Review

Total Synthesis and Chemical Biology of the Sarcodictyins

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The sarcodictyins A—F and eleutherobin comprise a family of marine-derived diterpenoids with potent cytotoxicities against various tumor cell lines. Investigations have revealed that several of these compounds exert their cytotoxic effects through tubulin binding in a mechanism analogous to that of the clinical anticancer drug Taxol™. The biological importance, challenging molecular architecture, and relative scarcity of these natural products have prompted several groups to undertake their total chemical synthesis. In this review, we summarize the current synthetic efforts and examine the preliminary structure–activity relationships which have emerged from early combinatorial libraries.

Key words sarcodictyin; eleutherobin; synthesis; tubulin; combinatorial library; anticancer

1. Introduction

Cancer is now the second leading cause of death in the United States, claiming over half a million lives each year. The personal and socioeconomic costs of the disease are staggering with 1.2 million new cases expected to be diagnosed this year alone at an annual cost of over 107 billion dollars.[1)] Yet, despite the overwhelming impact of cancer, it is increasingly becoming a survivable disease. In fact, the combined five year survival rate has risen to nearly 60% due, in part, to earlier detection and more effective treatment programs employing new chemotherapeutics in conjunction with surgery and radiation therapy.[1,2)] These new anticancer drugs have greater efficacy and safer toxicity profiles than previous generations of antineoplastics, and they are the result of decades of research within both the pharmaceutical and academic communities aimed at improving our understanding of cancer biology and finding strategies to selectively target the disease process.[3)] One of the great clinical successes of these efforts has been the development of the anticancer agent Taxol™ (Bristol-Myers Squibb) and its relative Taxotere™ (Rhone-Poulenc Rorer).[4)] Taxol (paclitaxel) (**1**, Fig. 1) is a diterpene originally isolated in 1971 as a cytotoxin from the bark of the Pacific Yew, *Taxus brevifolia*.[5)] Almost a decade after its isolation, Horwitz *et al*. ignited strong interest in this complex taxane with the discovery that its cytotoxicity is mediated through a novel mechanism of microtubule stabilization resulting in mitotic arrest and subsequent cell death.[6)] While previously discovered natural products were known to disrupt tubulin polymerization,[7)] this was the first example of cell cycle arrest occurring through induction of tubulin polymerization, and it established a new class of antitumor agents which has now grown to include the sarcodictyins and several other natural products.[8)]

2. Biology of Taxol and Related Microtubule Stabilizers

The mechanism of taxol induced cell cycle arrest has been comprehensively reviewed elsewhere[9)]; thus, we will only discuss it here briefly as it relates to the biology of the sarcodictyin family (*vide infra*). Normal cellular proliferation occurs through the coupling of DNA replication and cellular division under the careful regulation of the cell cycle.[10)] As illustrated in Fig. 2, the cell cycle is divided between the interphase (subdivided into G_1, S, and G_2) and mitotic phase (subdivided into prophase, metaphase, anaphase, telophase, and cytokinesis). A cell enters the cycle at G_1 of interphase and

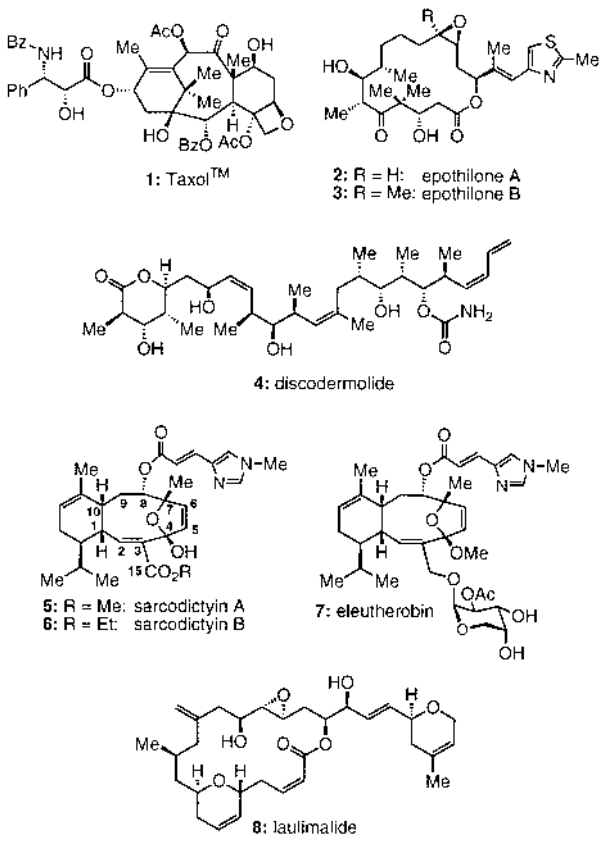

Fig. 1. Selected Natural Products with Tubulin Polymerization and Microtubule Stabilization Properties

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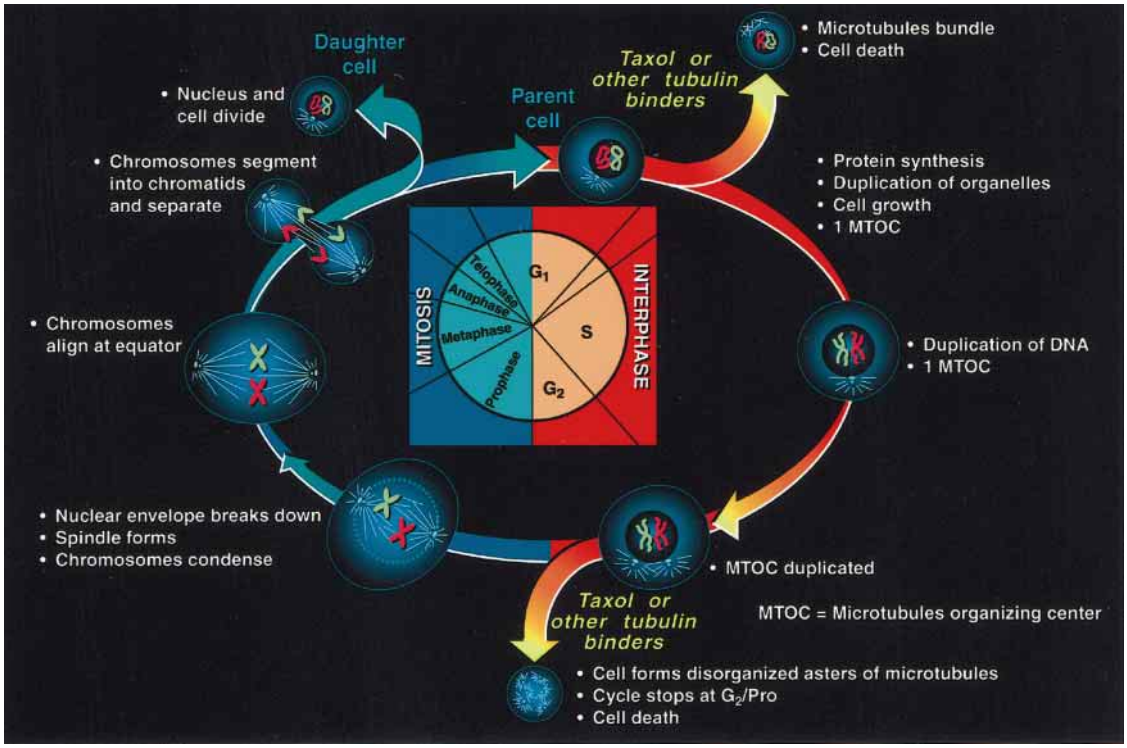

Fig. 2. Interaction of Taxol™ and Other Tubulin Binders with the Cell Cycle

undertakes increased protein synthesis and growth along with duplication of organelles in preparation for the S subphase where duplication of the DNA, histones, and microtubule organizing centers (MTOC) occurs.[11] After additional protein synthesis in the G_2 subphase, the cell enters mitosis at prophase with condensation of the duplicated chromatin into chromosomes, each consisting of two sister chromatids joined at a centromere. In the cytoplasm, the mitotic spindle begins to assemble from microtubules and, as the nuclear envelope disintegrates, a fraction of these microtubules extend outward from the spindle and attach to the kinetochores of the newly duplicated chromosomes.[12] This is followed by an alignment of the chromosomes across the cellular equator through dynamic tension exerted *via* the microtubules as the cell progresses through metaphase.[13] During the ensuing anaphase, the microtubules attached to chromosome kinetochores begin to shorten concomitant with migration of the mitotic spindles towards opposite poles, effecting segregation of the sister chromatids and initiating cellular elongation. This elongation, promoted by the action of additional microtubules not attached to chromatids, continues into telophase where nuclear envelopes reform and the mitotic spindles dis-

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appear.[14] Completion is marked by cytokinesis to produce two identical daughter cells with high genetic fidelity as assured by the checkpoints governing each step of the cell cycle.[15]

A careful orchestration of microtubule dynamics is fundamental to the proper function of the cell cycle[16]; consequently, the ability of taxol to nucleate tubulin polymerization at reduced concentrations in the absence of regulatory factors (*i.e.* GTP, Mg^{2+}) and subsequently prevent microtubule disassembly through stabilization allows it to interrupt cell division.[9,17] The mechanism by which taxol and related microtubule binders act on tubulin and microtubules has been comprehensively reviewed elsewhere.[9a,18] The application of such action to halting the uncontrolled proliferation of rapidly dividing tumor cells which often lack critical cell cycle checkpoints is readily appreciated. Specifically, incubation with moderate doses of taxol results in cell cycle arrest between G_2 and prophase as a result of the formation of asters not properly organized about the MTOC as required for normal chromosome segregation.[9a] At other points in the cycle, taxol promotes the irreversible formation of microtubule bundles. This sequestering of tubulin in nonfunctional aggregates coupled with a stabilization of the otherwise dynamic microtubule machinery results in a failure of cell division usually accompanied by apoptosis.[9] The clinical potential of this mechanism of action was quickly realized and great efforts were devoted to optimizing the efficacy and safety of taxol as well as addressing the crucial issue of supply.[9a,19] These efforts culminated in the FDA approval of taxol for ovarian cancer in 1992, and approval for other cancer types in subsequent years. Today, taxol is the drug of choice for many solid tumors including ovarian, breast, non-small cell lung, bladder, esophagus, head, and neck; and it has proven to be particularly effective at treating recurrent tumors as well as those unresponsive to previous first line therapies.[4]

