Applications of total synthesis toward the discovery of clinically useful anticancer agents

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This *tutorial review* provides a historical sampling of synthetic efforts undertaken in our laboratory, which have led to the total syntheses of a range of small molecule natural products of potential interest in oncology. It has become evident that natural products, and structures clearly derivable from natural products, have a remarkable record in the treatment of cancer at the clinical level. It is likely that, with the growing power of chemical synthesis, small molecule natural products will play a continuing role in providing lead anticancer compounds.

Introduction

The continuing search for procedures and agents of clinical value in oncology is driven by the widespread occurrence of the disease and by its always serious and often tragic consequences. The misery and devastation of cancer are such that a wide range of initiatives have been employed in the search for late stage palliation, improvements in quality of life, significant extensions of survival time and even cures. Clearly the cancer problem is multifaceted and, accordingly, calls for fundamental research to mine the depths of insights available from fields such as cell biology, tumor biology, immunology, and, more recently, genomics. Systematic rational approaches must surely hold out the best chances for ultimate long-term value.

Given the scope of the problem, its consequences for those affected, and its huge collateral damage to society at large, it is not surprising that other efforts are pursued in search of the goals enumerated above. These other means may often seem rather unsophisticated, and may even smack of desperation. One such avenue, often lacking specific validated molecular targets, has been that of screening a myriad natural product collections (from phytochemical, fermentation, marine and terrestrial sources). Such pharmacognosy-driven investigations typically begin with an early exploratory material-gathering phase, and advance to compound enrichment, purification, structure identification, and chemical modification stages. In some instances, a program in chemical synthesis may then be initiated.

Aside from those uncommon situations where chemical synthesis is vital for gaining reasonable scale access to the

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natural products because of the difficulty of their availability in nature, the total synthesis enterprise has traditionally initially been driven by intrinsic intellectual challenges of solving complicated problems in chemistry. Synthesis may also provide corroboration for structural assignment and may lead to evaluatable congeners based on the general matrix of the natural product itself. Total synthesis can also provide access to the natural product for other laboratories not involved in discovery. As we shall demonstrate herein, total chemical synthesis may also provide means for gaining access to structures, which could not be reached by using the natural product as a launching platform for molecular modification.

Often, the screening phase of natural products has been directed to gross cytotoxicity assays against immortalizable cancer cell lines. With the explosive growth in the teachings of cell biology, including cancer biology, initial screening can be directed more rapidly, and more efficiently (in terms of amounts of substrates required), and with more telling effect, against specific molecular targets such as enzymes, receptors and other biomodulators of critical cascades. While the traditional type of natural product-initiated research seems rather primitive from a biological perspective, amazingly its actual track record in addressing the missions enumerated above has been quite substantial.

Though discoveries which can appropriately be characterized as breakthroughs are, by their nature, few and far between, it can hardly be denied that, cumulatively, products arising from programs directed to the discovery of small molecule natural products (SMNPs) have had a significant and valuable impact in clinically based oncology. One need only cite the taxoids, the *Vinca* alkaloids, the anthracyclines, the thecans, the etoposides, the mitomycinoids, and various steroids (as well as steroid look-alikes), as being illustrative of the utility of natural sources in the discovery progression. Such SMNP derived agents have expanded the options employed by oncologists in the perplexingly difficult cancer treatment enterprise. Indeed, even at this writing, many of the more interesting post-2000 pipeline candidates have emerged through the SMNP route. Needless to say, the possibility of discovering large biomolecules (for instance antibodies) as useful agents in oncology remains exciting. However, in this paper we focus on SMNPs, and particularly on the emerging synergism of SMNP discovery and SMNP chemical synthesis in drug discovery.[1]

It is interesting to theorize why SMNPs have served as such a rich source of anticancer drugs. The question is clearly a complicated one in that, in most instances, one can only conjecture why SMNPs are being biosynthesized in the first place. Nevertheless, while the precise ''business plan'' for SMNPs often remains uncertain, it is not unlikely that many of them have been elaborated for their ability to react with large biomolecules, including proteins. While there is no reason to suppose any connectivity between the natural target (for instance, a particular protein or family of proteins) and the proposed pharma oncology target, it can still be argued that the SMNP enters the discovery process in a far advanced starting point relative to even the purposefully, let alone randomly, synthesized ''small'' organic structure lacking a natural product connection. In a similar vein, it should be appreciated that since the SMNP is being biosynthesized under enzymatic control, it is already established that it, and presumably closely related congeners, are not only recognized by proteins, but are incorporatable into active site pockets. Moreover, by definition the SMNP has been accommodated in some living organism. This fact already moves it further along the all critical bioavailability and pharmacokinetic progression than random entries. Finally, particularly in the case of potential oncology applications, one can theorize that the SMNP has been fashioned in some instance to shield its host from biopredators. There could well often be a relationship between antipredator activity and cytotoxicity attributes, hopefully with exploitable selectivity margins. Given all of these advantages, the remarkable record of SMNPs in oncology is perhaps understandable in that they start life with great advantages in terms of ''pedigree,'' ''combat testedness'' and host compatibility.

Much of the research of our laboratory is directed to chemical synthesis of compounds which might prove to be valuable in oncology. The effort, particularly in the last fifteen years, has moved along two avenues. We try to expand the science of synthesis to reach anticancer vaccines of potential value in mobilizing the host immune system in dealing with neoplastic transformation. This thrust has occasioned a large and continuing effort in building complex oligosaccharides and glycopolypeptides displaying carbohydrate centered tumor associated antigens.

The other arm of our effort, of a rather more diffuse nature, is that of total synthesis inspired by SMNPs. To gain entry to our ''radar screen,'' the SMNP with reputed anticancer activity must be endowed with a structure whose synthesis is also likely to stimulate new chemistry of value.

In this paper, we provide some vignettes, largely of a chemical nature, which describe how our SMNP total synthesis program, focusing on possible oncology applications, has developed over the years. We also provide instances of how total chemical synthesis of SMNPs and SMNP congeners may enhance initiatives in preclinical oncology. Before beginning the chemistry driven accounts, we present some thoughts about the emerging and exciting synergy between synthesis and SMNP discovery in the finding of new drugs.

Of course, the idea of building analogs of natural products for evaluation in the hope of finding better drugs in general is an old one, with long-term successes particularly in the fields of steroids, β-lactam antibiotics and statins. There is certainly no reason to suppose that the SMNP has been fully optimized in terms of its native biological mission, whatever that might be, let alone for its proposed pharma applications. Optimization through structural modification, by totally natural means, must await nature learning and implementing modified biosynthetic pathways. Hence, even evolutionarily driven ''optimization'' is in reality surely a compromise between what's desirable and what's feasible in terms of the realities of enzymatically-orchestrated biosynthesis in a particular timescale.

Happily, the unique magic of chemistry is such that it can expand the range of the feasible, rapidly and dramatically—in some cases in a single experiment. Alas, direct structural modification of the natural product through chemical means

may also have its constraints. First, there must exist reaction types to effect the changes. Moreover, the methodology for implementing the proposed modifications must be compatible with existing functional groups. This may be an important limitation at two levels. First, the partial synthesis steps required for the molecular modifications must be feasible in the context of the resident groups. Second, the final molecular changes that are being made must also be consistent with the properties of the resident groups.

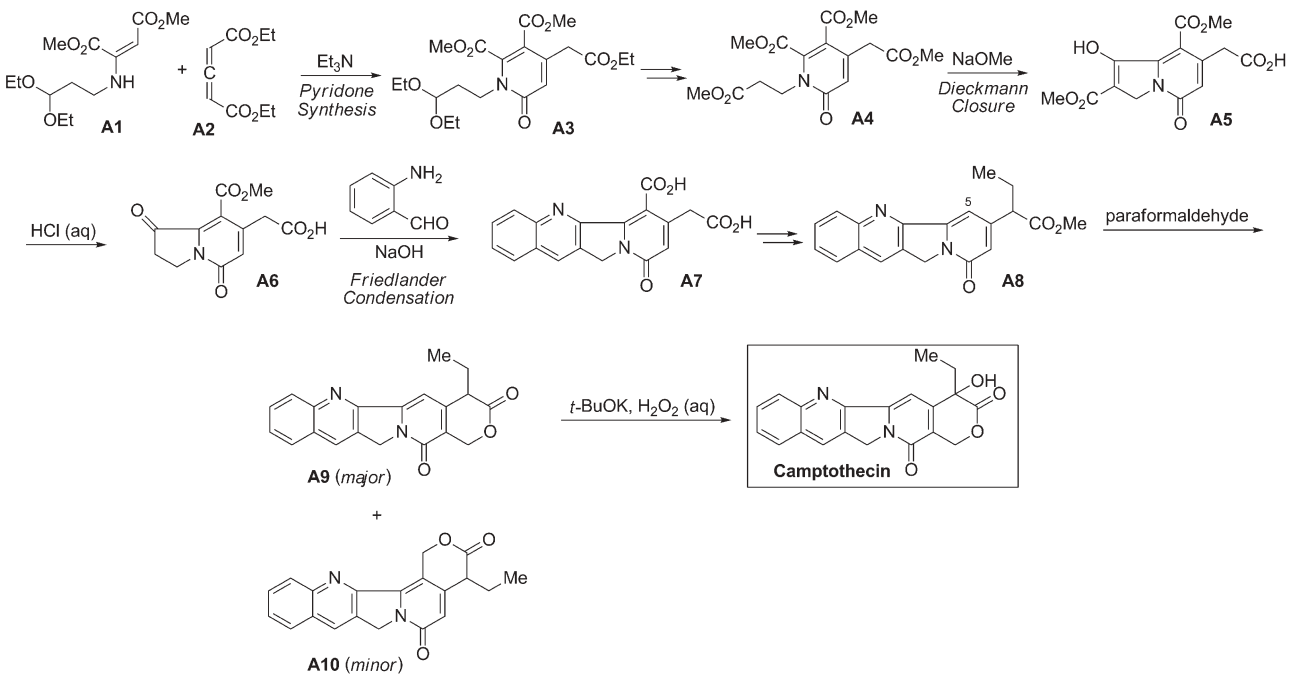
It is here that the concept of diverted chemical synthesis is so attractive in allowing for less restricted molecular editing. In this approach, far in advance of reaching the actual structure of the SMNP, one can anticipate desired future edits in the congener. Accordingly, one builds the edits into the structure being synthesized early on. In the same vein, one deletes potentially troublesome moieties that might interfere with proposed biological application or with the final feasibility of the molecular edits. In short, though chemical synthesis presently lacks the feature of amplifiability which is key to biology, chemistry comes with the huge advantage of being able, at least in principle, to address more quickly virtually *any* desired structural change in the pursuit of "molecular editing." This concept, which we term diverted chemical synthesis (DTS), requires little elaboration. The proposed molecular edits are introduced systematically by judicious melding of "building blocks" in a planned and orderly fashion. These edits may decrease or increase the complexity level of the SMNP thereby allowing exploration of high pedigree (*vide supra*) molecular space not available by starting with the SMNP itself.

This said, we proceed to revisit some case histories of earlier campaigns which, in the end, allowed us to accomplish our total synthesis goals. It goes without saying that each of these success stories recited below was really the summation of alternating advances and setbacks as the still far from mature theory underlying organic chemistry collides with sobering realities at the bench. Much interesting science often emerges from the challenges of adapting to disappointments which are an almost inevitable downside of bold undertakings.

Camptothecin

Following its isolation and structural elucidation in 1966,[2] the naturally occurring alkaloid camptothecin attracted considerable attention in the clinical community on the basis of its promising antitumor activity in a number of *in vitro* and *in vivo* settings. As a result of these early findings, the camptothecin challenge was undertaken in a number of chemical synthesis laboratories, culminating in the first total synthesis reported by Stork and Schultz in 1971.[3] Very shortly thereafter, our laboratory disclosed a total synthesis of this biologically compelling pentacyclic alkaloid.[4]

Our first-generation route made use of a then novel idea for assembling functionalized pyridines.[5] Indeed, the methodology had emerged from our interest in synthesizing camptothecin (Scheme 1). Thus, the diactivated allene, **A2**, was found, as predicted, to be highly susceptible to nucleophilic Michael addition of even a weakly nucleophilic vinylogous urethan enamine (**A1**). Carbon–carbon bond formation led to an adduct which, upon heating, smoothly underwent cyclization to provide the requisite pyridone triester, **A3**, albeit in only 45% yield. The latter was readily advanced to the stage of intermediate **A4** and, following Dieckmann closure, the bicyclic compound **A5** was in hand. Hydrolysis and selective decarboxylation were achieved simultaneously through exposure of **A5** to aqueous HCl, yielding ketone **A6**. With the C,D-ring system of camptothecin in place, we were able to efficiently install the quinolone portion of the molecule through a Friedlander condensation with



Scheme 1 First generation synthesis of camptothecin.

o-aminobenzaldehyde. Following esterification, decarboxylation, and alkylation, the monoester intermediate **A8** was obtained.

Treatment of **A8** with paraformaldehyde afforded desoxycamptothecin (**A9**), along with a minor regioisomer, which was determined to be isodesoxycamptothecin (**A10**), apparently resulting from hydroxymethylation at the competitive unsubstituted C_5 position of the pyridone. Remarkably, exposure of the anion of **A9** to aqueous hydrogen peroxide provided the natural product, camptothecin, albeit as the racemate, as shown below.

