Pure & Appl. Chem., Vol. 68, No. 11, pp. 2129–2136, 1996.
Printed in Great Britain.

Chemistry and biology of selected natural products

K. C. Nicolaou,* E. A. Theodorakis, C. F. Claiborne

Department of Chemistry, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, California 92037, and Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093

Abstract: Natural products often offer excitement, stimulation, challenges and opportunities for chemists, biologists and medical investigators. The study of their chemistry, biology and medicine provides, more often than not, rewards imagined and unimagined, and is still a major frontier in organic chemistry. In this article we summarize some of our recent work in this area and project ahead to the future of the field.

Introduction

Man's fascination with natural products goes back to ancient times (1). With the discovery of salicin from willow tree extracts and the development of aspirin in 1899, the art of exploiting natural products became a molecular science. The discovery of penicillin in 1928 and its subsequent development as a drug represents another milestone in the history of natural products, and marked the beginning of a new chapter in drug discovery, in which bacteria were added to the plant kingdom as sources for biologically active substances. Today, with marine organisms and other living creatures as additional sources of active compounds, the chemistry and biology of natural products represents a major path to drug discovery and development. Indeed a large portion of today's major drugs have their origins in nature. It is, therefore, not surprising that one of the most active and rewarding frontiers in modern chemistry is the study of the chemistry and biology of natural products.

In our laboratories the study of the chemistry and biology of natural products focuses on the following endeavors: (a) total synthesis; (b) molecular design of mimics or antagonists of the natural products; (c) chemical synthesis of the designed molecules; (d) molecular recognition experiments; (e) biological investigations; and (f) redesign and fine-tuning of molecular structure. The selection of the target molecules is of paramount importance and is based on criteria of novel architecture, important biological function and interesting mechanism of action. Thus, with the proper selection, one optimizes the opportunities for the discovery and development of new synthetic technology and strategies, and for useful contributions to chemistry, biology and medicine.

With this concept in mind, we highlight below a number of recent programs from our laboratories, which led to exciting developments within the area of chemistry and biology of natural products.

Calicheamicin γ_1^I

Calicheamicin γ_1^I (Fig. 1) belongs to a new class of extremely active antitumor agents, collectively known as enediynes (2). Isolated from soil bacteria, the enediynes are thought to exert their biological activities by generating highly reactive diradical species

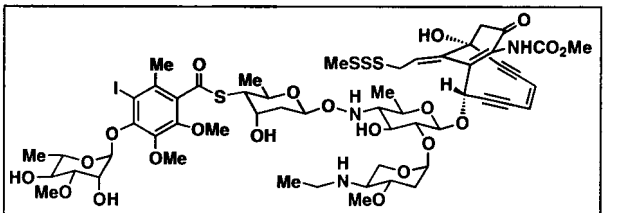

Fig. 1. Structure of calicheamicin γ_1^I

that are capable of damaging DNA (3). The molecular structure of calicheamicin γ_1^I can be seen as composed of three parts: (a) the 10-membered enediyne ring system which upon activation generates radicals via a Bergman cyclization (Fig. 2) (4); (b) the oligosaccharide domain which recognizes and binds to the minor groove of DNA, thereby serving as a delivery system; and (c) the trisulfide moiety which serves as the initiation point for the cascade of reactions leading ultimately to the diradical formation.

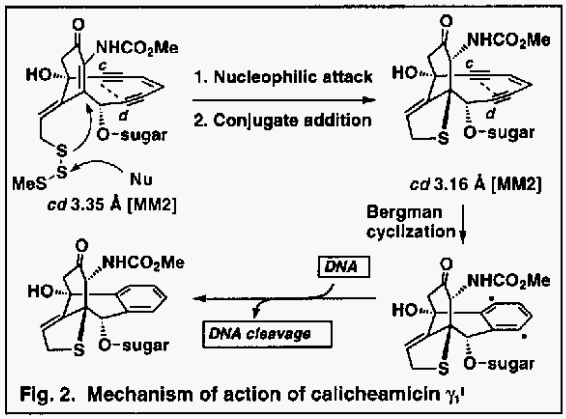

Fig. 2. Mechanism of action of calicheamicin γ_1^I

Due to its unusual and complex molecular structure, important biological activity and fascinating mode of action, calicheamicin γ_1^I became immediately an attractive and challenging target for total synthesis. The first total synthesis of calicheamicin γ_1^I was reported in 1992 from these laboratories (Fig. 3) (5,6). Our convergent strategy involved construction of the two key intermediates representing the oligosaccharide and enediyne parts of the molecule, followed by coupling and elaboration to the target compound (5).