The widespread clinical use of taxol since its introduction in 1992 has undoubtedly saved many lives, yet it has also served to expose its limitations. These include dose-limiting toxic side effects that undermine treatment regimens in many patients. Such side effects include haematological toxicity consisting mainly of neutropenia as well as neurotoxicity, which is principally manifested as peripheral neuropathy.[4c,20] In addition, the lipophilicity of taxol necessitates its formulation and delivery with polyethoxylated castor oil (Cremophor EL), which is known to elicit hypersensitivity reactions due to histamine release.[21] However, the most critical clinical limitation of taxol has resulted from the emergence of neoplastic phenotypes resistant to taxane-induced cell death. This acquired resistance emerges through at least two known mechanisms. First, as with other anticancer drugs, taxol is a substrate for the P-glycoprotein, an ATP dependent transmembrane transporter that is overexpressed on the surface of resistant neoplastic cells.[22] This P-glycoprotein functions as an energy dependent efflux pump effectively lowering the intracellular concentrations of cytotoxic natural products and other drugs thereby allowing cells to survive in the presence of otherwise toxic doses.[22] A second type of resistance is conferred by an over expression of tubulin isotypes (specifically I, II, and IVa), which are less susceptible to taxane induced polymerization and subsequent stabilization.[23]

As with other anticancer agents, these limitations can sometimes be overcome by employing adjunct therapies (*i.e.* granulocyte colony stimulating factor therapy to offset neutropenia) and optimized dosage schedules (to minimize neurotoxicity).[21] Even resistance can be attenuated using P-glycoprotein inhibitors although such agents generally have a very narrow therapeutic window.[24] Notwithstanding these options, a more general solution to the limitations of taxol is needed; consequently, large scale screening efforts have been undertaken to identify other natural product leads which have the same mechanism of action and cytotoxicity profile.[25] It is thought that by acting through a common mechanism, these new leads might share taxol's clinical benefits, but their distinct structures will endow them with unique and perhaps improved pharmacological profiles in terms of toxicity and susceptibility to resistance (especially that mediated by the P-glycoprotein). To date, these efforts have resulted in the identification of four novel structural types including the epothilones A (**2**) and B (**3**) isolated from a species of myxobacteria by Höfle, Reichenbach, and co-workers at Gesellschaft für Biotechnologische Forschung in Germany and independently by U.S. scientists at Merck.[26] Discodermolide (**4**) was isolated from the sponge *Discodermia dissoluta* as an immunosuppressant and subsequently shown to exhibit taxol-like activity,[27] while the marine-derived diterpenoids, eleutherobin (**7**),[28] and sarcodictyin A (**5**), B (**6**), C (**9**), D (**10**), E and F,[29—30] were isolated from separate species of soft coral. Finally, laulimalide (**8**) and isolaulimalide, isolated from several different species (*Cocospongia mycofijiensis*, *Hyattella* sp., and *Fasciospongia rimosa*) of coral over the last decade, were recently shown to share taxol's tubulin binding properties.[31] A number of groups, employing computational techniques and biological data, have proposed common pharmacophores among the members of this tubulin binding family including taxol, epothilone, discodermolide, and eleutherobin, which could account for their homologous modes of action and offer insights into the design of hybrids which might exhibit improved biological properties.[32]

3. Isolation, Structure, and Biological Activity of Sarcodictyin

The marine natural products **5**, **6**, and **7** all belong to the sarcodictyin category of the 2,11-cyclized cembranoid family of natural products which include the cladiellins (eunicellins), the briarellins, and the asbestinins.[33] All members of the sarcodictyin family possess a rigid oxygen-bridged bicyclo[8.4.0]tetradecatriene skeleton which is appended at C(8) with ester side chain, a hemiketal (sarcodictyins A—F, eleuthosides A and B, valdivone A and B) or ketal (eleutherobin) moiety at C(4), and a trisubstituted olefin between C(2) and C(3). The diterpenoids **5** and **6** were the founding members of this category, having been isolated in 1987 by Pietra *et al*. from the Mediterranean stoloniferan coral *Sarcodictyon roseum*.[29] The following year, the same group disclosed the structures of **9**, **10**, sarcodictyin E, and sarcodictyin F, also isolated from *Sarcodictyon roseum*.[30] In 1995, eight years later, the diterpene glycoside **7** was reported by Fenical *et al*. from an *Eleutherobia* species (possibly *E. albifora*) of soft coral found near Bennet's Shoul in Western Australia.[28] While possessing the same carbon skeleton as the sarcodic-

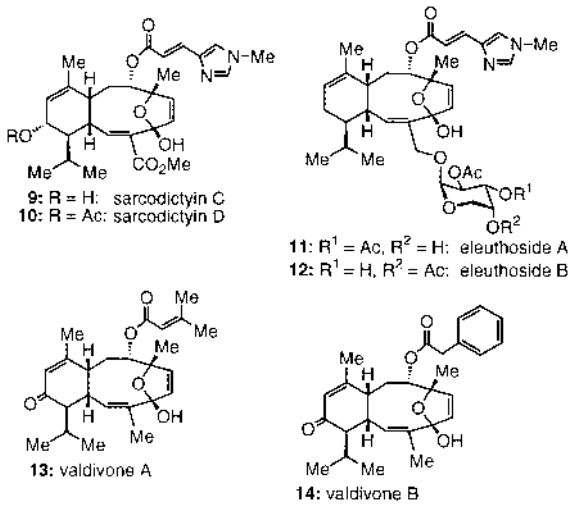


Fig. 3. Selected Natural Products from the Sarcodictyin Family

tyins, eleutherobin uniquely contained a β-linked 2-*O*-acetyl-D-arabinopyranose unit in place of the alkyl ester of the previous members. In 1996 eleuthoside A (**11**) and eleuthoside B (**12**), which are acetylated derivatives of eleutherobin, were reported by Kashman *et al*. as products isolated from a sample of *Eleutherobia aurea* found off the Kwazula-Natal Coast of South Africa.[34] Intriguingly, **5**, previously isolated from *Sarcodictyon roseum*, was also found in this sample of *Eleutherobia aurea*. Lastly, although appended with distinct side chains at C(8) as compared to other members, valdivone A (**13**) and valdivone B (**14**), which were isolated in 1968 and again in 1993 from *Eunicella stricta* and *Alcyonium valdivae* respectively, also possess the sarcodictyin carbon skeleton arguing for their inclusion in this class of compounds.[35]

3a. Biology of Sarcodictyins A—E Like the cases of other microtubule stabilizing natural products, the biological potential of the sarcodictyin family was not realized upon their initial isolation. In fact, while the cytotoxic properties of **5** and **6** were noted in the initial report of their isolation, it was not until a decade later that the antitumor activities of sarcodictyins A, B, C and E were recognized, and perhaps more importantly, it was not until then that their taxol-like mechanism of action was uncovered. Hence, in 1997, researchers at Pharmacia-Upjohn reported that sarcodictyins A, B, C, and E exhibited cytotoxicity against L1210 murine leukemia cells with IC_{50} values in the range of 408.5$\pm$21.3 nM to 911.7$\pm$393.5 nM *versus* taxol at 16.6$\pm$5.2 nM.[36] The necessary dose of these sarcodictyins to promote 90% tubulin polymerization was between 1.7 to 6.6 μM as compared to 4.4 μM for taxol.[36] Furthermore, all of the compounds were found to compete with ^{3}H-taxol in tubulin binding assays suggesting that they bind at the same or at overlapping sites.[36] Most important, however, was that the authors found sarcodictyins A, B and E to have a low resistance index (1.2 to 2.5) to the MDR (multi-drug resistant) cell line L1210/DX[36] suggesting that the sarcodictyin framework may be a poor substrate for the P-glycoprotein efflux pump. Later studies performed by Hamel and co-workers at the National Institutes of Health, employing synthetic sarcodictyins A and B (*vide infra*), confirmed many of these findings including induction of tubulin polymerization and competitive inhibition of taxol binding.[37] However, employing several more restrictive tubulin polymerization conditions (0 °C$\rightarrow$37 °C, with and without GTP or MAPs), they reported that the sarcodictyins were less potent tubulin polymerizers than eleutherobin or the previously isolated epothilones.[37] These researchers also disclosed the cytotoxicities of both sarcodictyin A and B in several other neoplastic cell lines, finding IC_{50} values in the range of 200—500 nM against prostate carcinoma, melanoma, breast carcinoma, and ovarian carcinoma. Encouragingly, both natural products retained complete activity against two resistant cell lines, overexpressing mutated tubulin isotypes.[37] This last finding complements the previous report by the Pharmacia-Upjohn researchers which found the sarcodictyins to be active against P-glycoprotein overexpressing tumor lines, opening the possibility of developing an agent active against both types of taxane resistant neoplastic diseases.