Following some disappointing findings at the clinical level in the 1970s, the camptothecin series enjoyed a resurgence of attention in the late 1980s, on the basis of two important developments. First, it was argued persuasively, by Wall and Wani *et al.*, the discoverers of camptothecin, that the administration of camptothecin as its sodium salt may have adversely impacted its ability to function at the clinical level.[6] Indeed, due to the low solubility and difficult formulatability of camptothecin, the compound had been administered as its lactone-opened sodium salt, which is not itself clinically active. It was conjectured that the *in vivo* lactonization that would be required to render the candidate biologically active might not necessarily occur as readily as had originally been anticipated. By this reasoning, the possibility of developing a camptothecin analog with enhanced water solubility and improved pharmacokinetics, which could be administered in the biologically active lactone form, presented itself as an option to be pursued. A second development which reinvigorated interest in this area was the finding, by Liu and colleagues, that camptothecin is a member of a growing class of topoisomerase inhibitors.[7] This important discovery would allow for more efficient searching of camptothecin analogs for biological activity at the *in vitro* level.

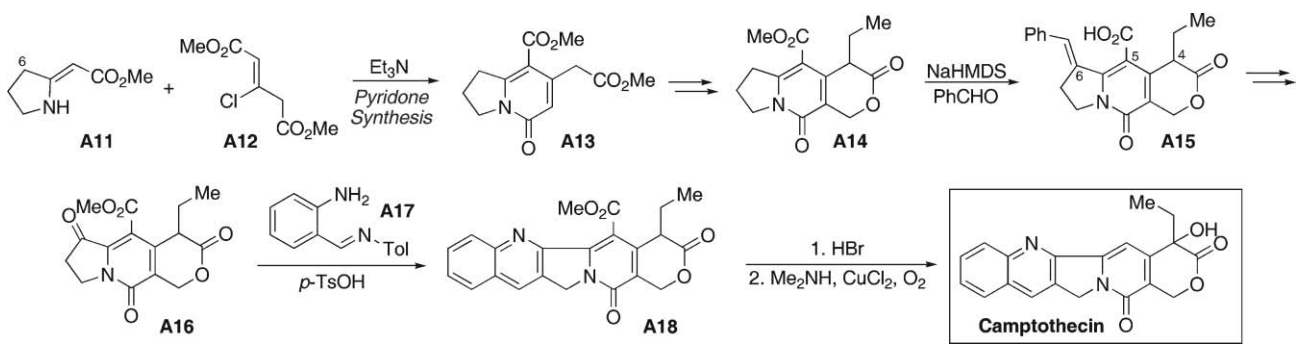
Given the revival of enthusiasm for the camptothecin family, some 20 years following the completion of the earliest total syntheses, we returned to the problem, seeking to reconfigure our initial route to allow for more efficient access to significant quantities of the drug and some select analogs. Thus, upon re-examining our earlier effort, we had identified, as a significant weakness, the penultimate step of the synthesis, in that hydroxymethylation of **A8** had provided a mixture of the desired desoxycamptothecin (**A9**) and its regioisomer, **A10**. Presumably, by blocking C_5 of the pyridone system at the stage of installation of the lactone, it would be possible to eliminate this competing side reaction. In this context, we had previously found, in the course of an analog synthesis, that the lactomethylation could indeed be achieved with a C_5 ester function in place.[8]

Another important advance had been realized in the intervening years. The pyridone synthesis, which we had developed in the context of our earlier camptothecin endeavor, required as one of the reactants a highly reactive and unstable dicarboethoxyallene substrate (*cf.* **A2**), and the yield of the reaction had suffered accordingly. We had subsequently found that this troublesome reagent could be produced, *in situ*, from a readily available chloroglutaconate precursor, in the presence of triethylamine. With these two specific advances at our disposal, and with the added benefit of further years of learning in the field of chemical synthesis, we endeavored to implement an improved second-generation route to the camptothecins.[9]

In an effort to maximize efficiency, we sought to perform the pyridone synthesis with the future C-ring of camptothecin already in place (Scheme 2). Thus, although we were not able to effect cyclization of an enamine substrate incorporating functionality at C_6, we did achieve reaction between **A11** and **A12**, to provide the pyridone **A13** in a very respectable 92% yield. With the C_5 blocking group in place, ethylation followed by hydroxymethylation with spontaneous lactonization proceeded in excellent yield to provide **A14** as the only regioisomer, as expected.

At this stage, the challenge would be that of functionalizing the C_6 position while minimizing competition from the C_4 center. A number of routes were investigated with varying degrees of success; however, in the end, the most efficient sequence involved treatment of **A14** with NaHMDS and benzaldehyde, as shown, to provide the benzylidene acid **A15** in 90% yield. Following ozonolysis and esterification, the intermediate ketone **A16** was in hand. As in the first-generation route, Friedlander condensation was employed for the installation of the A,B-ring system, as shown. The resultant pentacyclic intermediate, **A18**, was subjected to hot HBr-mediated decarboxylation, followed by oxidation at C_4, to provide DL-camptothecin in 39% overall yield from the abundantly available pyridone **A14**.

While we had indeed made excellent progress in our second-generation total synthesis of camptothecin, not surprisingly, other laboratories had organized to attack this problem. Indeed, these efforts led to the production of the required



Scheme 2 Second generation synthesis of camptothecin.

enantiomer of camptothecin with high stereocontrol. Two particular strategies which were particularly impressive were reflected in the works of Curran[10] as well as Fang.[11] Accordingly, having fought the good battle to maintain interest in camptothecin over the lean years (including having conducted some of the earliest telling SAR studies),[8] we were pleased to leave the concluding developmental phases to other laboratories whose total syntheses were rather more powerful than our own. Happily, a variety of camptothecin congeners known as "thecans" have entered oncological practice with clear success in otherwise difficultly treatable cancers of the pulmonary and digestive tracts. Parenthetically the thecans have proven to be rewarding for their consistently excellent illustrations of the SMNP discovery and DTS optimization synergies.

Mitomycins: mitomycin K and FR-900482

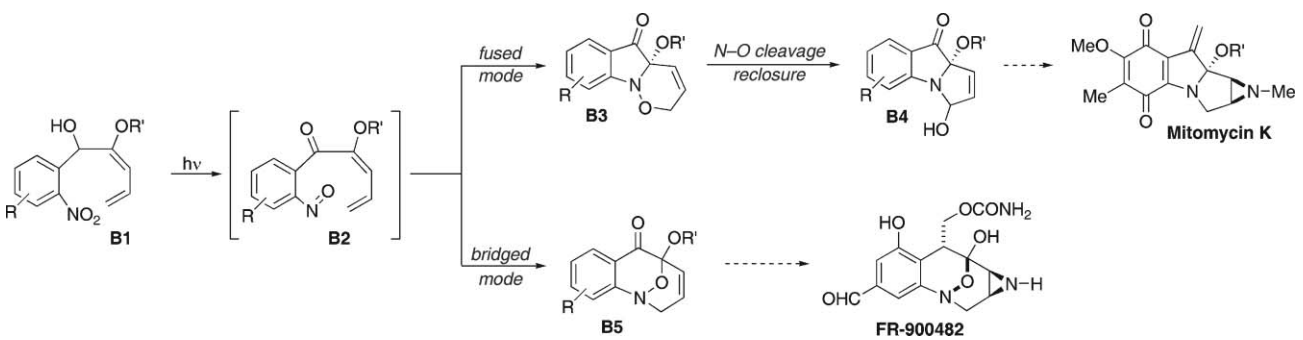
The members of the mitomycinoid class of natural products have commanded the attention of the scientific community over the decades since their initial isolation and elucidation. In addition to their varied and complex structures, many representatives of this family exhibit potent and potentially exploitable cytotoxic activity. One such member, mitomycin C, is a commonly prescribed antitumor agent, and several other constituents (and congeners thereof) were, at that time, being advanced toward clinical trials. From the earliest structure elucidation studies of Stevens,[12] followed by the landmark total synthetic efforts of Kishi,[13] as well as Fukuyama,[14] the mitomycins have provided a unique platform for groundbreaking advancements in the isolation and structure determination of SMNPs.

Our own long-term infatuation with the venerable mitomycin natural product family originally arose from an interest in exploring the assembly of pyrroloindole systems through activated cyclopropanes.[15] Although this initially conceived strategy ultimately failed to lead us to the mitomycins, it produced some then novel chemistry. However, our interest in the mitomycinoids occasioned a study directed to the total synthesis of mitomycin K.[16] These efforts also led to a total synthesis of FR900482.[17] A detailed account of our total mitomycinoid program is beyond the purview of this review, and can actually be found elsewhere.[18] Rather, in this context, we limit ourselves to a discussion of the details of the ultimately successful routes that were employed to reach the natural products, mitomycin K and FR900482.

Following the investigation of a number of ultimately unproductive approaches, we were drawn to what seemed to be a novel idea, wherein intramolecular nitroso Diels–Alder reaction might provide a viable route to our targets (Scheme 3). Under this paradigm, an intermediate of the type **B1** was envisioned as undergoing a photolytic redox reaction to generate a compound of the type **B2**, which would be predisposed toward Diels–Alder-like cyclization. From intermediate **B2**, two possible IMDA modes could be enviioned. Thus, the proposed IMDA might proceed in the "fused" mode, which would provide adduct **B3**. Following N–O cleavage and reclosure, **B3** would suffer conversion to **B4**, a potentially viable intermediate *en route* to the mitomycin K series. Alternatively, cyclization could occur *via* the "bridged" mode, to generate an FR900482-type adduct (**B5**). We anticipated that, given the short length of the proposed tether, steric rather than electronic considerations would prevail, and that formation of the fused adduct would be observed. In the event, a key model study confirmed this to be the case. Accordingly, we devised a strategy toward mitomycin K which would employ the nitroso-IMDA as the centerpiece of a concise, complexity-building strategy.

Thus, the IMDA precursor, **B8**, was readily assembled through coupling of aldehyde **B6** and the dienyllithio derivative, **B7**. We were pleased to find that, upon exposure of **B8** to photolytic conditions, the product isolated was not the hoped for direct IMDA product, **B10**, but rather the even more advanced **B11**. Apparently, **B10** had suffered photodissociation of the N–O bond, resulting in a diradicaloid structure which had undergone hydrogen atom migration and formation of the 5-membered C-ring (see **B11**). Presumably, the highly electron donating characteristic of the aromatic ring substituents served to promote fragmentation of the N–O bond.

In any event, with the rearranged tricyclic core system of mitomycin K in hand, we next sought to achieve the deoxygenation of C_3 and the installation of the aziridine functionality. Thus, **B11** was advanced to **B12** and, ultimately, to intermediate **B13**. Interestingly, we found the order of execution of the deoxygenation and aziridination events to be crucial. Thus, when free radical-induced deoxygenation was attempted with the aziridine already in place, the intermediate "carbinyl" radical was apparently prone to rapid fragmentation, leading to observed opening of the aziridine ring. By contrast, the triazoline intermediate, **B13**, was highly stable to free radical conditions, and upon treatment with tributyltin



Scheme 3 Nitroso-IMDA approach to the mitomycinoids.

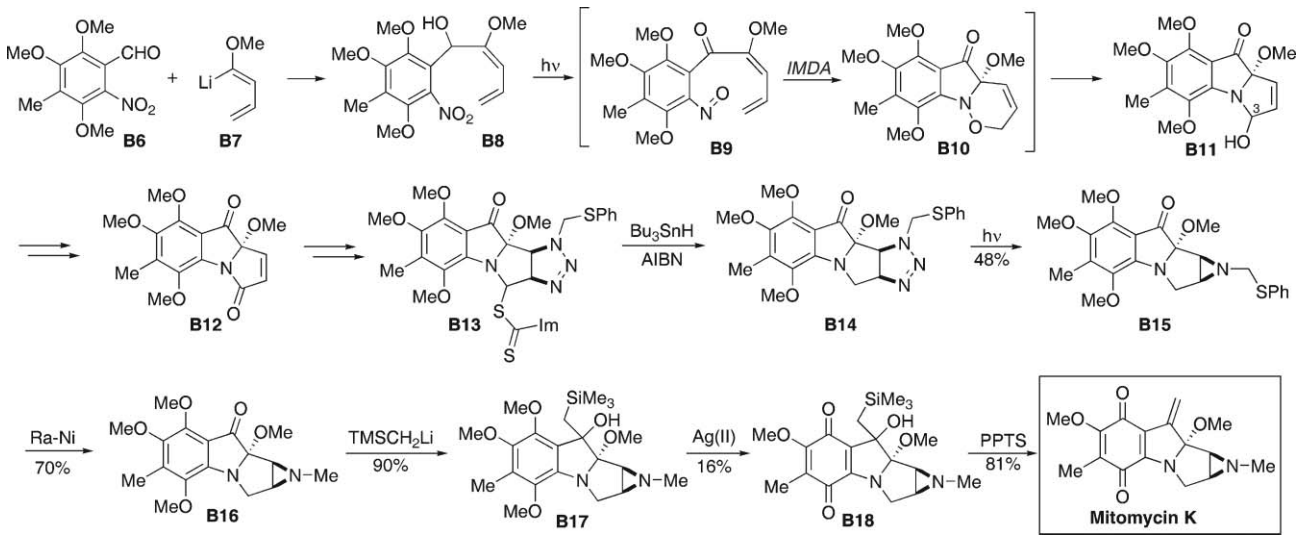
hydride and AIBN, deoxygenation proceeded smoothly to afford **B14**, which, under photolytic conditions, could be converted to the requisite thiophenylmethyl aziridine **B15**. At this stage, Raney Nickel desulfurization provided the *N*-methyl aziridine (**B16**) in 70% yield. In passing, we note that this method to reach **B16** from **B15** was worked out *de novo* to serve the total synthesis of mitomycin K.

