Marine Pyridoacridine Alkaloids: Structure, Synthesis, and Biological Chemistry

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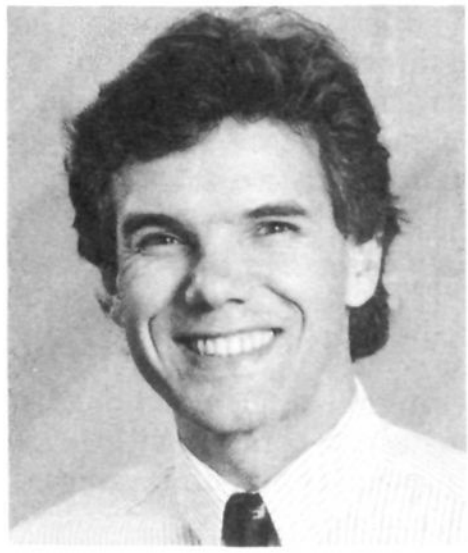


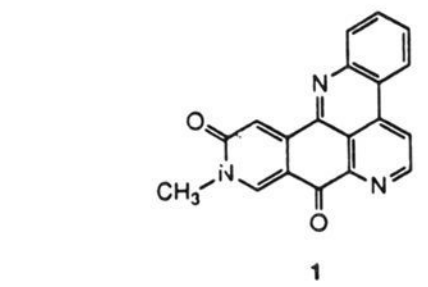
Tadeusz F. Molinski received his B.S., with honors, from Monash University, Melbourne, Australia, in 1978 and Ph.D. in synthetic organic chemistry from the Research School of Chemistry Australian National University, Canberra, in 1985 where he studied under John MacLeod. He completed 2 years of postdoctoral research with D. John Faulkner at Scripps Institution of Oceanography in 1987, followed by further postdoctoral work with Chris M. Ireland, at the University of Utah, and Trevor McMorris in the Department of Chemistry, University of California, San Diego. He joined the faculty of the chemistry department, University of California, Davis, in 1989 where he currently holds the rank of Assistant Professor. His research interests embrace the structural, bioorganic chemistry, and synthetic organic chemistry of new marine natural products.

I. Introduction

In 1983 two research groups, lead by Schmitz and Shoolery, reported the structure of a marine alkaloid, amphimedine (**1**, Chart I), which was the first of a new class of marine-derived alkaloids that collectively have come to be known as the "pyridoacridines".[1] Since then, over 40 additional examples have been published. The pyridoacridines are highly colored marine derived alkaloids based on the 11*H*-pyrido[4,3,2-*mn*]acridine skeleton, **2**,[2] however, the majority are more accurately described as "pyridoacridones", derivatives of the hypothetical iminoquinone, 8*H*-pyrido[4,3,2-*mn*]acridone (**3**). The former name, however, prevails in the literature and will be used here.

Several thorough reviews on marine natural products[3–9] and, in particular, alkaloids[10–13] have been published in recent years. This review constrains its scope to a more thorough discussion of the chemistry, total synthesis, biological chemistry, and biosynthesis of the pyridoacridine alkaloids. There is currently great interest in marine pyridoacridines due to their significant biological properties. Almost all have been reported as having significant cytotoxicity; however, several specific biological properties have also emerged

Chart I



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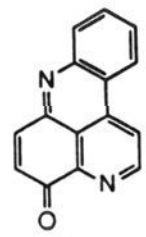

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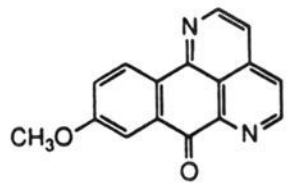

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for different compounds: inhibition of topoisomerase II,[14,15] anti-HIV activity,[16] Ca^{2+} release activity,[17] metal chelating properties,[18] and intercalation of DNA.[15,18] In addition, pyridoacridines have drawn attention because of their novel heterocyclic chemistry and an unprecedented distribution across several phyla of marine invertebrates.

Each of these natural products is based on a common tetracyclic heteroaromatic parent—pyrido[4,3,2-*mn*]-acridine (**2**).[19] Although alkaloids containing isomeric ring systems have been found in terrestrial plants (for example, eupomatidine (**4**) from the angiosperm *Eupomatia bennetti*[20,21]), it appears that the pyrido-[4,3,2-*mn*]acridine carbon skeleton is exclusive to marine invertebrates. For the purposes of this review, numbering of substituents and lettering of rings will refer to the parent structure **2**.

II. Chemical Properties and Occurrence

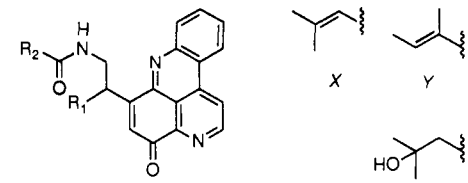
A. General Properties

Marine invertebrates, such as sponges and tunicates, are often ornately decorated with bright colors and patterns. Tropical compound tunicates (ascidians, Subphylum Urochordata) in particular can be richly pigmented in colors that vary from yellows, to deep reds, orange, and vivid blues and purples.[22] It is often found that pyridoacridines isolated from such tunicates are the pigments (zoochromes) responsible for their coloration.

Pyridoacridine alkaloids are pH indicators, a novelty which has not escaped the attention of marine natural products chemists who have become inured to working with other, less colorful natural products. The indicator property is correlated with the presence of at least two basic pyridine-like nitrogens and is probably associated with electronic perturbations an extended chromophore with charge-transfer properties. Simple indicator properties are absent in the less basic iminoquinones, such as cystodytin A (**5**) and diplamine (**6**) (Chart II). Alkaline solutions of the free base generally appear orange or red, while in acid solution they are green-blue to purple. Some quaternary ammonium alkaloids, like petrosamine (**7**), are deep blue or purple salts.

Pyridoacridines are typically isolated as refractory microcrystalline solids with reported melting points often in excess of 300 °C. In most cases this is probably due to their isolation as hydrochloride salts, unless provisions have been made for conversion to the free base. Only a few are optically active and this is always due to the presence of additional asymmetric side chain modifications. Otherwise, the majority of pyrido-acridines are planar heterocycles. Because of variability in oxidation states of the heterocyclic nucleus, pyri-doacridine alkaloids exhibit facile redox reactions. For example, the iminoquinone substructure **3** in many alkaloids is easily reduced by $NaBH_4$. Partially saturated nitrogen containing rings in pyridoacridine alkaloids are easily aromatized by air oxidation (autoxidation) upon storage or heating in solution. Finally, several pentacyclic pyridoacridines embody a 1,10-phenanthroline subunit, however, they do not react to form red complexes with Fe(II) salts. This lack of reactivity must be interpreted cautiously when assigning possible structures to new alkaloids as it does not provide evidence for lack of a 1,10-phenanthroline substructure.

Chart II



5 R_2 = *X* R_1 = H **10** R_2 = *X* R_1 = OH

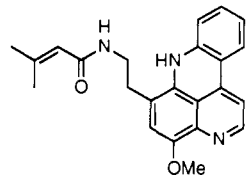
8 R_2 = *Y* R_1 = H **11** R_2 = *Y* R_1 = OH

9 R_2 = *Z* R_1 = H **12** R_2 = *X* R_1 = OMe

13 R_2 = *Y* R_1 = OMe

14 R_2 = *X* R_1 = $OOC(CH_2)_7CH{=}CH(CH_2)_7CH_3$

15 R_2 = *Y* R_1 = $OOC(CH_2)_7CH{=}CH(CH_2)_7CH_3$



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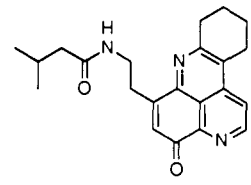

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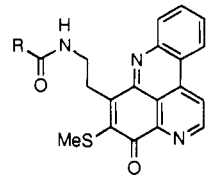
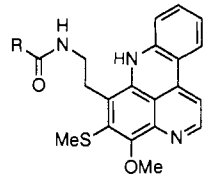


6 R_1 = CH_3

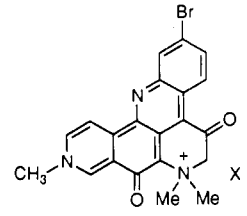
20 R_1 = CH_2CH_3

21 R_1 = $CH(CH_3)_2$



18 R_1 = CH_2CH_3

19 R_1 = CH_3



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B. Nuclear Magnetic Resonance

The assignment of structure by NMR in highly condensed heterocyclic aromatic compounds is complicated by the difficulty of defining the correct regioisomer from among many possibilities. Thus, methods that have been applied to new structure elucidations frequently draw on new powerful multi-pulse NMR methods (HMQC, HMBC, INADEQUATE, INAPT) for elucidating carbon frameworks. Good use has also been made of $^{1-3}J_{CH}$ coupling constant analysis in resolution of ambiguous structural assignments.[23] Naturally, single-crystal X-ray diffraction analysis has given definitive structures when suitable crystals were available. Because the ring system **2** is highly conserved, some general features in the appearance of the ^1H NMR

spectra are common to most of these alkaloids and useful in identifying a member of the pyridoacridine class. The disubstituted benzo ring A gives rise to a distinctive linear four proton coupled spin network (H1–H4, δ 7.0–9.0 ppm, J = 8–9 Hz) with H1 resonating at lowest field due to the deshielding acridine nitrogen. A second AB spin system (δ 8.5, 9.0 ppm, J = 5–6 Hz) is assignable to H5–H6—the α and β protons of a trisubstituted pyridine ring. Finally, a strong nOe (ca. 20%) is always seen between the two "bay region" protons, H4–H5, thus, linking these two nonscalar coupled substructures.

C. Phyletic Distribution

Pyridoacridine alkaloids have been found in sponges (phylum Porifera), tunicates (Urochordata), one anemone (Cnidaria), and one prosobranch (Mollusca). The fact that pyridoacridines have been *isolated from different species in more than one major phyla* is extraordinary, as cross phyletic distribution of marine secondary metabolites is rare. This has lent some support to the commonly expressed opinion that these alkaloids are actually products of marine microorganisms that can colonize invertebrates of several phyla in symbiotic unions. Despite many such parenthetic declarations in published papers describing new pyridoacridines, no evidence has appeared so far to confirm this hypothesis. It seems just as likely that these compounds are *de novo* products of the host organisms, perhaps overexpressed secondary metabolites of aromatic amino acid secondary metabolic pathways common to many marine organisms. The poor understanding in this area is a consequence of general lack of studies in alkaloid metabolism in marine organisms. (See the review by M.J. Garson in this issue of Chemical Reviews.) Clearly, additional research is needed.

III. Structural Chemistry

The following section describes the known pyridoacridines and the compounds are arranged by total ring count. A list of the pyridoacridine alkaloids reported to date (February, 1993), ordered by molecular formula and containing additional physical data (mp, $[\alpha]_D$) is provided in Table I. For convenience, synonyms for the same compound have been indicated. Pyridoacridines vary in structure by appendage of different side chains or fusion of rings to ring C, and occasionally, to the acridine nitrogen. Halogenation is rarely seen, and when present, this is always bromine at C2 in ring A (although, see batzellines and isobatzellines, Pyrroloacridines section III.E). As mentioned earlier, oxidation states of the rings vary, and sometimes partial saturation is seen in ring D, but more commonly, within additional rings appended to ring C.

A. Tetracyclic Alkaloids

The tetracyclic members of this class are archetypical pyridoacridines. The oxidation state may be that of a 10*H*-pyridoacridine **2** or the corresponding iminoquinone **3**. The latter oxidation state may be important in mediating the exceptional cytotoxicity associated with all marine alkaloids bearing the iminoquinone subunit.

The yellow tunicate *Cystodytes dellechiajei* from Okinawa afforded nine cytotoxic tetracyclic alkaloids, cystodytins A–I (**5** and **8–13**, Chart II).[17,24] The cystodytins A–C are the first pyridoacridine alkaloids isolated from a marine tunicate and the first tetracyclic members of this class. The common heterocyclic nucleus of cystodytins A–C (**5**, **8**, and **9**) is an iminoquinone-substituted at C10 with a 2-amidoethyl side chain. The *N*-acyl groups are derived from β,β′-dimethylacrylic, tiglic, and 3-hydroxy-3-methylbutanoic acids, respectively. Cystodytins D–I (**10–15**) are chiral, levorotatory compounds, due to the presence of a 2-amido-1-hydroxyethyl side chain, *N*-substituted with one of the above mentioned C_5 carboxylic acids. Cystodytins F–I (**12–15**) are substituted with an *O*-methyl ether or *O*-9-octadecenoate ester.[24] The isomeric pairs of cystodytin β,β′-dimethylacrylate and tiglate amides could not be separated and were characterized as 7:2 mixtures.

