Drug Discovery

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On the Reach of Chemical Synthesis: Creation of a Mini-Pipeline from an Academic Laboratory

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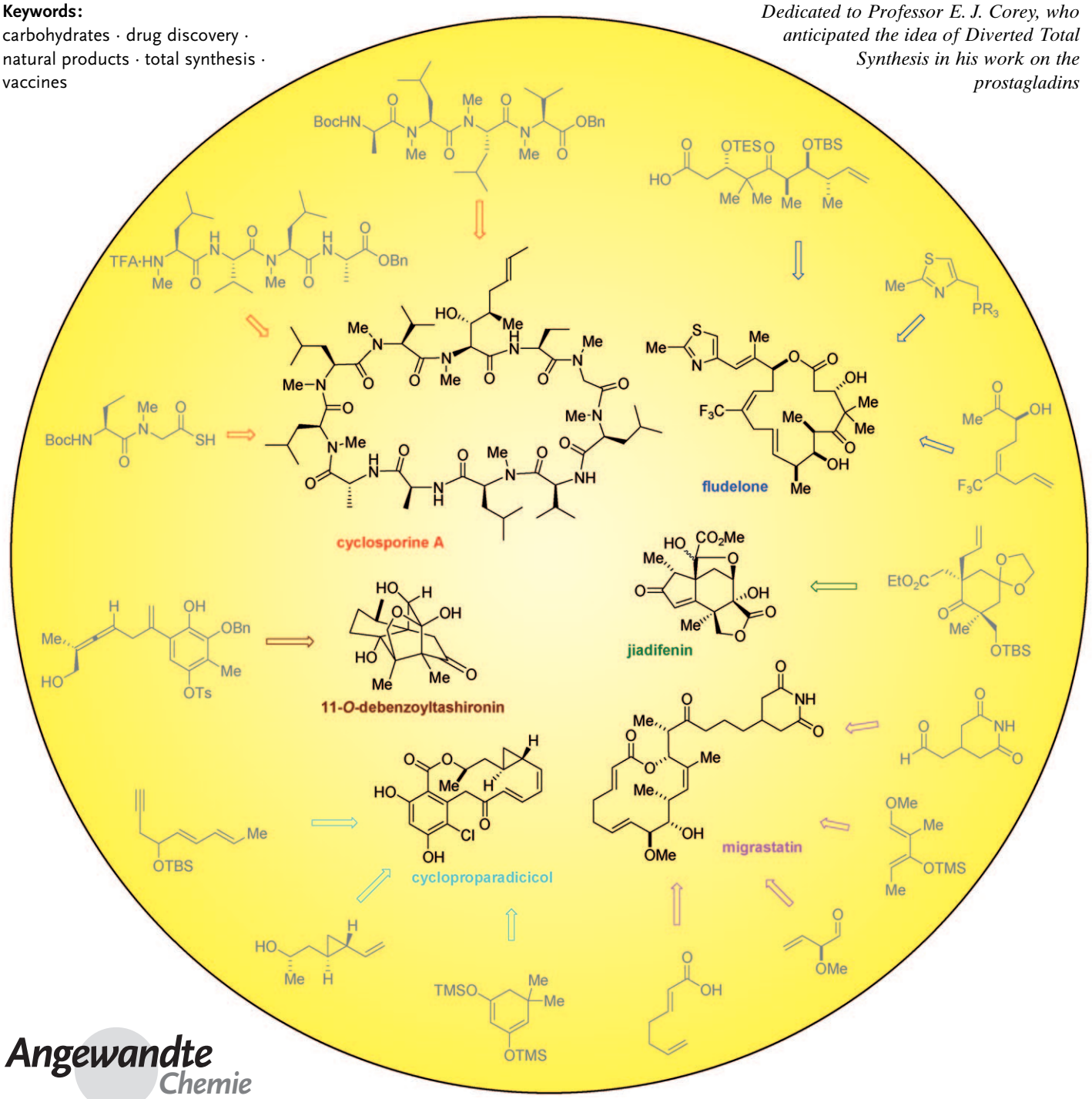
Dedicated to Professor E. J. Corey, who anticipated the idea of Diverted Total Synthesis in his work on the prostagladins



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In this retrospective, we recall some select cases of synergy between very challenging chemical synthesis and the identification of promising new candidates for pharmaceutics development. The progression from targets, often referred to as small molecules, to those of a size commonly associated with biologics (including glycoproteins) is also charted.