From **B16**, there remained the need to install the exocyclic olefin and to convert the dimethoxybenzene ring to the requisite *p*-quinone. The olefin would be implemented *via* a Peterson olefination protocol. Thus, **B16** was converted to **B17**, as shown. We had previously found this type of intermediate to be surprisingly stable to elimination in the context of the aromatic system, although elimination more readily occurred in the context of quinonoidal substrates. Thus, intermediate **B17** was oxidized to *p*-quinone **B18**, albeit in disappointingly low yield. As expected, PPTS-mediated elimination readily occurred to furnish the requisite exocyclic olefin of mitomycin K, as shown (Scheme 4).

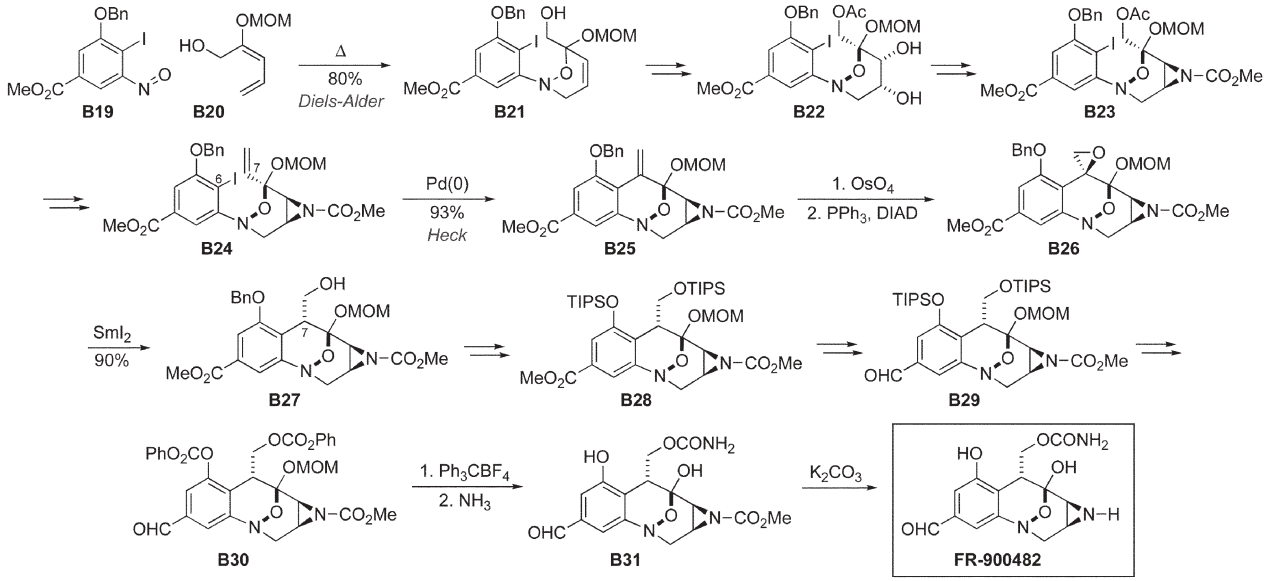
We now turn to the total synthesis of the mitomycinoid, FR-900482. We were determined not to approach the total synthesis of FR900482 by simply attempting a modest repackaging of our mitomycin K synthesis, pleasing as it was in its key steps. First, it was not clear that such a program could even be managed in a ''doable'' way. Furthermore, the learning experience provided by a fresh departure could be more substantial than attempting to repackage the mitomycin K synthesis to the FR series just to get there. Happily, a novel challenging strategy, which seemed to connect in a smooth way with the FR target, presented itself. Here, we would take recourse to an intermolecular nitroso-Diels–Alder reaction (see formation of **B21**, Scheme 5). From this matrix would be installed an aziridino moiety as well as a terminal vinyl group (see **B24**). The compelling question would be the feasibility of an intramolecular Heck reaction on substrate **B24** of uncommonly complex functionality (an N–O linkage as well as a particularly activated aziridine).

In the event, our synthesis of the proposed Heck precursor, **B24**, commenced with a then novel thermal intramolecular nitroso Diels–Alder reaction between *o*-nitroso iodide **B19** and diene **B20**, to provide the cycloadduct, **B21**, in good yield. This compound was advanced to diol **B22** and, thence, to the aziridine **B23**. Following conversion of the C_7 acetoxy group to a terminal olefin, the key substrate, **B24**, was in hand. We were quite pleased to observe that, upon treatment with palladium tetrakis(triphenylphosphine), substrate **B24** underwent the anticipated intramolecular Heck reaction to provide compound **B25** in 93% yield. The latter was readily advanced to the spiro-epoxide **B26**. Surprisingly, the stereospecific opening of this epoxide turned out to be somewhat of a challenge. Following a number of unsuccessful attempts, we discovered a viable solution to the problem, wherein treatment of **B26** with SmI_2 provided directly the β-hydroxymethyl functionality at C_7, in very good yield. Through a series of protecting group manipulations and oxidation state adjustments, we were ultimately able to gain access to compound **B31**. Finally, treatment of the latter with potassium carbonate led to removal of the aziridinyl ester, thus providing fully synthetic FR900482.

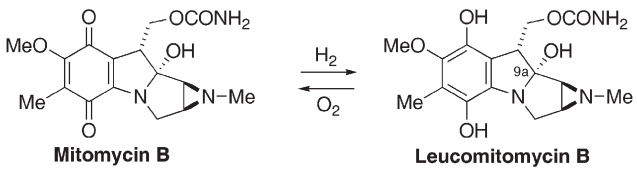
Having recited these total synthesis-driven studies, it is perhaps worth recalling the realization of a biomechanistic advance of some potential importance in the design in mitomycin drugs. Starting with mitomycin B, it was possible to generate the leuco form of the drug, and to demonstrate its surprising viability. Indeed, our laboratory was able to provide the first experimental characterization of leucomitomycin and to show that it has viability and can be oxidized to the quinonoidal (mitomycin) form without loss of the C_{9a} heterofunction, which would lead to rupture of the aziridine (Scheme 6).[19] Considerable evidence was brought to bear to suggest that drug activation of a leucomitomycin involves one-electron oxidation to trigger the unraveling cascade. Conversely, starting with the mitomycin drug, we conjectured that one-electron bioreduction may be involved in triggering the bioalkylating agent. The proof of structure of the DNA drug alkylation product was accomplished in a brilliant fashion by the collaborators Nakanishi, Tomasz, and Verdine.[20]



Scheme 4 Synthesis of mitomycin K.



Scheme 5 Synthesis of FR-900482.

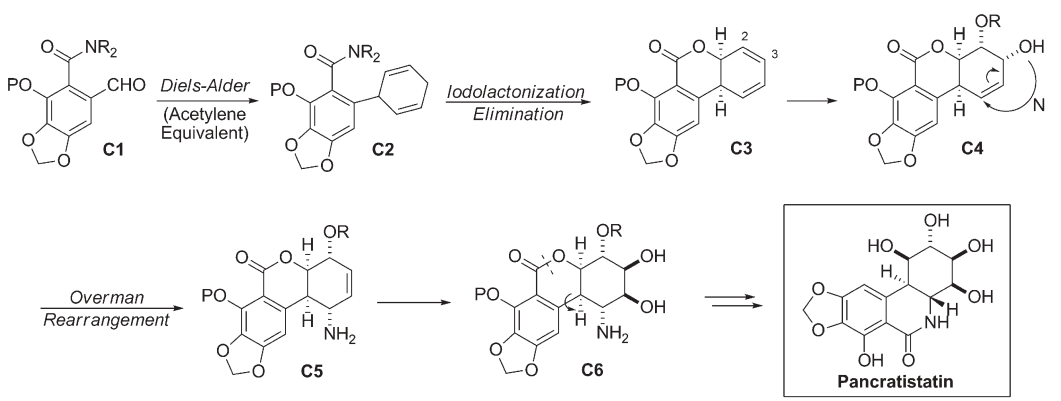


Scheme 6 Conversion of mitomycin B to leucomitomycin B.

Pancratistatin

Pancratistatin is a naturally occurring phenanthridone alkaloid, isolated by Pettit and co-workers from the root of the plant *Pancratium littorale*.[21] This compound showed promising levels of cytotoxic activity in preliminary investigations conducted at the National Cancer Institute.[22] On the basis of these reports, our laboratory undertook the total synthesis of pancratistatin. That we include our adventures in pancratistatin herein is not from the perspective of a recommended protocol. However, we hope that its value lies in demonstrating how total synthesis can be a learning platform, albeit often a painful one. Our pancratistatin effort serves as an example of how one survives even under trying circumstances.[23]

Our original synthetic plan anticipated, in retrospect naively, a concise, economically viable total synthesis of pancratistatin, commencing with aldehyde **C1** (Scheme 7). This readily accessible compound would be advanced to the stage of Diels–Alder adduct of the type **C2** which we envisioned might be converted to lactone **C3** by an iodolactonization–elimination sequence, as shown. We thought it would be a straightforward matter to install the *syn* diol functionality at C_2 and C_3. The resultant **C4** was expected to readily undergo Overman rearrangement to afford a compound of the type **C5**. The newly formed olefin of **C5** would be subjected to a second dihydroxylation to yield an intermediate (*cf*. **C6**) which would then be converted to pancratistatin *via* lactone ring-opening followed by ring closure of the acid onto the primary amine. Thus, our synthetic strategy appeared to be well formulated to allow for the efficient and stereoselective emplacement of each component of the densely functionalized core of pancratistatin. As will be seen, pancratistatin revealed itself to be a formidable target system. In practice many of the envisioned transformations turned out to be much more difficult than originally anticipated. Indeed, the completion of the pancratistatin synthesis required a number of painful



Scheme 7 Synthetic plan toward pancratistatin.

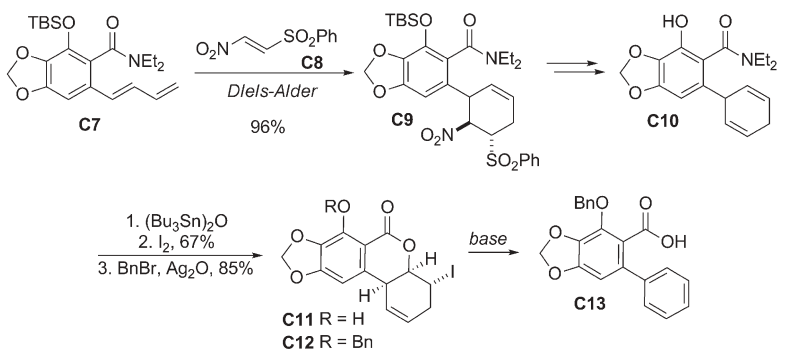
concessions at the level of conciseness and elegance. Nonetheless, obstacles were ultimately overcome and the final goal was eventually reached, albeit not in a fashion adaptable to large-scale synthesis.

Our synthesis commenced with diene **C7**, readily accessible from an aldehyde of the type **C1**. In the event, we were pleased to find that Diels–Alder reaction with dienophile **C8** proceeded in excellent yield to furnish adduct **C9**, which could be readily converted to the cyclohexyl diene substrate **C10**. At this stage, we hoped to accomplish iodolactonization followed by elimination to afford a lactone of the type **C3**. However, although the iodolactonization step proceeded without incident (**C10** to **C11**), all of our attempts to effect base-induced elimination of the iodide functionality were met with failure. Under all conditions evaluated, the dienyl system produced in the iodo elimination step rapidly underwent aromatization to provide the undesired carboxylic acid, **C13** (Scheme 8).

