A Selective Account of Effective Paradigms and Significant Outcomes in the Discovery of Inspirational Marine Natural Products[⊥,†]

Koneni V. Sashidhara, Kimberly N. White, and Phillip Crews*

Department of Chemistry and Biochemistry, University of California Santa Cruz, Santa Cruz, California 95064

Received December 24, 2008

Marine natural products continue to be a source of significant molecular structures that serve as a stimulus to seed further significant research. This account reviews some of the major advances in the study of marine biomolecules made at UC Santa Cruz over more than three decades. The continuing challenge of discovery and characterization of what we term "inspirational molecular structures" will be presented in a comprehensive fashion. Examples of privileged molecular structures and their impact on biomedicinal research will be an important theme. The three major groups of organisms explored include seaweeds, sponges, and marine-derived fungi, and the study of their active principles has greatly benefited from synergistic collaborations with both academic and biopharmaceutical groups. The concluding sections of this chronicle will touch on prospects for future outcomes involving new sources and strategies.

Introduction

The field of marine natural products chemistry has a rich history, and it offers continued promise for breakthroughs impacting many areas of science, especially chemical biology. In the early days our work was quite chemo-centric, but today it is interdisciplinary and benefits from synergistic collaborations with both academic and biopharmaceutical groups. A continuing core task involves the discovery and characterization of what we term "inspirational molecular structures". A major focus has been to exploit these structures as seeds for additional fundamental research alongside their development as potential therapeutic leads and/or application as molecular probes.

We have made substantial progress, especially since 1985, when the work of our group began to fully take shape; however there are still uninvestigated frontiers. Overall, there are eight initiatives that continue to guide our research, some of which are illustrated in Figure 1. These broadly based endeavors include (a) discovery and characterization of new small molecules emphasizing those from polyketide synthases (PKS), nonribosomal peptide synthetases (NRPS), and mixed PKS−NRPS pathways, (b) developing new and known structures as biomedically relevant leads, (c) engaging in crisp de novo structure elucidation accompanied by efficient dereplication, (d) using the natural products from coral reef sponges and marine-derived fungi (sourced from sponges or sediments) as stimuli for further inquiry, (e) engaging in careful taxonomic identification of all organisms explored, (f) biogeographical studies of sponges with high-value metabolites, (g) developing new methodologies for creating the libraries from macro and micro marine organisms, and (h) employing novel culture strategies for expanding the libraries. The sections that follow contain examples of insights obtained from exploration of these ideas. Of equal importance will be highlights showing the difficulties encountered and the significance of discoveries made.

To date, our laboratory has brought to light nearly 1000 compounds from marine sponges and marine-derived fungi. Years ago, a repository was created to house these compounds and several thousand of the following: (a) sponge material ready for processing, (b) crude extracts and semipure fractions, (c) preserved microbial cultures, and (d) sponge taxonomic voucher specimens. A large amount of information exists for these materials, and it is managed by powerful relational databases and web-based chemoinformatics. The pure compounds, compound rich mixture extracts, and our emerging repository of peak libraries constitute invaluable resources for detailed biomedical and related research, especially as new therapeutically relevant molecular targets are discovered. Also important is that the inherent chemical complexities of the structures in our repository provide robust materials for collaborative projects focused on new emerging technologies in chemical biology research.

It is relevant to discuss an early event that was the impetus for beginning a program of marine organic chemistry at UC Santa Cruz. Two significant books provided important foundation reading. The first was the monograph published in 1973 by Paul Scheuer with the title *Chemistry of Marine Natural Products*.[1] It described some 430 compounds organized by biogenetic chemical class and clearly showed the advantages of looking at the marine environment for new molecular structures. Sometime in 1974, one of us (P.C.) came across a book entitled *Poisonous and Venomous Marine Animals of the World*,[2] published in 1965, and was fascinated to read in the chapter on Porifera that extracts from sponges had been shown to possess antibiotic and antiparasitic properties. More exciting was the short section describing the chemistry of the bioactive principles; it contained only one word, "unknown", indicating that this could be a frontier topic for future research. As a phylum, sponges are an incredibly attractive research target because of their high biodiversity, widespread distribution, and unique aquiferous biology. Sponges are well known as hosts for a variety of microorganisms, and they provide a steady stream of nutrients for symbionts held within the choanocyte chambers. Typically, sponges pump a liter of water per cm^3 of tissue per hour. In addition, it is estimated that there are in excess of 5000 species of sponges, and this is undoubtedly a conservative estimate. For a variety of reasons, it took several years to become fully immersed in the chemistry of this phylum, in part because for eight years our attention was diverted by fascinating studies involving halogenated compounds from red seaweeds. Once attention had been refocused on sponges as a source of new chemical entities, the goal to engage in anticancer therapeutic lead discovery was also begun.

The other source for natural products currently being pursued by our group is marine-derived fungi. The natural history of this taxonomic group remains poorly understood with no reliable estimates of the overall numbers of species. In addition, the overall interest in the chemistry of fungi is growing because some consider

[⊥] Dedicated to Dr. David G. I. Kingston of Virginia Polytechnic Institute and State University for his pioneering work on bioactive natural products.

[†] Adapted from a Norman R. Farnsworth Research Achievement Award address, 49th Annual Meeting of the American Society for Pharmacognosy, Athens, Greece, August 3−8, 2008.

* To whom correspondence should be addressed. Tel: (831) 459-2603. Fax: (831) 459-4197. E-mail: phil@chemistry.ucsc.edu.

10.1021/np800817y CCC: $40.75
Published on Web 02/11/2009

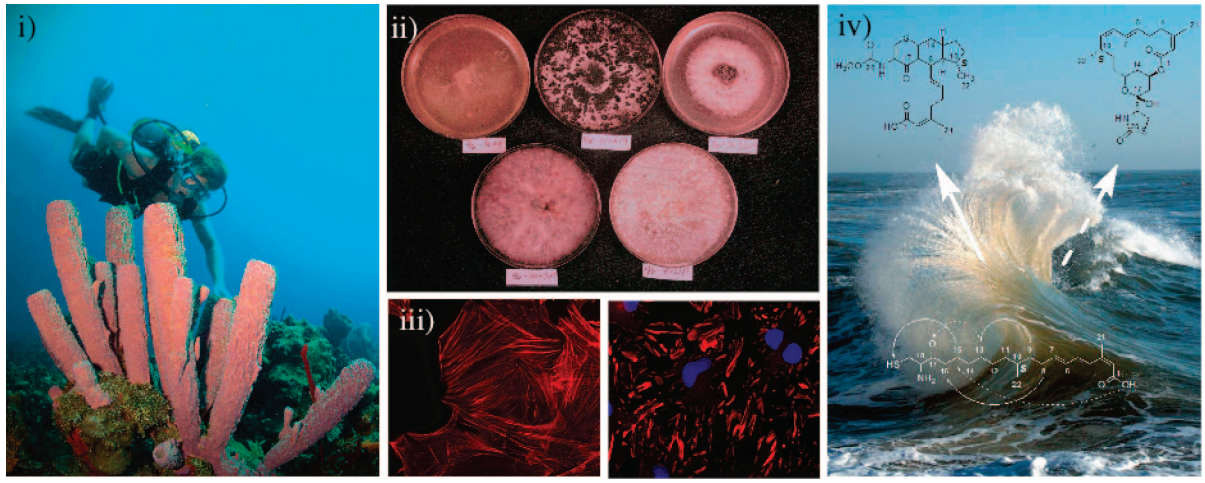


Figure 1. Overview of reseach initiatives: (i) sponges, (ii) fungi, (iii) cytoskeletal screens, and (iv) biosynthetic relationships.

Table 1. Selected Marine Natural Products in Development as Anticancer Drugs

clinical trial	name	class	source	target	discoverer
In Clinical Use	ectenaiscidin 743 (Yondelis)	NRP	tunicate	tubulin	PharmaMar, Rinehart
phase III	E7389 (halichondrin B inspired)[a]	PK	synthetic	tubulin	Eisai
phase II	dehydrodidemnin B (Aplidine)	PK-NRP	tunicate	ornithine decarboxylase	PharmaMar, Rinehart
phase II	soblidotin (aka TZT1027, dola-10 insp.)	NRP	synthetic	tubulin	Teikoku, Pettit
phase II	synthadotin (aka ILX651, dola-15 insp.)	NRP	synthetic	tubulin	ILEX
phase II	bryostatin 1	PK-NRP	bryozoan	PKC	GPC Biotech, Pettit
phase II	squalamine	aminosteroid	shark	angiogenesis	Zasloff
phase II	kahalalide F	NRP	mollusk	multiple	PharmaMar, Scheuer
phase I	PM02734 (kahalalide insp.)	NRP	synthetic	solid tumor	PharmaMar
phase I	Zalypsis (jorumycin insp.)[a]	alkaloid	synthetic	DNA	PharmaMar
phase I	E7974 (hemiasterlin insp.)[a]	NRP	synthetic	tubulin	Eisai
phase I	taltobulin (aka HTI286, hemiasterlin insp.)[a]	NRP	synthetic	tubulin	Wyeth, Andersen
phase I	salinosporamide A (aka NPI0052)	PK-NRP	bacteria	proteasome	Nereus, Fenical
phase I	spisulosine (aka ES285)	lipid	clam	Rho	PharmaMar
phase I	KRN-7000 (agelasphin insp.)[a]	lipid	synthetic	NKT	Koezuka-Kirin
phase I	NPI 2358 (halimide insp.)	alkaloid	synthetic	tubulin	Nereus, Fenical
phase I	LBH 589 (psammaplin insp.)[a]	alkaloid	synthetic	HDAC	Novartis
Discontinued					
phase II (<2004)	dolastatin 10	NRP	sea hare	tubulin	Pettit
phase II (<1999)	didemnin B	PK-NRP	tunicate	antineoplastic	Rinehart
phase II (<2004)	cemadotin (dola-15 insp.)	NRP	synthetic	tubulin	BASF, Pettit
phase II (<2002)	cryptophycin 52 (≈ arenastatin)[a]	NRP	synthetic	tubulin	Lilly, Valeriote
phase I (2004)	discodermolide[a]	PK	sponge	tubulin	Novartis, HBOI
phase I (2002)	LAF 389 (bengamide insp.)[a]	PK	synthetic	MetAP	Novartis, Crews
phase I (<2006)	LAQ 824 (psammaplin insp.)[a]	alkaloid	synthetic	HDAC	Novartis, Crews
phase I (<2000)	girolline (aka girodazole)[a]	alkaloid	sponge	protein synthesis	Potier

[a] Substances from marine sponges.

them among the world's greatest untapped resources for new biodiversity as well as chemodiversity.

The challenges encountered, especially in choosing specific taxa to study and with structure elucidation, along with the lessons learned are the major focus of this rather personal account. The research carried out on the UC Santa Cruz campus has involved a wide range of individuals including students from chemistry, oceanography, or biology programs, working alongside professional staff with skills at the interface of chemistry and biology. This brief perspective is intended to showcase the journey and important marine natural product milestone discoveries made at UC Santa Cruz over the last three decades.

Another Important Preamble

Research based on marine natural products with the ability to provide anticancer therapeutic leads is an important national priority. The current cancer statistics show that an expected 1 437 180 new cancer cases will be diagnosed and more than 565 650 Americans are expected to die of cancer (more than 1500 people a day) by the end of 2008.[3] This has been a motivating element in our research targeting solid tumor cancers, which together account for more than 65% of all cancer deaths in the U.S. It is clear that additional therapeutic interventions are needed, and the potential for marine natural products, especially from sponges, to make positive contributions now seems firm. There is an ever-expanding list of marine natural products or synthetics inspired by marine-derived compounds currently in or about to enter cancer clinical trials, as summarized in Table 1. One compound, ecteinascidin 743 (now called Yondelis or trabectedin) from the tunicate *Ecteinascidia turbinata*,[4] has emerged from this process. It has been approved by countries in the European Union to treat patients with advanced soft tissue sarcoma.[5]

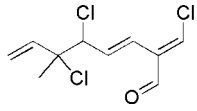
The additional entries of Table 1 illustrate the various marine natural product derived structural classes under evaluation in anticancer clinical trials. There are 16 compounds undergoing current trials, with 10 derived from total synthesis and nine created after a SAR study of a parent lead compound (indicated by "insp."). Strikingly, the list is also well represented by substances from marine sponges (indicated by "a"). Among the most complex structure listed is E7389, the subject of a phase III trial and a synthetic compound designed from the active core of the sponge

natural product halichondrin B.[6] Biosynthetic structural features from the NRPS or PKS−NRPS pathways are present in five of the six agents being evaluated in phase II trials. Of the nine compounds currently in phase I, five are synthetic agents based on sponge-derived products. One important seed compound of this group is psammaplin A, first discovered by our group[7] and eventually found to be a dual histone deacetylase (HDAC)[8] and DNA methyl transferase inhibitor. Its structure contributed to the design, by the medicinal chemistry group at Novartis, of LBH 589.[9] One entry of Table 1, NPI 2358,[10] a synthetic compound based on a unique diketopiperazine isolated from cultures of a marine-derived fungus, is in a phase I trial and underscores the potential of this group to contribute significant chemical structures.

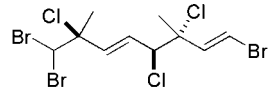
There are eight additional entries in Table 1 involving compounds based on marine natural products that were discontinued from clinical trials. One of these, discodermolide,[11] is a deep water sponge-derived natural product. Four other entries are either based on or related to sponge-derived natural products. These include cryptophycin 52[12] (based on arenarol[13]), LAF 389[14] (based on bengamide A,[15] extensively studied in our laboratory), LAQ 824[16] (inspired by psammaplin A,[7] as noted above), and girolline[17] (also known as girodazole). Overall, the list of Table 1 indicates that the possible mitigating factor of resupply or synthesis of a complex marine natural product is not a deterrent to advancing for clinical investigation marine biomolecules, which cannot be obtained in large amounts from nature. Finally, there are a number of marine natural product preclinical candidates under study worldwide, and some structures elucidated at UC Santa Cruz will be discussed later.