3b. Biology of Eleutherobin Similar to sarcodictyins A, B, and E, initial reports of eleutherobin's structure (**7**) in a 1995 patent were accompanied by disclosures of its potent cytotoxicity, albeit in the absence of a supporting mechanistic rationale.[28] Three years after this patent disclosure, Fenical *et al*. and their collaborators at Bristol-Myers Squibb revealed more detailed biological studies of eleutherobin, including evidence that it acted by mitotic arrest through tubulin polymerization.[38] Specifically, eleutherobin was shown to be cytotoxic to HCT116 human colon carcinoma cells (IC_{50}=10.7 nM *versus* taxol: IC_{50}=4.6 nM) and human ovarian carcinoma cells A2780 (IC_{50}=13.7 nM *versus* taxol: IC_{50}=6.7 nM).[38] In the NCI (National Cancer Institute) 60 cell line panel, eleutherobin exhibited a cytotoxicity profile similar to that of taxol with the NCI COMPARE program yielding an 84% correlation coefficient between the two.[38] Similar to sarcodictyins A—E, eleutherobin was able to displace ^{3}H-taxol from its binding site and induce tubulin polymerization *in vitro* at concentrations ranging from 2.5 μM to 10 μM at 37 °C. Moreover, the ultrastructure of glutaraldehyde-fixed polymerization products from incubation of 2.5 μM of eleutherobin or 2.0 μM of taxol were indistinguishable as illustrated in Fig. 4.[38] A morphological examination of HCT116 cells incubated with eleutherobin at moderate concentrations (EC_{50}=25 nM) showed mitotic arrest while those incubated with lower concentrations (3 to 10 nM) exhibited micronuclei.[38] Treatment of these cells with doses of eleutherobin ranging from 1 μM to 5 μM resulted in the formation of microtubule bundles similar to those obtained with taxol. Further evidence from flow cytometry experiments revealed that incubation of HCT116 cells with either taxol or eleutherobin (both at 50 nM) resulted in a G_2-M block.[38]

Experiments with eleutherobin against several taxane resistant cell lines yielded moderate results. The two cell lines evaluated were human colon carcinoma HCT116/(VM)46, which over expresses the P-glycoprotein, and the human ovarian carcinoma A2780/Tax 22 which employs mutated tubulin isotypes. Eleutherobin exhibited a 52-fold cross resistance (*versus* the parental line) to the HCT116/(VM)46 cell line as compared to a 100-fold resistance for taxol, suggesting that eleutherobin might be a substrate for the efflux pump.[38] This supposition was further supported by the finding that co-incubation with verapamil, a potent P-glycoprotein inhibitor, reverted this resistance. Similarly, against

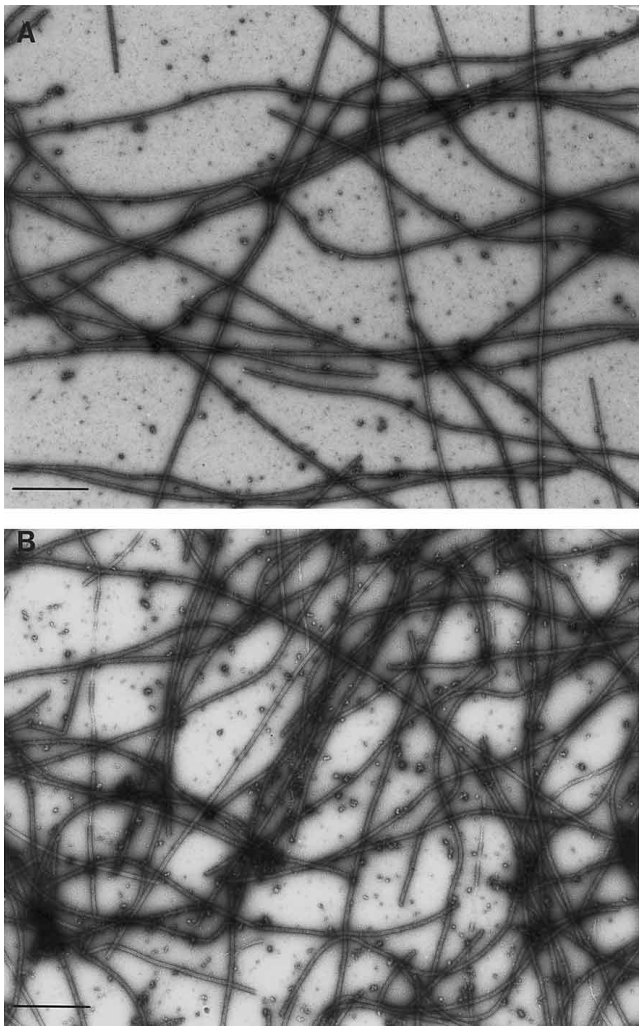


Fig. 4. Transmission Electron Microscopy of Tubulin Polymerization Products Obtained Following Incubation of Calf Brain Tubulin with Either 2.5 μM Eleutherobin (A) or 2.0 μM Taxol™ (B) at 37 °C for 30 min

Microtubules were fixed with glutaraldehyde and stained with 1% uranyl acetate. Bar = 1.0 μm. (Reprinted with permission from ref. 38.)

A2780/Tax 22 cells, eleutherobin exhibited 4.2-fold cross resistance as compared with 13-fold cross resistance for taxol. This confirms the relative homology of their binding sites as suggested by earlier competition experiments.[38]

As with sarcodictyins A and B, Hamel and co-workers recently disclosed additional biological properties of eleutherobin employing a synthetic sample (*vide infra*) in their studies.[37] While confirming most of the previously disclosed properties, these researchers also evaluated eleutherobin in a wider range of cytotoxicity assays showing it to have IC_{50} values in the range of 20—60 nM against prostate carcinoma, melanoma, breast carcinoma, and ovarian carcinoma.[37] Moreover, eleutherobin showed no susceptibility to resistance in ovarian carcinoma lines which overexpress mutated tubulin isotypes.[37]

4. Chemistry of the Sarcodictyin Family

Independent disclosures that the structurally homologous eleutherobin and sarcodictyins A—E exhibited potent cytotoxicities against various cancer cell lines *via* a taxol-like mechanism immediately excited interest in the scientific community.[39] Unfortunately, the relative scarcity of these rare marine natural products hampered further *in vitro* and *in vivo* biological testing. Accordingly, numerous synthetic groups took up the challenge of developing a total synthesis of one or more members. As of the writing of this review, there have been only two total syntheses reported, the first by our group[40—45] and the second by Danishefsky and coworkers[46—48] at Columbia University and The Sloan-Kettering Institute for Cancer Research. Additionally, Gennari *et al*. recently reported an advanced intermediate,[49] and several other groups have disclosed strategies through meeting abstracts.[50]

4a. The Nicolaou Total Synthesis of Sarcodictyins A and B At the outset of our efforts, we established three criteria for a successful total synthesis. First, we sought to develop a reliable and efficient route which would facilitate preparation of reasonable quantities of eleutherobin and sarcodictyins A and B for further biological investigations. Second, we hoped that such a route could be flexible enough to allow, in a convergent fashion, access to all three natural products from a common advanced intermediate. Finally, we desired a synthesis that employed late stage installation of key diversity elements yet was still versatile enough to allow for adaptation to an eventual solid phase-based approach to combinatorial libraries. Specifically, it was envisioned that achieving this last goal would allow for enlistment of both parallel and split-and-pool combinatorial approaches in the construction of sarcodictyin based libraries for structure–activity relationship studies.

With these goals in mind, we proceeded with a convergent retrosynthetic analysis as outlined in Chart 1 for **5** and **7**.[41—44] After initial cleavage of the (*E*)-*N*(6′)-methylurocanic acid residues, disconnection of the oxygen bridge at C(4) revealed ten-membered dieneones **15** and **16** with pendent hydroxyl groups suitably positioned to undergo, in the forward direction, spontaneous hemiketalization at the C(4) carbonyls, thus establishing the requisite oxygen-bridged bicycles. Consideration of how to effectively mask such dienones led to the proposition[40] of enynones **17** and **18** which were envisaged to be triggered into the anticipated cascades by selective reduction of the C(5) alkynes. Disconnection of the alkynylcarbonyl bond as the retron for an acetylide addition reduced the systems to monocyclic carvone derivatives **19** and **20**. At this point, the two retrosyntheses converged to intermediate **19** *via* a straightforward disconnection of the glycosidic bond of **20**.[42] Examination of key intermediate **19** suggested simplification of the appendages *via* a stereocontrolled acetylide addition on the top branch and a Knoevenagel condensation at the lower branch to give **21**, which could be derived from the readily available and inexpensive terpenoid (+)-carvone (**22**). An important, albeit less obvious, advantage of employing carvone as the starting material rested in the fact that while the absolute stereochemistry of **5** and **6** was known at the outset of our work that of **7** was not. Hence, the ready availability of both stereoisomers of carvone was reassuring in case of the unlikely event that the absolute stereochemistry of eleutherobin differed from that of sarcodictyins A and B.

As illustrated in Chart 2, work in the forward direction commenced with conversion of **22** into intermediate **29** following the straightforward, but slightly modified, procedure of Trost.[51] With the first ring of the sarcodictyin skeleton se-