Hydration of cystodytin A (**5**, 6% HCl aqueous 100 °C, 3 h) is reported to give cystodytin C (**9**).[17] When treated with diazomethane, **5** formed a methyl ether, **16**, (23%). This transformation is unusual as it constitutes a formal reductive methylation. The iminoquinone **5** is readily reduced in the ionization stage of a mass spectrometer (M + 2 ion in EIMS, MH + 2 for FABMS), as is typical for quinones. In remarkable contrast, cystodytin A could be hydrogenated over Adams catalyst in acetic acid to provide **17** by reduction of the side chain and disubstituted benzene ring, but leaving the iminoquinone intact.

Two reports disclose the structure of the relatively simple tetracyclic alkaloid, norsegoline (**50**), from the Red Sea tunicate, *Eudistoma* sp., along with shermilamine B (**40** = debromoshermilamine,[25] (see also section III.C, Hexacyclic Alkaloids, below).[26,27]

The vivid purple ascidian *Lissoclinum vareau* from Fiji contains two bright crimson pigments, the homologues varamine A (**18**) and B (**19**)[28] which cooccur with the antitumor pentathiepin alkaloid, varacin.[29] Varamines A and B have a parent tetracyclic aromatic ring system at the same oxidation level as the cystodytin A methylation product, **16**, however, the varamines also contain a methyl thioether substituent at C9. Assignment of the thiomethyl group followed from measurement of $^1J_{CH}$ from the ^{13}C satellites of the methyl ^1H NMR signal at δ 2.66 ppm which had a value of 141 Hz, typical for an *S*-methyl group. Ring C of varamine A, isoelectronic with hydroquinone, was readily oxidized by aqueous ceric ammonium nitrate to the iminoquinone **20** in quantitative yield.[28] The corresponding oxidation product, **6**, of varamine B (**19**) was identical with diplamine from the Fijian tunicate, *Diplosoma* sp.[30] Another homologue in this series, isobutyramide **21**, has been recently characterized from an unidentified Australian tunicate.[31]

B. Pentacyclic Alkaloids

The pentacyclic pyridoacridines can be classified into two groups: (a) those having one additional angular fused ring at C9,10 of the acridine system at ring C, and (b) those having linear ring fusion at C8,9 of ring C. Typical ring appendages include pyridine, tetradehydropyridine, pyridone, thiazine, or even a thiazole heterocycle. In some examples, a substituted 2-ethylamino side chain is also attached to the acridine C ring.

The bright yellow zoochrome, calliactine, from the Mediterranean anemone, *Calliactis parasitica*, has a

Table I

	name	no.	formula	phylum[a]	genus species	mp, °C	$[\alpha]_D$, deg	ref(s)
			Naturally Occurring Marine Pyridoacridines					
1	kuanoniamine A	41	$C_{16}H_7N_3OS$	U, M	tunicate[b] *Chelynotus semperi*	255–8		46
2	2-bromoleptoclinidone	29	$C_{18}H_8BrN_3O$	U	*Leptoclinides* sp.	>300		39
3	ascididemin	27	$C_{18}H_9N_3O$	U	*Didemnum* sp.	>300		38
4	meridine	30	$C_{18}H_9N_3O_2$	U	*Amphicarpa meridiana*	>250		14
5	"meridine tautomer"	31	$C_{18}H_9N_3O_2$	U	*Amphicarpa meridiana*			14
6	11-hydroxyascididemin	32	$C_{16}H_9N_3O_2$	U	*Leptoclinides* sp.	>250		14
7	calliactine	22	$C_{18}H_{12}N_4O$	C	*Calliactus parasiticus*			34
8	norsegoline	50	$C_{18}H_{14}N_2O_3$	U	*Eudistoma* sp.			26, 27
9	amphimedine	1	$C_{19}H_{11}N_3O_2$	P	*Amphimedon* sp.	>360		1
10	neoamphimedine	25	$C_{19}H_{11}N_3O_2$	P	*Xestospongia* c.f. *carbonaria*	>300		15
11	cyclodercitin	44	$C_{19}H_{14}N_3S$[c]	P	*Dercitus* sp.	298		18, 47
12	dercitamine	37	$C_{19}H_{16}N_4S$	P	*Stelleta* sp.	135		18, 47
13	stelletamine	46	$C_{20}H_{14}N_4S$	P	*Stelleta* sp.	280–2		18
14	kuanoniamine D	43	$C_{20}H_{16}N_4OS$	U, M	tunicate,[b] *Chelynotus semperi*	>300		46
15	nordercitin	36	$C_{20}H_{18}N_4S$	P	*Stelleta* sp.	176		18, 47
16	diplamine	6	$C_{20}H_{19}N_3O_2S$	U	*Diplosoma* sp.	202–4		30
17	petrosamine	7	$C_{21}H_{17}BrN_3O_2$[c]	P	*Petrosia* sp.	>330		43
18	shermilamine A	39	$C_{21}H_{17}BrN_4O_2S$	U	*Trididemnum* sp.	>300		25,48
19	debromopetrosamine	26	$C_{21}H_{18}N_3O_2$[c]	P	*Xestospongia* c.f. *carbonana*	>300		15
20	shermilamine B (debromoshermilamine A)	40	$C_{21}H_{18}N_4O_2S$	U	*Trididemnum* sp.	254		25, 48
21	dercitamide (= kuanoniamine C)	38	$C_{21}H_{18}N_4OS$	P	*Stelleta* sp.	192		18, 47
22	dercitin	34	$C_{21}H_{20}N_4S$	P	*Dercitus* sp.	168		18, 45
23	varamine B	19	$C_{21}H_{21}N_3O_2S$	U	*Lissoclinum* sp.			28
24	cystodytin A	5	$C_{22}H_{19}N_3O_2$	U	*Cystodytes dellechiajei*	182–4		17
25	cystodytin B	8	$C_{22}H_{19}N_3O_2$	U	*Cystodytes dellechiajei*	180–2		17
26	cystodytin D	10	$C_{22}H_{19}N_3O_3$	U	*Cystodytes dellechiajei*		−160[d]	24
27	cystodytin E	11	$C_{22}H_{19}N_3O_3$	U	*Cystodytes dellechiajei*		−160[d]	24
28	"isobutyramide"	21	$C_{22}H_{21}N_3O_2S$	U	tunicate[b]	202–4		31
29	cystodytin C	9	$C_{22}H_{21}N_3O_3$	U	*Cystodytes dellechiajei*	257–9		17
30	varamine A	18	$C_{22}H_{23}N_3O_2S$	U	*Lissoclinum* sp.			28
31	segoline A	47	$C_{23}H_{19}N_3O_3$	U	*Eudistoma* sp.	>300	−322	26, 27
32	segoline B	48	$C_{23}H_{19}N_3O_3$	U	*Eudistoma* sp.		+355	26, 27
33	isosegoline A	49	$C_{23}H_{19}N_3O_3$	U	*Eudistoma* sp.		−660	26, 27
34	cystodytin F	12	$C_{23}H_{21}N_3O_3$	U	*Cystodytes dellechiajei*		−133[d]	24
35	cystodytin G	13	$C_{23}H_{21}N_3O_3$	U	*Cystodytes dellechiajei*[b]		−133[d]	24
36	kuanoniamine B	42	$C_{23}H_{22}N_4OS$	U, M	tunicate,[b] *Chelynotus semperi*	>300		46
37	eilatin	51	$C_{24}H_{12}N_4$	U	*Eudistoma* sp.	>310		26, 27
38	eudistone B	53	$C_{27}H_{17}N_5O$	U	*Eudistoma* sp.		−177.8	50
39	eudistone A	52	$C_{27}H_{19}N_5O$	U	*Eudistoma* sp.			50
40	cystodytin H	14	$C_{40}H_{53}N_3O_4$	U	*Cystodytes dellechiajei*		−29[d]	24
41	cystodytin I	15	$C_{40}H_{53}N_3O_4$	U	*Cystodytes dellechiajei*		−29[d]	24
			Pyrroloquinones and Related Compounds					
42	batzelline C	66	$C_{11}H_9ClN_2O_2$	P	*Batzella* sp.			60
43	batzelline B	65	$C_{11}H_9ClN_2O_2S$	P	*Batzella* sp.			60
44	isobatzelline C	69	$C_{11}H_{10}ClN_3O$	P	*Batzella* sp.			51
45	isobatzelline D	70	$C_{12}H_{10}ClN_3OS$	P	*Batzella* sp.			51
46	batzelline A	64	$C_{12}H_{11}ClN_2O_2S$	P	*Batzella* sp.			60
47	isobatzelline A	67	$C_{12}H_{12}ClN_3OS$	P	*Batzella* sp.			51
48	isobatzelline B	68	$C_{12}H_{13}N_3OS$	P	*Batzella* sp.			51
49	prianosin B	58	$C_{18}H_{12}BrN_3O_2S$	P	*Prianos melanos*	250–1	+360	53
50	discorhabdin B	62	$C_{18}H_{12}BrN_3O_2S$	P	*Latrunculia* sp.	>360	+400	54
51	plakinidine C	56	$C_{18}H_{12}N_4O$	P	*Plakortis* sp.			56
52	discorhabdin C	61	$C_{18}H_{13}Br_2N_3O_2$	P	*Latrunculia* sp.			59
53	prianosin A (discorhabdin A)	57	$C_{18}H_{14}BrN_3O_2S$	P	*Prianos melanos*	>360	+248	52, 54
54	discorhabdin D	63	$C_{18}H_{14}N_3O_2S$[c]	P	*Latrunculia* sp.	>360	$[\alpha]_{546}$ −160	55
55	plakinidine A	54	$C_{18}H_{14}N_4O$	P	*Plakortis* sp.			56, 57
56	prianosin D	60	$C_{18}H_{15}N_3O_2S$	P	*Prianos melanos*	>300	+344	53
57	prianosin C	59	$C_{18}H_{15}N_3O_3S$	P	*Prianos melanos*	>300	+358	53
58	plakinidine B	55	$C_{19}H_{16}N_4O$	P	*Plakortis* sp.			56, 57
59	wakayin	71	$C_{20}H_{14}N_4O$	U	*Clavelina* sp.			61

[a] Phyla are abbreviated as follows: C, Cnidaria (anemone); M, Mollusca; P, Porifera (sponges); U, subphylum Urochordata (tunicates). [b] Unidentified. [c] Quarternary salt. Formula is for cation, only. [d] $[\alpha]_D$ for 7:2 mixture of geometric isomers.

long history and is probably the first noted marine pyridoacridine alkaloid. Calliactine attracted the attention of Lederer *et al.* in 1940[32] and later by Barbier;[33] however, structural work by both groups was hampered in part by low solubility along with difficulties in purification, and gave inconclusive results. In 1987 Cimino and co-workers reported their studies on the degradation and NMR spectroscopy of calliactine and its derivatives.[34] Calliactine is readily aromatized (presumably autoxidation–elimination with concomitant hydrolytic loss of ammonia) by boiling with dilute HCl to give "chlorocalliactine" or, with water, to give "neocalliactine" which in turn gave neocalliactine acetate upon treatment with acetic anhydride in