The Early Days: Some Triumphs and Annoying Diversions

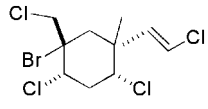
The tide pools in central California are teaming with red algae including the chemical-rich genus *Plocamium*. Our very first project in marine natural products chemistry utilized such alga and resulted in the isolation of cartilagineal[18] (**1**), an unusual polychlorinated monoterpene aldehyde from specimens of *P. cartilagineum* (Dixon), abundant in the intertidal zones north of Santa Cruz. This genus was selected for study because its crude extracts contained a host of new polyhalogenated monoterpenes, and some were toxic to goldfish. Some compounds were also found to be active in anti-insect screens carried out at the now defunct company Zoecon. The discovery of compound **1** was significant, as it was the first halogenated monoterpene to be reported from a red alga. This paper appeared slightly after a report of parallel research by the late Prof. Faulkner, resulting in the isolation of (3*R*,4*S*,7*S*)- *trans*,*trans*-3,7-dimethyl-l,8,8-tribromo-3,4,7-trichloro-1,5-octadiene[19,20] (**2**), the first halogenated monoterpene obtained from a southern California sea hare, *Aplysia californica*, which grazes on *P. cartilagineum* (aka *coccinium*).



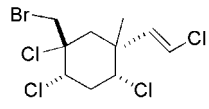
cartilagineal (**1**)



(3*R*,4*S*,7*S*)-*trans*,*trans*-3,7-dimethyl-1,8,8-tribromo-3,4,7-trichloro-1,5-octadiene (**2**)



violacene original (**3**)

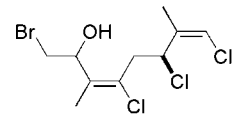


violacene revised (**4**)

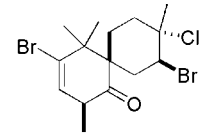
At the point in time when these simple polyhalo-monoterpenes were first being isolated and described, the NMR experimental methods now routinely used to accurately perform structure elucidation had not yet been developed.[21] This caused annoying problems; for example, the initial structure proposed for violacene[22] (**3**), isolated from *P. violaceum*, was eventually corrected to **4**, based initially on our analysis of ^{13}C NMR shifts and definitively on the X-ray data collected of crystals we obtained.[23]

We and others found that the study of any member of the *Plocamium* genus was guaranteed to give publishable results, and some of the relationships between the compounds observed versus the species studied provided results relevant to chemical ecology. These patterns, mostly taken from our work, included the following: (a) *P. cartilagenium* contained acyclic trihalo and pentahalo analogues, (b) the polyhalo monoterpenes of *P. violaceum* (summarized in Table 2) included alicyclic structures (**4** and plocamene B (**8**)) or acyclics (headed by preplocamane A (**13**)), (c) compounds from *P. oregonum* were dominated by Br- and Cl-containing acyclics (such as **2**), (d) *P. costatum* from Tasmania was also a source of acyclic oxygen-containing analogues (costatol (**15**)), and (e) *P. costatum* from the Australia Barrier Reef contained acyclics identical to those of *P. cartilagenium*.[24−27]

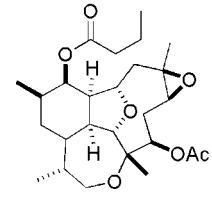
Once we learned that *P. violaceum* was cosmopolitan along the Pacific Coast of California and Oregon, the next logical step was to obtain and study it from diverse Pacific coastal habitats. Sampling was accomplished from 26 different collection sites, divided into three major geopolitical zones, as shown in Table 2: (a) southern Oregon, (b) northern California, and (c) central California. There were 11 polyhalogenated sesquiterpenes observed, and from a biosynthetic perspective, they could be organized into three different categories (Table 2): regular alicyclic isoprenoids (including **4**−**8**), rearranged alicyclic isoprenoid B (including **9**, **10**), and acyclic precursors preplocamenes (including **11**−**14**).[24−27] The relative ratios of these compounds were invariant at the individual collection sites, and the relative composition did not vary seasonally. While the morphology of *P. violaceum* was that same over the geographical range sampled, its biosynthetic machinery producing halomonoterpenes was quite different. Significantly, these data provided two principles: (a) there could be chemotype (CT) variations, and (b) there were advantages to conducting a biogeographical study. Our categorizations of the chemotypes for *P. violaceum* were defined by apparent biosynthetic relationships as follows: (a) collections made south of the Monterey Bay canyon largely afforded the preplocamenes, constituting chemotype (CT)-α; (b) another set, CT-*β*, was rich in the plocamene D family and devoid of the preplocamenes; (c) taxa designated as CT-*δ* had a preponderance of plocamene B members, but often possessed small amounts of the preplocamenes; and (d) CT-*γ* collections contained substantial mixtures of both plocamene B and plocamene D structures. An outcome of making these divisions was the CT information that could be used to guide the reisolation of a specific compound type. Thus, the best source for **13** would be CT-α at "Sea Rock Motel" or **4** from CT-*β* at "Pescadero Beach". As will be illustrated later, this phenomenon is also at work with sponge populations.



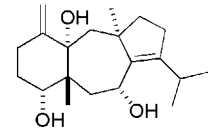
costatol (**15**)



kylinone (**16**)

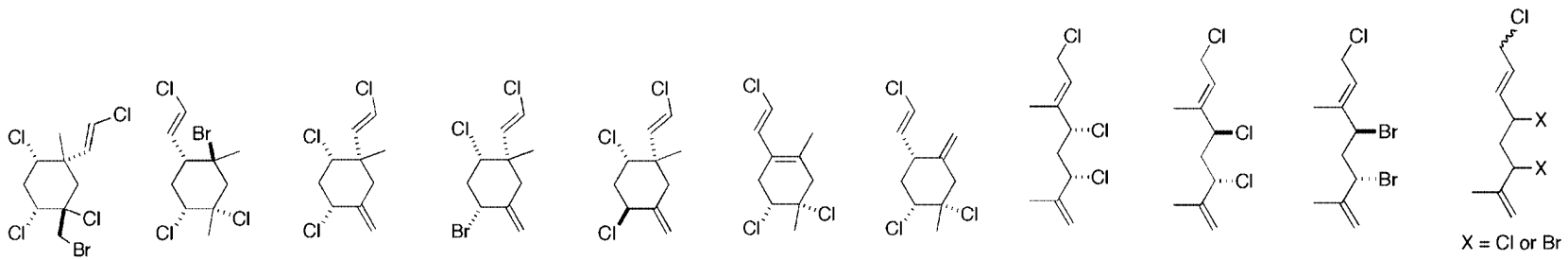


asbestinin epoxide (**17**)



dolastane triol (**18**)

Table 2. Our First Example of Organism Chemotypes through a Geographical Survey of *Placamium violaceum*[a]



violacene (**4**) | plocamene C (**9**) | plocamene D (**5**) | plocamene D′ (**6**) | *epi*-plocamene D (**7**) | plocamene B (**8**) | plocamene E (**10**) | pre-plocamene C (**11**) | pre-plocamene B (**12**) | pre-plocamene A (**13**) | **14**

location	violacene (**4**)	plocamene C (**9**)	plocamene D (**5**)	plocamene D′ (**6**)	*epi*-plocamene D (**7**)	plocamene B (**8**)	plocamene E (**10**)	preplocamene C (**11**)	preplocamene B (**12**)	preplocamene A (**13**)	**14**
Southern Oregon											
Cape Arago, North	rich	−	trace	−	trace	−	−	−	−	−	
Cape Arago, South	major	−	trace	−	−	moderate	minor	−	−	−	−
Simpson's Reef	major	−	trace	−	−	moderate	minor	−	−	−	−
Harris Beach	rich	−	−	−	−	minor	trace	−	−	−	−
Northern California											
Humbolt County											
Patrick's Point	−	−	moderate	moderate	−	−	−	−	−	−	−
Mendocino County											
Todd's Point	trace	trace	−	−	−	trace	moderate	minor	minor	−	−
Sea Rock Motel	−	−	−	−	−	−	−	−	−	rich	minor
Russian Gulch	−	−	−	−	−	−	−	moderate	minor	−	moderate
Central California											
San Mateo County											
Montara Lighthouse	moderate	minor	−	−	−	minor	moderate	−	−	−	−
Moss Beach	trace	trace	−	−	−	rich	minor	minor	−	−	−
Pescadero Beach	rich	−	−	−	−	trace	−	−	−	−	−
Bean Hollow	minor	−	minor	−	−	minor	−	−	−	−	−
Waddell Creek	rich	−	minor	−	−	minor	−	−	−	−	−
Santa Cruz County											
Davenport Landing	minor	minor	minor	−	−	moderate	minor	−	−	−	−
Bonny Doon	moderate	minor	minor	−	−	moderate	trace	−	−	−	−
Four Mile Beach	moderate	minor	minor	−	−	minor	trace	−	−	−	−
Pigeon Point (I)	minor	minor	trace	−	−	minor	minor	−	−	−	−
Pigeon Point (II)	minor	minor	−	−	−	minor	moderate	−	−	−	−
Monterey County											
Asilomar Beach	trace	−	−	−	moderate	−	−	minor	minor	minor	minor
Middle Reef Moss Beach	major	minor	−	−	−	minor	−	trace	trace	−	minor
Fanshell Beach	moderate	−	minor	−	−	minor	−	trace	trace	−	trace
Point Joe	−	−	−	−	−	−	−	trace	−	minor	minor
Pescadero Point	−	−	−	−	−	−	−	minor	minor	trace	minor
San Luis Obispo County											
San Simeon	moderate	−	−	−	−	minor	−	minor	minor	−	minor
Leffingwell Creek	major	trace	−	−	−	minor	−	−	−	−	−
Montana Del Oro	minor	minor	−	−	−	minor	−	trace	trace	−	minor

[a] rich = 80−100%, major = 60−79%, moderate = 35−59%, minor = 5−34%, trace = 0.1−5%, absent = −.

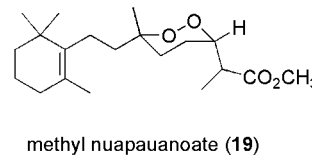
Our explorations in seaweed chemistry continued with the goal of exploring another ecological phenomenon. Collections of *Laurencia pacifica* (Kylin) and its associated epiphyte *Erythrocystis saccata* (J. Agardh) were gathered, and the idea was to compare the constituents of the host and epiphyte. A combination of spectroscopic and semisynthesis was used to characterize the total structure of kylinone[28] (**16**), a unique sesquiterpene, from the minor components of *Laurencia* extracts. The next element in this chemical ecology study involved a survey of sesquiterpenes from the epiphyte.[29] Overall, we examined three separate collections and found that the major components of the host and epiphyte were exactly parallel but varied as a function of collection location as follows: Stillwater cove, aplysin and debromoaplysin; Stillwater Cove re-collection, isolaurinterol and debromoisolaurinterol; Catalina Island, laurenisol and bromolaurenisol. The unmistakable observation that the sesquiterpenes of *E. saccata* exactly tracked those of the host was fascinating. The relative yields of sesquiterpenes from the epiphytes were much lower versus those from the host. The yields and structures were verified by GC-MS and NMR data, and the *E. saccata* was removed from the host by a surgical cut made well up on the epiphyte's thallus. We do not believe that these organisms actually engage in parallel de novo synthesis of the sesquiterpenes; however no follow-up experiments were ever conducted to provide further data to rationalize these observations.

Our initial attempts to build up collections of marine sponges for chemical study did not focus on Monterey Bay sponges. The extensive studies by Djerassi (Stanford University) indicated that Monterey Bay sponges were rich in sterols, and a strategic decision was made to pursue new chemistry from Caribbean coral reefs. One attractive specimen was initially identified as the sponge *Haliclona hogarthimi*. We successfully explored the terpenoids of that sample, culminating in the isolation of the unusual diterpene asbestinin epoxide[30] (**17**). This prompted a re-examination of the organism in its habitat, revealing that it actually was the gorgonian *Briareum asbestinum* (Pallus)!

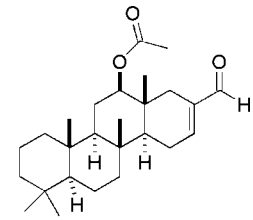
While our research focus had started the important shift to invertebrates, we were tantalized to pursue the constituents of dense mats of two intertwined brown algae that were abundant in the Caribbean Honduras Bay Islands. The extract obtained from the mixture identified as *Dictyota linearis* (C. Ag.) and *Dictyota divaricata* (Lamour) showed extreme toxicity to goldfish at 400 pg/mL (death in 90 min). Chemical investigation resulted in the isolation of the novel tricyclic diterpenes headed by a triol dolostane derivative (**18**).[31] While our results on these metabolites attracted interest, there were two lessons learned: pursuing mixed organism collections was not optimal to enable follow-up reisolation work, and dividing our resources between the study of seaweeds and sponges was not wise.

The Shift to Sponges: Building the Foundation

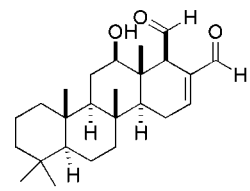
The decision in the middle 1980s to exclusively focus on marine sponges was concurrent to the start of a new UC collaborative venture, the Marine Chemistry and Pharmacology Program, funded by the California Sea Grant initiative. This innovative program facilitated compound isolation studies through pharmacological evaluation. As another important change, we shifted the expedition focus from Caribbean to Indo-Pacific habitats. The first compounds isolated were from sponges collected in the Kingdom of Tonga, and terpenoids dominated the initial discoveries. A large soft drab sponge from a Tongan coral reef sponge seemed incorrectly identified as a *Prianos* because a large number of Indo-Pacific *Diarcarnus* sponges were subsequently observed to contain the same compounds. Norterpene peroxides were isolated, with methyl nuapapuanote (**19**) being the first example of a norditerpene reported from a marine sponge.[32] Adding to this finding was that our study of the anti-inflammatory active extracts from another Tongan sponge *Hyrtios erecta* provided additional new sesterterpenes. These included the norterpenoid hyrtial[33] (**20**) and new scalaranes such as 12-deacetyl-12-*epi*-scalaradial[34] (**21**). The other astounding development was that 12 g of heteronemin (**22**) was isolated from 708 g of the dried *H. erecta*.[34] We were able to add a family of cytotoxic polyketide peroxides, such as xestin A (**23**), to our growing library of compounds through the study of an encrusting *Xestospongia* that was abundant in Fijian reefs.[35]



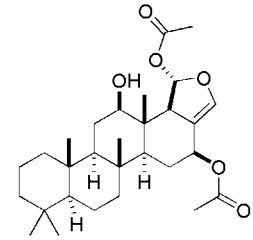
methyl nuapauanoate (**19**)



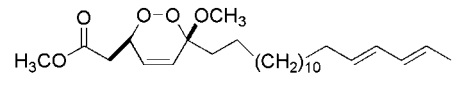
hyrtial (**20**)



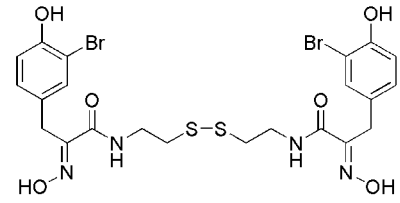
12-deacetyl-12-*epi*-scalardial (**21**)



heteronemin (**22**)



xestin A (**23**)



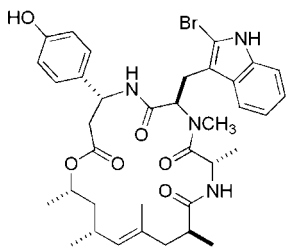
psammaplin A (**24**)

The next sets of bioactive natural products that we encountered were all nonterpenoid and often took considerable time to characterize. For example, psammaplin A (**24**) was obtained from the cytotoxic extract of *Psammaplysilla* sp. collected from Tonga. In 1987, we provided the first description of psammaplin A, having dense *N*-functionalization accompanied by S and Br heteroatoms.[36] The dual histone deacetylase and DNA methyltransferase activity we reported in 2003 has greatly stimulated interest in this compound series.[36] In fact, in 2007, 20 years after our first publication on this structure, there were 45 articles published based on the chemical biology study of **24**.

