Special Topics

Joys of Molecules. 2. Endeavors in Chemical Biology and Medicinal Chemistry

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J. Med. Chem., **2005**, 48 (18), 5613-5638• DOI: 10.1021/jm050524f • Publication Date (Web): 01 September 2005

Journal of
Medicinal
Chemistry

Volume 48, Number 18 September 8, 2005

2005 American Chemical Society A. C. Cope Award Address

Joys of Molecules. 2. Endeavors in Chemical Biology and Medicinal Chemistry†

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Received June 3, 2005

In 1969, as I was pondering the choice of a Ph.D. mentor in London (U.K.), two names came to mind: Sir Derek Barton, then at Imperial College, and Franz Sondheimer, then at University College. Delving deeper into their research, I recognized a sharp distinction between the two. Barton was primarily interested in natural products with biological activity; Sondheimer was dealing with annulenes and related polyunsaturated systems, interesting only from the theoretical point of view. While Barton's molecules were, for the most part, unsymmetrical and adorned with chiral centers, those of Sondheimer were beautifully symmetrical and usually flat (see, for example, 18-annulene (**2**), an aromatic analogue of benzene (Figure 1)). In the end, it was the architect in me that prevailed, and I decided to join, in September of 1969, Sondheimer's group where I would also have the privilege to be mentored by Peter J. Garratt. Parenthetically, it was only a month later that the Swedish Academy of Sciences chose Barton to share the 1969 Nobel Prize for chemistry, a fact that made me momentarily question my judgment at the time. Be that as it may, my time in the Sondheimer−Garratt training camp was marvelous and, most importantly, earned me a ticket to America and the good fortune to join, in 1972, Thomas J. Katz at Columbia University as a postdoctoral fellow. There, again, I worked on architecturally beautiful molecules of theoretical interest (such as benzvalene (**3**) (Figure 1), an isomer of benzene) before I joined the group of E. J. Corey at Harvard University in 1973.

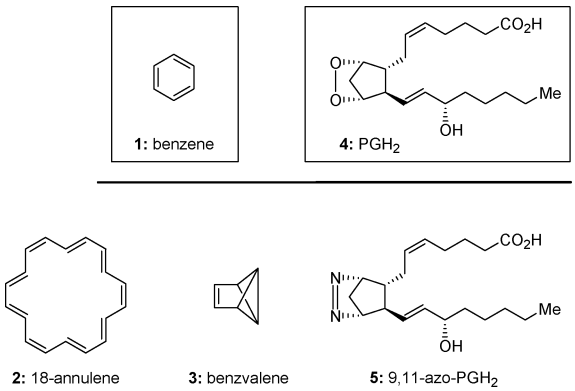

Figure 1. From aesthetically pleasing molecules to biology and medicine (1969−1975).

At Harvard I experienced the joys of biologically active molecules for the first time. I remember vividly Corey coming to my lab in late 1974, after a lecture by Bengt Samuelsson on biosynthetic studies on arachidonic acid metabolites, with a suggestion that I should make the diazo analogue (**5**, Figure 1) of PGH_2 (**4**, Figure 1), the unstable biosynthetic precursor of prostaglandins and thromboxanes. Together with my friend and labmate, Yoshi Machida, we did indeed synthesize this compound (**5**), which was sent to Samuelsson's laboratories in Stockholm for biological investigations. Soon thereafter we would receive the good news that our compound, which was much more stable than the labile PGH_2, was

† Dedicated to Professor Peter J. Garratt on the occasion of his 71st birthday.

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10.1021/jm050524f CCC: $30.25
Published on Web 09/01/2005

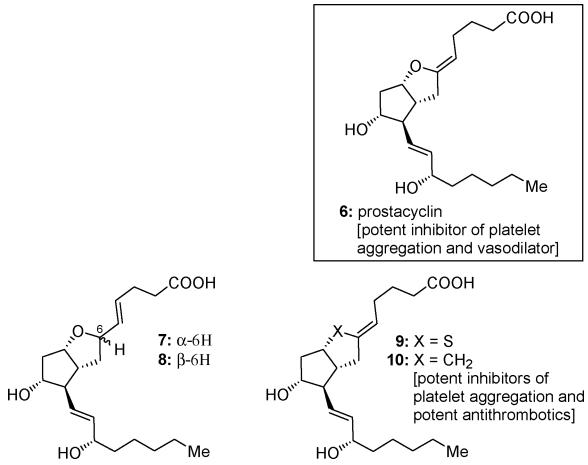


Figure 2. Prostacyclin and designed analogues as enabling tools for biology and medicine (antithrombotic agents and vasodilators).

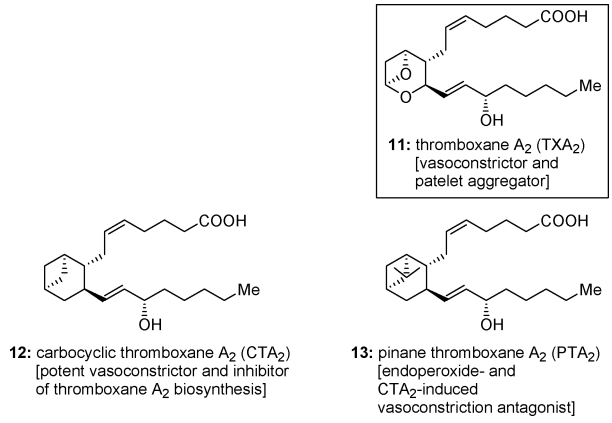


Figure 3. Thromboxane A_2 (TXA_2) and designed analogues (CTA_2 and PTA_2) as useful biological tools.

biologically active, aggregating human blood platelets and contracting smooth muscle with potencies several times higher than its naturally occurring endoperoxide counterpart (PGH_2).[1] I will never forget the thrill of biology as I learned of the amazing properties of this little molecule, designed after a natural product and made in the laboratory by chemical synthesis. Indeed, I feel fortunate to be able to relive that thrill almost every day as we design, synthesize, and test new compounds patented after some of nature's most intriguing and biologically active molecules. The Harvard experience had a profound influence on my career, for it provided me with an opportunity to appreciate the importance of biology and the enormous power of synthesis to enable it and shape it. The pursuit of novel natural products with important biological properties became the main theme of our research programs, which often amalgamated total synthesis campaigns with endeavors in chemical biology and medicinal chemistry.

While the campaigns in total synthesis and the new synthetic strategies and technologies that emerged from them are described in a separate article,[2] here I intend to reminisce on the chemical biology and medicinal chemistry aspects of these research programs in an effort to demonstrate the importance of natural products chemistry in biology and medicine and the crucial role of synthesis in bridging these disciplines for the benefit of mankind.

The disclosure of prostacyclin (PGI_2, **6**, Figure 2) by Sir John Vane and his group in 1976[3] coincided with my appointment as an Assistant Professor at the University of Pennsylvania (Penn). This coincidence became significant when, in the same year, we discovered a series of cyclizations, among which was the so-called selenoetherification reaction whereby cyclic ethers could be constructed from hydroxyalkene systems in a convenient manner.[4] It was a perfect match. Applying our new synthetic technology, we swiftly synthesized the isoprostacyclins **7** and **8** (Figure 2), which proved their stability but also their inability to mimic prostacyclin's antiaggregatory and smooth muscle relaxing properties, thus providing useful structure−activity relationships (SARs) in the field.[5] These opportunistic events positioned us well to make further contributions in the prostacyclin area. Thus, in 1977 and 1978 we designed and synthesized numerous prostacyclin analogues, including thiaprostacyclin (**9**, Figure 2)[6] and carboprostacyclin (**10**, Figure 2),[7] both of which were proven to be highly potent biological mimics of the natural product as antithrombotic agents and vasodilators. Together with the preparation of prostacyclin itself (**6**) from prostaglandin $F_{2\alpha}$,[8] these syntheses facilitated significantly the fast moving biological investigations of this important arachidonic acid metabolite by rendering this unstable and naturally scarce substance readily available, as well as providing a number of stable analogues as biological tools.

Our interest in the eicosanoid field soon expanded to the thromboxane A_2 (TXA_2, **11**, Figure 3) area, for this fascinating sibling of prostacyclin, equally important from a biological point of view, was also very unstable and scarce. Biosynthesized from PGH_2 (and its 15-hydroperoxide counterpart PGG_2), thromboxane A_2 was detected by Samuelsson in 1974 and shown to be a potent platelet aggregating and vasoconstricting agent; in other words, it had the opposite biological role to that of prostacyclin.[9] Being derived from a common precursor, these two molecules held the secrets of health and disease, and often life and death, for their delicate balance in the body is crucial to our normal physiology. Owing to the instability of thromboxane A_2, we designed and synthesized the two stable analogues carbocyclic thromboxane A_2 (CTA_2, **12**)[10] and pinane thromboxane A_2 (PTA_2, **13**),[11] both of which proved to be biologically active and useful as tools in biological studies. CTA_2 was found to be a potent vasoconstrictor (at 29 pM) and an inhibitor of the biosynthesis of thromboxane A_2 (at 200 nM) but not of that of prostacyclin, thus dissociating the vasoconstricting and aggregating properties of thromboxane A_2. PTA_2 also proved to be an inhibitor of thromboxane A_2 biosynthesis but not of prostacyclin production, acting as an antagonist for the vasoconstricting and platelet antiaggregatory actions of stable PGH_2 analogues while demonstrating no inhibitory activity against prostacyclin, making it a potential antithrombotic agent.[12] Overall, these designed molecules served as wonderful biological tools, facilitating studies in the field and paving the way for further developments to occur.[13]

The excitement generated by thromboxane A_2 and prostacyclin in the late 1970s was matched by that precipitated by the discovery of the leukotrienes by the

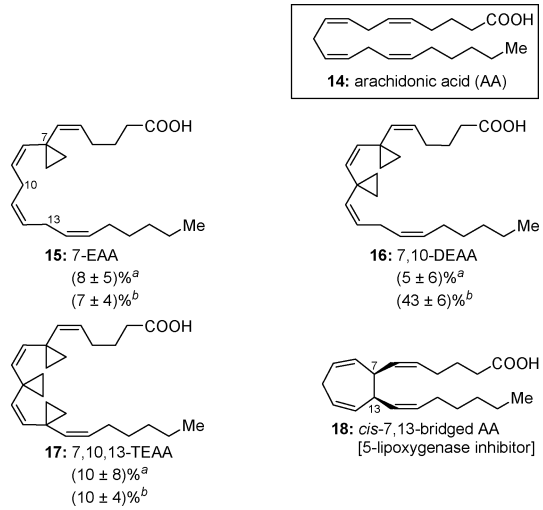


Figure 4. Arachidonic acid (AA), ethanoarachidonic acids (EAAs, DEAAs, and TEAAs) and bridged arachidonic acid (bridged-AA) as useful tools for biological investigations in the eicosanoid field. Footnote *a* in the figure indicates inhibition of LTC_4 and LTD_4 production in cat lung at 5 mM. Footnote *b* indicates antagonism of LTD_4 constriction in cat coronary artery at 5 mM.

Samuelsson group.[14] These developments ushered in a new era in the eicosanoid field that extended from chemistry to biology and from the domain of arachidonic acid (**14**, 5,8,11,14-eicosatetraenoic acid, Figure 4) and related polyunsaturated fatty acids to their mono-, di-, and trihydroxylated metabolites and their epoxide precursors and amino acid derivatives. The natural scarcity and chemical sensitivity of these bioactive molecules made chemical synthesis an indispensable tool for their study. Indeed, laboratory preparation not only facilitated their structural elucidation but more importantly rendered not only these precious natural products but also their analogues readily available for thorough biological investigations.[15] Corey and his group at Harvard played a dominant role in this regard. Our contributions complemented theirs in certain ways and will be briefly touched upon here.

A number of modified arachidonic acids were designed and synthesized as potential modulators of the arachidonic acid cascade, their promise stemming from both their rigidified structures and the deactivation of specific methylene group positions along the fatty acid chain.[16] Indeed, some of these compounds exhibited inhibitory activities against 5- and 12-lipoxygenases and phospholipase A_2, as well as modulation of the biosynthesis and action of certain leukotrienes (e.g., **15**−**18**, Figure 4).[17]

The hydroxylated eicosatetraenoic acids (HETEs), 5(*S*)-HETE (**19**),[18] 12(*S*)-HETE (**20**),[19] and 15(*S*)-HETE[20] (**21**, Figure 5) were also synthesized by stereoselective routes in order to render them available for biological investigations, for they were implicated in inflammation.

Leukotriene B_4 (**23**) is a novel arachidonic acid metabolite[21] whose natural scarcity and potent chemotactic activities dictated its chemical synthesis. Our strategy for its total synthesis was both stereoselective and flexible enough to deliver various analogues of the natural product, including the ones (**24**−**29**) shown in Figure 6.[22,23] In addition, the cyclopropane analogue of leukotriene A_4 (**30**) was synthesized and, unlike its

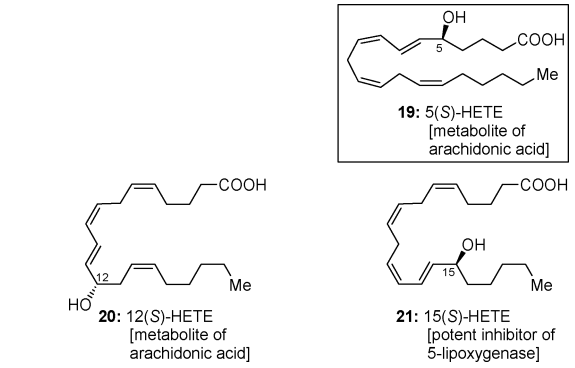


Figure 5. Hydroxytetraenoic acids (HETEs) as useful tools for biological investigations.

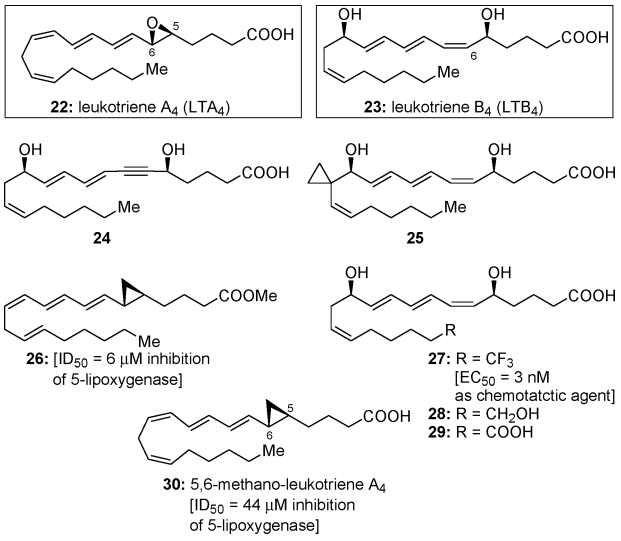


Figure 6. Leukotrienes A_4 and B_4 and analogues thereof for biological investigations.

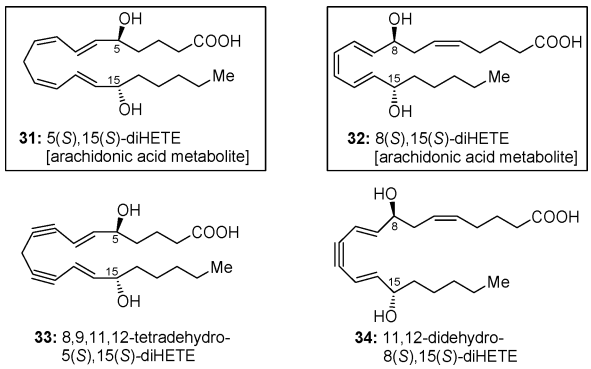


Figure 7. Synthesized dihydroeicosatetraenoic acids (diHETEs) and analogues thereof for biological investigations.

parent compound (**22**), proved to be quite stable.[24] Its biological activity included selective inhibition of 5-lipoxygenase.[25]

In addition to leukotriene B_4, the two dihydroxy-eicosatetranoic acids 5(*S*),15(*S*)-DiHETE (**31**) and 8(*S*),15(*S*)-DiHETE (**32**) (Figure 7) were also synthesized stereoselectively by a route that also delivered the acetylenic analogues 8,9,11,12-tetradehydro-5(*S*),15(*S*)-diHETE (**33**) and 11,12-didehydro-8(*S*),12(*S*)-diHETE (**34**) (Figure 7).[26] These and other linear eicosanoids were synthesized by a general strategy that exploited the cis/trans diene system(s) common to their structures. This structural motif was traced in

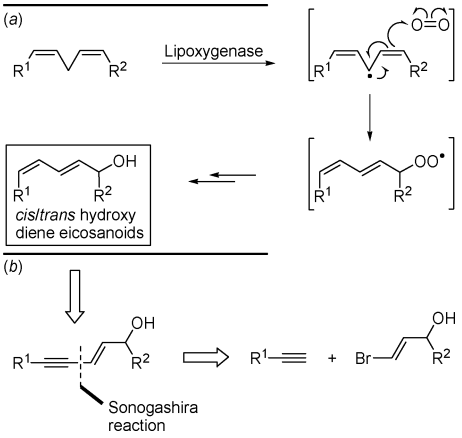


Figure 8. (a) Biosynthesis of *cis*/*trans*-hydroxydiene eicosanoids and (b) retrosynthetic analysis enabled by the Sonogashira coupling reaction.