Chart III

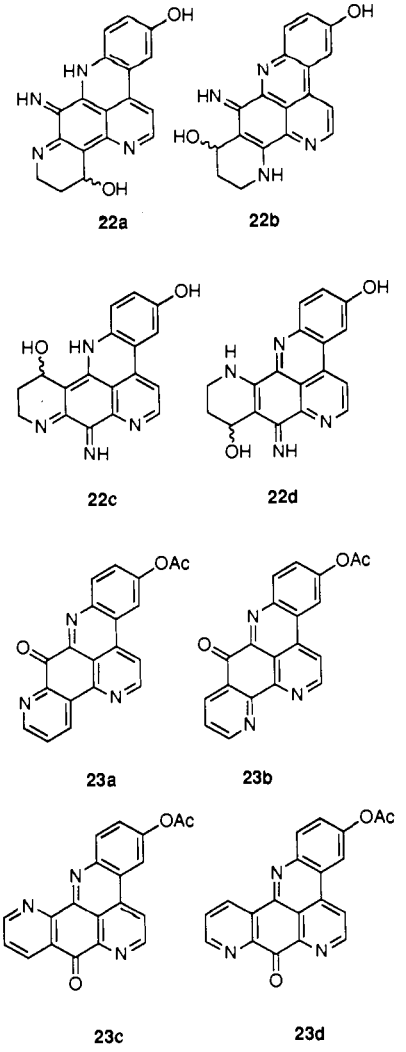
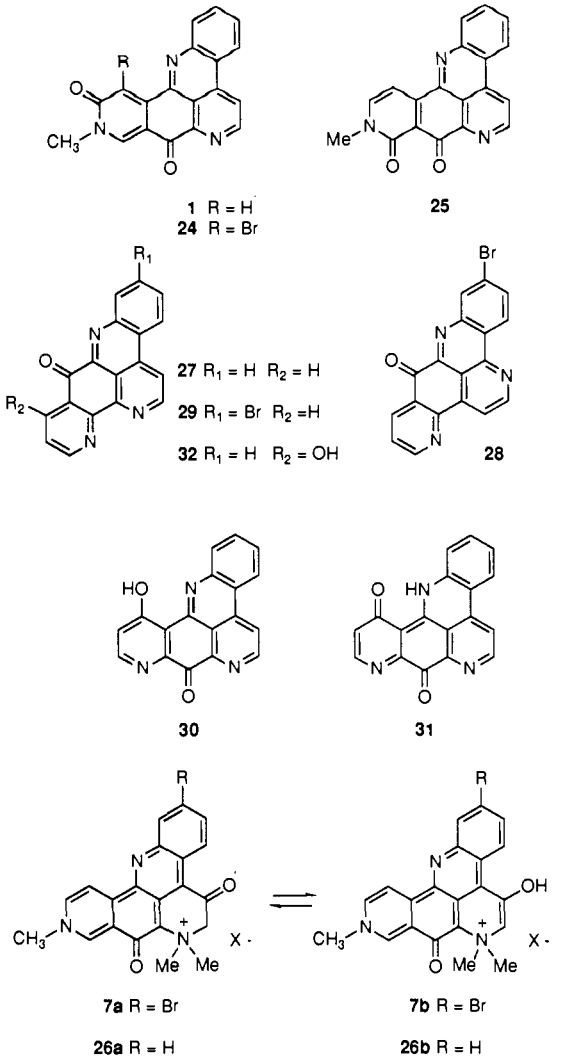


Chart IV



pyridine. The molecular formula $C_{18}H_{21}N_4O$ was established for calliactine and several possible structures were advanced (**22a–d**, Chart III). In addition, four possibilities for the structure of neocalliactine acetate, **23a–d**, were proposed, however, definitive assignments have not yet been reported on either of these compounds.

Amphimedine (**1**) was the first pyridoacridine to be fully characterized and the structure represents the first elucidated for this class of alkaloid.[1] Amphimedine is a sparingly soluble yellow pigment from a Guamanian sponge described as *Amphimedon* sp. The use of the powerful, but relatively insensitive 2D ^{13}C–^{13}C INADEQUATE NMR technique,[35–37] allowed critical bond connections to be made across quaternary carbon atoms in amphimedine and provided an unambiguous structure. Amphimedine is selectively brominated (Br_2, acetic acid) to give the monobromo derivative **24**. Researchers at SmithKline Beecham and Scripps Institution of Oceanography have reported neoamphimedine (**25**), along with amphimedine (**1**) and debromopetrosamine (**26**), from the Micronesian sponge *Xestospongia* cf. *carbonaria*[15] (Chart IV). Neoamphimedine (**25**) is a regioisomer of **1**.

Kobayashi and co-workers described ascididemin (**27**) from a species of *Didemnum* collected in Okinawa.[38] The structural proof relied on extensive use of long-range ^1H–^{13}C correlation (COLOC) data and critical comparison with the properties of amphimedine (**1**). It is noteworthy that ascididemin, like related pyridoacridine alkaloids which embody a 1,10-phenanthroline ring system, does not form a bright red complex with Fe^{2+} that is characteristically observed with 1,10-phenanthroline, itself.

In 1987 Schmitz and co-workers isolated 2-bromoleptoclinidone from *Leptoclinides* sp. collected in Truk Lagoon.[39] 2-Bromoleptoclinidone, one of three known halogenated pyridoacridines, was assigned structure **28** based on interpretation of long range 2D ^1H–^{13}C NMR correlation data and the absence of a color reaction with Fe^{2+}; however, this structure was later revised. The correct structure **29** was shown to have an alternate pyrido ring orientation.[40] This was confirmed by selective long-range 1D ^1H–^{13}C INAPT experiments[41] and debromination of 2-bromoleptoclinidone to ascididemin (**27**).[40]

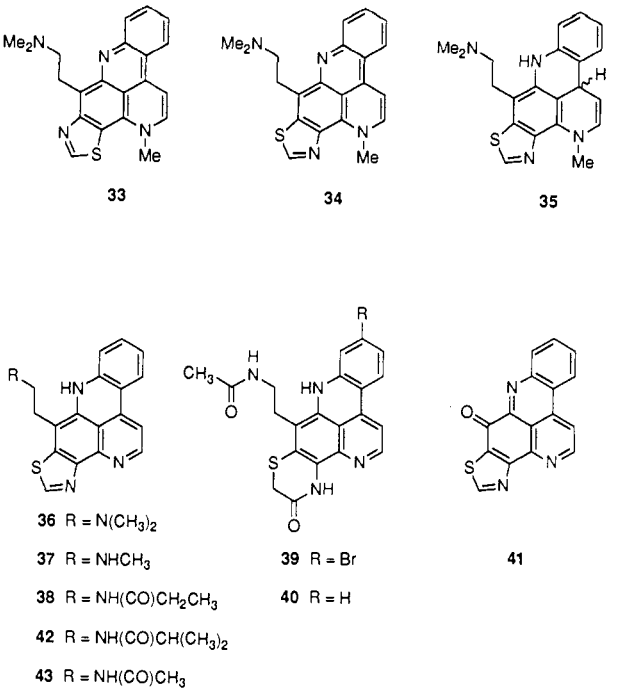
Pyridoacridines are not restricted to marine invertebrates from tropical waters. Meridine (**30**), a pentacyclic phenolic alkaloid, was obtained from a South Australian temperate water tunicate, *Amphicarpa meridiana*[14] and, more recently, from a Caribbean sponge, *Corticum* sp.[42] The structure of meridine was determined by single-crystal X-ray diffraction analysis.[14] A cooccuring isomer, **31**, was also isolated from *A. meridiana* by silica gel chromatography and char-

acterized by spectroscopic analysis. The regiochemistry in **31** was assigned on the basis of nOe studies, however, this finding is unusual as the isomer **31**, a vinylogous amide, must exist as a relatively nonlabile tautomer because it slowly undergoes complete isomerization to meridine (**30**) upon standing in $CDCl_3$. Rapid tautomerism of pyridoacridines has been observed previously in the case of petrosamine (**7**, see below[43]). In the same communication, Schmitz *et al.* also reported a regioisomer of meridine, 11-hydroxyascididemin (**32**) from *Leptoclinides* sp.,[14] the tunicate which also yielded 2-bromoleptoclinidone. All three natural products, **30**, **31**, and **32**, are clearly related to calliactine and neocalliactine acetate. Comparison of the data for the three structures with published data for neocalliactine acetate (**23**)[34] allowed Schmitz *et al.* to narrow down the possible structures of neocalliactine acetate to **23a** or **23b**.

When viewed underwater, the Caribbean sponge *Petrosia* sp. appears jet-black due to its deep, dark pigmentation. Samples collected from Belize and immersed in methanol imparted a deep green-blue color to the solvent, but when the extract was diluted with water, the color changed to purple.[44] These observations guided the isolation of the brominated pigment petrosamine (**7**) from *Petrosia* sp., along with tryptamine.[43] Petrosamine, as revealed by single-crystal X-ray crystallography in the solid state, exists as the chloride salt of a quaternized pyridone–acridine ring system, **7a**. Correlation of the solvent-dependent changes in the UV spectrum and NMR spectra suggested that the remarkable color changes observed by varying solvent polarity were associated with shifts in the position of a keto-enol equilibrium, favoring the enol form, **7b**. When ^1H NMR spectra of petrosamine were measured in hydroxylic solvents (D_2O, CD_3OD) the methylene proton signals of **7** rapidly disappeared due to deuterium exchange.[43] As mentioned earlier, debromopetrosamine (**26**) was isolated along with neoamphimedine (**25**) and amphimedine (**1**), from the Micronesian sponge *Xestospongia* cf. *carbonaria*.[15] Comparison of the latter sponge with the original sponge sample, from which amphimedine (**1**) was obtained,[1] suggests the two specimens are identical.[15]

The deep-water sponge *Dercitus* sp. yields the dark violet pigment dercitin, together with dimethylindolinium chloride.[45] The structure **33** (Chart V) originally proposed on the basis of spectroscopic data, including 2D INADEQUATE, and chemical conversion contains a novel benzothiazole moiety. This structure was later revised to **34**[18] by interpretation of the magnitudes of long range carbon–proton coupling constants. The earlier assignment error arises from inherent difficulties in interpretation of 2D ^1H–^{13}C NMR spectra in highly condensed heteroaromatic compounds; in this case, the placement of nitrogen and sulfur of the thiazole ring with the correct position relative to other acridine substituents. The solution lies in correct assignment of the benzothiazole ring junction carbons. The thiazole proton exhibits different $^3J_{CH}$ to each of the two ring junction quaternary carbons, thus providing unambiguous assignment of the respective ^{13}C signals.[18,46] The ease with which a partially reduced pyridoacridine system can be aromatized was demonstrated when the dihydropyridoacridine, **35** obtained by reduction of

Chart V



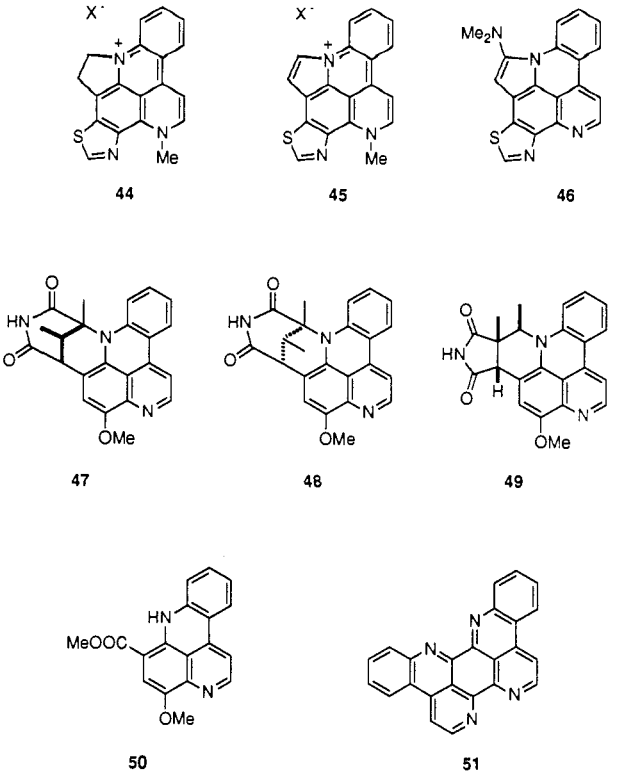
dercitin by sodium borohydride, rapidly autoxidized back to dercitin during workup.