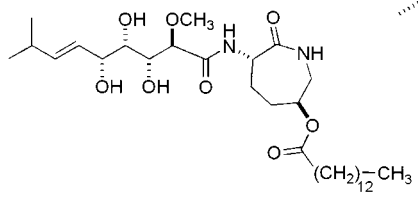
A new collaborative venture with a group at Syntex Research (Dr. T. Matthews) opened the door to extensive study of Fijian sponge metabolites. A parasite assay target, the nematode *Nippostrongylus braziliensis*, provided the pathway to identify a host of very inspirational natural products that we and others would continue to study for many decades. These included reports of specific compounds (by year) as follows: jasplakinolide[37] (**25**), 1986; bengamide A[15] (**26**), 1986; latrunculin A[38] (**27**), 1987; melyne A[39] (**28**), 1988; bengazole A[40] (**29**), 1988; mycothiazole[41] (**30**), 1988; fijianolide B[42] (**31**), 1988; suvanine[43,44] (**32**), 1988; plakinidine A[45] (**33**), 1990; xestoaminol A[46] (**34**), 1990, and fascaplysin A[47] (**35**), 1991. The structures represented in this list were varied and unprecedented at the time of their disclosure. Overall these discoveries illustrated that the use of an antiparasitic disease model

screen could provide significant outcomes. Unfortunately, we were not able to build on these rich findings because of the disinterest of the biopharmaceutical sector to engage in antiparasitic drug development. As will be discussed next, we were able to shift the focus to a cytotoxicity screening paradigm, resulting in outcomes that attracted wider outside interest.

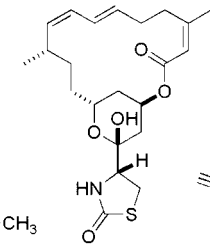
there would be two or three expeditions that would yield hundreds of sponges, and a premium was always placed on obtaining tropical sponges from less well-studied orders. Initially, the input of a UC Santa Cruz collaborating taxonomist, Dr. M. C. Diaz, was important, and eventually this task was transferred to Dr. R. W. M. van Soest at the University of Amsterdam. The difficult challenge of complet-



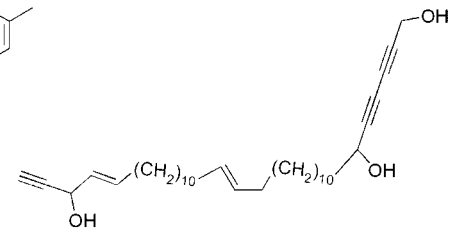
jasplakinolide (**25**)



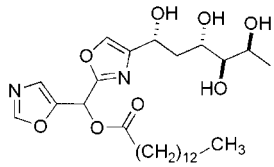
bengamide A (**26**)



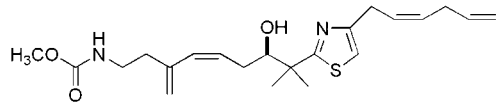
latrunculin A (**27**)



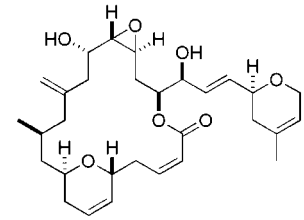
melyne A (**28**)



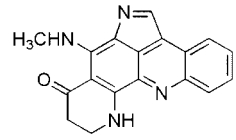
bengazole A (**29**)



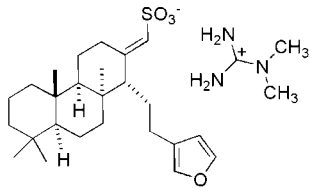
mycothiazole (**30**)



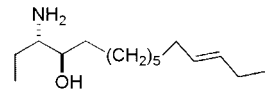
fijianolide B (**31**)



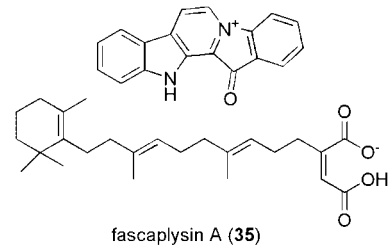
plakinidine A (**33**)



suvanine (**32**)



xestoaminol A (**34**)



fascaplysin A (**35**)

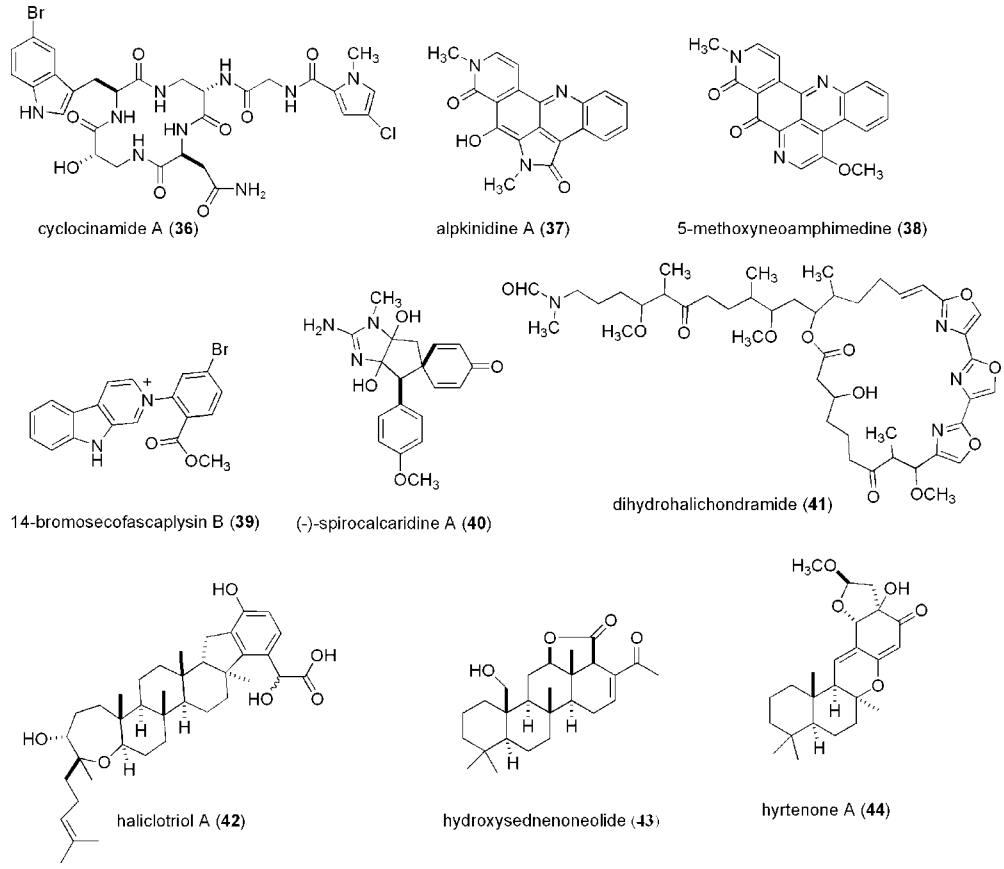
Our work on sponge-derived cytotoxic compounds began to take shape once a collaboration was established (with Prof. Valeriote, Henry Ford Cancer Center, Detroit) that employed a novel pharmacology paradigm to develop anticancer therapeutic leads from marine natural products.[48] The key tool was the solid tumor selective assay to assess differential activity among solid tumor cells (murine C38, human H-125, H-116, U251N, MCF-7, LnCaP, OVCAR), leukemia cells (murine L-1210, human CCRF-CEM), and normal cells (bone marrow committed progenitor cells). This approach is mechanism-blind, and the goal is to search for materials that will kill solid tumor cells while exhibiting less toxic effects against leukemia or normal cell lines. Having a powerful yet simple screen, it became possible to rapidly prioritize work on a variety of sponge extracts. As an important proof of concept, Valeriote and Moore collaborated using this approach in the successful evaluation of the cryptophycin family (see Table 1), subsequently evaluated in a phase I anticancer clinical trial.[49] One significant early lead we found from this screen included fascaplysin A[47] (**35**), discussed above in the antiparasite discovery effort, which was further assessed by another unique strategy referred to as a clonogenic assay. This experimental design allows determination of a cytotoxic effect at different concentrations for the targeted tumor over an extended period of time and provides the essential data to plan an in vivo trial.

Empowered by this screen we have devoted considerable effort to unearth and pursue materials that are solid-tumor selective. Often, this work began during an expedition to gather organisms to provide the extracts and compounds for investigation. Typically, each year ing formalities with foreign governments was successfully dealt with to allow collections to occur in diverse coral reef regions ranging from Papua New Guinea, Fiji, Vanuatu, Solomon Islands, Madagascar, to Venezuela.

These expeditions afforded a continuing stream of significant lead compounds discovered from sponges. Foremost among this group were sponge-derived heterocycles, many of which were nitrogen containing. A sample of these included cyclocinamide A[50] (**36**), alpkinidine A[51] (**37**), the 5-methoxy neoamphimedine[51] (**38**), 14-bromosecofascapysin B[52] (**39**), spirocalcaridine A[53] (**40**), and dihydrohalichondramide[54] (**41**). Additional compounds of interest and devoid of nitrogen functionality included haliclotriol A[55] (**42**), hydroxysednenoneolide[56] (**43**), and hyrtenone A[57] (**44**). Accompanying each of these compounds was a number of analogues for which the accompanying bioactivity properties provided more information about the active pharmacophore.

Sponges: Current Milestone Discoveries

Jasplakinolide[37] (**25**), also called jaspamide,[58] has been of continuing importance to our research. Rather unusual is **25**, which can be isolated in reasonable yield from two Indo-Pacific sponges in separate taxonomic orders, *Jaspis splendens* (order Tetractinomorpha) and *Auletta* cf. *constricta* (order Ceractinomorpha). It is a potent actin filament stabilizer and also inducer of actin polymerization.[59] More significantly, jasplakinolide has emerged as an important molecular tool even though it was unsuccessful in progressing through the steps required for preclinical development as an anticancer chemotherapeutic. Reflecting its widespread use



are the annual literature citations on jasplakinolide in cell biology studies, which are very large (>50 papers/year).

Once the collaborative arrangement with Prof. Valeriote (Ford Cancer Center) was fine-tuned, eye-catching results began to emerge. These broad-based strategies that are now standard tools in our collaborative program consist of (i) discovery and structural elucidation of new biomolecules (at UCSC), (ii) implementation of in vitro anticancer screening (at Ford Cancer Center), (iii) advanced pharmacological evaluation (at Ford Cancer Center and at the NCI), and (iv) field biology (at UCSC).

Recently, four compounds have emerged as the most important entities for continued study and are shown in Figure 2. Heading this list is psymberin[60] (**45**), a sponge-derived PKS−NRPS biosynthetic product whose structure and astounding cytotoxicity profile were published after a 10-year campaign to isolate it from *Psammocinia bulbosa*. Although we described the sponge-derived polyketide fijianolide B[42] (**31**) decades ago, it took a sustained effort to obtain additional SAR and therapeutic understanding. We recently revised the structure of mycothiazole[61] (**30**) and are making good progress in the preclinical evaluation of this nanomolor active compound. Even though the latrunculins have been studied for almost 30 years, we have identified a new analogue, 18-*epi*-latrunculol A[62] (**46**), of current interest, because this cytotoxin did not exhibit microfilament-disrupting activity, common to all other latrunculin analogues.

Psymberin (45). The significant physical and biological properties of (+)-psymberin (**45**), identical to those independently reported for (+)-irciniastatin A (**45**),[63] make this a "privileged" molecular structure. Leukemia cell lines are relatively insensitive to **45**, whereas impressive activities are observed against solid tumors: (+)-psymberin (e.g., LC_{50} < 2.5 nM vs MDA-MB-435 breast cancer line),[64] Pettit's data for (+)-irciniastatin (e.g., GI_{50} = 5.2 nM vs MCF-7 breast cancer line),[63] and de Brabander's data for synthetic (+)-psymberin (e.g., IC_{50} = 1 nM vs PC3 prostate cancer line).[65] The bioactivity of two synthetic psymberin diastereomers plus evaluation of the designed compound psympederin[65] underscore that the unaltered (+)-psymberin structure has the best activity. The biological properties of (+)-**45** are also distinct versus the structurally related (+)-pederin[66−68] and its multitude of analogues. A multifaceted process was used for the experimental therapeutics evaluation of (+)-**45**. A clonogenic dose−response study (see above) was conducted using HCT-116 cells carried out at 2, 24, and 168 h with 90% cell kill as follows: 2 h ≥3 ng/mL, 24 h ≥2 ng/mL, and 168 h ≥20 pg/mL. These data predicted (a) the in vivo HCT-116 cell therapeutic effect could be observed either as a bolus or on chronic administration, and (b) exposure of tumor cells to **45** must be above 3 ng/mL for 2 h, 2 ng/mL for 24 h, or 20 pg/mL for 7 days. The maximum tolerated dose (MTD) of **45** = 125 μg/kg for SCID mice and 25−50 μg/kg (NCI Developmental Therapeutics Program). Finally, HCT-116 tumor bearing SCID mice treated with (+)-**45** using a bolus injection (125, 62, and 31 μg/mouse) showed that the highest dose was toxic, while the second and third doses gave %T/C values of 75% and 86%, respectively, at 23 days. This demonstrates modest but encouraging therapeutic efficacy of (+)-**45**. The NCI-DTP program hollow fiber assay using multiple solid tumor cell lines[69] also gave a positive outcome: overall score = 34 (active score ≥20). The follow-up xenograft testing has also begun at the NCI.

