped with chemical groups to remove excess reagents and impurities from reaction mixtures, thus facilitating the advancement of the growing synthetic intermediate to the next stage. Steven V. Ley (University of Cambridge,



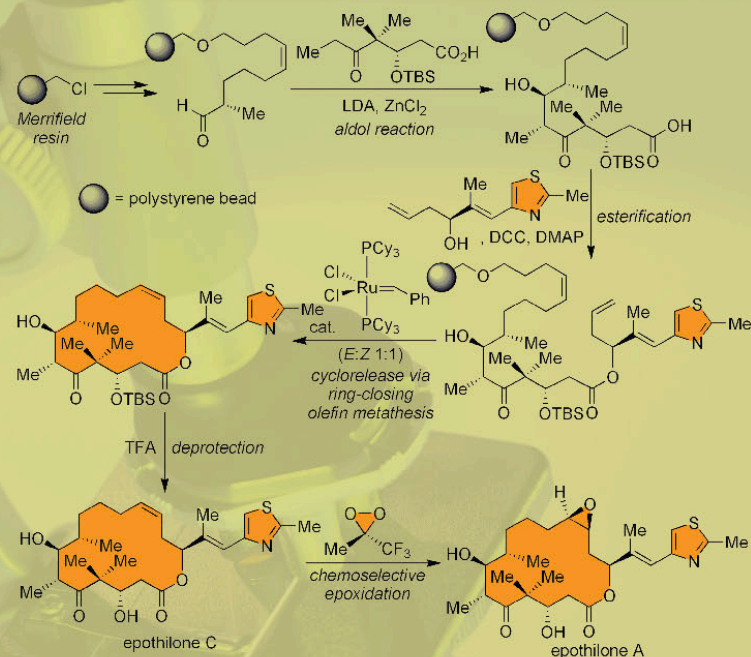
Steven V. Ley



Space-filling models of epothilones A (top) and B (bottom)



Box 4 Nicolaou's solid-phase synthesis of epothilones A and C

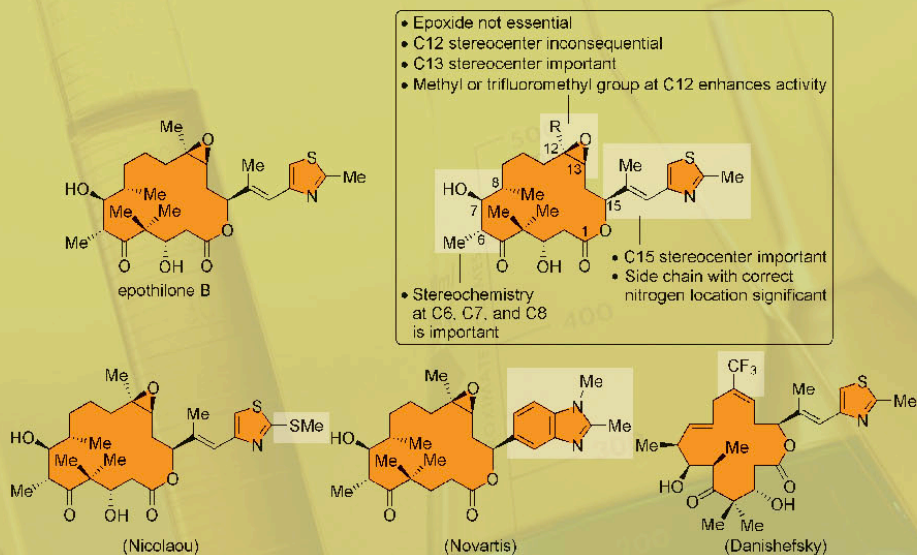




Samuel J. Danishefsky

K. C. Nicolaou

Box 5 SAR results for the epothilones and selected designed analogues



Epothilones

2 5 8

UK) has been a pioneer in developing such solid-phase reagents and strategies, now finding their way into common laboratory practices.

Epothilone B, the most potent of the naturally occurring epothilones, has been synthesized in the laboratory by several groups, including those of Danishefsky and Nicolaou. The worldwide efforts in the chemical synthesis of the epothilones enabled extensive chemical biology investigations and allowed their structure-activity relationships (SARs) to be established (Box 5). Today, several epothilones are undergoing late-stage clinical trials, spearheaded by companies such as Novartis, Bristol-Myers Squibb, Schering AG, and Hoffmann-La Roche, with the hope that at least some will soon be approved as medicines to help patients afflicted by the dreaded disease of cancer.



Nissim Calderon



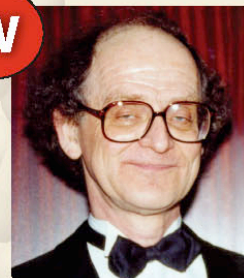
Yves Chauvin

Mo



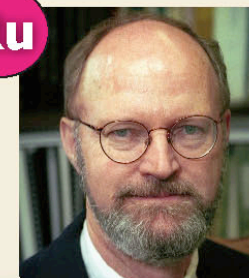
Richard R. Schrock

W



Thomas J. Katz

Ru



Robert H. Grubbs

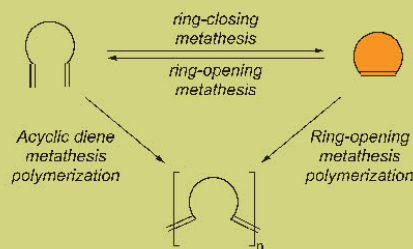
Metathesis reactions, such as those used in the synthesis of the epothilones, revolutionized organic synthesis in the 1990s, but the roots of these processes can be traced back nearly fifty years. There are three main variations of the metathesis reaction (see adjacent): alkene metathesis, alkyne metathesis, and enyne metathesis. Alkene metathesis involves the formal cleavage of the alkene reactants, and the interchanging and recombining of the alkylidene fragments to give two new alkenes. The intermolecular cross-metathesis process is used in the petrochemical industry for the production of linear alkenes. If the two alkenes are within the same molecule, a ring-closing metathesis (RCM) reaction results, usually with the expulsion of a small alkene by-product. Metathesis reactions can also be used to prepare polymers from either cyclic alkenes or acyclic dienes. This process is widely used in both academic and industrial laboratories.

Alkyne metathesis, using metal alkylidyne catalysts, works in an analogous manner. Enyne metathesis involves the formal cleavage of the alkene bond, and the addition of the fragments to the ends of the alkyne, forming a diene product. Again, this reaction can be employed in both cross- and ring-closing variants, however, unlike the alkene and alkyne metatheses, there is no by-product expelled in this process.

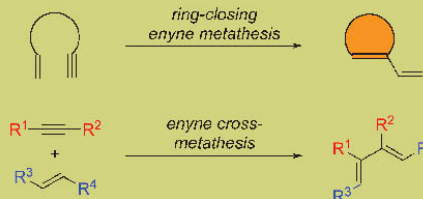
The olefin metathesis reaction was recognized by Nissim Calderon of the Goodyear Tire and Rubber Company, who coined the term from the Greek for 'change places'. The generally accepted mechanism for alkene metathesis is based upon the suggestions made by the French chemist Yves Chauvin, with key experimental evidence later provided by the groups of Thomas J. Katz (Columbia University, USA) and Robert H. Grubbs (then at the University of Michigan, now at California Institute of Technology, USA). In 1976, Katz and his group described the first well-defined metathesis catalyst, a tungsten Fischer carbene. This milestone was followed in 1990 by the introduction of a molybdenum-based catalyst by Richard R. Schrock (Massachusetts Institute of Technology, USA), and in 1995 and 1999 by Grubbs' first- and second-generation ruthenium-based catalysts. Today, chemists have a large number of catalysts at their disposal, most frequently based on ruthenium or molybdenum, developed by a number of investigators. Each catalyst has its own characteristics, and the choice of which to utilize is dictated by the particular reaction being considered. Grubbs, Schrock, and Chauvin were awarded the 2005 Nobel Prize in Chemistry for their work on the development of the metathesis reaction as a tool for organic synthesis.

As mentioned above, metathesis processes are used in the petrochemical industry for processing hydrocarbons. The cross-metathesis process for the production of polymers was one of the first examples of olefin metathesis observed and is still widely used today. The use of RCM in the pharmaceutical industry is beginning to emerge. For example, Boehringer Ingelheim Pharma in Germany used RCM in the preparation of almost 400 kg of a drug candidate for the treatment of hepatitis C, BILN 2061 ZW.

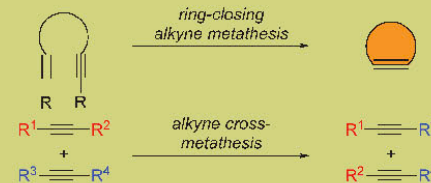
Alkene metathesis



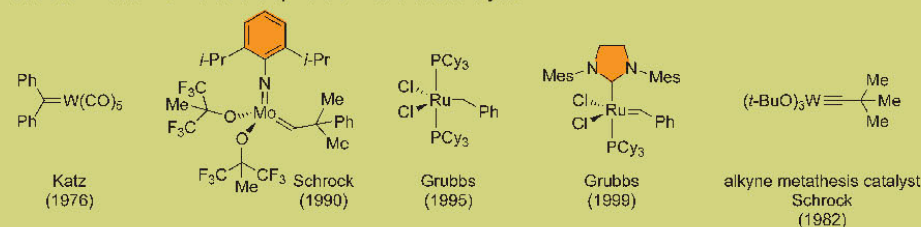
Enyne metathesis



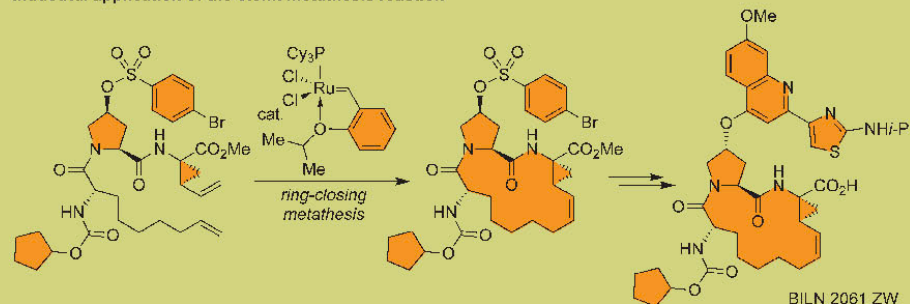
Alkyne metathesis

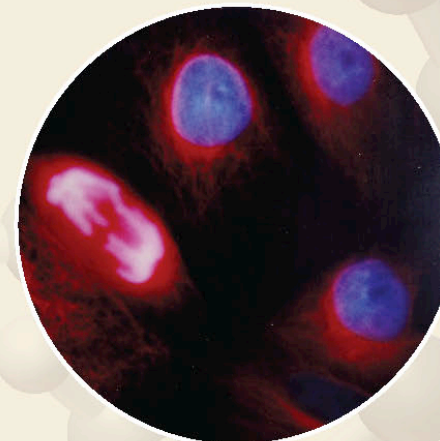
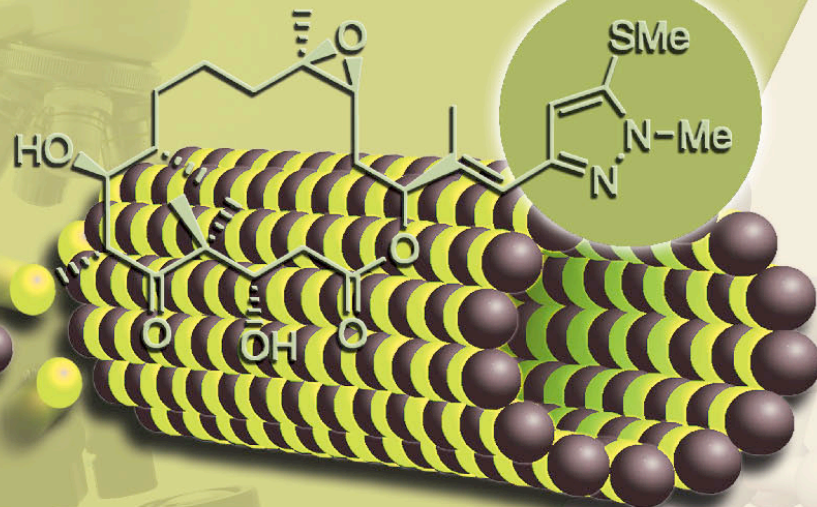


Selected milestones in the development of metathesis catalysts



Industrial application of the olefin metathesis reaction





Further Reading

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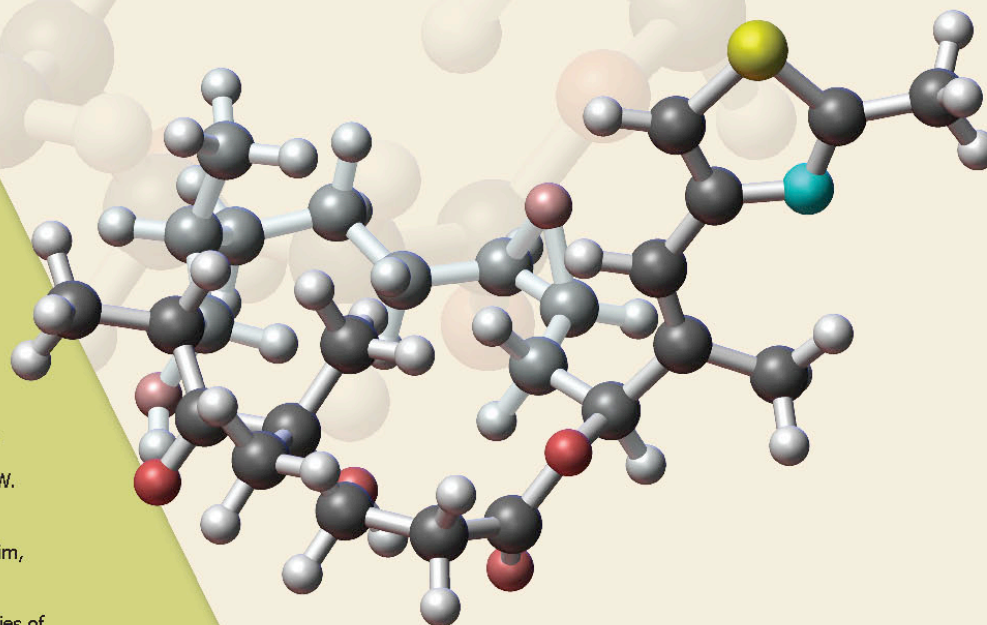
K. C. Nicolaou, F. Roschangar, D. Vourloumis, Chemical Biology of Epothilones, *Angew. Chem. Int. Ed.* **1998**, *37*, 2014–2045.

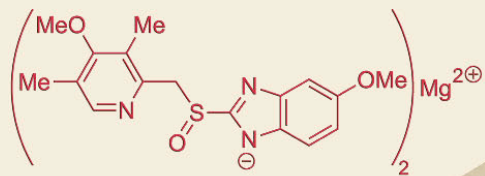
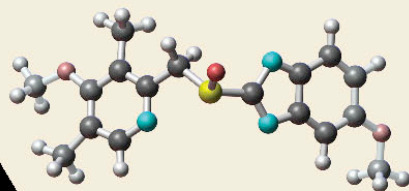
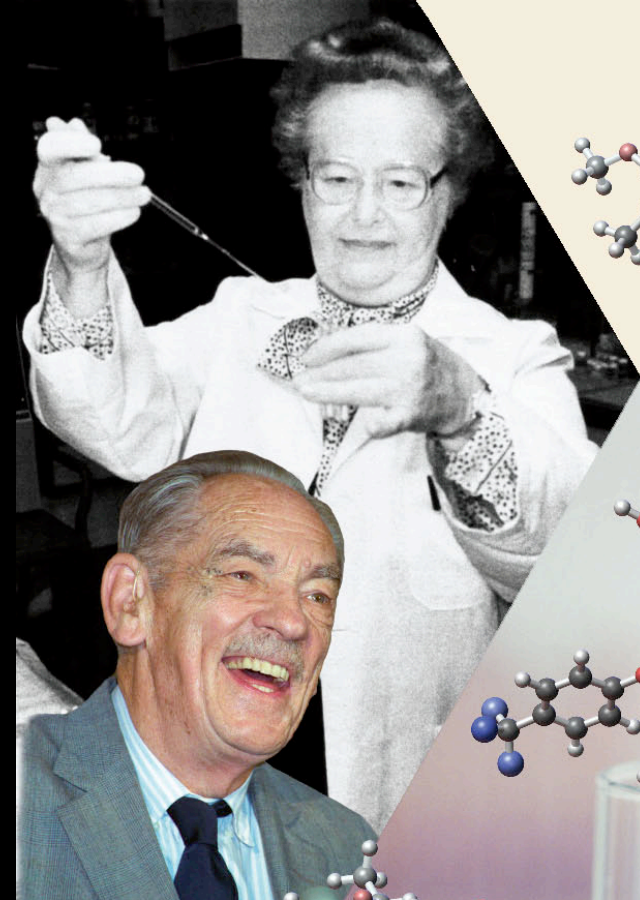
K. C. Nicolaou, A. Ritzén, K. Namoto, Recent Developments in the Chemistry, Biology and Medicine of the Epothilones, *Chem. Commun.* **2001**, 1523–1535.

Handbook of Combinatorial Chemistry, Vols. 1 & 2, K. C. Nicolaou, R. Hanko, W. Hartwig, Eds., Wiley-VCH, Weinheim, **2001**.

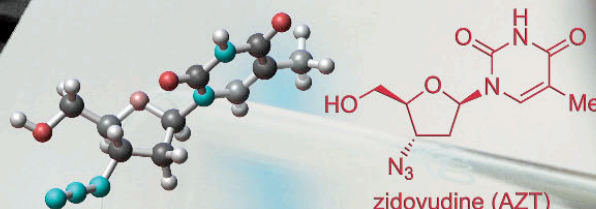
K. C. Nicolaou, S. A. Snyder, *Classics in Total Synthesis II*, Wiley-VCH, Weinheim, **2003**, pp. 161–210.

A. Rivkin, T.-C. Chou, S. J. Danishefsky, On the Remarkable Antitumor Properties of Fludelone: How We Got There, *Angew. Chem. Int. Ed.* **2005**, *44*, 2838–2850.

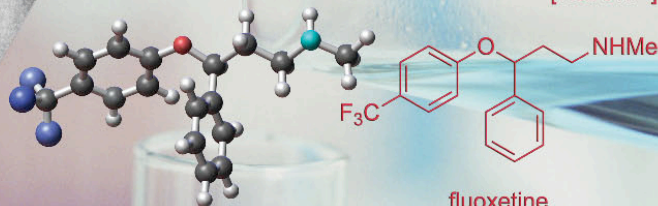




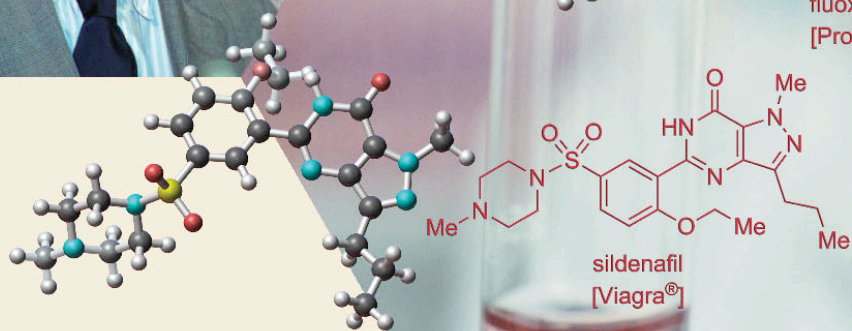
omeprazole
[Losec®, Nexium®]



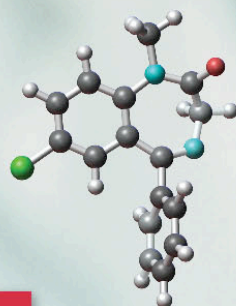
zidovudine (AZT)
[Retrovir®]



fluoxetine
[Prozac®]



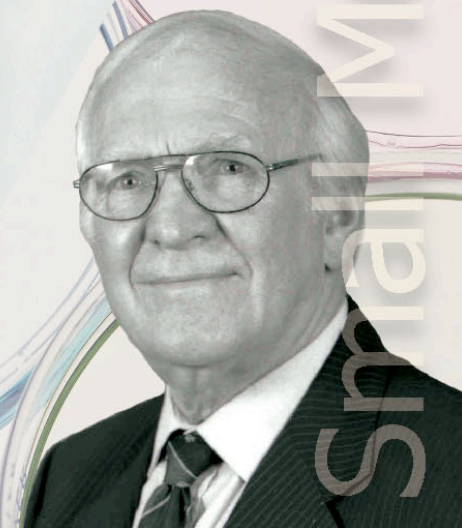
sildenafil
[Viagra®]



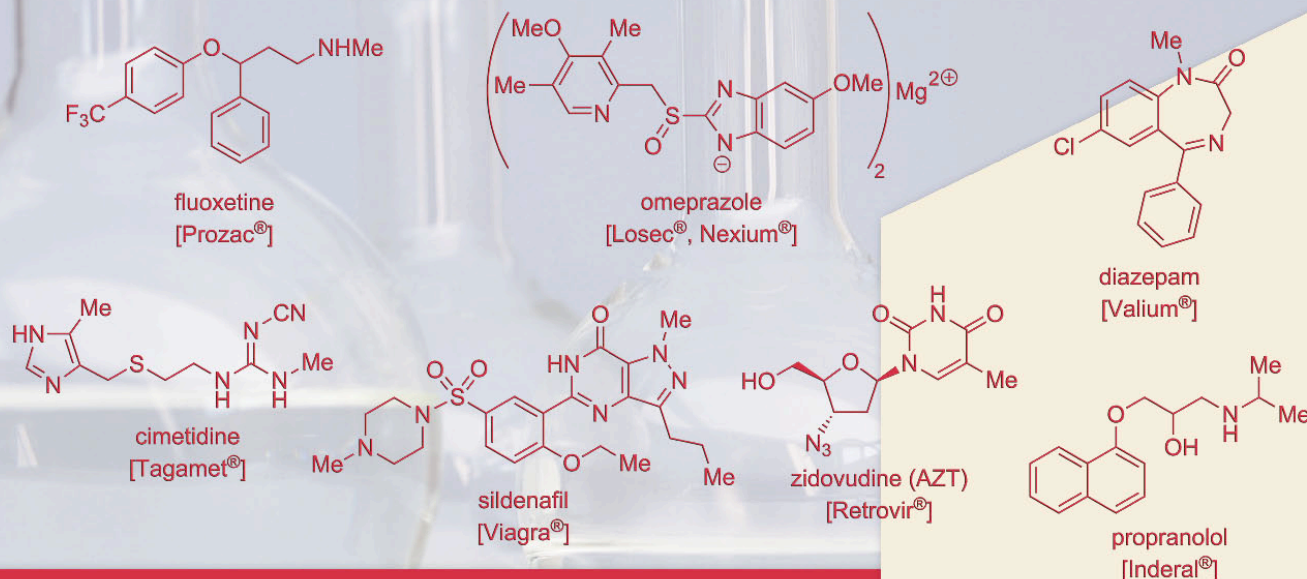
diazepam
[Valium®]

Small Molecule Drugs

Chapter 33



Small Molecule Drugs



Modern Drug Discovery and Development

While the practice of drug discovery and development in the modern pharmaceutical industry may vary from company to company, it follows the general pattern described below, consisting of five distinct phases. The overall process is challenging, time-consuming, and very expensive (Box 1).