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1. Introduction

1.1. Small Molecule Natural Products in Drug Discovery

The natural product estate has served as an invaluable resource in the search for structurally novel lead agents of potential therapeutic value. Although recent years have witnessed a dramatic decline in the level of the pharma industry's commitment to the natural product-based drug development concept,[1] it must be acknowledged that a remarkable number of new chemical entities (NCEs) approved over the past two decades have nonetheless been small molecule natural products (SMNPs) or SMNP-based.[2] Indeed, a significant portion of approved drugs are either natural products themselves or have a clear connection to a parent natural product. By way of example, Taxol,[3] rapamycin,[4] and vancomycin[5] were first isolated from natural sources, while cabergoline and Zocor[6] were developed through structural modifications of biologically active natural products (Scheme 1). At times, the central pharmacophore of the SMNP may be transferred to an entirely novel structural setting, as was the case with Lipitor.[7] We refer to these types of agents as SMNP-inspired.

Notwithstanding a lack of enthusiastic commitment on the part of the pharmacology industry to natural products research, SMNPs have continued to be a valuable source of lead compounds in drug discovery. Why is this? It seems likely that, as a consequence of their biosynthesis and maintenance in living hosts, SMNPs inherently possess some particular attributes that also render them promising therapeutic agents. Presumably, SMNPs are being biosynthesized and evolutionarily optimized for the purposes of interacting with proteins, such as enzymes or receptors. Needless to say, therapeutic agents are typically designed to bind to exactly these types of biomolecules. Furthermore, the SMNP possesses a distinct advantage at the outset of the drug development process, in that, by definition, it has been housed in a living system. In the light of the high failure rate of drugs on grounds of host incompatibility, it seems clear that the importance of sustainability in a biological host should not be overlooked.

Of course, even given the potential advantages offered by SMNPs, it is nonetheless rather rare that the natural product itself will be found to be the ideal therapeutic agent. Typically, the structural framework of the biologically active SMNP may be viewed as a useful advanced starting point en route to the optimal drug candidate.[8] This is because the biological target of the SMNP is unlikely to bear a compelling relationship with the specific bio-targets that would be of maximum interest in a pharmaceutical setting. Moreover, the SMNP produced by the host must be viewed as a consensus structure, representing optimization of biological function, but subject to the practical constraints imposed by the host system's biosynthetic capabilities. Left strictly to the host's own devices, such new biosynthetic pathways are not readily altered.

One should thus view the biologically active SMNP as representing valuable, but not necessarily optimized, pharmacophoric space. It is the goal of medicinal chemistry to identify the key structural features that are responsible for the desired biological activity. Moreover, it is important to identify the functionalities within the SMNP that are unnecessary or even undesired from the pharmaceutical application standpoint. Thus, in a sense, the chemist has major advantages over nature in the time frame in which total synthesis molecular editing can occur.

1.2. Total Synthesis and Diverted Total Synthesis in the Drug Discovery Process

It is in the delineation of the various structural components of the SMNP that one may begin to appreciate the incredible power of chemical synthesis and, in particular, the process which we term "diverted total synthesis" (DTS).[9] Through recourse to de novo chemical synthesis, it may be possible to overcome the constraints imposed by the biosynthetic pathway, thereby gaining entry to structural motifs that would not be accessible through manipulation of the natural product itself. The notion of DTS, outlined in Figure 1, is pleasingly straightforward. Thus, an investigation might be launched toward the total synthesis of a biologically compelling natural product, **C**. On the way to the natural product, one may synthesize an advanced intermediate, **B**. It could be of interest to use intermediate **B** to reach point **D**, which represents chemical space of a higher order of complexity