In a second report from the same laboratories the structures of four minor congeners from *Dercitus* sp. and another sponge *Stelleta* sp., both of the family Pachastrellidae, were revealed.[47] The structures shown here (**36**, **37**, and **44**) are those that have been revised by reinterpretation of spectroscopic data for the same reasons that dercitin was revised.[18,46] Nordercitin (**36**) and dercitamine (**37**) are related to **34** as *N*-methyl homologues (Chart V). Reductive methylation of dercitamine (HCOOH, HCHO) gave nordercitin (**36**). Dercitamide (**38**) contains a propionamido side chain and is identical with kuanoniamine C (see below). Cyclodercitin (**44**) is a hexacyclic quaternary salt (see below).

The purple colonical tunicate *Tridemnum* sp. yielded two related alkaloids, shermilamine A (**39**) and shermilamine B (**40**), described in two reports.[25,48] The first report describes shermilamine A and shows the structure (solved by single-crystal X-ray crystallography) of a pentacyclic pyridoacridine–thiazinone system, brominated at C2 (acridine numbering), while the second report reveals shermilamine B (**40**) as the debromo-analog of **39**. A footnote in the second report,[25] however, states that the ^1H NMR and ^{13}C NMR data reported first[48] for shermilamine A are actually those for shermilamine B, while the MS data reported for A are those of a mixture of A and B. Readers should note the second report contains corrected spectroscopic data for both compounds and a different numbering scheme. Two different melting points are reported in the two papers for shermilamine A (**39**, mp 135 °C and mp >300 °C), perhaps corresponding to the free base and the salt.[25,48]

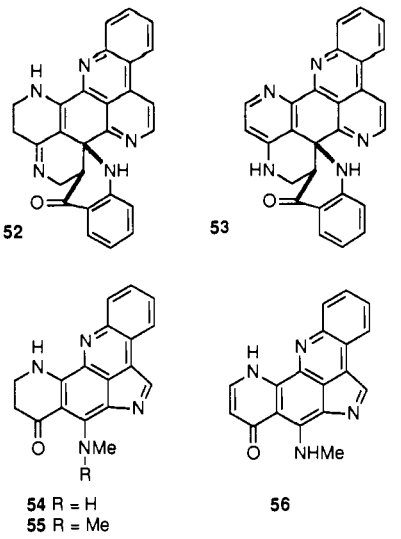
The kuanoniamines A–D (**41**, **42**, **38**, **43**), along with shermilamine B (**40**), were found in the lamellarid mollusc, *Chelynotus semperi*, and its prey, an unidentified tunicate, both collected in Pohnpei.[46] Kuanoniamine C is identical with dercitamide (**38**) and the latter

Chart VI



44 45 46 47 48 49 50 51

Chart VII



52 53

54 R = H
55 R = Me

56

name should take precedence.[18,47] Dercitamide (38), along with meridine (30), is the only pyridoacridine found in both a sponge and a tunicate. Kuanoniamines B (42) and D (43) are homologues of kuanoniamine C, bearing isovaleramide and acetamide side chains, respectively. Kuanoniamine A (41) differs from the other three alkaloids in lacking the 2-amidoethyl side chain and contains an iminoquinone structure analogous to those found in 2-bromoleptoclinidone and ascididemin. Dercitamide (38, = kuanoniamine C) and kuanoniamine D (43), together with shermilamine B (40), have also been isolated from dark purple tunicates of the genus *Cystodytes*, collected in Pohnpei[18] and in the Mediterranean Sea.[49]

C. Hexacyclic and Heptacyclic Alkaloids

Two hexacyclic alkaloids have been reported from the deep-water sponges *Dercitus* sp. and *Stelleta* sp. Cyclodercitin (44, Chart VI) is found along with dercitin (34) and related compounds in *Stelleta* sp.[47] The sixth ring in cyclodercitin is formally derived by cyclization of the 2-aminoethyl side chain to the acridine nitrogen, while the pyridine ring is substituted with an *N*-methyl group. When dissolved in TFA-*d*, cyclodercitin spontaneously autoxidizes to the hexacyclic pyrrolo compound 45. More recently, the hexacyclic alkaloid stelletamine (46), was obtained as a minor component of *Stelleta* sp.; the structure was determined by X-ray analysis.[18]

As mentioned earlier, six alkaloids were reported from the Red Sea tunicate, *Eudistoma* sp., including the known shermilamine B (40),[25] and the first optically active pyridoacridine alkaloids.[26,27] Segoline A (47) and isosegoline A (49) are regioisomeric hexacyclic alkaloids, each containing a rare cyclic imide.[26] The structure of 47 was established by single-crystal X-ray diffraction spectroscopy while 49 was determined by interpretation of NMR spectroscopic data. Segoline B (48) was assigned as the diastereoisomer of 47 in which the bridge across the cyclic imide ring is inverted. This was supported by the strongly bisignate CD curves for 47 and 48 which are almost exactly opposite in sign. Norsegoline (50) has already been mentioned under Tetracyclic Alkaloids. The structure of eilatin (51), an unusual pyridoacridine "pseudo-dimer" was also solved by X-ray diffraction and is the only known heptacyclic pyridoacridine alkaloid.

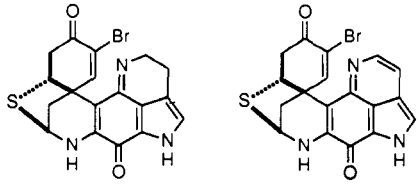
D. Octacyclic Alkaloids

Chiral pyridoacridines are rare. Eudistones A (52) and B (53, Chart VII) are two optically active octacyclic alkaloids from the Seychelles tunicate *Eudistoma* sp.[50] which also cooccur with ascididemin (1).[38] The structures of 52 and 53 differ from other members of this class in that an additional dihydroisoquinolone bicyclic ring system is fused to a quaternary sp^3 carbon of the acridine system. The relative stereochemistry of the carbon skeleton was determined by comparison of NMR coupling constants with values predicted from molecular modeling. The two compounds were correlated by autoxidation; bubbling air through a solution of eudistone A (52) in DMSO at 60 °C aromatized the dihydropyridine ring to provide eudistone B (53).[50] The circular dichroism spectrum of eudistone B (53) exhibited a strong bisignate Cotton effect, however, the absolute configuration of the two compounds remains unknown.

E. Pyrroloacridines and Related Compounds

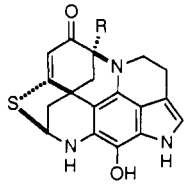
This review would be incomplete without mentioning a small group of marine alkaloids whose structures, although not strictly within the definition of "pyridoacridine", bear structural similarities to the preceding compounds. Like the pyridoacridines, these minor compounds are mostly cytotoxic. The batzellines, isobatzellines,[51] prianosins,[52,53] discorhadbins,[54,55] and plakinidines[56,57] are sufficiently reminiscent of the more commonly encountered pyridoacridines that they warrent description here. Although the structural similarities in these compounds are suggestive of a common

Chart VIII

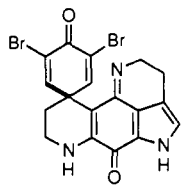


57

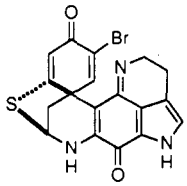
58



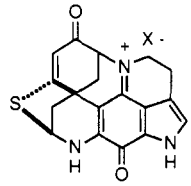
59 R = OH
60 R = H



61



62



63

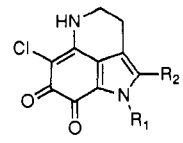
biosynthetic origin, proof of this hypothesis awaits definitive studies.

Pyrroloacridines have similar ring construction to the pyridoacridine alkaloids; however, they contain a fused-pyrrole ring rather than the familiar trisubstituted pyridine ring. The first examples of this class are plakinidine A (**54**) and B (**55**), obtained from a Vanuatu species of *Plakortis* sponge.[57] A second report by Ireland *et al.* described the isolation and characterization of the same compounds, together with a new plakinidine C (**56**), as constituents of *Plakortis* sp., collected in Fiji.[56] Two-dimensional ^{13}C–^{13}C INADEQUATE[36] was used to verify most of the structural elements of plakinidine B (**55**).[56,58]

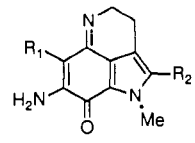
Bioassay guided purification of the methanol–toluene extract of the tropical green sponge *Prianos melanos* from Okinawa gave a cytotoxic pigment, prianosin A (**57**, structure defined by X-ray crystallography[52]) and prianosin B (**58**), C (**59**) and D (**60**, Chart VIII).[53] Several independent reports from the group at the University of Canterbury described the identification of discorhabdins, compounds similar to prianosins. The first report described the isolation and structure elucidation by X-ray, of discorhabdin C (**61**) an achiral spiro-alkaloid.[59] Discorhabdin A (identical to prianosin A, **57**) was isolated together with discorhabdin B (**62**), from three species of *Latrunculia* sponge collected in the temperate waters around New Zealand.[54] More recently, the quaternary iminium salt, discorhabdin D (**63**), was isolated from both *Latrunculia brevis*, from New Zealand and *Prianos* sp., from Okinawa.[55] Each discorhabdin or prianosin contains an α,β-unsaturated cyclohexenone, spiro-fused to a tetracyclic heterocyclic ring system at various levels of oxidation. Each ring system, with the exception of discorhabdin C (**61**) is also bridged with a tetrahydrothiophene ring.

Three simple pyrroloquinolines, batzellines A–C (**64**–**66**, Chart IX), were obtained from a deep-water

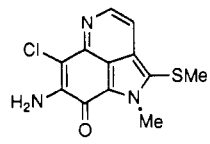
Chart IX



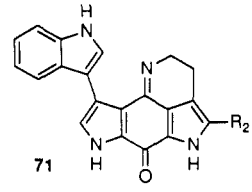
64 R_1 = Me R_2 = SMe
65 R_1 = H R_2 = SMe
66 R_1 = Me R_2 = H



67 R_1 = Cl R_2 = SMe
68 R_1 = H R_2 = SMe
69 R_1 = Cl R_2 = H



70



71

collection of *Batzella* sp. from the Bahamas.[60] Each alkaloid has a tetrahydroquinolone nucleus, further bridged across both rings by a trisubstituted pyrrole ring. Once again, an iminoquinone substructure is evident along with the rare appearance of chlorine in a condensed heterocyclic alkaloid. Four additional alkaloids, isobatzellines A–D (**67**–**70**) were later reported from the same sponge.[51] In spite of the names, isobatzellines are not strictly isomeric with batzellines, but differ in replacement of one of the *o*-quinone carbonyl groups with an amino group. Compared to A, isobatzelline D (**70**) has lost the elements of hydrogen from the piperidine ring to give an aromatic structure.[51] This oxidation is facile; autoxidation of isobatzelline A (**67**) to D (**70**) could be observed within a few hours during thin-layer chromatography, or by treatment with DDQ. Isobatzelline A (**67**) was converted to batzelline A (**64**) by diazotization–substitution in aqueous nitrous acid.

Wakayin (**71**) is a unique indolo alkaloid found in the Fijian tunicate, *Clavelina* sp.[61] The structure of wakayin was obtained by interpretation of DQFCOSY, HMBC, and HMQC spectra. Compound **71** has a superficial resemblance to discorhabdins, prianosins, and the simple iminoquinone, isobatzelline C (**69**), however, an additional remnant of tryptophan catabolism is now clearly evident in the biogenesis of this alkaloid.