Determination of the disease pathogenesis and development of an assay. Before chemists become involved, the drug discovery process begins with biologists and physicians, who must unravel the complex pathways that lead to a specific disease. Ideally, this research will identify a particular target such as an enzyme or a receptor, modulation of which is expected to cure or manage the disease. Biochemists must then develop an assay to determine the effects of small molecules on the target. The complexity of these assays can vary dramatically, from simple biochemical tests, to cultures of viruses, bacteria, or tumor cells. Simpler assays are easier to perform and can be used to screen hundreds of thousands of compounds quickly; more complex systems may give a more reliable indication of the efficacy of a compound under physiological conditions. Validation of the biological target must be carried out as early as possible in the development process. This involves confirming that interference with the selected biological target will indeed result in benefits to the patient.

Finding a lead compound. With an assay available, chemists can begin to search for compounds as potential leads for further refinement. Such preliminary screening often looks for a single property in the compounds tested, selecting, for example, only those compounds that

In this chapter, we will focus not on a story of a natural product, but rather on some important classes of designed small molecule drugs. As in so many other fields, chemical synthesis plays a vital role in drug discovery and development. We will begin with a brief overview of the modern drug discovery and development process, highlighting the different roles played by chemists, and then relate the stories of various medications to treat mental illnesses, viral infections, gastrointestinal disorders, heart conditions, and sexual dysfunction.

Box 1 The drug discovery and development process

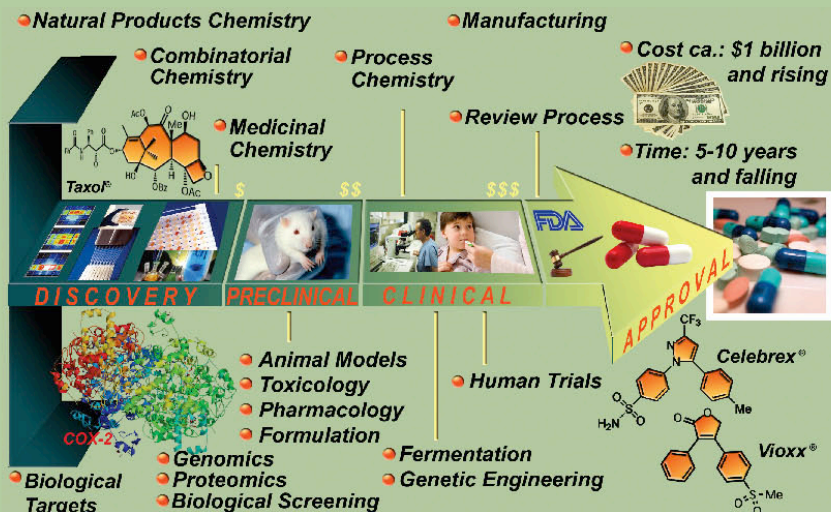


exhibit significant activity in the biological assay. As we have seen in the preceding chapters, identifying leads from nature can be highly rewarding, and some believe natural products offer the best opportunities for drug discovery, although isolating and determining their structures can be a laborious and expensive process. Alternatively, chemists can design and synthesize any number of compounds, often with the help of robotics and combinatorial techniques (see Chapter 29). This latter strategy has the advantage that the structures of the molecules are already well-defined, although the compounds prepared in this manner are often of limited structural complexity. Over the last few decades, pharmaceutical companies, as well as specialist suppliers, have amassed vast libraries of compounds which are screened to provide leads for developing new drugs.

Optimizing a lead compound.

Once a suitable lead compound has been identified, chemists can begin refining its chemical structure to improve both its activity in the assay and its physicochemical properties in a process called rational drug design. The relationship between the structure of the molecule and its biological activity is explored by designing, synthesizing, and testing a series of analogues. The resulting structure-activity relationships (SARs) are then used to design the next generation of compounds in an iterative process. Computer modeling can be useful at this stage, particularly if structural information about the biological target is available (e.g. from x-ray crystallography). X-Ray snapshots of ligands

bound to their targets are invaluable to the drug designer as they reveal the interactions between the compound and its receptor at the molecular level. Those regions of the molecular structure found to be essential to the binding and biological activity of the compound are identified and optimized, while other regions can be fine-tuned to give the most desirable physical and chemical properties. These properties include factors such as bioavailability, solubility and stability, towards chemical and biological degradation and other types of metabolism *in vivo*. These features are as important to the success of the drug as the biological activity. For example, solubility is essential for proper absorption and distribution of the drug within the body and, if a drug is to be administered orally, it should ideally be stable to the acidic environment of the stomach. If ideal physical properties cannot be installed in the drug candidate, considerable effort is required at the formulation stage to address this problem.

Developing a drug. Once a drug candidate has been identified, it progresses to the next stage of development. From this point on, considerably larger quantities of material are required. Initially, batches of up to a few kilograms must be prepared for use in further tests, such as toxicology

- **Chemistry**
- **Biology**
- **Medicine**



and animal studies, in order to determine how the molecule behaves in a living system (*in vivo*). Many drug candidates fail at this stage due to unforeseen problems with toxicity or metabolism. A compound that is active in biochemical assays may even have no activity *in vivo*. If the drug candidate successfully crosses these hurdles, production must be scaled-up even further to provide a large and reliable supply. This often requires a complete reevaluation of the synthetic route that was used in the discovery process, in which the emphasis is on flexible syntheses that can be employed to produce many structural analogues as rapidly as possible. In a manufacturing process, the overall efficiency (in terms of cost and time) and reproducibility of the synthetic route, together with the degree of purity of the final product, are crucial to the success of the venture. Process development chemists also seek to limit the use of toxic or dangerous materials and solvents, to ensure personnel safety and to minimize the environmental impact of the synthesis. Meanwhile, chemists also play an important role in drug formulation, which helps the body make the best use of the active component, allows administration to be as simple and convenient as possible, and improves the shelf life of the final product. With a reliable and plentiful supply provided by pilot plant production, the drug candidate can then move into clinical trials.

Clinical trials. While clinical trials do not involve chemists directly, we

will provide a brief outline here for the sake of completeness. These trials must produce objective evidence of the safety and efficacy of the new drug, as well as its advantages over existing treatments, before regulatory authorities can approve it for general clinical use. There are three phases of clinical trials. In Phase I trials, the drug is given to a small group of healthy subjects to determine safe and tolerable dosage levels and investigate drug metabolism. Phase II clinical trials typically involve several hundred patients, and are used to establish effective doses. If the trials so far have been successful, the candidate drug then enters Phase III clinical trials, usually involving several thousand patients at various sites. The new treatment is compared to existing treatments and sometimes to a placebo, usually in double-blind studies in which even the doctor does not know which treatment is being used. Following analysis of all the data collected during trials, the regulatory authorities, such as the Federal Drug Administration (FDA) in the United States, decide whether to approve the new drug. Many treatments fail during clinical trials, a rather unfortunate and costly occurrence, considering the years of effort that have already been invested to discover and advance the drug candidate to this stage by the pharmaceutical company. Those that get through, however, can make all the difference, often between life and death, for the patients who receive them. Post-marketing studies (Phase IV clinical trials) may also be a condition of product approval, but are typically undertaken by companies even in the absence of a regulatory mandate. Phase IV studies provide information on the effects of the drug over a much larger patient population and longer timescale

Patients and medicine

than is possible during Phase II and III trials. Adverse side effects detected during Phase IV studies can result in the restriction or withdrawal of a drug.

Drugs for the Mind

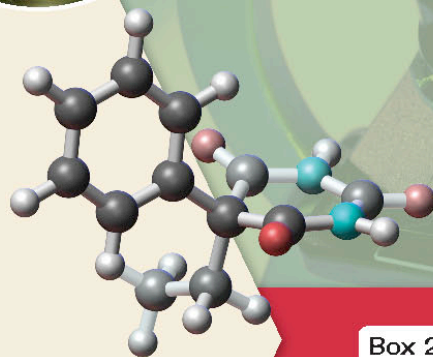
Mental illnesses are particularly difficult to diagnose and treat. Unlike other types of disease, such as infections and cancer, significant controversy surrounds the diagnosis and treatment of many mental health conditions. The exact nature of depression and the point at which anxiety stops being a normal response to stress and becomes a clinical problem are examples of the ambiguities surrounding mental health. To further complicate matters, medical opinions can vary among practitioners as to the mental health status of an individual patient, and, on a larger scale, general consensus of diagnosis among practitioners can vary greatly between different countries.

Much of this difficulty stems from our poor understanding of the functioning of the brain, as compared with our relatively advanced knowledge of other organs. The brain is particularly problematic to study, as it is the most fragile and sensitive organ and, unlike the liver for example, its function cannot easily survive physical and chemical investigations such as sampling and analysis. Mental illnesses involving problems such as anxiety, personality disorders, or cognitive function, are also very poorly suited for investigation using animal models. An

additional problem for both diagnosis and treatment is the presence of the blood-brain barrier (a physical barrier caused by the low permeability of capillary walls in the blood supply of the brain), which prevents many substances from crossing between the brain and the blood stream. As a result, few mental health problems have a physical diagnosis based on blood tests or scans; even Alzheimer's disease, which currently affects over four million Americans, can be diagnosed definitively only by autopsy.

Having established some of the challenges to treating mental health, we will now trace the development of selected classes of drugs that have been used to alleviate such conditions. From a historical perspective, our ability to treat these illnesses has been ever increasing, as has the specificity of the drugs, giving hope for the future of therapy in this field.

Barbiturates. Our discussion of drugs for the mind begins with the barbiturate class. Barbiturates were the first class of drugs for the mind developed by the fledgling pharmaceutical industry. Prior to their introduction, the only treatments available for psychiatric disorders were opiates and alcohol. The pioneer of barbiturates was German chemist Adolf von Baeyer, who we first encountered

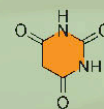


Ball and stick model of the molecule of phenobarbital (Luminal®)

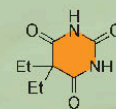


Adolf von Baeyer

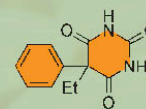
Box 2 Selected barbiturates



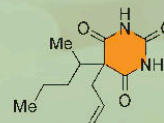
barbituric acid



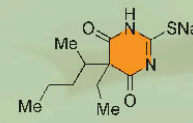
diethylbarbital
[Veronal[®]]
Bayer



phenobarbital
[Luminal[®]]
Bayer



secobarbital
[Seconal[®]]
Eli Lilly



thiopental sodium
[Sodium Pentothal[®]]
Abbott

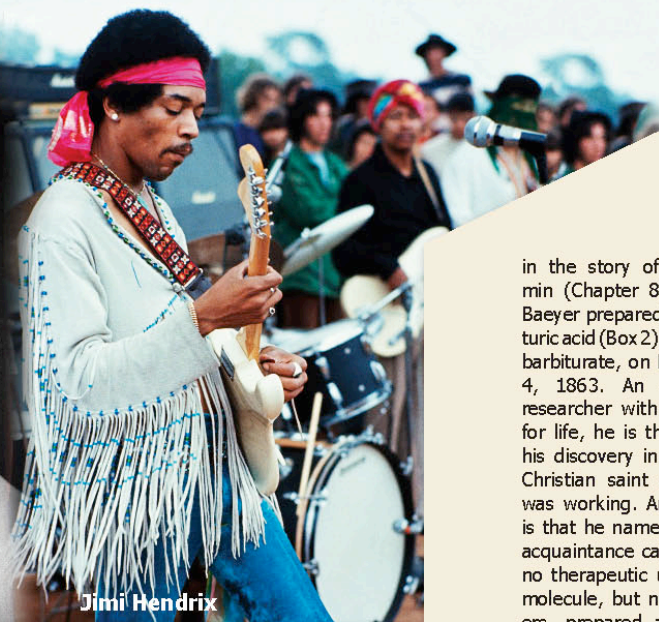
Drugs for the Mind

- Barbiturates
- Phenobarbital



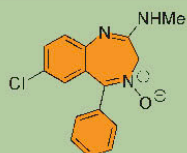


Marilyn Monroe

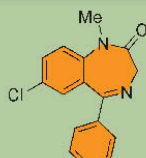


Jimi Hendrix

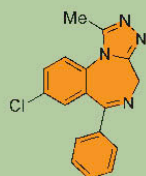
Box 3 Selected benzodiazepines



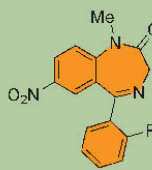
chlordiazepoxide
[Librium[®]]
Hoffmann-La Roche



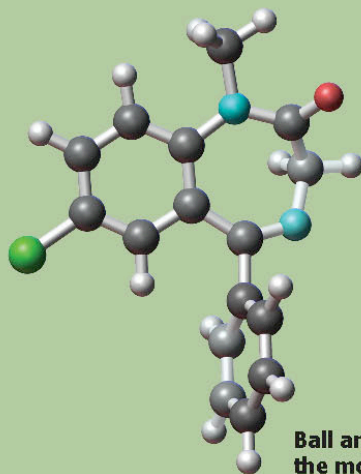
diazepam
[Valium[®]]
Hoffmann-La Roche



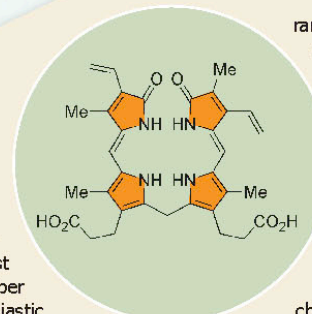
alprazolam
[Xanax[®]]
Pfizer



flunitrazepam
[Rohypnol[®]]
Hoffmann-La Roche



Ball and stick model of
the molecule of diazepam
(Valium[®])



Bilirubin

in the story of haemin (Chapter 8). von Baeyer prepared barbituric acid (Box 2), the first barbiturate, on December 4, 1863. An enthusiastic researcher with a famous zest for life, he is thought to have named his discovery in honor of St. Barbara, a Christian saint on whose feast day he was working. An alternative explanation is that he named his compound after an acquaintance called Barbara. Baeyer had no therapeutic use in mind for this new molecule, but nevertheless he, and others, prepared a number of derivatives such that when Emil Fischer (see Chapter 3, Glucose) and his colleague Joseph von Mering recognized the medicinal potential of barbiturates in 1903, a number of analogues were already available.

Fischer and von Mering were studying the effects of diethylbarbital (Box 2) and discovered that it induced sleep in dogs. This led to diethylbarbital, marketed under the trade name Veronal[®], being widely used as a sedative and sleeping pill, as well as being the first treatment for anxiety. Many other barbiturates (Box 2) followed in quick succession, the most famous being phenobarbital. Marketed as Luminal[®], phenobarbital was introduced by Bayer (Adolf von Baeyer's fledgling pharmaceutical company) in 1912. In addition to its sedative activity, phenobarbital was found to be an effective anticonvulsant and was used to treat epilepsy, a disease for which it is still used today, albeit

rarely. Phenobarbital offered considerable improvement over potassium bromide, which had been used for epilepsy therapy since its discovery in 1857 by Sir Charles Locock, as the latter can cause dermatitis and psychosis. Phenobarbital was also used to treat neonatal jaundice. The characteristic yellow color of the jaundiced infant is caused by high levels of bilirubin in the blood.

Bilirubin arises as a breakdown product of normal hemoglobin catabolism when red blood cells die. The characteristic colors of urine and feces are due to the excretion of further breakdown metabolites of bilirubin. Although neonatal jaundice is usually harmless and self-correcting, rare complications can lead to neurological damage. This application of phenobarbital has now been superseded by phototherapy, which was discovered accidentally in the 1950s and is much safer than drug therapy. Today, phenobarbital is still used regularly in veterinary medicine to treat convulsions.

In all, some 2,500 different barbiturates have been used over the last century. These drugs have variable potencies and durations of action, with effects ranging from mild sedation to complete anesthesia. Approximately twenty barbiturates are still licensed in the United States as sedatives, anesthetics, and anxiolytics (drugs for anxiety). The barbiturates are central nervous system (CNS) depressants that have effects similar to alcohol. They enhance the activity of the neurotransmitter gamma-aminobutyric acid (GABA) at the GABA_A subgroup of receptors by altering the structure of the receptor so

that GABA can bind more easily. This type of interaction is known as allosteric activation. Activation of the GABA_A receptors opens an ion channel in the neuron membrane, allowing chloride ions to enter the cell, interfering with its function. Alcohol operates in part by the same mechanism, hence the cross-tolerance and dependence that is observed in addicts to these drugs.

Despite the benefits brought by the barbiturate drugs, their use is waning due to some serious problems. Barbiturates quickly induce both physical and psychological dependence, and long-term users develop a high tolerance for the drug, eroding its safety margin because greater doses are then needed to produce the same effect. Barbiturates have been implicated in the deaths of a number of high profile celebrities, including actress Marilyn Monroe and guitarist Jimi Hendrix. Accidental barbiturate overdoses are common and frequently occur when the patient also consumes alcohol. The dangers of barbiturate overdose were highlighted by a case of industrial negligence in 1940. The company Winthrop was manufacturing phenobarbital and the antibiotic sulfathiazole in the same plant. Contamination of a batch of sulfathiazole with phenobarbital led to many deaths amongst patients who had unknowingly taken high doses of the barbiturate along with their antibiotic. This disaster led to the introduction of strict new rules governing the manufacture of drug substances.

Benzodiazepines. The benzodiazepines (Box 3) quickly gained popularity in place of barbiturates. During clinical trials, psychiatrists noted that the benzodiazepines could relieve anxiety without significantly affecting cognitive function, which was a significant advantage over earlier treatments. Their action is also more specific than that of barbiturates, leading to

fewer side effects and allowing lower doses to be prescribed. As a result, fewer accidental or deliberate overdoses were recorded with these agents. Within fifteen years of the launch of the benzodiazepines, the most popular of these drugs, Valium® (diazepam), was the most widely prescribed pharmaceutical in the world and, by 1987, an astounding 2.8 billion tablets of Valium® were being manufactured each year. Astonishingly, the first member of the benzodiazepine class, Librium®, was nearly discarded without ever being tested!

Leo H. Sternbach, a chemist at the New Jersey (USA) facility of Hoffmann-La Roche, first discovered what eventually became Librium® in 1957. Sternbach had fled his native Poland for Switzerland in the 1930s, joining Hoffmann-La Roche. However, fearing a Nazi invasion, the company opted to evacuate all their vulnerable employees to the United States in 1941. As a postdoctoral scholar in Krakow (Poland), Sternbach had worked on a class of dye molecules called benzheptoxdiazines. In the USA, he sought to exploit his experience with these compounds in his search for new drugs to replace the barbiturates. He found that all the compounds he made contained six-membered rings, rather than the seven-membered ring structures he had supposed, and were inactive in the assays the company was using. When he treated one such compound with methylamine (MeNH₂), it underwent a ring expansion to give a crystalline material (Box 4),

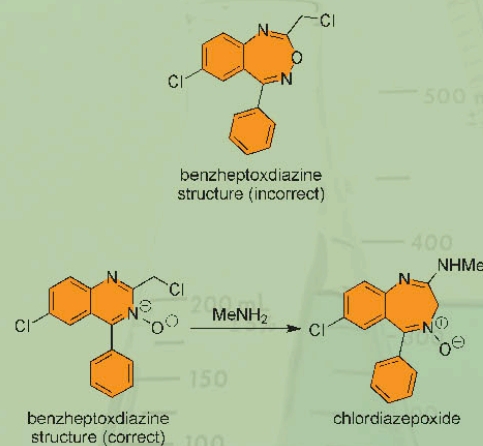
Benzodiazepines

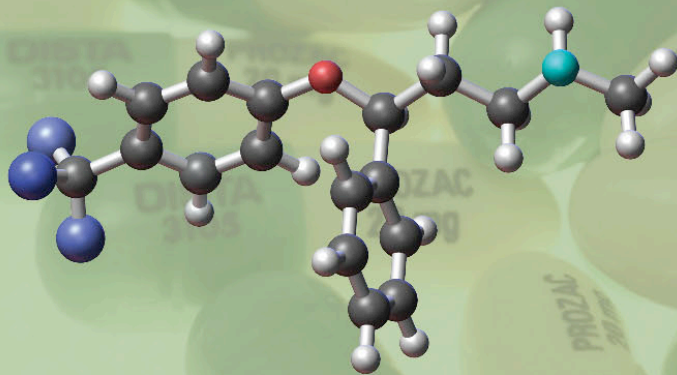
- Librium®
- Valium®



Leo H. Sternbach

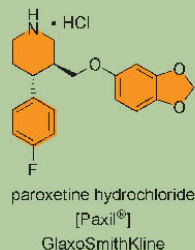
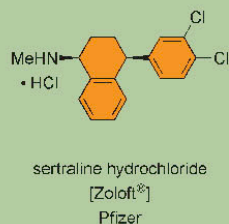
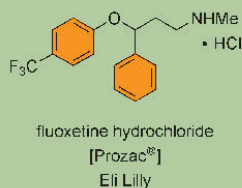
Box 4 Benzheptoxdiazines — structure and conversion to benzodiazepines





Ball and stick model of the molecule of fluoxetine (Prozac®)

Box 5 Selective serotonin reuptake inhibitors (SSRIs)



which he labeled Ro 5-0690 and set aside. Eighteen months later, in 1957, the laboratory was being cleared and an assistant asked if a sample of Ro 5-0690 should be sent for biological evaluation or discarded. Fortunately, Sternbach chose the former, and soon received positive results from Lowell O. Randall, the head of pharmacology at Hoffmann-La Roche. During Phase III trials, the full potential of the drug became clear, as patients being treated for anxiety showed marked improvements in their mental health without loss of cognitive function or motor coordination. Hoffmann-La Roche launched Librium® in 1960, followed three years later by Valium®, a more active congener. In 1981, Xanax®, a still better drug, was launched. All three drugs proved highly lucrative for Hoffmann-La Roche and have benefited millions of patients for many years.