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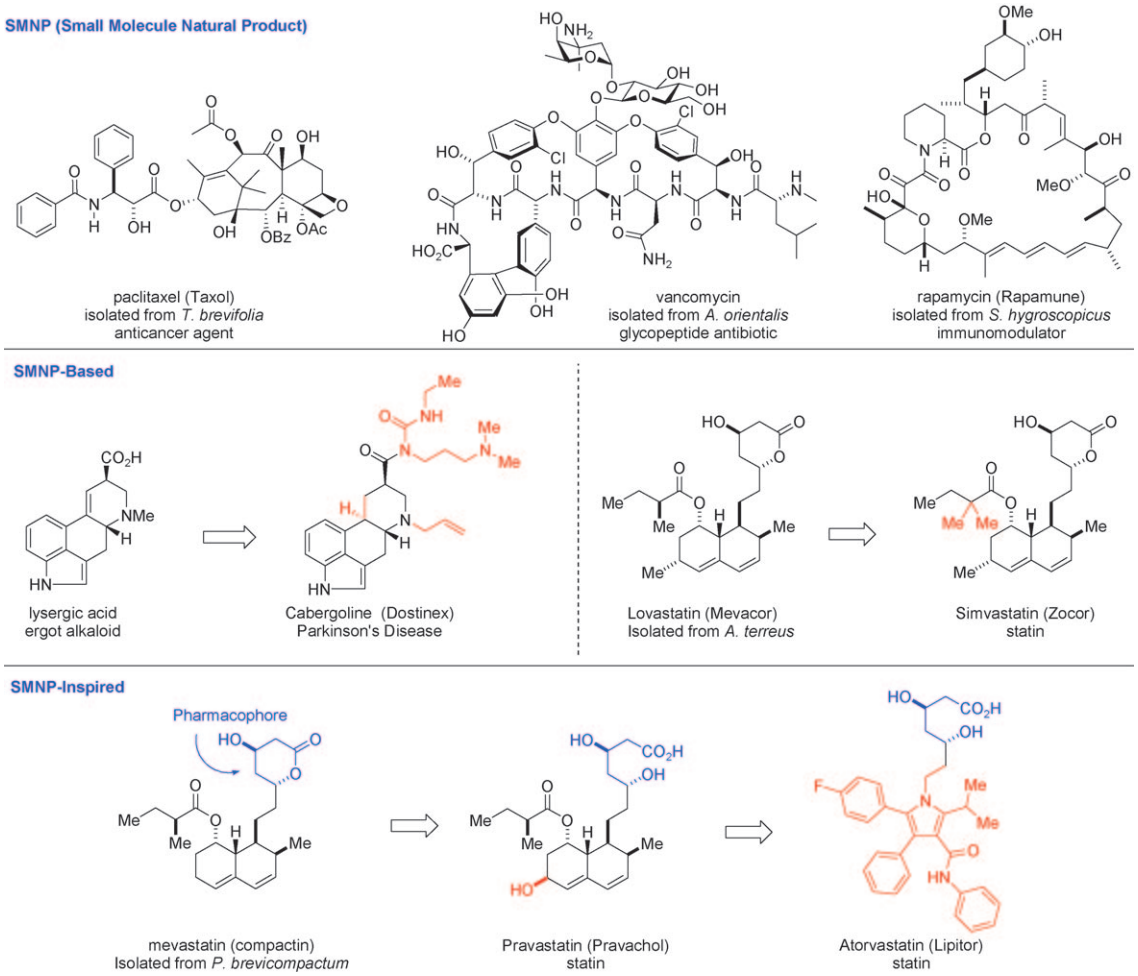
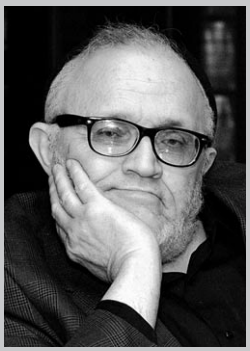


Scheme 1. Examples of small molecule natural products in drug discovery.

than is encountered in the natural product itself, or to advance to point **E**, which is of a lower order of chemical complexity. Due to limitations in synthetic methodology, often neither of these structure types can be reached from the natural product itself. As such, through recourse to DTS, one might begin to assemble a SAR (structure–activity relationship) profile of the natural product. The logic underpinning the strategy of DTS is the perception that SMNPs may generally be viewed as privileged, high-pedigree structures.