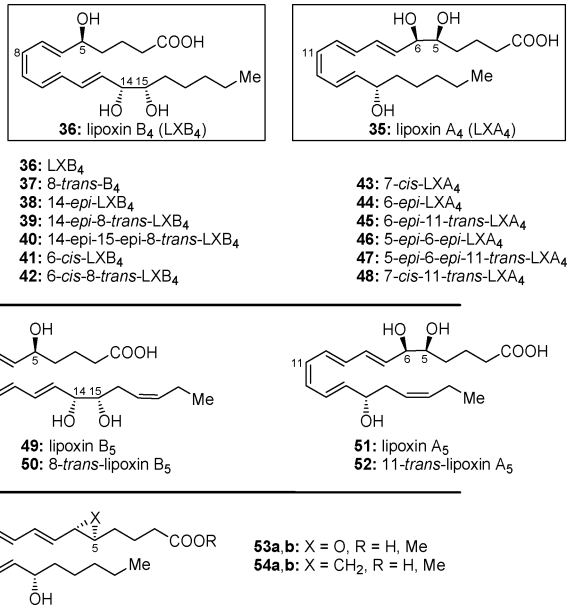


Figure 9. Naturally occurring lipoxins A_4, B_4, A_5, and B_5, biogenetic precursor lipoxin epoxide, and analogues thereof for structural elucidation and biological investigations.

a retrosynthetic fashion back to a conjugated eneyne system, which was constructed using a Sonogashira coupling reaction in which a terminal acetylene was coupled to a vinyl halide under Pd^0-Cu^I catalyzed conditions. This general strategy is shown in retrosynthetic format in Figure 8.[15]

The lipoxins, a series of naturally occurring trihydroxy eicosatetra- and eicosapentaenoic acids (Figure 9), have been shown to possess a number of disease relevant activities such as inhibition of leukotriene function and induction of chemotaxis.[27] In the 1980s, the structural elucidation and biological investigation of these metabolites demanded their chemical synthesis because of the extremely small quantities available from biosynthetic sources. Applying a strategy similar to that for the synthesis of the other hydroxylated eicosanoids (Figure 8), a number of lipoxins (**36**−**52**, Figure 9) were synthesized for comparison with the naturally derived materials, enabling their structural elucidation and facilitating explorations in their chemical biology.[28,29] In addition to the trihydroxylated lipoxins, lipoxin epoxides **53a**,**b** were made available by chemical synthesis and shown to lead, stereoselectively, to lipoxins A_4 (**35**) and B_4 (**36**) under biogenetic conditions.[30] As in the case of leukotriene A_4, the corresponding 5,6-cyclopropane analogues of lipoxin epoxide (**54a**,**b**, Figure 9) were also synthesized[28b] as potential inhibitors of the biosynthesis of the lipoxins.

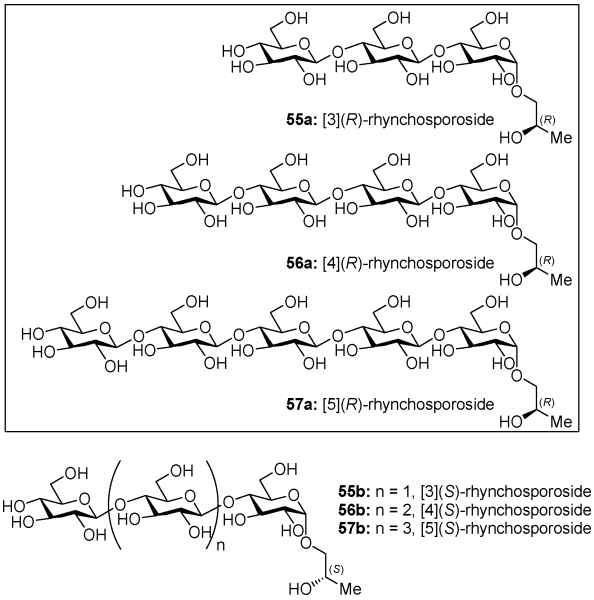
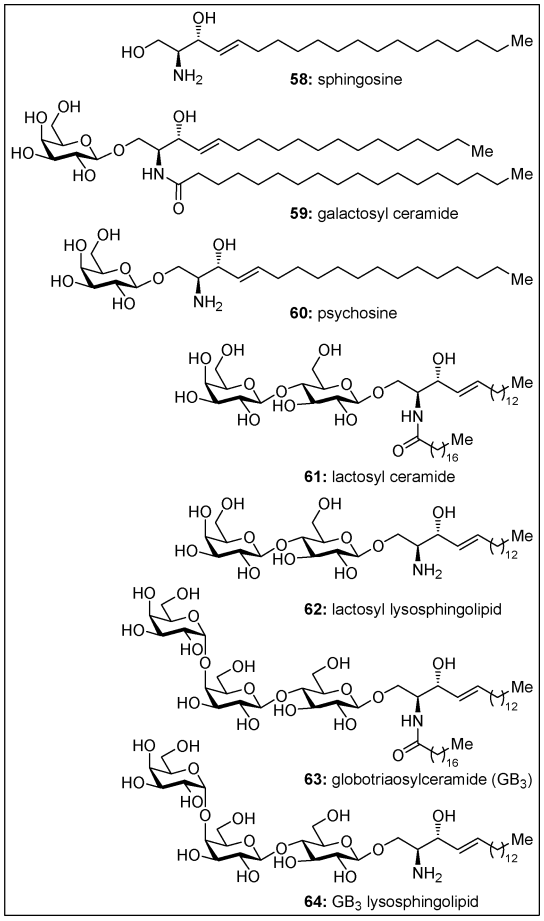


Figure 10. Rhynchosporosides made readily available by chemical synthesis for biological studies.

The abundance of carbohydrates in nature and their undeniable importance in biology have made them attractive synthetic targets to many investigators for well over a century. Their appeal did not escape our attention, much of which has, for the last several years, focused on new synthetic technologies for their chemical synthesis, molecular design, and chemical biology studies.[31] Herein, I will briefly recount the chemical biology investigations that were enabled by the synthesis of natural and designed carbohydrate-based molecules.

Produced by *Rhynchosporidium secalis*, the rhynchosporosides are a family of fungal metabolites that cause scald disease in barley, rye, wheat, and other grasses.[32] In light of their biological importance and natural scarcity, we initiated a program directed toward the synthesis of the higher homologues of the family, a task accomplished in 1985 (**55**−**57**, Figure 10). Bioassays on the synthesized compounds confirmed impressive destructive potencies for the [3](*R*)-, [4](*R*)-, and [5](*R*)-rhynchosporosides (**55a**−**57a**), causing massive tip wilt and necrosis in young barley plants, while their [*n*](*S*) counterparts (**55b**−**57b**) proved to be inactive or considerably less active in this regard.[33]

Glycosphingolipids such as those shown in Figure 11 (**58**−**64**) are key constituents of the membranes of most cell types and, as such, are recognized as fundamental mediators of cell−cell recognition and communication, cell-growth regulation, and antibody interactions. Following their synthesis and structural characterization,[34−36] a series of these compounds was studied in order to determine their biological properties. These investigations showed that these synthetic sphingolipids do not inhibit superoxide anion generation, leukotriene B_4 production, or aggregation induced by either fMetLeuPhe or A23187 in intact human neutrophils.[37]



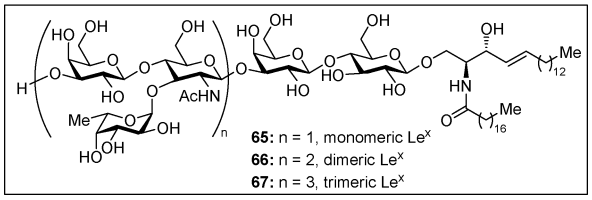


Figure 11. Sphingosine and selected glycosphingolipids rendered readily available by chemical synthesis for biological studies.

Glycosphingolipids carrying the Lewis antigen X (Le^x) determinant are known to accumulate in a wide variety of human cancers. Three members of this family of compounds (**65**−**67**, Figure 11) were prepared in our laboratories, showcasing once again the crucial role of chemical synthesis in rendering available scarce, but important, naturally occurring substances for biological studies.[38]

Sialyl Le^x-type molecules were identified as binding ligands for ELAM-1, a biological receptor of considerable importance given its association with inflammation and related disorders.[39,40] Furthermore, these ligands, and in particular sialyl Le^x (**68**) and sialyl dimeric Le^x (**69**), were identified as tumor-associated oligosaccharides, making them potential candidates for tumor cell targeting (Figure 12). Following their synthesis, spectroscopic and modeling studies were performed on these compounds in collaboration with the Wong group at Scripps in order to aid in the understanding of their structure−function relationship.[41] Furthermore, the Ca^{2+} binding properties of the sialyl Le^x, and deoxygenated fucose analogues thereof, were examined by electrospray mass

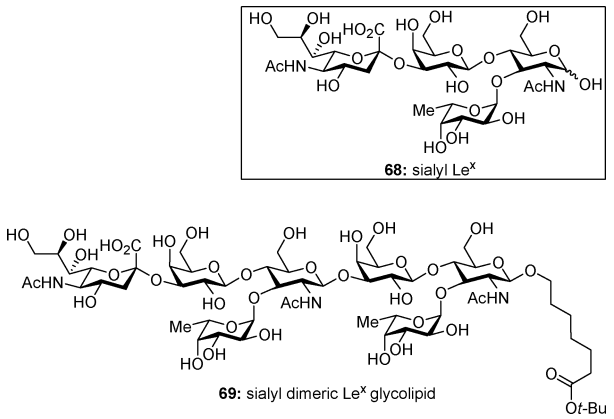


Figure 12. Sialyl Le^x and sialyl dimeric Le^x derivative made available by chemical synthesis for biological investigations.

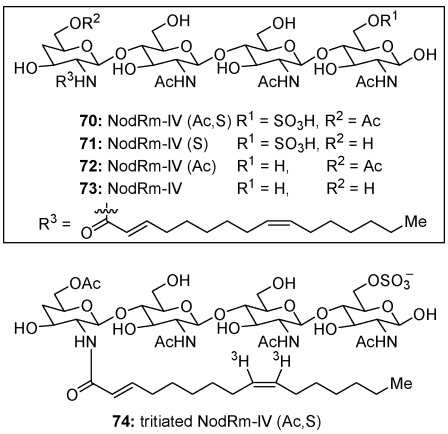


Figure 13. NodRm-IV factors rendered available by chemical synthesis for biological studies.

spectrometry. These studies provided evidence that Ca^{2+} is coordinated primarily to the GalGlcNAc moiety of sialyl Le^x.[42,43]

The *Sinorhizobium meliloti* nodulation factors (NFs) elicit certain symbiotic responses in alfalfa (*Medicago sativa*) roots. An important limitation in the study of SARs of the naturally occurring NFs, beyond their natural scarcity, was the inability to obtain pure compounds. The reason for this deficiency was that all rhizobial species produce a mixture of NFs with different substitution patterns (Figure 13).[44] Our synthetic studies in the field rendered these precious products (**70**−**73**, Figure 13) available in pure form for further biological investigations.[45] Furthermore, the characterization of binding sites for NF in roots of the symbiotic host plant *Medicago truncatula* was investigated by the use of the synthetic tritiated analogue (**74**, Figure 13), showing that the binding was independent of both the *O*-acetyl and the sulfate groups and did not depend on the degree of unsaturation of the fatty acid component of the molecule.[46] However, both the acetate and sulfate moieties appear to be essential for biological activity, since the synthetic compounds **70** and **71** exhibited nodulation at the same low concentrations (down to 100 pM) as the natural samples, whereas their desulfated (**72**) or deacetylated (**73**) counterparts had more than 100-fold decreased activity. In addition, in order to probe the effect of the long-chain *N*-acyl group on biological activity, a series of synthetic unnatural NF analogues was tested, resulting in the determination of the opti-

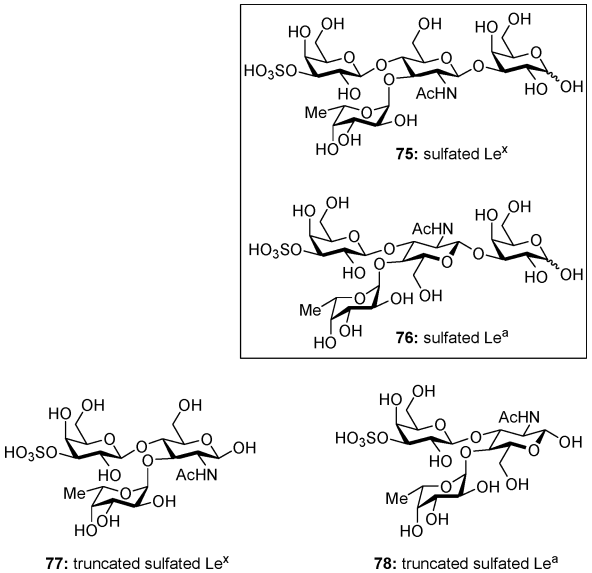


Figure 14. Sulfated Le^x and Le^a and analogues thereof for biological investigations.

mum length for the chain (C_{16}) and of the preferred nature of the unsaturation (2*E*,9*Z* unsaturated).[44]

In light of the important roles of selectins through their expression on the surface of leukocytes and consequent recruitment to inflammation sites, extensive studies were carried out on these biomolecules, leading to the isolation of two sulfated tetrasaccharides, sulfated Le^x (**75**) and sulfated Le^a (**76**) (Figure 14). These scarce substances were prepared in our group, along with the truncated trisaccharide analogues **77** and **78**,[47] and tested, in addition to other Le oligomers, for their ability to support E-selectin binding when converted into neoglycosphingolipids, as well as for their ability to inhibit E-selectin binding to immobilized lipid-linked sialyl Le^a, Le^x, and sulfated Le^a pentasaccharides.[48] Initial results indicated that sulfated Le^a tetra- and pentasaccharides were the most potent oligosaccharide ligands for human E-selectin of those tested at the time. Subsequent studies targeted the binding specificity of the leukocyte-adhesion molecule L-selectin (leukocyte homing receptor) toward the structurally defined sulfated oligosaccharides. The results of these investigations, which were carried out in collaboration with the Feizi group in the U.K., revealed that the sulfated tetrasaccharides exhibited high binding affinities toward L-selectin in the quantitative microwell binding assay, while their trisaccharide siblings were considerably less active in the same assay.[49]

Isolated from the mycelial walls of the fungus *Phytophthora megasperma*, heptasaccharide phytoalexin elicitor (HPE, **79**, Figure 15) boasts considerable molecular complexity and significant biological activity.[50] This substance exhibits potent phytoalexin elicitor activity with nanomolar binding properties toward its receptor. Our solid-phase chemical synthesis of this oligosaccharide, in 1997,[51] was notable not only for its efficiency but also because of its flexibility to deliver analogues for biological investigations.

The marine natural products plakosides A (**80**) and B (**81**) (Figure 16) possess structural features uncommon to other glycosphingolipids.[52] These potent immunosuppressive agents were targeted for synthesis

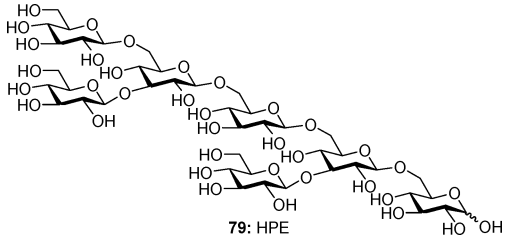


Figure 15. Heptasaccharide phytoalexin elicitor (HPE) was made readily available by chemical synthesis for biological studies.