Given their similar physiological effects, it is perhaps unsurprising that benzodiazepines have a similar mechanism of action to the barbiturates, although the processes involved are not identical. The benzodiazepines appear to affect only those GABA_A receptors located in the subcortical nuclei, and not those in the brain stem or spinal cord, in accord with the profound tranquilizing effect accompanied by only minor impairment of motor and cognitive function. The benzodiazepines and GABA are thought to augment each other's binding to the GABA_A receptors, although the precise details remain a mystery.

Unfortunately, the benzodiazepines have also become drugs of abuse and,

although they were not initially believed to be addictive, they have since been shown to induce a strong physical dependence in patients who take them for prolonged periods. It has been claimed that a large number of alcohol abusers and illicit drug users may also be or have been reliant on benzodiazepines. A lesser known example of an abused benzodiazepine is the so-called 'date-rape' drug, Rohypnol® (Box 3). Despite being illegal in many countries, this highly potent CNS depressant is available on the streets of most major cities. Although more commonly used as a recreational drug, Rohypnol® is also used in the sinister practice in which an unwitting victim is drugged and then robbed or raped. The effects of the drug can prevent the victim from clearly recalling the incident, leading to difficulties in detecting and prosecuting the crime.

The high incidence of benzodiazepine abuse has its roots in misguided prescribing practices during the early life of these drugs. They were often prescribed by family doctors ill-equipped to diagnose pathological anxiety accurately. The widespread use of Valium® led to the drug becoming known as "mother's little helper". As this name implies, addiction to benzodiazepine sedatives was much more common in women than men. Fortunately, more selective, non-addictive anxiolytic drugs have since been developed (see below), and the use of benzodiazepines has shown a marked decline. However, as they are effective and safe for short-term treatment, their continuing use is probably secured, at least for the foreseeable future.

Selective serotonin reuptake inhibitors. Selective serotonin reuptake inhibitors (SSRIs, Box 5) were launched as antidepressants in the late 1980s with the introduction of Prozac® (fluoxetine hydrochloride), manufactured by the pharma-

ceutical company Eli Lilly. Within two years of its launch, Prozac® had become the most highly prescribed antidepressant ever and, by 1999, two years before its patent expired, it accounted for around 25 % of Lilly's \$10 billion annual revenues.

Depressive disorders are common and on the increase, probably due in part to both improved diagnosis and an increase in the numbers of patients seeking help; indeed, 10–20 % of the populations of most Western countries are estimated to take regular medication to combat negative feelings. Between 1991 and 2001, antidepressant prescriptions in the UK increased by fifteen million to twenty-four million per annum, and so much Prozac® is now taken that it has been detected in rivers and groundwater.

The predecessors of the SSRIs were the monoamine oxidase inhibitors (MAOIs) and the tricyclic antidepressants, both of which were introduced in the 1950s. Interestingly, iproniazid (Marsilid®, Box 6), the first MAOI, was originally used to treat tuberculosis before its antidepressant effects were noted. However, these drugs are not selective enough, and patients must endure serious side effects, including hypertension, blurred vision, and headaches. The relative lack of such side effects associated with SSRIs has contributed to their popularity.

While depression is difficult to study, it is believed to be linked to neurotransmitter imbalances in the brain of the patient. Serotonin is stored at nerve junctions (synapses) and released when needed. It is then destroyed by enzymes or taken up by the receiving cell. Those suffering from depressive disorders have unusually low

Antidepressants

- Prozac®
- Zoloft®
- Paxil®

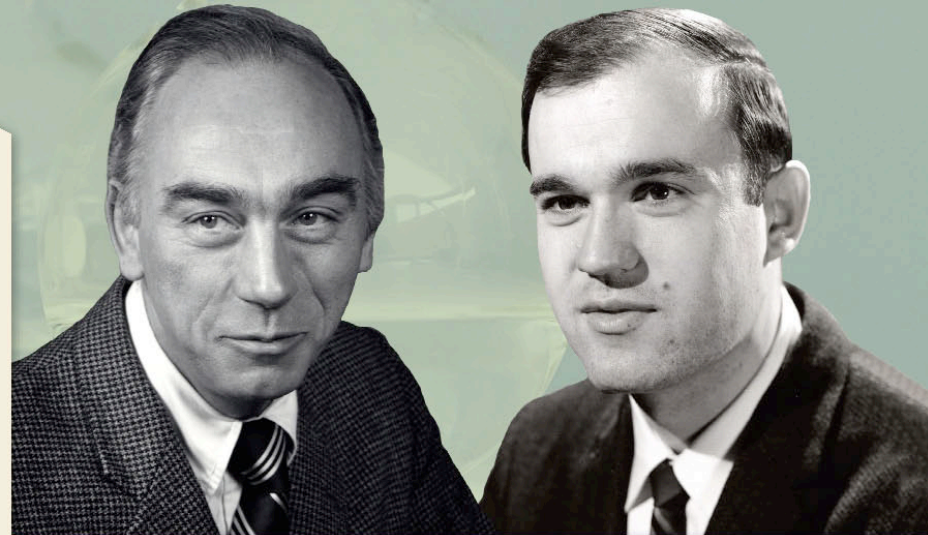
serotonin levels. SSRIs act to inhibit the action of the pump that removes serotonin, leading to a rise in the concentration of the neurotransmitter in the synapse. This mechanism explains the short-term effects of these drugs; however, in the longer term SSRIs appear to induce permanent changes in brain structure, making the patients less prone to depressive events, an effect that is more difficult to explain.

Prozac® was discovered in the USA by a team of scientists at Eli Lilly in the 1960s in an early example of rational drug design. Chemists Bryan B. Molloy and Klaus K. Schmiegel synthesized and tested a number of compounds based on the template of the antihistamine drug diphenhydramine hydrochloride (marketed as Benadryl® in the USA) (Box 6). One of these compounds was found to inhibit serotonin uptake specifically, and, after several years of development, Eli Lilly announced their findings in 1974. Branded Prozac®, the drug was launched in 1987. The competitors Zoloft® (Pfizer) and Paxil® (SmithKlineBeecham, now GlaxoSmithKline) were launched in 1991 and 1992, respectively, and have also become hugely successful (Box 5).

The SSRIs have not been free of controversy. Ironically, the lack of side effects associated with Prozac® has been a contributing factor. The apparent safety of Prozac® led to rather liberal prescrib-

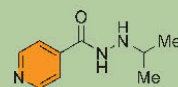
Bryan B. Molloy

Klaus K. Schmiegel

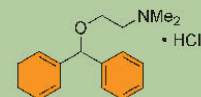


Box 6

Structures of iproniazid and diphenhydramine hydrochloride

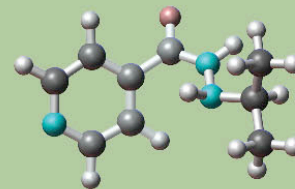


iproniazid
[Marsilid®]
Hoffmann-La Roche

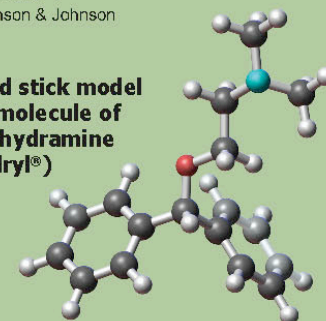


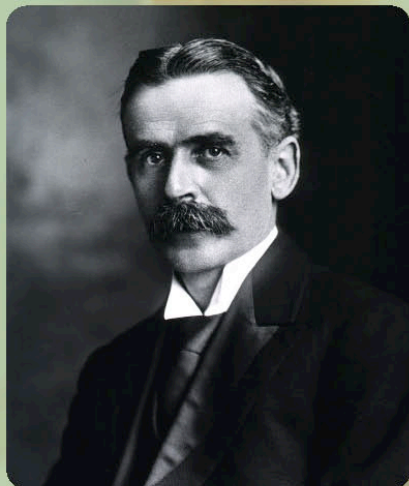
diphenhydramine hydrochloride
[Benadryl®]
Pfizer/Johnson & Johnson

Ball and stick model of the molecule of iproniazid (Marsilid®)



Ball and stick model of the molecule of diphenhydramine (Benadryl®)





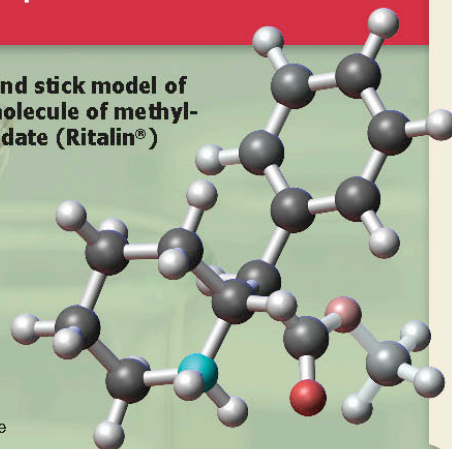
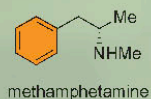
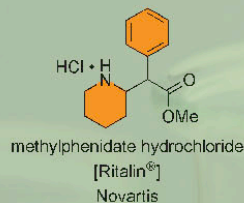
Sir George F. Still



Charles Bradley

Box 7 Ritalin® and selected amphetamines

Ball and stick model of the molecule of methylphenidate (Ritalin®)



ADHD
● Ritalin®

ing practices, and prompted some to voice concerns over its widespread use. One issue was the belief that it was being prescribed to those who were not clinically ill, but rather experiencing a tough period in their life. Other disturbing reports concern changes in character in those who have taken SSRIs, including violent behavior and an increased risk of suicide. This led the FDA to demand that stronger warnings be placed in the drug packaging. Supporters of the drugs argue that untreated depression carries a greater risk of suicide, and Eli Lilly strongly denies any link between Prozac® treatment and suicide or violent behavior. Recently, there have also been reports that some SSRIs can lead to dependence if used for long periods. Despite these controversies, SSRIs remain the first line of treatment for clinical depression.

Amphetamines. Amphetamines (Box 7) are the final class of drugs for the mind discussed in this section. They are stimulants based on the structure of α -methylphenethylamine (amphetamine), the parent member. Many derivatives have been prepared and, unfortunately, several members of the class are drugs of abuse, used recreationally to produce euphoric sensations. They are also used clinically to treat behavioral problems and narcolepsy. Arguably one of the most controversial drugs for the mind is the amphetamine Ritalin®, discussed below.

Ritalin® (methylphenidate hydrochloride, Box 7). The difference between true behavioral problems that affect children's social development and learning and their natural propensity to become excited or distracted can be very difficult to define, leading to problems in diagnosing such conditions. In 1902, British pediatrician Sir George F. Still described twenty children in his care as "...passionate, defiant, spiteful..." and lacking in "...inhibitory volition..." and suggested that this behavior was due to neurological damage, rather than bad parenting. This theory gained credence following the viral encephalitis epidemic of 1917–1928. Clinicians noted similar behavioral patterns in children who had been affected by the virus and were assumed to have suffered mild brain damage. An indication of the potential benefits of stimulants came in 1937 when Charles Bradley, working at a Rhode Island (USA) hospital for children with behavioral problems, described how amphetamines he had prescribed to treat severe headaches seemed to cure the youngsters of their behavioral difficulties. By the 1950s, children with the symptoms described above were labeled 'minimally brain dysfunctional', reflecting the prevailing theory on behavioral problems. In 1955, the FDA approved Ritalin®, made by Ciba-Geigy (now part of Novartis), for the treatment of various psychological disorders, including depression, chronic fatigue, and narcolepsy. Following the expiration of their patent in 1967, the company sought to introduce Ritalin® for the treatment of children with minimal brain dysfunction. Ciba-Geigy also sponsored a great deal of

research into behavioral disorders in children. By 1975, one million children in the United States had been diagnosed with a behavioral disorder; around half of these were prescribed medication, with some 265,000 receiving Ritalin®. Since then, the number of children diagnosed with what is now known as attention deficit hyperactivity disorder (ADHD) has increased to startling levels, as has the frequency of Ritalin® prescription. Approximately 3.5 million children in the United States have been diagnosed with ADHD, corresponding to about five percent of all children under the age of eighteen; the diagnosis is three times more common in boys. Notably, the rate of ADHD diagnosis in Europe is roughly one tenth of that in the United States, underscoring the differences in prevailing clinical opinion, and, perhaps, culture. ADHD is also becoming an increasingly common diagnosis in adults, with Ritalin® being one of the fastest growing drugs prescribed.

It may seem curious that treating hyperactive individuals with a stimulant should give a calming effect, especially when the same drug induces euphoria and excitement in healthy subjects. The frontal lobes of the brain regulate behavior and are rich in receptors for the neurotransmitters dopamine and norepinephrine. ADHD sufferers have reduced electrical activity and blood flow in these areas, and also low dopamine levels. Ritalin® is known to affect the release and uptake of dopamine and norepinephrine. Induction of an increase in dopamine levels in the frontal lobes may explain its curative effects in ADHD patients, however, the details of the mechanism of action are, as yet, unknown.

Critics of Ritalin® therapy contend that the drug is grossly overprescribed, and has become an easy option for pacifying obstreperous or distracted chil-

dren who are more in need of better parenting or behavioral therapy. Misdiagnosed ADHD may also mask other serious problems, including neglect or abuse. It is claimed that Ritalin® makes children zombie-like and stifles their creativity and intellect. Many parents of afflicted children disagree and credit Ritalin® for making their home life more bearable.

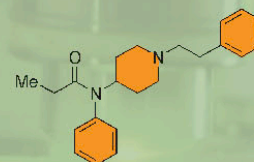
Numerous other drugs for the mind have been discovered and are in use today; some of these are shown in Box 8.



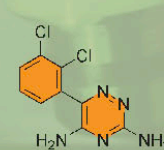
Drugs to Tackle Viruses

Viruses are amongst the smallest and simplest infective agents. Far from being a handicap, their structural simplicity makes them particularly difficult to fight. Viruses comprise a core of genetic material (DNA or RNA) and a few enzymes enclosed in a protein shell. Some are also surrounded by a lipoprotein envelope, but they have essentially no metabolism. Viruses cause a broad range of diseases in animals, plants and even bacteria. Examples of viruses include the rhinovirus and adenovirus, which cause the common cold and sore throat, respectively, and the herpes family, which cause a variety of conditions from glandular fever (caused by the Epstein-Barr virus) to recurring sores on the skin. Hepatitis, measles, mumps, rubella, polio, and influenza are among the many other examples of viral

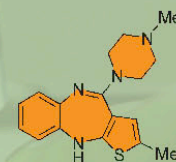
Box 8 Other selected drugs for the mind



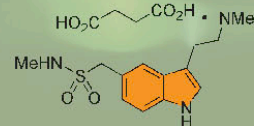
fentanyl
[Sublimaze®]
(anesthesia)
Janssen



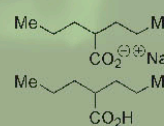
lamotrigine
[Lamictal®]
(bipolar disorder,
epilepsy)
GlaxoSmithKline



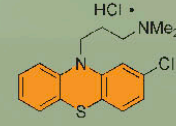
olanzapine
[Zyprexa®]
(schizophrenia)
Eli Lilly



sumatriptan succinate
[Imitrex®]
(migraine)
GlaxoSmithKline



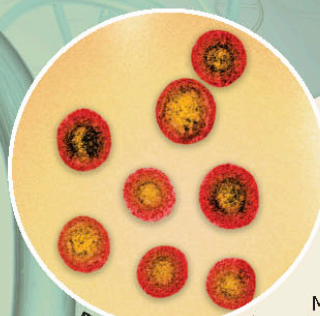
divalproex sodium
[Depakote®]
(bipolar disorder,
epilepsy, migraine)
Abbott



chlorpromazine hydrochloride
[Thorazine®]
(antipsychotic)
GlaxoSmithKline

Gertrude B. Elion

George H. Hitchings



Epstein-Barr virus

diseases. The most deadly viral infections include the hemorrhagic fevers caused by the tropical African Ebola or Marburg viruses, and the human immunodeficiency virus (HIV) that causes AIDS (acquired immunodeficiency syndrome).

Aside from the progress made with vaccines, discussed in greater detail in the next chapter, comparatively little progress has been made in finding effective drugs to treat viral infections. While this volume contains many chapters describing treatments for bacterial, fungal, and parasitic infections, and cancer, we have yet to discuss an antiviral agent. The root of this disparity can be found partly in the lack of suitable targets for such drugs. Bacteria, fungi, parasites, and tumor cells must all maintain complex metabolisms. If these are interrupted by drugs, the cells either die or are disabled and made vulnerable to the immune system. In contrast, viruses have no intrinsic metabolism. They hijack the host's own metabolic machinery to reproduce, so targeting the virus selectively is a significant challenge.

However, there have been notable successes in this field, and some are described below.

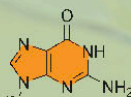
Zovirax® (acyclovir). The launch of acyclovir (Zovirax®, Box 9) in 1980 marked a turning point in antiviral therapy. For the first time, a drug was available to

treat viral infections, those caused by the varicella zoster (which causes chickenpox and shingles) and herpes simplex viruses. Acyclovir was discovered by a team led by Gertrude B. Elion. Elion's lifelong collaboration with her boss, George H. Hitchings, led to the development of rational drug design, revolutionizing the pharmaceutical industry. These talented scientists were awarded the 1988 Nobel Prize in Physiology or Medicine, "for their discoveries of important principles for drug treatment." Elion and Hitchings began working for Wellcome Laboratories (originally part of the British firm Burroughs Wellcome and now part of GlaxoSmithKline) in Tuckahoe (New York, USA) in the 1940s. They began searching for compounds that would interfere with bacterial metabolism, a strategy that became known as the antimetabolite approach. Hitchings earned his Ph.D. from Harvard University for work on the chemistry of purines. The purines adenine and guanine represent one of the two types of bases found in nucleic acids, the other being the pyrimidines thymine, cytosine, and uracil (Box 9). Both purines and pyrimidines would figure prominently in the research of Hitchings and Elion. They began preparing a range of analogues of the naturally occurring purines, either by modifying the natural structures or by synthesizing them from scratch, and tested the compounds for antibacterial activity. The discovery of the structure of DNA, announced by James D. Watson and Francis H. D. Crick in 1953, further fueled their line of research, aimed

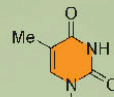
at disrupting cell division.

During the 1950s and 1960s, the pair discovered numerous useful drugs, including antibiotics such as trimethoprim and 6-mercaptopurine

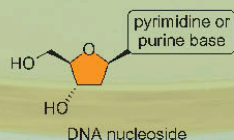
Box 9 Acyclovir and the structures of nucleosides



purines

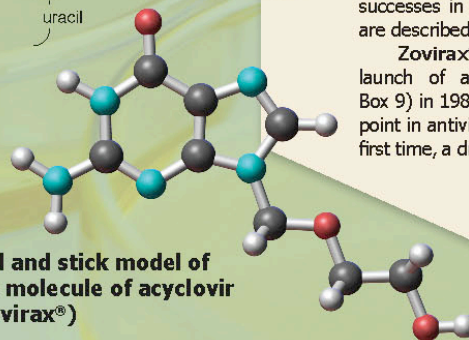


pyrimidines



Antivirals

- Zovirax®
- Retrovir®



Ball and stick model of the molecule of acyclovir (Zovirax®)



(6-MP, which was used to treat childhood leukemia, see Chapter 22, Cyclosporin, FK506, & Rapamycin). These purines inhibit cellular reproduction by mimicking the natural purine DNA bases. The synthetic purines either become incorporated into DNA during replication and thus corrupt it, or inhibit an enzyme involved in DNA synthesis or manipulation, leading to a disruption of cell division. This approach is particularly suited for attacking rapidly dividing cells such as bacteria or cancer cells. Following their successes against bacteria and cancer, the next step was to apply the antimetabolite approach to antiviral therapy. In the 1970s, Hitchings had been promoted away from active research, and Elion, now head of experimental therapy at Wellcome Laboratories (relocated to North Carolina, USA), presided over a flourishing antiviral program. In an extension of the purine and pyrimidine analogue program, the team now began to prepare more sophisticated compounds that mimicked more of the structure of the nucleoside. Nucleosides are composed of a purine or pyrimidine base and a sugar moiety. Acyclovir, an analogue of the guanosine nucleoside, was first synthesized by Howard J. Schaeffer and his team, under Elion's guidance. Acyclovir is actually a pro-drug that is converted into the active compound (the corresponding triphosphate) in the body. A viral thymidine kinase enzyme initiates this process, converting acyclovir into the monophosphate derivative; host cell kinases then complete the transformation. The viral kinase is many times more efficient than those in the host, giving the drug its required selectivity. Acyclovir triphosphate inhibits the enzyme



Luc Montagnier

DNA polymerase, blocking DNA synthesis and preventing viral replication. The drug candidate quickly progressed through trials and the regulatory process, and was launched in 1980 as Zovirax®. Since then, it has been used to treat Epstein-Barr virus infections, shingles, chickenpox, genital herpes, and cold sores, as well as rarer herpes infections. Today, weaker formulations are also available without prescription, particularly for the treatment of cold sores.