1.3. Biologicals as Targets for Total Synthesis

Although the concepts of total synthesis and diverted total synthesis are traditionally understood in the context of small molecule natural products (SMNPs), they are also relevant to the study of much larger biomolecules, such as proteins and oligosaccharides. As a result of major methodological developments in other laboratories, as well as our own, the distinction between small molecules and biologicals is per-



Samuel J. Danishefsky received his B.S. degree at Yeshiva University. His first academic position was at the University of Pittsburgh, where he joined as Assistant Professor in 1963. In 1980, he moved to Yale University and was named Eugene Higgins Professor in 1981. He became Sterling Professor at Yale in 1990. In 1993, he moved back to New York as Professor of Chemistry (now Centenary Professor) at Columbia University and as the Kettering Professor at Memorial Sloan-Kettering Cancer Center. In 1996, he shared the Wolf Prize in Chemistry with Gilbert Stork. In 2006, he was the recipient of the Franklin Medal in Chemistry, the Bristol Myers Squibb Lifetime Achievement Award in Chemistry, and the National Academy of Sciences Award in the Chemical Sciences.

Rebecca M. Wilson received her undergraduate training in biochemistry at Tufts University. She went on to the University of California at Berkeley and then to Caltech, where she conducted research with Professor David MacMillan, with a focus on the development of organocatalytic enantioselective intramolecular Diels–Alder reactions. After receiving her masters, she worked in the small-molecule project group at Amgen Pharmaceuticals, where she dealt with analytical and regulatory matters. In 2004, she joined the program of Samuel Danishefsky at Memorial Sloan-Kettering Cancer Center, where she is involved in chemical synthesis and its emerging role in vaccines and small-molecule drugs.

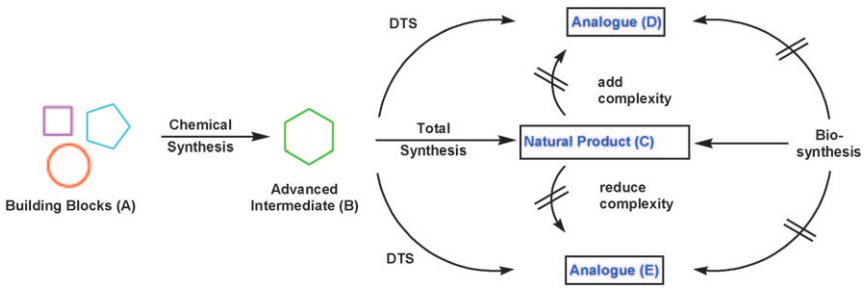


Figure 1. Diverted total synthesis (DTS).

haps becoming much less apt. Potentially valuable so-called biologicals can now be prepared through chemical synthesis, and the same principles of diverted total synthesis that are utilized in the development of small molecule therapeutic agents may similarly be applied to biological agents. Ultimately, once a particular target structure in the biologic domain is identified as being worthy of further development, it is likely that biology, with its remarkable powers of amplification, will be far more effective than chemical synthesis at producing the molecule. However, in many instances, synthesis is very much competitive at the initial discovery stage, where purity, rather than scale, is the critical factor.

1.4. An Academic Pipeline

Our laboratory has long been fascinated with the chemical synthesis of small molecule natural products and biologicals of potential therapeutic value.[9,10] We select our targets for their intriguing structural features, as well as for their purported biological activity. Upon completion of the total synthesis of the natural product itself, we seek to confirm the reported activity and to prepare congeners through DTS. Through carefully chosen and highly interactive collaborative efforts, we evaluate the biological effects of various perturbations of the molecular framework and, on the basis of these findings, we continue to fine-tune the drug platform. As an academic research group, we of course do not have the resources to build extensive "libraries" of compounds. Rather, by focusing on SMNPs, we hope to take advantage of "molecular pedigree" to perhaps compensate for numbers of compounds which can be thrown at a problem. Nonetheless, our carefully designed, small-scale drug pipelines have thus far produced some very promising candidates for further exploration. A number of these candidates have entered clinical trials.