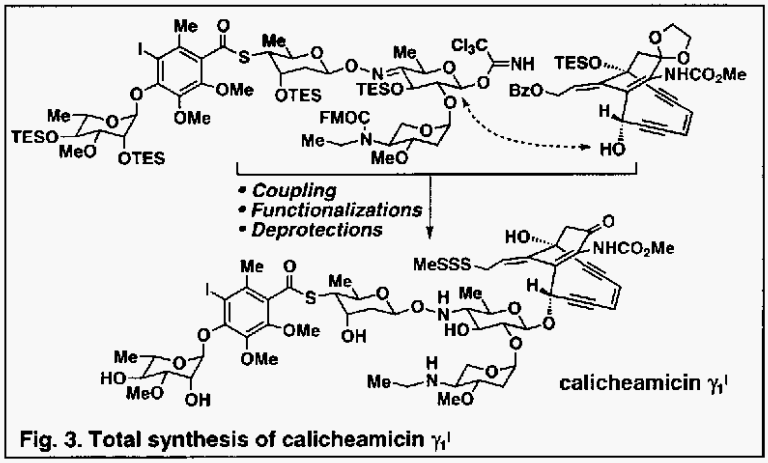

Fig. 3. Total synthesis of calicheamicin γ_1^I

Using the chemistry developed for the total synthesis, several designed molecules were synthesized (7). Among them, an analog termed calicheamicin θ_1^I (Fig. 4) was found to be extremely potent (in some cases up to 1000 times more potent than the natural compound) against tumor cells (8). The mechanism of action of this synthetic material, believed to be the most cytotoxic non-peptidic agent known, involves apoptosis initiated by double-strand DNA cuts (Fig. 4). Using similar strategy and principles, we designed and synthesized a series of potent compounds based on the structure and mechanism of action of dynemycin A, another member of the enediyne family (7). These designed molecules, together with the naturally occuring substances, serve as useful tools in biology and hold promise as new anticancer agents.

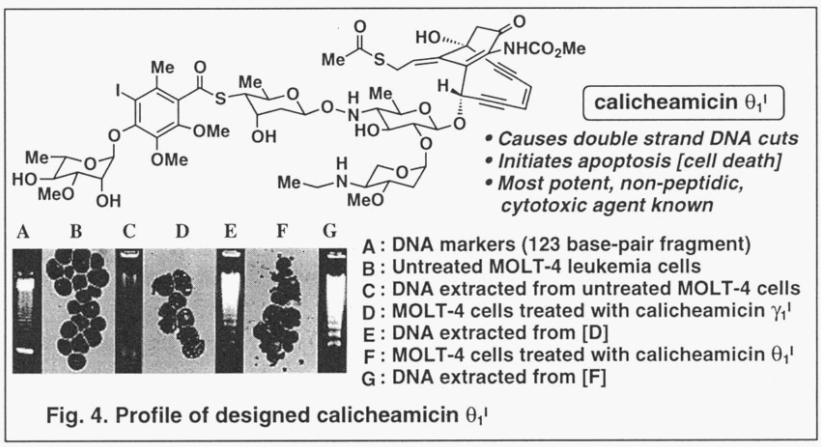

Fig. 4. Profile of designed calicheamicin θ_1^I

Rapamycin

One of the most significant advances of modern medicine is the advent of organ transplantation. This revolution in medicine was made possible with the use of immunosuppressive agents such as cyclosporin, FK506 and rapamycin (Fig. 5) (9). Although the story of this fascinating molecule commences with its isolation in the early 1970's (10), it did not attract serious attention from the scientific community until the 1980's, when the powerful immunosuppressive properties of cyclosporin and FK506 were discovered.

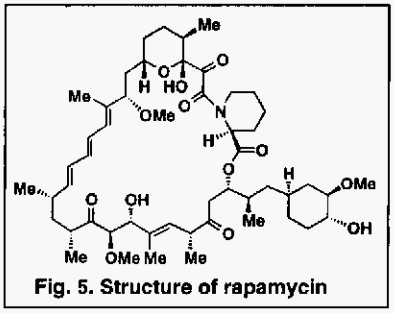

Fig. 5. Structure of rapamycin

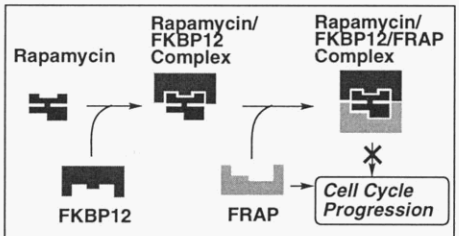

Fig. 6. Mechanism of action of rapamycin

Rapamycin's striking resemblance to the structure of FK506 moved it to the forefront of chemical and biomedical research as a challenging synthetic target, as a probe for immunological studies, and as a potential drug candidate in organ transplant operations.

Rapamycin suppresses the immune system (blocks T cell proliferation) by sequentially binding to two different proteins (11). It initially forms a complex with FKBP12 which then proceeds to bind FRAP (or TOR, or RAFT1), a protein essential for the proliferation of T cells, thus preventing it from carrying out its biological functions (Fig. 6).