Retrovir® (AZT, zidovudine). In the early 1980s, a viral illness began to claim lives in clusters around the world. Fear spread quickly as many of the victims were apparently fit and healthy young men. The illness was completely new and displayed many varied symptoms. Eventually it became clear that the deaths were not due to the virus directly, but to a variety of opportunistic infections, including PCP (*Pneumocystis carinii* pneumonia), Kaposi's sarcoma (rare forms of pneumonia and skin cancer, respectively), and toxoplasmosis (an infection caused by the protozoan *Toxoplasma gondii*). People with healthy immune systems are normally able to fight off such conditions, but this virus invades and destroys the patient's CD₄ (or T₄) cells, compromising their immune systems. In 1984, Luc Montagnier of the Pasteur Institute (Paris, France) identified a retrovirus (a virus with an RNA genome

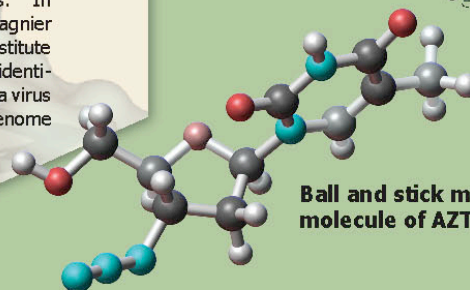
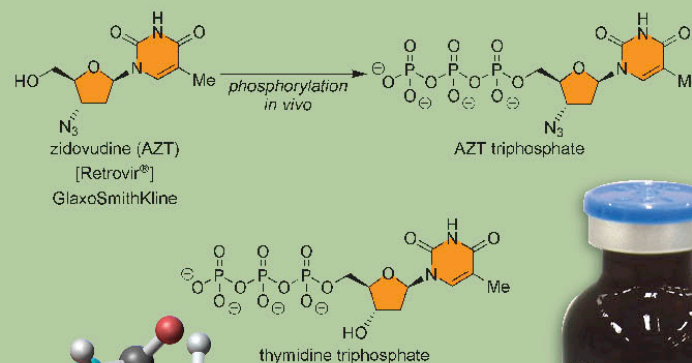
James D. Watson



Francis H. D. Crick



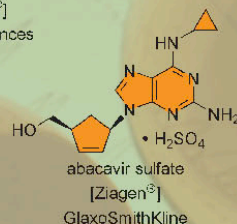
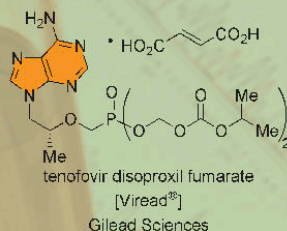
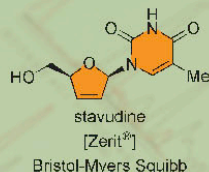
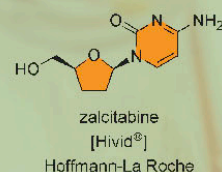
Box 10 AZT, its active triphosphate, and thymidine triphosphate



Ball and stick model of the molecule of AZT (Retrovir®)



Box 11 Selected nucleoside reverse transcriptase inhibitors (NRTIs)



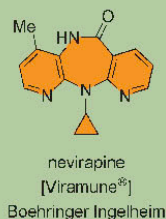
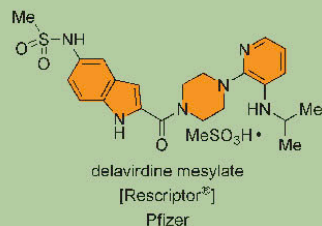
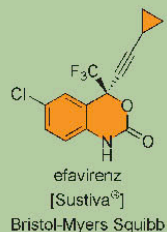
rather than a DNA genome) in patients suffering from this new disease. His discovery was followed quickly by similar reports from Abraham Karpas (University of Cambridge, UK), Jay A. Levy (University of California, San Francisco, USA) and Robert C. Gallo (National Cancer Institute, USA). Initially, each group named the virus differently, but a short time later a consensus was reached and it was universally named the human immunodeficiency virus (HIV). Today, researchers know a great deal about HIV infection and the onset of AIDS; however, the worldwide spread of the virus and problems of drug resistance continue to challenge scientists and patients fighting the disease. In 2006, the UNAIDS organization estimated that 38.6 million people are infected with HIV worldwide, and that in the preceding year there were 4.1 million new HIV infections and 2.8 million deaths from AIDS. So far, the epidemic has been concentrated in sub-Saharan Africa. Some drugs have been developed to fight HIV infection, but they have yet to give us the upper hand in eradicating this disease.

The first drug to be launched for the treatment of HIV was azidothymidine (AZT, Retrovir®, or zidovudine). This compound (Box 10), an analogue of the thymidine nucleoside, was first synthesized and tested by the Elion-Hitchings team in the 1960s as an anti-cancer drug. Although it was unsuccessful in this regard, it was not

forgotten, and, in the 1980s, it was found to suppress viral replication. It was approved by the FDA for treating HIV in 1987. AZT works by inhibiting the viral enzyme reverse transcriptase. The HIV genome is encoded in RNA, and must be transcribed into DNA in order to be incorporated into the genome of the host cell. This process is called reverse transcription, as it is literally the reverse of the normal cellular transcription process. As with acyclovir, AZT is converted into its triphosphate and incorporated into the growing DNA chain. This event terminates the growing DNA polymer as AZT has an azide group in place of the hydroxyl group needed for attachment of the next DNA unit. In the early days of HIV therapy, AZT offered a crucial lifeline, but its use is now limited to a few specialist applications because it is relatively toxic and often ineffective due to viral resistance that has since developed. Indeed, the high mutation rate of the HIV virus makes drug resistance an especially challenging problem. A host of other reverse transcriptase inhibitors, both nucleoside- (NRTIs, Box 11) and non-nucleoside-based (NNRTIs, Box 12), have since been developed to replace AZT.

HIV protease inhibitors. In the 1970s, cancer researchers began studying retroviruses because they were known to cause various cancers. They found that retrovirus proteins are not made individually, but in amalgamated polyproteins. To release the individual proteins the viruses possess protein-cleaving enzymes, which are not activated until after the virus particles bud from the host cell. If these enzymes could be inhibited, the virus would be rendered incapable of invading new hosts. The emerging gene tech-

Box 12 Selected non-nucleoside reverse transcriptase inhibitors (NNRTIs)



Protease Inhibitors

- **Invirase®**
- **Norvir®**
- **Crixivan®**

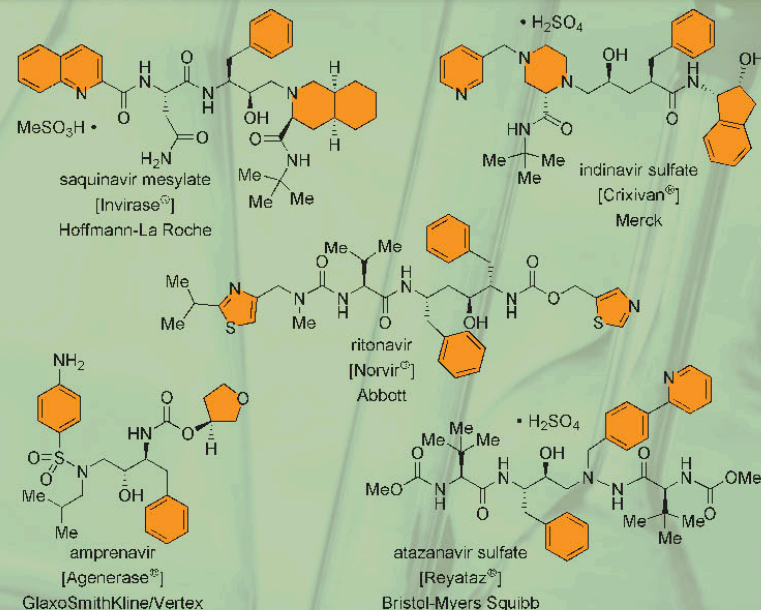
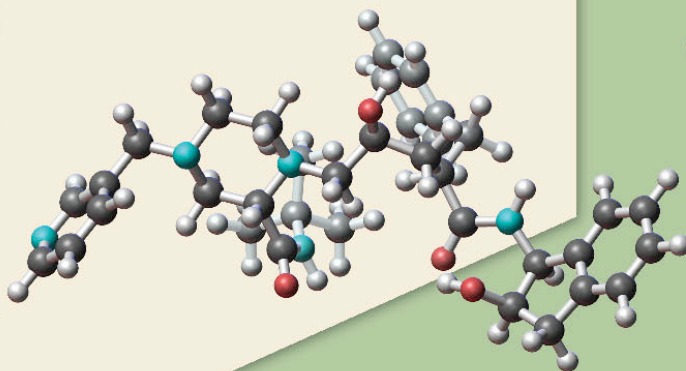
nologies of the 1980s allowed scientists to sequence the complete HIV genome, which they used to identify viral proteins. In 1986, several independent groups isolated HIV protease and confirmed that the enzyme was an aspartic protease, meaning that the amino acid residue aspartate occupies a key position in the active site. The enzyme also bore distinct similarities to the digestive enzymes renin and pepsin. In 1988, Irving Sigal, a talented biochemist working for Merck, showed that mutating the HIV protease led to the production of immature viruses incapable of infecting new cells, thus confirming the potential of this enzyme as a drug target. Within a year, two groups led by Manuel Navia (Merck) and Alexander Wlodawer (Frederick Cancer Research Facility, Maryland, USA) independently determined the three dimensional structure of HIV protease by x-ray crystallography, revealing a dimeric enzyme shaped like a walnut, with the active site between the two halves.

Medicinal chemists now had more than enough information to begin searching for HIV protease inhibitors. Indeed, they had a head start as several potent renin inhibitors were already known. They also knew that the role of the enzyme was to cleave the peptide chain at the amide bond between phenylalanine and proline residues, so compounds that mimic the structure of this junction (e.g. peptides or their analogues) could potentially act as inhibitors. However, finding peptide-like molecules that do not undergo degradation by our own proteases was a significant challenge. Aided by the sharp tools of chemical synthesis, structural biology, and computer modeling, medicinal

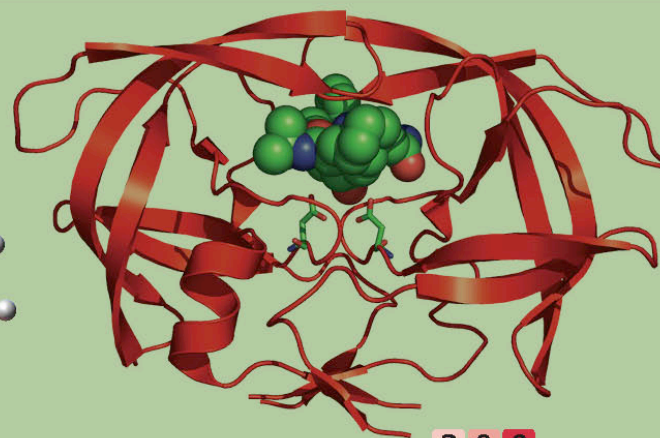
chemists gave this problem their urgent attention. The enormous efforts directed towards this research led to an early triumph for structure-driven drug design with the approval, in December 1995, of the Hoffmann-La Roche drug saquinavir (Invirase[®], Box 13), the first HIV protease inhibitor to be approved for clinical use. In the following spring, two further drugs, ritonavir (Norvir[®], Abbott Laboratories) and indinavir (Crixivan[®], Merck), were launched. Today, many more inhibitors are available (for examples, see Box 13), and these drugs play a vital role in treating those living with HIV. However, they are not without side effects, including lipodystrophy (the redistribution of body fat), kidney stones, and depression.

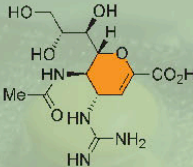
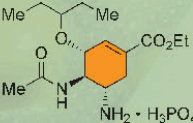
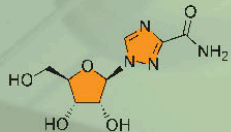
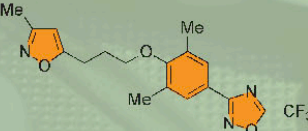
Combination therapy. Treating HIV was initially found to be extremely difficult because the virus mutates rapidly, enabling it to develop resistance to drugs remarkably quickly. By the mid-1990s, drug resistance was dominating the scientific literature on HIV, but in 1996, at an international AIDS conference in Vancouver (Canada), a number of speakers described a new strategy

Ball and stick model of the molecule of indinavir (Crixivan[®])



Below: X-Ray crystallographic structure of HIV-1 protease in complex with saquinavir. The small molecule inhibitor is shown in space-filling format with hydrogen atoms omitted for clarity (green: carbon, red: oxygen, blue: nitrogen).



Generic name (Trade name)	Structure or description	Mode of action	Marketer	Indication
zanamivir [Relenza®]		inhibition of influenza virus neuraminidase, alteration of virus particle aggregation and release	GlaxoSmithKline	treatment of influenza
oseltamivir phosphate [Tamiflu®]		prodrug - after hydrolysis of the ethyl ester to afford the free carboxylic acid, the drug works by inhibition of influenza virus neuraminidase, alteration of virus particle aggregation and release	Roche	treatment of influenza
formivirsen sodium [Vitravene®]	antisense drug (designed DNA fragment)	designed DNA fragment that binds tightly to a region of the virus RNA that has been identified as important in viral protein production	ISIS/ Novartis	treatment of CMV (cytomegalovirus) eye infections
interferon alpha-2b [PEG-Intron®]	naturally occurring small protein - made through biotechnology	interferons are glycoprotein secreted by cells in response to viral infection, synthetic inducers, or biological inducers. They inhibit protein synthesis and other aspects of viral replication	Schering-Plough	treatment of hepatitis C virus - used in combination with ribavirin
ribavirin [Rebetol®]		nucleoside analogue - exact mechanism of action unknown	Schering-Plough	treatment of hepatitis B and C - used in combination with interferon alpha-2b
pleconaril (drug still at clinical trial phase)		binds to a pocket on rhinovirus thus inhibiting uncoating of the virus	ViroPharma/ Schering-Plough	treatment of common cold



David Ho

to avoid resistance. Among them was David Ho (Aaron Diamond AIDS Research Center, New York City, USA) who described treating HIV sufferers with 'cocktails' of drugs. In this approach, patients were given three or four drugs of different types simultaneously. These patients showed marked reductions in viral load (the number of viral particles in the blood), even to the point that the virus was almost undetectable, and experienced less frequent lapses due to drug resistance. This combination therapy has been adopted widely, and has shown significant success in slowing the progression of the disease and preventing the emergence of resistance, although there is still no cure for HIV. Current combination regimes typically consist of two NRTIs and one NNRTI or a protease inhibitor. Combination therapy has since been applied in the treatment of other conditions, including tuberculosis (a re-emerging epidemic) and certain cancers that are prone to drug resistance. Ho was hailed as the Man of the Year by Time Magazine in 1996 for pioneering this therapeutic approach.

Influenza Drugs

While this section has mainly focused on HIV treatments, there have also been advances in the treatment of many other viral infections. A number of these are summarized in Box 14. Some have arisen from the enormous research efforts driven by HIV, while others repre-

Influenza Drugs

- Relenza®
- Tamiflu®

sent new innovative ways to tackle viruses. Zanamivir (Relenza®) and oseltamivir phosphate (Tamiflu®) are both licensed for the treatment of influenza, and are further examples of what can be achieved when rational drug design, using information obtained from x-ray crystallography studies of the corresponding protein target, is employed. Oseltamivir is an orally available prodrug in which the ethyl ester is hydrolyzed to the corresponding carboxylic acid *in vivo*. Due to its poor bioavailability, zanamivir must currently be administered by inhalation. Both drugs function by inhibiting the activity of the neuraminidase enzymes of the influenza virus, blocking the release of the virus from infected cells. All strains of the influenza virus display the glycoprotein enzymes haemagglutinin and neuraminidase on their surface, and these enzymes are critical to the replication strategy of the virus. Haemagglutinin, of which sixteen subtypes are known, is responsible for the initial binding of the virus to the cell that is being infected. After completion of the viral replicative cycle, neuraminidase (nine known subtypes) enables the newly formed virus particles to be released from the infected cell and infect new ones, propagating the spread of the virus throughout the organism. Influenza strains are characterized by the different subtypes of these two enzymes. Oseltamivir was used successfully to help combat the influenza epidemic caused by the H5N1 (haemagglutinin subtype five, neuraminidase subtype one) virus strain ('bird flu') in Southeast Asia in 2005. Fears that mutation of this strain may lead to an influenza pandemic on the scale of the Spanish flu pandemic which swept the world in 1918–1919 (and claimed far more lives than did World War I) has led to the stockpiling of Tamiflu® (oseltamivir) by governments around the globe, and much controversy about the manufac-

ture and supply of this drug. Tamiflu® and Relenza® have spurred the development of further neuraminidase inhibitors, now entering clinical trials.

Drugs for the Gastrointestinal Tract

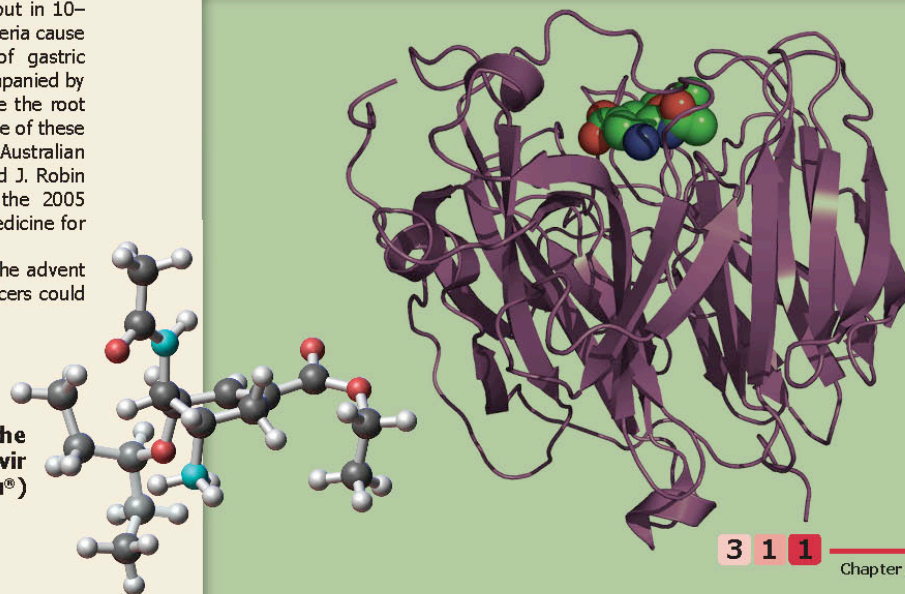
Many people suffer from gastrointestinal ailments caused by excess gastric acid (mainly hydrochloric acid) coming into contact with tissue that would not normally be exposed to such a harsh chemical environment. Examples include persistent acid reflux disease in which gastric acid enters the esophagus, causing painful heartburn and permanent damage, and also peptic ulcers (where damage to the protective stomach lining allows acid to attack the tissue beneath). Ulcers can eventually perforate, leading to dangerous bleeding. Until the 1980s, stress and lifestyle were blamed for the majority of gastric complaints, however, many have now been found to be caused by bacteria. The presence of *Helicobacter pylori* bacteria in the stomach is common, and is usually asymptomatic, but in 10–15 % of those infected the bacteria cause ulceration. Direct treatment of gastric symptoms is now usually accompanied by a course of antibiotics to tackle the root causes of the condition. The role of these bacteria was discovered by two Australian scientists, Barry J. Marshall and J. Robin Warren, who were awarded the 2005 Nobel Prize in Physiology or Medicine for their work.

H₂-Antagonists. Before the advent of this class of drugs, peptic ulcers could

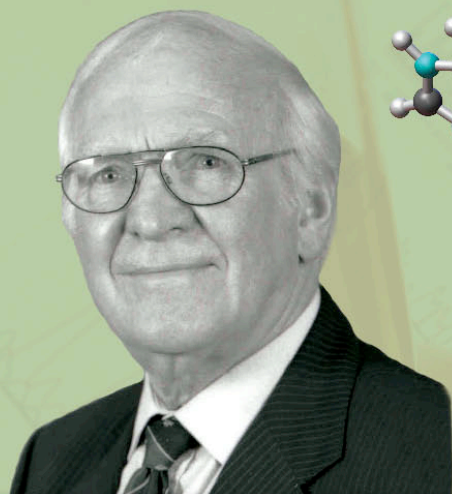


J. Robin Warren and Barry J. Marshall

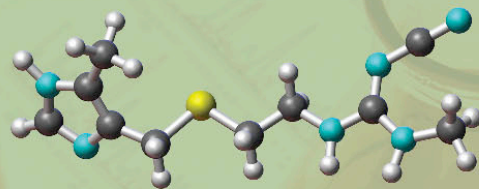
Below: X-Ray crystallographic structure of N1 neuraminidase monomer of the H5N1 avian virus in complex with oseltamivir (space-filling model), which is bound following hydrolysis of the ethyl ester to the carboxylic acid



Ball and stick model of the molecule of oseltamivir (Tamiflu®)



Sir James W. Black



Ball and stick model of the molecule of cimetidine (Tagamet®)

Box 15

Selected H₂-antagonists and the structure of histamine

H₂-Antagonists

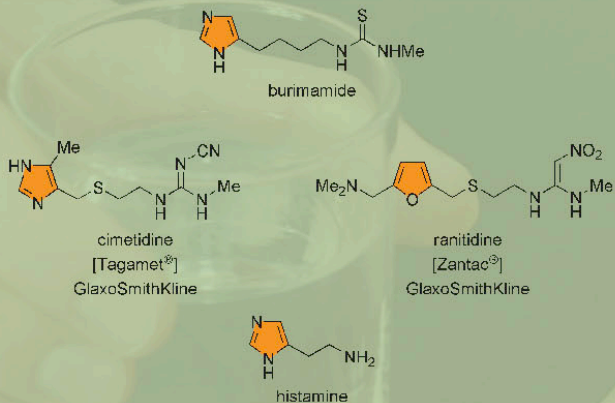
- Tagamet®
- Zantac®

not be treated effectively. Patients were confined to bed, fed a bland diet, and given antacid tablets. Healing was slow and frequently incomplete, and many would eventually require surgery. The development of the H₂-antagonists began with the work of Sir James W. Black, who determined the link between histamine (Box 15) and increased acid secretion. Histamine is a chemical mediator that plays a key role in many physiological processes, including allergy and inflammation. The wide range of physiological effects of this compound make specificity paramount in any program aimed at disrupting only one of its roles. Four types of histamine receptor (H₁–H₄) are now known, each regulating a different task. Following work by Heinz Schild, Black and his colleagues characterized the H₂ receptor and showed that histamine binding at this site stimulated gastric secretions. Black and his team at the pharmaceutical company of SmithKline and French (UK) began to search for a suitable antagonist (a molecule that inhibits the normal physiological function of a receptor) for the H₂ receptor, with a view to suppressing excess stomach acid. Between 1964 and 1968, the team prepared over two hundred synthetic histamine mimics, but only one compound showed the potential to prevent histamine secretion. After years of further refinement, they prepared burimamide, a thiourea derivative (Box 15). Hailed as a major breakthrough, their results were published in the journal *Nature* in 1972. However, burimamide was

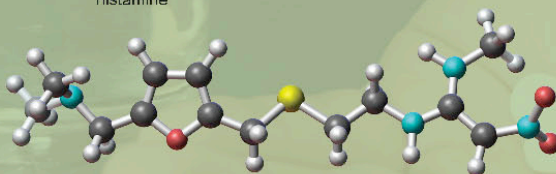
not sufficiently potent for oral administration and required injection. In the search for a better drug, a further series of compounds was designed and prepared by a group of chemists from the original team, Graham J. Durant, John C. Emmett, and C. Robin Ganellin. In 1972, they discovered a cyanoguanidine analogue that they called cimetidine (Tagamet®, Box 15). This time the candidate sailed through the approval process and was launched in 1976 as an anti-ulcer drug. By 1979, Tagamet® was the top-selling pharmaceutical in North America. Sir James Black's vision in pioneering the design of molecules to fit known receptors revolutionized the drug discovery process and led to the award of the 1988 Nobel Prize in Physiology or Medicine (shared with Hitchings and Elion).