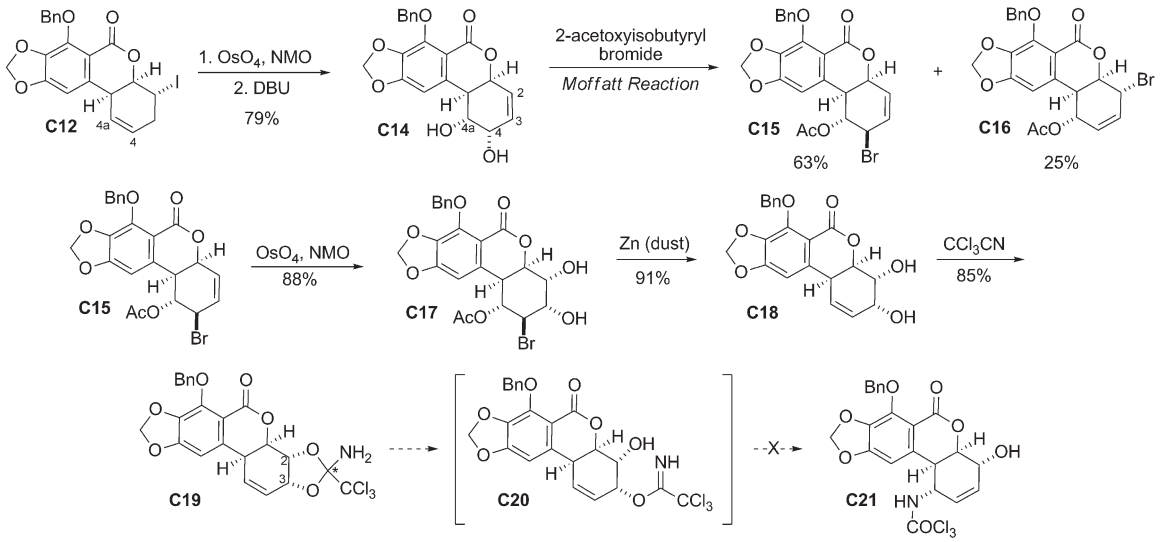
In light of these disappointing findings, we sought to protect the C_4–C_{4a} olefin temporarily such that aromatization would no longer be possible. In this context, the iodolactone **C12** was subjected to dihydroxylation and, following exposure to DBU, hydrogen iodide was eliminated from **C12** without incident to furnish the C_2–C_3 olefin of **C14**. At this stage, the goal was to be the conversion of intermediate **C14** to the Overman rearrangement precursor (*cf*. **C18**). The overall transformation (**C14** to **C18**) would require a somewhat awkward reversal of functionalities, wherein the C_2–C_3 olefin would be converted to a *syn*-diol, while the C_4–C_{4a} diol would be re-entered as an olefin. In the event, diol **C14** was subjected to Moffatt conditions, to afford the *trans*-acetoxy bromide, **C15**. Disappointingly, this reaction also afforded a 25% yield of the undesired intermediate **C16**. In any case, **C15** was treated with OsO_4 and NMO to furnish the requisite C_2–C_3 diol, as shown. Finally, treatment of the resultant intermediate, **C17**, with Zn (dust) provided only the anticipated product, **C18**, in which reductive elimination had been selective for the *trans*-bromoacetate (C_4–C_{4a}), in preference to the *trans*-bromohydrin (C_4–C_3).

With intermediate **C18** in hand at last, we were now prepared to install the imidate at C_3, in anticipation of a subsequent Overman rearrangement. In practice, treatment of **C18** with trichloroacetonitrile provided the cyclic orthoamide, **C19**, as an isomeric mixture (see asterisk), rather than the expected imidate **C20**. Nonetheless, on the basis of past precedent, we had reason to hope that, under the proper conditions, intermediate **C19** could be transiently converted to imidate **C20** *in situ*, thus rendering it a viable substrate for the Overman rearrangement. We were thus disappointed to find that the conversion of **C19** to **C21** failed to occur under any of the conditions examined. Interestingly, isomerically pure cyclic orthoamide **C19** was found to equilibrate (see asterisk) upon thermolysis in *tert*-butylbenzene, suggesting the transient viability of either the hoped-for imidate intermediate **C20** or the regioisomer in which the imidate was formed at C_2. If the **C20** intermediate was, indeed, formed, it would appear that re-formation of the cyclic orthoamide occurred at a rate that eliminated the possibility of rearrangement. If, in fact, the incorrect C_2 imidate regioisomer was solely formed in the equilibration process, then rearrangement would of course not be feasible. In any event, compound **C19** was clearly not a viable substrate for the desired Overman rearrangement (Scheme 9).

Given the proclivity of the diol of **C18** to form a cyclic orthoamide, it was clear that the C_2 hydroxyl would need to be properly masked in order to permit the formation of a C_3 imidate substrate. Thus, intermediate **C17** was advanced to compound **C22**, in which the C_2 hydroxyl was protected as a benzyl group. Exposure of the latter to NaH and trichloroacetonitrile furnished the rearrangement precursor, **C23**. In the event, under pyrolytic conditions, the long sought rearrangement did occur in 56% yield to provide the desired adduct, **C24**, as shown. We were also pleased to find that an OsO_4-mediated dihydroxylation reaction proceeded in good yield to afford the required diastereomer of **C25**. At this stage, treatment of the lactone with potassium carbonate yielded an intermediate, believed to be the amino acid **C26** which, upon exposure to DCC yielded the dibenzyl ether of pancratistatin. Reduction of the latter, as shown, afforded fully synthetic pancratistatin (Scheme 10). Happily following our inaugural total synthesis of pancratistatin, the compound has been synthesized efficiently in other laboratories. The pancratistatin problem stands as a reminder to the synthetic chemist of the flexibility and retrofitting that are often required when one's best-laid plans come face to face with the reality. The complexities associated with manipulating multifaceted and often unpredictable chemical systems should not be underestimated at the planning stage and survival strategies should always be close at hand.



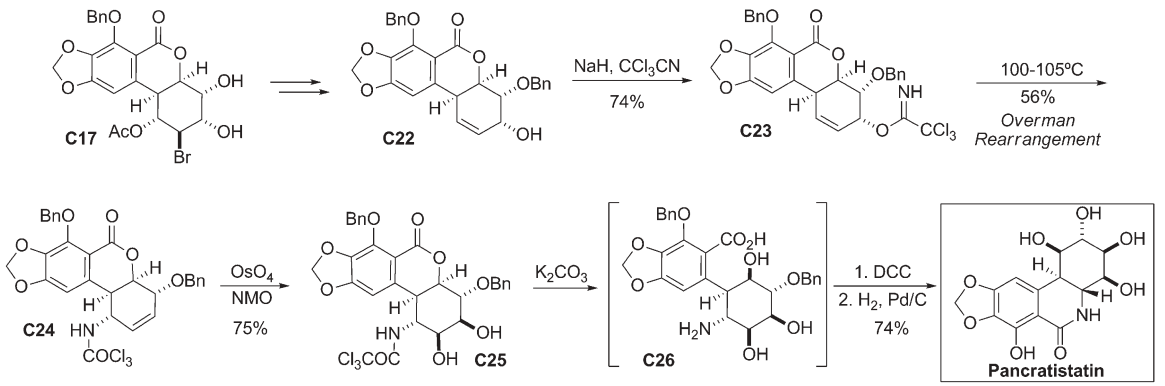
Scheme 8 Unsuccessful lactonization–elimination sequence.



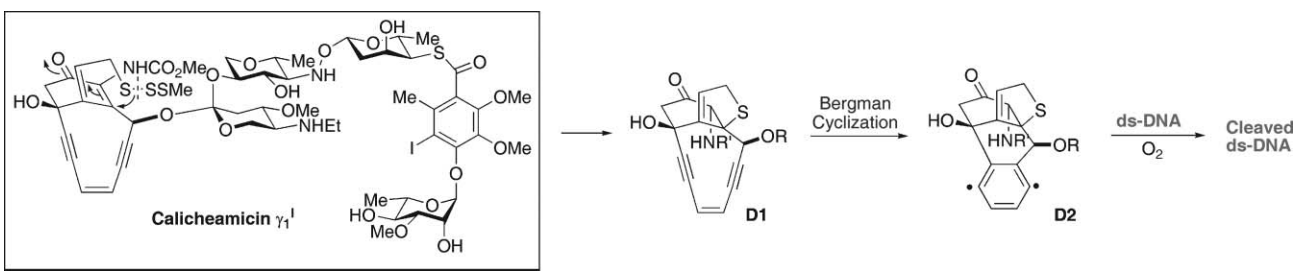
Scheme 9 Attempted Overman rearrangement of **C19**.

Calicheamicin γ_1^I

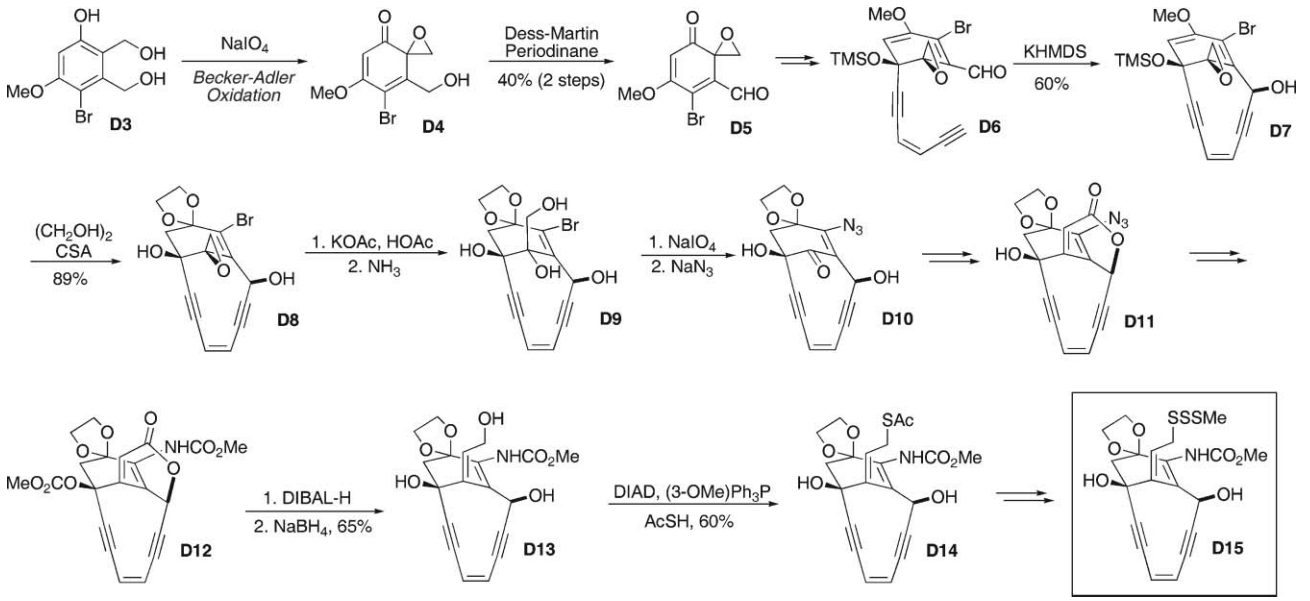
That we would launch a total synthesis effort directed at the enediyne antibiotics was clear from the moment that we had the privilege to view the fascinating architecture of their stalwart prototypes, esperamicin and calicheamicin γ_1.[24,25] The design of these SMNP structures, including an unprecedented enediyne "warhead" region, a safety catch trisulfide region to prevent premature misfiring of the warhead, and a brilliantly chiseled oligosaccharide/aromatic guiding domain, demonstrate the creativity of the universe of natural products. Following suitable reductive cleavage of the polysulfide and formation of a dihydrothiophene anchor, the enediyne moiety reorganizes itself following the dicta of Bergman cyclization, to produce **D2** from **D1** (Scheme 11). Diradicaloid **D2** can be looked upon as a form of "radiation" through organic chemistry. Moreover, the total synthesis problem gained in currency due to the remarkable sequence specificity through which the 1,4-diyl cleaves double stranded DNA. The potency of these drugs seemed to put them in a class by themselves. The cleavage site selectivity accrues from the recognition of the oligosaccharide domain with specific DNA substructural patterns. In light of its unique structure and biological mode of action, it is not surprising that a number of groups launched



Scheme 10 Completion of the inaugural total synthesis of pancratistatin.



Scheme 11 Mechanism of action of calicheamicin γ_1^I.



Scheme 12 Synthesis of calicheamicinone (**D15**).

efforts directed at the synthesis of calicheamicin γ_1^I. The first total synthesis was accomplished by Nicolaou and colleagues in 1992.[26]

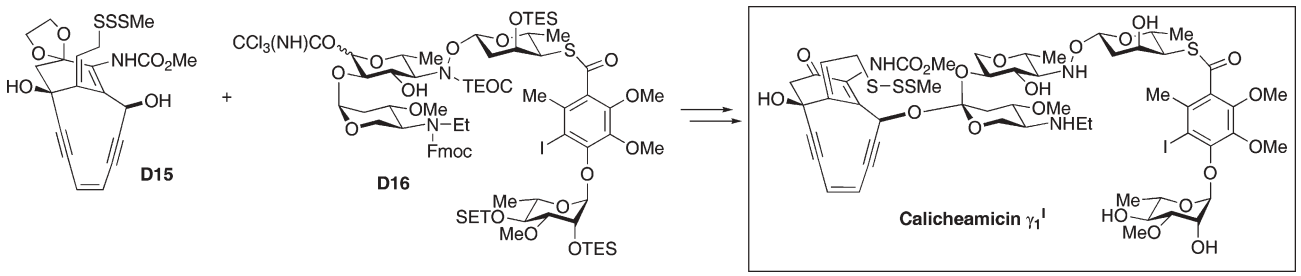
Our first subgoal was the total synthesis of the aglycon of calicheamicin, which we termed calicheamicinone (Scheme 12).[27] The total synthesis commenced with triol **D3**. A key feature of our plan was the use of a spiroepoxide linkage, generated through a Becker–Adler oxidation, to, in effect, protect a vicinal dicarbonyl substructure. Oxidation of the Becker–Adler intermediate generated aldehyde **D5**. In what was certainly one of the concise steps of the synthesis, compound **D5** was subjected to attack by the anion of diethynylethylene. It was anticipated and found that the acetylide anion preferentially attacked the keto function with high stereocontrol, because the otherwise more reactive aldehyde group had been converted to its carbonyl amide intermediate through the action of the secondary amide. Following silylation of the tertiary alcohol, compound **D6** was in hand. The step which created the calcheamicinone skeleton by chemical synthesis involved conversion of **D6** to its acetylide anion, which attacked the aldehyde with surprisingly high stereoselectivity, producing **D7**. With the calicheamicinone skeleton in hand, and in fully functionalized form, the synthesis continued with interchange of the enol ether function to a ketal (see **D8**). The epoxy group was ultimately converted to a diol (**D9**) which in turn was cleaved to generate the keto function. Azidolysis of the vinylogous acid bromide generated **D10**, and subsequently the unsaturated urethane function shown in **D12**. Following intramolecular Horner–Emmons closure, the critical allylic alcohol group was introduced (**D13**). Construction of the trisulfide was accomplished to complete the synthesis of the calicheamicinone ketal, **D15**.