The challenging structure and potential importance of rapamycin in medicine provided a strong impetus for the development of a total synthesis and offered ample opportunity for development of new synthetic technology and strategies. Among them is the double Stille coupling

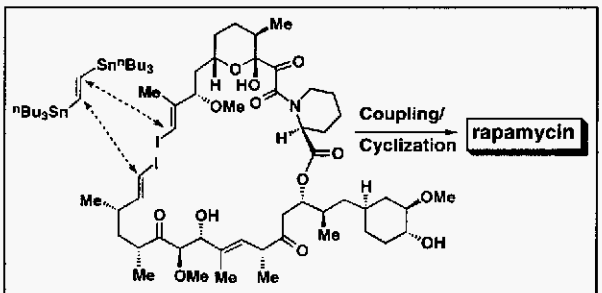

Fig. 7. Total synthesis of rapamycin

reaction ("stitching cyclization") featured in our total synthesis (12), which was used to construct simultaneously the conjugated triene and macrocyclic ring systems of the molecule (Fig. 7). Three other total syntheses of rapamycin have followed (12,13).

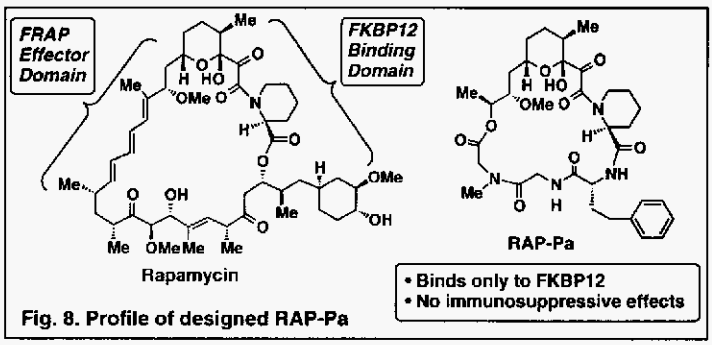

Fig. 8. Profile of designed RAP-Pa

In order to probe the structure-activity relationships and develop new biological tools for immunochemistry and potential agents for immunosuppression, a number of rapamycin analogs were designed and synthesized. Figure 8 shows one such molecule, (RAP-Pa), whose hybrid structure was based on the structures of rapamycin (FKBP12 binding domain) and cyclosporin (peptide domain) (14). This compound binds, as expected, only to FKBP12, and could serve as a powerful tool in studying the mode of action of these molecules.

Taxol™

The modern history of Taxol™ (Fig. 9) began in the early 1960's when A. Barclay, a botanist from the United States Department of Agriculture (USDA), collected samples of the Pacific Yew tree (*Taxus brevifolia*) from a forest in Washington State as part of a major initiative to search for natural sources

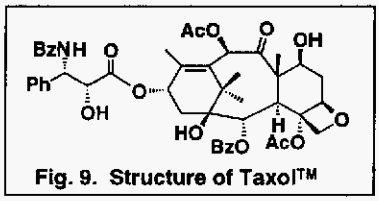

Fig. 9. Structure of Taxol™

of anticancer agents (15). His samples found their way, after initial biological screening, to the laboratories of M. C. Wani and M. Wall, two chemists who isolated and determined its cytotoxic properties and elucidated its chemical structure with the help of X-ray crystallographers P. Coggen and A. T. McPhail (16). Taxol's™ development was at first slow, until its unique mode of action was determined by S. B. Horwitz and her group (17). This group reported that Taxol™ binds to microtubules causing their stabilization and thus preventing cell mitosis. Since the microtubules are the main components of the mitotic spindle which must smoothly separate into two new mitotic spindles during mitosis, Taxol's™ binding inhibits this process and thus kills the cells at the G2 phase (prior to mitosis) (Fig. 10)(18).

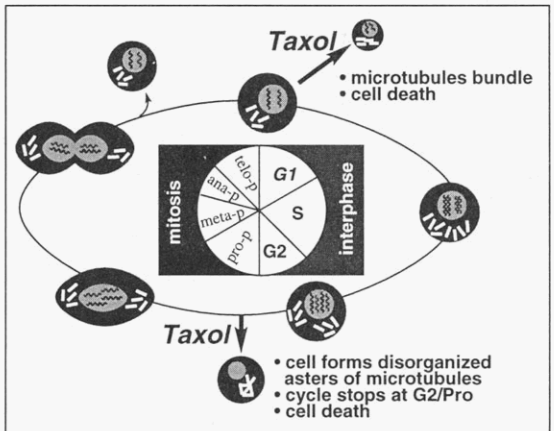

Fig. 10. Mechanism of action of Taxol™

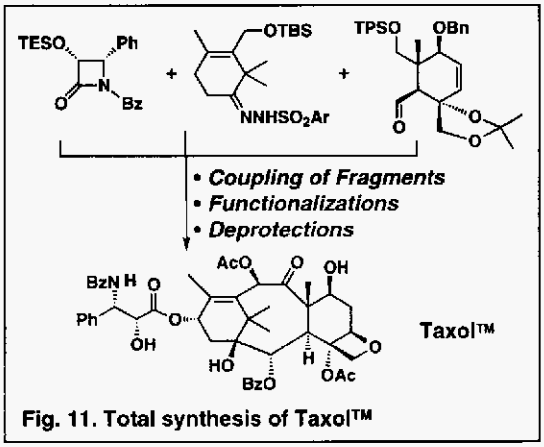

Fig. 11. Total synthesis of Taxol™

Alerted to the great promise of Taxol™ and its derivatives and the initially short natural supplies of the drug, we launched a program directed towards its total synthesis. The synthetic strategy developed in these laboratories relied on a convergent approach, whereby two fragments representing rings A and C were constructed, each *via* a Diels-Alder reaction (19). These fragments were then joined together through a Shapiro reaction and the elaborated product was cyclized using a McMurry coupling reaction to produce, after appropriate functionalizations, attachment of the side chain, and deprotections, Taxol™ in its naturally occurring form (Fig. 11)(19,20).