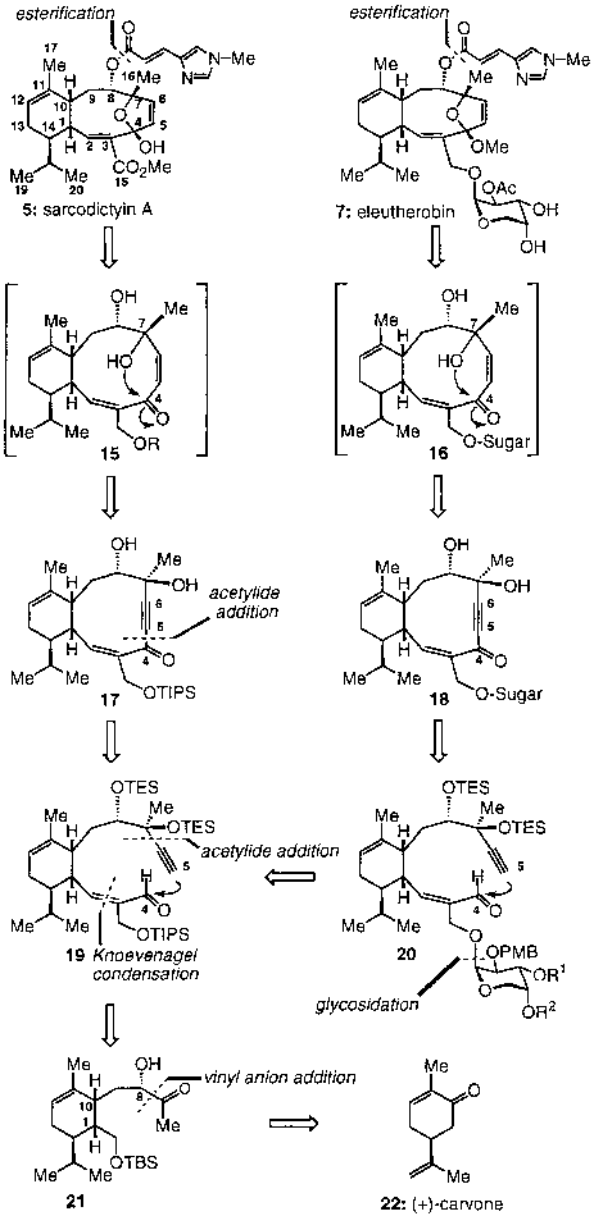


Chart 1. Retrosynthetic Analysis of **7** and **5**

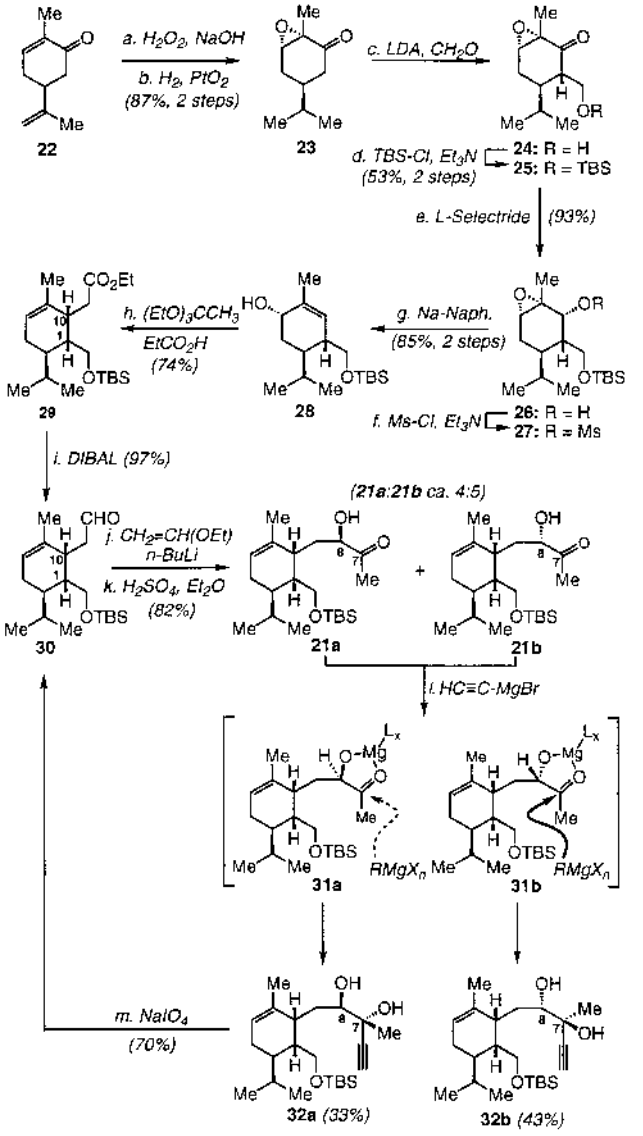


Chart 2. The Nicolaou Synthesis of Key Intermediate **32b** from **22**

cured, attention then turned towards elaborating the two appendages off of C(1) and C(10) to allow for the eventual construction of the oxygen bridged bicycle.[40,43] Initially, attention was focused on extending the C(10) side chain by reduction of the ethyl ester group to an aldehyde which was then subjected to addition of the lithio-derivative of ethyl vinyl ether and subsequent acid-catalyzed hydrolysis, giving a mixture of α-hydroxyketones **21a** and **21b**. The mixture (inseparable by conventional chromatography) was treated with ethynyl magnesium bromide effecting chelation-controlled Grignard addition to the C(7) carbonyls, as shown for **31a** and **31b** (see Chart 2), to produce exclusively diastereomeric diols **32a** and **32b**, which were readily separable. While diol **32b** possessed the correct stereochemistry and was carried forward, diol **32a** was recycled back to aldehyde **30** by treatment with $NaIO_4$. It should be noted that in an earlier approach[40] (not shown) we had achieved an analogous, but completely stereoselective, conversion of **30** to **21b** which required six steps and featured a Sharpless asymmetric epoxidation. However, the brevity of the recycling approach shown here proved significantly more efficient.[43]

The final stage prior to cyclization to the ten-membered enynone was elongation of the C(1) appendage which initially entailed a series of protecting group manipulations transforming **32b** to **35** as shown in Chart 3.[43] The revealed alcohol at C(2) was then oxidized to the corresponding aldehyde, and the remaining carbons of the lower appendage were installed *via* a selective Knoevenagel condensation with ethyl cyanoacetate to provide cyanoester **37**. In anticipation of the upcoming cyclization, this cyanoester was reduced to the corresponding hydroxyaldehyde **38** which was protected as its silyl ether **39**. In the cyclization event, deprotonation of acetylide **39** with LHMDS resulted in smooth conversion to macrocycle **40** which was immediately oxidized to enynone **41**. Before formation of the oxygen bridge, the hydroxyls at C(7) and C(8) needed to be deprotected to give **42**. With **42** in hand, the proposed reduction-hemiketalization strategy was ready to be put to the test. Gratifyingly, upon selective hydrogenation of the C(5) alkyne, the putatively formed

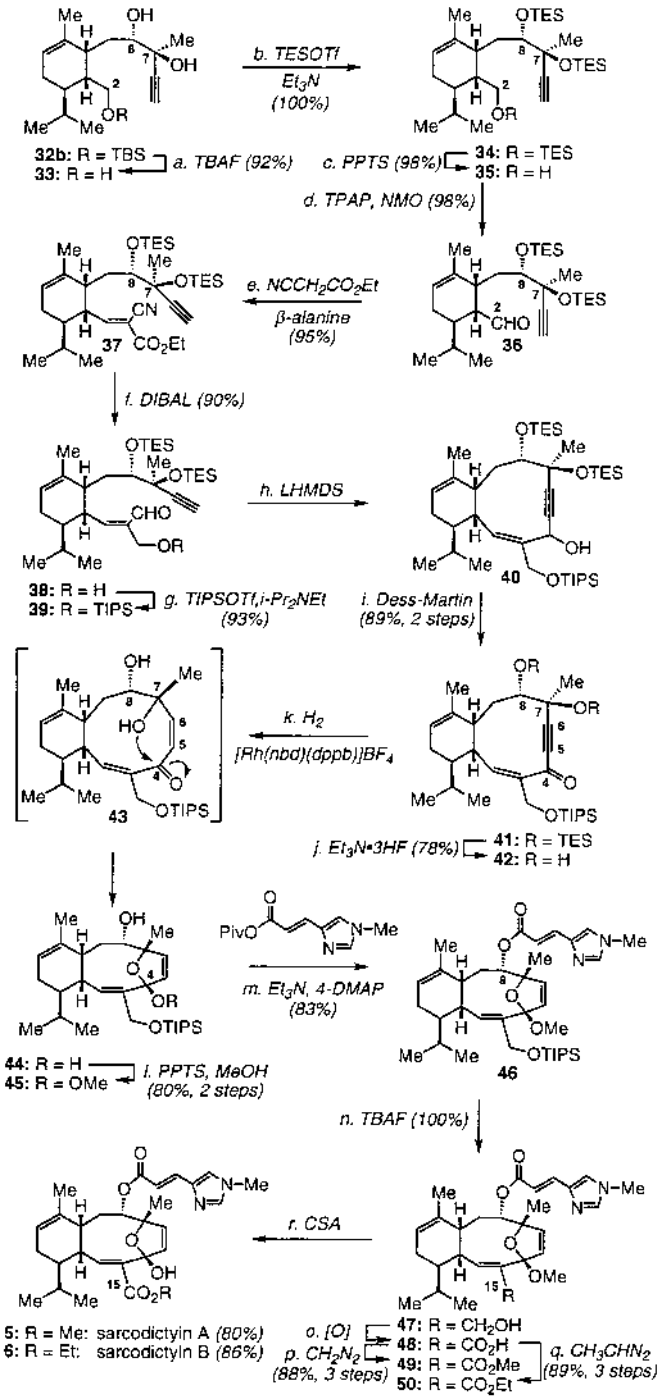


Chart 3. The Nicolaou Synthesis of **5** and **6**

dienone **43** underwent spontaneous collapse to hemiketal **44** which was subsequently protected as the methyl ketal by treatment with PPTS in MeOH. The only complication encountered during this cascade was some undesired over-reduction of the C(5) olefin to give the corresponding saturated product. The problem of over-reduction was carefully attenuated by screening a series of hydrogenation catalysts, and it was found that $[Rh(nbd)(dppb)]BF_4$ was most effective, giving over 10 : 1 selectivity for the desired **44** *versus* the corresponding over-reduction product, while Lindlar's catalyst proved least effective with a ratio of only 2 : 1.[43]

With the successful construction of tricyclic compound **45** the completion of the synthesis was at hand, requiring only elaboration of the C(8) side chain and the C(15) ester. The free hydroxyl at C(8) was esterified with the mixed anhydride of (*E*)-*N*(6′)-methylurocanic acid[40,52] to give ester **46** which was then subjected to desilylation with TBAF to give the primary alcohol **47**. Conversion of this alcohol to the corresponding carboxylic acid **48** was accomplished *via* a two-step oxidation procedure utilizing Dess-Martin periodane followed by $NaClO_2$. The resulting acid was esterified with diazomethane and diazoethane (**49** and **50**) followed by removal of the methyl ketal at C(4) to give sarcodictyin A (**5**) and sarcodictyin B (**6**), respectively.[43]