Meanwhile, the rival firm Glaxo was also searching for H₂-antagonists. Following a quantitative structure-activity relationship (QSAR) strategy, they refined their design, eventually discovering ranitidine (Zantac®, Box 15), which was launched in 1981. Despite being the second drug to make it to the market, Zantac® was also hugely successful, becoming the world's top-selling drug in 1988. The patents on both drugs have now expired and lower doses are available in over-the-counter medications in many countries. Interestingly, the former rivals subsequently joined forces to form the global pharmaceutical giant GlaxoSmithKline (GSK).

Proton Pump Inhibitors. In the late 1980s, the relatively small Swedish pharmaceutical firm Astra launched a new type of drug to compete with the H₂-antagonists. Their drug, called Losec® (omeprazole, Box 16), blocks a gastric H⁺/K⁺-ATPase, an enzyme that pumps acid into the stomach, and is, therefore, a highly effective antiulcer drug. Losec® was originally marketed as the racemate (an equal



Ball and stick model of the molecule of ranitidine (Zantac®)



mixture of both enantiomeric forms), but was later reformulated as Nexium®, which contains only the active (S)-enantiomer. This 'purple pill' has been the subject of one of the biggest pharmaceutical advertising campaigns of recent times in the USA, contributing to its huge success and the emergence of AstraZeneca (as the company became known following a merger with the pharmaceutical arm of Zeneca) as a major player in the pharmaceutical industry. The combined sales of omeprazole (Losec® and Nexium®) in its various guises can now be measured in the tens of billions of dollars.

Proton Pump Inhibitors

- Losec®
- Nexium®

we encountered the Nobel Laureate Sir James Black. Before Tagamet®, however, this highly successful scientist discovered the β -blockers as treatments for high blood pressure and angina. The story of this discovery begins over two centuries earlier, with the first description of angina in a medical text. In 1768, William Heberden accurately stated, "They who are afflicted with it are seized while they are walking (more especially if it be uphill and soon after eating) with a painful and most disagreeable sensation in the breast, which it seems as if it would extinguish life, if it were to increase or continue." Progress in unraveling the intricate physiology of the heart and the etiology of heart disease was slow during the eighteenth and nineteenth centuries. In 1928, Chester S. Keefer and William H. Resnik published a comprehensive explanation of angina. They confirmed that the cause was oxygen starvation in the coronary tissues, and that this could arise from several underlying conditions, from coronary artery disease and vasospasm, to decreased oxygen saturation in the blood. Heart attacks (myocardial infarctions) were directly linked

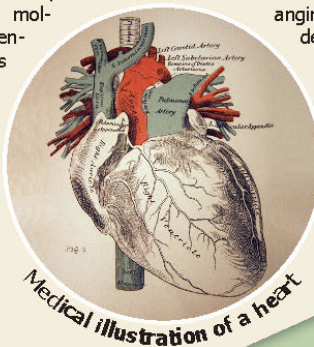
to coronary artery obstructions and angina, and the risk of sudden death in angina sufferers was highlighted.

This work provided the physiologic basis for Black's work in treating these conditions. His great insight was

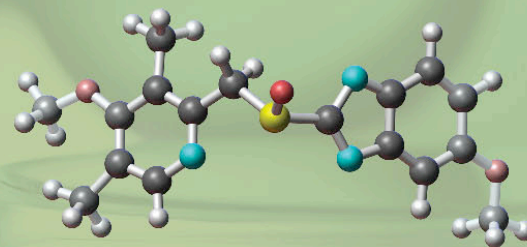
Drugs for the Heart

There is little need to reiterate the significance of drugs used to treat heart conditions. The story of a number of cholesterol-lowering agents (Chapter 26, Mevacor®, Zartagocic acids, and the CP molecules) highlighted the catastrophic impact of cardiovascular disease on modern society, and outlined some of the measures taken to combat heart problems. Many of the hypocholesteremic agents described in Chapter 26 were natural products, or derivatives thereof. In contrast, several other cardiovascular medications comprise rationally designed, small molecule drugs. We shall concentrate here on two classes that have both historical significance and enduring application.

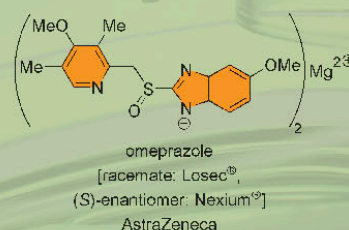
β -Blockers. In the story of Tagamet® above,



Medical illustration of a heart

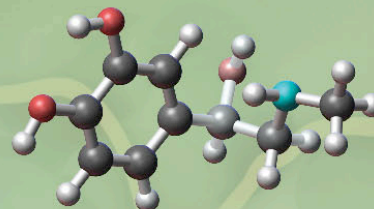
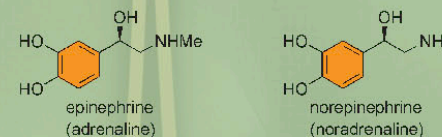


Ball and stick model of the molecule of (S)-omeprazole (Nexium®) [monomeric unit]

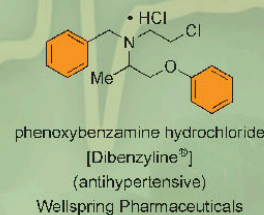


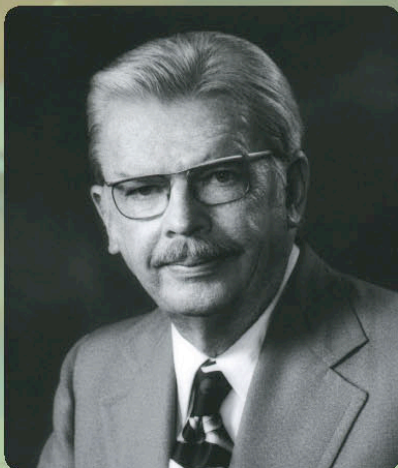
Box 17

Structures of epinephrine (adrenaline), norepinephrine (noradrenaline), and phenoxybenzamine hydrochloride (Dibenzylamine®)



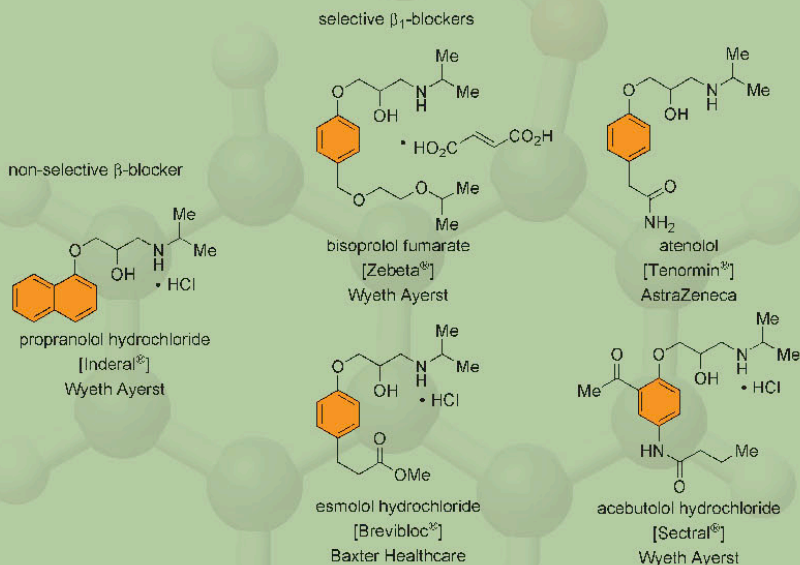
Ball and stick model of the molecule of epinephrine (adrenaline)





Raymond P. Ahlquist

Box 18 Selected β -blockers



Drugs for the Heart

- β -Blockers
- Propranolol
- Adrenaline

to connect the clinical knowledge of heart conditions (he was a clinician himself by training) with the growing body of knowledge of the pharmacology of the autonomic nervous system.

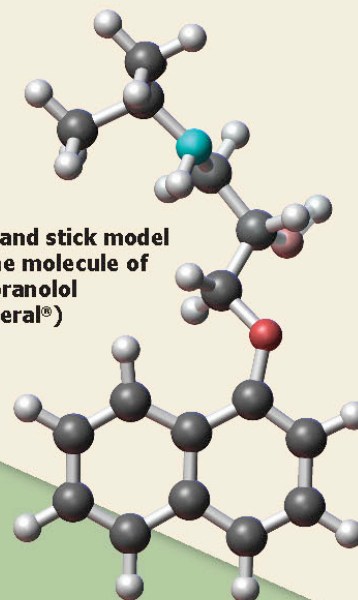
William Bates reported the discovery of adrenaline (or epinephrine, Box 17) in the *New York Medical Journal* in 1886. It was isolated and purified by Polish physiologist Napoleon Cybuski and American pharmacologist John Jacob Abel in 1895 and 1897, respectively. Japanese scientist Jokichi Takamine independently isolated a mixture of adrenaline and noradrenaline (norepinephrine, Box 17) in 1901 and was granted a US patent. He would make

a fortune from marketing this mixture under the name Adrenalin®. Animal studies showed that adrenaline causes tachycardia and increased blood pressure. Early anti-adrenaline drugs (e.g. phenoxybenzamine, Box 17) were found to reverse the rise in blood pressure, but to have no effect on heart rate. This dichotomy led Walter

B. Cannon and Arturo Rosenblueth to conceive the sympathin hypothesis, invoking different molecular modifiers, in 1939. Unfortunately, this erroneous idea became entrenched, and delayed the discovery of the true nature of the action of adrenaline for many years.

In 1948, Raymond P. Ahlquist proposed that different receptors were responsible for the various effects of adrenaline. He named the receptors alpha (α) and beta (β), names that are still used today. Such was the support for the sympathin hypothesis that Ahlquist found it difficult to publish his results, yet today he is hailed as a visionary scientist. Black was intrigued by the implications of Ahlquist's work, and, in 1958, embarked on a research program to design drugs to reduce the oxygen demand of the heart. This strategy was at odds with standard practice, which focused on vasodilators such as nitroglycerin. Calcium channel blockers, another important class of heart drugs, were originally developed as vasodilators. Black believed that oxygen consumption in the heart was determined by both systemic arterial pressure and heart rate. Disrupting the former is too dangerous because it can cause heart attacks, but reducing heart rate, which is controlled by the autonomic nervous system,

Ball and stick model of the molecule of propranolol (Inderal®)



would have the desired effect. Working at Imperial Chemical Industries (UK) with medicinal chemist John Stephenson, Black began making and testing compounds to block the β -adrenergic receptors. Following a long search, a potent β -antagonist, named nethalide, was discovered, but, while it was effective *in vivo*, it also induced tumors in mice and thus was abandoned. Fortunately, a safer compound, propranolol (Box 18), was found and launched as a drug in 1964 under the brand name Inderal®. Since then, propranolol has changed the face of cardiac medicine. It reduces the incidence of heart attacks in susceptible individuals, treats angina, and lowers dangerously high blood pressure. Curiously, propranolol also dilutes memories, removing the strongest emotions. This occurs because adrenaline is produced in the body in times of excitement or trauma, however, propranolol blocks its action and therefore dulls the memory of the event. Propranolol has been an enduring success, and is still in use today, despite the advent of newer drugs. Many of the newer generation of β -blockers (Box 18) are selective antagonists for the β_1 -adrenergic receptors (those found in the heart) and do not affect the β_2 -receptors found in smooth muscle. As a result, these drugs have fewer side effects.

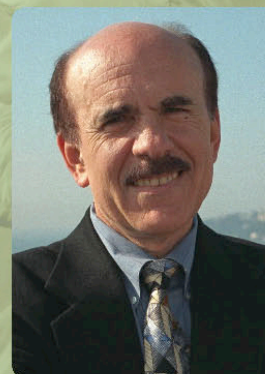
Nitroglycerin. Nitroglycerin is closely associated with the name of Nobel. Alfred Nobel was born in Stockholm (Sweden) in October 1833. His father, Immanuel Nobel, was an engineer and inventor who had an interest in the development of explosives. Alfred was highly intelligent and, by age seventeen, was fluent in five languages. His interests spanned science, poetry, and English literature. The young Alfred was sent on an extended tour of scientific institutions, which his father hoped would focus

his interests and energy on the physical sciences so he could enter the family business. In Paris, he worked with the famous chemist Théophile-Jules Pelouze. Here he met a young Italian, Ascanio Sobrero, who had prepared an extremely explosive liquid, trinitroglycerin (nitroglycerin), by the reaction of glycerin with a mixture of nitric and sulfuric acids. Sobrero believed nitroglycerin to be worthless, as it was too dangerous and unstable to be of practical use. He also noted that exposure to the vapor of this compound resulted in severe headaches, hinting at the medicinal application that would follow. Alfred Nobel was convinced that he could find some way to control the detonation of nitroglycerin, and thus make his fortune.

Meanwhile, Immanuel Nobel had enjoyed great success in St. Petersburg (Russia), where he designed and sold the first naval mines during the Crimean War. However, at the end of the war the market for explosives diminished and Immanuel was bankrupted. Two of his sons remained in St. Petersburg and later became rich in the oil industry. Immanuel and his two other sons, Alfred and Emil, returned to Sweden, where Alfred began investigating a way to tame nitroglycerin. His success did bring fortune, but it came at a high price; Emil Nobel was amongst those killed by an explosion at the factory. Explosions were so frequent that the Stockholm authorities banned Nobel from working within the city limits. He moved to a barge on Lake Mälaren where he found a way to stabilize the explosive by mixing nitroglycerin with



Robert F. Furchgott



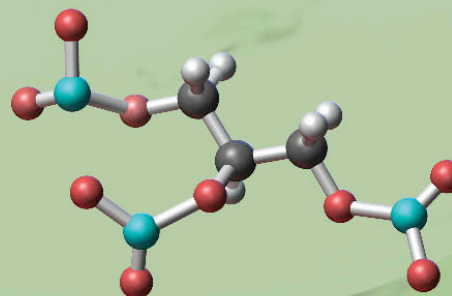
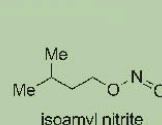
Louis J. Ignarro



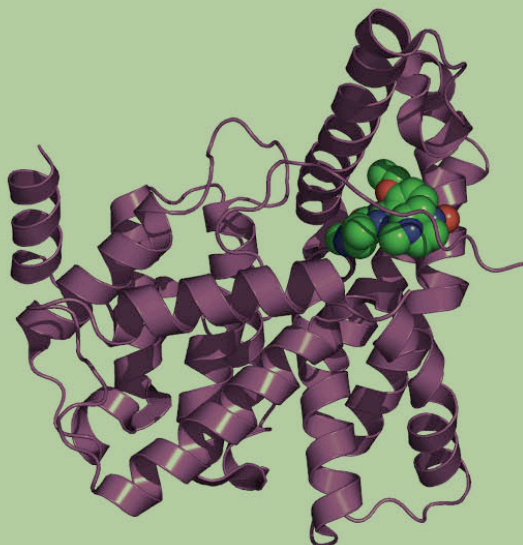
Ferid Murad

Box 19

Structures of isoamyl nitrite and glycerin trinitrate (trinitroglycerin, nitroglycerin)



Ball and stick model of the molecule of trinitroglycerin (nitroglycerin)



X-Ray crystallographic structure of the catalytic domain of human PDE5A1 in complex with sildenafil (Viagra®) (space-filling model)

Drugs for ED

- **Viagra®**
- **Cialis®**
- **Levitra®**

silica to form a paste that could be molded into charges for use in demolition. The new explosive, called dynamite, was hugely successful in both civil and military applications. Contrary to public perception, Alfred did not initially seem overly disturbed by the potential military application of dynamite and went on to develop other important military explosives. While he was a staunch pacifist who campaigned against war, he believed that peace could be guaranteed only by the invention of a weapon so powerful that it would act as a deterrent.

Nobel endured ill health throughout his life, and in old age he suffered from heart disease and angina. In Paris in 1890, his doctors prescribed nitroglycerin, but he refused to believe it could help and mocked the name, Trinitrin, given to the medicine, believing it was used to avoid alarming patients. The British physician Sir Thomas Lauder Brunton first noted the beneficial effects of organic nitrites in treating angina in 1867. He originally studied amyl nitrite, but nitroglycerin (Box 19) soon became the treatment of choice. In an ironic twist, after over one hundred years of clinical use, the 1998 Nobel Prize in Physiology or Medicine was awarded to Robert F. Furchgott, Louis

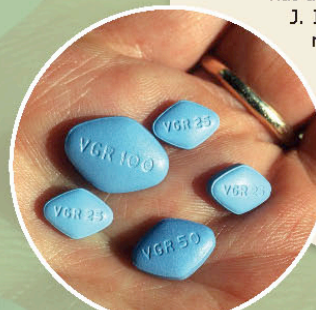
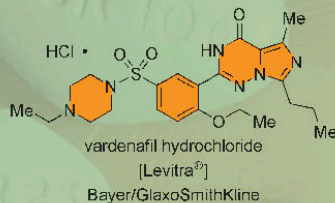
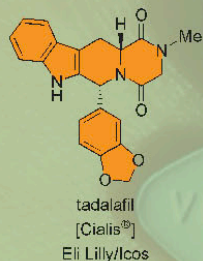
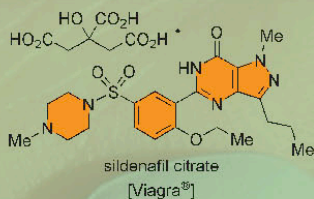
J. Ignarro and Ferid Murad for determining the mechanism of action of nitroglycerin. They discovered that it stimulates the release of the vasodilator nitric oxide (NO) from endothelial cells of the blood vessels. The NO diffuses into the smooth muscle where it triggers

the relaxation of the myofilaments, causing the blood vessels to widen and thus reduce blood pressure. Nitroglycerin is still in use today, although newer and safer drugs have largely supplanted it.

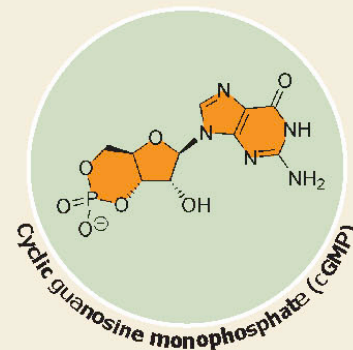
Drugs to Sexual Dysfunction

The final drug to be described in this chapter was initially investigated as a treatment for angina, but, while it would fail in this regard, it would open up a new field of medical intervention. In 1992, sildenafil citrate (later branded Viagra®) was being tested as an angina treatment by Pfizer (Sandwich, UK). It was not doing well in early clinical trials; however, an unexpected new property was discovered, saving sildenafil from obscurity. Volunteers in clinical trials are required by law to report every reaction they feel, no matter how insignificant it seems. A number of volunteers overcame their initial embarrassment to report experiencing an increased tendency to get erections. With this result, the drug was reinvestigated as a treatment for impotence, or erectile dysfunction (ED) as it became known. This time the trials progressed successfully, and Viagra® was launched in 1998. Within weeks, more than one million prescriptions had been written for Viagra® in the United States alone. After just one full year on the market, sales of Viagra® had exceeded \$1 billion.

Box 20 Drugs to treat sexual dysfunction



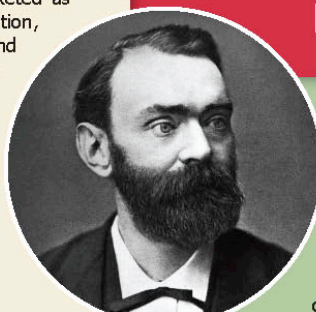
Viagra® pills



Alfred Nobel and the Nobel Prize

Viagra® (Box 20) is a selective and potent inhibitor of the enzyme phosphodiesterase type 5 (PDE5). PDE5 cleaves and inactivates the chemical messenger cyclic guanosine monophosphate (cGMP). cGMP causes smooth muscle relaxation in the erectile tissue, increasing blood flow. The release of nitric oxide into the blood during sexual arousal triggers the production of cGMP, and it has been suggested that an inability to produce enough nitric oxide causes erectile dysfunction. Inhibiting PDE5 leads to increased levels of cGMP, facilitating erection. The effectiveness of Viagra® may also be enhanced by psychological factors, but such effects are difficult to quantify and are still under investigation. Recent reports suggest that Viagra® can also increase blood flow to the sexual organs in women, making it an effective treatment for female sexual dysfunction. Two other PDE5 inhibitors have subsequently been marketed as treatments for erectile dysfunction, Cialis® (tadalafil, Eli Lilly/Icos) and Levitra® (vardenafil hydrochloride Bayer/GlaxoSmithKline). In 2004, these three drugs boasted combined sales of \$2.4 billion.

In June 2005, sildenafil (marketed as Revatio®) was approved as a treatment for



Alfred Nobel

Box 21 The Nobel Prize

Alfred Nobel bequeathed his substantial fortune for the purposes of awarding annual prizes in the fields of Chemistry, Physics, Physiology or Medicine, Literature, and Peace, reflecting his diverse interests. These five original Nobel Prizes, as the awards became known, were supplemented by the addition of the Nobel Memorial Prize in Economics in 1968. The

prizes have been awarded since 1901, and are the most prestigious awards given in each category. Initially, the awards honored the most important work carried out in each field in the previous year, however, the scope has since widened, and the prizes are often given many years after the work has been done. Many of the protagonists in this volume have been awarded the Nobel Prize for their groundbreaking research.