This Review will attempt to illuminate, through example, our own laboratory's quest to develop viable drug candidates, both in the biological and small molecule realm, through total synthesis, diverted total synthesis, and collaborative biological exploration. The first section of this Review will describe recent advances in our laboratory toward the synthesis, DTS, and biological evaluation of a range of SMNP-based compounds, including the epothilones, migrastatins, radicicols, panaxytriol, and neurotrophically active agents. The remainder of the Review will focus on selected programs underway in the realm of larger molecules ("biologics"). In particular, we describe the development of a carbohydrate-based cancer vaccine program, as well as our progress toward the total synthesis of two highly complex, biologically active glycoproteins: erythropoietin alpha (EPO) and human follicle stimulating hormone (hFSH).

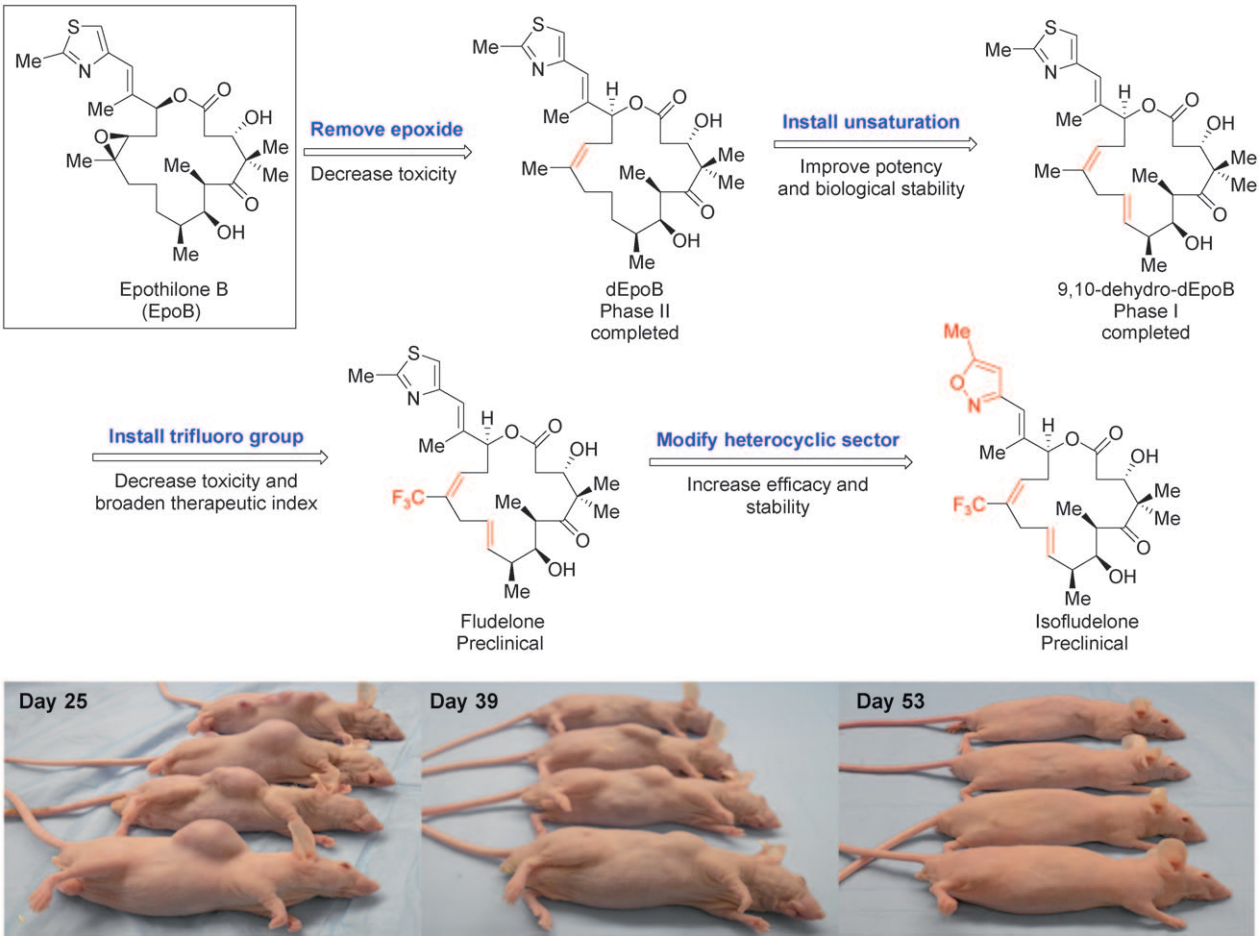
2. Total Synthesis and DTS of Small Molecule Natural Products