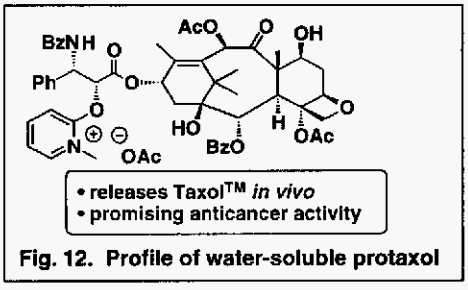

Fig. 12. Profile of water-soluble protaxol

Among the many taxoid analogs that were synthesized in our laboratories, a water soluble protaxol with a 2-methylpyridinium group at the 2' position (2'-MPA, Fig. 12) proved to be a highly promising compound as a potentially improved form of Taxol™ (21). This latter compound is currently under investigation as a new anticancer agent.

Balanol

The recent isolation and structural elucidation of balanol (Fig. 13) (22), represents a significant advance in the quest for effective inhibitors of protein kinase C (PKC). PKC mediated signal transduction is known to lead to a variety of cellular responses, including gene expression and cell proliferation (23), and activated PKC (when bound to ATP) has been implicated in diverse diseases such as cancer, cardiovascular disorders, asthma, inflammation, diabetes, and HIV infection (24).

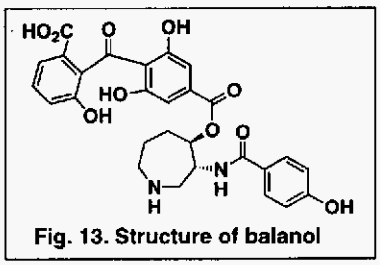

Fig. 13. Structure of balanol

The biological mode of action of balanol is thought to involve competitive binding against ATP at the catalytic domain of PKC, thus inhibiting protein phosphorylation and signal propagation

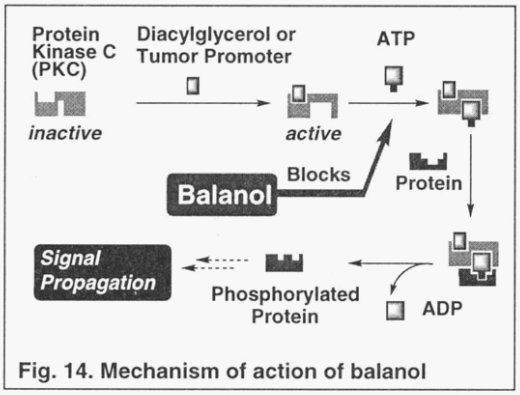

Fig. 14. Mechanism of action of balanol

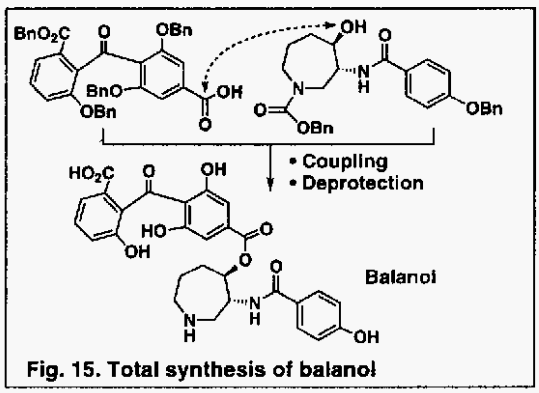

Fig. 15. Total synthesis of balanol

(Fig. 14) (25). Consequently, the identification of potent and selective PKC inhibitors may not only serve to further illuminate the mechanism of signal transduction, but may also result in the development of novel drugs with considerable therapeutic value.

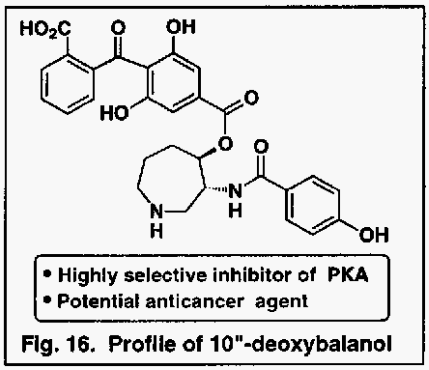

Fig. 16. Profile of 10"-deoxybalanol

Due to the biomedical importance of this molecule, we initiated a program directed towards the synthesis not only of the natural balanol, but also of several designed analogs. Our strategy (26), involved coupling of the two components shown in Fig. 15, which after functional group deprotection delivered balanol in enantiomerically pure form (26,27). A combination of molecular design, total synthesis and biological evaluation of designed balanoids, led to the development of the 10"-deoxybalanol (Fig. 16) which was found to be a very specific PKA inhibitor (28). Such molecules serve as useful tools for elucidating signal transduction pathways and they may also hold considerable promise as potential anticancer agents.