4b. The Nicolaou Total Synthesis of Eleutherobin With the successful total synthesis of the first two members of the sarcodictyin family,[40,43] our attention turned toward the glycosidated diterpene eleutherobin (**7**). As mentioned previously, we hoped to develop a convergent approach wherein both eleutherobin and sarcodictyins A and B might be derived from the same advanced intermediate. The question, however, was at what stage to diverge the two syntheses towards their respective targets. Ideally, it was hoped that tricyclic alcohol **47** (Chart 3) might be glycosidated to allow a late stage divergence. Unfortunately, preliminary results revealed that such a glycosidation occurred with no anomeric selectivity. This lack of selectivity was in line with subsequent reports from the Daniskefsky group that model studies of a similarly proposed glycosidation yielded stereorandom results.[47] To avoid this problem, we decided to effect a more stereocontrolled glycosidation somewhat earlier in the sequence as outlined in Chart 4.[41,44] A suitably protected arabinose donor (**51**) was constructed as previously described[41,44] and this donor was reacted with alcohol **38** in the presence of TMSOTf. At this stage, we discovered the α/β anomeric ratio was highly solvent- and temperature-dependent, allowing for selective formation of either the α- or the β-anomer.[44] At the extremes, the use of 2 : 1 dioxane:toluene at 0 °C gave 8 : 1 selectivity for the desired β-anomer **52**, whereas using hexane at −78 °C resulted in a 1 : 8 ratio in favor of the undesired α-anomer.[44] Satisfied with the 8 : 1 selectivity for **52**, we proceeded forward to effect completion of the synthesis of eleutherobin in a somewhat analogous fashion to that of the sarcodictyins A (**5**) and B (**6**). Formation of enynone **54** was accomplished by deprotonation of acetylide **52** followed by oxidation of the resulting ten-membered macrocycle. Prior to hydrogenation, the PMB group on the arabinose unit was cleaved, and the free hydroxyl was subsequently acetylated giving **55**, which was subjected to the action of $Et_3N \cdot 3HF$ to free the two pendent hydroxyls at C(7) and C(8). Hydrogenation, this time over Lindlar's catalyst, resulted in smooth conversion to the tricyclic hemiketal **57** which upon treatment with PPTS in MeOH was converted to methyl ketal **58**. Finally, the (*E*)-*N*(6′)-methylurocanic acid side chain was installed and the remaining silyl groups of the arabinose moiety were cleaved to complete the first total synthesis of **7**.[41,44]

4c. The Danishefsky Total Synthesis of Eleutherobin The Danishefsky group also reported a construction of the tricyclic core[46] of the sarcodictyin family and, subsequently, a total synthesis of **7**.[47] Their approach commenced from the terpenoid (*R*)-(−)-α-phellandrene as outlined in Chart 5.[46] Similar to our own strategy, they required the installation of

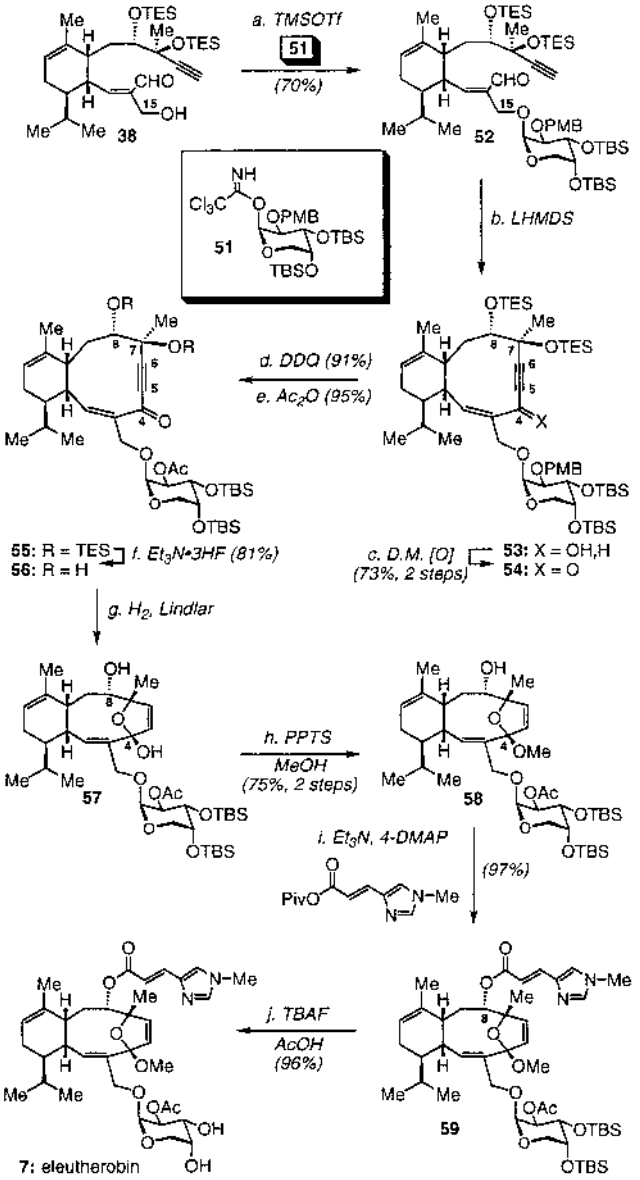


Chart 4. Completion of the Nicolaou Total Synthesis of **7**

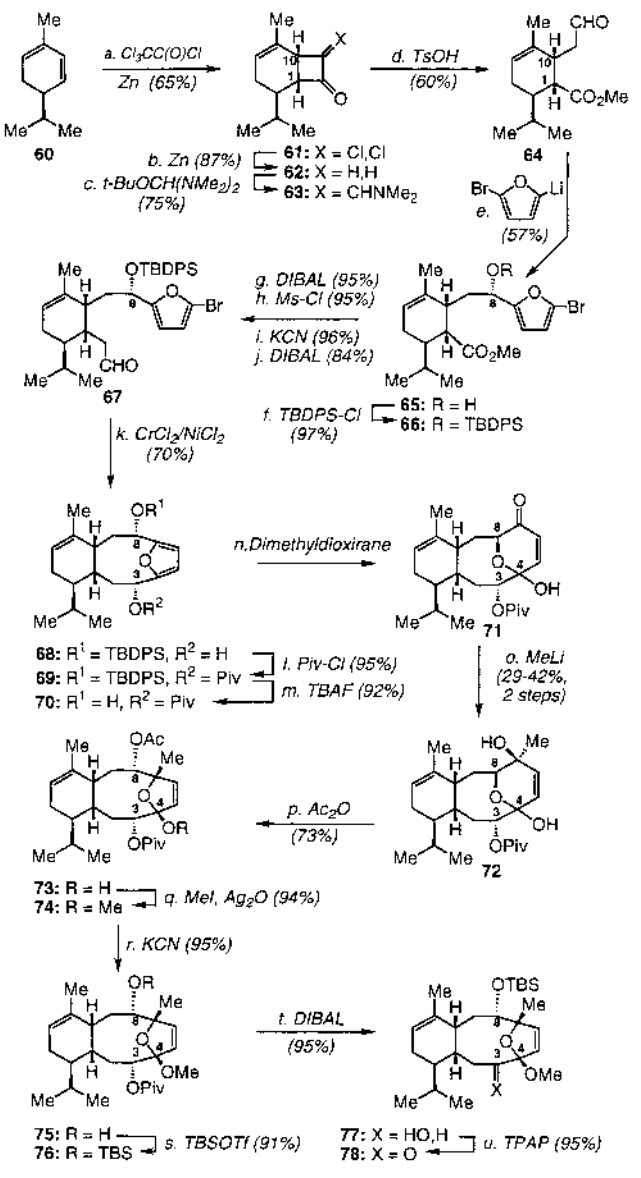


Chart 5. The Danishefsky Synthesis of Tricyclic Intermediate **78** from *R*-(−)-α-Phellandrene

two *cis*-fused appendages onto the (*R*)-(−)-α-phellandrene skeleton. This was elegantly and rapidly accomplished through a regio- and stereoselective [2+2] photoaddition giving rise to α-dichloroketone **61** which was dechlorinated with zinc to cyclobutanone **62** and subsequently converted to **63** *via* a Bredereck-type transformation. The transformed cycloadduct **63** then underwent acid-catalyzed fragmentation upon treatment with TsOH to give aldehyde-ester **64**.

Elaboration of the top appendage commenced with a moderately stereoselective addition of monolithiated 2,5-dibromofuran yielding α-hydroxy furan **65** [plus the C(8)-epimer] which was protected to afford silyl ether **66**.[46] Prior to closure of the eight-membered ring, the appendage off the C(1) carbon needed to undergo a one-carbon homologation as outlined in Chart 5 (**66**→**67**). With **67** in hand, closure of the eight-membered ring was stereoselectively effected through a Nozaki-Kishi reaction. Before attempting the key oxidative ring expansion, several protecting group manipulations were effected converting **68** into **70**, which upon treatment with dimethyldioxirane, afforded the pyranose containing intermediate **71**. The latter compound (**71**) was treated with methyllithium to effect stereoselective methylation of the carbonyl at C(7) giving rise to **72**, albeit in modest yield. Treatment of **72** with acetic anhydride resulted in rearrangement of the pyranose moiety to the sarcodictyin carbon skeleton, presumably through trapping of the secondary alcohol at C(8) under equilibrating conditions.[46] In preparation for the completion of the synthesis, substrate **73** was subjected to a series of protecting group manipulations followed by oxidation of the C(3) alcohol to the corresponding ketone of **78**. In a subsequent communication[48] (not shown), the team reported an improved conversion of (**69**→**75**) employing silyl protection of the C(4) alcohol prior to methyllithium addition followed by TsOH-catalyzed rearrangement in an overall yield of 60—70%.