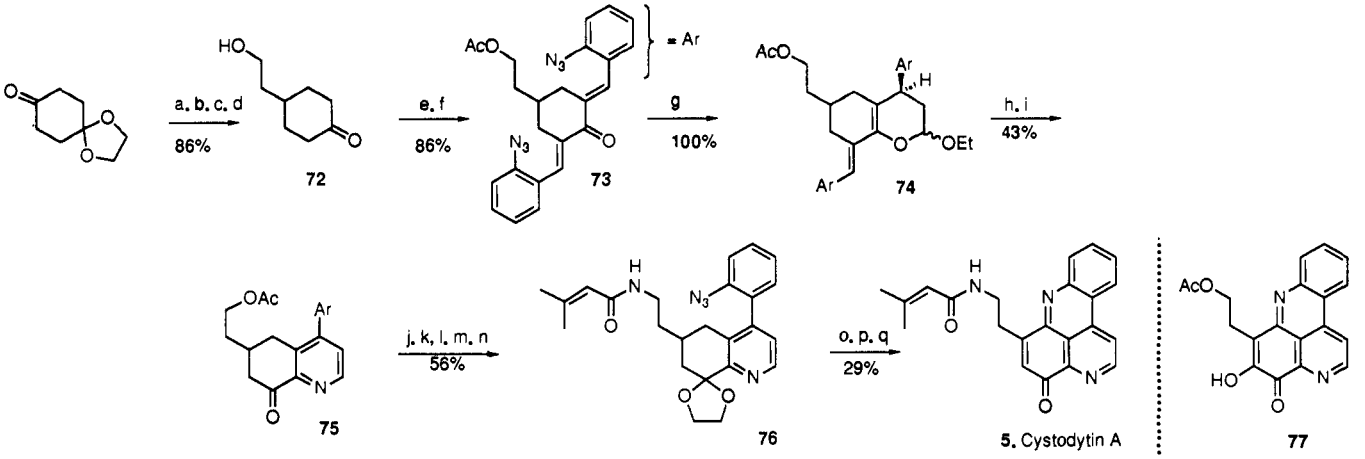
IV. Total Synthesis

Several papers have appeared describing total synthesis of pyridoacridine alkaloids or progress toward related pyridoacridine heterocycles. The following section will focus mainly on the total syntheses of natural products, with only brief mention of related synthetic work.

A. Cystodytins A (5) and B (8)

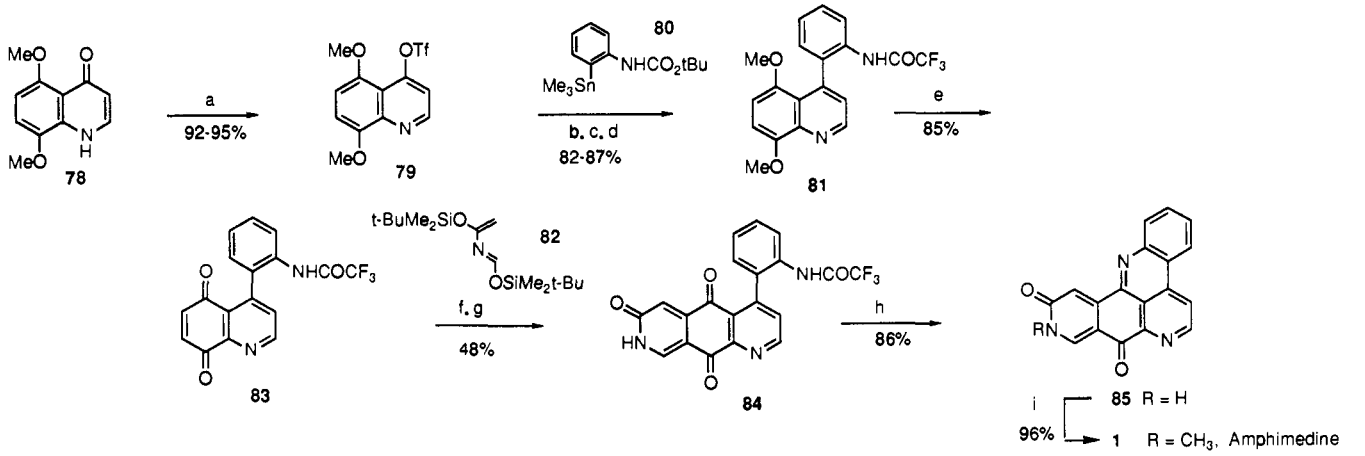
Recently Ciufolini *et al.* completed the total syntheses of cystodytins A (**5**) and B (**8**) utilizing an efficient intramolecular photochemical nitrene insertion into an aryl substituted dihydroquinolone (Scheme I[62,63]). The half-ethylene ketal of cyclohexane-1,4-dione was condensed with dimethyl(carbethoxymethyl)phosphonate, and the resulting α,β-unsaturated ester was reduced in

Scheme I[a]



[a] (a) $(MeO)_2POCH_2COOEt$, NaH, DME; (b) H_2, Pd on C; (c) LAH, Et_2O; (d) HCl (aq), THF; (e) *o*-$C_6H_4(N_3)CHO$, aq EtOH, NaOH; (f) Ac_2O, pyridine; (g) $CH_2{=}CHOEt$, $(CH_2Cl_2)_2$, cat. Yb(fod)$_3$, reflux; (h) $HONH_2{\cdot}HCl$, MeCN, reflux; (i) O_3, MeOH, −78 °C, then Me_2S, −78 °C to 25 °C; (j) $(CH_2OH)_2$, $(EtO)_3CH$, cat. CSA, reflux; (k) K_2CO_3, MeOH; (l) MsCl, Et_3N, CH_2Cl_2; (m) Gabriel synthesis; (n) β,β-dimethylacryloyl chloride, Et_3N, CH_3Cl_2; (o) 4 N HCl, aq, THF, 50 °C; (p) *hν*, Pyrex, PhCl, 110 °C; (q) DDQ, 25 °C.

Scheme II[a]



[a] (a) $(Tf)_2O$, 2,6-lutidine, CH_2Cl_2; (b) $Pd(PPh_3)_4$, 2 mol %, dioxane, LiCl, 3 equiv, 5–7 h, 100 °C; (c) TFA; (d) TFAA, $(iPr)_2NEt$; (e) CAN, 2.4 equiv, CH_3CN/H_2O, 23 °C, 15 min; (f) THF, 23 °C, 16 h; (g) pyridinium·HF, 5 equiv; (h) 6 M HCl, THF aq. 70–80 °C, 3 h; (i) Me_2SO_4, K_2CO_3, DMF.

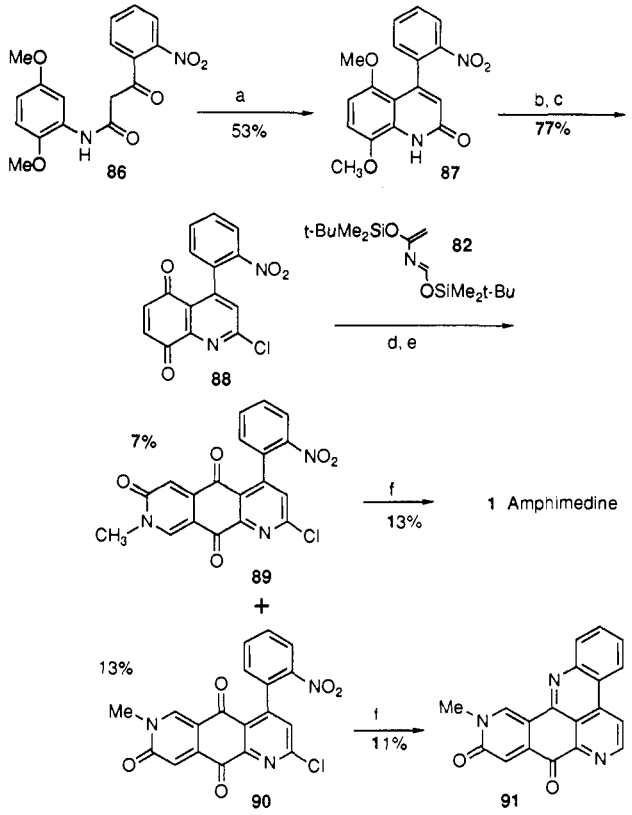
two steps (H_2, Pd; LAH) and deprotected to give alcohol **72**. Double Knoevenagel condensation of **72** with *o*-azidobenzaldehyde and acetylation afforded **73**, which added ethyl vinyl ether in the presence of Yb(III) catalyst to give ethyl ketal **74** as a 1:1 mixture of diastereomers. Condensation of **74** with hydroxylamine and concomitant aromatization followed by ozonolysis delivered pyridine **75**. Elaboration of the acylamide side chain was achieved after protection of the ketone as the ethylene ketal, by Gabriel synthesis and coupling of the resultant primary amine with β,β′-dimethylacryloyl chloride to provide precursor **76**. The stage was set for the final annelation and hydrolysis of the ketal protecting group (HCl, aqueous) was followed by photolytic triplet nitrene insertion and DDQ oxidation, affording cystodytin A (**5**). Cystodytin B (**8**) was synthesized in a likewise manner from the amine precursor to **76**, except tigloyl chloride was used in the acylation. In a parallel scheme, compound **77** was produced and suggested as a potentially useful intermediate in the synthesis of cystodytin analogs.[63] Indeed, it would appear that the iminoquinone, **77**, is ideally suited for short syntheses of diplamine (**6**) and varamines A (**18**) and B (**19**) starting with Michael addition of methyl thiolate to the iminoquinone ring followed by elimination.

B. Amphimedine (1)

Three total syntheses of amphimedine have been published.[64-67] In addition, several papers have appeared outlining synthetic approaches to tetracyclic or pentacyclic alkaloid skeletons.[68-72]

The first synthesis of amphimedine by Echavarren and Stille exploited an aza-Diels–Alder reaction and palladium-catalyzed cross coupling of aryl organostannanes with electrophilic aryl triflates (Scheme II[64]). The easily prepared quinoline **78** was converted to the corresponding 4-*O*-triflate **79** and condensed with the aryl stannane **80** in the presence of palladium(0), followed by refunctionalization to the trifluoroacetamide **81**. Diels–Alder addition of the aza diene **82** to **83** (obtained by oxidation of **81** with ceric ammonium nitrate) afforded quinone **84**. Compound **85**, obtained by hydrolysis of the trifluoroacetamide group in **84** with concomitant ring cyclization, was smoothly methylated to synthetic amphimedine (**1**, 23% overall yield), identical in all respects with the natural product.[1]

Scheme III[a]



[a] (a) 80% H_2SO_4, aq, 75 °C, 30 min; (b) $PCl_5/POCl_3$, 70 °C, 45 min; (c) CAN, CH_3CN aq, 0 °C, 15 min; (d) $CHCl_3$, 35 °C, 8 h; (e) CH_3I, K_2CO_3, tris[2-(2-methoxyethoxy)ethyl]amine, DMF, rt, 1 h; (f) H_2, 10% Pd/C, Et_3N, MeOH, rt, 20 h.

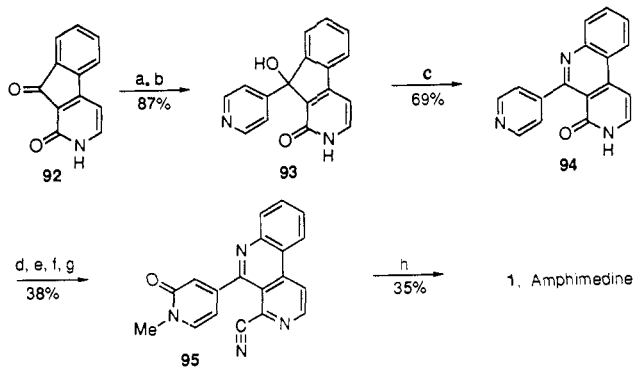
The second amphimedine synthesis, due to Kubo and Nakahara, also employs an aza-Diels–Alder reaction, although the yield was lower (Scheme III[65]). (*o*-Nitrobenzoyl)acetanilide (**86**), prepared by condensation of (*o*-nitrobenzoyl)acetic ester with 2,5-dimethoxyaniline, was cyclized in 80% H_2SO_4 to provide quinolone **87**. Treatment of **87** was with $PCl_5/POCl_3$, followed by oxidative demethylation with ceric ammonium nitrate gave quinone **88**. Diels–Alder addition of **88** to **82** gave a complex mixture which was directly methylated and separated by chromatography to provide the two regioisomers **89** and **90**. Amphimedine (**1**) was obtained from compound **89** by hydrogenolysis–condensation over palladium on charcoal, while the same treatment applied to **90** gave **91**, a new isomer of amphimedine.

A short, efficient route to amphimedine, elaborated by Prager and Tsopelas, utilizes an azido-ring expansion of a fluorenol and begins with **92** (Scheme IV[66,67]). Treatment of **92** with 4-pyridyllithium gave fluorenol **93**, followed by single-step azide substitution/rearrangement/N_2 elimination to provide the tetracyclic phenanthrone **94**. Completion of the synthesis by refunctionalization of **94** to the α-cyano precursor **95**, followed by cyclization in polyphosphoric acid afforded amphimedine (**1**).