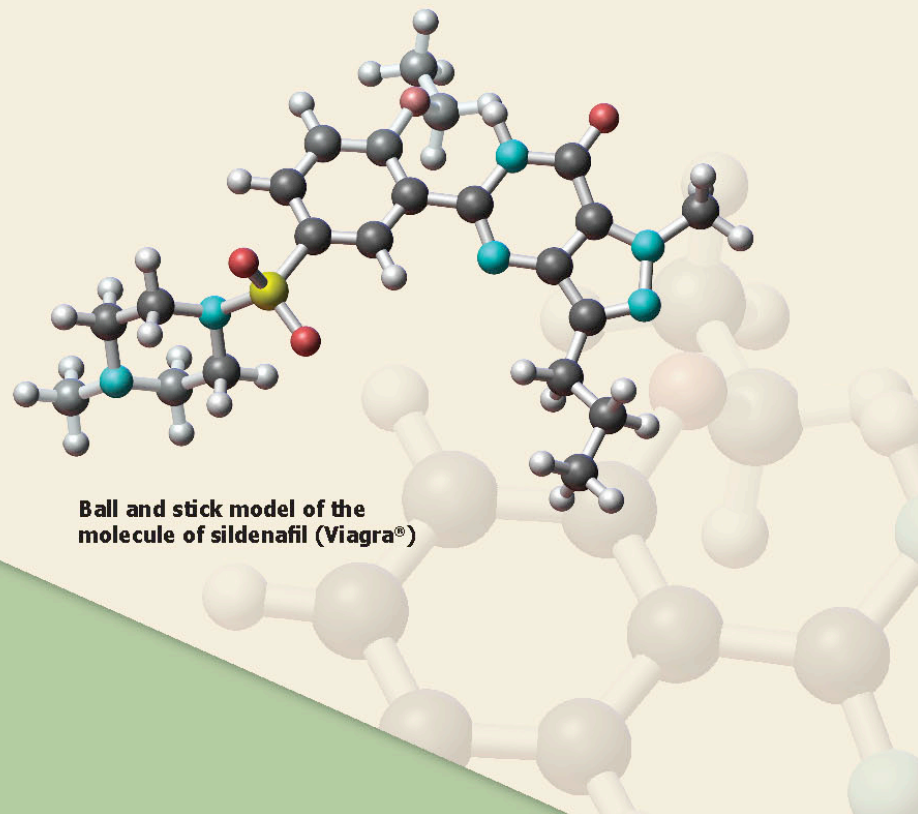
Nobel Prize medal



body. By inhibiting the PDE5-mediated breakdown of cGMP in the smooth muscle of the pulmonary vasculature, sildenafil relaxes the arterial wall, decreasing pulmonary vascular resistance and alleviating the symptoms of PAH.

pulmonary arterial hypertension (PAH), a progressive disease characterized by increasing pulmonary vascular resistance, ultimately leading to right ventricular failure of the heart. The connection between erectile dysfunction and PAH may seem tenuous at first, but the same mechanism of action of sildenafil is operative in both cases. PDE5 is primarily distributed in arterial wall smooth muscle in the penis and lungs, thus sildenafil induces vasodilation selectively in these areas of the

This chapter has focused on selected important pharmaceuticals, and indicates the central role of organic chemistry in the drug discovery and development process. Of course, the small molecule drugs discussed above are but a few of the many wondrous medications we have available today. The number of treatments and their power to cure will surely increase in the future as science and technology continue to open new opportunities for drug designers to exploit.



Ball and stick model of the molecule of sildenafil (Viagra®)

Further Reading

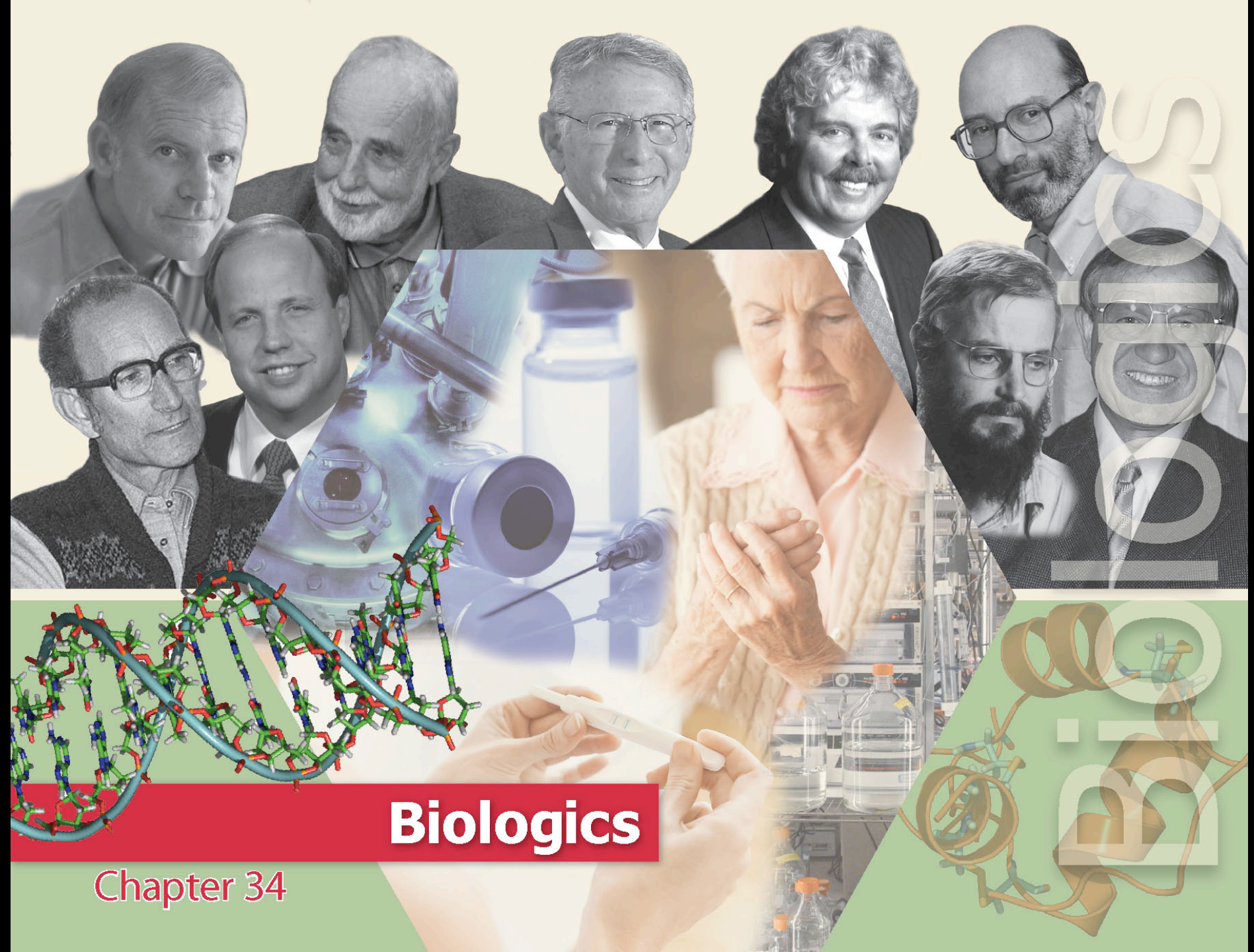
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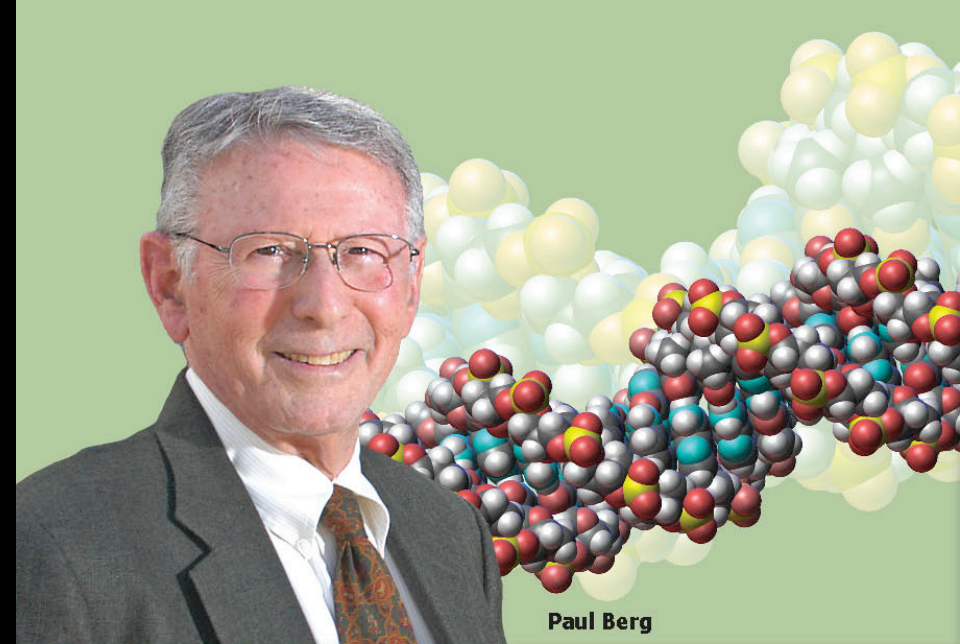
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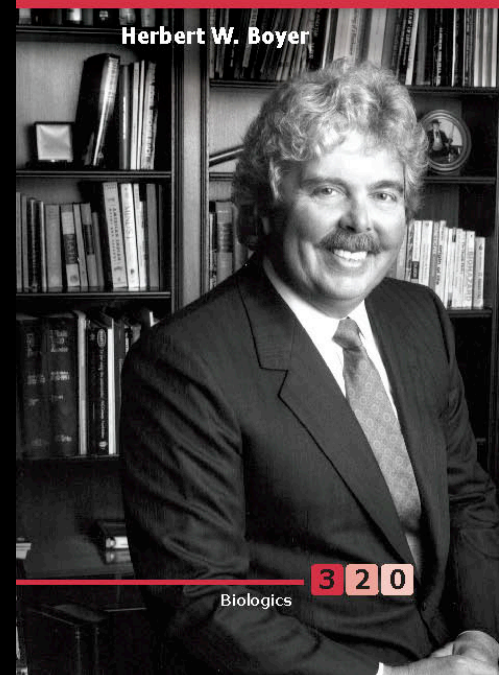


Biologics

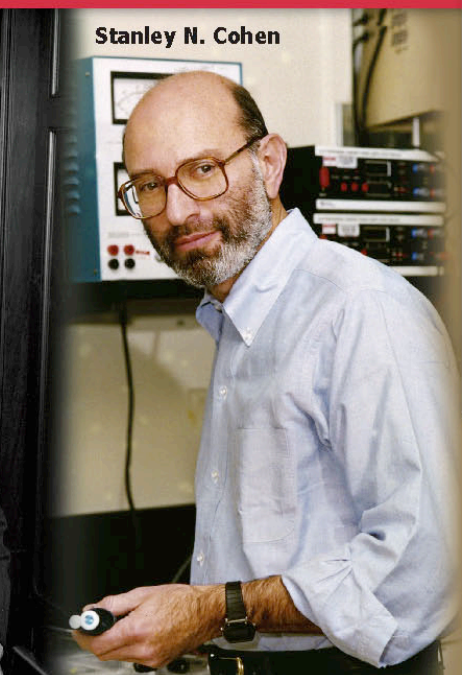
Chapter 34



Paul Berg



Herbert W. Boyer

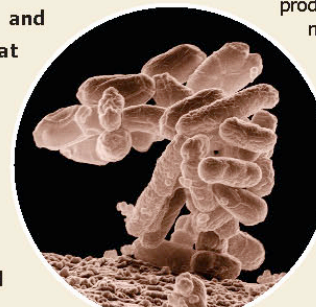


Stanley N. Cohen

Even the largest natural products discussed in this book are relatively tiny compared to many of the giant biopolymers produced by living organisms. Such polymers include proteins, nucleic acids, and polysaccharides, and it is only recently that we have been able to produce some of them in the laboratory. Until the development of modern biotechnology, these macromolecules had to be harvested from natural sources, a costly and time-consuming process plagued by problems of scale and purification. Transference of disease by products contaminated with, for example, viruses and prions was an additional concern. Relying on natural sources also limited our ability to adapt and manipulate the molecules in question. In this chapter, we will see how the boom of modern biotech-

nology in the 1970s and 1980s led to a host of new techniques which provided solutions to so many of these issues. We will then discuss a number of macromolecules that have been brought to the market as lifesaving medications, collectively known as biologics.

The United States government defines biotechnology (biotech) as any technique that uses living organisms (or parts of organisms) to make or modify products, including foods, medicines, and soap powders, to name but a few items. Under this very broad definition man has been using biotechnology for thousands of years. Activities such as bread, wine, and cheese production all involve the use of colonies of living microorganisms to assist in making the end-product.

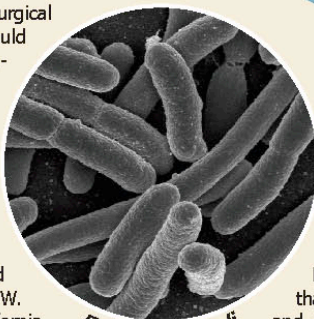


Escherichia coli

More recently, antibiotics such as penicillin have been, and continue to be, manufactured using fermentation biotechnologies. However, the triggering of the biotech revolution has only been possible due to the major breakthroughs made in DNA-based technologies over the last few decades, some of which are highlighted below.

The 1970s witnessed the emergence of genetic engineering at the molecular level. Initial research conducted by biochemist Paul Berg (Stanford University, USA) showed that DNA from different species

could be integrated with surgical precision to form what would become known as recombinant DNA. Paul Berg's fundamental work in establishing this field led to him being one of the three recipients of the 1980 Nobel Prize in Chemistry. These DNA-manipulating techniques were further developed by a biochemist, Herbert W. Boyer (University of California, San Francisco, USA), and a geneticist, Stanley N. Cohen (Stanford University, USA). In recombinant DNA technologies, sections of DNA that code for an important protein can be identified, spliced from the DNA chain, and inserted into a vector which carries the DNA to a new host where it is replicated. Specially adapted, fast growing bacteria from the *Escherichia coli* (*E. coli*) strain are the most commonly employed hosts. As a consequence of their normal reproductive cycles, the host cells replicate the introduced DNA and begin to synthesize the targeted protein which can be harvested and purified ready for its intended use. In principle, large amounts of the recombinant protein can be produced in factories using vats of genetically engineered organisms.



Escherichia coli

In a great coup in the late 1970s, a chemist turned venture capitalist, Robert A. Swanson, courted Herbert Boyer and persuaded him to set up the first modern biotechnology company, Genentech (South San Francisco). The first meeting between Swanson and Boyer, which took place in 1976, has become legendary. It is said that Swanson had

become excited upon reading about Boyer's pioneering recombinant DNA work, so much so that he placed a call to Boyer and requested an immediate meeting. Boyer, absorbed by his science, somewhat grudgingly agreed to give the young venture capitalist a ten minute window in his busy schedule. Swanson's vision and enthusiasm was contagious and the ten minute meeting grew into a three hour discussion. By the time their conversation concluded on that day, Genentech had been conceived. Genentech's initial triumph was to manufacture the first human protein (somatostatin, a growth hormone-releasing inhibitory factor) using a microorganism (*E. coli*). By the end of the 1970s, Genentech scientists had also produced recombinant human insulin and a human growth hormone. Genentech's portfolio allowed it to go public in 1980, and it has gone from strength to strength as a market leader in biologics. In 1982, the Food and Drug Administration (FDA) granted approval to Genentech for the first genetically engineered drug – human insulin produced by bacteria. In addition to marketing

its own products, Genentech has also worked closely, through licensing agreements, with many of the biggest pharmaceutical companies in the world to rapidly bring recombinant products

● Recombinant DNA Technologies

Robert A. Swanson



David Goeddel and Genentech's President Robert A. Swanson examining fermentation vats for the mass production of human insulin and human growth hormone



César Milstein

Georges J. F. Köhler



Modern pregnancy tests use monoclonal antibodies to detect minute traces of the peptide hormone human chorionic gonadotropin (hCG) in the blood or urine to indicate the presence or absence of an implanted embryo.



Niels K. Jerne



H. Gobind Khorana



Marvin H. Caruthers



Robert W. Holley

to the market. The Genentech formula has become the industry standard and many of the biotechnology firms in operation today aspire to emulate the success of Genentech. Indeed, many scientists and venture capitalists strive to repeat this success story as a means to progress their discoveries into marketable drugs.

Besides recombinant proteins, another important class of engineered biomolecules is monoclonal antibodies. Monoclonal antibodies are homogenous populations of

of monoclonal antibodies" won Georges J. F. Köhler (Basel Institute for Immunology, Switzerland) and César Milstein (Medical Research Council Laboratory of Molecular Biology, Cambridge, UK) a share of the 1984 Nobel Prize in Physiology or Medicine. They shared this prize with Niels K. Jerne (also at Basel Institute for Immunology), who was recognized "for theories concerning the specificity in development and control of the immune system." Monoclonal antibodies are now widely used as analytical tools in basic research and medical diagnosis. Modern pregnancy tests use monoclonal antibodies to detect minute traces of the peptide hormone human chorionic gonadotropin (hCG) in the blood or urine to indicate the presence or absence of an implanted embryo. The use of mono-

immune system proteins designed to bind specifically to a single site on a desired molecular target (antigen). They are produced using special tumor cells (called hybridomas) that have been fused with antibody producing cells. These tumor cells multiply rapidly and indefinitely, generating potentially large amounts of antibodies. Because the tumor cells themselves are all clones of a single parent cell, the antibodies they produce are all identical. The "...discovery of the principle for production

clonal antibodies is also becoming increasingly important in the treatment of certain diseases, and this chapter will highlight one such application (see also Chapter 23, Calicheamicin γ_1).

The discovery of recombinant proteins and monoclonal antibodies, to name but two applications, would not have been possible without concomitant advances in the areas of DNA sequencing, amplification, and chemical synthesis. Paul Berg's fellow recipients of the 1980 Nobel Prize

in Chemistry, Walter Gilbert (Harvard University, USA) and Frederick Sanger (Cambridge University, UK), independently developed methods for determining the nucleotide order of DNA fragments, in essence enabling the genetic code to be read in the laboratory. These DNA sequencing techniques are of key importance in the Human Genome Project and related endeavors. In 1983, Kary B. Mullis and co-workers at the biotechnology company Cetus developed a method for enzymatically replicating DNA sequences *in vitro*. Known as the polymerase chain reaction (PCR), this technique enables a small amount of the DNA sequence to be amplified rapidly and exponentially. Today, PCR is used routinely in biological research and other applications; for example, the basis of one type

- **DNA Structure**
- **DNA Synthesis**
- **DNA Sequencing**
- **PCR**

DNA encodes can be engineered. Also in 1983, Marvin H. Caruthers (University of Colorado, Boulder, USA) developed a chemical method whereby fragments of DNA, in a predetermined sequence of 5–75 base pairs, could be constructed *in vitro*. Within a short time, both Caruthers and Leroy



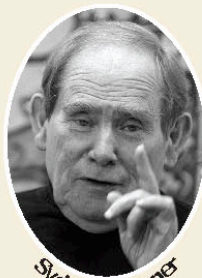
Frederick Sanger



Walter Gilbert



Marshall W. Nirenberg

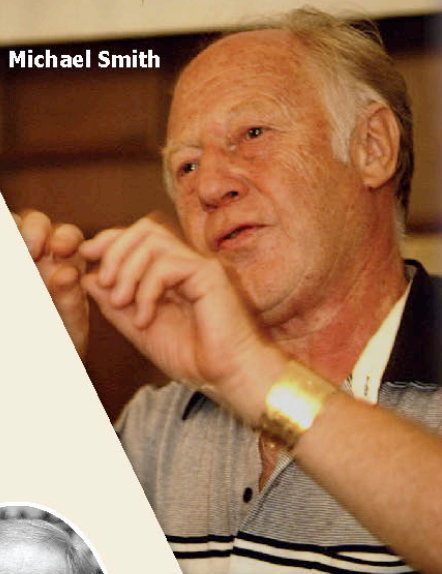


Sydney Brenner

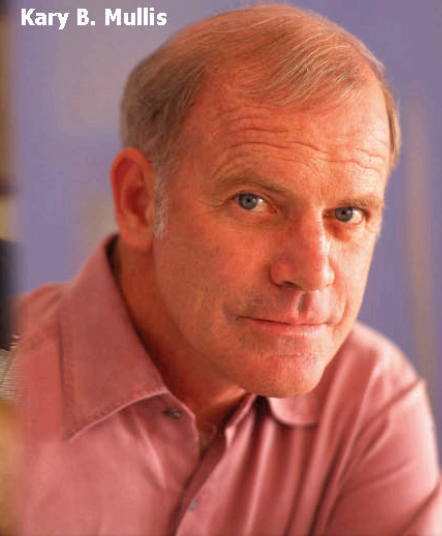
of HIV test involves PCR multiplication of viral RNA, while in another application PCR is used in forensic science to detect and profile minute residual pieces of DNA left at a crime scene. Mullis shared the 1993 Nobel Prize in Chemistry with Michael Smith (University of British Columbia, Canada). Smith pioneered site-directed mutagenesis, the technique by which mutations can be created at defined sites in DNA sequences, and thus specific changes to the amino acid sequence of the protein for which the

Hood (then at the California Institute of Technology, USA) had invented instruments that automated this DNA synthesis process. This work built upon the foundations laid by H. Gobind Khorana (then at the University of Wisconsin, USA, later at the Massachusetts Institute of Technology, USA), who pioneered methods for the chemical synthesis of oligonucleotides (short sequences of DNA or RNA nucleotides) in solution. Khorana's work, which followed earlier studies by Sydney Brenner (who would be

Michael Smith



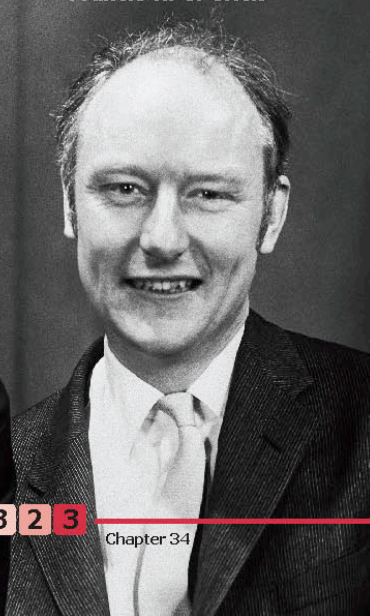
Kary B. Mullis



James Watson



Francis H. C. Crick





Dr. Edward Jenner vaccinating young boy

Box 1 The emergence of vaccines

Date of introduction of first vaccine	Disease
1796	smallpox
1885	rabies
1897	plague
1923	diphtheria
1926	pertussis
1927	tetanus
1927	tuberculosis (BCG)
1935	yellow fever
1955	poliomyelitis (injectable)
1962	poliomyelitis (oral)
1964	measles
1967	mumps
1970	rubella
1981	hepatitis B
2006	human papillomavirus



Frederick G. Banting

awarded a share of the 2002 Nobel Prize in Physiology or Medicine for later studies on the genetic regulation of organ development), Francis H. C. Crick and others, was also instrumental in elucidating the nature of the genetic information contained in the DNA double helix model proposed by Watson and Crick. Khorana shared the 1968 Nobel Prize in Physiology or Medicine with Robert W. Holley (Cornell University, USA) and Marshall W. Nirenberg (National Institutes of Health, Bethesda, Maryland, USA) "for their interpretation of the genetic code and its function in protein synthesis."