Zaragozic Acid A (Squalestatin S1)

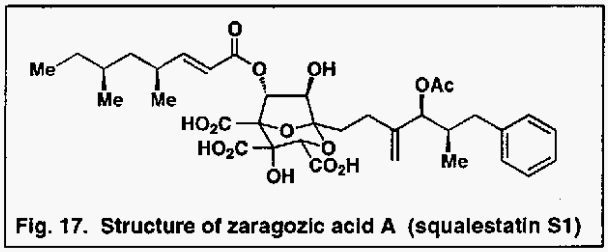
Fig. 17. Structure of zaragozic acid A (squalestatin S1)

The fascinating story of zaragozic acid A (squalestatin S1) (Fig. 17) commenced in 1992 with its isolation and characterization independently by two different companies: Merck (29) and Glaxo (30). The chemistry and biology of zaragozic acid A (squalestatin S1) is associated with cholesterol lowering and coronary heart disease (CHD). Approximately half of the human body's cholesterol requirements are satisfied by endogenous biosynthesis and considerable effort has been devoted in recent years to control this source of cholesterol by blocking specific steps in the cholesterol biosynthetic pathway (31). The first cholesterol-committed step in this pathway is the dimerization of farnesyl pyrophosphate to presqualene pyrophosphate. Zaragozic acid A interferes at this point by inhibiting the action of squalene synthase, the enzyme that is responsible for this transformation (Fig. 18). In addition, this remarkable compound exhibits potent antifungal activity and is an inhibitor of *ras*-farnesyl transferase, which is implicated in carcinogenesis.

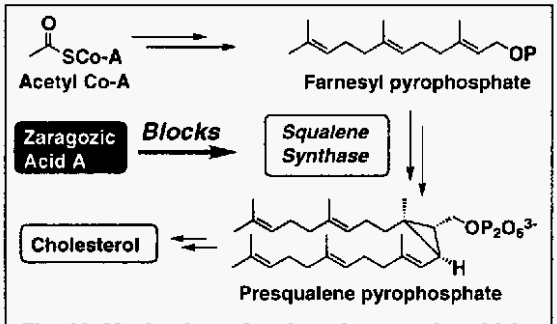

Fig. 18. Mechanism of action of zaragozic acid A

Zaragozic acid A contains a highly oxygenated and unique bicyclic core with three carboxylic acid groups extending from it. Its total

synthesis presented an unusual challenge and provided an opportunity to develop new synthetic strategies (32, 33). Our convergent synthetic approach began with achiral precursors and involved the construction, coupling and elaboration of the three fragments shown in Fig. 19. Instrumental to the success of this synthesis were a Sharpless asymmetric dihydroxylation reaction and a novel acid-catalyzed rearrangement of a spiro[3.9]bicyclo[4.3.0]nonane system to the [3.2.1] bicyclic skeleton of zaragozic acid A (32).

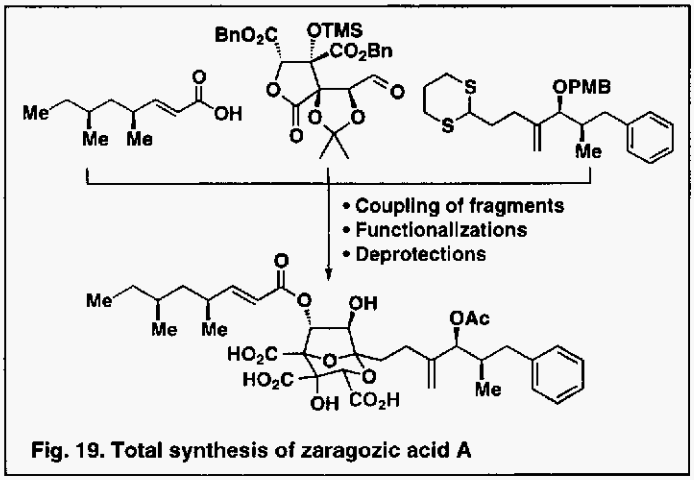

Fig. 19. Total synthesis of zaragozic acid A

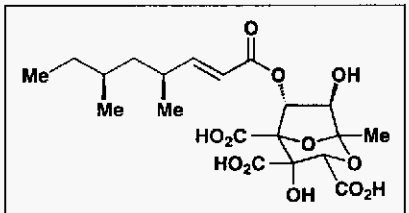

Fig. 20. Designed zaragozic acid analog

A number of designed zaragozic acid analogs have also been synthesized and are currently under biological investigation, while others are under construction (Fig. 20) (34). It should be mentioned at this juncture that, in view of toxicity effects, the naturally occurring compound (like many other lead compounds) may not reach the clinic and therefore synthetic or semisynthetic mimics assume high priority as potential drug candidates in this area. Such molecules may also play important roles as biological tools.