Fijianolide B (31). This marine-derived polyketide was characterized simultaneously in 1988 at UCSC[42] as (−)-fijianolide B and at the University of Hawaii as laulimalide.[70] Significantly, **31** and analogues promote microtubule stabilization. Research needed to further demonstrate the preclinical potential for this molecular structure has been completed recently. These involved (a) obtaining a biogeographical understanding of the most reliable sponge chemotypes as a source of (−)-**31** and new analogues, (b) scaling up the isolation of (−)-**31** to launch in vivo trials in tumor-bearing mice, and (c) extending the record of SAR through biological screening of new fijianolides possessing functionality not previously created through synthesis. The cytotoxicities exhibited by (−)-**31** illustrate its significance and include natural (−)-**31** (KB IC_{50} = 29 nM[71] and MDA-MB-435 IC_{50} = 5.7 nM[72]) versus synthetic

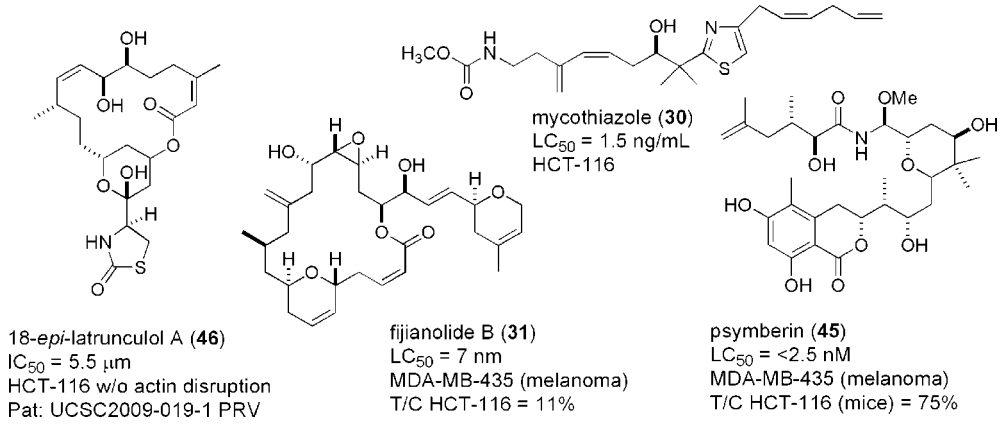


Figure 2. High-priority sponge-derived natural products.

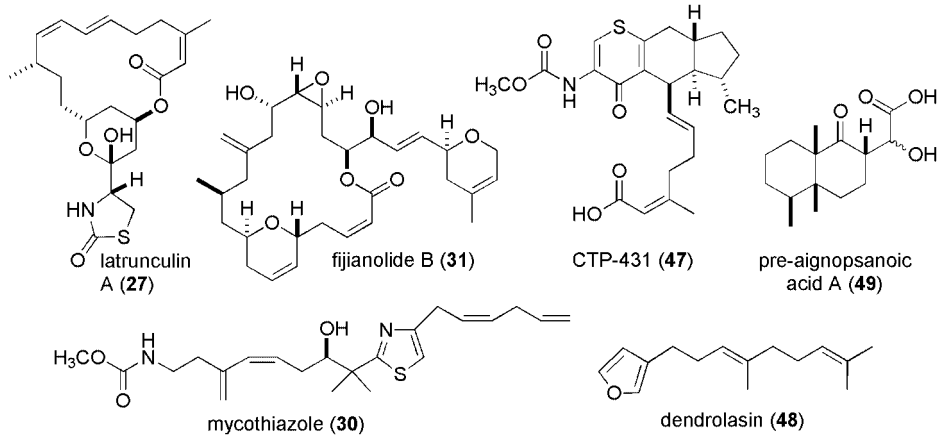
(−)-**31** (MDA-MB-435 IC_{50} = 2 nM). These impressive biological data have motivated 11 total syntheses for (−)-**31** from eight different research groups.[73] In addition, five different teams have prepared 35 synthetic congeners of (−)-**31**.[74−84] None of the synthetic analogues obtained to date have exhibited greater in vitro potency in comparison to (−)-**31**. The in vivo assessment demonstrated that **31** significantly inhibited the growth of HCT-116 tumors. SCID mice implanted with tumor cells were treated with **31** starting 3 days after tumor inoculation and followed until day 30. Bolus compound administration (iv, daily for 5 days) at 12.5 and 25 mg/kg/day showed that the best activity was achieved at 25 mg/kg/day. The minimal %T/C values were 80% at day 9 for the lower dose and 11% at day 11 for the higher dose; body weights of mice receiving all doses increased throughout the 30 days and were identical to untreated controls. These results support that further in vivo therapeutic evaluation of **31** is merited, and we recommend this compound as a clinical candidate in the treatment of solid cancer tumors.[85]

Mycothiazole (30). This rare sponge-derived metabolite has an appealing structure and compelling bioactivity properties uncovered by Dr. Valeriote (Ford Cancer Center). The IC_{50} values against H116 cells in liquid culture are 1.8 and 1.2 ng/mL (median value of 1.5 ng/mL). The MTD has been determined to be approximately 3 mg/kg. Clonogenic dose−response studies have shown that the 2 and 24 h values are >10 μg/mL, and the 7-day study is ongoing. The NCI mean graph data for mycothiazole (NSC 647640) are encouraging and indicate that it is selective against several tumor cell lines such as DMS 114 (small-cell lung cancer) and NCI-H23 (non-small-cell lung cancer). The closest COMPARE analysis match in the NCI database is methotrexate (NSC 740, formerly amethopterin), an antimetabolite used clinically to treat certain cancers, severe psoriasis, and adult rheumatoid arthritis. Recent studies by Prof. Nagle (University of Mississippi, unpublished data) suggest that mycothiazole inhibits HIF-1 (hypoxia-inducible factor 1) activation in breast and prostate tumor cells. It also inhibits hypoxia-induced secreted VEGF (vascular endothelial growth factor) at low nM concentrations.[86] Several research groups have accomplished partial and total syntheses of mycothiazole subunits and analogues.[87] However, no total synthesis of mycothiazole with the revised stereochemistry from *E* to *Z* at C-14, 15 has been completed. It has been proposed by others completing the synthesis and biological evaluation of simplified mycothiazole analogues that the nature of the heterocyclic moiety seems to modulate the cytotoxic activity (against HCT-15 colon cancer cells).[88]

18-*epi*-Latrunculol A (46). Almost 30 years ago Kashman, while engaged in the study of the Red Sea sponge *Negombata magnifica* (old genus designation *Latrunculia*), isolated and studied the two seminal compounds latrunculin A (**27**) and latrunculin B.[89,90] These compounds have a macrolide 1,3-fused to a tetrahydropyran containing a 2-thiazolidinone side chain. Interestingly, latrunculin A shares a carbon skeleton with the terrestrial myxobacterium-derived anticancer agent epothilone B.[91] We justified additional study of latrunculin analogues because of (a) their mixed PKS/NRPS biogenetic origin, (b) their potent actin inhibition properties (latrunculin A is a widely used small-molecule molecular probe), and (c) their potent cytotoxicity against cancer cell lines. Surprisingly, in spite of the situation that latrunculins have been described from several sponges and can be accessed through total synthesis, few comprehensive experimental therapeutic studies have been conducted on this family. We recently completed such a study that involved isolation and evaluation of 13 analogues.[62] The striking activity profile for **46** is intriguing, and apparently an 18*S* configuration of its thiazolidinone ring diminishes the anti-actin effect without eliminating the cytotoxicity properties. This pattern does not appear to hold for the latrunculin B series, as the microfilament-disrupting activities were similar in analogues where the configuration changes from *R* to *S*. As a final observation, the activity profile of **46** seems to be similar to that of oxolatrunculin B,[92] as each may inhibit cancer cell line growth by an actin-independent pathway.

Chemotypes of *Cacospongia mycofijiensis.* Obtaining and examining biogeographical-based collections of sponges whose extracts have exhibited solid tumor selectivity in the in vitro cytotoxicity disk diffusion assay can be quite rewarding.[93,94] Applying this approach facilitated gaining a comprehensive understanding of the variations in the constituents of *Cacospongia mycofijiensis*. Prior to reinvestigations undertaken in 2002, we believed that the components of this sponge varied among four different biosynthetic categories, and lead structures are shown in Table 3. The list here includes dendrolasin[95] (**48**, sesquiterpene), fijianolide B[42] (**31**, polyketide), latrunculin A[89] (**27**, mixed PKS−NRPS), and mycothiazole[41] (**30**, mixed PKS−NRPS). Two parallel projects provided fresh insights, and this occurred through a study of samples in our repository alongside obtaining additional sponge material from new sites. The serendipitous isolation of CTP-431[96] (**47**) transpired during the reinvestigation of a Fijian collection of *C. mycofijiensis*. We believe that CTP-431, possessing a very distinctive structure, is biosynthetically related to latrunculin A. The other development came about during our survey of 15 individual specimens from the pooled northern Papua New Guinea collection of this sponge. The discovery of preaignopsanoic acid (**49**)[97] from two of these was exciting, as this structure defines an entirely new sesquiterpene class, distantly related to the 4,9-friedodrimane family. We now recognize, as shown in Table 3, that six different structural families can be isolated from *C. mycofijiensis*, and a maximum of five occur in taxa from a single geographical zone. Understanding about the variation in major (and minor)

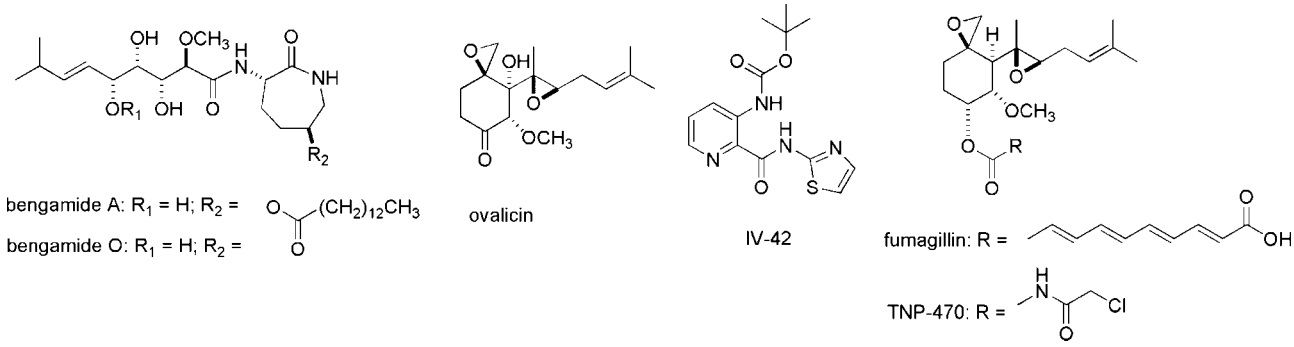
Table 3. Biogeographical Variations in the Constituents of *Cacospongia mycofijiensis*[a,b]



collection site	fijianolides	CTP-431	latrunculins	aignopsanes	mycothiazole	dendrolasin
Fiji[c]	yes	yes	yes	no	yes	yes
Vanuatu[d,e]	yes	no	yes	no	yes	no
Solomon Islands	no	no	yes	no	no	yes
Papua New Guinea	yes	no	yes	yes	yes	no
Tonga	no	no	yes	no	yes	no
Indonesia	yes	no	yes	no	yes	no

[a] Previously known as *Spongia mycofijiensis.* [b] Sanders, M. L.; van Soest, R. W. M. *Biologie* **1996**, *88*, 117−122. [c] Kakou, Y.; Crews, P. *J. Nat. Prod.* **1987**, *50*, 482−484. [d] Quinoa, E.; Kakou, Y.; Crews, P. *J. Org. Chem.* **1988**, *53*, 3642−3644. [e] Crews, P.; Kakou, Y.; Quinoa, E. *J. Am. Chem. Soc.* **1988**, *110*, 4364−4368.

Table 4. Proof of Concept Examples of Natural and Synthetic MetAP Specific Inhibitors



compound	inhibition of enzyme activity: MetAP1 (IC_{50} μM)	inhibition of enzyme activity: MetAP2 (IC_{50} μM)	source
bengamide A	2.0	11	sponge
bengamide O	3.0	>50	sponge
ovalicin	NA	0.0004	fungus
IV-43	2.0	>300	synthetic
fumagillin	NA	0.03	fungus
TNP-470	NA	0.001	synthetic

components among various sponge chemotypes can be used in a variety of circumstances including (a) planning the successful reisolation of specific bioactive constituents, (b) executing traditional biosynthetic studies involving the injection of labeled biosynthetic precursors, or (c) developing molecular genetics studies to define a biosynthetic gene cluster.

Probing the Molecular Targets of the Bengamides. Few structural changes are tolerated in the bengamide A framework in order to maintain maximum cytotoxicity.[98] Interestingly, the profile in the NCI 60 cell line panel for bengamides A and B (see **26**) were unique versus all the standard antitumor compounds in the NCI database.[99] The significant in vivo antitumor activity observed for bengamides A, B, and LAF389 lead to the launch of a clinical evaluation of the latter, but it was eventually terminated, in part due to unpredictable cardiovascular toxicity.[100]

A more complete understanding of the antitumor mechanism of action for bengamide A is continuing to emerge. From a historical perspective, the bengamides[101] represent the second class of MetAP inhibitors to be described.[102] The family of enzymes known as methionine aminopeptidases (MetAPs) catalyzes the removal of *N*-terminal methionine from newly synthesized proteins.[103] Prokaryotic organisms typically have only one type of MetAP, whereas eukaryotes and humans have two isoforms, MetAP1 and MetAP2. Currently, both isoforms are considered relevant as a target for cancer chemotherapy. MetAP2 has been identified as the possible target for the fungal-derived antiangiogenic compounds shown in Table 4, ovalicin and fumagillin, which also effect T cell activation.[104,105] The finding that these compounds inhibit MetAP2 and not MetAP 1 was also considered to be significant. Other inhibitors of MetAP2 have been reported as potential therapeutic

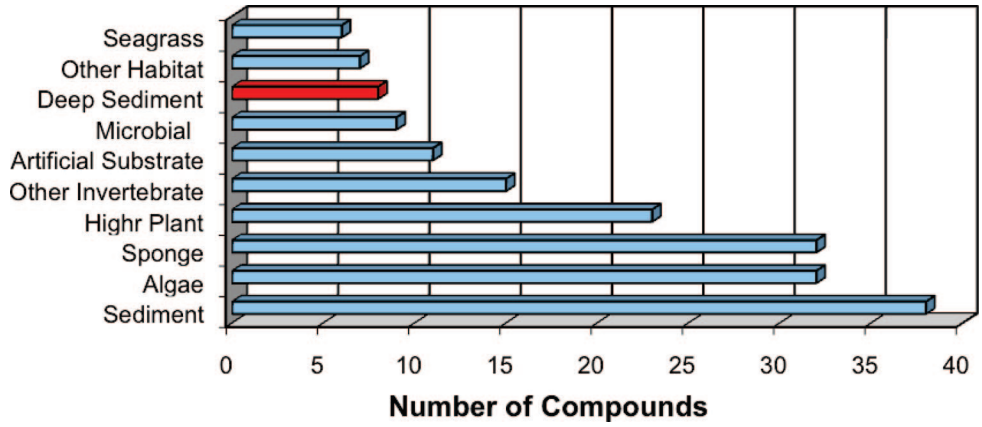


Figure 3. Histogram of marine-derived fungal compounds versus source.