In addition to the selected few scientists discussed above, it should be remembered that a great many other researchers have contributed to the advancement of these fields. Today we can decipher the genetic code of an organism, make DNA strands, produce proteins, and even genetically modify plants and animals, all for the benefit of humanity. To be



Lady Mary Wortley Montagu

sure, many more breakthroughs, scientific and technological, are waiting in the wings.

Vaccines

Vaccines are the oldest and broadest category of biologic drug. Their use has succeeded in eliminating or substantially reducing the devastating impact of many terrible diseases. Today they are used routinely in the developed world, where immunization, especially in childhood, is largely taken for granted. The principle of immunization is much older than many believe. The term 'vaccine' was coined by Edward Jenner in the eighteenth century, but he was not the first to use this technique. Smallpox was probably the first disease to be prevented with vaccines, and it is the only one to have been eradicated in this way. The origins of the practice can be traced to ancient India and China, where physicians used material derived from smallpox sufferers to inoculate healthy people. In China, dried and powdered smallpox pustules were inhaled, while in India smallpox scabs were placed on scratched skin. In 1718, Lady Mary Wortley Montagu, wife of the British ambassador to the Ottoman Empire, reported that the Ottomans inoculated themselves using fluid from the pustules. She endeavored to have this practice adopted in the UK, and even inoculated her own son.

In 1796, Edward Jenner, an English country doctor,

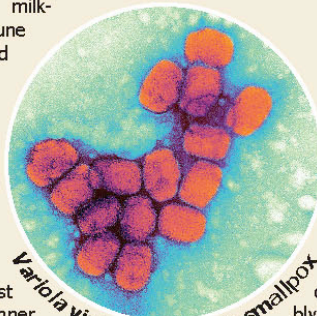


Louis Pasteur

● Vaccines ● Antibodies

noted that several local milkmaids seemed to be immune to smallpox. He attributed this protection to their exposure to the related, but milder, cowpox. If the milkmaids contracted cowpox from their daily contact with cows, they could no longer catch smallpox. In an experiment that would make most people balk today, Jenner took infectious fluid from a milkmaid, Sarah Nemes, and inoculated a local eight-year-old, James Phipps. Forty-eight days later, when Phipps had recovered from the cowpox, Jenner exposed the child to smallpox. Fortunately, he did not develop any symptoms of the more serious disease. This experiment also gave rise to the term vaccine, named from *vaccina*, the infectious cowpox agent. The great biologist and chemist Louis Pasteur built on Jenner's success, developing vaccines for a number of diseases of viral and bacterial origin, including rabies, cholera and anthrax. Since then, a broad range of vaccines has been developed, increasingly making use of better-characterized formulations (Box 1).

The first public mass vaccinations were carried out as early as 1805, when the Mexican and Guatemalan authorities organized a systematic anti-smallpox inoculation program. This foresight allowed the Americas to be declared smallpox-free long before the rest of the world. A global program did not begin until 1956, when the World Health Organization (WHO) undertook a campaign to eradicate smallpox. They discovered that high coverage and a second line of rapidly targeted vaccinations, to respond to local outbreaks, were needed



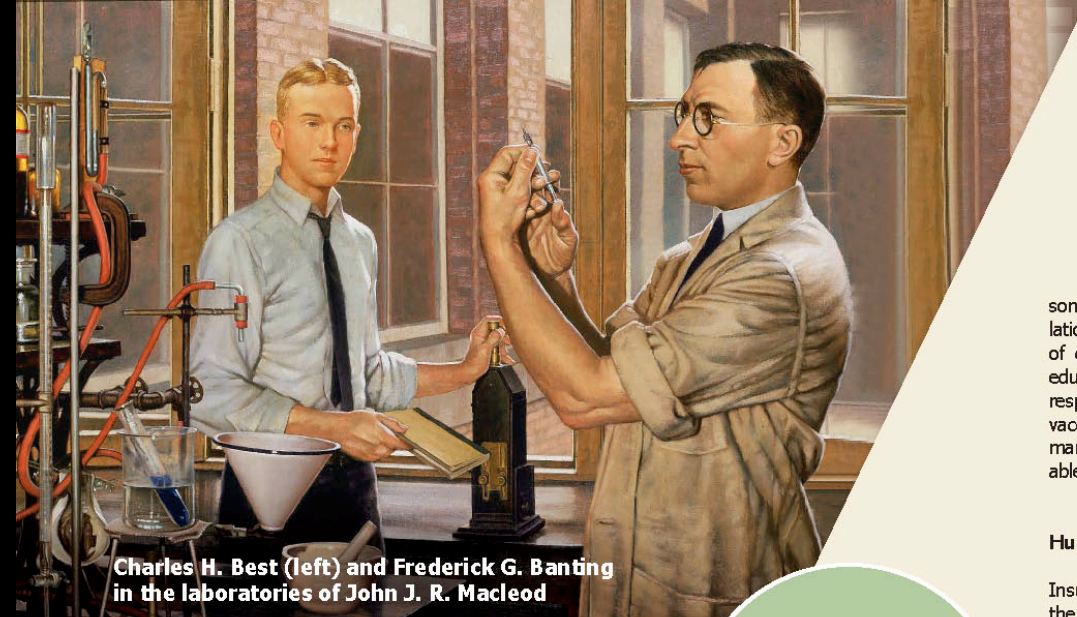
Varicella virus that causes smallpox

for success. In 1979, the WHO announced that smallpox had been eradicated. Expanded WHO plans to eradicate other diseases, most notably poliomyelitis, for which cheap vaccines are available, are now underway.

Vaccines work by preparing our immune systems to fight off the infection efficiently. The vaccine comprises foreign matter that triggers an immune response. Macrophages and lymphocytes attack the foreign material and B-lymphocytes produce antibodies against the invading proteins. When the initial response has passed, memory cells (specialized B-cells) persist in the blood. If the same (or similar) infective agent enters the bloodstream again, the memory cells proliferate rapidly and mount a powerful immune response, preventing the infection from taking hold. Depending on the type of vaccine used, the memory cells may give life-long protection, or they may persist for only a short time, and thus require booster vaccinations. Some details of a number of vaccine types are shown in Box 2.

Vaccines are undoubtedly the most powerful and cost-effective of public health measures, however they are not without controversy. The safety of the vaccines and their formulations must be closely monitored to maintain confidence in vaccination programs. For example, suggested links between some vaccines and autism have led to reduced vaccination uptake in

Vaccine type	Immunization mode	Strength of immunity	Example
Inactivated/killed	The infecting organism is killed (by soaking in formalin) prior to being injected into the patient	Not strong, booster shots are required	Typhoid vaccine and the Salk poliomyelitis vaccine
Acellular	Part of the organism is injected (e.g. flagella, protein cell wall)	Not strong, booster shots are required	<i>Haemophilus influenzae</i> B (HIB-B) vaccine
Live attenuated/weakened	Weakened organism injected, once inside body multiplies causing a strong immune response. Most dangerous because can mutate back to active form and become infective.	Strong, lifelong protection	Measles, mumps, rubella vaccines
Toxoid	Toxin from organism injected after treatment to render it harmless (e.g. by addition of aluminium salts)	Low level immunity, often administered with an agent that enhances immune response	Tetanus and diphtheria vaccines
Subunit	Genetic engineering used to produce harmless bacteria (or yeast) host cells, which carry part of genetic code of the infective pathogens	Variable, usually good, protection	Hepatitis B vaccine
Similar	Like Jenner's original, a similar but milder pathogen is used to protect the patient	Strong, usually lifelong protection	Bacillus Calmette-Guérin (BCG) vaccine for tuberculosis



**Charles H. Best (left) and Frederick G. Banting
in the laboratories of John J. R. Macleod**



Schack A. S. Krogh

some countries, leaving populations vulnerable to outbreaks of disease. In addition to proper education, developed nations have a responsibility to increase the availability of vaccines in the developing world, where many children are still dying from preventable diseases.

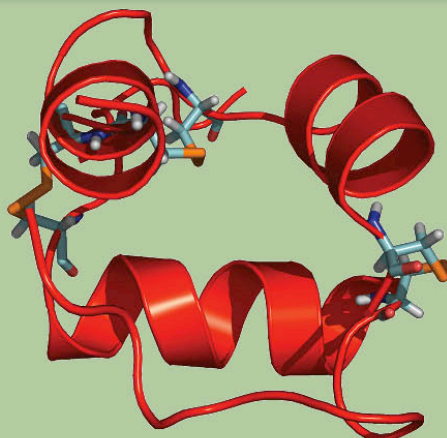
the worldwide cost of insulin alone was \$6.75 billion in 2004. This expense cannot be met in many countries, and thus diabetics are left to suffer the consequences such as loss of limbs, blindness, heart disease and kidney failure. Hence, there is the pressing need to discover and develop newer sources of insulin that can satisfy the demand and provide the drug less expensively (to all those patients who need it).

Insulin originally took only twelve months from first isolation to commercialization, due to the dire need for diabetes treatments and the ratherless stringent controls placed on drugs in the early twentieth century. Two Canadian scientists, Frederick G. Banting and Charles H. Best, working in the laboratory of John J. R. Macleod at the University of Toronto isolated insulin in 1921. After some initial reluctance they agreed to collaborate with the pharmaceutical industry, and Connaught Laboratories (now part of Sanofi Aventis) and Eli Lilly began producing insulin. The academic scientists also offered royalty-free licenses to a number of European institutions. Danish Nobel Laureate August Krogh, whose wife was a diabetic, exploited this opportunity by founding Nordic Insulin Laboratories (now Novo Nordisk). Through the Medical Research Council, the UK became the first country to approve insulin injections for diabetics, in 1922. Eli Lilly followed suit by winning approval in the USA in 1923 with a much larger scale production. Macleod and Banting received a share of the 1923 Nobel Prize in Medicine or Physiology for their work. The brief gap between the discovery and this award indicates the perception of the importance of their research. At that time, the structure of insulin was unknown, even though it is a relatively

Humalin® (human insulin)

Insulin provides an excellent gauge of the development of therapies using large biomolecules over the last century. It is a polypeptide hormone produced by β -cells in the pancreas, which regulates blood glucose levels. Diabetes mellitus, the disease that results from a failure to control glucose levels, occurs in two distinct types. Type 1 usually begins in childhood and is characterized by a lack of insulin production. To survive, type 1 diabetics must receive daily injections of insulin. Type 2 diabetes accounts for nearly ninety percent of all cases and, although it is not caused by insulin deficiency, approximately one third of sufferers benefit from insulin therapy. Diabetes is becoming ever more prevalent. The WHO estimates that the number of diabetics worldwide grew from 30 million in 1985 to 177 million in 2000, and that 300 million people will suffer from the disease by the year 2025. It also estimates that nine percent of all deaths are caused by diabetes, with the vast majority of these being considered premature. The cost of treating diabetes is, therefore, enormous;

● **Diabetes** ● **Humalin® (Insulin)**



Solution-phase average structure of the highly active monomeric des-[Phe(B25)] human insulin mutant as determined by NMR spectroscopy and molecular dynamics calculations. The one intrastrand and two interstrand disulfide bonds are depicted in stick format.

small protein, having just fifty-one amino acids (twenty-one in chain A and thirty in chain B, the two chains being joined by two disulfide bonds). The structure was eventually solved in 1969 by Professor Dorothy Crowfoot Hodgkin at the University of Oxford (UK) using x-ray crystallography. She had devoted thirty-five years of work to the problem, beginning in 1934 when she was given a sample of ten milligrams of insulin by another prominent Oxford chemist, Sir Robert Robinson. It took one year to grow suitable crystals, and a further thirty-four years of data analysis in the pre-computer era to determine the full structural details of insulin.

For more than half a century, the demand for insulin was met using supplies extracted from animals. Bovine (cow) and porcine (pig) insulin molecules differ only slightly from the human form and can usually be substituted without problems. However, the growing numbers of diabetics and problems with allergic reactions to the animal products fueled a search for alternative insulin supplies. Insulin is too large for laboratory chemical synthesis to yield practical quantities, so the breakthrough in production had to wait until the advent of genetic engineering (see above). In 1978, the fledgling biotechnology company Genentech collaborated with the City of Hope National Medical Center (California, USA) to produce human insulin using recombinant DNA technology. They synthesized the gene for insulin and introduced it into the genome of *E. coli* bacteria. Fermentation of the bacteria led to the production of large quantities of the hormone, which could be extracted using techniques similar to those developed for penicillin production. At that time, the production

● **Anemia**
● **EPOGEN®**
(Erythropoietin)

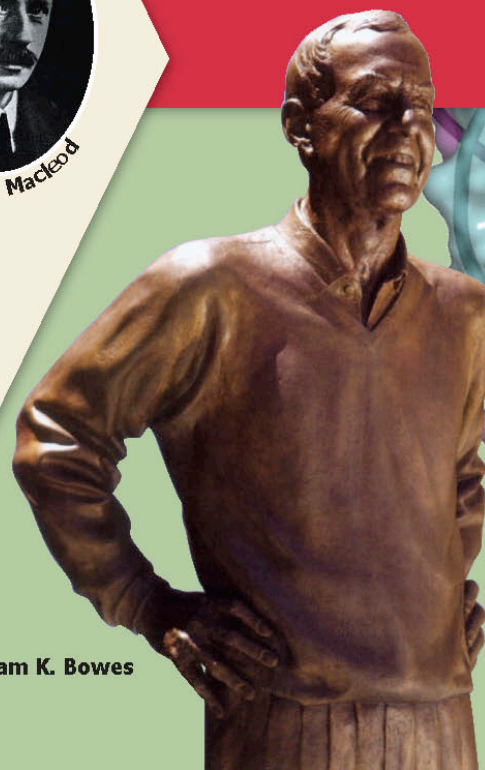
of active peptides by genetic engineering was forbidden, so the company prepared the two chains in separate fermenters and joined them chemically after purification. Before such a drug could be launched, the regulatory authorities demanded strict testing to ensure the material was safe. The chemical and biological properties of the recombinant DNA and the active protein had to be determined and the process for joining the two chains understood fully. The folding of the synthetic peptide also had to be examined in detail. Clinical trials began in 1980 and, in 1982, the UK approved the first genetically engineered drug. It was named Humalin® and was produced by Eli Lilly under a licensing agreement from Genentech. This collaboration heralded a new trend in which established pharmaceutical firms use their infrastructure to carry out the development and marketing of discoveries made in smaller, risk-taking biotechnology companies. A new era in drug discovery was on the horizon. Following the launch of Humalin®, analogues of insulin with more favorable pharmacological properties, such as delayed effect and lengthened duration of action, were introduced. More than twenty such drugs are now available. A number of companies are now developing other drug treatments for type 2 diabetes and, in 2005, the FDA approved a new drug to help control blood glucose in patients for whom insulin therapy alone is insufficient. Symlin®, produced



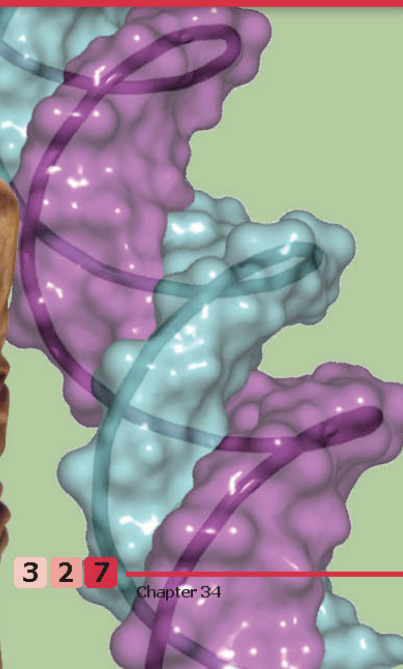
John J. R. Macklod



Dorothy Crowfoot Hodgkin



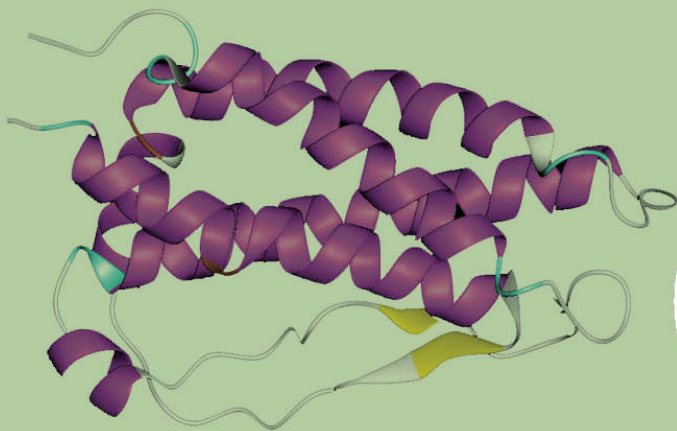
William K. Bowes





Fu-Kuen Lin

Solution-phase averaged structure of human erythropoietin as determined by NMR spectroscopy



by Amylin Pharmaceuticals (USA), is a synthetic analogue of amylin, another pancreatic hormone that contributes to glucose maintenance. And Januvia®, developed by Merck, is the first of a number of emerging small molecule drugs to be approved by the FDA for the treatment of diabetes.

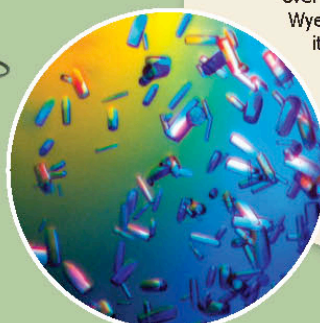
EPOGEN® (erythropoietin)

Erythropoietin (EPO) is a peptide hormone produced in the kidneys (and liver in infants). It stimulates the production of red blood cells from bone marrow and plays pivotal roles in regulating hemoglobin biosynthesis and red blood cell levels. The protein contains 165 amino acids and has four sugar groups attached to it. EPO was first isolated from urine in the 1970s. The gene encoding EPO was identified shortly thereafter, as it was clear that this protein had many potential medical applications, and by using the recombinant DNA techniques described in our introduction to this chapter EPO production was initiated in vitro. The competition in this field was fierce and led to one of the largest patent disputes in history. In 1996, Amgen finally triumphed over the Genetic Institute (now part of Wyeth) to secure full patent protection for its product, named EPOGEN®, seven years after it was first launched.

Amgen (the acronym stands for Applied Molecular Genetics) was founded in 1980 by a small group of scientists and investors led by the venture capitalist William K.

Bowes, who recruited the first CEO of this fledgling company, George B. Rathmann. Amgen's first major achievement came in October 1983, when Fu-Kuen Lin successfully cloned the EPO gene. Lin's team then developed a method for producing EPO in a form and quantity that enabled its use in the treatment of anemia. The resulting molecule entered clinical trials in December 1985 and, in 1987, Amgen received its first patent on what would become the company's first medicine. On June 1, 1989, the FDA approved EPOGEN® for anemia management associated with end-stage renal disease. EPOGEN® became biotech's first blockbuster drug (annual sales of at least \$1 billion); its commercial success was key in enabling Amgen to flourish from its humble beginnings in the (then) small town of Thousand Oaks, California. Today, Amgen is one of the world's biggest biotechnology companies, rivaling established 'Big Pharma' companies in size and capitalization, and continues to be a leading innovator in the identification, isolation, purification, and use of human proteins as therapeutic targets.

EPO is used to treat severe anemia. Those receiving cancer chemotherapy or HIV combination therapy can suffer serious anemia, which can be overcome using EPO injections. Sufferers of chronic kidney disease can also benefit from EPO. Furthermore, patients preparing for some surgeries can be given EPO prior to the operation in place of blood transfusions during the procedure. Unfortunately, its effects are exploited by some endurance athletes (such as cyclists, distance runners, and skiers) whose performance can be significantly enhanced by the EPO-induced increase in red cell count, and, consequently, oxygen supply. Abuse of EPO in this way is very dangerous because the associated thickening of the blood can cause heart



Crystals

attacks and strokes, especially when the athlete becomes dehydrated. Indeed, EPO has been implicated in the deaths of a number of high-profile athletes. A legitimate way of gaining this advantage is to train at high altitude (above 6000 feet), where the body adapts to the thinner air by producing more EPO naturally.

In 2004, sales of the α -EPO variant produced by Johnson & Johnson under license from Amgen were \$6.2 billion in the USA alone. Sales of other variants outside the USA contributed a further \$4.8 billion. This partnership agreement has, however, been far from smooth due to alleged license and patent infringements, an increasing phenomenon between competing pharmaceutical companies. Amgen has recently developed darbepoetin (Aranesp®), an engineered EPO analogue that has two extra sugar units attached to its peptide backbone. These extra groups increase its potency while slowing down its clearance from the body, allowing for marked reductions in dosing.