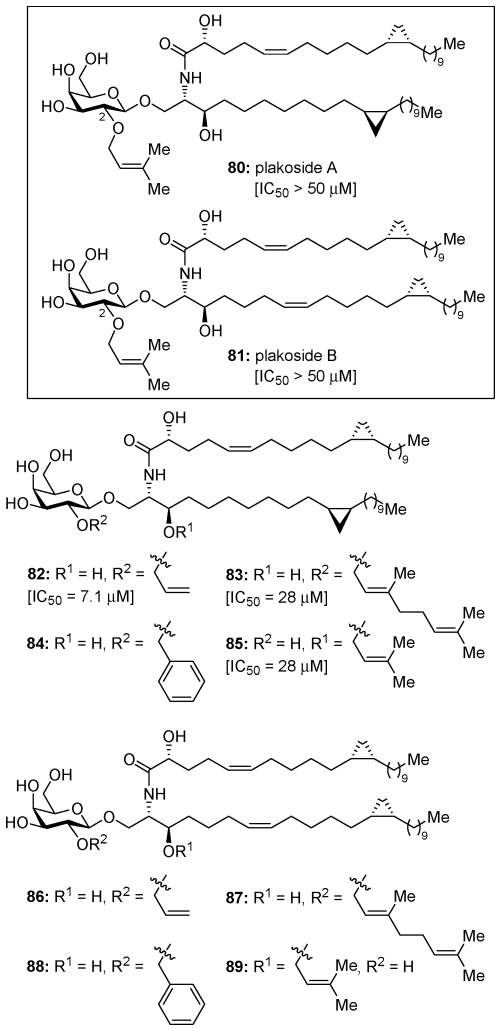
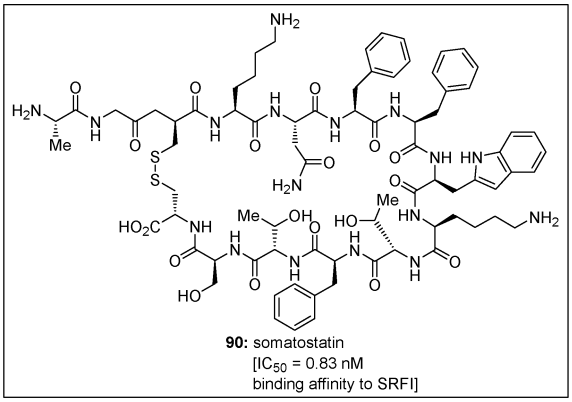
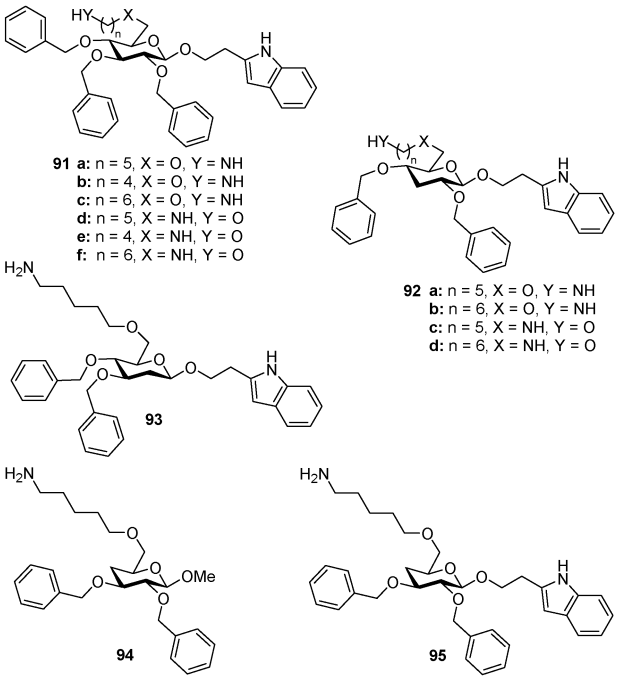


Figure 16. Plakosides and analogues thereof rendered readily available by chemical synthesis for biological investigations. IC_{50} values refer to inhibition of the mixed-lymphocyte-reaction (MLR) proliferation assay.[53]

and biological investigations, with the prenyloxy group on the C-2 position of the carbohydrate moiety and the free hydroxy group on the sphingosine chain being identified as sites for introducing structural diversity. The ensuing chemical biology studies revealed some interesting SARs (see Figure 16) and allowed useful conclusions to be made regarding their potential as therapeutic agents.[53]

On the basis of the biological properties of somatostatin (**90**, Figure 17),[54] we decided in 1990, together with Ralph Hirschmann, to investigate the possibility of using carbohydrate frameworks as templates for the construction of peptide mimetics.[55] Thus, the substituted





Sugar peptidomimetics			IC_{50} [μM]	
O-linked	*N*-linked	*n*	*O*-linked	*N*-linked
91a	**91d**	5	15.0	14.0
91b	**91e**	4	—	—
91c	**91f**	6	11.0	5.1
92a	**92c**	5	8.4	10.0
92b	**92d**	6	6.6	34.0
93		5	35.0	
94		5	—	
95		5	47.0	

Figure 17. Somatostatin and carbohydrate-based peptidomimetics. IC_{50} values refer to binding affinities to the SRIF receptor.

glucose-based analogues **91**−**95** (Figure 17) were designed and synthesized retaining crucial amino acid side chains in the proper spatial orientation but differing from the natural substance in that they were devoid of both the peptide backbone and the biochemically fragile peptide bonds (see Figure 17). These compounds were pioneering in that they proved to be forerunners of several new designs that were to follow. Indeed, their biological evaluation revealed micromolar binding affinities to the peptide hormone somostatin receptor (SRIF) in a dose-dependent manner.[56,57]

In 1999 we synthesized the stereochemically homogeneous dodecasaccharide **96** (Figure 18), one of the largest oligosaccharides to be constructed on solid phase.[58] Our synthesis employed a modification of our

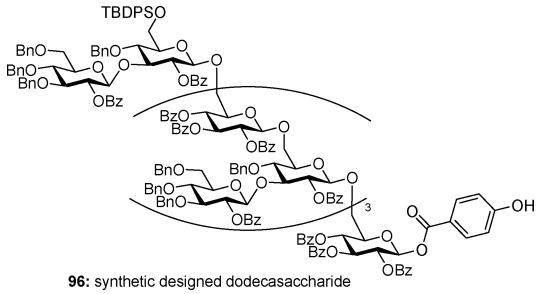


Figure 18. Designed dodecasaccharide constructed by solid-phase synthesis.

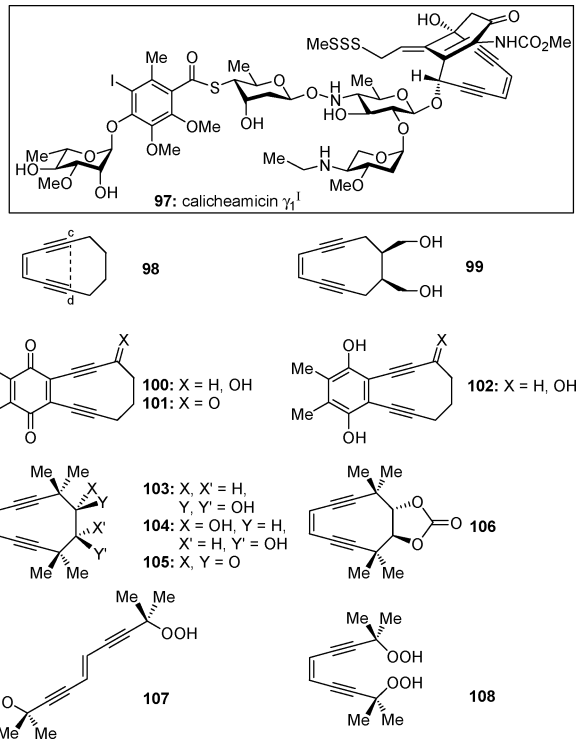


Figure 19. Calicheamicin γ_1^I and selected designed enediynes capable of cleaving DNA.

previously developed solid-phase chemistry employing photolabile linkers.[51]

The discovery of the first enediyne antitumor antibiotics in 1987 ushered in a new era of molecular design of DNA-cleaving molecules that were also endowed with potent cytotoxic properties. Among them, calicheamicin γ_1^I (**97**, Figure 19) captured our imagination not only as a challenging synthetic target but also as a unique prototype for mimicking with simpler structures.[59] Our molecular designs in this area were multifaceted. They included the parent 10-membered ring enediyne hydrocarbon **98** and the more water-soluble dihydroxy 10-membered ring enediyne **99** (Figure 19), the first man-made enediyne with DNA-cleaving properties whose action did not require any additives.[60,61] Following the mechanism of action of calicheamicin, this molecule cleaves double-stranded DNA through a benzenoid diradical generated at 37 °C (half-life $t_{1/2} = 11.8$ h) by a Bergman-type cycloaromatization reaction as shown in Figure 20.

In an effort to control the reactivity of the enediyne moiety toward the Bergman cycloaromatization through redox chemistry, the conjugated systems **100**−**102** (Figure 19) were designed and synthesized.[62] As ex-

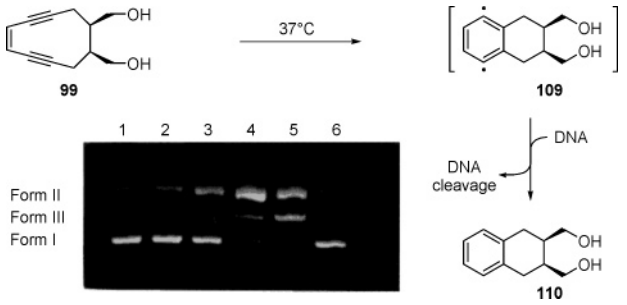


Figure 20. Mechanism of the DNA-cleaving action of cyclic conjugated enediynes (i.e., **99**). Electrophoresis gel was φX174. Form I DNA (50 μM per base) was incubated with compound **99** in Tris-acetate buffer (pH 8.5, 50 mM) at 37 °C for 12 h and analyzed by agarose gel electrophoresis: (lane 1) DNA alone; (lanes 2−5) DNA + **99** at 1.0, 10, 100, and 500 μM, respectively; (lane 6) DNA + **110** at 2 mM. Reproduced with permission from the *Journal of the American Chemical Society*.[60] Copyright 1988 American Chemical Society.

pected on electronic grounds, the quinone **100** was more reactive than its dihydroquinone counterpart **102**, as well as its eneynone derivative **101**, cleaving double-stranded DNA at 37 °C and killing leukemia MOLT-4 cells with IC_{50} values down to 5.0×10^{-7} M for **100** (compared to $\sim 10^{-5}$ M for **102**). The stability of dihydroquinones raises the possibility of their use as prodrugs capable of serving as pregenitors to their more reactive quinone counterparts in vivo upon oxidation, a process that could, in principle, occur in the body.

Along the same lines, the 10-membered ring enediynes **103**−**106** (Figure 19) were designed, synthesized, and tested for their DNA-cleaving properties. Copublished with the Bergman group,[63] these investigations revealed a number of interesting aspects of these molecules, including the DNA-cleaving properties of **103** and **104** at 50 °C, the higher stability of the cyclic carbonate derivative **106** compared to its diol counterpart **104** toward cycloaromatization, and the extrusion of two molecules of carbon monoxide from diketone **105**, leading, in the presence of molecular oxygen, to the intriguing and DNA-cleaving hydroperoxides **107** and **108** (Figure 19).

From our studies with these molecules, it became clear that the reactivity of the cyclic enediynes toward Bergman cycloaromatization can be modulated by molecular fine-tuning through ring size and substituent adjustments. A frequently reliable indicator for the propensity of this radical generating reaction was the "cd" distance (see structure **98**, Figure 19); shortening of this distance to less than 3.25 Å triggers cyclization.[64]

More complex enediyne systems were designed and synthesized along the road to calicheamicin γ_1^I when we developed the synthetic technology to construct the core structure of the molecule. Thus, the calicheamicinones shown in Figure 21 were made available for biological investigations, which revealed their potent, but nonselective, DNA-cleaving properties at pH 6.0−8.5, causing single and double strand cuts at millimolar concentrations and potent cytotoxicities.[65] In 1994, 2 years after the completion of the total synthesis of calicheamicin γ_1^I (**97**),[66] and as a climactic accomplishment, we reported our synthetic preparation of, and investigations into, calicheamicin θ_1^I (**113**, Figure 21).[67] This rationally designed enediyne proved to be ex-

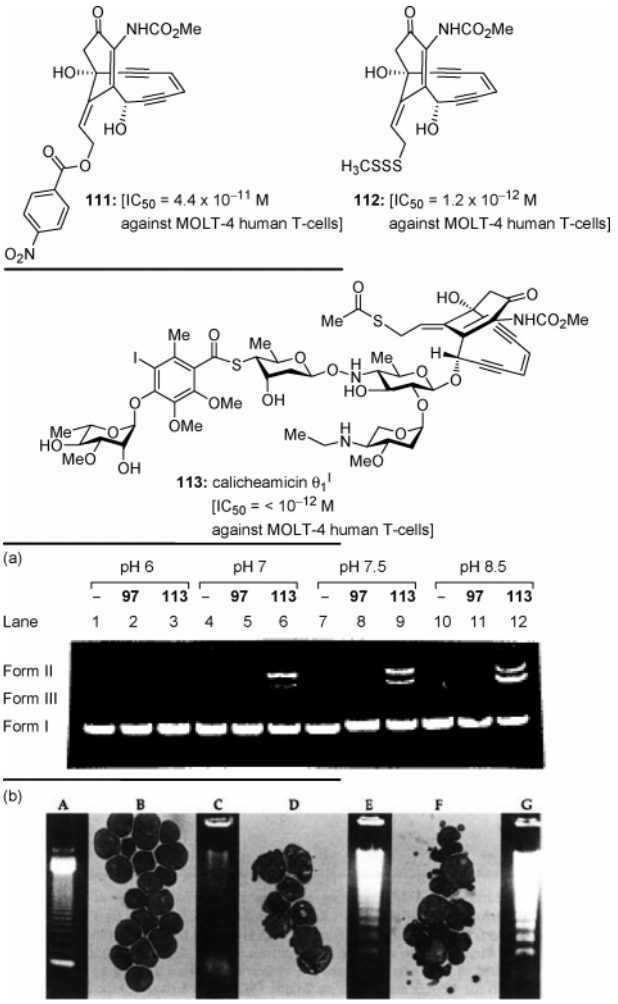


Figure 21. Calicheamicinone model systems and calicheamicin θ_1^I and its DNA-cleaving and cytotoxic properties. IC_{50} values refer to the MOLT-4 human T-cell carcinoma transformed cell line. (a) Interaction of calicheamicin θ_1^I (**113**) with double-stranded DNA. Shown are agarose gel electrophoresis patterns of supercoiled CJX174 DNA cleavage by calicheamicins θ_1^I (**113**) γ_1^I (**97**). Designed calicheamicin θ_1^I causes double strand cleavage of supercoiled DNA with higher potencies than natural calicheamicin γ_1^I under mildly basic conditions. Supercoiled closed circular form I φX174 DNA (200 ng) was incubated with 10 nM **97** or **113** in different pH buffer solutions in a total volume of 10 μL (dimethyl sulfoxide (DMS0)/4 mM Tris buffer (1:99), 4 mM ethylenediaminetetraacetic acid (EDTA)) for 12 h at 37 °C. Electrophoresis was carried out by using 1% agarose gel with added ethidium bromide. Lanes 1, 4, 7, and 10 are controls: (form I) supercoiled DNA; (form II) nicked DNA; (form III) linear DNA. (b) Apoptotic morphology and nuclear DNA degradation induced by calicheamicin θ_1^I and its natural counterpart, calicheamicin γ_1^I: (A) molecular weight markers consisting of multiples of a 123 base-pair fragment (Gibco BRL); (B) untreated MOLT-4 leukemia cells; (C) DNA extracted from untreated MOLT-4 cells; (D) MOLT-4 cells incubated with 10^{-10} M calicheamicin γ_1^I; (E) DNA extracted from MOLT-4 cells exposed to 10^{-8} M calicheamicin γ_1^I; (F) MOLT-4 cells incubated with 10^{-10} M calicheamicin θ_1^I; (G) DNA extracted from MOLT-4 cells exposed to 10^{-8} M calicheamicin θ_1^I. Solutions of calicheamicins θ_1^I and γ_1^I in DMSO, 10^{-3} M, were diluted in culture medium to the appropriate concentrations and added to cell cultures. MOLT-4 cells (10^5) were incubated with 10^{-10} M final concentrations of **113** or **97** for apoptosis morphology assays and with 10^{-8} M final concentrations for DNA degradation assays. After incubation for 4 h at 37 °C, cells were harvested and processed[68] to visualize apoptotic morphology and DNA degradation.