In work which is not reviewed herein,[28] we were able to assemble the entire arylpentasaccharide domain and, in a fateful and maximally convergent glycosylation, the coupling of **D15** and **D16** was accomplished, leading shortly thereafter to fully synthetic calicheamicin (Scheme 13).[29]

Dynemicin A

About at the time that we were involved in the terminating phases of the calicheamicin effort, we noted in the literature a new enediyne antibiotic, dynemicin A.[30] Again, it was clear from the moment we saw this compound that an engagement directed to its total synthesis would be inevitable. Indeed, it was necessary to direct a significant effort to the dynemicin goal.[31,25] In 1995, Myers and associates disclosed the first total synthesis of dynemicin A.[32]

In devising our route to dynemicin A, we thought that it would be inappropriate to attempt to force the calicheamicin strategy onto this new target, simply because there were certain



Scheme 13 Completion of the total synthesis of calicheamicin.

common characteristics (particularly, the enediyne). Rather, to us, the striking differences between dynemicin and calicheamicin suggested that a new approach to the problem would be appropriate in the light of the challenging set of new structural issues to be solved. In the planning exercise, we focused on the presence of the oxirane at the fusion of the A,B-ring system. It is the presence of this oxirane which enables the survival of the enediyne linkage, without suffering cyclization and 1,4-diyl formation. In gross terms, we aimed at generating two components. The first would be the homophthalic anhydride, **E1**, which would be a cycloaddition coupling partner with the other component, **E2**. The latter would contain the serious part of the dynemicin structure. Somehow these structures would be merged to produce dynemicin. An interesting route was proposed and reduced to experimental reality for reaching this highly complex subunit **E2**. Our hope was that **E2** was to serve as the dienophile in an overall Diels–Alder type construction, using **E1** as the diene equivalent (Scheme 14).

Most pleasing to us was the use of a perhaps non-obvious intramolecular Diels–Alder reaction to advance upon **E13** (Scheme 15). The reader will note that strong preference for *endo* addition in the IMDA reaction of the readily available **E3** leads to **E4**. Oxidative cleavage of the secondary benzyl ether generates a primary alcohol which traps the *cis* disposed aldehyde as its hemiacetal (see **E6**). Following ammoniolysis, **E6** is smoothly converted, giving rise to **E7**, wherein the remote stereochemical relationship governing C_4 and C_7 is under tight control. The reader will also take note that a rare benzophenone acetal was used to promote β-face attack by the TIPS acetylide, a key nucleophilic alkylation step (**E8** to **E9**). The latter was advanced to the epoxy bis-iodoacetylide **E10** and, following bis-Sonogashira interpolative cross coupling, gave **E11** and then **E12**. Oxidative de-aromatization converts **E12** to **E13**. Anionic-like Diels–Alder reaction of **E13** and **E14** set the stage for conversion to dynemicin itself.

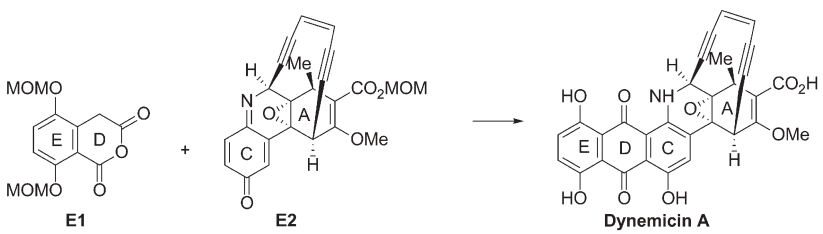
Taxol

Isolated by Wall and coworkers in 1964, from the bark of *Taxus brevifolia*,[33] taxol drew a considerable amount of attention from the oncology community, arising from early reports of potent cytotoxicity against a range of cancer cell lines.[34] As is not always the case, these promising *in vitro* findings were mirrored in extensive preclinical investigations against tumors embedded in xenografts. From these studies, taxol was advanced to human clinical trials. It has been approved for the treatment of a number of cancer types, and stands today as one of the most widely prescribed antitumor drugs in use. In addition to its remarkable antitumor properties, taxol caught the fancy of the synthetic chemistry community, since its complex, densely functionalized core structure posed an almost irresistible challenge to many practitioners of synthesis. In 1994, Nicolaou[35] and Holton[36] independently and virtually concurrently published the first total syntheses of taxol by totally different routes.

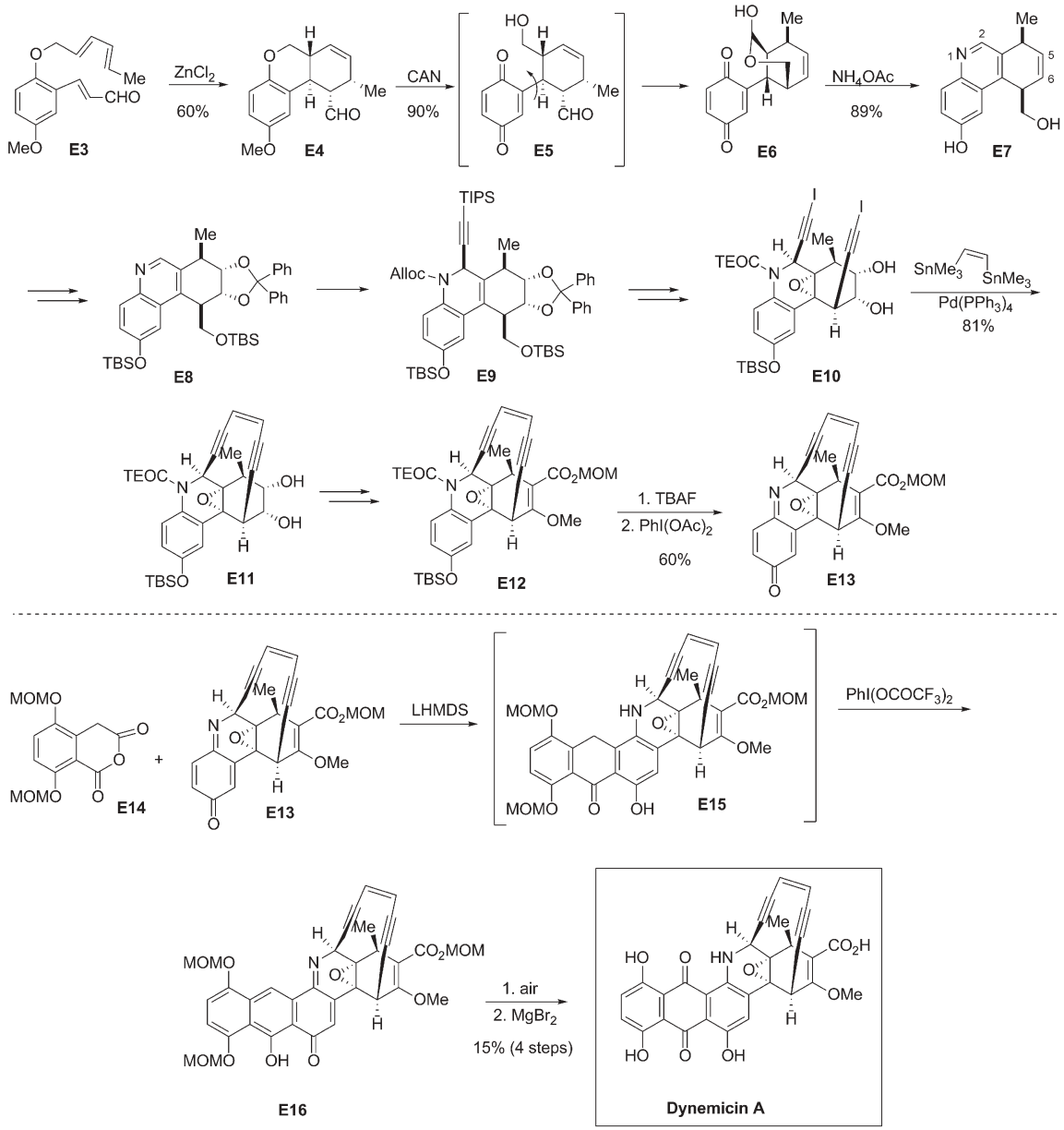
The highly complex structure of taxol is such that *de novo* total synthesis was never, to our thinking, a reasonable possibility for the manufacture of taxol. In practice, the drug is obtained through semisynthesis of baccatin III, a direct biological precursor, which is isolable in large quantities from renewable regions of a number of plants. However, although the goal of accomplishing the total synthesis of taxol remained primarily an academic-level pursuit, we anticipated that chemical synthesis could, at least in principle, serve the unique purpose of providing access to appropriate quantities of chemically modified analogs which might prove of interest. Of course, if "diverted total synthesis" is one of the goals of a synthetic program, the route should be designed accordingly. For instance, any likely pharmacophores may well be installed at an early stage of the synthesis, to allow maximal freedom to divert the route toward structurally simplified analogs. In the context of the taxol effort, it had been supposed that the oxetane functionality might play a critical role in its biological activity. Accordingly, with the added incentive of analog synthesis in mind, we sought to implement a synthetic strategy wherein the oxetane would be installed at an early stage. At the outset of our investigations, it remained to be seen as to whether the presence of this rather sensitive functionality would prove problematic over the course of the chemical synthesis.[37]

We further hoped to accomplish the total synthesis of taxol in an enantioselective fashion, in order to gain access to the required antipode known as taxol. In planning the project, we discerned a connection, if somewhat far-fetched, between taxol and the more trodden chemical space of steroids, as well as terpenoids. In this regard, we viewed the (*S*)-Wieland–Miescher ketone (**F1**), itself readily obtained through an L-proline-catalyzed asymmetric aldol condensation, as a promising starting point for our taxol-inspired synthetic endeavors. A strategy presented itself, wherein the putative A and C rings would be joined through a vinyllithium addition to an aldehyde (Scheme 16, see formation of **F5**). We further envisioned that the otherwise daunting B-ring might then be fashioned through an intramolecular Heck reaction.

In the event, enantiomerically pure **F1** was advanced to become the key coupling precursor, aldehyde **F2**. The latter was treated with vinyllithium **F4**, itself derived from vinyl



Scheme 14 Synthetic strategy toward dynemicin A.

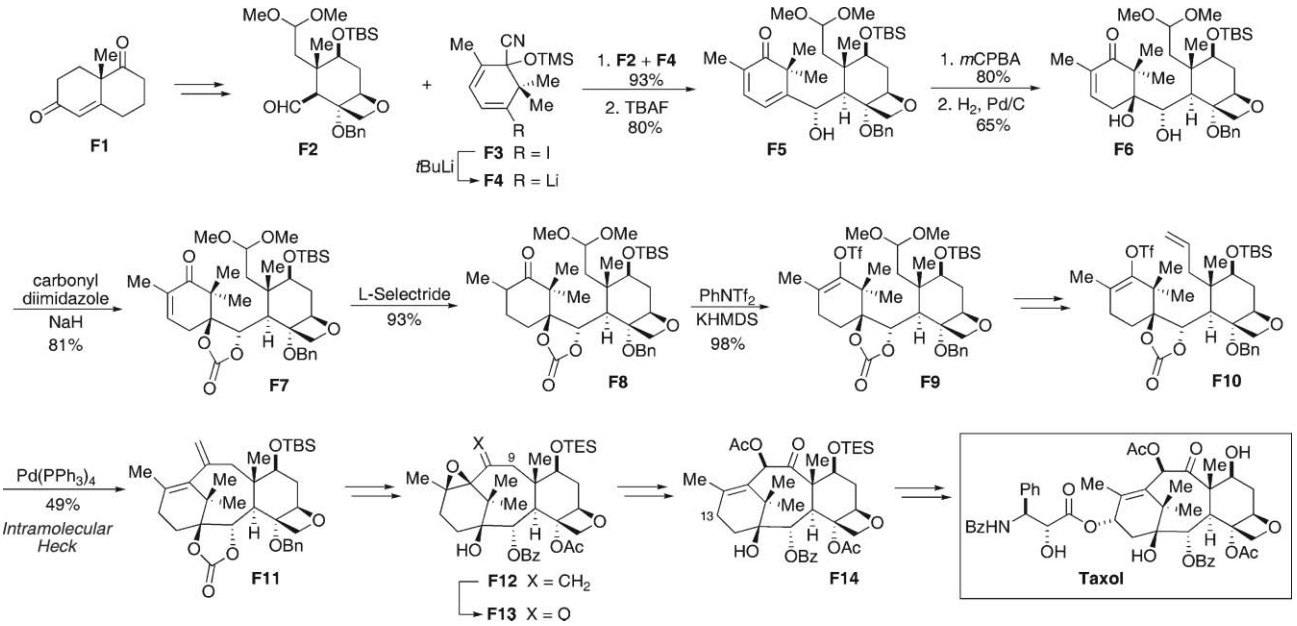


Scheme 15 Synthesis of dynemicin A.

iodide **F3**, to afford, following ketone deprotection, the adduct **F5**. Directed epoxidation, followed by reduction, provided the diol **F6**, which was readily protected as the cyclic carbonate, **F7**, as shown. Finally, conjugate reduction furnished the saturated ketone, **F8**. In light of the considerable steric congestion inherent in intermediate **F8**, we were pleased to find that vinyl triflate formation proceeded smoothly and in excellent yield. Following conversion of the dimethyl acetal to a terminal olefin, the key intermediate, **F10**, was in hand. The stage was now set for us to attempt the critical Heck ring closure. Gratifyingly, the intramolecular Heck reaction proceeded, as hoped, even on this highly functionalized intermediate, to furnish the tetracyclic adduct, **F11**, as shown.