C. 2-Bromoleptoclinidone (29)

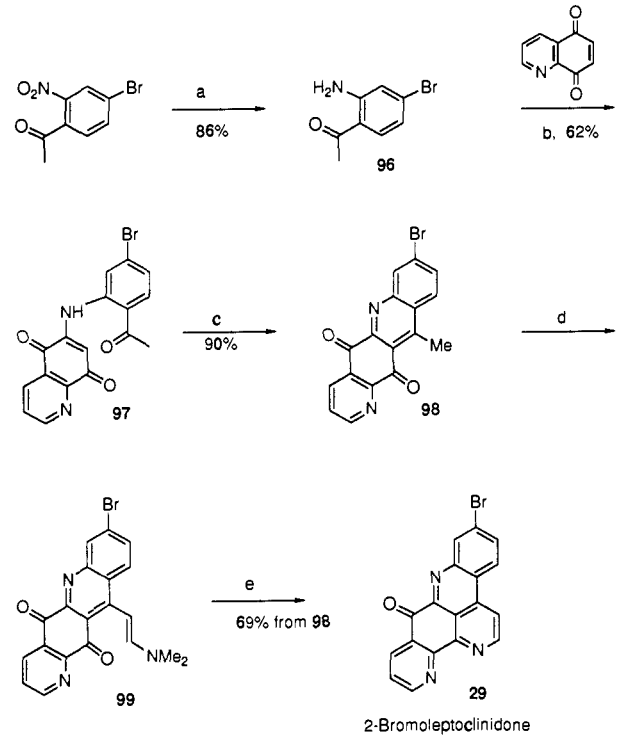
The synthesis of 2-bromoleptoclinidone (**29**), by Bracher, began with amine **96** prepared by selective reduction of 4-bromo-2-nitroacetophenone (Scheme V[73]). Oxidative amination of *p*-quinolinequinone by

Scheme IV[a]



[a] (a) Me_3SiCl, Et_3N, THF, 60 °C; (b) 4-pyridyllithium, −40 to 20 °C, 2 h; (c) NaN_3, PPA, 45 °C, 20 h; (d) PCl_5, DMF (cat.), in $POCl_3$, 180 °C, 20 h; (e) $MeOSO_2F$, 1.3 equiv, 20 °C, 40 min; (f) KOH, $K_3[Fe(CN)_6]$, 2 equiv, 20 °C, 10 h; (f) CuCN, DMSO, 150 °C, 4 h; (h) PPA, 90 °C, 5 h.

Scheme V[a]



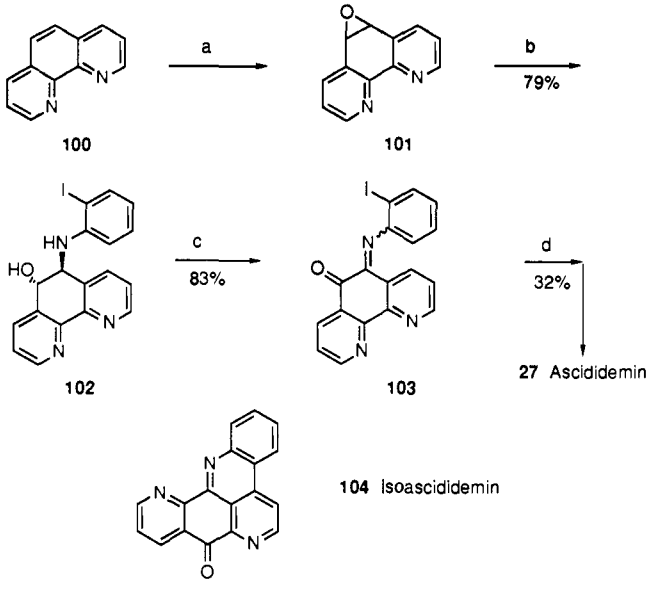
[a] (a) Fe, AcOH; (b) O_2, $Ce^{IV}(SO_4)_2$; (c) $AcOH/H_2SO_4$; (d) $Me_2NCH(OCH_2CH_3)_2$; (e) NH_4Cl, AcOH.

96 in the presence of air and Ce(IV) gave an intermediate **97** that cyclized to **98** when heated in a mixture of concentrated sulfuric and acetic acids. The enamine **99** was obtained by condensation of **98** with dimethylformamide diethyl acetal which, in turn, provided 2-bromoleptoclinidone (**29**) upon heating with ammonium chloride in refluxing acetic acid.

D. Asciidemin (27)

Two syntheses of asciidemin (**27**) have been published. The first synthesis by Bracher is essentially the same route described above for the total synthesis of 2-bromoleptoclinidone (see Scheme V), only it uses 2-nitroacetophenone for the oxidative amination.[74] A novel synthesis, reported by Moody *et al.* and published in two accounts, begins with 1,10-phenanthroline (**100**, Scheme VI).[75,76] Epoxidation of **100** with aqueous

Scheme VI[a]



[a] (a) NaClO, aq; (b) *o*-$IC_6H_4NH_2$, Et_3Al, CH_2Cl_2; (c) $BaMnO_4$, CH_2Cl_2; (d) *hν*, quartz, H_2SO_4.

bleach gave the oxidophenanthroline (**101**) in excellent yield. Lewis acid catalyzed epoxide ring opening with 2-iodoaniline gave amino alcohol **102** which was oxidized with barium permanganate to *o*-iminoquinone **103** in 66% overall yield. Isomerization–cyclization of **103** was achieved by carrying out photolysis in concentrated sulfuric acid and, after chromatography, ascididemin (**27**) was obtained in 32% yield.

The reported synthesis of the unnatural isomer, isoascididemin (**104**) is of some interest as it may provide an entry into several angularly fused pentacyclic pyridoacridines, such as meridine (**30**).[77]

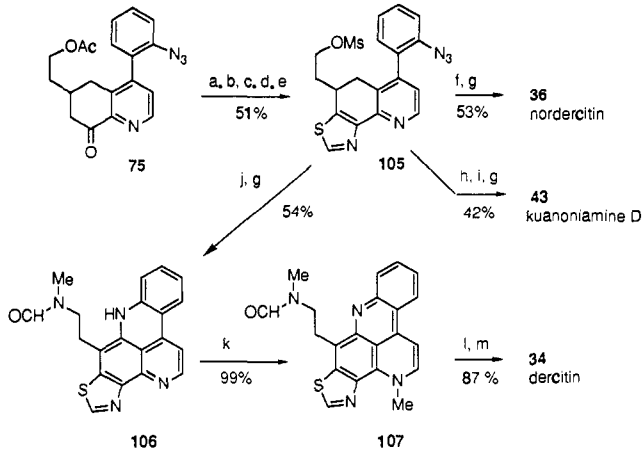
E. Dercitin (34), Nordercitin (36), and Kuanoniamine (43)

The successful strategy employed by Ciufolini[63] in the preparation of cystodytins A (**5**) and B (**8**), has recently been extended to the total syntheses of dercitin (**34**), nordercitin (**36**), and kuanoniamine D (**43**, Scheme VII).[78] Fusion of thiazole to the intermediate ketone **75** (see Scheme I) was achieved by bromination at the α-position, followed by Taumann reaction with thiourea, deamination of the resulting 2-aminothiazole with isoamyl nitrite and refunctionalization of the side chain to afford mesylate **105**, the common intermediate for the syntheses of the three alkaloid targets. Kuanoniamine D (**43**) was obtained from **105** after Gabriel synthesis, acetylation of the resulting primary amine, and photolysis. Alternatively, displacement of mesylate **105** with dimethylamine and subsequent photolysis gave nordercitin (**36**). Finally, dercitin (**34**) was prepared in good yield by displacement of **105** with *N*-sodio-*N*-methylformamide, followed by photolysis to the intermediate **106**, selective *N*-methylation of the pyridine ring (MeI, K_2CO_3) to **107** and reduction of the formyl group in two steps ($POCl_3$, $NaBH_4$).

F. Batzelline C (66) and Isobatzelline (69)

The syntheses of batzelline C (**66**) and isobatzelline C (**69**) took advantage of a common late intermediate,

Scheme VII[a]



[a] (a) pyridinium tribromide, AcOH, 50 °C; (b) $H_2N(CS)NH_2$, EtOH, 35 °C, 5 min; (c) *i*-AmONO, DMF, 80 °C; (d) K_2CO_3, MeOH; (e) MsCl, Et_3N, CH_2Cl_2, 0 °C; (f) Me_2NH, aq. DMF; (g) *hν*, 9:1 chlorobenzene/acetophenone, 110 °C; (h) Gabriel synthesis; (i) Ac_2O, py; (j) MeNHCHO, NaH, 0 °C; (k) MeI, K_2CO_3, PhH; (l) $POCl_3$; (m) $NaBH_4$, DME.

114 (Scheme VIII).[79] 2,4-Dimethoxy-5-nitrobenzaldehyde is converted in four steps to the *N*-carbamate (**108**). Curtius reaction of **108** and capture of the incipient isocyanate with 2-(trimethylsilyl)ethanol led to dicarbamate **109**. Removal of the benzyl group by hydrogenolysis and introduction of the indole ring by heating with 4-chloroacetoacetate in ethanol gave **110**. Reduction to the dihydroindole **111** with sodium cyanoborohydride allowed selective chlorination to be carried out at the position *ortho* to the methoxyl group, followed by reoxidation to the indole **112** with DDQ. Elaboration of the lactam ring was carried out in several steps to provide **113a** which was reduced to the common precursor **114** of batzelline (**66**) and isobatzelline (**69**). The later natural products were obtained, respectively, by (i) *O*-methyl deprotection and autoxidation (batzelline C, **66**) or (ii) ceric ammonium nitrate oxidative demethylation and amination of the liberated *o*-quinone with ammonium chloride (isobatzelline C, **69**).[79]

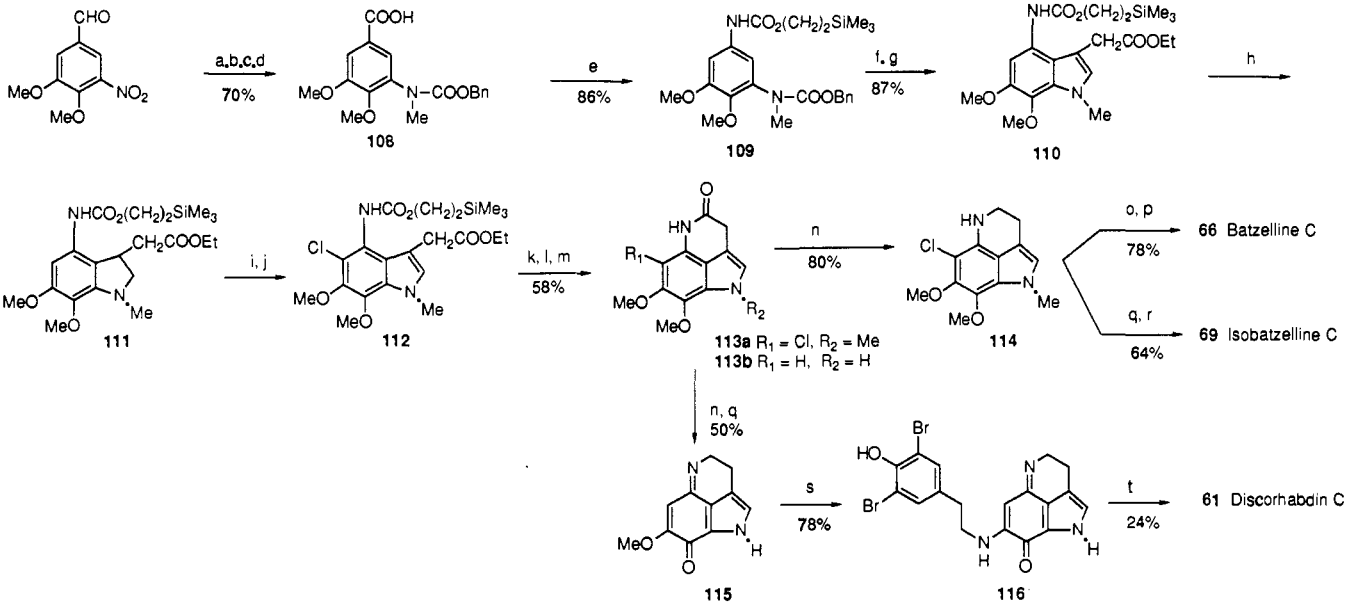
G. Discorhabdin C (61)