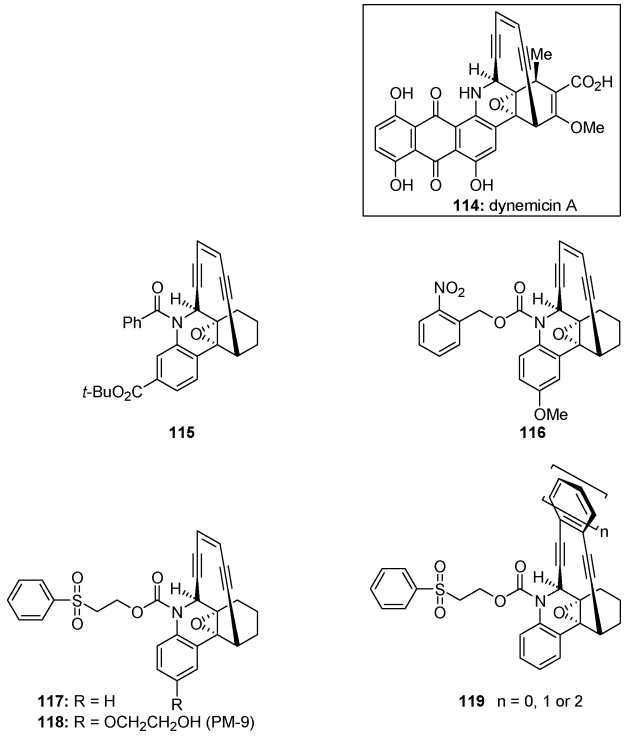


Figure 22. Dynemicin A and selected designed biological mimics.

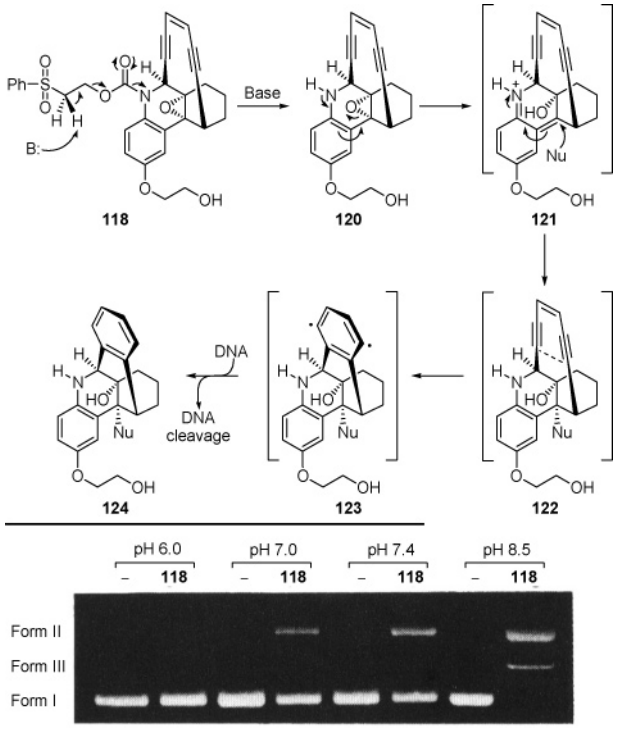


Figure 23. Mechanism of DNA-cleaving action of designed enediyne PM-9 and electrophoresis gel of interaction of supercoiled DNA with PM-9 (enediyne **118**). φX174 DNA (50 μM per base pair) was incubated for 24 h at 37 °C with **118** in various buffer solutions and analyzed by electrophoresis (1% agarose gel, ethidium bromide stain). Lanes 1, 3, 5, and 7 are DNA controls at pH 6.0, 7.0, 7.4, and 8.5, respectively. Lanes 2, 4, 6, and 8 are compound **118** (1000 μM) at pH 6.0, 7.0, 7.4, and 8.5 with DNA, respectively: (form I) supercoiled DNA; (form II) nicked DNA; (form III) linear DNA. Reprinted with permission from *Science* (http://www.aaas.org) (Nicolaou, K. C.; Dai, W.-M.; Tsay, S.-C.; Estevez, V. A.; Wrasidlo, W. Designed Enediynes. A New Class of DNA-Cleaving Molecules with Potent and Selective Anticancer Activity. *Science* **1992**, *256*, 1172−1178). Copyright 1992 AAAS.

tremely potent and exhibited selective DNA-cleaving properties. The triggering mechanism of this designed compound featured a thioacetyl group in place of the trisulfide moiety of the natural product (**97**). This small, but important, change allowed this molecule to be effective under a much wider range of activation conditions. Thus, under near-physiological conditions, calicheamicin θ_1^I caused single- and double-strand cuts of DNA and induced apoptosis of tumor cells (see Figure 21b) at very low concentrations and in the absence of any additives, suggesting a significant improvement as a cytotoxic agent over the natural calicheamicin (which was essentially inactive under the same conditions). Indeed, when tested against a broad range of cell lines, calicheamicin θ_1^I (**113**) was found to be superior to the natural product in terms of its biological properties in several circumstances. Of particular significance was its powerful cytotoxicity against breast, colon, ovarian, prostate, and the SK-MEL 28 melanoma cell lines.[67]

Equally exciting was dynemicin A (**114**),[69] another highly potent antitumor antibiotic whose exotic structure and mechanism of action inspired extensive investigations in the fields of chemistry, biology, and medicine. Our activities in this area focused on the design and synthesis of simpler biological mimics of the natural product containing various substitution patterns. Figure 22 presents a number of examples from the dozens of molecules constructed and tested for DNA cleavage and cytotoxicity.[70−73] Of particular interest were those enediynes equipped with base-sensitive activators (i.e., **117**−**119**, Figure 22) and photosensitive triggering devices (e.g., **116**, Figure 22).[70] Designed on the basis of the mechanism of action of dynemicin A, these molecules exhibited potent DNA-cleaving properties and cytotoxicity against various tumor cells as exemplified by the phenomenally active compound code-named PM-9 (**118**, Figure 22), whose springing into action could be brought about in vitro by base (see DNA cleavage in Figure 23) and in vivo by enzymatic or chemical means. It is interesting to note that this relatively simple enediyne **118** was found to have a potency comparable to that of calicheamicin γ_1^I, yet **118** was much more accessible by chemical synthesis. It is noteworthy that in the case of calicheamicin γ_1^I (**97**) its indiscriminate cytotoxicity was tamed by conjugation to a tumor-targeting antibody, leading to the development of MyloTarg, a clinically used anticancer agent against certain types of leukemia. It is, therefore, not inconceivable that PM-9 may serve as a suitable molecular "warhead" for similar conjugates as potential targeted therapeutic agents.

Figure 24 summarizes our dynemicin A designs and SARs within this structural motif.[70] These mechanistically based rational designs varied considerably, leading to a number of stable small molecules equipped with easily activated triggering devices, a "warhead" and handles for attachment to appropriate delivery systems. It is not unimaginable that the basic principles demonstrated in these chemical biology studies may one day be translated into powerful chemotherapeutic agents, especially in light of the recent successes in the antibody and nanotechnology fields.

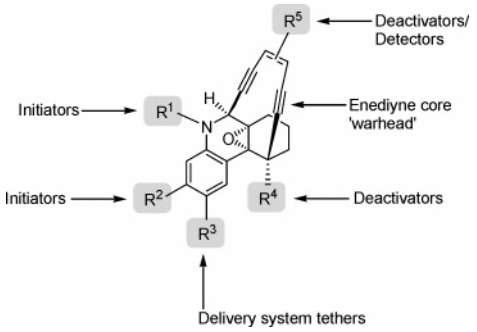


Figure 24. Dynemicin A inspired enediyne designs and SARs with regard to DNA cleavage.

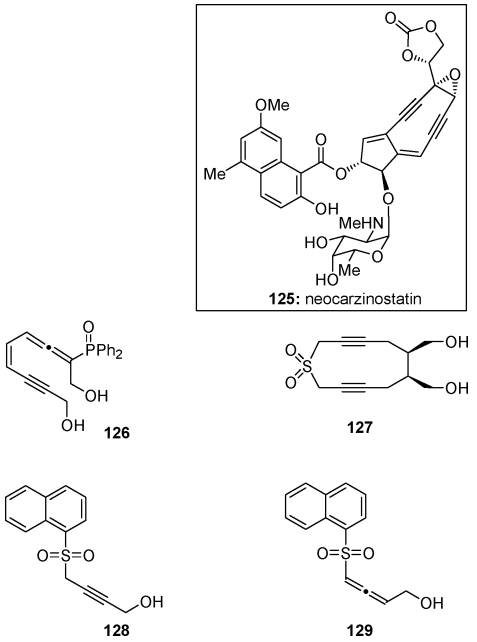


Figure 25. Designed molecules with DNA-cleaving properties related to neocarzinostatin.

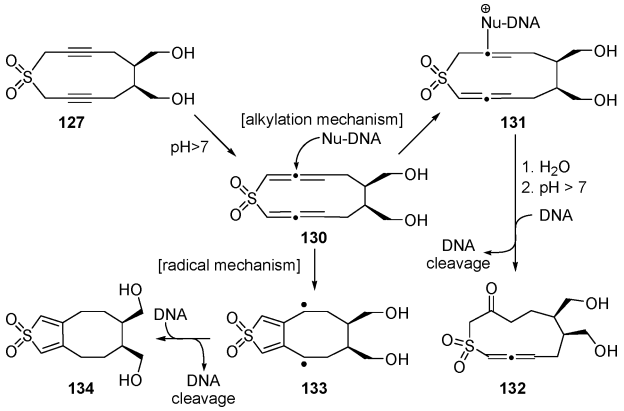


Figure 26. Mechanistic rationale for the design and action of DNA-cleaving molecules based on the propargylic sulfone structural motif.

A series of propargylic and conjugated allenic sulfones and phosphine oxides were designed as potential DNA-cleaving molecules[74,75] based on the radical mechanistic rationale shown in Figure 26.[59,74] Indeed, a plethora of such compounds were synthesized and proven to be capable of damaging the genetic material, although their mode of action most likely involves nucleophilic alkylation rather than radical chemistry (which provided the main motivation for their design), as supported by model studies.[74] A small collection of these bioactive molecules together with the structure of neocarzinostatin chromophore (a naturally occurring substance whose mechanism of DNA-cleaving action was inspirational to these studies) is shown in Figure 25.

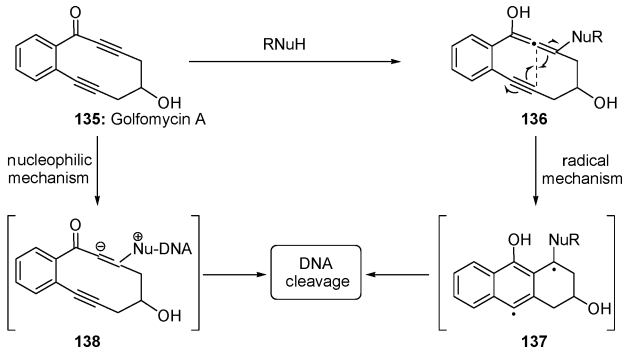
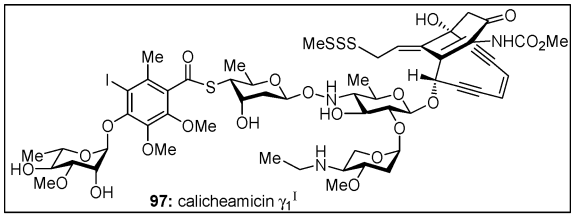


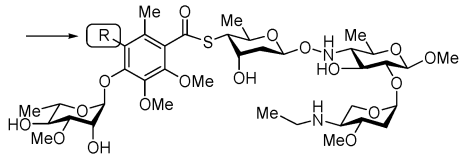
Figure 27. Mechanistic rationale for the design and action of golfomycin A via radical or nucleophilic pathways.

Of particular interest among the series of small-molecule DNA-cleaving agents prepared in our laboratories during the enediyne era was golfomycin A (**135**), a designed molecule also inspired by the mechanism of action of neocarnizostatin and named after the student who synthesized it.[76] The chemistry of this enynone proved to be quite intriguing, as witnessed in the cascade depicted in Figure 27. The propensity of golfomycin A to undergo facile nucleophilic attack is presumed to be due to strain relief and energy gain that accompanies the cycloaromatization reaction involved.

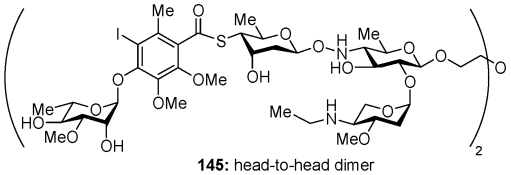
As part of the calicheamicin γ_1^I campaign, we had the opportunity to study DNA−carbohydrate interactions.[59] To examine the effect of the iodine residue of the aromatic ring of calicheamicin on its DNA binding affinity, we synthesized the series of oligosaccharides **139**−**144** (Figure 28) featuring different substitution patterns on the aromatic ring (i.e., I, Br, Cl, F, Me, H) and compared their binding affinities to that of calicheamicin γ_1^I (**97**) in a competitive binding assay.[77] Carried out in collaboration with the Joyce group,[68] this study revealed the essential role of the iodine residue to the potent and TCCT-selective DNA-binding properties of the oligosaccharide domain with the following order of decreasing affinity: I > Br > Cl ≥ Me ≥ F ≫ H (see Figure 28). Direct comparison of the iodine to hydrogen substitution revealed the loss of ca. 2.3 kcal mol^{-1} of binding energy. Furthermore, extensive NMR spectroscopic studies of DNA−oligosaccharide complexes formed from synthetic calicheamicin γ_1^I oligosaccharide (**139**) and computer modeling led to the design of the head-to-head (**145**, Figure 28) and head-to-tail (**146**) oligosaccharide dimers, whose chemical synthesis allowed a study of their interactions with duplex DNA fragments.[78] These investigations revealed extremely high binding affinities of these dimers to TCCT-rich sequences of DNA with the molecules wrapping themselves within the minor groove of the TCCT-rich sites of the DNA helix, as shown in Figure 29.[79]

In an interesting study carried out in collaboration with the Vogt group at Scripps, it was found that synthetic oligosaccharide **145** (Figure 28) inhibited transcription factor binding to DNA in a sequence-

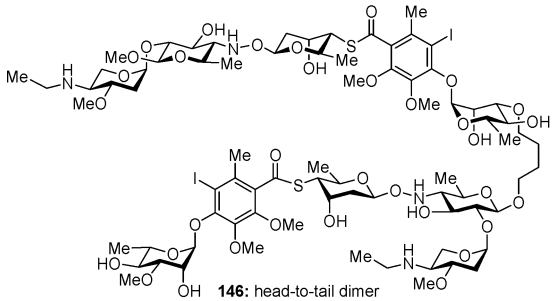




Ligand	K_{obs} [μM]	ΔG [kcal mol^{-1}]	ΔΔG [kcal mol^{-1}]
97: calicheamicin γ_1^I	0.135 ± 0.006	–9.7	–
139: R = I	4.1 ± 0.4	–7.6	2.1
140: R = Br	10.0 ± 1.7	–7.1	2.6
141: R = Cl	32.0 ± 3.3	–6.4	3.3
142: R = F	44.0 ± 3.1	–6.2	3.5
143: R = Me	38.0 ± 5.0	–6.3	3.4
144: R = H	180.0 ± 19.0	–5.3	4.4



145: head-to-head dimer
[10^{-8} – 10^{-9} M^{-1} binding affinity on TCCT-sites]



146: head-to-tail dimer
[10^{-8} – 10^{-9} M^{-1} binding affinity on TCCT-sites]

Figure 28. Calicheamicin γ_1^I and oligosaccharides for DNA binding affinities.

selective manner; it also interfered with transcription by polymerase II in vitro. Exhibiting effective concentrations in the micromolar range in these assays, this dimeric oligosaccharide proved to be significantly more active than the corresponding monomer (**139**, Figure 28).[78a]

In an effort to develop totally synthetic and selective DNA-cleaving molecules, we designed and synthesized the calicheamicin–dynemycin hybrid structures **148** and **149** by merging the racemic mixture of PM-9 with the calicheamicin oligosaccharide fragment (**147**, not shown) (Figure 30). Interestingly, neither of these hybrid molecules exhibited the high potency and selectivity of calicheamicin γ_1^I as a DNA-cleaving agent, underscoring the importance of precise docking of the molecular "warhead" into the minor groove of DNA for optimal cleavage.[80]

In our campaign to synthesize paclitaxel (Taxol) (**150**) in the early 1990s, we had the opportunity to design and synthesize a number of novel taxoids for biological studies. Among them was the benzenoid paclitaxel

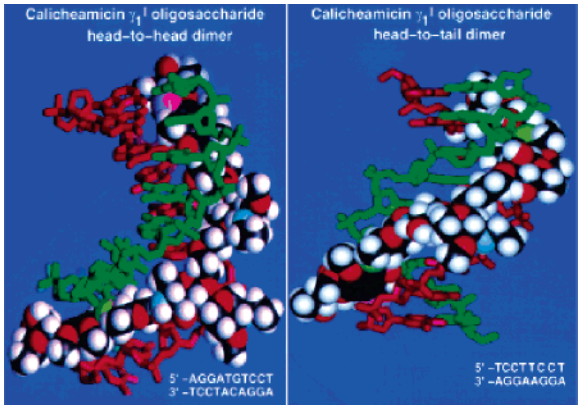


Figure 29. Computer-generated molecular models of oligosaccharide–DNA complexes: (right) head-to-tail dimer **146** bound to 5′-TCCTTCCT-AGGAAGGA-3′; (left) head-to-head dimer **145** bound to 5′-AGGATGTCCT-AGGACATCCT-3′. The DNA strands are displayed in green and red. Color code for the oligosaccharide atoms is as follows: C, black; H, white; O, red; N, blue; S, yellow; I, purple. Modeling studies and interactive docking were done on an SGI Indigo-2 workstation with Insight II (Biosym Technologies, Inc., San Diego, CA). Pictures were created using AVS (AVS Inc., Waltham, MA) and locally developed modules on a DEC Alpha 3000/500 computer with a Kubota Pacific Denali graphics card. Reproduced with permission from *Journal of the American Chemical Society*.[78b] Copyright 2002 American Chemical Society.