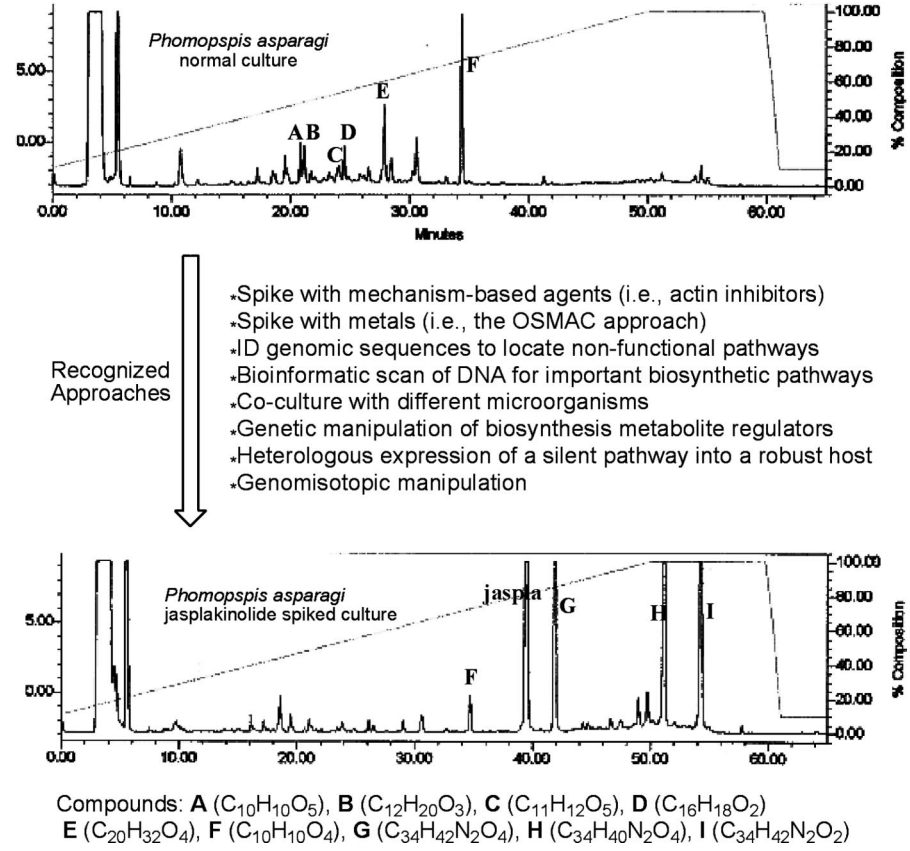


Figure 4. Strategies to turn on nonfunctional biosynthesis pathways in microorganism cultures.

agents for cancer.[106,107] The subsequent design of the MetAP1-specific fumagillin analogue TNP-470[108] is a key, new development. Unlike fumagillin, bengamide A (**26**) inhibits both MetAP1 and MetAP2, and the same is true for the Novartis bengamide synthetic analogue, LAF389.[109] Another compound, bengamide O, appears to have a different profile against the MetAP isoforms.[110]

The bengamides decreased the tyrosine kinase activities of *c*-Src both in vitro and in vivo and eventually delayed cell cycle progression through G2/M.[110] It is hypothesized that the clinical toxicity observed for bengamides (and by implication for other nonspecific MetAP inhibitors) could arise from global inhibition of *N*-terminal methionine processing. It has also been shown that blocking MetAP2 similarly inhibits the noncanonical Wnt signaling pathway.[111] The results obtained from experiments with fumagillin (a selective MetAP2 inhibitor) imply a potential connection between inhibiting Src family kinases and blocking noncanonical Wnt signaling.[111]

Overall, there have been very few specific MetAP1 inhibitors discovered to date. The sponge compound bengamide O may provide one such example. Alternatively, there are synthetic pyridine-2-carboxylic derivatives, such as IV-43 (Table 4), recently discovered through high-throughput screening of a library of 12 800 synthetics.[112] There is encouragement that MetAP1 may serve as a useful anticancer drug target. A next step in the quest to fully understand the molecular target and action of the bengamides will be to pursue additional therapeutic work with bengamide O and similar functionalized analogues.

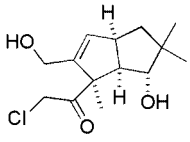
Marine-Derived Fungi

Our hypothesis, formulated in 1993, that sponges could harbor fungal spores that, after culturing, would be a prolific source of natural products was the motivation to begin research in this area. Another stimulus to begin study of microorganism natural products were the estimates that approximately one-half of the world's

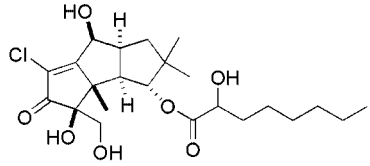
biomass is microbial.[113] Further encouraging was the belief that the biodiversity of marine-derived fungi, especially from the water−sediment interface and the anoxic environment below this zone, offered new opportunities for finding diverse species of fungi. Microbial ecologists are now asking questions about the populations of microorganisms endemic to these two potentially different communities. There are now a host of publications from various laboratories including our own clearly illustrating that communities of marine-derived fungi capable of producing diverse natural products when cultured can be found from the many different marine environments.

An understanding of the biological and chemical fundamentals of marine-derived fungi is still evolving. Currently, the best known habitat for marine fungal diversity consists of mangrove areas, which have contributed 50% of over 450 species discovered up to 2000.[114] The 2006 estimates of marine fungi worldwide indicated 2000 possible species, 800 of which are known to be saltwater obligate.[115] In spite of this biological understanding, it is not yet possible to predict the chemical signatures expected from taxonomically identified cultures of marine-derived filamentous fungi, which makes this group an exciting one for continuing chemical study.

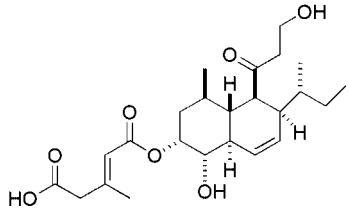
Our emphasis has always been on exploring marine-derived filamentous fungi grown in saltwater culture. An important part of the historical record is represented by two, almost simultaneous, early proof-of-principle results showing that chemical study of sponge-derived fungi would be rewarding. These initial discoveries included our report of chloriolins A (**50**)[116] and B (**51**), which are halogenated sesquiterpenes produced during the saltwater culture of an unidentified fungus from *Jaspis splendens* collected in Papua New Guinea in 1993. A similar benchmark finding was published in 1993 from the Kitagawa laboratory and involved the isolation of trichoharzin (**52**),[117] a polyketide from the culture of salt obligate strain *Trichoderma harzianum* obtained from a *Mycale* sponge.



chloriolin A (**50**)



chloriolin B (**51**)



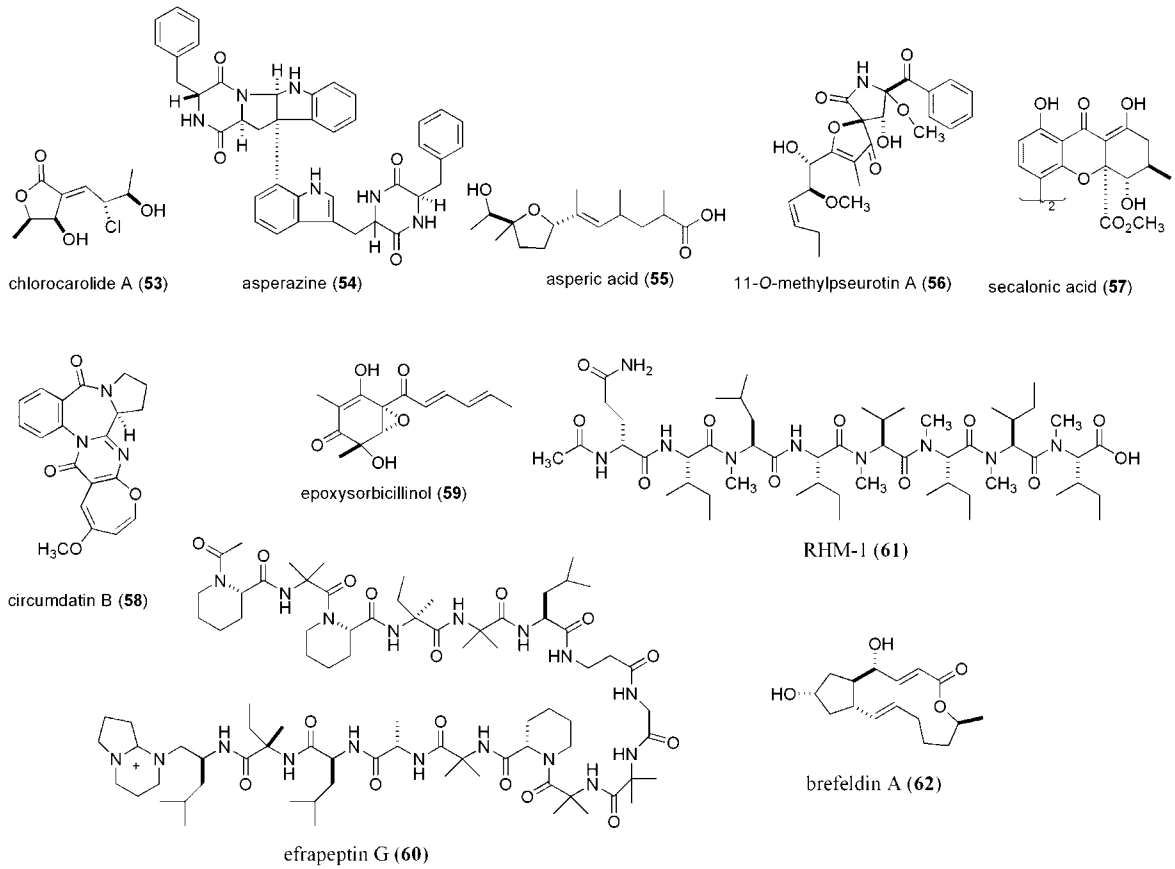
trichoharzin (**52**)

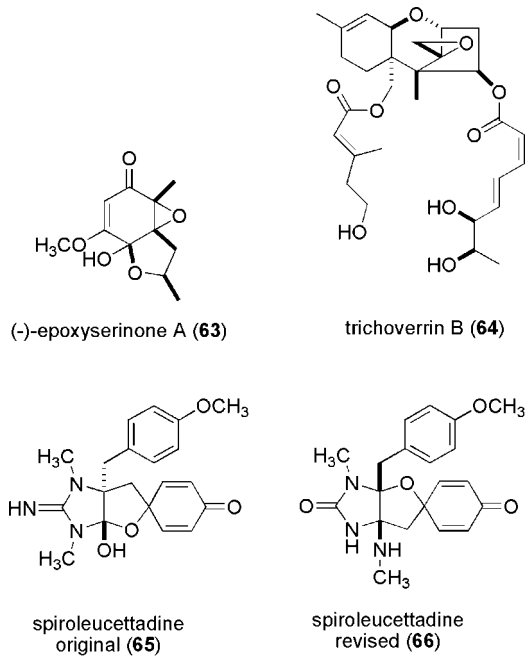
These two discoveries of sponge-derived fungi capable of producing unique compounds in saltwater culture were forerunners of further successful research conducted in our laboratory. For example, our saltwater culture of sponge-derived *Aspergillus* strains were found to be a source of another set of halogenated metabolites, such as chlorocarolide A (**53**).[118] We have found it rewarding to investigate other strains of marine-derived *Aspergillus*, and three significant new compounds discovered included the alkaloid asperazine (**54**),[119] the polyketide asperic acid (**55**), and the polyketide 11-*O*-methylpseurotin A[120] (**56**). Two other *Aspergillus* strains also provided us with additional known compounds including secalonic acid[121] (**57**) and circumdatin B[122,123] (**58**). Our study of cultures from *Trichoderma longibrachiatum* provided a very different end point as compared to the example above, because epoxysorbicillinol[124,125] (**59**) and related polyketides were isolated. The opportunity to accumulate different classes of structurally unusual bioactive peptides that were challenging to characterize occurred through a multiyear investigation of additional sponge-derived fungi. The most complex products were linear pentadecapeptides, headed by efrapeptin G[126] ($C_{63}H_{143}N_{18}O_{18}$) (**60**) from a sponge-derived *Acremonium* strain that also afforded linear peptides of the RHM family[127] ($C_{53}H_{97}N_9O_{11}$) (**61**) that are highly *N*-methylated octapeptides. Three of the efrapeptins (E, F, and G) were nM-active in cytotoxicity assays against H125 cells and warrant further therapeutic study. A different sponge derived fungus, *Metarrhizium* sp., was observed to produce six cyclic, mildly cytotoxic depsipeptides of the destruxin class, accompanied by the well-studied polyketide cytotoxin brefeldin A (**62**).[128]

Recently, we began to explore marine sediments as a source of additional fungal strains. Such a shift in focus is now amply justified when scanning the current literature especially the histogram of marine-derived fungi as a source of new compounds shown in Figure 3. This compilation, revealing some eye-catching patterns, traces the record up to early 2007. Through 2002, just 4% of the 273 marine-derived fungal compounds discovered were obtained from shallow-water sediments.[129] Today, there are more than 500 hundred unique compounds reported from the culture of marine-derived fungi from all sources.[130] Strikingly, the instances of compounds obtained from shallow-water sediments have recently surpassed those from the previously popular sources, sponges and algae. Indicating the next frontier could be deep-water sediments, which have not been extensively explored.