2.1. Epothilones

Originally isolated from the *Sorangium cellulosum* myxobacterium,[11] epothilone B (EpoB) was reported to exhibit potent in vitro cytotoxicity. Like taxol, EpoB promotes the stabilization of microtubule polymerization, which results in the interruption of the cell division process and ultimately leads to cell apoptosis. However, unlike taxol—and, in fact, unlike most clinically available anticancer agents—the epothilones do not appear to suffer from a loss of effectiveness associated with the onset of multidrug resistance (MDR).[12] At a clinical level, the onset of MDR—the causes of which are not yet fully understood—can have disabling consequences for therapeutic prognosis. Clearly, a chemotherapeutic agent that would retain its cytotoxicity in the face of otherwise multidrug resistant tumors would be of great value for those individuals for whom the currently available treatments are no longer viable.

Our own early involvement in the epothilone program led to the completion of the inaugural synthesis of EpoB[13] and the related epothilone, EpoA.[14] Preliminary in vivo studies revealed EpoB to be highly toxic in mice, even at subtherapeutic dosages. Suspecting that this nonspecific toxicity might be a consequence of the epoxide linkage at C12–C13 of the natural product, we sought to "edit" out this structural feature. Thus, dEpoB (EpoD), itself a biogenetic precursor to EpoB, was prepared through DTS (Scheme 2). Indeed, this compound was found to be much less toxic than the parent natural product, and has been shown to be very well tolerated in a number of in vivo settings. In addition, although dEpoB is markedly less potent than EpoB, it does retain its efficacy against MDR cell lines. On the basis of promising preclinical findings, dEpoB was advanced to clinical trials. Phase II trials in breast cancer have been completed, and the compound is now being evaluated for other indications.

In our second-generation analogues, we hoped to regain some of the potency that had been lost in proceeding from EpoB to dEpoB. It was hypothesized that biological stability and potency could be bolstered through the introduction of structural features that might confer rigidity to the molecule. In the end, this hoped-for rigidity was achieved through the



Scheme 2. Diverted total synthesis of the epothilones and chemotherapeutic effect of fludelone against extra-large MX-1 xenografts in nude mice (30 mg kg^{-1}, Q12Dx4, 6 h infusion, $N=4$). The fludelone family of preclinical anticancer agents was invented and brought forward at MSKCC in the laboratory of Samuel Danishefsky. A particular fludelone, KOS-1803, was jointly developed by MSKCC and the Kosan Biosciences company. The results of these joint MSKCC–Kosan investigations have been published extensively in authoritative journals and meeting abstracts. In my opinion (S.J.D.) the data show remarkable promise at the preclinical level. The KOS-1803 findings, available to me, point to an impressive therapeutic index and describe cures. I feel that KOS-1803 shows significant promise as an anticancer agent and, accordingly, merits further preclinical and clinical development. Kosan Biosciences, for which S.J.D. was on the scientific advisory board, was acquired by a major pharmaceutical company. That company now has control over the development of KOS-1803.

installation of a second olefin at the C9–C10 position. The newly synthesized analogue, termed 9,10-dehydro-dEpoB, was found to exhibit significantly enhanced potency in in vivo mouse settings.[15a,b] Furthermore, 9,10-dehydro-dEpoB demonstrates increased serum stability in comparison with dEpoB. 9,10-dehydro-dEpoB has been evaluated in phase I clinical trials and is currently being pursued for other indications.