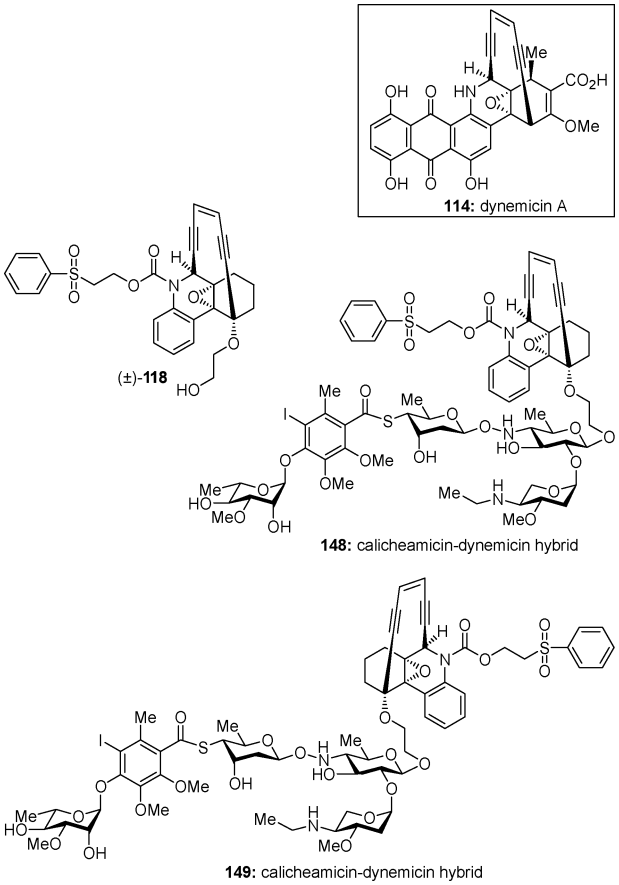
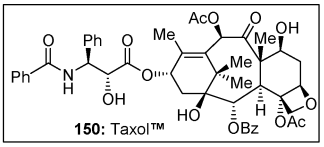


Figure 30. Dynemicin A and designed analogues thereof.

analogue **151** (Figure 31) which, although not as potent as paclitaxel, showed significant cytotoxicity against certain tumor cells.[81,82] Interestingly, the diastereoisomer of **151**, taxoid **152** (Figure 31), showed essentially no cytotoxicity under the same conditions. This high-

Cytotoxicities of Taxol™ analogues

Taxoid	IC_{50} (Ovcar-3)
150 (Taxol™)	6.20×10^{-10} M
151	2.80×10^{-7} M
152	inactive



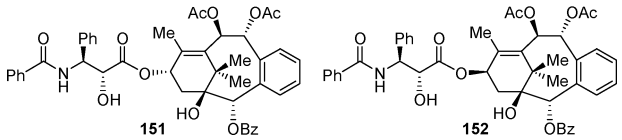


Figure 31. Benzenoid paclitaxel analogues **151** and **152** tested against tumor cell line Ovcar-3.

R =		IC_{50} (M)
Taxol™	**150**	6.2×10^{-10}
	153	3.7×10^{-9}
	154	6.7×10^{-10}
	155	4.5×10^{-8}
NMe_2	**156**	2.2×10^{-6}
	157	$>1 \times 10^{-4}$

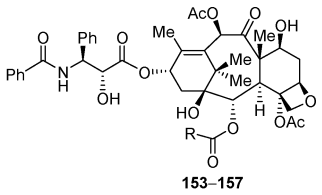


Figure 32. Paclitaxel and analogues thereof. IC_{50} values refer to MOLT-4 human T-cell leukemia cell line.

lights the tight constraints of the SARs around the structure of taxanes, particularly with regard to stereochemistry.

The C-2 analogues **153**−**157**, carrying varying ester groups at the C-2 positions, are representative of several that were constructed by semisynthesis and tested for their cytotoxic properties. While their biological properties proved to be comparable to those of paclitaxel,[83,84] the entire project provided useful SARs within the taxoid field (Figure 32).

In further studies aimed at circumventing the insolubility of paclitaxel in aqueous media, we designed and synthesized several water-soluble paclitaxel derivatives including those shown in Figures 33 and 34.[85] These water-soluble taxoids were designed as prodrugs according to the indicated mechanistic rationales, and indeed, they performed as expected, releasing paclitaxel in human blood plasma and exhibiting pharmacological profiles similar to or, in certain aspects, better than that of the natural product itself (see Figures 33 and 34).[86] The 2′-methylpyridinium acetate **168** (2′-MPA) and 7-methylpyridinium acetate **169** (7-MPA) (Figure 34), in particular, proved very interesting.[84,86,87] Thus, while 2′-MPA showed an improved in vivo pharmacological profile compared to paclitaxel and formed helical structures in aqueous media, 7-MPA served well to define the taxoid conformation (as a highly clustered structure) in water in an NMR spectroscopic study.[87]

During this campaign we also had the opportunity to develop a number of fluorescent paclitaxel derivatives (see Figure 35) that served well as imaging devices in chemical biology studies.[88]

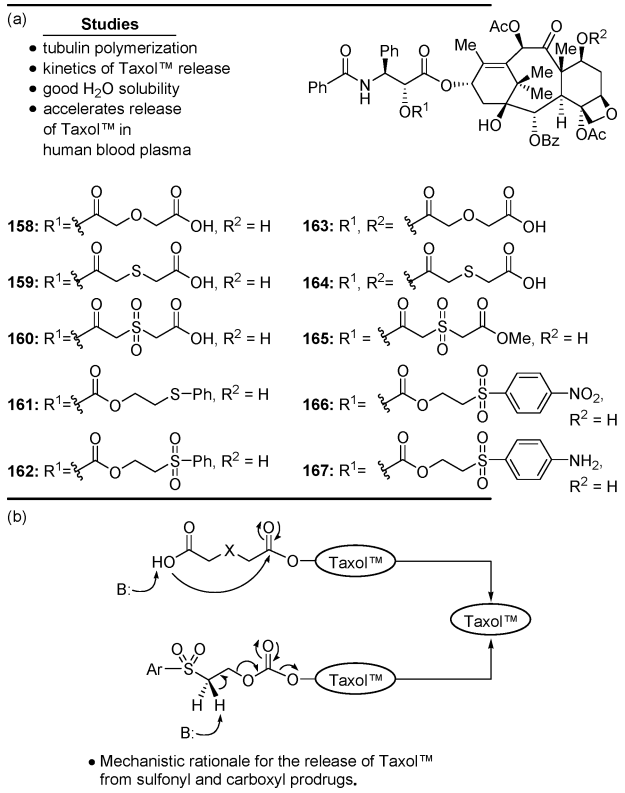


Figure 33. (a) Water-soluble paclitaxel sulfonyl and carboxyl prodrug candidates and (b) mechanism of action.

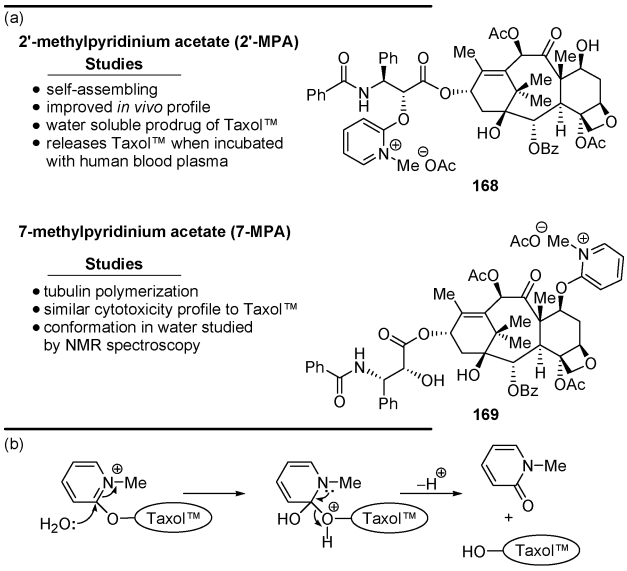


Figure 34. (a) Water-soluble paclitaxel pyridinium prodrug candidates and (b) mechanism of action.

The epothilone class of natural products, represented by epothilones A and B (Figure 36), appeared on the scene of chemistry and biology in 1996 when their structures were disclosed, although their discovery had occurred a few years earlier, first in Germany[89] and subsequently in the U.S.[90] The excitement surrounding this class of antitumor agents intensified when their paclitaxel-like mechanism of action was recognized and their high potency against drug-resistant tumor cells, including paclitaxel-resistant cell lines, became apparent.[91] Our contributions to the epothilone field were

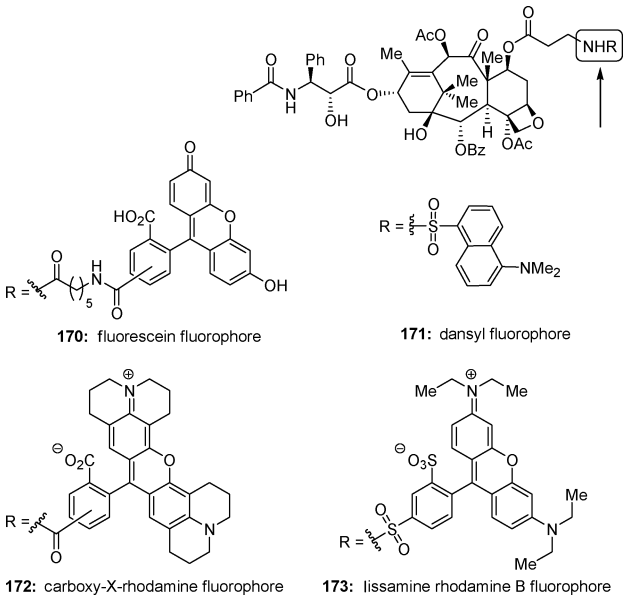
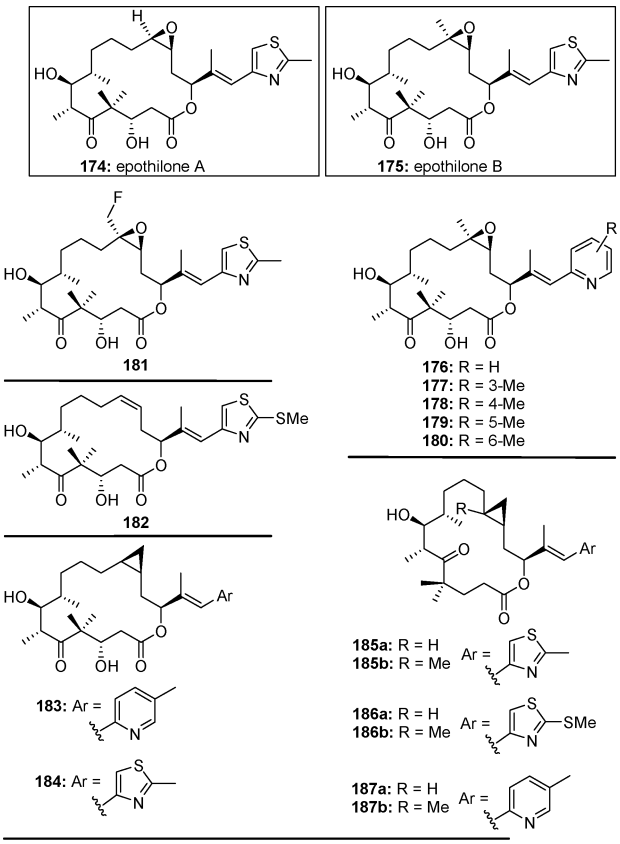


Figure 35. Fluorescent paclitaxel derivatives synthesized for imaging studies.

described in over 30 publications and several patents and, in addition to the total synthesis of a number of naturally occurring members of the family, included the design and construction of numerous synthetic analogues.[92] Employing our technology developed for their total synthesis, we synthesized hundreds of epothilone analogues, using both combinatorial and target-oriented strategies. Biological evaluation of these analogues as tubulin polymerization inducers and tumor cell killers led to a clear SAR picture.[92k] Among the most interesting designed epothilones are those depicted in Figure 36. Thus, it was shown that the thiazole ring could be replaced by a pyridine moiety as long as the nitrogen atom maintained its position adjacent to the bridge connecting the heterocycle to the mainframe of the molecule as shown in structure **176** (Figure 36). It was also interesting to determine that from the Me-substituted pyridine epothilones, only the 4- and 5-Me analogues (**178** and **179**, Figure 36) maintained high potency, as opposed to the 3- and 6-substituted analogues (**177** and **180**, Figure 36), which were found to exhibit significantly lower tubulin polymerization and cytotoxic potencies.[92t]

Another interesting lead came in the form of the methylthioepothilone C (**182**, Figure 36) and its $\Delta^{12,13}$ trans counterpart (not shown), which exhibited striking tubulin binding properties and cytotoxicities.[92m] These observations led to the design and synthesis of methylthioepothilone B (**188**, Figure 38), whose remarkable pharmacological profile elevated it to drug candidate status and propelled it into clinical trials as a promising anticancer agent.[92aa,ab]

Another notable epothilone analogue is 26-fluoroepothilone B (**181**, Figure 36) whose pharmacological investigation revealed promising action against several tumor cell types. Most notably, this compound exibited potent cytotoxicity against human prostate cancer cell lines as well as against models of human prostate tumors in nude mice. Significantly, this fluoroepothilone B produced a prolonged alteration in prostate tumor mitotic and apoptotic indices that may translate into



		Inhibition of carcinoma cell growth		
Compound	TP [%]	1A9 IC_{50} [nM]	1A9PTX10 IC_{50} [nM]	1A9PTX22 IC_{50} [nM]
174	76	2.2	20	5.9
175	98	0.13	1.0	0.31
176	79.5	0.1	1.0	0.15
177	0	>300	>300	>300
178	90.3	0.1	0.36	0.1
179	92	0.15	0.6	0.15
180	45	9.0	72	18
181	93	0.2	0.4	0.2
182	92	9.0	22	28
183		2.5	19.2	4.22
184		0.25	9.0	2.0
185a		10.1	17.2	4.7
185b		15	52	5.0
186a		1.2	3.2	0.8
186b		3.5	5.7	11.5
187a		0.6	5.9	1.4
187b		1.74	10.9	5.6

Figure 36. Epothilones A and B and selected analogues thereof and selected biological properties (TP, tubulin polymerization (%); IC_{50} values (nM) against ovarian carcinoma cell growth for parental 1A9 and for β-tubulin mutant cells 1A9PTX10 and 1A9PTX22).