It took several years for our laboratory to develop inexpensive apparatus for the collection of deep-water sediments, and this work began before the patterns of Figure 3 were fully recognized. Our collection apparatus was fashioned along the lines of "mud grabbers" developed by the Fenical (UC San Diego) laboratory.[131] Deployment of it has occurred during all of our recent expeditions in both the Caribbean and remote Indo-Pacific sites. Our 2004 disclosure of three new pentaketide anserinone analogues, headed by (−)-epoxyanserinone B[132] (**63**) from a deep-water sediment-derived *Penicillium* sp., constituted an encouraging first finding. A similar recent report of 10 nitrogen-containing metabolites from a deep-water sediment-derived *Chromocleista* strain provided a further demonstration of the benefits from this approach.[133] Finally, our recent disclosure[134] of tyrosol carbamate isolated from the culture of a deep-water sediment-derived *Arthrinium* sp. completes the current record of the small amount of published work in this area. Taken together, these reports substantiate that secondary-metabolite-producing fungal strains can be obtained from deep-water habitats.

A continuing challenge in the quest of isolating diverse molecular structures from marine-derived fungi is to avoid pursuing cultures ladened with common metabolites. In 2004, toward the end of our study of the sponge-derived fungus *Myrothecium verrucaria*, rich in its production of trichoverrin B[135] (**64**) and related macrolides, we attempted to challenge these cultures with conditions that might alter the profile of secondary metabolites. At that juncture, the "OSMAC" (one strain many compounds) paradigm, repopularized by Bode and Zeeck in 2002,[136] seemed to be a straightforward strategy, as it involved the systematic alteration of culture conditions to generate new metabolites. As an ultimate test of OSMAC we used harsh conditions, specifically the addition of Cu^{2+} salts to the saltwater cultures, but this did not significantly alter the production of trichoverroids. During other studies we had applied another obvious OSMAC strategy, varying the seawater concentration during culturing of marine-derived fungal strains, but rarely observed major shifts in the metabolite profiles. Our most comprehensive study of this type involved the saltwater culturing of terrestrial ATTC-derived strains of *Coriolus consors* known for producing sesquiterpenes such as coriolin A.[136]





A refinement introduced during a next phase of our studies was to consider OSMAC alternatives beyond those involving simple changes in culture conditions to generate new profiles of secondary metabolites. The idea here was to add natural product modulators of fundamental cell biology processes to the saltwater cultures. Our first choice was to add actin or tubulin inhibitors. Shown in Figure 4 is a proof-of-concept result accomplished by spiking cultures of the marine-derived fungus *Phomopsis asparagi* with actin inhibitors such as jasplakinolide (results shown here) or swinholide A (data not shown). The outcome was the same in both instances and involved the diminished production of five simple oxygen-containing compounds labeled in Figure 4 as **A**−**E** accompanied by the appearance of new, unobserved alkaloid actin inhibitors including chaetoglobosin-510 (**I**), -540 (**H**), and -542 (**G**). The prospects for using other strategies to turn on what we term nonfunctional biosynthetic pathways are also summarized in Figure 4. Several of these approaches have been recently reviewed by Gross, who discussed current understanding in manipulating what is termed "orphan biosynthesis pathways".[137]

Structure Elucidation: Some Comments and Challenges

The tools for structure elucidation of complex marine natural products have continued to evolve. Most would agree that organic structure analysis should now be uncomplicated. In the past decade there have been some spectacular advances, including innovations in multidimensional NMR pulse sequences,[138] user-friendly apparatus for ultrahigh-resolution mass spectrometry,[139] widespread availability of tandem mass spectrometry,[126,139] refinements in the use of chiroptical data,[140] and the possibility of completing X-ray studies on poor-quality crystals.[141] Representative of such recent triumphs from our laboratory using one or more of these tools include reports on the structures of marine natural products such as (a) the cytotoxic alkaloid asperazine[119] ($C_{40}H_{36}N_6O_4$), (b) the polypeptide cyclolithistid A[142] ($C_{54}H_{86}ClN_{11}O_{15}$), (c) a mixed PKS−NRPS metabolite, isomotuporin[143] ($C_{40}H_{57}N_5O_{10}$), or (d) an atropoisomeric dimer, dicurcuphenol A[144] ($C_{30}H_{42}O_2$).

The decision matrix guiding our trajectory to the desired end point, an unequivocal proposal of a total structure, is shown in Figure 5. Engaging in aggressive dereplication represents a recent addition to our toolbox. This process uses partial data sets plus carbon framework substructure conclusions as they are accumulated for input to search a variety of commercial and proprietary databases. We have found it useful to aggressively engage in dereplication at all stages of the structure elucidation even when the compound under investigation has no literature precedents. As further discussed next, such dereplication efforts were successfully employed in our concise structure elucidations.

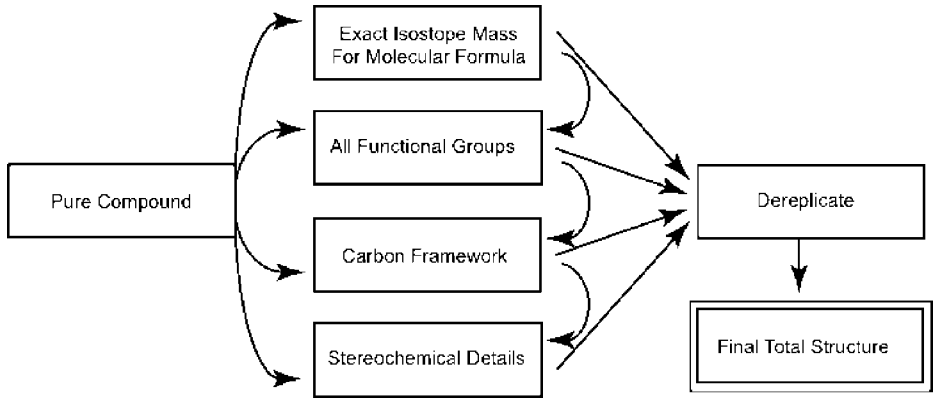


Figure 5. Overall flow of information in structure elucidation.

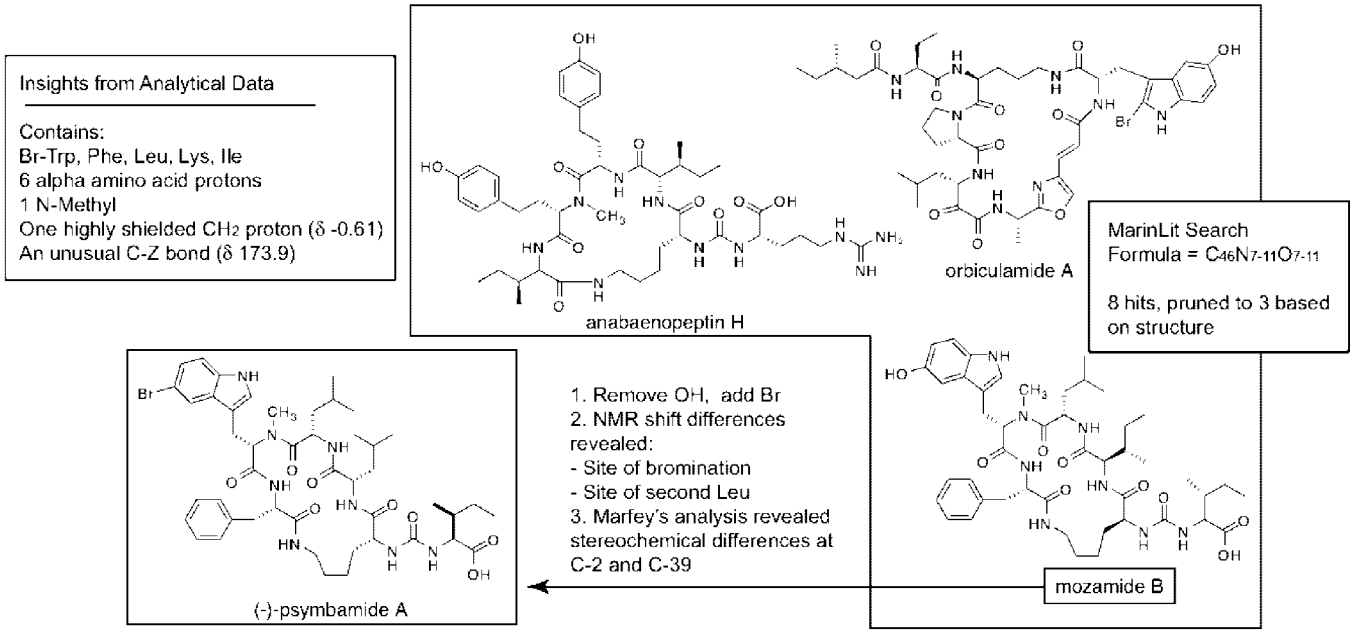


Figure 6. An aggressive application of dereplication.

We unexpectedly encountered a complex NRPS peptide, subsequently named (−)-psymbamide A.[145] This occurred during isolation work on six specimens of *Psammocinia* aff. *bulbosa* being processed to reisolate (+)-psymberin (**45**). Based on taxonomic considerations and molecular formula data, the characterization of pysmbamide A ($C_{46}H_{65}BrN_8O_8$) began by considering a possible structural relationship of it to (+)-cyclocinamide A[50] (**36**), of molecular formula $C_{29}H_{33}BrClN_9O_8$, which had been previously isolated from this sponge. The key insights from the analytical data shown in Figure 6 were clearly incompatible with this initial idea, but resonances for a 5-bromotryptophan could be assigned. Collectively, the partial atom count obtained by HRMS and NMR guided the subsequent dereplication step. A partial formula range consisting of $C_{46}N_{7-11}O_{7-11}$ was the seed for dereplication searches in MarinLit, with the N and O count based on the assignment of six α-amino acid protons, an indole NH, and an unusual C=O (at $\delta_C = 174$). A high unsaturation number, 18, was required by the molecular formula, and a cyclic peptide structure seemed attractive to account for the remaining unsaturation that could not be assigned to the substructure collection. This partial formula search of Marinlit[146] yielded eight hits, each of which was a cyclic peptide, and three seemed especially appealing. These are shown in Figure 6, and included were mozamide B[147] ($C_{46}H_{66}N_8O_9$), anabaenopeptin H ($C_{46}H_{70}N_{10}O_{10}$),[148] and orbiculamide A[149] ($C_{46}H_{62}BrN_9O_{10}$). The two structures possessing phenylalanine and tryptophan groups were sponge-derived, and the non-halogen-containing member of this pair was considered further. Attention was focused on mozambide B after a quick calculation showed replacing the OH in its molecular formula by a Br gave the formula for pysmbamide A. Finally, the structural differences between these two compounds were pinpointed by comparing their respective ^1H and ^{13}C NMR chemical shifts.

There is an important cautionary note that now needs discussion. Some critically thinking individuals, especially those involved in the total synthesis of complex natural products, recognize that even when all of the modern tools of structure elucidation discussed above are applied, errors can be made[150] and other considerations may be needed. Rather astounding is that errors in reported structures of natural products continue to abound, and this was highlighted in a recent review[151] noting that from 1990 to early 2004 more than 300 revisions were made. This suggests that accurate organic structure analysis is not yet routine.

Another important, but rarely discussed, challenge occurs when few hydrogen atoms are present in the molecular scaffold. The obvious steps of using protons sprinkled throughout a carbon framework as reporter groups to highlight direct and/or through-space magnetic couplings followed by drafting lists of molecular frameworks will be compromised when the H count is sparse. In this regard, we now understand when the ratio of H/C is less than 1, NMR data sets will be less useful and other methods must be used. This realization prompted our laboratory to consider the parallel evaluation of experimental ^{13}C NMR shifts with those from

density functional theory (DFT) calculations as a means to distinguish among sets of substructures.[62,152] The other essential approach in such a situation is to obtain X-ray crystallographic data. A recent example from our laboratory that illustrates this situation involves correction of the structure of spiroleucettadine from **65**[153] to **66**.[152] Repeated efforts to synthesize spiroleucettadine failed,[154−156] and questions emerged about the correctness of the original structure. Eventually we concluded that the low ratio of H/C = 0.8 in the core of spiroleucettadine compromised the original structure elucidation process. Reisolation of spiroleucettadine accompanied by DFT calculations to evaluate the experimental ^{13}C NMR shifts favored high-scoring structure **66**.[152] This proposal was ultimately confirmed via X-ray analysis of crystalline spiroleucettadine and underscores the validity of DFT calculations in structure elucidation.

Future Prospects

This account has highlighted the spectacular ability of marine organisms, both macro and micro, in producing exotic compounds. It is the extreme structural novelty coupled with new modes of biological activity that continue to make the study of marine natural products a rewarding venture. Only a small percentage of all marine organisms have been investigated for their potential to produce novel structural scaffolds. Further, the rich and biodiverse reefs of the Indo-Pacific Wallacea region have not received much attention. It is clear that the subject of marine bioorganic chemistry continues to be driven by inspirational structures, and as such it is thriving.

In looking toward the future there are several circumstances that are evident. There are new strategies being developed by many laboratories throughout the world that will continue to provide motivating new developments involving oceanographic sampling and other unique laboratory approaches to discover new molecular structures. There are a host of continuing challenges to overcome that will require much additional research. The most interesting to our laboratory involves the long-standing and burning question regarding the origin of sponge and other invertebrate natural products. What is the true producer? Some believe it is the assemblage of invertebrate associates, while others are convinced that specific strains of hetrotrophic bacteria or cyanobacteria play a key role. In the past, we have engaged in such studies and debates but have not arrived at firm answers. Devoting more attention to developing marine-based approaches to the culture of marine microorganisms is of obvious importance.

Our work on the products of marine-derived fungi grown in culture was also briefly treated in this account. We believe that investigations on marine microorganisms will continue to grow and should provide rewarding outcomes. Especially worthwhile could be the further study of the sponge-derived bacterial communities. We have just begun to explore one aspect of this subject, and the current focus is on marine-derived myxobactera. Absolutely intriguing are highly cited observations of parallel structures from terrestrial myxobactera and marine sponges. At the top of our list are (a) jasplakinolide[37] (sponge, *Jaspis splendens* and *Auletta constricta*) versus chondramide A[157] (mycobacteria, *Chondromyces crocatus*), (b) latrunculin A[89] (sponge, *Cacospongia mycofijiensis* and *Negombata magnifica*) versus epothilone B[91] (mycobacteria, *Sorangium celullosum*), and (c) bengamide E[158] (sponge, *Jaspis coriacea*) versus bengamide E analogues[159] (mycobacteria, *Mycoccus virescens*). We look forward to obtaining insights from future research to explain these circumstances.

Acknowledgment. This research was generously supported over the years by grants from the NIH National Cancer Institute, the National Science Foundation, and the California Sea Grant Program. The efforts of a host of research students and staff at UC Santa Cruz were essential in completing the research discussed. The list is much too long to reproduce here, and these names can be found in the papers from our laboratory that are cited herein. Special thanks to K. Tenney (UCSC) for design of the graphical abstract, to Dr. T. Johnson and Dr. W. Inman for their input, and to the BOYSCAST program, DST, Government of India, for a fellowship (K.V.S.).