Brevetoxin B

The brevetoxins are a family of marine neurotoxins associated with the "red tide" occurences (35). These phenomena occur periodically around the world and are responsible for catastrophic killings of fish and other marine life as well as human poisoning, through contaminated food consumption.

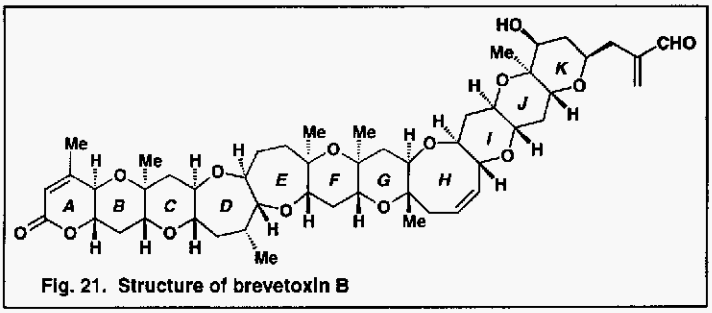

Fig. 21. Structure of brevetoxin B

Within this family, brevetoxin B (Fig. 21), occupies a prominent position, as it was the first member to be discovered. Its highly complex structure, reported in 1981 (36), represented at the time an unprecedented molecular architecture, consisting of a series of eleven *trans*-fused ether rings.

The biological mode of action of brevetoxin B involves binding and activation of sodium channels (Fig. 22) (37). By virtue of its length (30 Å) and hydrophobic properties, this molecule is able to enter the cell membrane and bind to the sodium channels, thus changing their conformation. This allows an influx of sodium ions, resulting in several unpleasant neurological and physiological effects and even death by asphyxiation (38).

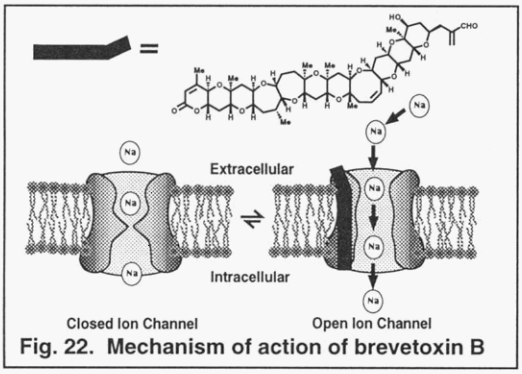

Fig. 22. Mechanism of action of brevetoxin B

The unique structure of brevetoxin B together with its interesting biological activity and harmful environmental consequences, prompted us to initiate a program directed towards its total synthesis.

Towards the achievement of this goal, we had to

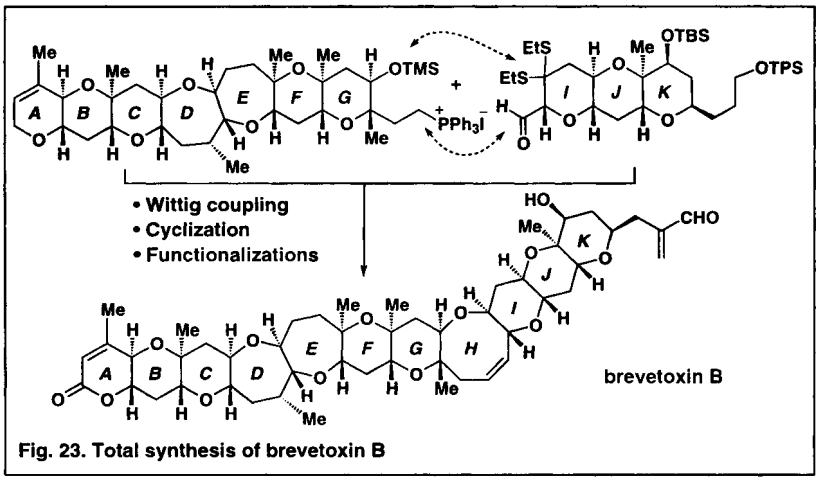

Fig. 23. Total synthesis of brevetoxin B

develop a number of new synthetic reactions and methods for the construction of cyclic ethers, thereby expanding the collection of tools for organic synthesis. In our successful final strategy, two large segments representing rings ABCDEFG and IJK were synthesized and coupled together, to produce, after cycli-zation and final functionalizations, the natural product in its proper enantiomeric form (Fig. 23) (39).

The developed methodology allowed the synthesis of designed analogs of brevetoxin B. A shorter analog, named truncated brevetoxin B [AFGHIJK] (Fig. 24), was synthesized (40) in order to test the hypothesis put forward by Baden and Gawley regarding the relationship between the required length of the molecule and the binding to its receptor (37). The biological tests with the synthetic molecule supported this hypothesis by not exhibiting significant binding to the sodium channel (41). Continued studies in this area are expected to advance our knowledge of the structure and function of ion channels and to make significant contributions to neurobiology in general.