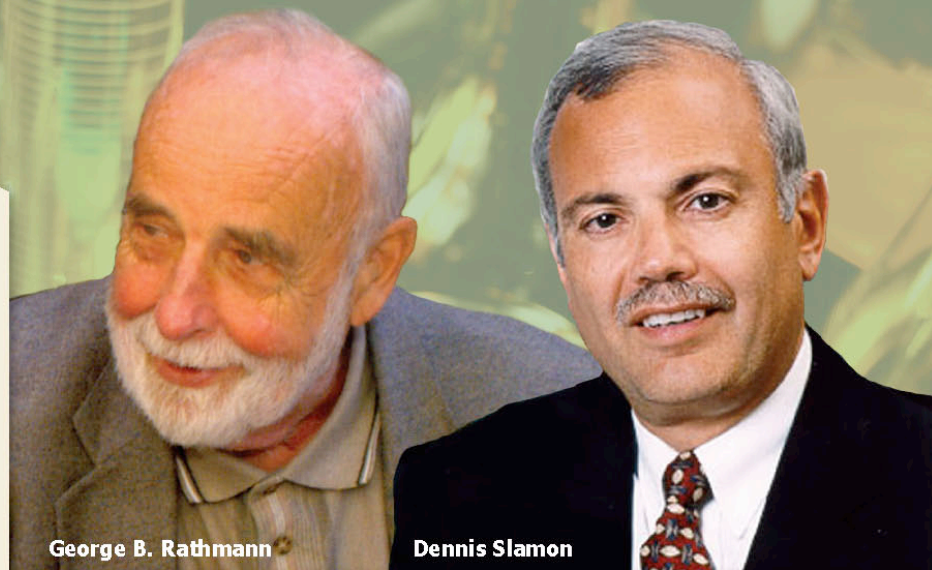
The story of EPO shows how the lessons learned from insulin have been applied to produce a new blockbuster protein drug, however it also highlights the controversies that can arise from commercializing genetic technology and naturally occurring hormones.

Herceptin® (trastuzumab)

In 1987, Dr. Dennis Slamon and colleagues at UCLA (University of California, Los Angeles, USA) published an article in *Science* in which they linked overexpres-

● **Breast Cancer** ● **Herceptin®**

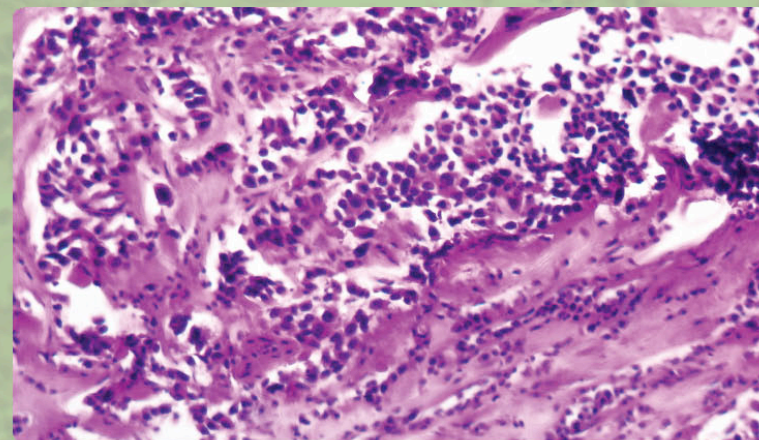
sion of the gene coding for a protein called HER2 (human epidermal growth factor receptor 2) with an aggressive form of breast cancer. Under normal circumstances HER2 transmits signals from outside the cell to the nucleus, directing cell growth. When cancer develops, cellular growth rates change and natural checks and balances controlling the cell cycle fail. The gene that encodes for the protein HER2 is called an oncogene, a gene having the potential to cause a normal cell to become cancerous. While it had been postulated for many years that oncogenes might be associated with growth factors, it was not until the early 1980s that Genentech scientists first proved that this hypothesis was correct when they found and studied an oncogene that was a mutated form of the epidermal growth factor (EGF) cell surface receptor gene. These researchers quickly broadened their investigation and began to search for other oncogenes related to the EGF-receptor gene. They named the first one they found HER2, and, using cloning technology, they were subsequently able to identify the protein for which HER2 coded. It was from a collaboration with the Genentech specialists that Dennis Slamon found high levels of HER2 oncogene expression in tumor samples taken from approximately 25 % of patients with metastatic breast cancer. Slamon found that the HER2 oncogene caused breast cancer cells to produce abnormally high concentrations

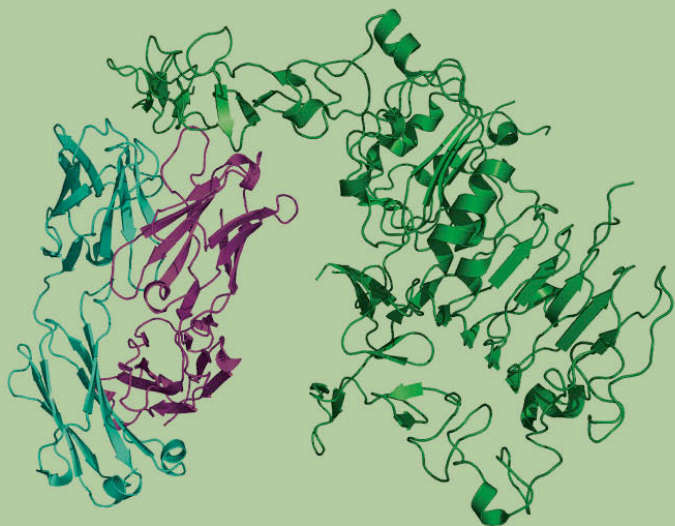


George B. Rathmann

Dennis Slamon

Breast cancer cells





Crystal structure of the human HER2 receptor (green) complexed with the trastuzumab (Herceptin®) antigen-binding fragment (magenta/cyan)



Herceptin®

of the HER2 protein and that in patients suffering from this overproduction the disease was particularly aggressive. Furthermore, women with HER2-positive metastatic breast cancer had a greater likelihood of recurrence, a poorer prognosis, and decreased survival rates in comparison to women with HER2-negative cancer.

To exploit the therapeutic potential of this knowledge, the Genentech team added copies of the HER2 oncogene into a normal cell line, thereby turning it into a cancerous one. The scientists then developed a monoclonal antibody that bound to the extracellular component of the HER2 cell surface receptor protein. Binding of the antibody to the HER2 receptor induces cell cycle arrest, reducing the proliferation of the tumor cells. Mice with tumors that overexpressed the HER2 oncogene were treated with this monoclonal antibody and as a result their tumors shrunk. After a great deal of further refinement, which included making the antibody compatible with the human immune system, Herceptin® (trastuzumab) was born in 1990. Following eight years of clinical trials (and that on a fast track approval), Herceptin® was approved by the FDA for the treatment of patients with HER2 protein overexpressing breast cancer in September 1998.

Herceptin® is generally used in combination with Taxol® (see Chapter 25) in order to attack the cancer on more than one front, thus giving

patients increased chances of recovery. Herceptin® is thought to possess three separate and distinct types of activity: it blocks tumor cell growth, labels the cancer cells for destruction so the body's immune system becomes involved in fighting the cancer, and works synergistically with chemotherapy to destroy HER2-positive cancer cells. The worldwide distribution and marketing of Herceptin® was aided by a deal signed between Genentech and Hoffmann-La Roche in 1998.

Despite its success in treating breast cancer, it has emerged that the use of Herceptin® can result in the development of cardiac dysfunction in a minority of patients; as a result, those treated with Herceptin® have to be carefully monitored. In some countries, notably the UK, Herceptin® has provoked controversy for an entirely different reason. Herceptin® is considerably more expensive than many traditional chemotherapeutic agents and there is ongoing debate as to whether the drug is an affordable option. In 2006, after an acrimonious legal battle in the highest courts of the country, campaigners won the right for all British women suffering from the early stages of HER2-positive metastatic breast cancer to receive Herceptin®. This ruling went against the judgment of the National Institute for Health and Clinical Excellence, the regulatory body charged with appraising which medicines and treatments should be available on the National Health Service (NHS). Critics of this ruling contend that the more liberal use of Herceptin® will inevitably lead to deleterious cutbacks elsewhere in the cancer treatment budget of the NHS. Nevertheless, Herceptin® represents a new direction in targeted therapy using monoclonal antibodies, and, perhaps, one of the first steps on the road to truly personalized medicine.

- **Rheumatoid Arthritis**
- **Enbrel®**



Enbrel® (etanercept)

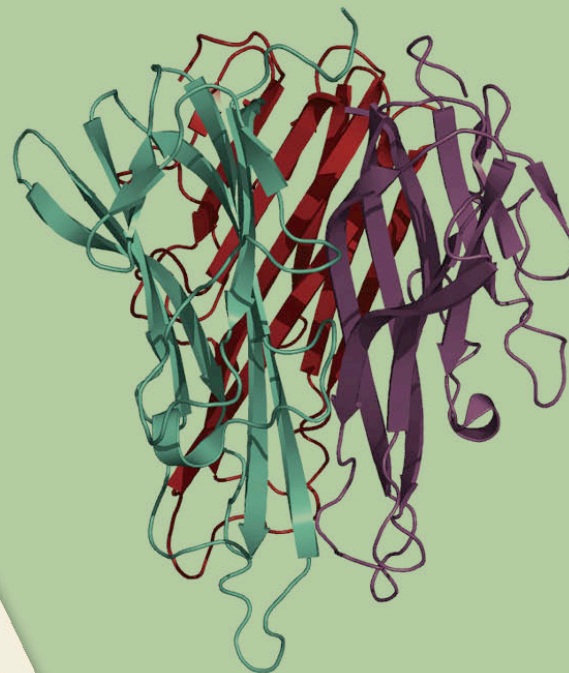
Rheumatoid arthritis is a disease in which the body's immune system turns upon itself and attacks apparently healthy tissue, leaving the sufferer physically debilitated and living in constant pain. The exact cause of arthritis is unclear; environment, genetic traits, hormonal factors, and pathogens (viruses and bacteria) have all been implicated. Rheumatoid arthritis results in the joints becoming inflamed and, ultimately, seriously and irrevocably damaged. Damage occurs when the immune system is activated, prompting mediatory cells to migrate to the joints where they initiate an inflammation cascade (see Chapter 15, Prostaglandins & Leukotrienes). The inflammation irritates the locality, thus perpetuating the cycle of trigger followed by exaggerated immune response. Long-term prospects for the patient are grim. Cartilage begins to wear down and swelling of the joint lining (the synovium) means that the joint becomes increasingly damaged and painful. Rheumatoid arthritis affects two million Americans and, contrary to popular belief, can strike at any time in one's life. More women than men suffer from this condition and women are less likely to go into remission with treatment than their male counterparts. Many different approaches exist for the treatment of rheumatoid arthritis. Some drugs target the pain and inflammation directly [e.g. nonsteroidal anti-inflammatory drugs (NSAIDs), topical pain relievers, corticosteroids, and narcotic pain relievers], whilst others attempt to modify the disease progression completely. This latter class of drugs works by disrupting the immune response, and notable recent additions to this category are biologics.

Researchers at the biotechnology company Immunex developed etanercept

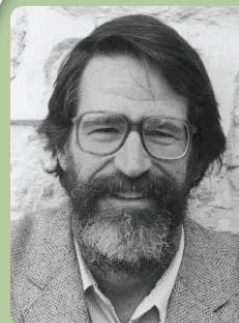
as a recombinant protein for the treatment of inflammatory diseases. In 1998, the FDA approved etanercept for treatment of the symptoms of rheumatoid arthritis in patients whose disease had failed to respond to prior medication, and the drug was introduced to the market in 1999 under the trade name Enbrel®. Such was the immediate impact and success of Enbrel® that Immunex could not manufacture enough of the drug to keep up with demand. At the same time, the failure of other drug candidates in clinical trials had left the company in a perilous financial position. Seizing this opportunity, Amgen acquired Immunex, and the rights to Enbrel®, in a takeover bid in 2002. Following the merger, Amgen was able to bring additional manufacturing facilities on line, quickly clearing the 40,000-plus waiting list for the drug. Amgen now co-markets Enbrel® in a licensing agreement with Wyeth.

Enbrel® is a so-called dimeric fusion protein in which active domains of two human proteins were fused to generate a new protein that is 934 amino acids long and can be manufactured using recombinant DNA techniques. One protein is the extracellular ligand-binding portion of the receptor for tumor necrosis factor alpha (TNF α). The second protein is a human immunoglobulin (IgG1), from which a portion called the Fc domain has been incorporated into Enbrel®. The net effect is an artificially engineered antibody. Enbrel® works by binding to TNF α , thereby blocking its normal interaction with cell surface TNF α .

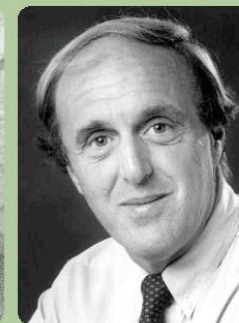
● **Humira®**
● **Remicade®**
Phage display
● **Kineret®**
● **Orencia®**



Above: X-Ray crystallographic structure of the TNF α trimer, the binding target of etanercept (Enbrel®) and related biologic therapeutics



George P. Smith



Richard A. Lerner



Sir Gregory Winter



Drugs that relieve pain

Further Reading

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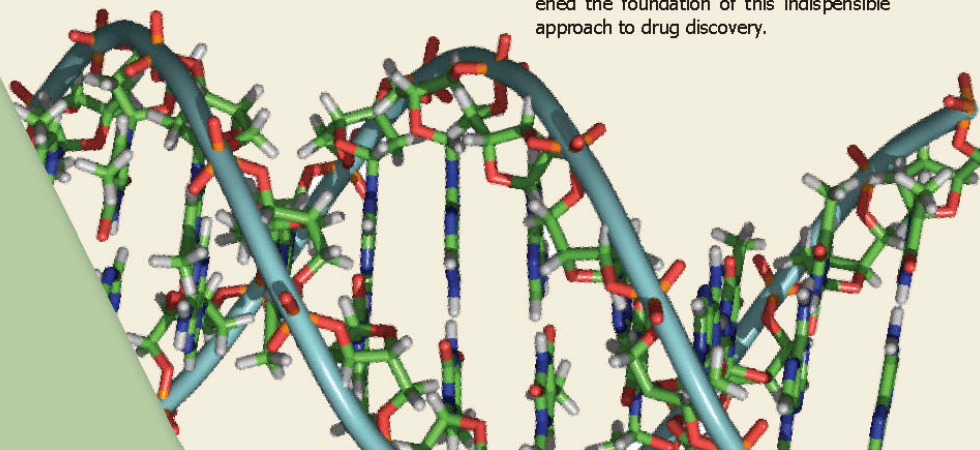
receptors. $\text{TNF}\alpha$ is a cytokine, a chemical intimately involved in the normal inflammatory and immune responses. Elevated levels of $\text{TNF}\alpha$ are found in the tissues of patients suffering from rheumatoid arthritis. By binding to and deactivating $\text{TNF}\alpha$, Enbrel® disrupts the biochemical cascade leading to inflammation, thus alleviating the symptoms of rheumatoid arthritis.

Since its launch, Enbrel® has brought some much needed relief to hundreds of thousands of rheumatoid arthritis patients worldwide. Its indications have been broadened so that it is now regularly used to treat juvenile arthritis, psoriatic arthritis, ankylosing spondylitis, and severe plaque psoriasis. A number of other biologics for the treatment of arthritis are also currently on the market. These products include Humira® (adalimumab, Abbott Laboratories) and Remicade® (infliximab, Centocor Pharmaceuticals), both of which also block $\text{TNF}\alpha$ activity, Kineret® (anakinra, Amgen), which blocks the cytokine interleukin-1, and Orencia® (abatacept, Bristol-Myers Squibb), which blocks activation of T-cells. Despite the fact that patients suffering from rheumatoid arthritis speak of these drugs in

glowing terms, they are not without their disadvantages. Proteins are not stable to many conditions including stomach acid, therefore these drugs need to be refrigerated and injected subcutaneously. Furthermore, some patients taking these biologics suffer from depression of their immune response, making them more susceptible to infections.

Phage display, a technique for generating large libraries of antibodies, has been pivotal in the discovery of some of these therapeutic antibodies. George P. Smith (University of Missouri, USA), Richard A. Lerner (The Scripps Research Institute, La Jolla, USA), and Sir Gregory Winter (University of Cambridge, UK) contributed to the discovery and development of this powerful method.

We hope that on reading this survey of a few milestones in this area you will be left feeling some of the same excitement and hope that is driving scientists and biotechnology companies to invest ever more time and money in inventing new biologic drugs. Biologics are certain to have a bright future, but it is fair to say that future success in treating diseases will come not from finding one single solution to all problems, but from our ability to innovate and continually build a strong cross-disciplinary approach to research, one in which both chemistry and biology play equally important roles. The human genome project has certainly demonstrated and strengthened the foundation of this indispensable approach to drug discovery.



Epilogue

The stories in this book have been about molecules that changed the world, their chemistry, biology and medicine, and the protagonists that discovered, synthesized and studied them. Because of our background and expertise, this book was written from a perspective amplifying the contributions of chemistry, especially chemical synthesis. We are sure that biologists and medical doctors or even other chemists would have different angles from which to approach the subject. To be sure, however, the fascination and impact of these molecules, and others like them, on society and the excitement of the science behind them remain undisputed.

We hoped that we have conveyed from our perspective the historical development of the art and science of chemical synthesis, its awesome power to create new materials for all imaginable and unimaginable applications for the benefit of humankind, and its importance in shaping the world as we know it today. Due to space and time limitations we focused primarily on medicine and certain other aspects where fine chemicals are playing a role in our lives such as dyes, perfumes, vitamins and agrochemicals. But molecules and chemistry offer so much more. Their central role within the sciences and our world make them enabling, indispensable, and rewarding beyond the boundaries of imagination.

Thus, it was the advent of chemistry and chemical synthesis that led not only to the magic of modern medicine, but also to the refinement of petroleum and the production of gasoline and other energy sources as well as fine chemicals that

shaped the world in the twentieth century. Polymers and plastics for all purposes are also products of technologies based on the fundamentals of chemistry and chemical synthesis as are so many other high tech materials from which emerged all kinds of useful tools and devices such as liquid crystal displays, computer chips, engines, instruments of communication, and transportation vehicles. And it is chemistry again that will provide solutions to our future energy and material needs beyond petroleum.

This rich harvest speaks volumes for the future especially if we assume, as it has been said, that the twenty-first century belongs to chemistry and biology. Indeed these sciences stand at a threshold of major developments from which will flow untold, even more revolutionary, benefits to society. Cures for cancer, Alzheimer's disease and viral infections are but a few of such expectations. New energy sources and novel materials for medical devices and other special needs are more good things to come. The appeal of such eventualities and the excitement of being part of such scientific, technological, and medical breakthroughs provide enormous incentives and attractions to young men and women who will surely find the challenges ahead both stimulating and rewarding. We would like to think that, through the pages of this book, we have provided the inspiration for them to choose such careers and the encouragement to pursue them with vigor and enthusiasm fit for a journey full of discoveries and wisdom such as described in the following passage from the Greek poet C.V. Cavafy, that we like to leave you with.

Ithaca

*When you start on your journey to Ithaca,
Then pray that the road is long,
Full of adventure, full of knowledge,
Do not fear the Lestrygonians
And the Cyclopes and the angry Poseidon.
You will never meet such as these on your path,
If your thoughts remain lofty, if a fine
Emotion touches your body and your spirit.
You will never meet the Lestrygonians,
The Cyclopes and the fierce Poseidon,
If you do not carry them within your soul,
If your soul does not raise them up before you.
Then pray the road is long.
That the summer mornings are many,
That you will enter ports seen for the first time
With such pleasure, with such joy!
Stop at Phoenician markets,
And purchase fine merchandise,
Mother-of-pearl and corals, amber and ebony,
And pleasurable perfumes of all kinds,
Buy as many pleasurable perfumes as you can;
Visit hosts of Egyptian cities,
To learn and learn from those who have knowledge.
Always keep Ithaca fixed in your mind.
To arrive there is your ultimate goal.
But do not hurry the voyage at all.
It is better to let it last for long years;
And even to anchor at the isle when you are old,
Rich withal that you have gained on the way,
Not expecting that Ithaca will offer you riches.
Ithaca has given you the beautiful voyage.
Without her you would never have taken the road.
But she has nothing more to give you.
And if you find her poor, Ithaca has not defrauded you.
With the Great Wisdom you have gained, with so much experience,
You must surely have understood by then what Ithacas mean.*

—C. P. Cavafy

MOLECULES THAT CHANGED THE WORLD

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AND ITS IMPACT ON SOCIETY

Both the lay person and the dedicated student alike will take pleasure in reading fascinating stories about legendary molecules such as those of Aspirin®, penicillin and Taxol®, and will discover that learning about chemistry, biology, and medicine, the molecules of nature, and the history and practice of chemical synthesis can be both intriguing and inspirational. The specialist and teacher will find the scientific content of the text enriching and highly rewarding. Teachers can choose to cover the aspects of the text appropriate for their target audience, whether it is at the high school, undergraduate or graduate level. Readers with no formal background in science can be introduced to the impact of chemistry on society in the broader sense, while synthetic chemists will be reminded of just how far their science has evolved and take joy in reminiscing about the bountiful harvest of their labors.



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THE ART OF TOTAL SYNTHESIS



“But a deeper and more careful inquiry reveals to us that absolutely new ideas are of the very rarest occurrence; almost the whole work of human genius consists in assimilating what others have thought, in combining what others have imagined separate, in recasting the form of their thought, and so producing what seems a perfectly new thing, and yet is only the old under a new aspect. No instance of this is more signal than that of a great composer in music (in total synthesis). The gift of original melody (synthesis), as it is called is rare and precious. The possessor of it is justly considered a genius. But no melody could possibly speak to us except a combination of perfectly well known elements. The only originality is in their assimilation and reproduction.”

J. P. Mahaffy, 1910

J. P. Mahaffy, *What Have the Greeks Done for Modern Civilisation*, G. P. Putnam's Sons, New York, 1910, pp. 214–215, 246–247. *see Alfred, Lord Tennyson, “Locksley Hall” 1835.



THE ART OF TOTAL SYNTHESIS



“...the theory of composing words (molecules) is easy enough, and yet the art a mystery, which only very few can ever attain in perfection,—each, too, after his own fashion, and stamped with his own genius,—so the theory of conversation (total synthesis) may be reduced to a small number of general observations, and yet the perfect practice of it is a mystery, which defies analysis—one of the myriad manifestations of human genius which all can admire but no one can ever explain.”

J. P. Mahaffy, 1887



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