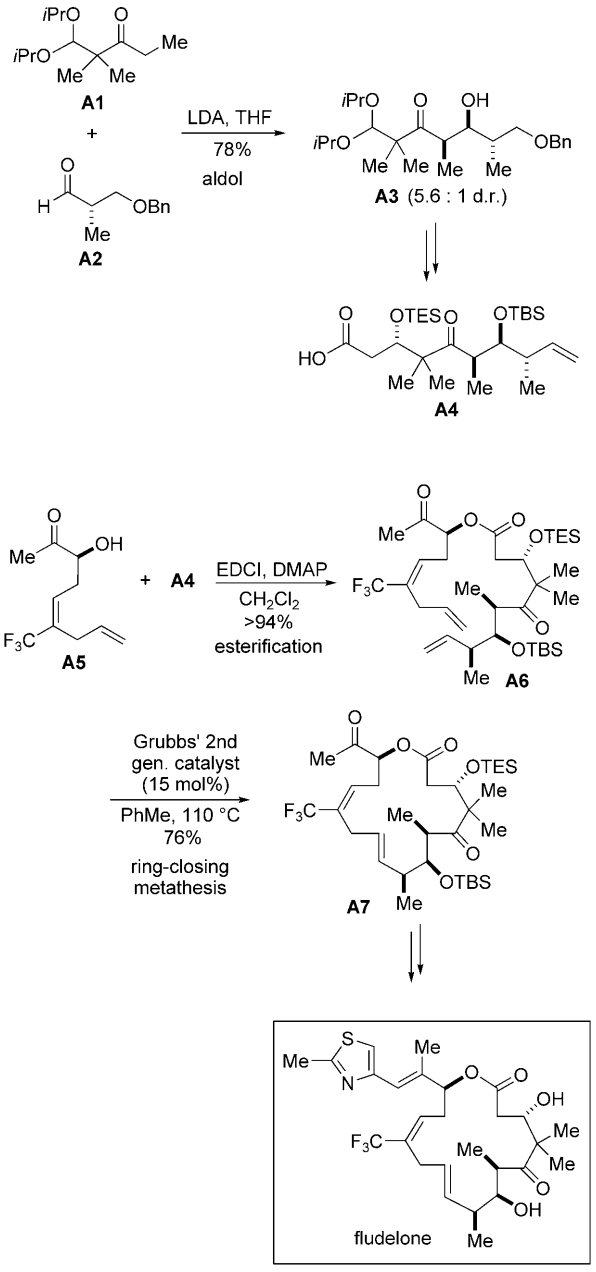
Presumably as a consequence of its enhanced potency, 9,10-dehydro-dEpoB is also more toxic than is dEpoB. Accordingly, lower dosages are tolerated in vivo and in the treatment of some particularly refractory tumors, 9,10-dehydro-dEpoB is unable to achieve the hoped-for levels of tumor eradication. In our next-generation epothilone series, we would seek to mitigate this toxicity and thereby broaden the therapeutic index. Remarkably, it was found that when the C12 methyl group was replaced with a trifluoromethyl functionality, a dramatic improvement in terms of therapeutic index ensued. The trifluoromethyl analogue, termed fludelone, is markedly less toxic than 9,10-dehydro-dEpoB.[15] Despite the corresponding decrease in potency, the therapeutic index of fludelone is far superior to that of the parent compound. Indeed, in in vivo mouse settings, fludelone is capable of eradicating particularly refractory tumors against which 9,10-dehydro-dEpoB is not nearly as effective.