At this stage, it would be necessary to convert the exocyclic olefin to a ketone functionality. In practice, this "mopping up" phase proved surprisingly difficult. Standard oxidizing reagents, such as ozone and osmium tetroxide, preferentially reacted at the tetrasubstituted olefin. Consequently, we resorted to a sequence wherein we would temporarily mask the problematic internal olefin. On the basis of model studies, we were aware that removal of the TBS group would prove troublesome in the final stages of the synthesis. Thus, following the replacement of the TBS functionality with the more labile TES group, the compound was exposed to *m*CPBA and selectively underwent epoxidation at the tetrasubstituted olefin, albeit in only 45% yield.

Following a series of protecting group manipulations, compound **F12** was in hand. At this stage, upon exposure of the exocyclic methylene group to the action of osmium tetroxide followed by subsequent cleavage of the diol with lead tetraacetate, **F12** was successfully converted to the ketone **F13**, as shown. Having at last achieved the selective



Scheme 16 Synthesis of taxol.

functionalization of the Heck reaction derived exocyclic olefin, we were able to unmask the tetracyclic olefin through exposure of **F13** to SmI_2. Following the C_9 oxidation protocol developed by both Holton and Nicolaou, we ultimately gained access to intermediate **F14**. Allylic oxidation followed by sodium borohydride reduction allowed for the stereoselective emplacement of the C_{13} hydroxyl function. Finally, following the precedent of Ojima and coworkers,[38] the C_{13} side chain was installed, to provide fully synthetic, optically active taxol.

As matters had transpired, the gross outline of our original plan had been reduced to practice and the total synthesis of nature's enantiomer of taxol had been accomplished for the first time without recourse to a protocol of degradation and reconstitution (relay synthesis). Nonetheless, the complexity of the chemistry was such that, in the end, our fond hopes of finding new and promising taxoids through total synthesis were frustrated.

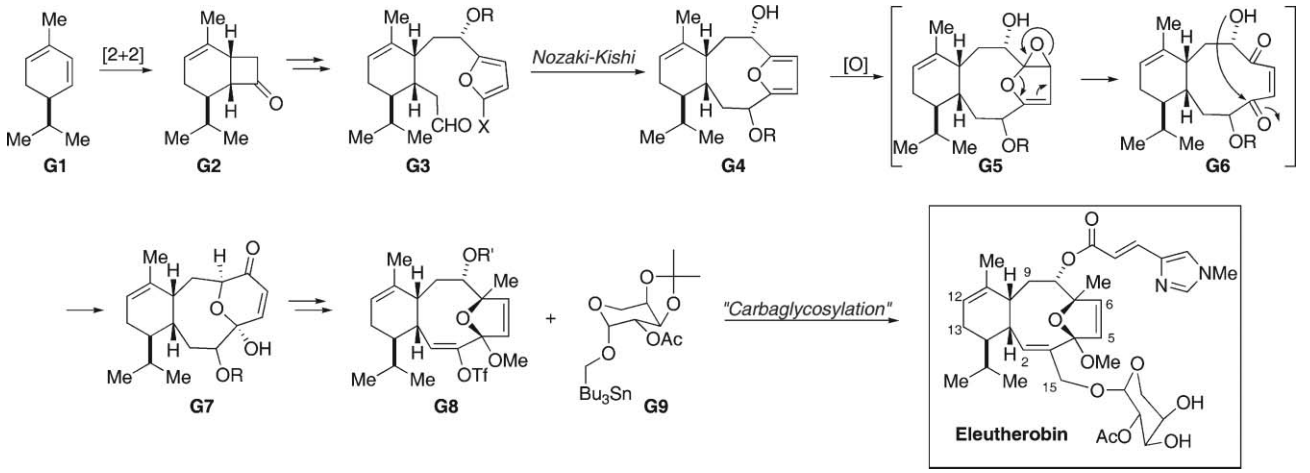
Eleutherobin

At approximately the time that the taxol total synthesis/analog program was winding down, there appeared a fascinating natural product, eleutherobin, characterized by Fenical and associates,[39] following isolation of the agent from a marine soft coral *Eleutherobia*, from the coast of western Australia. Fenical showed that the *in vitro* cytotoxicity properties of eleutherobin were quite comparable with those of taxol. On the basis of tubulin binding studies, it seemed likely that this new agent shared in the mechanism of action of taxol. The first total synthesis of eleutherobin was disclosed by Nicolaou *et al.* in 1997.[40]

Fresh from the perspective of the bittersweet experience in our taxol synthesis project, we viewed eleutherobin as an opportunity to reach a tubulin-directed, naturally occurring anticancer agent, by total synthesis through some interesting organic chemistry. It was also our hope that a synthetic route directed toward eleutherobin might also be productive in allowing us to explore chemical space which was not available from the natural product itself.[41]

A concept which fascinated us in a projected thrust to reach eleutherobin is shown in Scheme 17, in broad terms. The starting point was to be (*S*)-α-phellandrene (**G1**). It would provide the absolute configuration at its isopropyl-bearing carbon to reach nature's enantiomeric version of eleutherobin, *i.e.* (*S*) at C_{14}. In the broadest of terms, the starting material **G1** would be advanced to **G3** by a sequence of steps involving a [2 + 2] cycloaddition reaction followed by an appropriately managed fragmentation. A decisive step in converging on eleutherobin was seen to be a Nozaki–Kishi type closure, in the course of bringing us from **G3** to **G4**. Through nucleophilic alkylation, a methyl group would be introduced at the carbonyl carbon, destined to become C_7. Thus, following appropriate manipulations, we hoped to advance **G4** to the level of **G8**. At this point, an exciting possibility presented itself for attachment of the arabinose derived pentose sector (**G9**) to the eleutherobin core structure (**G8**) (*vide infra*).

In practice, dichloroketene served well as a [2 + 2] cycloaddition agent, enabling us to advance from **G1** to **G2**. Activation of the methylene group of the cyclobutenone, through the equivalent of formylation, led in time to **G10**. As anticipated, the aldehyde of **G10** served as a substrate for accepting the 2-lithio-5-bromofuran nucleophile. **G11**, thus produced, was subjected to a carbon degradation, and adjustment of oxidation level, thus reaching **G12**. Happily, this compound did serve as a substrate for a Nozaki–Kishi ring closure, allowing us to reach **G13** with high stereoselectivity. Cleavage of the silyl ether, followed by epoxidation of the furan double bond, and subsequent rearrangement, brought us to compound **G15**. The latter was advanced to **G18** as shown, which was converted to the eleutherobin aglycon as its vinyl triflate (see **G19**, Scheme 18).



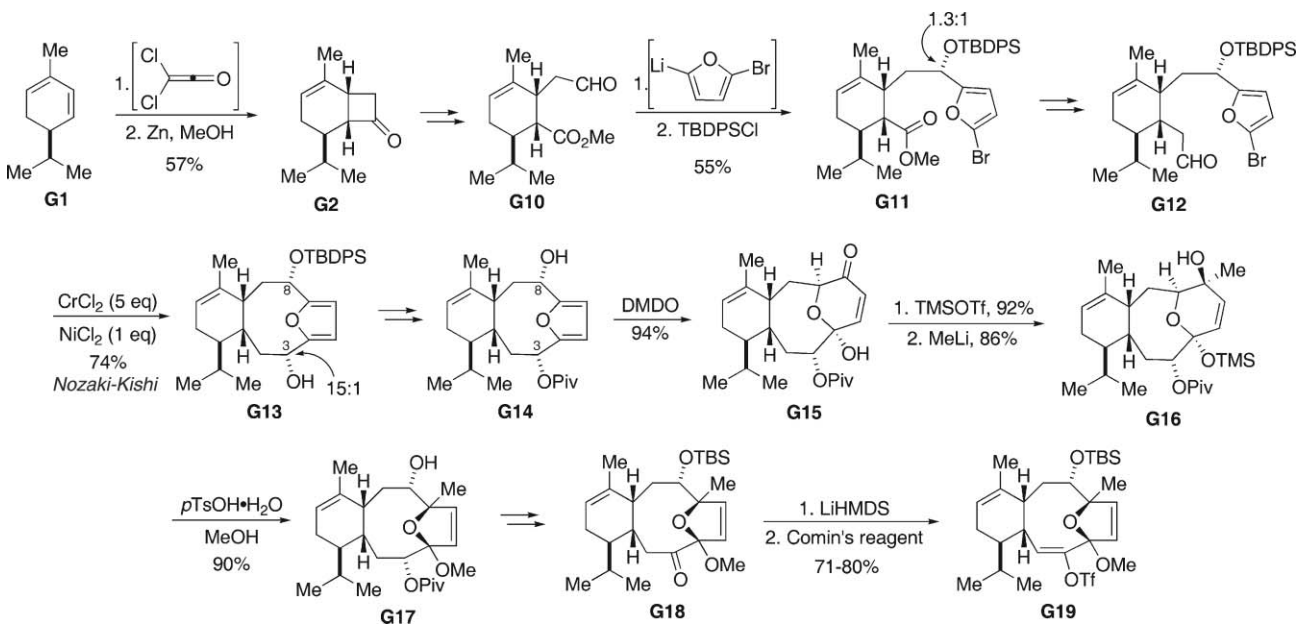
Scheme 17 Synthetic plan.

In the most venturesome step of a generally high-risk synthesis, the glycoside **G21** was established through a formal "sp^3 Stille-type" reaction, leading to the furanosidyl methyl derivative. Coupling of **G19** and **G21** produced **G22**, thus enabling conversion to eleutherobin as shown in Scheme 19.

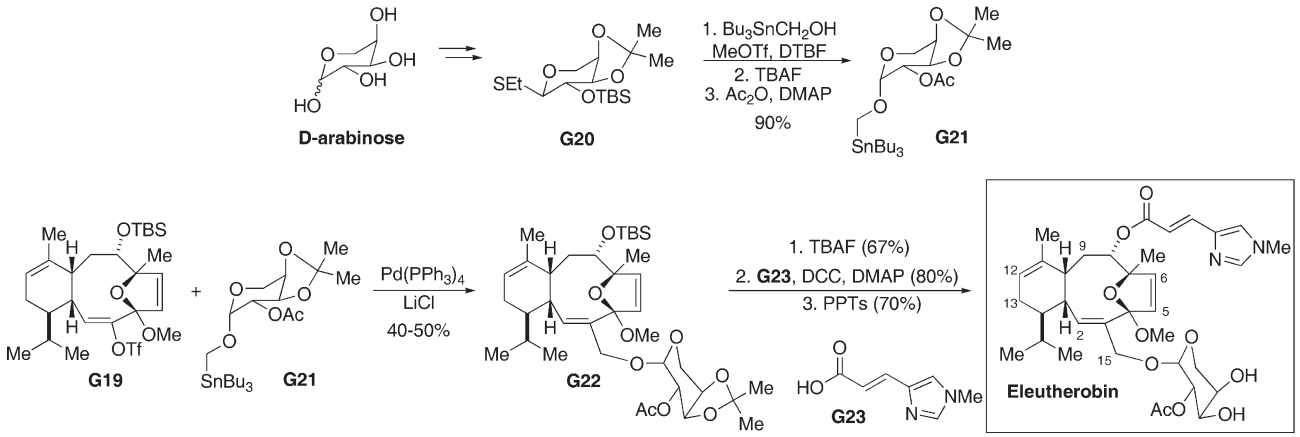
Since eleutherobin was present from natural sources to only a very sparing extent, we optimized the synthesis, and built up close to half of a gram of fully synthetic drug. The compound was then evaluated in *in vivo* screens using xenograft models. As it turned out, the results were extremely disappointing. It was opined that ester cleavage was apparently a very important liability in realizing the biological activity of eleutherobin in an *in vivo* setting. It seemed that the urocanic acid moiety of eleutherobin was critical for its cytotoxic activity. Yet, the agent apparently seemed quite vulnerable to the action of esterases. To deal with this problem would have required a radical modification of eleutherobin; *i.e.* replacement of the ester of the linkage to the urocanic moiety—no small undertaking. Given the emergence of still another naturally occurring family of tubulin directed anticancer agents, the eleutherobin project was brought to closure. It is well, however, to point out that as part of this process, the actual proof of structure of eleutherobin in terms of the connectivity of the arabinose sector with the terpenoid-like domain was rigorously established for the first time.

Epothilones

In addition to taxol and eleutherobin, discussed earlier, another tubulin-directed family of cytotoxic agents, the epothilones, engaged our attention shortly before the end of the century. Isolated from myxobacteria of the genus *Sorangium*,[42] the epothilones initially garnered serious attention in that they appear to retain their cytotoxicity against multidrug resistant (MDR) cell lines.[43] This unique attribute seemed particularly noteworthy in light of the fact that many



Scheme 18 Synthesis of carbaglycosyl acceptor, **G19**.



Scheme 19 Synthesis of eleutherobin.