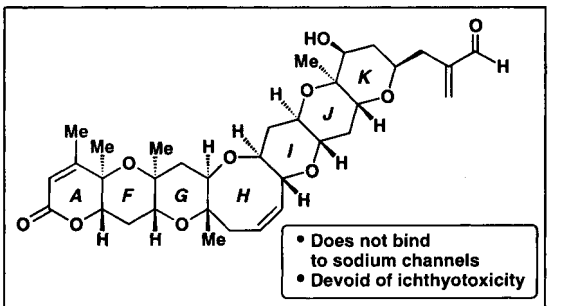

Fig. 24. Profile of truncated brevetoxin B [AFGHIJK]

Conclusion

In this article, a number of projects in the authors laboratories involving studies in the chemistry and biology of natural and designed molecules have been briefly discussed. In all these projects, synthetic chemistry has played a major role, not only in synthesizing the natural compounds, but also in rendering available synthetic molecules whose biological properties were investigated. While a number of these molecules are assisting investigations to probe biological issues, others became drug candidates. The future of the science that blends chemistry and biology of natural products looks, indeed, very exciting. With the increasing power of chemical synthesis, the prospects of discovery and invention of new chemical entities and new phenomena appear greater than ever. Chemists are destined to play a major role in this rapidly moving frontier. They should not, however, forget that ground-breaking organic synthesis should also be practiced for its own sake, for new advances in the latter field are still needed.

Acknowlegdment. We would like to express our gratitude and appreciation to all our co-workers, whose names appear in the references, for their contributions to the research described in this article. Our programs have been financially supported by the National Institutes of Health, Merck, Sharp and Dohme, Glaxo, Inc., Schering Plough, Pfizer, Hoffman La Roche, and the ALSAM Foundation.

References

1. J. Mann, *Murder, Magic and Medicine*, Oxford University Press, 1994.
2. K. C. Nicolaou and W.-M. Dai, *Angew. Chem. Int. Ed. Engl.* **30**, 1387 (1991).
3. M. D. Lee, et al, *J. Am. Chem. Soc.* **109**, 3464 (1987). M. D. Lee, et al, *J. Am. Chem. Soc.* **109**, 3466 (1987).