An examination of intermediate **78** reveals the necessity for a one-carbon homologation at C(3) in order to complete the full framework of eleutherobin. Presumably daunted by

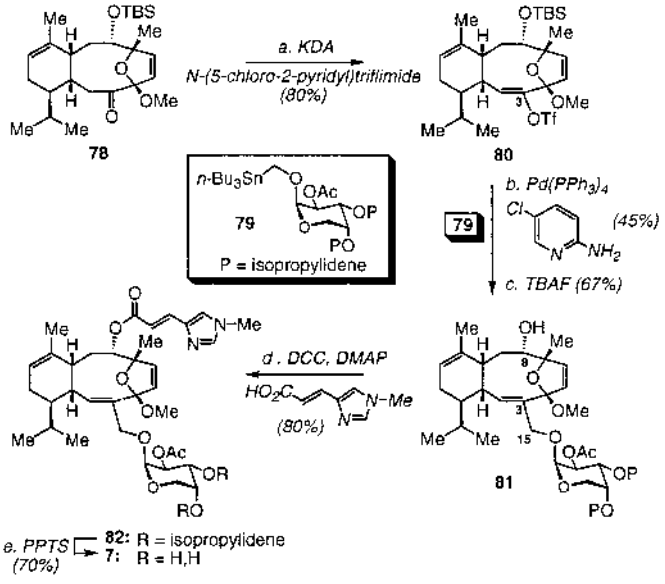


Chart 6. Completion of the Danishefsky Total Synthesis of **7**

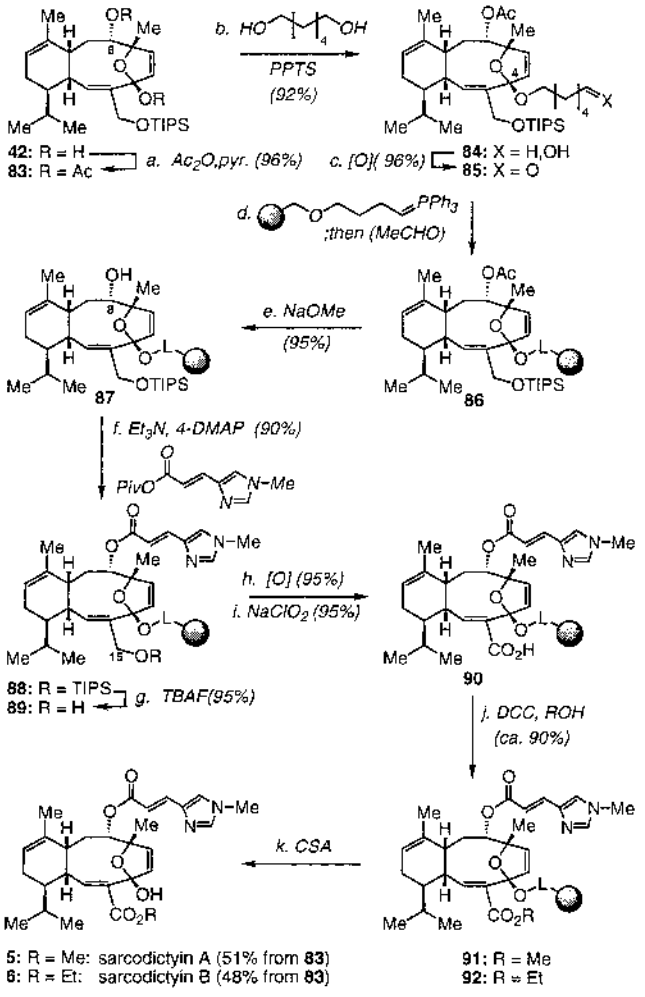


Chart 7. The Nicolaou Solid Phase Synthesis of **5** and **6**

the difficulty of selectively glycosidating the homologated skeleton, the group employed a daring strategy (Chart 6) by attempting to install a pre-glycosidated variant of the C-15 carbon through an sp^3 version of the Stille coupling employing alkylstannane **79** (performed with the desired β-configuration) and vinyl triflate **80**.[47] In the event, the expected coupling proceeded in 45% yield upon heating the coupling partners with $Pd(PPh_3)_4$, LiCl, and 2-amino-5-chloropyridine in THF to give, after desilylation, **81**, which was easily converted to **7** *via* a DCC-mediated esterification of the (*E*)-*N*(6′)-methylurocanic acid side chain and subsequent deprotection of the arabinose moiety.[47]

5. Synthesis and Biological Evaluation of Designed Sarcodictyin Libraries

With the task of the total synthesis completed, the next phase of the sarcodictyin research focused on using the chemistry developed to effect the construction of diversity libraries suitable for exploring structure–activity relationships. Past experience within our laboratories with the epothilone family had demonstrated the increased efficiency that solid phase-based combinatorial chemistry could bring to the task of creating diversity libraries,[53] and, as such, our first goal was to adapt our solution phase synthesis to a solid phase approach.[45]

The first step in such an approach was to develop a linking strategy wherein an advanced intermediate from the synthesis could be tethered so as to allow for maximum installation of diversity elements while maintaining a reliable and efficient cleavage protocol.[45] After considerable experimentation, it was decided that tricyclic hemiketal **42** would be employed as the library scaffold and be linked through a mixed ketal moiety at C(4) as outlined in Chart 7.[45] Hence, **42** was first peracetylated to give **83** and then treated with 1,6-hexanediol and PPTS to provide **84**. In preparation for resin loading, the terminal hydroxy group of **84** was oxidized to the corresponding aldehyde (**85**), which upon treatment with a resin-bound ylide underwent smooth loading *via* olefination to give resin-bound sarcodictyin scaffold **86**. To confirm the viability and fidelity of this linking strategy, the total syntheses of both **5** and B **6** were completed from intermediate **86** in a fashion analogous to the solution phase synthesis. Briefly, treatment of **86** with NaOMe revealed the free C(8) hydroxyl group which was esterified with the (*E*)-*N*(6′)-methylurocanic acid side chain followed by deprotection and oxidation of the C(15) alcohol to carboxylic acid **90**. DCC-mediated esterification of **90** with either methanol or ethanol gave **91** or **92**, respectively, which were independently cleaved from the resin *via* treatment with CSA to provide samples of **5** and **6** identical to those previously obtained by solution phase synthesis.[40,43]

With the linker-cleavage protocol verified, attention turned towards library construction.[45] Examination of the sarcodictyins suggested three key elements of diversity for consideration as follows: the side chain appended to C(8), the hemiketal at C(4), and the C(15) ester moiety. As outlined in Chart 8, we sought to address each of these elements in a sequential fashion.[45] Thus, the previously linked scaffold **87** was first esterified at the C(8) hydroxyl moiety employing five distinct (R_1) acyl donors to give **93**. After desilylation of the C(15) alcohol, intermediate **94** was split along three pathways. The first entailed acylation (R_2) of the C(15) hydroxyl followed by transketalization release with a series of alkyl alcohols (R_3) ultimately yielding structures of the general type **100**. The second pathway involved oxidation of the C(15) hy-