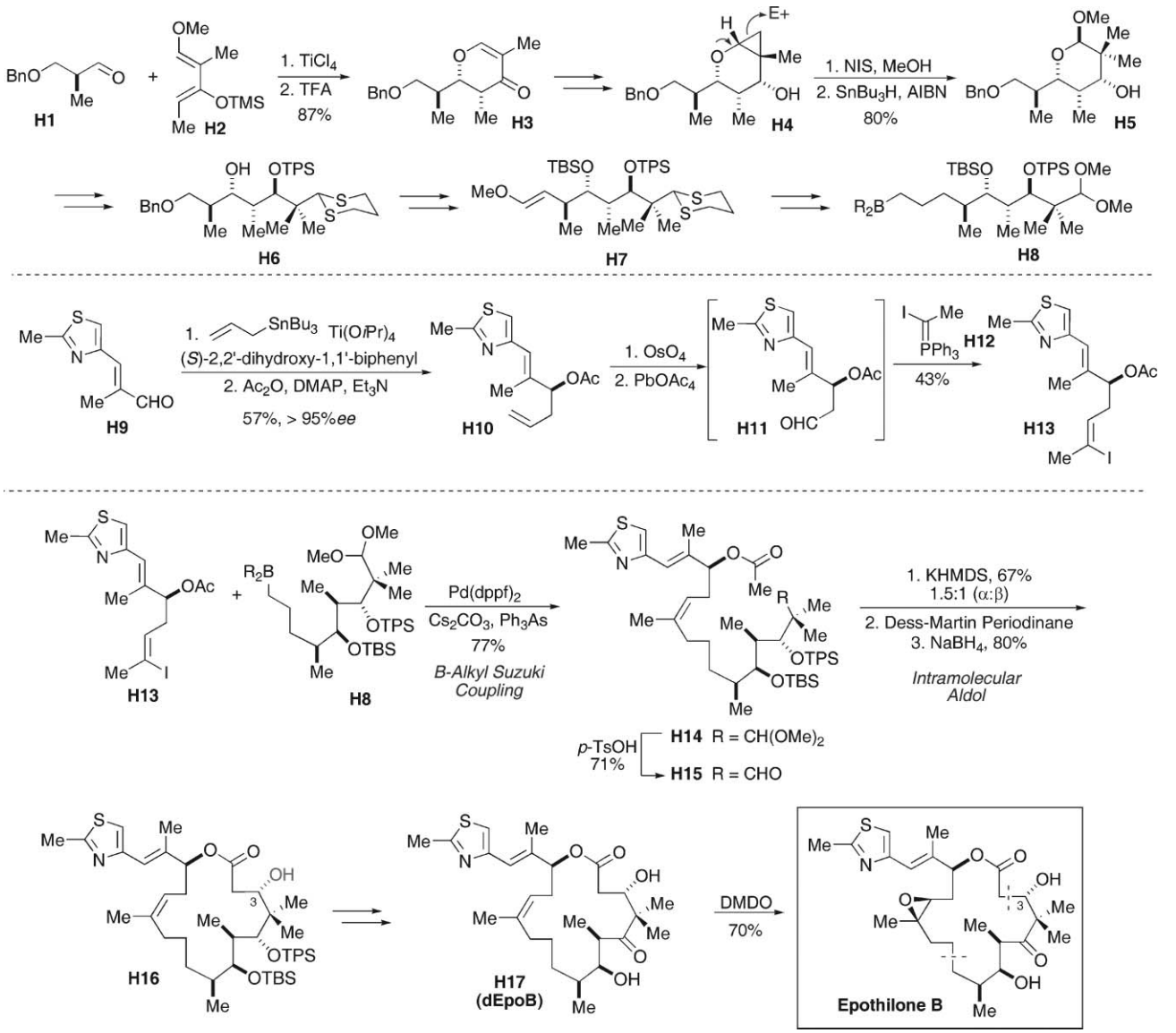
commonly administered anticancer drugs, including taxol, vinblastine, and adriamycin, are susceptible to the disabling onset of multiple drug resistance in clinical settings, thereby compromising their efficacy in the long-term treatment of various cancers. The exciting reports of Bollag *et al*. prompted a number of groups, including our own, to explore synthetic routes to these natural products. Having gained access to meaningful quantities of synthetic material, again we hoped to explore the biological activity of the natural products, and congeners thereof, in more sophisticated *in vivo* models.

In 1996, we disclosed the inaugural total synthesis of epothilone A (EpoA)[44] and, the following year, we completed the total synthesis of epothilone B (EpoB).[45] The latter had been reported to possess superior *in vitro* activity in comparison to EpoA. Our initial forays in total synthesis paved the way for critical investigations aimed at discerning the biological properties of the epothilones, with the assistance of a number of critically enabling collaborative efforts. Indeed, we are currently engaged in an ongoing and fruitful program, which is devoted to reaching novel epothilones through total synthesis. In the past decade since the disclosure of the first synthesis of EpoA, we have developed a number of improved routes which provide ready access to analogs for these ongoing investigations. Without retelling the details of the progression which may be found elsewhere,[46] it is appropriate to note that our epothilone endeavor has, thus far, yielded three particularly promising drug candidates. dEpoB has been in Phase II clinical trials against breast cancer, while 9,10-dehydro-dEpoB is now being evaluated in Phase II settings against breast cancer. Most recently, the fludelones, remarkable variants of the epothilones, are highly promising compounds which are currently advancing through preclinical evaluations.[47] Herein, we confine ourselves to a description of our first-generation synthesis of epothilone B (EpoB), which, from the standpoint of general synthetic logic (as opposed to practicality) was the most interesting. It was our intention to implement a convergent route to EpoB, wherein two relatively complex fragments would be merged at a late stage of the synthesis. Along these lines, we identified two seemingly logical points of disconnection (see EpoB structure, Scheme 20), which would lead us back to fragments of the type **H8** and **H13**. It was hoped that these intermediates would first be joined through a B-alkyl Suzuki coupling, then a rather bold idea, in order to provide an intermediate that would be amenable to intramolecular aldol ring-closing reaction. The rationale for this venturesome idea involved exploiting the non-enolizability of the aldehyde in providing access to the required ester enolate.

In the event, the synthesis of the polypropionate sector (**H8**) commenced with Lewis acid-catalyzed Diels–Alder cyclocondensation between the virtually enantiopure aldehyde **H1** and diene **H2**. In keeping with precedent from our earlier work, the resident C_2 stereocenter on **H1** provided facial control during the cycloaddition, and would ultimately enable access to **H8** with enantiomeric definition. Cycloadduct **H3** was advanced to the cyclopropyl glycoside **H4**. Although we were never able to achieve a direct, non-oxidative cyclopropyl ring opening (E = H), we eventually did develop a viable two-step sequence, allowing for installation of the required *gem*-dimethyl functionality in good overall yield. Thus, NIS-mediated oxidative cyclopropane ring opening, conducted in methanol, yielded an intermediate iodomethyl compound which, upon exposure to tributyltin hydride, was reduced to **H5**. Protection of the secondary alcohol, followed by cleavage of the glycosidic bond, afforded the dithiane intermediate **H6**. The latter was advanced to the stage of the alkyl borane coupling partner, **H8**, *via* the intermediate enol ether, **H7**, in a straightforward manner. Thus, our chemistry in the eighties, directed to the Lewis-acid catalyzed Diels–Alder cyclocondensation (LAC-DAC) reaction, had served us well in this setting.

Our next challenge was that of synthesizing, in enantioenriched form, the *Z*-vinyl iodide, **H13**, with geometric control about the trisubstituted olefin. The sequence commenced with a catalytic, asymmetric addition of allyl nucleophile to aldehyde **H9**. The reaction proceeded with >95% ee to provide, following acetylation, intermediate **H10**. Selective dihydroxylation of the terminal olefin, and subsequent oxidative cleavage of the resulting glycol, furnished the unstable aldehyde **H11**. We were pleased to find that this compound smoothly underwent Wittig reaction with phosphorane **H12** to provide the requisite coupling partner, vinyl iodide **H13** as only the *Z*-isomer.

With the fully functionalized fragments in hand, we were prepared to attempt the crucial B-alkyl Suzuki coupling. We note that, at the time of these investigations, the success of this



Scheme 20 Inaugural total synthesis of epothilone B (EpoB).

type of coupling reaction was far from assured. In searching the literature, we could not find precedent either for the intermolecular coupling of a B-alkyl (as opposed to B-alkenyl) fragment of the complexity level of **H12** for coupling with a vinyl iodide such as **H13**, in which the olefin is not part of a β-iodoenoate system. Thus, it was quite gratifying to find that, in the presence of an appropriate palladium source, B-alkyl Suzuki coupling of **H8** and **H13** proceeded smoothly to produce the geometrically pure *Z*-olefinic adduct **H14** in 77% yield.

Hydrolysis of the acetal linkage provided aldehyde **H15**, which, upon exposure to base (KHMDS), indeed underwent intramolecular aldol cyclization and gave rise to the macrolactone as a 1.5 : 1 (α : β) mixture of alcohol isomers. Fortunately, this disappointing selectivity was readily remedied through recourse to a simple oxidation–reduction sequence, which provided **H16** with high stereoselection favoring formation of C_3 alcohol exclusively in the desired *S*-configuration. This intermediate was advanced to 12,13-desoxy-epothilone B (termed dEpoB) **H17**, itself a cytotoxic agent which has shown promise in clinical settings. Upon exposure to dimethyldioxirane (DMDO), **H17** readily underwent regio- and stereoselective epoxidation at the C_{12}–C_{13} olefin to afford the natural product epothilone B (EpoB), as shown. Elsewhere, we have described the combined chemical synthesis, medicinal chemistry, and pharmacology program which ensued from the synthesis shown in Scheme 20.[46]

Radicicols

Originally isolated from *Monocillium bonorden* in 1953,[48] radicicol recently had come to our attention on the basis of reports of its high binding affinity and inhibition of the heat shock protein 90 (Hsp90) molecular chaperone.[49] Hsp90 mediates the folding of a number of oncogenic proteins (such as Raf1 and Her2). Thus, inhibition of this protein could be of value in the treatment of cancer. Our own interest in small molecule Hsp90 inhibitors had commenced with our efforts

toward the natural product geldanamycin, which is known to bind to Hsp90 at levels as low as 1.2 μM. We considered radicicol—which binds to Hsp90 at only 20 nM, but lacks the potential liability (from a drug perspective) of the quinone moiety (which could be a source of free-radical induced toxicity)—to be an excellent candidate for total synthesis and rigorous biological investigation.

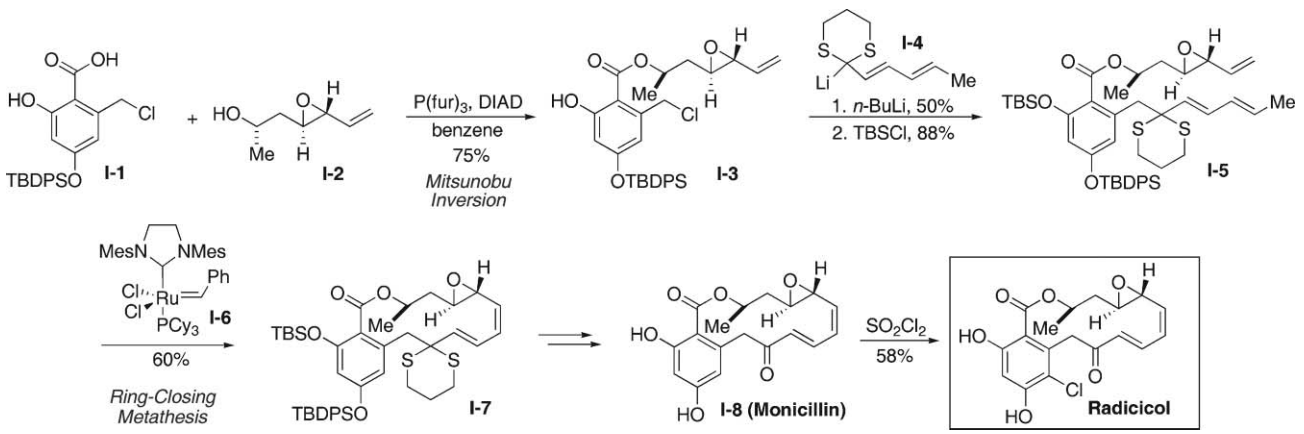
We thus sought to develop a route to fully synthetic, optically active radicicol.[50] An optimally convergent synthesis was envisioned to entail the merger of three fragments: a functionalized benzoic acid (**I-1**), an enantiomerically defined epoxy alcohol (**I-2**) and a dienyl system possessing a masked ketone functionality (**I-4**). In the event, each of these subunits was prepared without incident. The first two fragments, **I-1** and **I-2** were joined through a Mitsunobu inversion to provide **I-3** in 75% yield, as shown. We note that the successful implementation of this coupling strategy had required us to identify reaction conditions that would suppress formation of the phthalamide of **I-1**. In the end, formation of the undesired phthalamide side product was completely eliminated through the judicious use of a relatively non-nucleophilic phosphine source [$P(fur)_3$] and a non-polar solvent (benzene). Next, lithium dithiane (**I-4**) addition to the benzylic chloride proceeded, albeit in a somewhat disappointing 50% yield and, following protection of the aromatic alcohol, intermediate **I-5** was in hand. Macrocycle formation was achieved through a ring-closing metathesis reaction, which made use of the Grubbs' catalyst (**I-6**), providing intermediate **I-7** in 60% yield. Following Pummerer-like removal of the dithiane and cleavage of the silyl protecting groups, the natural product monocillin I (**I-8**) was in hand. This resorcylic macrolide is also isolated from species of the *Monocillium* genus, and is reported to exhibit a number of antifungal and antibiotic properties. Finally, chlorination of **I-8** proceeded with the desired regioselectivity, to provide optically active radicicol, as shown (Scheme 21).