4. R. R. Jones and R. G. Bergmann, *J. Am. Chem. Soc.* **94**, 660 (1972). T. P. Lockhart and R. G. Bergman, *J. Am. Chem. Soc.* **103**, 4091 (1981).
5. K. C. Nicolaou, C. W. Hummel, E. N. Pitsinos, M. Nakada, A. L. Smith, K. Shibayama and H. Saimoto, *J. Am. Chem. Soc.* **114**, 10082 (1992).
6. For another synthesis see: S. A. Hitchcock, S. H. Boyer, M. Y. Chu-Moyer, S. H. Olson and S. J. Danishefsky, *Angew. Chem. Int. Ed. Engl.* **33**, 858 (1994).
7. K. C. Nicolaou, W.-M. Dai, S.-C. Tsay, V. A. Estevez and W. Wrasidlo, *Science* **256**, 1172 (1992).
8. K. C. Nicolaou, T. Li, M. Nakada, C. W. Hummel, A. Hiatt and W. Wrasidlo, *Angew. Chem. Int. Ed. Engl.* **33**, 183 (1994).
9. M. K. Rosen and S. L. Schreiber, *Angew. Chem. Int. Ed. Engl.* **31**, 384 (1992).
10. D. C. N. Swindells, P. S. White and F. A. Findlay, *Can. J. Chem.* **56**, 2491 (1978). J. A. Findlay and L. Radics, *Can. J. Chem.* **58**, 579 (1980).
11. S. H. Snyder and D. M. Sabatini, *Nature Medicine* **1**, 32 (1995).
12. K. C. Nicolaou, T. K. Chakraborty, A. D. Piscopio, N. Minowa and P. Bertinato, *J. Am. Chem. Soc.* **115**, 4419 (1993).
13. For other syntheses see: D. Romo, S. D. Meyer, D. D. Johnson and S. L. Schreiber, *J. Am. Chem. Soc.* **115**, 7906 (1993). C. M. Hayward, D. Yohannes and S. J. Danishefsky, *J. Am. Chem. Soc.* **115**, 9345 (1993). A. B. Smith III, S. M. Condon, J. A. McCauley, J. L. Leaser Jr., J. W. Leahy and R. E. Maleczka Jr, *J. Am. Chem. Soc.* **117**, 5407 (1995).
14. T. K. Chakraborty, H. P. Weber and K. C. Nicolaou, *Chemistry & Biology* **2**, 157 (1995).
15. T. Junod, *Life* **15**, 71 (1992).
16. M. C. Wani, H. L. Taylor, M. E. Wall, P. Coggen and A. T. McPhail, *J. Am. Chem. Soc.* **93**, 2325 (1971).
17. S. B. Horwitz, J. Fant and P. B. Schiff, *Nature* **277**, 665 (1979). E. Nogales, S. G. Wolf, I. A. Khan, R. F. Luduena, et al. *Nature*, **375**, 424 (1995).
18. K. C. Nicolaou, W.-M. Dai and R. K. Guy, *Angew. Chem. Int. Ed. Engl.* **33**, 15 (1994).
19. K. C. Nicolaou, Z. Yang, J. J. Liu, H. Ueno, P. G. Nantermet, R. K. Guy, C. F. Claiborne, J. B. Renaud, E. A. Couladouros, K. Paulvannan and E. J. Sorensen, *Nature* **367**, 630 (1994).
20. For another synthesis see: R. A. Holton, et al, *J. Am. Chem. Soc.* **116**, 1597 (1994). R. A. Holton, et al, *J. Am. Chem. Soc.* **116**, 1599 (1994).
21. K. C. Nicolaou, R. K. Guy, E. N. Pitsinos and W. Wrasidlo, *Angew. Chem. Int. Ed. Engl.* **33**, 1583 (1994).
22. P. Kulanthaivel, et al, *J. Am. Chem. Soc.* **115**, 6452 (1993).
23. Y. Nishizuka, *Science* **258**, 607 (1992).
24. D. Bradshaw, C. H. Hill, J. S. Nixon and S. E. Wilkinson, *Agents Actions* **38**, 135 (1993).
25. A. Ohshima, M. Yanagisawa, A. Katoh, T. Fujii, T. Sano, S. Matsukuma, T. Furumai, M. Fujiu, K. Watanabe, K. Yokose, M. Arisawa, T. Okuda, *J. Antibiot.* **47**, 639 (1994).
26. K. C. Nicolaou, M. E. Bunnage, K. Koide, *J. Am. Chem. Soc.* **116**, 8402 (1994).
27. For other syntheses, see: J. W. Lampe, P. F. Hughes, C. K. Biggers, S. H. Smith, H. Hu, *J. Org. Chem.* **59**, 5147 (1994). N. Vicker, et al., *J. Chem. Soc. Perkin I* in press..
28. K. Koide, M. E. Bunnage, L. G. Paloma, J. R. Kanter, S. S. Taylor, L. L. Brunton and K. C. Nicolaou, submitted.
29. J. D. Bergstrom, et al., *Proc. Natl. Ac. Sci. U.S.A.*, **90**, 80 (1993).
30. P. J. Sidebottom, et al., *J. Antibiot.* **45**, 648 (1992).
31. I. Abe, J. C. Tomesh, S. Wattanasin and G. D. Prestwich, *Nat. Prod. Rep.* **11**, 279 (1994).
32. K. C. Nicolaou, A. Nadin J. E. Leresche, S. La Greca, T. Tsuri, E. W. Yue and Z. Yang, *Angew. Chem. Int. Ed. Engl.* **33**, 2187 (1994). K. C. Nicolaou, A. Nadin, J. E. Leresche, E. W. Yue and S. La Greca, *Angew. Chem. Int. Ed. Engl.* **33**, 2190 (1994).
33. For other syntheses see: E. M. Carreira and J. DuBois, *J. Am. Chem. Soc.* **116**, 10825 (1994). D. A. Evans, J. C. Barrow, J. L. Leighton, A. J. Robichaud and M. J. Sefkow, *J. Am. Chem. Soc.* **116**, 12111 (1994).
34. K. C. Nicolaou, E. W. Yue, S. La Greca, A. Nadin, Z. Yang, J. E. Leresche, T. Tsuri, Y. Naniwa and F. DeRiccardis, *Chem. Eur. J.* **1**, 0000 (1995).
35. D.M. Anderson, *Scientific American*, **271**, 62 (1994).
36. Y.-Y. Lin, M. Risk, S. M. Ray, D. Van Engen, J. Clardy, J. Golik, J. C. James and K. Nakanishi, *J. Am. Chem. Soc.* **103**, 6773 (1981).
37. K. S. Rein, D. G. Baden and R. E. Gawley, *J. Org. Chem.* **59**, 2101 (1994). K. S. Rein, B. Lynn, R. E. Gawley, and D. G. Baden, *J. Org. Chem.* **59**, 2107 (1994).
38. *Toxic Dinoflagellates*, D. M. Anderson, A. W. White and D. G. Baden, Eds.; Elsevier; New York, 1985.
39. K.C. Nicolaou, E.A. Theodorakis, F.P.J.T. Rutjes, J. Tiebes, M. Sato, E. Untersteller and X.-Y. Xiao, *J. Am. Chem. Soc.* **117**, 1171 (1995). K.C. Nicolaou, F.P.J.T. Rutjes, E.A. Theodorakis, J. Tiebes, M. Sato and E. Untersteller, *J. Am. Chem. Soc.* **117**, 1173 (1995).
40. K.C. Nicolaou, J. Tiebes, E.A. Theodorakis, F.P.J.T. Rutjes, M. Sato, E. Untersteller, *J. Am. Chem. Soc.* **116**, 9371 (1994).
41. R. E. Gawley, K. S. Rein, G. Jeglitsch, D. J. Adams, E. A. Theodorakis, J. Tiebes, K. C. Nicolaou and D. G. Baden, *Chemistry & Biology* **2**, 0000 (1995).