References and Notes

(1) Scheuer, P. J. *Chemistry of Marine Natural Products*; Academic Press, Inc.: New York, 1973.
(2) Halstead, B. W. *Poisonous and Venomous Marine Animals of the World*; U.S. Government Printing Office: Washington, DC, 1965.
(3) See the American Cancer Society's website: cancer.org/docroot/STT/stt_0_2001.asp?sitearea=STT&level=1 (accessed 10/16/08).
(4) Rinehart, K. L.; Holt, T. G.; Fregeau, N. L.; Stroh, J. G.; Keifer, P. A.; Sun, F.; Li, L. H.; Martin, D. G. *J. Org. Chem.* **1990**, *55*, 4512–4515.
(5) Blay, J. Y.; von Mehren, M.; Samuels, B. L.; Fanucchi, M. P.; Ray-Coquard, I.; Buckley, B.; Gilles, L.; Lebedinsk, C.; ElSayed, Y. A.; Le Cesne, A. *Clin. Cancer Res.* **2008**, *14*, 6656–6662.
(6) Hirata, Y.; Uemura, D. *Pure Appl. Chem.* **1986**, *58*, 701–710.
(7) Quinoa, E.; Crews, P. *Tetrahedron Lett.* **1987**, *28*, 3229–3232.
(8) Kim, D. H.; Shin, J.; Kwon, H. J. *Exp. Mol. Med.* **2007**, *39*, 47–55.
(9) Qian, D. Z.; Kato, Y.; Shabbeer, S.; Wei, Y. F.; Verheul, H. M. W.; Salumbides, B.; Sanni, T.; Atadja, P.; Pili, R. *Clin. Cancer Res.* **2006**, *12*, 634–642.
(10) LoRusso, P.; Yee, L.; Papadopoulos, K. P.; Tolcher, A. W.; Romero, O.; Wang, D.; Heath, E. I.; Lloyd, G. K.; Longenecker, A.; Neuteboom, S. C.; Federico, K.; Cropp, G.; Spear, M. A.; Mita, M. *Mol. Cancer Ther.* **2007**, *6*, 3437S–3437S.
(11) Gunasekera, S. P.; Gunasekera, M.; Longley, R. E.; Schulte, G. K. *J. Org. Chem.* **1990**, *55*, 4912–4915.
(12) Panda, D.; Jordan, M. A.; DeLuca, K.; Wilson, L. *Mol. Biol. Cell* **1998**, *9*, 1585.
(13) Schmitz, F. J.; Lakshmi, V.; Powell, D. R.; Vanderhelm, D. *J. Org. Chem.* **1984**, *49*, 241–244.
(14) Sarabia, F.; Sanchez-Ruiz, A. *Tetrahedron Lett.* **2005**, *46*, 1131–1135.
(15) Quinoa, E.; Adamczeski, M.; Crews, P.; Bakus, G. J. *J. Org. Chem.* **1986**, *51*, 4494–4497.
(16) Zhao, M.; Qian, D.; Hartke, C.; Rudek, M. A.; Pili, R.; Baker, S. D. *Clin. Cancer Res.* **2003**, *9*, 6261S–6261S.
(17) Lavelle, F.; Zerial, A.; Fizames, C.; Rabault, B.; Curaudeau, A. *Invest. New Drugs* **1991**, *9*, 233–244.
(18) Crews, P.; Kho, E. *J. Org. Chem.* **1974**, *39*, 3303–3304.
(19) Faulkner, D. J.; Stallard, M. O.; Fayos, J.; Clardy, J. *J. Am. Chem. Soc.* **1973**, *95*, 3413–3414.
(20) Faulkner, D. J.; Stallard, M. O. *Tetrahedron Lett.* **1973**, 1171–1174.
(21) Kane, V. V.; Martin, A. R.; Peters, J. A.; Crews, P. *J. Org. Chem.* **1984**, *49*, 1793–1796.
(22) Mynderse, J. S.; Faulkner, D. J. *J. Am. Chem. Soc.* **1974**, *96*, 6771–6772.
(23) Vanengen, D.; Clardy, J.; Khowiseman, E.; Crews, P.; Higgs, M. D.; Faulkner, D. J. *Tetrahedron Lett.* **1978**, 29–32.
(24) Crews, P. *J. Org. Chem.* **1977**, *42*, 2634–2636.
(25) Crews, P.; Campbell, L.; Heron, E. *J. Phycol.* **1977**, *13*, 297–301.
(26) Crews, P.; Kho-Wiseman, E. *J. Org. Chem.* **1977**, *42*, 2812–2815.
(27) Crews, P.; Kho-Wiseman, E.; Montana, P. *J. Org. Chem.* **1978**, *43*, 116–120.
(28) Selover, S. J.; Crews, P. *J. Org. Chem.* **1980**, *45*, 69–72.
(29) Crews, P.; Selover, S. J. *Phytochemistry* **1986**, *25*, 1847–1852.
(30) Selover, S. J.; Crews, P.; Tagle, B.; Clardy, J. *J. Org. Chem.* **1981**, *46*, 964–970.
(31) Crews, P.; Klein, T. E.; Hogue, E. R.; Myers, B. L. *J. Org. Chem.* **1982**, *47*, 811–815.
(32) Manes, L. V.; Bakus, G. J.; Crews, P. *Tetrahedron Lett.* **1984**, *25*, 931–934.
(33) Crews, P.; Bescansa, P.; Bakus, G. J. *Experientia* **1985**, *41*, 690–691.
(34) Crews, P.; Bescansa, P. *J. Nat. Prod.* **1986**, *49*, 1041–1052.
(35) Quinoa, E.; Kho, E.; Manes, L. V.; Crews, P.; Bakus, G. J. *J. Org. Chem.* **1986**, *51*, 4260–4264.
(36) Pina, I. C.; Gautschi, J. T.; Wang, G. Y. S.; Sanders, M. L.; Schmitz, F. J.; France, D.; Cornell-Kennon, S.; Sambucetti, L. C.; Remiszewski, S. W.; Perez, L. B.; Bair, K. W.; Crews, P. *J. Org. Chem.* **2003**, *68*, 3866–3873.
(37) Crews, P.; Manes, L. V.; Boehler, M. *Tetrahedron Lett.* **1986**, *27*, 2797–2800.
(38) Kakou, Y.; Crews, P.; Bakus, G. J. *J. Nat. Prod.* **1987**, *50*, 482–484.
(39) Quinoa, E.; Crews, P. *Tetrahedron Lett.* **1988**, *29*, 2037–2040.
(40) Adamczeski, M.; Quinoa, E.; Crews, P. *J. Am. Chem. Soc.* **1988**, *110*, 1598–1602.
(41) Crews, P.; Kakou, Y.; Quinoa, E. *J. Am. Chem. Soc.* **1988**, *110*, 4365–4368.

(42) Quinoa, E.; Kakou, Y.; Crews, P. *J. Org. Chem.* **1988**, *53*, 3642–3644.
(43) Manes, L. V.; Crews, P.; Kernan, M. R.; Faulkner, D. J.; Fronczek, F. R.; Gandour, R. D. *J. Org. Chem.* **1988**, *53*, 570–575.
(44) Manes, L. V.; Naylor, S.; Crews, P.; Bakus, G. J. *J. Org. Chem.* **1985**, *50*, 284–286.
(45) Inman, W. D.; O'Neill-Johnson, M.; Crews, P. *J. Am. Chem. Soc.* **1990**, *112*, 1–4.
(46) Jimenez, C.; Crews, P. *J. Nat. Prod.* **1990**, *53*, 978–982.
(47) Jimenez, C.; Quinoa, E.; Crews, P. *Tetrahedron Lett.* **1991**, *32*, 1843–1846.
(48) Subramanian, B.; Nakeff, A.; Tenney, K.; Crews, P.; Gunatilaka, L.; Valeriote, F. *J. Exp. Ther. Oncol.* **2005**, *5*, 195–204.
(49) Moore, R. E.; Corbett, T. H.; Patterson, G. M. L.; Valeriote, F. A. *Curr. Pharm. Des.* **1996**, *2*, 317–330.
(50) Clark, W. D.; Corbett, T.; Valeriote, F.; Crews, P. *J. Am. Chem. Soc.* **1997**, *119*, 9285–9286.
(51) Thale, Z.; Johnson, T.; Tenney, K.; Wenzel, P. J.; Lobkovsky, E.; Clardy, J.; Media, J.; Pietraszkiewicz, H.; Valeriote, F. A.; Crews, P. *J. Org. Chem.* **2002**, *67*, 9384–9391.
(52) Segraves, N. L.; Robinson, S. J.; Garcia, D.; Said, S. A.; Fu, X.; Schmitz, F. J.; Pietraszkiewicz, H.; Valeriote, F. A.; Crews, P. *J. Nat. Prod.* **2004**, *67*, 783–792.
(53) Crews, P.; Clark, D. P.; Tenney, K. *J. Nat. Prod.* **2003**, *66*, 177–182.
(54) Braet, F.; Spector, I.; Shochet, N.; Crews, P.; Higa, T.; Menu, E.; de Zanger, R.; Wisse, E. *BMC Cell Biol.* **2002**, *3*, 7.
(55) Crews, P.; Harrison, B. *Tetrahedron Lett.* **2000**, *56*, 9039–9046.
(56) Stessman, C. C.; Ebel, R.; Corvino, A. J.; Crews, P. *J. Nat. Prod.* **2002**, *65*, 1183–1186.
(57) Amagata, T.; Whitman, S.; Johnson, T. A.; Stessman, C. C.; Loo, C. P.; Lobkovsky, E.; Clardy, J.; Crews, P.; Holman, T. R. *J. Nat. Prod.* **2003**, *66*, 230–235.
(58) Zabriskie, T. M.; Klocke, J. A.; Ireland, C. M.; Marcus, A. H.; Molinski, T. F.; Faulkner, D. J.; Xu, C. F.; Clardy, J. C. *J. Am. Chem. Soc.* **1986**, *108*, 3123–3124.
(59) Senderowicz, A. M. J.; Kaur, G.; Sainz, E.; Laing, C.; Inman, W. D.; Rodriguez, J.; Crews, P.; Malspeis, L.; Grever, M. R.; Sausville, E. A.; Duncan, K. L. K. *J. Natl. Cancer Inst.* **1995**, *87*, 46–51.
(60) Cichewicz, R. H.; Valeriote, F. A.; Crews, P. *Org. Lett.* **2004**, *6*, 1951–1954.
(61) Sonnenschein, R. N.; Johnson, T. A.; Tenney, K.; Valeriote, F. A.; Crews, P. *J. Nat. Prod.* **2006**, *69*, 145–147.
(62) Amagata, T.; Johnson, T. A.; Cichewicz, R. H.; Tenney, K.; Mooberry, S. L.; Media, J.; Edelstein, M.; Valeriote, F.; Crews, P. *J. Med. Chem.* **2008**, *51*, 7234–7242.
(63) Pettit, G. R.; Xu, J.-P.; Chapuis, J.-C.; Pettit, R. K.; Tackett, L. P.; Doubek, D. L.; Hooper, J. N. A.; Schmidt, J. M. *J. Med. Chem.* **2004**, *47*, 1149–1152.
(64) Cichewicz, R. H.; Valeriote, F. A.; Crews, P. *Org. Lett.* **2004**, *6*, 1951–1954.
(65) Jiang, X.; Williams, N.; De Brabander, J. K. *Org. Lett.* **2007**, *9*, 227–230.
(66) Cardani, C.; Ghiringhelli, D.; Mondelli, R.; Quilico, A. *Tetrahedron Lett.* **1965**, *6*, 2537–2545.
(67) Furusaki, A.; Watanabe, T.; Matsumoto, T.; Yanagiya, M. *Tetrahedron Lett.* **1968**, 6301–6304.
(68) Matsuda, F.; Tomiyoshi, N.; Yanagiya, M.; Matsumoto, T. *Tetrahedron* **1988**, *44*, 7063–7080.
(69) Hall, L. A.; Krauthauser, C. M.; Wexler, R. S.; Hollingshead, M. G.; Slee, A. M.; Kerr, J. S. *Anticancer Res.* **2000**, *20*, 903–911.
(70) Corley, D. G.; Herb, R.; Moore, R. E.; Scheuer, P. J.; Paul, V. J. *J. Org. Chem.* **1988**, *53*, 3644–3646.
(71) Corley, D. G.; Herb, R.; Moore, R. E.; Scheuer, P. J.; Paul, V. J. *J. Org. Chem.* **1988**, *53*, 3644–3646.
(72) Mooberry, S. L.; Tien, G.; Hernandez, A. H.; Plubrukarn, A.; Davidson, B. S. *Cancer Res.* **1999**, *59*, 653–660.
(73) Mulzer, J.; Ohler, E. *Chem. Rev.* **2003**, *103*, 3753–3786.
(74) Gallagher, B. M.; Fang, F. G.; Johannes, C. W.; Pesant, M.; Tremblay, M. R.; Zhao, H. J.; Akasaka, K.; Li, X. Y.; Liu, J. K.; Littlefield, B. A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 575–579.
(75) Pryor, D. E.; O'Brate, A.; Bilcer, G.; Diaz, J. F.; Wang, Y. F.; Wang, Y.; Kabaki, M.; Jung, M. K.; Andreu, J. M.; Ghosh, A. K.; Giannakakou, P.; Hamel, E. *Biochemistry* **2002**, *41*, 9109–9115.
(76) Ahmed, A.; Hoegenauer, E. K.; Enev, V. A. S.; Hanbauer, M.; Kaehlig, H.; Ohler, E.; Mulzer, J. *J. Org. Chem.* **2003**, *68*, 3026–3042.
(77) Wender, P. A.; Hegde, S. G.; Hubbard, R. D.; Zhang, L.; Mooberry, S. L. *Org. Lett.* **2003**, *5*, 3507–3509.
(78) Mooberry, S. L.; Randall-Hlubek, D. A.; Leal, R. M.; Hegde, S. G.; Hubbard, R. D.; Zhang, L.; Wender, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 8803–8808.
(79) Paterson, I.; Bergmann, H.; Menche, D.; Berkessel, A. *Org. Lett.* **2004**, *6*, 1293–1295.
(80) Gallagher, B. M.; Zhao, H. J.; Pesant, M.; Fang, F. G. *Tetrahedron Lett.* **2005**, *46*, 923–926.
(81) Paterson, I.; Menche, D.; Hakansson, A. E.; Longstaff, A.; Wong, D.; Barasoain, I.; Buey, R. M.; Diaz, J. F. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2243–2247.
(82) Wender, P. A.; Hilinski, M. K.; Soldermann, N.; Mooberry, S. L. *Org. Lett.* **2006**, *8*, 1507–1510.
(83) Wender, P. A.; Hilinski, M. K.; Skaanderup, P. R.; Soldermann, N. G.; Mooberry, S. L. *Org. Lett.* **2006**, *8*, 4105–4108.
(84) Faveau, C.; Mondon, M.; Gesson, J. P.; Mahnke, T.; Gebhardt, S.; Koert, U. *Tetrahedron Lett.* **2006**, *47*, 8305–8308.
(85) Johnson, T. A.; Tenney, K.; Cichewicz, R. H.; Morinaka, B. I.; White, K. N.; Amagata, T.; Subramanian, B.; Media, J.; Mooberry, S. L.; Valeriote, F. A.; Crews, P. *J. Med. Chem.* **2007**, *50*, 3795–3803.
(86) Nagle, D. G.; Zhou, Y.-D. Abstract presented at the 6th U.S. Japan Symposium, Utah, July 1−6, 2007.
(87) Mahler, G.; Serra, G.; Manta, E. *Synth. Commun.* **2005**, *35*, 1481–1492.
(88) Mahler, G.; Serra, G.; Dematteis, S.; Saldana, J.; Dominguez, L.; Manta, E. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1309–1311.
(89) Kashman, Y.; Groweiss, A.; Shmueli, J. *Tetrahedron Lett.* **1980**, *21*, 3629–3632.
(90) Kashman, Y.; Groweiss, A.; Lidor, R.; Blasberger, D.; Carmely, S. *Tetrahedron* **1985**, *41*, 1905–1914.
(91) Hofle, G. H.; Bedorf, N.; Steinmetz, H.; Schomburg, D.; Gerth, K.; Reichenbach, H. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1567–1569.
(92) Ahmed, S. A.; Odde, S.; Daga, P. R.; Bowling, J. J.; Mesbah, M. K.; Youssef, D. T.; Khalifa, S. I.; Doerksen, R. J.; Hamann, M. T. *Org. Lett.* **2007**, *9*, 4773–4776.
(93) Robinson, S. J.; Riener, M.; Tenney, K.; Loveridge, S. T.; Mooberry, S. L.; Valeriote, F. A.; Crews, P. *Planta Med.* **2008**, *74*, 1078–1078.
(94) Rubio, B. K.; Robinson, S. J.; Avalos, C. E.; Valeriote, F. A.; de Voogd, N. J.; Crews, P. *J. Nat. Prod.* **2008**, *71*, 1475–1478.
(95) Quilico, A.; Piozzi, F.; Pavan, M. *Tetrahedron* **1957**, *1*, 177–185.
(96) Johnson, T. A.; Amagata, T.; Oliver, A. G.; Tenney, K.; Valeriote, F. A.; Crews, P. *J. Org. Chem.* **2008**, *73*, 7255–7259.
(97) Johnson, T.; Amagata, T.; Oliver, A. G.; Tenney, K.; Ang, K.-H.; McKerrow, J.; Crews, P. *J. Org. Chem.*, submitted.
(98) Kinder, F. R.; Versace, R. W.; Bair, K. W.; Bontempo, J. M.; Cesarz, D.; Chen, S.; Crews, P.; Czuchta, A. M.; Jagoe, C. T.; Mou, Y.; Nemzek, R.; Phillips, P. E.; Tran, L. D.; Wang, R. M.; Weltchek, S. *J. Med. Chem.* **2001**, *44*, 3692–3699.
(99) Thale, Z.; Kinder, F. R.; Bair, K. W.; Bontempo, J.; Czuchta, A. M.; Versace, R. W.; Phillips, P. E.; Sanders, M. L.; Wattanasin, S.; Crews, P. *J. Org. Chem.* **2001**, *66*, 1733–1741.
(100) Dumez, H.; Gall, H.; Capdeville, R.; Dutreix, C.; Van Oosterom, A. T.; Giaccone, G. *Anti-Cancer Drugs* **2007**, *18*, 219–225.
(101) Adamczeski, M.; Quinoa, E.; Crews, P. *J. Org. Chem.* **1990**, *55*, 240–242.
(102) Towbin, H.; Bair, K. W.; DeCaprio, J. A.; Eck, M. J.; Kim, S.; Kinder, F. R.; Morollo, A.; Mueller, D. R.; Schindler, P.; Song, H. K.; van Oostrum, J.; Versace, R. W.; Voshol, H.; Wood, J.; Zabludoff, S.; Phillips, P. E. *J. Biol. Chem.* **2003**, *278*, 52964–52971.
(103) Lowther, W. T.; Matthews, B. W. *Biochim. Biophys. Acta Protein Struct. Mol. Enzym.* **2000**, *1477*, 157–167.
(104) Griffith, E. C.; Su, Z.; Turk, B. E.; Chen, S. P.; Chang, Y. H.; Wu, Z. C.; Biemann, K.; Liu, J. O. *Chem. Biol.* **1997**, *4*, 461–471.
(105) Sin, N.; Meng, L. H.; Wang, M. Q. W.; Wen, J. J.; Bornmann, W. G.; Crews, C. M. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 6099–6103.
(106) Lu, J.; Chong, C. R.; Hu, X. Y.; Liu, J. O. *J. Med. Chem.* **2006**, *49*, 5645–5648.
(107) Hu, X. Y.; Addlagatta, A.; Matthews, B. W.; Liu, J. O. *Angew. Chem., Int. Ed.* **2006**, *45*, 3772–3775.
(108) Logothetis, C. J.; Wu, K. K.; Finn, L. D.; Daliani, D.; Figg, W.; Ghaddar, H.; Gutterman, J. U. *Clin. Cancer Res.* **2001**, *7*, 1198–1203.
(109) Hu, X.; Dang, Y.; Tenney, K.; Crews, P.; Tsai, C. W.; Sixt, K. M.; Cole, P. A.; Liu, J. O. *Chem. Biol.* **2007**, *14*, 764–774.
(110) Hu, X.; Dang, Y.; Tenney, K.; Crews, P.; Cole, P. A.; Liu, J. O. *Chem. Biol.* **2007**, *11*, 561–568.
(111) Zhang, Y.; Yeh, J. R.; Mara, A.; Ju, R.; Hines, J. F.; Cirone, P.; Griesbach, H. L.; Schneider, I.; Slusarski, D. C.; Holley, S. A.; Crews, C. M. *Chem. Biol.* **2006**, *13*, 1001–1009.
(112) Hu, X. Y.; Addlagatta, A.; Lu, J.; Matthews, B. W.; Liu, J. O. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 18148–18153.
(113) Whitman, W. B.; Coleman, D. C.; Wiebe, W. J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 6578–6583.
(114) Sridhar, K. R. *Curr. Sci.* **2004**, *86*, 1586–1587.
(115) Das, S.; Lyla, P. S.; Khan, S. A. *Curr. Sci.* **2006**, *90*, 1325–1335.
(116) Cheng, X. C.; Varoglu, M.; Abrell, L.; Crews, P.; Lobkovsky, E.; Clardy, J. *J. Org. Chem.* **1994**, *59*, 6344–6348.