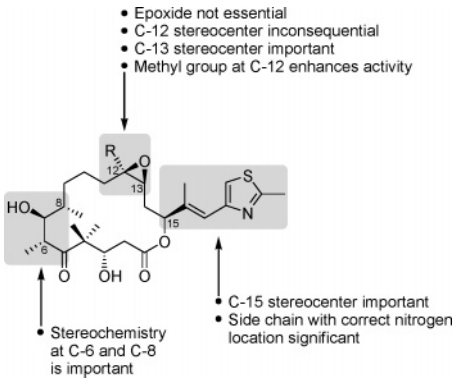
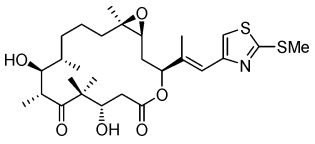


Figure 37. SARs within the epothilone family.

clinically useful anticancer responses in human patients. From these studies, this analogue was judged to



188: methylthioepothilone B
IC_{50} = 0.17 nM, against 1A9
IC_{50} = 0.26 nM, against 1A9PTX10
IC_{50} = 0.25 nM, against 1A9PTX22

Figure 38. Methylthioepothilone B, a clinical drug candidate for cancer chemotherapy (IC_{50} values (nM) against 1A9 parental human ovarian carcinoma cell line and against β-tubulin mutant cells 1A9PT10 and 1A9PT22].

be superior to paclitaxel in mice with regard to its therapeutic index.[92x]

A most intriguing finding was the fact that both the cis- and the trans-$\Delta^{12,13}$ isomers and their β-epoxides were active in the tubulin polymerization and cytotoxicity assays with comparable potencies. Interestingly, these potencies extended to the corresponding β-cyclopropanes (e.g., **185**−**187**, Figure 36), confirming the fact that the epoxide oxygen is not a prerequisite for biological activity; rather, it was the overall conformation of the macrocycle that mattered in the expression of the bioaction of these molecules.[92z] These observations, and those pertaining to the location of the basic nitrogen atom of the side chain heterocycle, proved to be consistent with the binding model of the epothilones to their receptor that emerged from subsequent X-ray crystallographic studies of tubulin−epothilone complexes.[93] That molecular design and chemical synthesis anticipated the model to the extent that it did (see SARs, Figure 37)[91k] is a testimony to the power of this combination in rational drug design.

Eleutherobin (**189**, Figure 39)[94] and its siblings, eleuthosides A and B,[95] appeared on the scene as novel marine natural products in the 1996−1997 period. Their potent cytotoxicities against several tumor cell lines were quickly linked to their paclitaxel-like tubulin polymerization properties, creating considerable interest with regard to their potential as anticancer agents.[96] The previously discovered sarcodictyins A (**190**) and B (**191**) (Figure 39),[97] also isolated from marine organisms, added to the excitement, for their structures and biological properties were recognized as being similar to those of eleutherobin. The scarcity of these naturally occurring substances coupled with their promising pharmacological profiles prompted several investigators to pursue their study.[98] Besides the total synthesis of all members of the eleutherobin/sarcodictyin class,[99−101] our contributions included the design and synthesis of a series of analogues for biological investigations. Their construction involved a combination of solution and solid phase chemistry.[102] Comprising over 60 members, this compound library facilitated the establishment of certain SARs as shown in Figure 40. Some of the most potent compounds to be identified from this research program are those shown in Figure 39 (sarcodictyin analogues **192**, **193**, and **194**). Interestingly, the potency of these compounds in certain cytotoxicity assays did not always correlate well with their tubulin polymerization potencies, a fact that suggested a dual mechanism of action, one based on their tubulin binding properties and another endowed to them by their ability to form oxonium-type alkylating species under physiological conditions.[103]

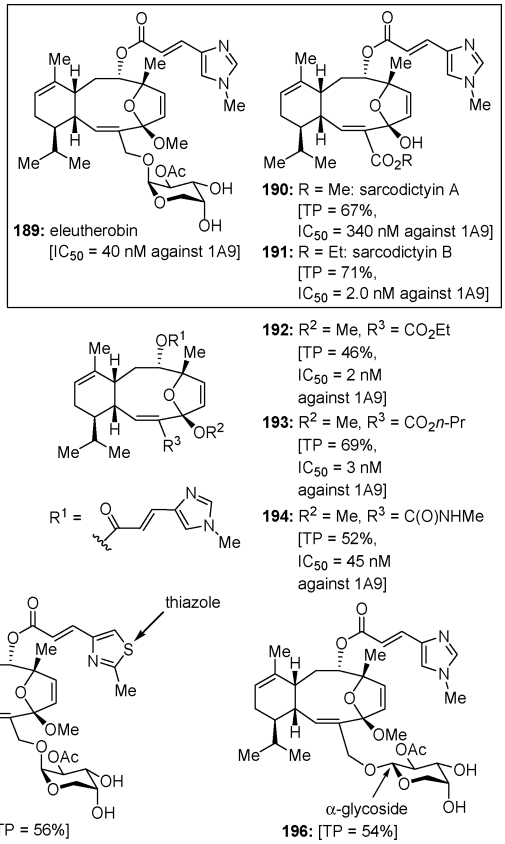


Figure 39. Eleutherobin, sarcodictyins A and B, and selected designed analogues. A library of over 60 compounds was constructed and biologically evaluated in tubulin polymerization and cytotoxicity assays (TP, tubulin polymerization (%) with drug concentrations of 100 μM and incubation times of 90 min; IC_{50} values (nM) against human ovarian 1A9 tumor cells).

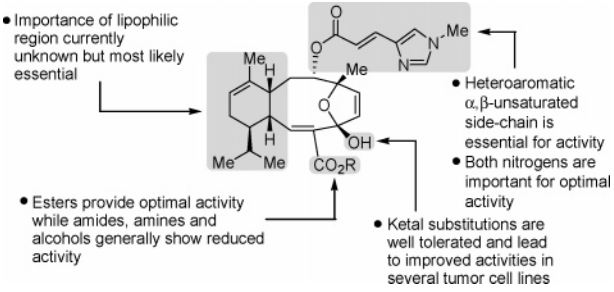


Figure 40. SARs for the sarcodictyin family of natural products.

Further to the medicinal and mechanistic studies, these investigations provided sufficient quantities of eleutherobin for in vivo pharmacological studies, which enabled the evaluation of this promising, but scarce, natural product as a potential drug candidate.

During the campaign to synthesize vancomycin (**197**, Figure 41), the "antibiotic of last resort",[104] we had the opportunity to develop a number of powerful technologies for target-oriented and combinatorial synthesis that enabled the construction of a series of monomeric and dimeric designed analogues of this complex molecule.[105] Particularly rewarding was our "target-accelerated" combinatorial synthesis strategy[106] that led, among the more than 75 library members synthesized, to the discovery of several novel vancomycin dimers endowed with impressive antibacterial properties. The two shown

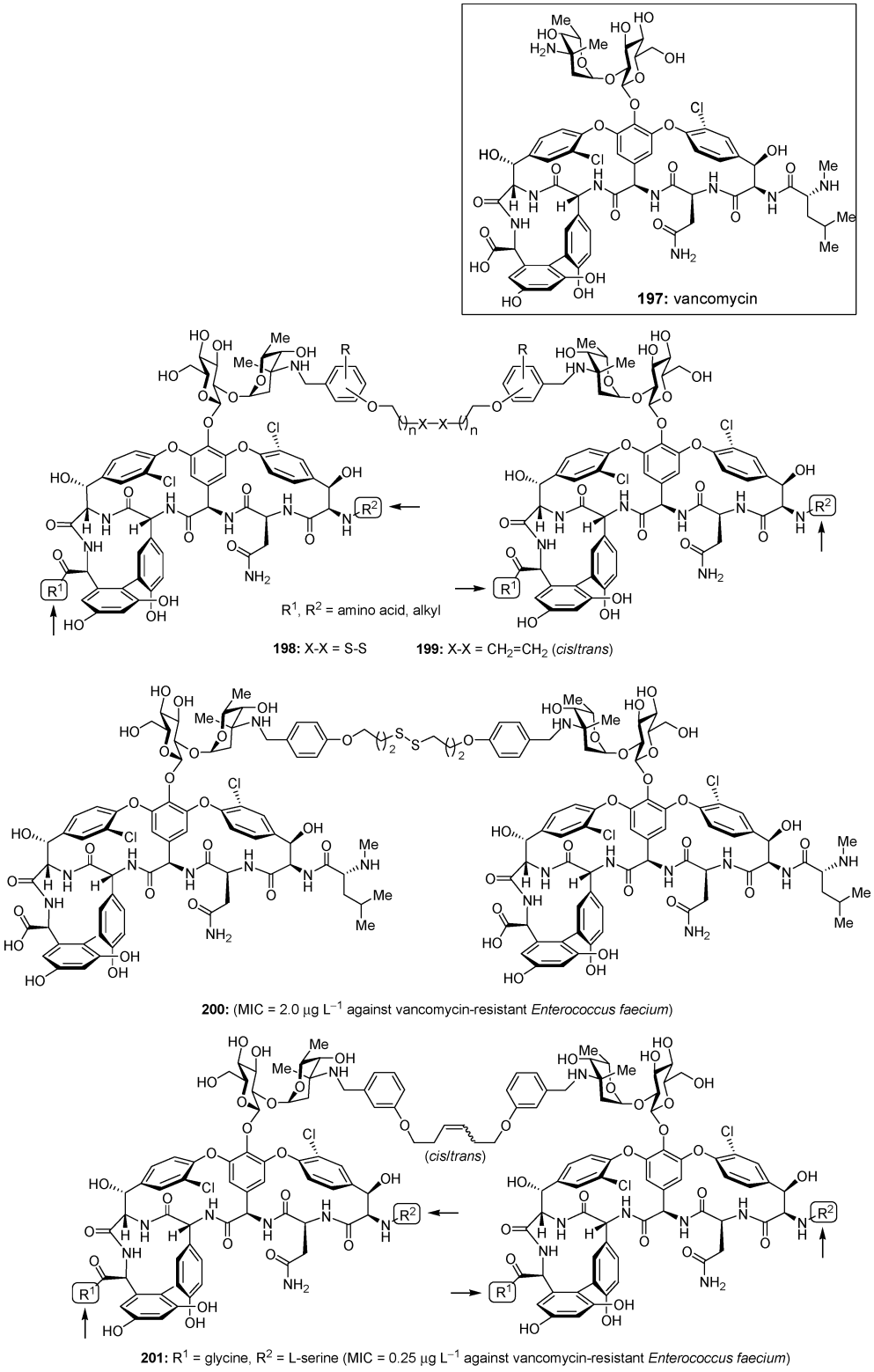


Figure 41. Vancomycin and vancomycin dimer libraries.

in Figure 41, compounds **200** and **201**, are most notable for their high potency against vancomycin-resistant bacteria (**200**, MIC = 2.0 μg mL^{-1} against vancomycin-resistant *Enterococcus faecium*; **201**, MIC = 0.25 μg mL^{-1} against vancomycin-resistant *Enterococcus faecium*).

The integrins are a family of cell surface proteins involved in cell−cell and cell−matrix adhesion.[107] Peptide and antibody antagonists of integrin $\alpha_v\beta_3$ block angiogenesis and inhibit tumor growth. As part of a program directed toward developing non-peptide small-molecule integrin antagonists,[55,108] we designed and synthesized a 12-membered series of *o*-nitroaryl ether, thioether, and amine non-peptide mimetics for biological evaluation (Figure 42). In collaboration with the Cheresh group at Scripps, these compounds were screened against a variety of integrins, including $\alpha_v\beta_3$, $\alpha_{IIb}\beta_3$, and $\alpha_v\beta_5$, for their binding affinity and selectivity toward

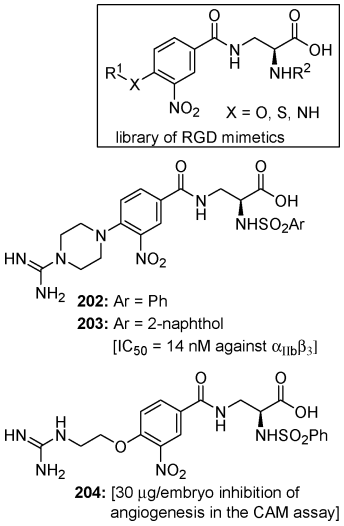


Figure 42. Designed integrin antagonists and antiogenesis inhibitors. IC_{50} values refer to inhibitions of the $\alpha_{IIb}\beta_3$-receptor, and CAM is chick chorioallantoic membrane.

these biological targets as well as for their ability to inhibit cell adhesion. A number of selected compounds were also tested for their ability to inhibit angiogenesis in vivo using the chick chorioallantoic membrane (CAM) assay. From those compounds tested, *o*-nitroarylamines **202** and **203** proved to be the most potent and selective inhibitors of $\alpha_{IIb}\beta_3$ ($IC_{50} = 14$ nM), whereas *o*-nitroaryl ether **204** (Figure 42) exhibited impressive in vivo inhibition of angiogenesis (at 30 μg/embryo) in the CAM assay.[109] These compounds may prove to be useful as tools or lead compounds for further chemical biology studies and other investigations directed at new chemotherapeutic agents.

Owing to the benzopyran structural motif being found in thousands of natural products, a benzopyran-type library of well over 10000 members (general structure **205**; specific examples **206**−**211**; Figure 43) was designed and synthesized using novel synthetic technologies. Cycloloading of certain prenylated phenols onto a novel phenylselenyl bromide resin allowed for the efficient construction and manipulation of the dimethylbenzopyran framework by solid-phase synthesis.[110] Chemical biology studies employing these compound libraries resulted in several discoveries, including those highlighted in Figure 44.

Initial screening of the library against certain bacterial strains in collaboration with a Bayer group led to the discovery of several lead compounds, including **212** (Figure 44). This lead compound was then optimized by redesigning and synthesizing focused libraries, leading to **213** (Figure 44), a highly potent antibacterial agent active at a level as low as $IC_{50} = 2$ μg/mL against a number of bacterial strains, including methicillin-resistant *Staphylococcus aureus* (MRSA).[111]

In another study and in collaboration with the Cassida group, we identified lead compound **214** (Figure 44) which, after optimization through medicinal-type chemistry, led to compound **215** (Figure 44), a potent inhibitor of the mitochondrial enzyme complex NADH−ubiquinone oxidoreductase ($IC_{50} = 18$ nM). Inhibitors such as **215** were found to exhibit cytostatic activity against a range of cancer cell lines.[112]

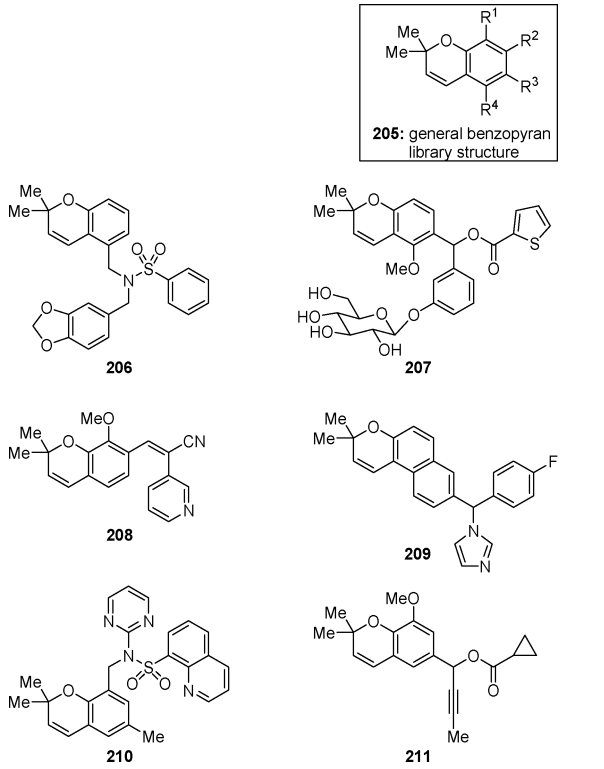


Figure 43. General benzopyran library (over 10 000 members) structure and representative examples of compound members.

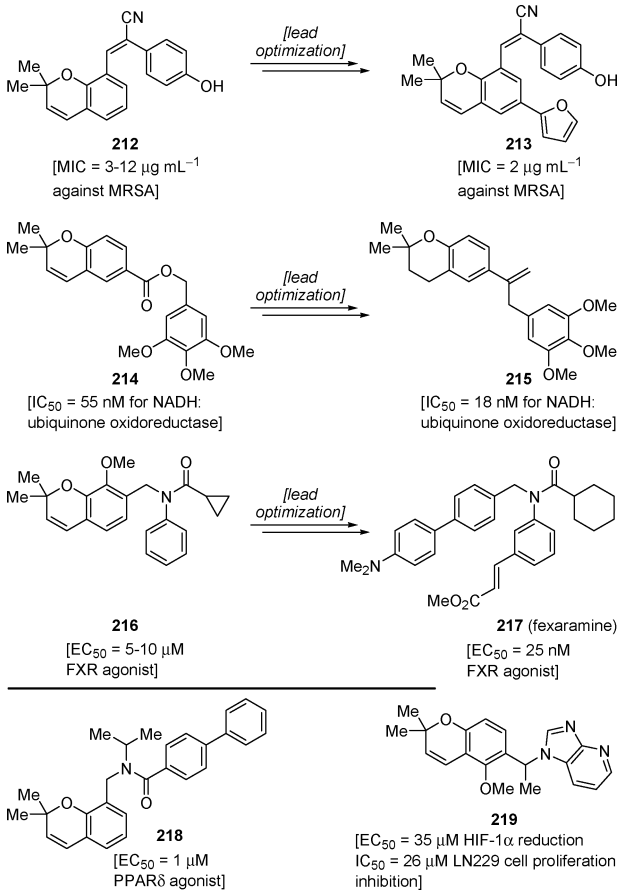


Figure 44. Selected chemical biology applications of the benzopyran library.