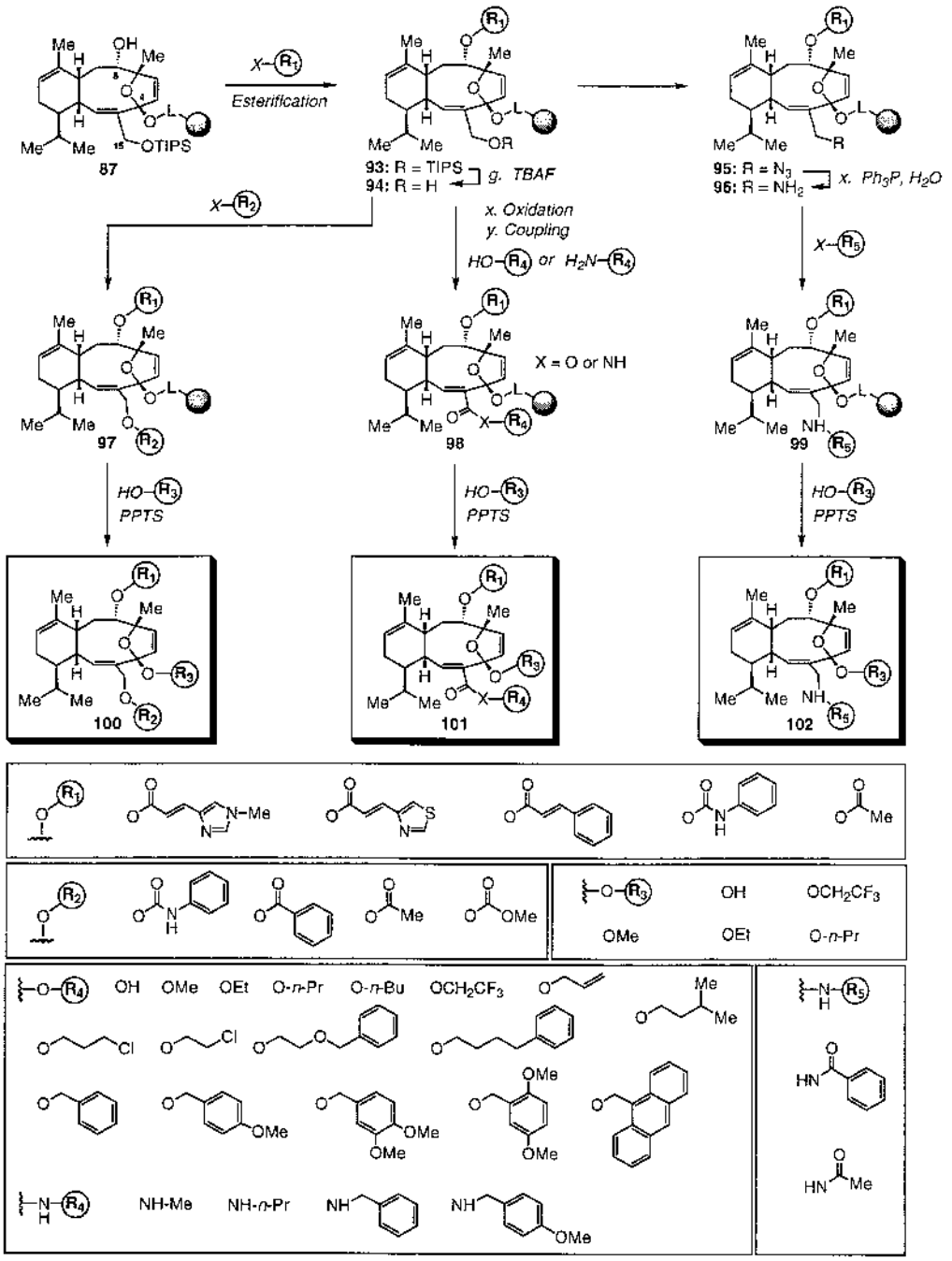


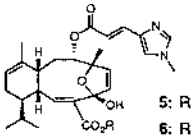
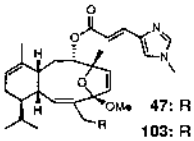
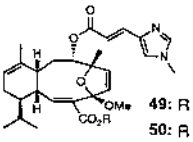
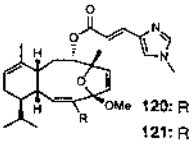
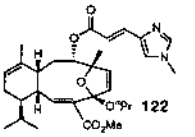
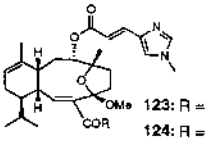
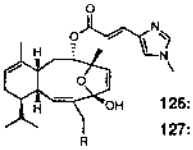
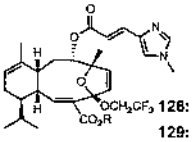
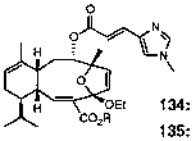
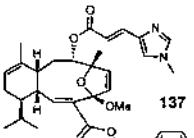
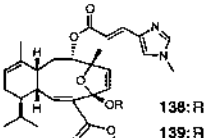
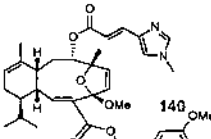
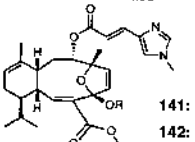
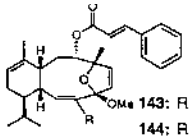
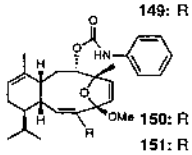
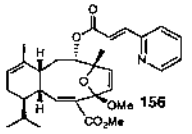
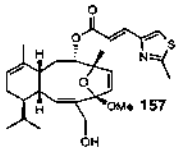
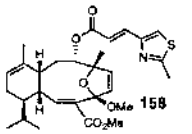
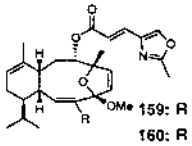
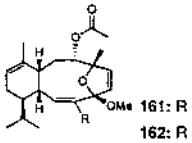
Chart 8. The Nicolaou Solid Phase Synthesis of Sarcodictyin Libraries

droxyl of **94** to the corresponding carboxylic acid, which was converted to either an amide or an ester (**98**) by coupling with a series of amines (R_4) and alcohols (R_4), respectively. These analogs were then released by transketalization as before, giving rise to **101**. The final path involved conversion of alcohol **94** to the corresponding amine **96** which was acylated (R_5) giving structures of the form **99**, release of which by transketalization provided **102**.

This resin-based approach allowed rapid access to a host of diverse substitutions on the sarcodictyin skeleton.[45] Additionally, we complemented this solid phase approach with the solution phase construction of additional analogs.[42] In concert, these approaches yielded the library shown in Table 1,[42,45] which was evaluated using colorimetric tubulin polymerization assays as described elsewhere.[25,54] Promising analogs were also tested in cytotoxicity assays employing ovarian cancer cells (1A9) and two taxol resistant cell lines (1A9PTX10 and 1A9PTX22). These results are summarized in Table 2.[45,54,55]

The structure–activity relationships derived from these studies are summarized diagramatically in Figure 5. The most obvious finding was the importance of the α,β-unsaturated hetero-aromatic side chain for both tubulin binding and antiproliferative activities. Replacement of the natural (*E*)-*N*(6′)-methylurocanic acid side chain with cinnamate (**143**—**149**), phenyl carbonate (**150**—**155**), or acetate (**161**—**163**) resulted in complete loss of tubulin polymerizing ability.[45] Moreover, the natural (*N*)-methylimidazole hetero-aromatic

Table 1. Structures and Tubulin Polymerization Properties of Sarcodictyin Analogs

Ref.	Structure	%Tubulin polymerization
42	 5: R = Me	67
42	6: R = Et	71
42	 47: R = OH	37
42	103: R = F	27
42	104: R = OAc	37
42	105: R = OBz	12
42	106: R = N_3	24
42	107: R = OCONHPh	24
45	108: R = NHAc	30
45	109: R = NHBz	5
42	 49: R = Me	72
42	50: R = Et	46
45	110: R = Bn	61
45	111: R = CH_2CH_2Cl	40
45	112: R = $(CH_2)_2CH_2Cl$	30
45	113: R = CH_2CF_3	31
45	114: R = $(CH_2)_3CH_2Ph$	36
45	115: R = ^{n}Bu	52
45	116: R = ^{n}Pr	69
45	117: R = CH_2CH_2OBn	54
45	118: R = $CH_2CH{=}CH_2$	51
45	119: R = $(CH_2)_2CH(CH_3)_2$	10
42	 120: R = CHO	4
45	121: R = $CH(OMe)_2$	74
42	 122	79
45	 123: R = NHBn	75
45	124: R = NHMe	52
45	125: R = NH^nPr	15
45	 126: R = OH	2
45	127: R = OAc	34
45	 128: R = $(CH_2)_2CH_2Cl$	22
45	129: R = CH_2CH_2Cl	21
45	130: R = ^{n}Bu	23
45	131: R = CH_2Ph	38
45	132: R = $(CH_2)_3CH_2Ph$	1
45	133: R = Pr	21
42	 134: R = Me	85
45	135: R = CH_2CH_2OBn	28
45	136: R = $(CH_2)_2CH(Me)_2$	20
45	 137	22
45	 138: R = Me	26
45	139: R = Et	18
45	 140	16
45	 141: R = Me	48
45	142: R = Et	24
45	 143: R = CO_2Bn	1
45	144: R = CONHMe	3
45	145: R = CONHBn	2
45	146: R = CH_2NHAc	6
45	147: R = CH_2NHBz	5
45	148: R = CO_2Me	6
45	149: R = *p*-MeOBnNH	3
45	 150: R = CO_2Me	4
45	151: R = CONHMe	2
45	152: R = CONHBn	2
45	153: R = CO_2Bn	1
45	154: R = CH_2NHAc	1
45	155: R = CH_2NHBz	2
45	 156	18
45	 157	5
45	 158	42
45	 159: R = CO_2Et	4
45	160: R = CHO	9
45	 161: R = CO_2Me	0
45	162: R = CH_2NHAc	4
45	163: R = CHO	3

The tubulin polymerization measurements were performed at 37 °C as described elsewhere[25a,45,54] with drug concentrations of 100 μM and incubation times of 90 min.

displays optimal activity, whereas substitution with pyridine (**156**), thiazole (**158**), or oxazole (**159**) resulted in a decrease in activity.[42,45)]

In addition to the side chain, the C(4) hemiketal and the C(15) ester were also probed to determine their relevance for activity. In general, tubulin binding properties were largely unaffected by conversion of the hemiketal at C(4) to a mixed alkyl ketal (**49**, **122**, **134**) although a moderate decrease was noted for **50**. Remarkably, however, several of these ketals (**49**, **50**, **134**) exhibited marked improvements in antiproliferative properties as compared to the parent sarcodictyins A and B. In fact, in the ovarian carcinoma cell lines these ketals had antiproliferative activities which were in most cases at least ten-fold greater than the natural products, and moreover, their susceptibility to resistance was minimal as determined by comparisons between the resistant and parental cell lines (overexpressing mutated tubulin isotypes). Encouraged by these results, researchers at NIH further investigated the activity of compound **49**, which was the C(4) methyl ketal of sarcodictyin A. As shown in Table 3, compound **49** exhibited improved activities in taxol susceptible prostate, melanoma, and breast cancer lines as compared to natural sarcodictyins A and B; and, quite dramatically, in ovarian cancer cell lines (both parental and resistant), compound **49** displayed cytotoxicities ten-fold greater than the sarcodictyins (**5**, **6**), 2-fold greater than **7**, and equal to **2** and **1**.

Table 2. Cytotoxicity Data for Selected Sarcodictyin Analogs

Cmpd.		Induction of tubulin polymerization %Tubulin Polymerization	Inhibition of carcinoma cell growth IC_{50} (nM) 1A9	1A9PTX10	1A9PTX22
1	taxol	65	2	50	40
2	epothilone A	73	2	19	4
3	epothilone B	97	0.04	0.035	0.04
5	sarcodictyin A	67	240	140	360
6	sarcodictyin B	71	2	160	80
156		18	430	1800	>2000
158		42	300	244	180
159		4	>2000	800	385
47		37	800	>2000	>2000
103		27	1850	>2000	>2000
104		37	1050	>2000	1620
127		34	1400	>2000	>2000
106		47	>2000	>2000	1800
108		30	800	1600	1200
49		72	70	4	84
50		46	2	1	60
110		61	360	1210	540
111		40	95	85	100
112		30	200	350	290
114		38	110	90	120
115		52	25	35	31
116		69	3	4	5
117		54	80	91	85
118		51	9	12	10
120		4	600	400	600
121		74	30	45	60
122		47	>2000	>2000	1800
123		75	500	1400	700
124		52	45	65	60
134		85	110	13	160
135		28	110	400	440
136		20	500	1240	1000
137		22	1400	1300	1400
138		26	640	1300	900
141		48	110	90	130

Experiments were performed as described elsewhere[45,54,55)] using ovarian carcinoma parental cell line 1A9 and two parental derived resistant lines, PTX10 and PTX22.