With fully synthetic radicicol in hand, we were able to confirm its remarkable inhibitory activity against the Hsp90 chaperone. However, biological evaluations revealed radicicol to be ineffective in *in vivo* animal settings. We suspected that this failure might be attributable to induced instability *in vivo*, as well as nonspecific cytotoxicity arising from its epoxide functionality. We were concerned that, as in the epothilones, the presence of an epoxy linkage could prohibitively limit the exploitable therapeutic advantage. The presence of the dienyl epoxide also raised concerns to us regarding shelf stability and pharmacostability of the drug.

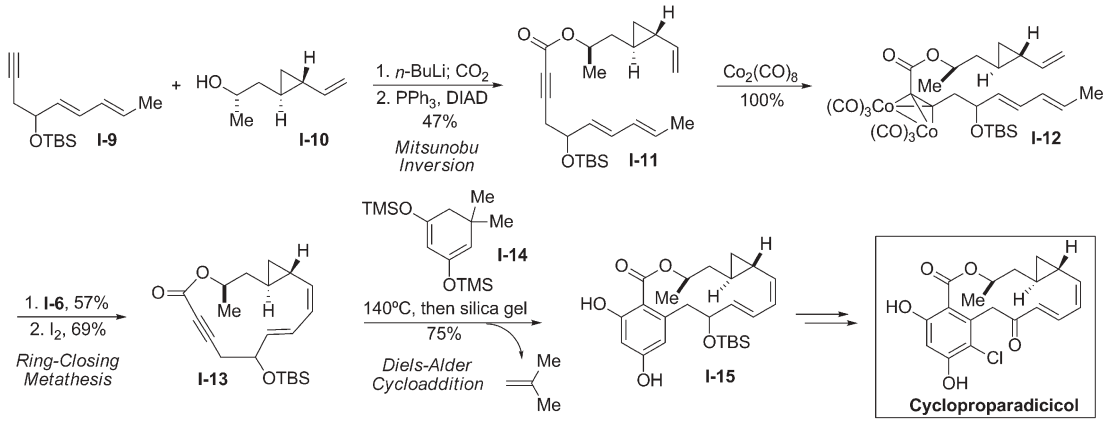
With these considerations in mind, we sought to design an analog that would retain the potency of radicicol, while alleviating some of the potential *in vivo* complications of an epoxide. We elected to exchange the epoxide moiety for a cyclopropyl group through diverted total synthesis, and thus set as our target a compound that we term, perhaps loosely, as "cycloproparadicicol."

Our first-generation synthesis of cycloproparadicicol borrowed heavily from the general strategy which worked in the synthesis of radicicol.[51] However, despite the convergence of the route, we noted several deficiencies in the synthesis, mainly in the yields of the dithiane addition and olefin metathesis steps. Our modified second-generation synthetic route, which we hoped would deliver substantial quantities of fully synthetic material for extensive investigations, would make use of a novel type of Diels–Alder dienophile (*i.e.* an "ynolide") to allow for the construction of the aromatic sector of the resorcinylic system through non-conventional means (Scheme 22).[52]

In the event, the terminal alkyne of substrate **I-9** was subjected to carbonylation and, following Mitsunobu inversion of alcohol **I-10**, the alkynoate ester **I-11** was in hand. Interestingly, the ring closing metathesis of **I-11** proved problematic. A variety of metathesis conditions led only to recovery of starting material. We reasoned that perhaps the failure of the substrate to cyclize could be attributable to the constraints imposed by the linear acetylenic functionality, which could further disrupt the reaction by unproductively coordinating with the catalyst. In an effort to address these issues, we sought to mask the potentially troublesome acetylene functionality through temporary engagement as a dicobalt carbonyl complex. The hope was that a compound of the type **I-12** would be more geometrically inclined to undergo ring closing metathesis, and would furthermore not suffer from unproductive substrate-catalyst interactions. In the event, upon exposure to Grubbs' catalyst **I-6**, intermediate **I-12** readily underwent cyclization, providing the macrocyclic



Scheme 21 First generation synthesis: radicicol.



Scheme 22 Second generation synthesis: cyclopropradicicol.

adduct in 57% yield. The alkyne was reinstated upon exposure of the dicobalt complex to iodine, and intermediate **I-13** was in hand. At this stage, we were prepared to examine the viability of our plan to utilize a Diels–Alder reaction to install the aromatic sector at this late stage of the synthesis. In the event, we were pleased to find that the acetylenic moiety, generally rather unreactive as a dienophilic coupling partner, was indeed able to participate in Diels–Alder reaction with diene **I-14** to afford the aromatic resorcinylic macrolide **I-15** in a 75% yield. From this intermediate, cyclopropradicicol was prepared in two trivial steps.

This streamlined, second-generation synthesis, which took a rather non-conventional approach to the installation of the aromatic sector, has allowed for the preparation of gram quantities of synthetic cyclopropradicicol, which has been shown to bind the Hsp90 molecular chaperone at approximately 160 nM. Importantly, this modified congener of radicicol does display efficacy in animal models, thus lending some experimental encouragement to our original hypothesis regarding the liability of the dienyl epoxide moiety in *in vivo* settings. The viability of cyclopropradicicol as a lead candidate for development is currently being actively investigated.

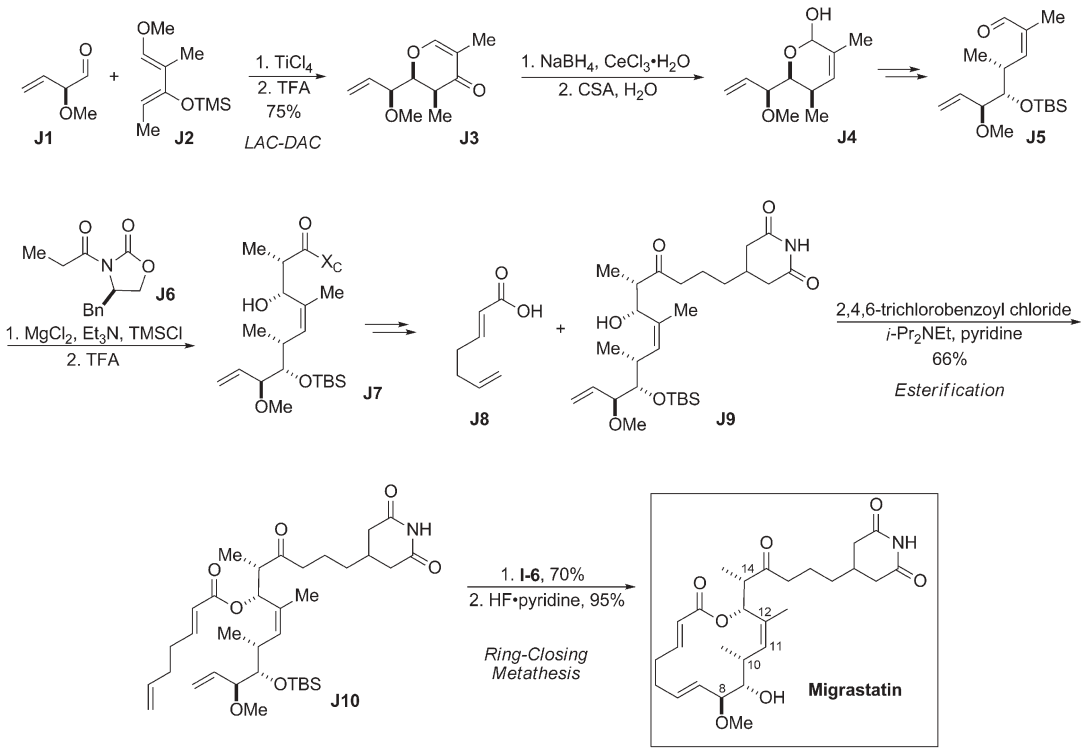
Migrastatin

Migrastatin, a macrolide isolated from two strains of *Streptomyces*, had been identified as a small molecule inhibitor of tumor cell migration.[53] Given the importance of *in vivo* cellular motility to the phenomenon of tumor metastasis, we wondered whether a cell migration inhibitor, such as migrastatin, might serve as a valuable lead chemotherapeutic agent. While migrastatin is not cytotoxic *per se*, a potent and selective inhibitor of tumor cell migration could well serve as a useful agent in preventing the spread of cancer in clinical settings.

We thus set out to design a synthetic route which would ideally provide opportunities for the facile preparation of structurally simplified congeners through diverted total synthesis, while maintaining maximal efficiency.[54] We further set the requirement that the synthesis be accomplished in an asymmetric fashion, to provide access to optically active migrastatin. In this regard, we envisioned an opening phase Lewis acid-catalyzed Diels–Alder cyclocondensation (LAC-DAC), which would provide controlled access to the future C_8, C_9, and C_{10} stereocenters of the macrolide (Scheme 23). The previously undescribed β,γ-unsaturated aldehyde, **J1**, was prepared in optically active form in three steps from commercially available dimethyl-2,3-*O*-isopropylidene-L-tartrate. Lewis acid-mediated cyclocondensation of **J1** with diene **J2**, followed by exposure to acid, provided the cycloadduct **J3** in 75% yield. It will be noted that, in addition to enabling stereocontrolled access to the three contiguous stereocenters, this reaction served to set the stage for formation of the requisite trisubstituted C_{11}–C_{12} *Z*-olefin of migrastatin. Luche reduction of the enone, followed by Ferrier rearrangement of the resultant allylic acid, furnished lactol **J4**. This intermediate was smoothly converted to the enal **J5** through a straightforward sequence involving reductive ring opening, followed by protection of the secondary alcohol and, finally, oxidation of the primary alcohol. At this stage, we hoped to install the C_{13}–C_{14} bond through an *anti*-selective aldol reaction. In the event, treatment of **J5** with propionyl oxazolidinone **J6** in the presence of $MgCl_2$ and TMSCl, provided the desired aldol product, **J7** as a single isomer. The chiral auxiliary was subsequently removed and, following installation of the glutarimide side chain through a Masamune–Roush variant of the Horner–Wadsworth–Emmons reaction with subsequent Stryker-mediated enone reduction, intermediate **J9** was in hand.

Following a number of thwarted efforts to accomplish esterification with acid **J8**, we eventually realized success through employment of a modified Yamaguchi procedure, which delivered the ring-closing metathesis precursor, **J10**, in 66% yield, as shown. In the event, macrocyclization was readily accomplished through exposure to Grubbs' metathesis catalyst, **I-6**, and, following deprotection of the C_9 alcohol, fully synthetic, optically active migrastatin was in hand.

Fully synthetic migrastatin was evaluated in a wound healing assay, and the results confirmed the initial reports of the biological activity of isolated migrastatin. Through the process of diverted total synthesis, we have subsequently prepared and evaluated a range of structurally simplified migrastatin congeners, many of which have exhibited significantly enhanced tumor cell migration inhibitory properties in comparison with the parent compound itself. Notably, these



Scheme 23 Synthesis of migrastatin.

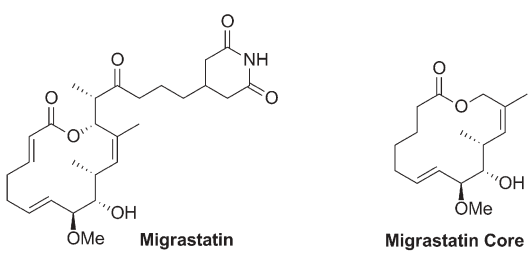
studies have revealed that the observed activity is not dependent on the presence of the glutarimide side chain. The removal of this side chain, and with it the complexity-adding stereocenters at C_{13} and C_{14}, actually leads to *increased* levels of cell migration inhibition, as determined by the wound healing assay (see migrastatin *vs*. migrastatin core, Scheme 24). These interesting findings certainly provide compelling support for the value of diverted total synthesis as a tool for identifying superior agents of potential therapeutic value. The migrastatin program is being vigorously pursued in our laboratory, as, through iterative cycles of analog synthesis and collaborative biological studies, we are expanding our understanding of the biological properties of this very promising class of molecules.

Conclusions

By reliving, if only briefly, the cases described herein, we hope to have shown, by example, how the special capacities of organic synthesis can be applied to the goal of discovering new oncolytic agents based on leads from SMNPs. These problems at once challenged the creative capacities of synthetic organic chemistry, and directed them to settings where successes could well enjoy applications to other types worthy scientific undertakings.

We believe that teachings of some value in the venerable art/science of organic synthesis can be garnered from studying the experiments discussed above. Of course, to enhance such a learning experience, one must return to the original papers and to their historiographies. These snapshots were provided as inducements to do so.

We remain hopeful and even confident that the future will bring with it increasing opportunities by which the growing power of organic synthesis and its underlying logic can be brought to bear upon intellectually fascinating causes. To us, the concept that any structure of which the mind (often prompted by the ingenuity of nature) can conceive might actually be synthesizable in a chemical laboratory, provides to us one of the most exciting callings in all of science. Needless to say, the real heroes of these vignettes were our creative colleagues who designed the most novel approaches, and fought the often lonely battles to reduce them to experimental reality. It is to them that we dedicate these recollections.



Scheme 24 Migrastatin core.

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