In a similar chemical biology endeavor with the Evans group at the Salk Institute for Biological Studies (La

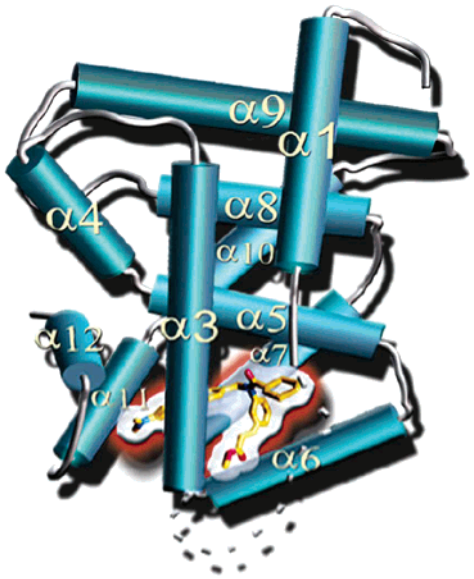


Figure 45. X-ray derived structure of the FXR-fexaramine (**217**) complex.[113]

Jolla, CA), we were able to screen the same benzopyran library against several nuclear receptors. Using the farnesoid X receptor (FXR), a bile acid sensor involved in modulating cholesterol metabolism, the library yielded several leads. A follow-up screen using a focused library of around 200 benzopyrans provided a range of low-affinity agonists for FXR (EC_{50} = 5−10 μM), of which **216** (Figure 44) was among the most promising.[113] Further optimization of this lead led to a number of nanomolar ligands for this protein, with compound **217** (Figure 44) being the most potent. Named fexaramine, this high-affinity ligand (EC_{50} = 25 nM) facilitated important structural biology studies. Thus, the complex formed from **217** and FXR yielded to X-ray crystallographic analysis, leading to a high resolution (2.8 Å) structural elucidation of not only the protein itself but also its binding site with a bound fexaramine molecule (see Figure 45).[113] In a separate study with the Salk group involving screening against the PPARδ receptor, a number of lead compounds such as **218** (Figure 44) were identified and are currently being optimized, aiming for selective and potent ligands for this important biological target.[114]

Recently, a screen of the benzopyran library in collaboration with the Van Meir group at Emory University led to the discovery of an inhibitor of the hypoxia-inducible factor 1 (HIF-1) pathway. This pathway, which is central to a cell's response to a low-oxygen state, has become a target for the treatment of solid tumors. Benzopyran **219** (Figure 44) was found to inhibit production of the HIF-1α protein (EC_{50} = 35 μM) and cell proliferation in the LN229 glioblastoma human cancer cell line (IC_{50} = 26 μM).[115] This lead is currently being optimized because a potent inhibitor of this biological target may prove to be useful in cancer chemotherapy.

These results demonstrated the usefulness of the benzopyran library in ligand discovery for chemical biology studies and elevated this project to an exemplary status, for it not only provided the opportunity for the invention of new and enabling synthetic technologies but also precipitated important discoveries in biology.

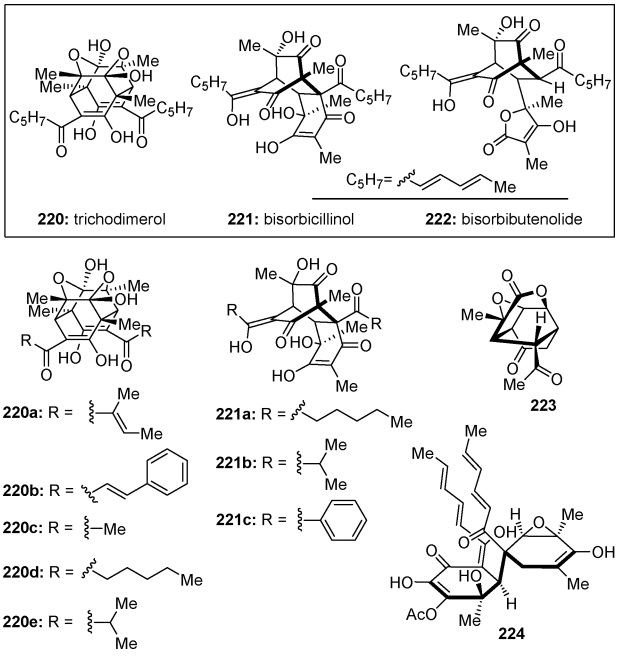


Figure 46. Bisorbicillinoids and synthesized analogues thereof.

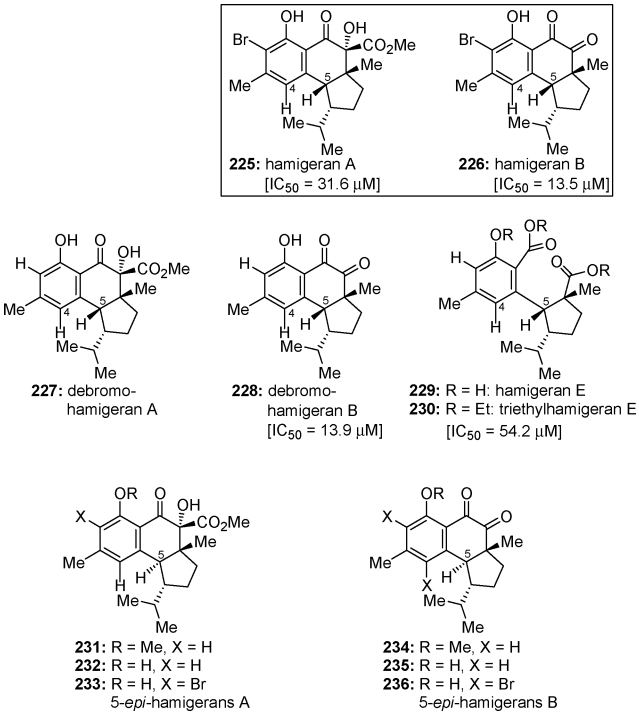


Figure 47. Hamigerans A and B and designed analogues thereof. IC_{50} values refer to in vitro cytotoxicity against leukemia P-388 tumor cells.

Indeed, the record of this library as a platform for discovery in biology and medicine bodes well for its potential for future contributions to these areas, for there remain so many biological targets to be explored through screening and optimization studies.

The bisorbicillinoids[116] (trichodimerol (**220**),[117] bisorbicillinol (**221**),[117c] and bisorbibutenolide (**222**);[118] Figure 46) exhibit significant biological properties. Thus, **220** is an inhibitor of lipopolysaccharide-induced production of tumor necrosis factor α (TNF-α) in human monocytes, while **221** and **222** show antioxidant properties. Our biomimetic total syntheses of these novel natural products were accompanied by the construction of a series

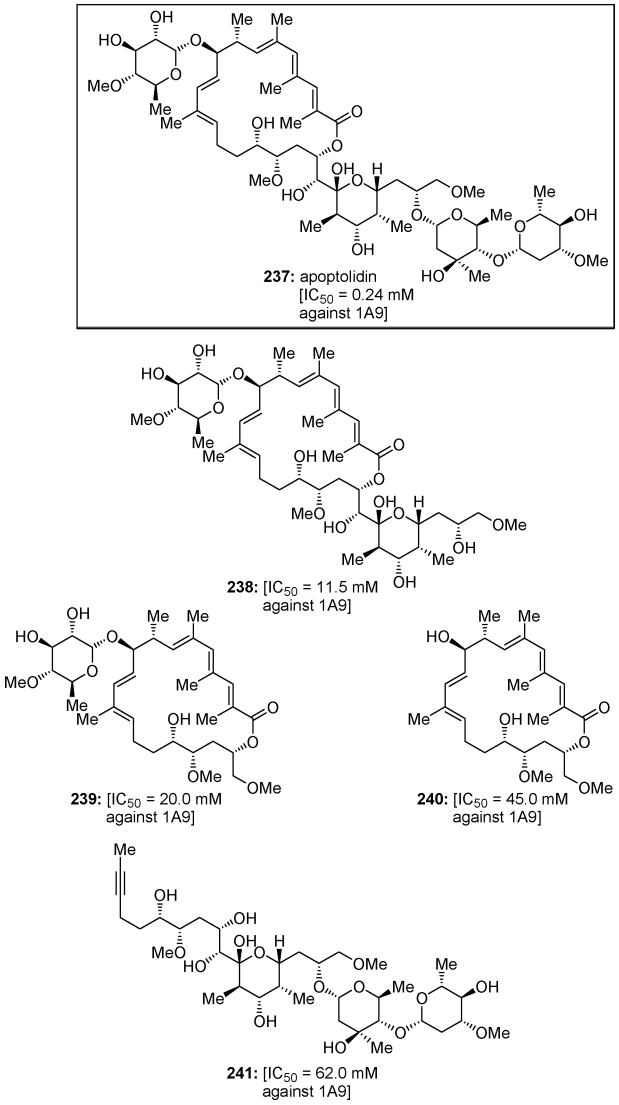


Figure 48. Apoptolidin and analogues thereof for SARs. IC_{50} values refer to 1A9 human ovarian carcinoma cells.

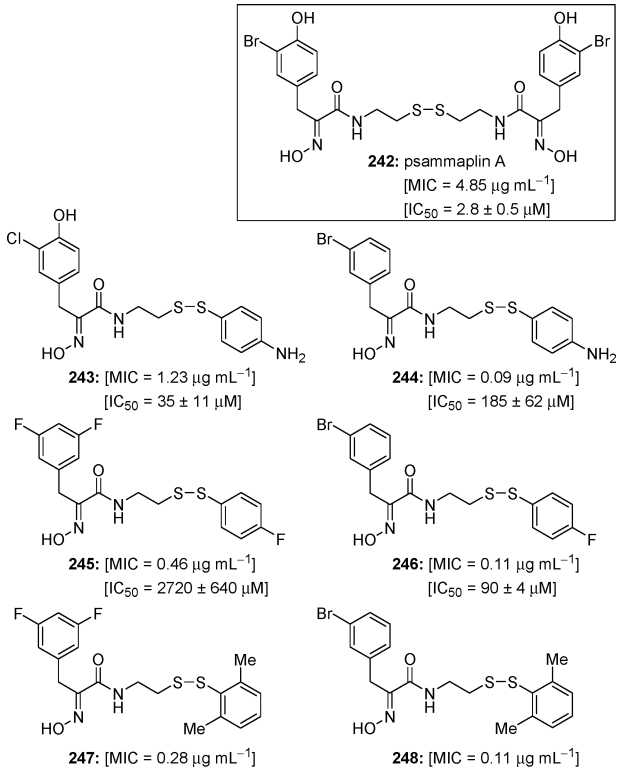


Figure 49. Psammaplin A and designed analogues with potent antibacterial activities. MICs are the averages of the following nine bacterial strains: *Staphylococcus aureus* ATCC 6538, *S. aureus* ATCC 13709, *S. aureus* ATCC 29213, *S. aureus* ATCC 25923, *S. aureus* ATCC 700698, resistant to methicillin and with heterogeneous susceptibility to vancomycin, *S. aureus* ATCC 43300 resistant to methicillin, *S. aureus* ATCC 700787 resistant to methicillin and intermediate susceptibility to vancomycin, *S. aureus* ATCC 700788 resistant to methicillin and intermediate susceptibility to vancomycin, and *S. aureus* ATCC 700789 resistant to methicillin and intermediate susceptibility to vancomycin. IC_{50} values (μM) refer to inhibition of *Mycobacterium tuberculosis* detoxification enzyme mycothiol-*S*-conjugate amidase (MCA).

of designed and accidental structural analogues, a small collection (**220a**−**e**, **221a**−**c**, **223**, **224**) of which is shown in Figure 47.[119] Such compounds may prove to be useful tools in future biological studies or as potential leads for ligand development against certain disease-related biological targets.

The biological properties of the hamigerans (e.g., **225**, **226**, Figure 47) range from cytotoxicity against P-388 leukemia cells (e.g., 4-bromohamigeran B, $IC_{50} = 13.5$ μM) to antiviral activity against herpes and polio viruses [e.g., hamigeran B, $IC_{100} = 132$ μg/disk).[120] As part of our total synthesis program in this area, we constructed at least 15 hamigeran analogues, some of them shown in Figure 47 (**227**−**229**, **231**−**236**),[121] for further biological studies.

Apoptolidin (**237**, Figure 48)[122] constituted a highly valued and challenging synthetic target because of its potent cytotoxicity and complex molecular architecture. Its impressively selective induction of apoptosis in rat glia cells transformed with adenovirus E1A and E1B19K oncogenes, and hence its potential in medicine, elevated considerably its appeal as a synthetic target, prompting us to undertake its total synthesis.[123] As part of that campaign, we established key SARs as shown in Figure 48. Thus, it was interesting to watch the molecule progressively lose its potency against 1A9 human ovarian carcinoma as it was stripped from its carbohydrate units (see compounds **238**−**240**, Figure 48) or its polyunsaturated macrocycle (see structure **241**, Figure 48).[123a]

Psammaplin A (**242**, Figure 49)[124] is a unique, marine-derived natural product with antibiotic activity.[125] Its symmetrical nature about the disulfide bond allowed for a special strategy of combinatorial synthesis to be applied to analogue construction. Coined "combinatorial scrambling strategy", this technology enabled the rapid synthesis and biological evaluation of a library of over 3800 analogues, leading to an informative set of SARs (see Figure 50) from which emerged a number of highly potent psammaplins, examples of which are those shown in Figure 49.[126] It is notable that all of these analogues (**243**−**248**) are considerably more potent than the naturally occurring substances against a panel of bacterial strains. Some of them (**243**−**246**, Figure 49) were found to be inhibitors of mycothiol-*S*-conjugate amidase (MCA) from *Mycobacterium tuberculosis*.[127] Since MCA has been proposed as a target for the development of new types of antituberculars and other antimycobacterial agents, these findings may prove to

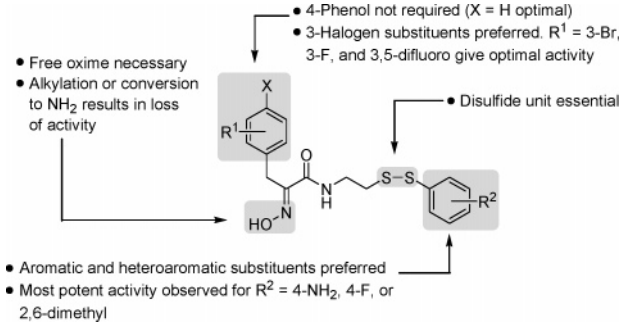


Figure 50. SARs of heterodimer analogues of psammaplin A.

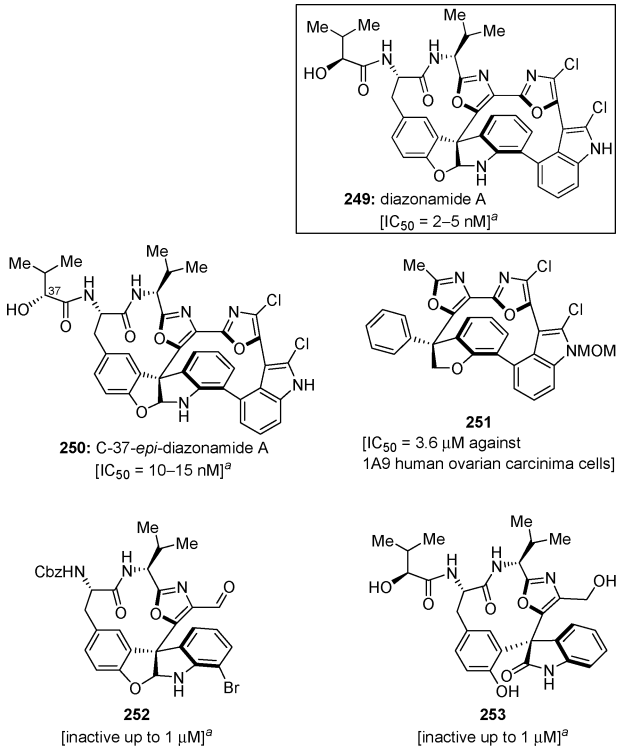


Figure 51. Diazonamide A and analogues thereof. Footnote *a* in the figure indicates that the compounds were tested in growth inhibition assays with 1A9 human ovarian carcinoma, PC-3 human prostate carcinoma, MCF-7 human breast carcinoma, A549 human lung carcinoma cell lines and the drug-resistant cell lines derived from the 1A9 parental cells A2780/AD10 and 1A9/PTX10.

be useful in the search for new drugs for the treatment of tuberculosis and other related diseases.