In an attempt to further capitalize on the improvement acquired by ketalization, we employed optimized compound **49** as parent scaffold to screen substituents of the alkyl group of the C(15) ester. Replacement of the methyl ester of **49** with slightly larger groups such as Et (**50**), *n*-Pr (**116**), *n*-Bu (**115**), and allyl (**118**) resulted in modestly decreased tubulin polymerizing properties. Surprisingly however, these modest tubulin polymerization losses did not translate into reduced antiproliferative properties as compared to **49**, and, in certain cases (**116**, **118**), these alkyl substitutions resulted in improved cytotoxicities over the already optimized compound **49**. The introduction of haloalkyl (**111**—**113**) or larger substituted aromatic groups (**137**—**142**) into the C(15) ester resulted in significantly decreased tubulin polymerizing ability as well as loss of cytotoxicity. Additionally, we attempted several complete substitutions for the C(15) ester (still maintaining the C(4) methyl ketal) including conversion to an amide (**123**—**125**), reduction to the alcohol (**47**, **104**, **105**, **107**), transformation to an amine (**108**, **109**), and other miscellaneous substitutions (**103**, **106**, **120**, **121**). Generally, these drastic changes resulted in decreased tubulin polymerization properties and loss of antiproliferative activities with a few notable exemptions. Namely, conversion to the dimethyl acetal (**121**) and the *N*-methylamide (**124**) resulted in structures slightly less active than the parent structure **49**, but

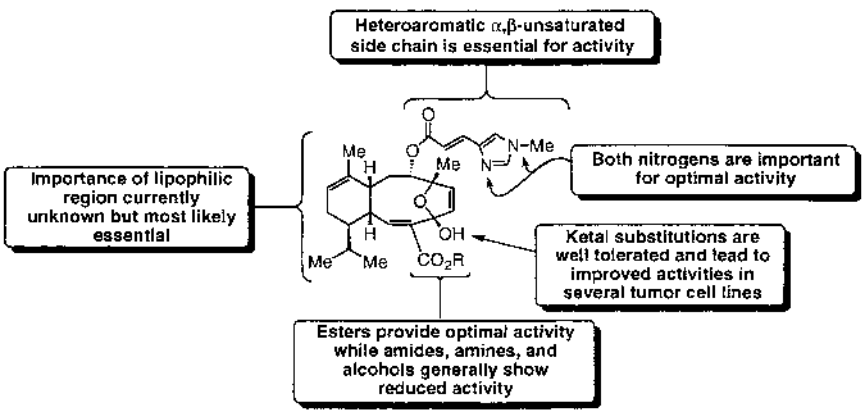


Fig. 5. Structure–Activity Relationships for Sarcodictyin Family of Natural Products

Table 3. Cytotoxicity Data for Structure **49**

Cmpd.	Inhibition of carcinoma cell growth PC3	LOX-IMV1	MCF-7	1A9	1A9PTX10	1A9PTX22
	IC_{50} (nM)					
1 taxol	4	6	2	4	60	60
2 epothilone A	10	10	5	10	40	10
3 epothilone B	0.9	0.9	0.4	1	3	1
5 sarcodictyin A	200	400	300	300	200	300
6 sarcodictyin B	200	500	400	300	300	300
7 eleutherobin	20	30	10	40	60	30
49 analog	50	80	300	20	20	10

Experiments were performed as described elsewhere[37] using PC3 prostate carcinoma, LOX-IMV1 melanoma, MCF-7 breast carcinoma, 1A9 ovarian carcinoma parental cell lines as well as PTX10 and PTX22 resistant ovarian carcinoma cell lines.

still ten-fold more active than the natural products sarcodictyins A (**5**) and B (**6**) in both susceptible and resistant ovarian carcinoma lines.

While not exhaustive, these current structure activity studies have served to reveal several important trends. Specifically, the discovery that introduction of a methyl ketal at the C(4) position of the sarcodictyins drastically improves *in vitro* antiproliferative properties in a variety of neoplastic cell lines warrants further investigation, especially since it might be possible to optimally complement this substitution by perturbing the alkyl group of the C(15) ester (as suggested by several compounds such as **116** and **118**). From a structural standpoint, it is intriguing to examine these optimal analogs (**49**, **50**, **115**—**118**), especially as they compare to the natural product eleutherobin. While both eleutherobin and these optimized analogs share a C(4) methyl ketal, they differ significantly at the C(15) position where eleutherobin contains a sugar and the others, an ester. Yet, in spite of these C(15) differences, their overall antiproliferative properties are quite similar, leading to the proposition that the carbohydrate moiety of eleutherobin may not be essential for activity.[37] This proposition is in agreement with the findings, by Ojima and co-workers, that the carbohydrate of eleutherobin lies outside the common pharmacophore developed to account for the activities of taxol, epothilone, discodermolide, and eleutherobin.[32a,32b] In light of this, future libraries might be designed to examine a more careful gradient of C(15) substitutions from the methyl ester of sarcodictyin A (**5**) to the arabinose moiety of eleutherobin (**7**) to search for compounds with improved pharmacological properties and possibly higher antiproliferative activities.

A second subtle, yet intriguing trend also emerged from these structure–activity studies. Namely, for several of the more active compounds, tubulin polymerizing properties did not vary uniformly with antiproliferative properties. For example, compounds **50** and **118** were significantly less potent tubulin polymerizers (46% and 51%, respectively) than the natural product sarcodictyin A (67%), yet their antiproliferative properties were significantly greater (IC_{50} 1—60 nM) than those of sarcodictyin A (IC_{50}=200—300 nM). There are several plausible explanations for this puzzling pattern. The first possibility is that these analogs may undergo intracellular metabolic transformations to produce more active tubulin binders *in vitro*.[37] Secondly, it may be that the structural changes which serve to moderately impair tubulin binding

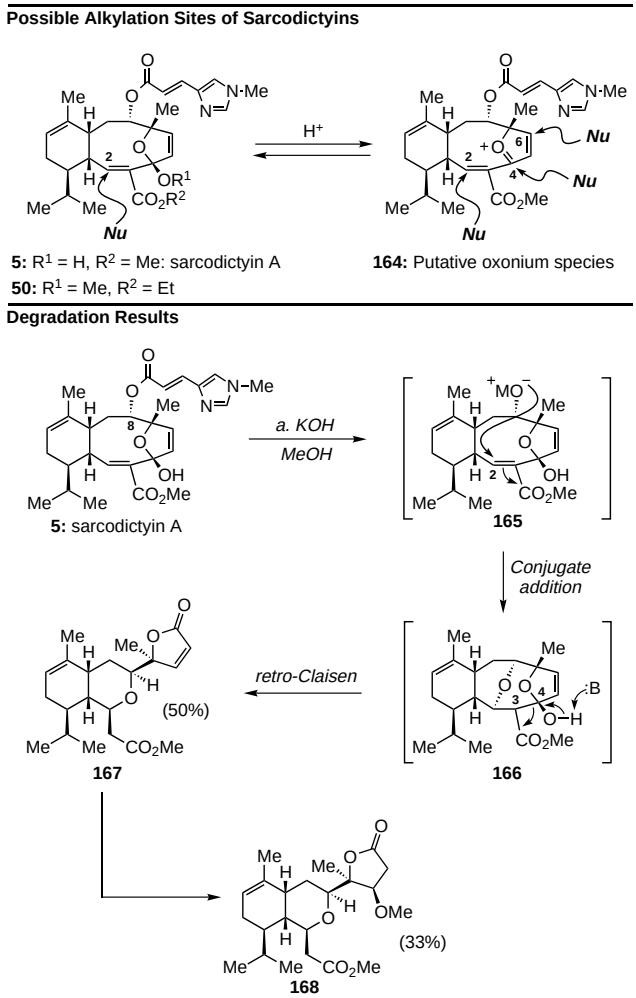


Chart 9. Alkylating Potential of Sarcodictyin Family of Natural Products and Supporting Degradation Reaction Cascades

abilities actually enhance transport of the agents into cells, resulting in increased intracellular concentrations and consequently greater cytotoxicities.[37] A third possibility is that these compounds are exhibiting markedly improved microtubule stabilizing effects *in vitro* as compared to the natural products.[37] Finally, these inconsistencies might be a result of these compounds acting at a second as yet unidentified target or through a second unrelated mechanism of action such that the observed cytotoxicities are actually composite values.[37,42] The implications of the latter possibility led us to a closer examination of the sarcodictyin skeleton. While as of yet unconfirmed, one possibility is outlined at the top of Chart 9.[42] Examination of the structures of sarcodictyin A (**5**) and compound **50** illustrates the possible formation (under mildly acidic conditions) of the putative oxonium species **164** revealing a number of electrophilic sites *e.g.* C(2), C(4), or C(6) (in addition to the already electrophilic C(2) site of the parental structures). Such sites might be susceptible to attack by endogenous nucleophiles (*i.e.* proteins or DNA) leading to altered cellular function and possible cell death. Evidence for the electrophilicity of the C(2) position was provided by Pietra *et al*. during degradation studies of **5** as shown in Chart 9.[29] Thus, treatment of **5** with methanolic base resulted in hydrolysis of the C(8) ester which was fol-

lowed by intramolecular Michael addition of the free hydroxyl group onto the C(2) position forming the bis-(oxygen-bridged) species **166**. The latter compound (**166**) underwent spontaneous fragmentation *via* a retro-Claisen reaction to give a mixture of **167** and **168**. Studies aimed at better elucidating the potential biological relevance of this and other possible mechanisms are needed.

6. Conclusion

In summary, recent activities in these and other laboratories have resulted in the total chemical synthesis of the natural products **7** and **5** and **6** in both solution as well as on solid phase. Such studies have increased the relatively scarce supply of each natural product in addition to facilitating the construction of a first generation diversity library to provide insights into the structure–activity relationships of the sarcodictyins. Among these insights, the most exciting was the realization that the antiproliferative activity of the sarcodictyins can be markedly improved by modest structural changes in the C(4) and C(15) regions of the molecule's skeleton. Further studies in this area may lead to optimization of these effects and the emergence of new biological tools and drug candidates for cancer chemotherapy.

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