Yamamura and co-workers successfully extended their approach to the tricyclic batzelline nucleus to complete the first total synthesis of discorhabdin C (**61**). Borane reduction of intermediate **113b** gave a lactam that was oxidized to the indolo–iminoquinonone **115** (Scheme VIII). Coupling of **115** with 3,5-dibromotyramine gave **116** which underwent oxidative electrochemical spiro annulation to provide **61**.[80] A second synthesis of **61** by Kita and co-workers also exploited aryl participation in spiro annulation through hypervalent iodine-mediated phenolic coupling.[81,82]

V. Biological Activity

Most pyridoacridines show *in vitro* cytotoxicity against cultured tumor cells (L1210 murine leukemia, P388, etc.) or antineoplastic activity in whole animal experiments. Rather than enumerate the summary data (IC_{50}'s) for *in vitro* cytotoxicity, which can be found with most reports on structure elucidation, this review will focus on more detailed studies which clarify aspects

Scheme VIII[a]



[a] (a) Fe, HCl; (b) CbzCl, $NaHCO_3$; (c) MeI, NaH/DMF; (d) Jones oxidation; (e) Curtius reaction—; (i) $CO(Imd)_2$/THF, rt, (ii) NaN_3, (iii) toluene, reflux, (iv) 2-(trimethylsilyl)ethanol, 60 °C; (f) H_2, Pd on C; (g) $ClCH_2COCH_2CO_2Et$, EtOH, reflux, 60 °C; (h) $NaBH_3CN$, AcOH; (j) NCS, CH_2Cl_2; (j) DDQ, CH_2Cl_2; (k) AcOH–$HClO_4$ (20:1), rt; (l) KOH/ aq MeOH, rt; (m) DCC, THF; (n) BH_3·SMe_2/THF; (o)BBr_3, CH_2Cl_2; (p) O_2, H^+, H_2O; (q) CAN, CH_3CN; (r) NH_4Cl, EtOH; (s) 3,5-dibromotyramine, $NaHCO_3$, EtOH; (t) anodic oxidation.

of molecular mechanisms of action. Two general properties of pyridoacridine alkaloids emerge from the few published studies on mechanism: (a) they are DNA intercalating agents, and (b) nucleic acid intercalation is further modulated by binding to other receptors (topoisomerase, transition metals, etc.).

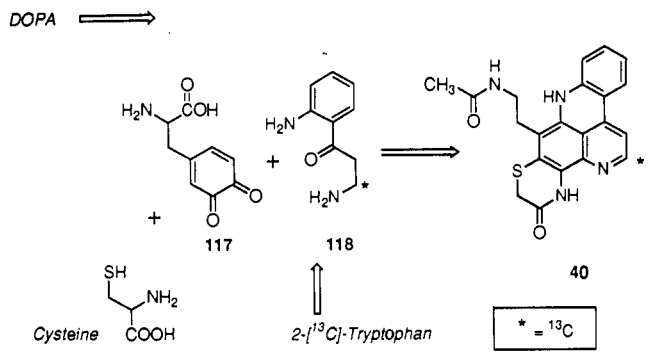
Dercitin (**34**) inhibits a variety of cultured cell clones at nanomolar concentrations and shows antitumor activity in mice and modest antiviral activity against Herpes simplex and A-59 murine corona virus at micromolar concentrations.[45] A thorough study by Burres *et al.* showed that dercitin inhibits both DNA and RNA synthesis by up to 83% at 400 nM, but protein synthesis was effected to a lesser extent.[83] Dercitin also bound to calf thymus DNA and relaxed supercoiled *φX174* DNA at 36 nM and inhibited DNA polymerase and DNase nick translation at 1 nM. Collectively these results suggested that inhibition of enzyme activity by dercitin was of secondary importance, and its activity was entirely consistent with potent intercalation of nucleic acids. Topoisomerase was not significantly inhibited by **34**.[83] A second report compared the activity of several analogs of **34** and postulated the nature of functional subunits (pharmacophores) in dercitin responsible for antiviral and antitumor activities. Potential new structural types for anti-HIV drugs were proposed based on dercitin.[16]

Researchers at SmithKline and Beecham have recently devised a specific screen for topoisomerase II inhibitory natural products using a genetically engineered yeast strain.[15] In contrast to dercitin, neoamphimedine (**25**) was found to be a potent inhibitor of purified mammalian topoisomerase II (IC_{50} 1.3 μM), but not of topoisomerase I. Compound **25** was also shown to intercalate DNA with a K_M of 2.8×10^5 M^{-1} and a binding site size of 1.8 base pairs per molecule of **25** (cf. ethidium bromide, 1.6×10^6 M^{-1} under the same conditions). Interestingly, the isomeric amphimedine (**1**), along with petrosamine (**7**) and debromopetrosamine (**26**), had little effect on topoisomerase I or II activity, despite showing comparable cytotoxicity. The authors postulated that the cytotoxicity of **25** toward mammalian cells can be explained by DNA damage resulting from native topoisomerase II inhibition; however, the other pyridoacridines may elicit cytotoxicity through as yet unidentified mechanisms associated with DNA processing.[15] Ascididemin (**27**) and shermilamine B (**40**) also inhibited topoisomerase II, albeit at higher concentration (75 and 30 μM, respectively), while shermilamine A (**39**) and meridine (**30**) were inactive.[14] Wakayin (**71**) was marginally inhibitory toward topoisomerase II (250 μM); however, it showed an interesting differential cytotoxicity against mammalian cell clones that is indicative of DNA damage or interference with DNA processing.[61]

Binding to DNA has also been demonstrated for other pyridoacridines. Faulkner *et al.* reported that the fluorescence spectrum of kuanoniamine D (**43**) was quenched upon addition of calf thymus DNA and the emission wavelength of 534 nm (excitation at λ 350 nm) changed to 593 nm.[18] Both findings were suggestive of alkaloid binding to DNA. The two "bay region" nitrogens in 2-bromoleptoclinidone (**29**) are ideally disposed to present the two donor nitrogen atoms of a bidentate ligand to metals. Alkaloid **29** forms an octahedral Ru(II) complex which intercalates into DNA, and the DNA–Ru(II) complex of **29** induces photoactivated single strand cleavage of supercoiled pBR322 DNA under visible light irradiation.[84]

Faulkner, He, and co-workers determined that kuanoniamine D (**43**) forms 2:1 Co(II) and Cu(II) complexes with stability constants of 2.5×10^{10} M^{-2} and 1.3×10^{10} M^{-2}, respectively.[18] Fluorescence quenching also indicated weaker but significant complexation of **43** with Fe(II) and Zn(II), in contrast to reports showing that red Fe(II) complexes are not formed between 1,10-phenanthroline type pyridoacridine alkaloids and Fe(II) salts.[34,38,39]

Scheme IX



Finally, it is of interest to note that cytotoxic cystodytins A (**5**) and B (**8**) were found to be potent Ca^{2+} release agents and stimulated calcium release from the sarcoplasmic reticulum at 36 and 13 times the potency of caffeine, respectively.[17]

VI. Biosynthesis

Like most heterocyclic alkaloids, the pyridoacridines are products of aromatic amino acid metabolism. The tunicate *Cystodytes dellechiajei* from the Mediterranean Sea produces kuanoniamine D (**43**) and shermilamine B (**40**). Through feeding experiments *in situ* and with cell-free extracts of the tunicate, Steffan *et al.* have shown that both L-[5-^{3}H]tryptophan and [7,8-$^3H_2$]DOPA are precursors to shermilamine B.[49] Feeding of liposomal[2-^{13}C]tryptophan (100 mg) to *C. dellechiajei* followed by harvesting after 4 months, resulted in ^{13}C label incorporation at C6 of shermilamine B (**40**) as shown by enhanced signals in the corresponding ^{13}C NMR spectrum of the purified product. The authors proposed a biosynthetic pathway to **40** (Scheme IX) involving tryptophan, DOPA, and cysteine. In this scheme DOPA is oxidized to the *o*-quinone **117** and catabolism of tryptophan provides the intermediate, kynuramine **118**, for *N*-ring formation while cysteine is elaborated to the thiazinone ring.

VII. Concluding Remarks

Over 40 pyridoacridine alkaloids have been characterized and there is little doubt that additional structural variations on this common theme will shortly appear. New pyridoacridine structures provide fertile ground for the design and execution of heterocyclic synthesis. Although some total syntheses of pyridoacridine alkaloids have employed classical methods, newer synthetic strategies are emerging for efficient assembly of tetracyclic and pentacyclic ring systems.

The biological activity of pyridoacridine alkaloids suggests that, in addition to intercalation of DNA, there may be other factors involved in, say, topoisomerase II inhibition. For comparison, the anthraquinone glycoside doxorubicin, used in antitumor therapy, shares with the iminoquinone neoamphimedine (**25**)[15] the properties of DNA intercalation, DNA single-strand cleavage, and topoisomerase II inhibition.[85] The quinone subunit in doxorubicin mediates production of free radicals which are partly responsible for its activity. Given that iminoquinones are potentially good Michael acceptors and/or redox couples, it is anticipated that future studies on mechanism of action will examine their participation in enzyme inhibition in this light.

An intriguing fact which compels further investigation is that the majority of pyridoacridines come from species in two disparate phyla, sponges, and tunicates. The hypothesis that symbiotic microbes are involved in the biosynthesis is one possibility, but it has not been tested. In any case, it would be of great interest to determine the reasons for the similarity of biosynthesis of pyridoacridines in both types of organisms as such research will help to fill an existing void in the body of knowledge of marine alkaloid biosynthesis.

VIII. Abbreviations

CAN	ceric ammonium nitrate
Cbz	carbobenzoxy
COLOC	^{1}H–^{13}C correlation by long-range coupling
COSY	^{1}H–^{1}H correlation spectroscopy
DDQ	dichlorodicyanoquinone
DMF	dimethylformamide
DOPA	dihydroxyphenylalanine
HETCOR	^{1}H–^{13}C heteronuclear correlation (one bond)
HMBC	heteronuclear multiple bond correlation
HMQC	heteronuclear multiple quantum coherence
INADEQUATE	incredible natural abundance double quantum transfer experiment
INAPT	selective insensitive nucleus enhancement by polarization transfer
nOe	nuclear Overhauser effect
THF	tetrahydrofuran

IX. Acknowledgments

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X. References

(1) Schmitz, F. J.; Agarwal, S. K.; Gunasekera, S. P.; Schmidt, P. G.; Shoolery, J. N. *J. Am. Chem. Soc.* **1983**, *105*, 4835–4836.
(2) Patterson, A. M.; Capell, L. T.; Walker, D. F. *The Ring Index*, 2nd ed.; American Chemical Society: Washington, DC, 1960.
(3) Faulkner, D. J. *Nat. Prod. Rep.* **1984**, *1*, 251–280.
(4) Faulkner, D. J. *Nat. Prod. Rep.* **1984**, *1*, 551–598.
(5) Faulkner, D. J. *Nat. Prod. Rep.* **1986**, *3*, 1–33.
(6) Faulkner, D. J. *Nat. Prod. Rep.* **1987**, *4*, 539–576.
(7) Faulkner, D. J. *Nat. Prod. Rep.* **1988**, *5*, 613–663.
(8) Faulkner, D. J. *Nat. Prod. Rep.* **1990**, *7*, 269–309.
(9) Faulkner, D. J. *Nat. Prod. Rep.* **1992**, *9*, 323–364.
(10) Christopherson, C. In *Alkaloids: Chemistry and Pharmacology*; Brossi, A., Ed.; Academic Press: Orlando, 1985; Vol. 24; pp 25–110.
(11) Fenical, W. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; John Wiley and Sons: New York, 1986; Vol. 4, pp 275–330.
(12) Kobayashi, J.; Ishibashi, M. In *Alkaloids: Chemistry and Pharmacology*; Brossi, A., Cordell, G. A., Ed.; Academic Press: San Diego, 1992; Vol. 41, pp 41–124.
(13) Wrobel, J.; Wojtasiewicz, K. In *The Alkaloids: Chemistry and Pharmacology*; Cordell, J. A., Ed.; Academic Press: San Diego, 1992; Vol. 42, pp 249–297.
(14) Schmitz, F. J.; Deguzman, F. S.; Hossain, M. B.; Vanderhelm, D. *J. Org. Chem.* **1991**, *56*, 804–808.
(15) Carté, B. K.; Faucette, L. F.; Faulkner, D. J.; Hemling, M. E.; Caranfa, M. J.; MacKenzie, L.; Hofmann, G. A.; McCabe, F. L.; Offen, P. H.; Troupe, N.; Johnson, R. K.; Hertzberg, R. *Biochemistry*, submitted for publication.