(117) Kobayashi, M.; Uehara, H.; Matsunami, K.; Aoki, S.; Kitagawa, I. *Tetrahedron Lett.* **1993**, *34*, 7925–7928.
(118) Abrell, L. M.; Borgeson, B.; Crews, P. *Tetrahedron Lett.* **1996**, *37*, 2331–2334.
(119) Varoglu, M.; Corbett, T. H.; Valeriote, F. A.; Crews, P. *J. Org. Chem.* **1997**, *62*, 7078–7079.
(120) Boot, C. M.; Gassner, N. C.; Compton, J. E.; Tenney, K.; Tamble, C. M.; Lokey, R. S.; Holman, T. R.; Crews, P. *J. Nat. Prod.* **2007**, *70*, 1672–1675.
(121) Yamazaki, M.; Maebayas, Y.; Miyaki, K. *Chem. Pharm. Bull.* **1971**, *19*, 199–201.
(122) Rahbaek, L.; Breinholt, J.; Frisvad, J. C.; Christophersen, C. *J. Org. Chem.* **1999**, *64*, 1689–1692.
(123) Ookura, R.; Kito, K.; Ooi, T.; Namikoshi, M.; Kusumi, T. *J. Org. Chem.* **2008**, *73*, 4245–4247.
(124) Sperry, S.; Samuels, G. J.; Crews, P. *J. Org. Chem.* **1998**, *63*, 10011–10014.
(125) Wood, J. L.; Thompson, B. D.; Yusuff, N.; Pflum, D. A.; Matthaus, M. S. P. *J. Am. Chem. Soc.* **2001**, *123*, 2097–2098.
(126) Boot, C. M.; Amagata, T.; Tenney, K.; Compton, J. E.; Pietraszkiewicz, H.; Valeriote, F. A.; Crews, P. *Tetrahedron* **2007**, *63*, 9903–9914.
(127) Boot, C. M.; Tenney, K.; Valeriote, F. A.; Crews, P. *J. Nat. Prod.* **2006**, *69*, 83–92.
(128) Tamura, G.; Ando, K.; Suzuki, S.; Takatsuk, A.; Arima, K. *J. Antibiot.* **1968**, *21*, 160–161.
(129) Bugni, T. S.; Ireland, C. M. *Nat. Prod. Rep.* **2004**, *21*, 143–163.
(130) Ebel, R. In *Frontiers in Marine Biotechnology*; Proksch, P., Muller, W. E. G., Eds.; Horizon Bioscience: Wymondham, U.K., 2006; pp 73−143.
(131) Wilson, E. K. *Chem. Eng. News* **2003**, *81*, 37–38.
(132) Gautschi, J. T.; Amagata, T.; Amagata, A.; Valeriote, F. A.; Mooberry, S. L.; Crews, P. *J. Nat. Prod.* **2004**, *67*, 362–367.
(133) Park, Y. C.; Gunasekera, S. P.; Lopez, J. V.; McCarthy, P. J.; Wright, A. E. *J. Nat. Prod.* **2006**, *69*, 580–584.
(134) Gautschi, J. T. T., K.; Compton, J.; Crews, P. *Nat. Prod. Commun.* **2007**, *2*, 541–546.
(135) Amagata, T.; Rath, C.; Rigot, J. F.; Tarlov, N.; Tenney, K.; Valeriote, F. A.; Crews, P. *J. Med. Chem.* **2003**, *46*, 4342–4350.
(136) Bode, H. B.; Bethe, B.; Hofs, R.; Zeeck, A. *ChemBioChem* **2002**, *3*, 619–627.
(137) Gross, H. *Appl. Microbiol. Biotechnol.* **2007**, *75*, 267–277.
(138) Berger, S.; Braun, S. *200 and More NMR Experiments: A Practical Course*, 3rd ed.; Wiley-VCH: Weinheim, 2004.
(139) Williams, D. E.; Lapawa, M.; Feng, X. D.; Tarling, T.; Roberge, M.; Andersen, R. J. *Org. Lett.* **2004**, *6*, 2607–2610.
(140) Zuber, G.; Goldsmith, M.-R.; Hopkins, T. D.; Beratan, D. N.; Wipf, P. *Org. Lett.* **2005**, *7*, 5269–5272.
(141) Allingham, J. S.; Tanaka, J.; Marriott, G.; Rayment, I. *Org. Lett.* **2004**, *6*, 597–599.
(142) Clark, D. P.; Carroll, J.; Naylor, S.; Crews, P. *J. Org. Chem.* **1998**, *63*, 8757–8764.
(143) Wegerski, C. J.; Hammond, J.; Tenney, K.; Matainaho, T.; Crews, P. *J. Nat. Prod.* **2007**, *70*, 89–94.
(144) Cichewicz, R. H.; Clifford, L. J.; Lassen, P. R.; Cao, X. L.; Freedman, T. B.; Nafie, L. A.; Deschamps, J. D.; Kenyon, V. A.; Flanary, J. R.; Holman, T. R.; Crews, P. *Bioorg. Med. Chem.* **2005**, *13*, 5600–5612.
(145) Robinson, S. J.; Tenney, K.; Yee, D. F.; Martinez, L.; Media, J. E.; Valeriote, F. A.; van Soest, R. W. M.; Crews, P. *J. Nat. Prod.* **2007**, *70*, 1002–1009.
(146) Blunt, J. W.; Munro, M. H. G. *Marinlit*; University of Canterbury: Christchurch, NZ, July 2006.
(147) Schmidt, E. W.; Harper, M. K.; Faulkner, D. J. *J. Nat. Prod.* **1997**, *60*, 779–782.
(148) Itou, Y.; Suzuki, S.; Ishida, K.; Murakami, M. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1243–1246.
(149) Fusetani, N.; Sugawara, T.; Matsunaga, S.; Hirota, H. *J. Am. Chem. Soc.* **1991**, *113*, 7811–7812.
(150) Li, J.; Burgett, A. W. G.; Esser, L.; Amezcua, C.; Harran, P. G. *Angew. Chem., Int. Ed.* **2001**, *40*, 4770–4773.
(151) Nicolaou, K. C.; Snyder, S. A. *Angew. Chem., Int. Ed.* **2005**, *44*, 2050–2050.
(152) White, K. N.; Amagata, T.; Oliver, A. G.; Tenney, K.; Wenzel, P. J.; Crews, P. *J. Org. Chem.* **2008**, *73*, 8719–8722.
(153) Ralifo, P.; Crews, P. *J. Org. Chem.* **2004**, *69*, 9025–9029.
(154) Aberle, N.; Ovenden, S. P. B.; Lessene, G.; Watson, K. G.; Smith, B. J. *Tetrahedron Lett.* **2007**, *48*, 2199–2203.
(155) Li, C. M.; Danishefsky, S. J. *Tetrahedron Lett.* **2006**, *47*, 385–387.
(156) Chang, J. J.; Chan, B.; Ciufolini, M. A. *Tetrahedron Lett.* **2006**, *47*, 3599–3601.
(157) Holzinger, A.; Lutz-Meindl, U. *Cell Motil. Cytoskel.* **2001**, *48*, 87–95.
(158) Thale, Z.; Kinder, F. R.; Bair, K. W.; Bontempo, J.; Czuchta, A. M.; Versace, R. W.; Phillips, P. E.; Sanders, M. L.; Wattanasin, S.; Crews, P. *J. Org. Chem.* **2001**, *66*, 1733–1741.
(159) Hoffman, H.; Haag-Richter, S.; Kurz, M.; Tietgen, H. U.S. Patent, 7,153,846 B2, 2006.

NP800817Y