During our quest for diazonamide A (**249**, Figure 51),[128] a scarce marine natural product with potent antitumor properties,[129] we had the opportunity to design and synthesize a series of analogues for chemical biology studies.[128b] Thus, in addition to the natural product itself (**249**), its C-37 epimer (**250**) and the simpler analogues **251**−**253** (Figure 51) were synthesized and tested against a number of tumor cell lines, including 1A9 human ovarian carcinoma and its paclitaxel-resistant sibling 1A9/PTX10 cell line. Interestingly, only compound **250**, containing the complete structural framework of the natural product, showed comparable activity (IC_{50} = 10−15 nM) against several cell lines (1A9 human ovarian carcinoma, PC-3 human prostate carcinoma, MCF-7 human breast carcinoma, and A549 human lung carcinoma cell lines and the two

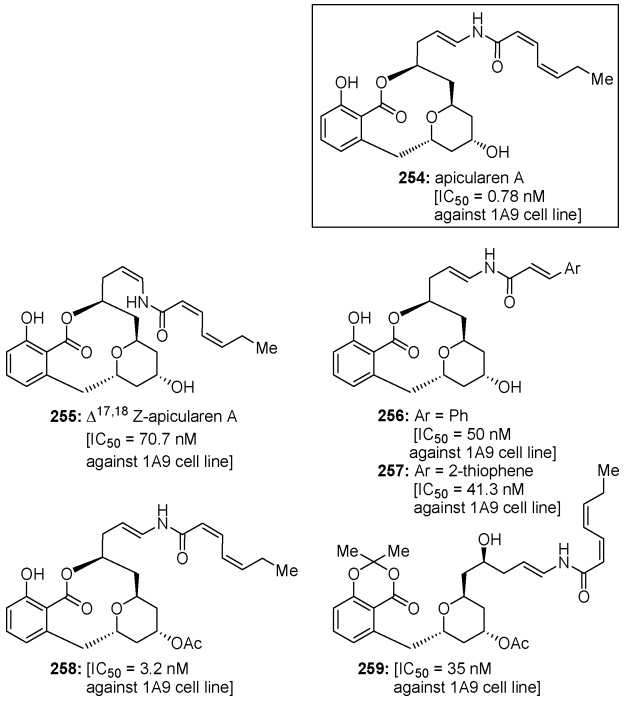


Figure 52. Apicularen A and designed analogues (16 analogues synthesized). IC_{50} values refer to cytotoxicity against the 1A9 human ovarian carcinoma cells.

drug-resistant cell lines derived from the 1A9 parental cells: A2780/AD10 and 1A9/PTX10) to that exhibited by synthetic diazonamide A (IC_{50} = 2−5 nM against every cell line tested from those mentioned above except for the A2780/AD10 line). These studies suggested that one macrocycle alone is not enough to endow the molecule with potent cytotoxicity against the tested cells.

Apicularen A (**254**, Figure 52) is a prominent member of the benzolactone acylenamide class of natural products with potent cytotoxic properties.[130] As part of our program directed toward its total synthesis, we designed and synthesized a focused analogue library for chemical biology studies. These investigations led to some interesting SARs within the apicularen family and the identification of a number of bioactive analogues, including compounds **255**−**259** (Figure 52). These studies underscored the importance of the acylenamide structural motif for biological activity and suggested the possibility of simpler structures (e.g., **259**) as mimics of the natural product.[131,132]

Lateriflorone (**260**, Figure 53)[133] and gambogin (**261**, Figure 53)[134] are members of the *Garcina* family of natural products whose intriguing molecular architectures and biological properties elevated them to a highly attractive status as synthetic targets. Our program within this area resulted in the total synthesis of three members of the class, namely, *O*-methylforbesione,[135] *O*-methyllateriflorone (**262**),[136] and gambogin (**261**) (Figure 53).[137] The developed technology was applied to the construction of several analogues of these natural products, including those shown in Figure 53 (**262**−**266**). These designed molecules enabled the establishment of certain SARs within these structural motifs and are path-pointing with regard to future chemical biology and medicinal chemistry investigations. Particularly interesting were the lateriflorone analogues **263** and **264**, which exhibited potencies comparable to that of

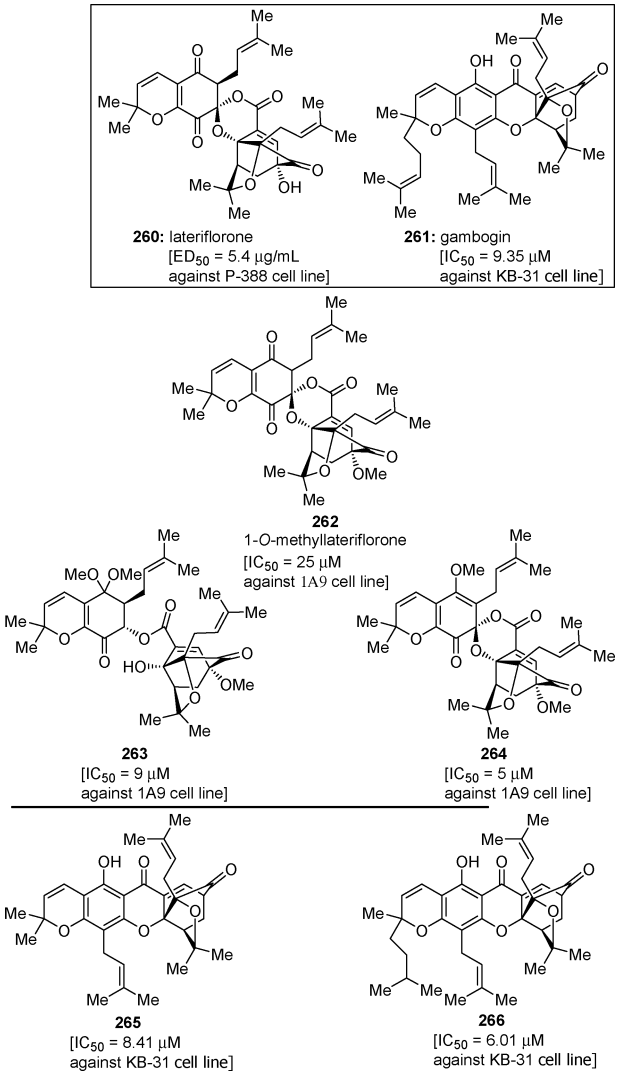


Figure 53. Lateriflorone, gambogin, and analogues thereof. ED_{50} and IC_{50} values refer to cytotoxicity against cancer cell line P-388, ovarian cancer cell line 1A9, and human epidermoid cancer cell line KB-31.

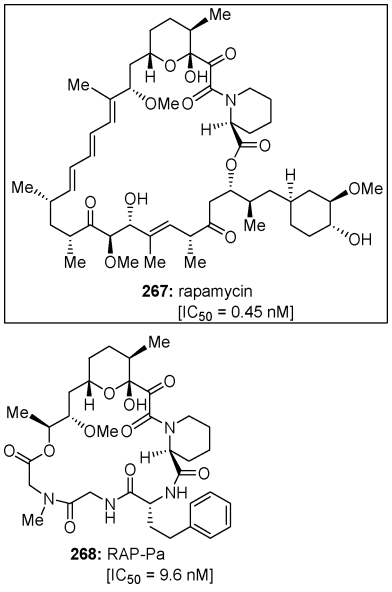
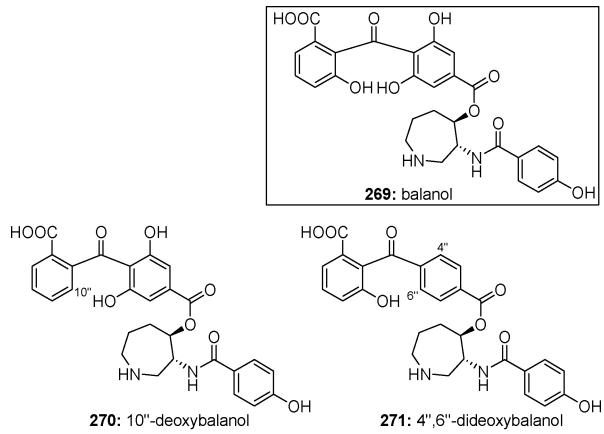


Figure 54. Rapamycin and rapamycin-based FKBP12 ligand PAR-Pa. IC_{50} values refer to binding affinities for FKBP12.



Kinase	269	270	271
PKA	4.7 ± 1.3	3.9	3.4
PKC	5.3 ± 1.8	640	80

Figure 55. Balanol and designed analogues. Inhibition constants are given by $K_i = [(IC_{50})(K_d)]/(L + K_d)$, where K_d is the apparent affinity for ATP and L is the concentration of ATP. Values are shown in nM.

their natural product (**260**),[136] and the analogues of gambogin **265** and **266**, which also proved to be equipotent to the naturally occurring counterpart (**261**).[137]

The naturally occurring immunosuppressive agent rapamycin (**267**, Figure 54) is a powerful tool for studying signal transduction mechanisms and other biological phenomena.[138] This synthetically challenging molecule was synthesized in our laboratories in 1993.[139] As part of this campaign and with the aim of designing FKBP12 binding ligands whose complexes may or may not bind to the second cellular target involved in the mechanism of action of rapamycin, we designed and synthesized the rapamycin-based ligand RAP-Pa (**268**).[140] This compound proved to bind tightly to FKBP12, but in contrast to rapamycin, it showed no affinity in IL-6 dependent B-cell proliferation, confirming that both the binding domain and the effector domain of the molecule are required to produce the biological effect.

As part of our program directed toward the total synthesis of balanol (**269**, Figure 55), a highly potent inhibitor of protein kinase C (PKC), we designed and synthesized a series of analogues of the natural product for biological studies.[141] Among them, the 10″-deoxy (**270**) and 4″,6″-dideoxy (**271**) balanols (Figure 55) showed potent and selective inhibitory activity against cPKA (**270**, IC_{50} = 6.3 nM; **271**, IC_{50} = 5.5 nM) versus PKC (**270**, IC_{50} = 834 nM; **271**, IC_{50} = 111 nM) (see also K_i constants, Figure 55), demonstrating the ability to differentiate between these two kinases, despite their homology[142] (Figure 55). These inhibitors may serve as useful biological tools in deciphering signal transduction pathways.

During the campaign[143] to synthesize brevetoxin B (**272**, Figure 56), the marine neurotoxin associated with the "red-tide" phenomena, we had the opportunity to probe the importance of the length of the molecule to its biological properties. Thus, employing the synthetic technologies developed in this program, we were able to construct the truncated brevetoxin B analogue **273** (~20 Å long)[144] and, in collaboration with the Baden group, determined[145] that it was not ichthyotoxic at micromolar concentrations and exhibited decreased receptor binding affinity compared to brevetoxin B,

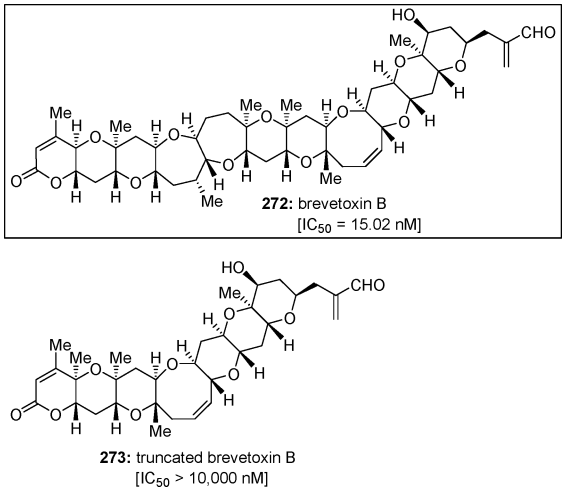


Figure 56. Brevetoxin B and truncated analogue. IC_{50} values refer to synaptosome binding activity against 1.8 nM [^{3}H]-PbTx-3.

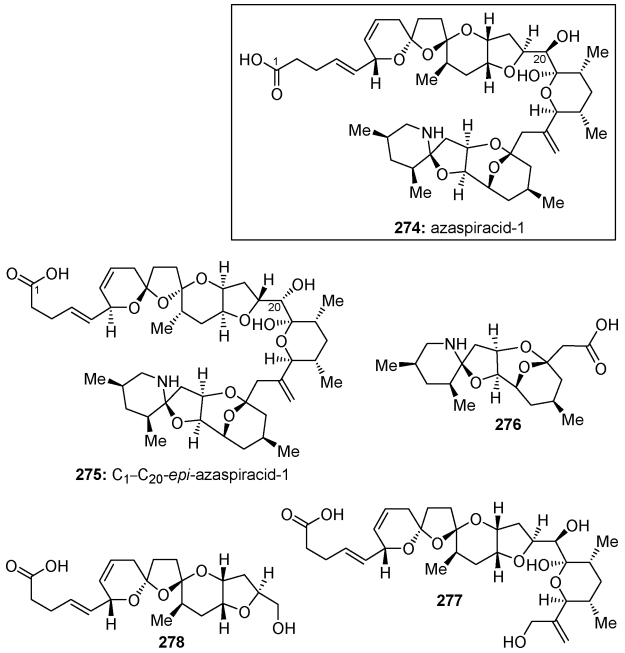


Figure 57. Azaspiracid 1, C_1–C_{20}-*epi*-azaspiracid-1 and truncated analogues for biological investigations.

causing only a shift of activation potential without affecting mean open times or channel inactivation. These findings supported the hypothesis that the strong binding of brevetoxin B to sodium channels hinges heavily not only on its rigid framework but also on its length (~30 Å).

Azaspiracid-1 (**274**, AZA-1, Figure 57), the notorious poison found in mussels and synthesized in our laboratories in 2004[146] through a campaign that also resulted in the revision of its originally proposed structure,[147] provided us with the opportunity to study its chemical biology through analogue construction. Thus, by synthesizing a number of epimeric and truncated azaspiracids such as **275**–**278** (Figure 57), we were able to establish the first SARs within the azaspiracid class with regard to their toxic effects. Of these compounds only AZA-1 (**274**) and its diastereomer C_1–C_{20}-*epi*-AZA-1 (**275**) exhibited significant toxicity in mice, with compound **275** being one-quarter as toxic as **274**. The lack of toxicity of the severely truncated analogues **276**–**278** (Figure 57) implies that the entire structure of AZA-1, or at least a major part of it, is required for biological activity.[148] Further studies, now made possible through this work, are directed toward the elucidation of the mechanism of action of azaspiracid-1.

I hope the projects briefly highlighted above provide further illustrations of the ways that natural products chemistry, aided by its powerful ally, chemical synthesis, contributes to biology and medicine. Thus, total synthesis endeavors offer unique avenues to enrich the supplies of scarce bioactive substances and therefore enable their thorough biological investigation. Furthermore, such campaigns provide opportunities for the design and synthesis of variations of natural substances for testing as potential improvements. As a conclusion, it cannot be denied that whether in search of biological tools or drug candidates, the combination of leads from nature, chemical synthesis, and rational design as means to reach them remains unsurpassed.

Acknowledgment. In acknowledging my many coworkers whose names may or may not appear on the cited papers, I express one more joy: the pleasure of having been able to share with them all the joys described in this article and of the A. C. Cope Award. That the American Chemical Society decided to bestow on me this special honor is due, to a large measure, to the brilliant contributions of my co-workers, and it reflects on them as much as it does on me. I also express my humble thanks and appreciation to my wife, Georgette, and to my children, Colette, Alexander, Christopher, and P. J., for their understanding and unconditional love, without which my life would not have been so enjoyable and rewarding. My deep gratitude and appreciation are also extended to my long-serving staff members Vicky Nielsen Armstrong and Janise Petrey for their crucial contributions over the years and to Dr. Theocharis Koftis for his help with this manuscript. I gratefully acknowledge the National Institutes of Health, The Skaggs Institute for Chemical Biology, and my many friends from the pharmaceutical and biotechnology industries for their financial support over the years.

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JM050524F