(16) Taraporewala, I. B.; Cessac, J. W.; Chanh, T. C.; Delgado, A. V.; Schinazi, R. F. *J. Med. Chem.* **1992**, *35*, 2744–52.
(17) Kobayashi, J.; Cheng, J.; Wälchli, M. R.; Nakamura, H.; Hirata, Y.; Sasaki, T.; Ohizumi, Y. *J. Org. Chem.* **1988**, *53*, 1800–1804.
(18) Gunawardana, G. P.; Koehn, F. E.; Lee, A. Y.; Clardy, J.; He, H. Y.; Faulkner, D. J. *J. Org. Chem.* **1992**, *57*, 1523–1526.
(19) IUPAC; nomenclature: pyrido[9,9,1:4′,3′,2′]acridine. The total number and relative orientation of rings for these alkaloids vary. Accordingly, structures drawn in compliance with The Rules will change orientation. For the purpose of uniformity, all alkaloids are drawn here with the same orientation as amphimedine (1), with respect to the pyridoacridine nucleus.
(20) Taylor, W. C.; Carroll, A. R. *Aust. J. Chem.* **1991**, *44*, 1615–26.
(21) Taylor, W. C. In *Alkaloids: Chemistry and Pharmacology*; Brossi, A., Ed.; Academic Press: Orlando, 1985; Vol. 24, pp 1–23.
(22) Monniot, C.; Monniot, F.; Laboute, P. *Coral Reef Ascidians of New Caledonia*; Orstom Editions: Paris, 1991; pp 247.
(23) Marchand, J. L. In *Methods in Stereochemical Analysis*; Verlag Chemie International: Deerfield Beech, FL, 1983; Vol. 2.
(24) Kobayashi, J.; Tsuda, M.; Tanabe, A.; Ishibashi, M.; Cheng, J. F.; Yamamura, S.; Sasaki, T. *J. Nat. Prod.* **1991**, *54*, 1634–1638.
(25) Carroll, A. R.; Cooray, N. M.; Poiner, A.; Scheuer, P. J. *J. Org. Chem.* **1989**, *54*, 4231–4232.
(26) Rudi, A.; Benayahu, Y.; Goldberg, I.; Kashman, Y. *Tetrahedron Lett.* **1989**, *29*, 3861–3862.
(27) Rudi, A.; Kashman, Y. *J. Org. Chem.* **1989**, *54*, 5331–5337.
(28) Molinski, T. F.; Ireland, C. M. *J. Org. Chem.* **1989**, *54*, 4256–4259.
(29) Davidson, B. S.; Molinski, T. F.; Barrows, L. R.; Ireland, C. M. *J. Am. Chem. Soc.* **1991**, *113*, 4709–4710.
(30) Charyulu, G. A.; McKee, T. C.; Ireland, C. M. *Tetrahedron Lett.* **1989**, *30*, 4201–4202.
(31) Searle, P. A.; Molinski, T. F. Unpublished results.
(32) Lederer, E.; Tessier, G.; Huttrer, C. *Bull. Soc. Chim. Fr.* **1940**, *7*, 608.
(33) Barbier, M. *Naturwissenschaften* **1982**, *69*, 341.
(34) Cimino, G.; Crispino, A.; De Rosa, S.; De Stefano, S.; Gavagnin, M.; Sodano, G. *Tetrahedron* **1987**, *43*, 4023–4030.
(35) Bax, A.; Freeman, R.; Kempsell, S. P. *J. Am. Chem. Soc.* **1980**, *102*, 4849.
(36) Bax, A.; Freeman, R.; Frenkiel, T. A.; Levitt, M. H. *J. Magn. Reson.* **1981**, *43*, 478.
(37) Mareci, T. H.; Freeman, R. *J. Magn. Reson.* **1982**, *48*, 158.
(38) Kobayashi, J.; Cheng, J.; Nakamura, H.; Ohizumi, Y.; Hirata, Y.; Sasaki, T.; Ohta, T.; Nozoe, S. *Tetrahedron Lett.* **1988**, *29*, 1177–1180.
(39) Bloor, S. J.; Schmitz, F. J. *J. Am. Chem. Soc.* **1987**, *109*, 6134–6136.
(40) de Guzman, F. S.; Schmitz, F. J. *Tetrahedron Lett.* **1989**, *30*, 1069–1070.
(41) Bax, A.; Ferretti, J. A.; Nashed, N.; Jerina, D. M. *J. Org. Chem.* **1985**, *50*, 3029–3034.
(42) McCarthy, P. J.; Pitts, T. P.; Gunawardana, G. P.; Kelly-Borges, M.; Pomponi, S. *J. Nat. Prod.* **1992**, *55*, 1664–1668.
(43) Molinski, T. F.; Fahy, E.; Faulkner, D. J.; Van Duyne, G. D.; Clardy, J. *J. Org. Chem.* **1988**, *53*, 1340–1341.
(44) Molinski, T. F. Personal observation.
(45) Gunawardana, G. P.; Kohmoto, S.; Gunasekera, S. P.; McConnell, O. J.; Koehn, F. E. *J. Am. Chem. Soc.* **1988**, *110*, 4356–4358.
(46) Carroll, A. R.; Scheuer, P. J. *J. Org. Chem.* **1990**, *55*, 4426–4431.
(47) Gunawardana, G. P.; Kohmoto, S.; Burres, N. S. *Tetrahedron Lett.* **1989**, *30*, 4359–4362.
(48) Cooray, N. M.; Scheuer, P. J.; Parkanyi, L.; Clardy, J. *J. Org. Chem.* **1988**, *53*, 4619–4620.
(49) Steffan, B.; Brix, C.; Pütz, W. *Tetrahedron*, submitted for publication.
(50) He, H. Y.; Faulkner, D. J. *J. Org. Chem.* **1991**, *56*, 5369–5371.
(51) Sun, H. H.; Sakemi, S.; Burres, N.; McCarthy, P. *J. Org. Chem.* **1990**, *55*, 4964–4966.
(52) Kobayashi, J.; Cheng, J.; Ishibashi, M.; Nakamura, H.; Ohizumi, Y.; Hirata, Y.; Sasaki, Y.; Lu, H.; Clardy, J. *Tetrahedron Lett.* **1987**, *28*, 4939–4942.
(53) Cheng, J.-F.; Ohizumi, Y.; Wälchli, M. R.; Nakamura, H.; Hirata, Y.; Sasaki, T.; Kobayashi, J. *J. Org. Chem.* **1988**, *53*, 4621–4624.
(54) Perry, N. B.; Blunt, J. W.; Munro, M. H. G. *Tetrahedron* **1988**, *44*, 1727–1734.
(55) Perry, N. B.; Blunt, J. W.; Munro, M. H. G.; Higa, T.; Sakai, R. *J. Org. Chem.* **1988**, *53*, 4127–4128.
(56) West, R. R.; Mayne, C. L.; Ireland, C. M.; Brinen, L. S.; Clardy, J. *Tetrahedron Lett.* **1990**, *31*, 3271–3274.
(57) Inman, W. D.; O'Neill-Johnson, M.; Crews, P. *J. Am. Chem. Soc.* **1990**, *112*, 1–4.
(58) Zabriskie, T. M.; Mayne, C. L.; Ireland, C. M. *J. Am. Chem. Soc.* **1988**, *110*, 7919–7920.
(59) Perry, N. B.; Blunt, J. W.; McCombs, J. D.; Munro, M. H. G. *J. Org. Chem.* **1986**, *51*, 5476–5478.
(60) Sakemi, S.; Sun, H. H.; Jeffort, C. W.; Bernardinelli, G. *Tetrahedron Lett.* **1989**, *30*, 2517–2520.
(61) Copp, B. R.; Ireland, C. M.; Barrows, L. R. *J. Org. Chem.* **1991**, *56*, 4596–4597.
(62) Ciufolini, M. A.; Byrne, N. E. *Tetrahedron Lett.* **1989**, *30*, 5559–5562.
(63) Ciufolini, M. A.; Byrne, N. E. *J. Am. Chem. Soc.* **1991**, *113*, 8016–8024.
(64) Echavarren, A. M.; Stille, J. K. *J. Am. Chem. Soc.* **1988**, *110*, 4051–4053.
(65) Kubo, A.; Nakahara, S. *Heterocycles* **1988**, *27*, 2095–2098.
(66) Prager, R. H.; Tsopelas, C.; Heisler, T. *Aust. J. Chem.* **1991**, *44*, 277–285.
(67) Prager, R. H.; Tsopelas, C. *Heterocycles* **1989**, *29*, 847–848.
(68) Subramanyam, C.; Noguchi, M.; Weinreb, S. M. *J. Org. Chem.* **1989**, *54*, 5580–5585.
(69) Meghani, P.; Mills, O. S.; Joule, J. A. *Heterocycles* **1990**, *30*, 1121–1129.
(70) Labarca, C. V.; MacKenzie, A. R.; Moody, C. J.; Rees, C. W.; Vaquero, J. J. *J. Chem. Soc., Perkin Trans. 1* **1987**, 927–929.
(71) Gellerman, G.; Rudi, A.; Kashman, Y. *Tetrahedron Lett.* **1992**, *33*, 5577–5580.
(72) Ali, N. M.; Chattopadhyay, S. K.; McKillop, A.; Perretgentil, R. M.; Ozturk, T.; Rebelo, R. A. *J. Chem. Soc., Chem. Commun.* **1992**, 1453–1454.
(73) Bracher, F. *Liebigs Ann. Chem.* **1990**, 205–206.
(74) Bracher, F. *Heterocycles* **1989**, *29*, 2093–2095.
(75) Moody, C. J.; Rees, C. W.; Thomas, R. *Tetrahedron Lett.* **1990**, *31*, 4375–4376.
(76) Moody, C. J.; Rees, C. W.; Thomas, R. *Tetrahedron* **1992**, *48*, 3589–3602.
(77) Gómez-Bengoa, E.; Echavarren, A. M. *J. Org. Chem.* **1991**, *56*, 3497–3501.
(78) Bishop, M. J.; Ciufolini, M. A. *J. Am. Chem. Soc.* **1992**, *114*, 10081–10082.
(79) Tao, X. L.; Nishiyama, S.; Yamamura, S. *Chem. Lett.* **1991**, 1785–1786.
(80) Nishiyama, S.; Cheng, J. F.; Tao, X. L.; Yamamura, S. *Tetrahedron Lett.* **1991**, *32*, 4151–4154.
(81) Kita, Y.; Tohma, H.; Inagaki, M.; Hatanaka, K.; Yakura, T. *J. Am. Chem. Soc.* **1992**, *114*, 2175–2180.
(82) Kita, Y.; Tohma, H.; Inagaki, M.; Hatanaka, K.; Kikuchi, K.; Yakura, T. *Tetrahedron Lett.* **1991**, *32*, 2035–2038.
(83) Burres, N. S.; Sazesh, S.; Gunawardana, G. P.; Clement, J. J. *Cancer Res.* **1989**, *49*, 5267–74.
(84) Goulle, V.; Lehn, J. M.; Schoentjes, B.; Schmitz, F. J. *Helv. Chim. Acta* **1991**, *74*, 1471–1476.
(85) Calabresi, P.; Chabner, B. A. In *The Pharmacological Basis of Therapeutics*, 8th ed.; Goodman Gilman, A., Rall, T. W., Nies, A. S., Taylor, P., Eds.; Pergamon: New York, 1990; pp 1242.