Finally, through modification of the heterocyclic sector of the epothilone framework, an iso-fludelone derivative (termed KOS-1803) has been prepared.[15d] This compound exhibits remarkable potency and stability, and has been found to achieve complete remission and therapeutic cures in certain mouse xenograft models in as few as four doses, administered at relatively infrequent intervals (up to 12 days).

Iso-fludelone and fludelone are both highly promising candidates for further development. It is our belief that the epothilone program, undertaken in our laboratory and enhanced through carefully selected collaborative efforts, serves as an excellent example of the power of total synthesis and diverted total synthesis to identify lead candidates for

further development. Through small, but carefully considered, perturbations of the epothilone framework, we were able to modify the natural product so as to gain access to increasingly powerful anticancer drug candidates. Needless to say, the structural "edits" highlighted in Scheme 2 could not have been easily accomplished through modification of the natural product (EpoB) itself. Only through recourse to the principles of DTS was it possible to efficiently gain access to adequate quantities of these promising analogues for further evaluation.

The convergent total synthesis of optically active fludelone is presented in Scheme 3.[15] As shown, the synthetic route makes use of a diastereoselective aldol reaction to produce the β-hydroxyketone, **A3**, which incorporates the three contiguous stereocenters of fludelone. This intermediate is advanced to acid **A4**, and esterification with alcohol **A5** yields the metathesis precursor, **A6**. Ring-closing metathesis (RCM) exclusively affords the desired *trans* isomer, **A7**, which, upon installation of the heteroaromatic sector, provides fludelone itself. The iso-fludelone analogue is made in a very similar manner.



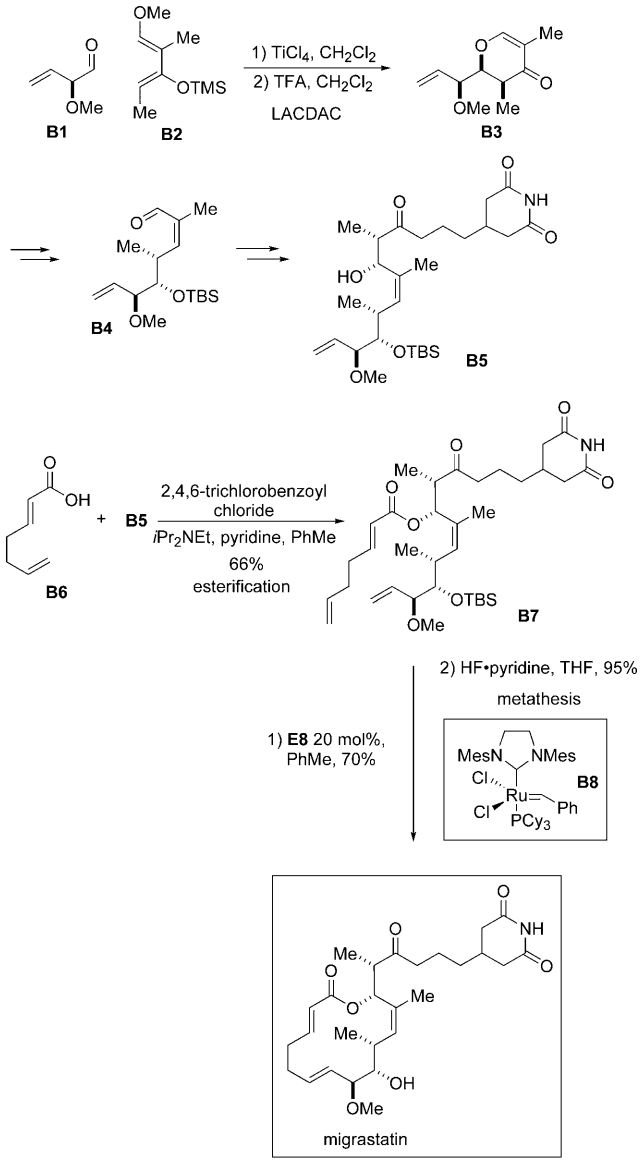
Scheme 3. Synthesis of fludelone. LDA = lithium diisopropylamide, THF = tetrahydrofuran, EDCI = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, DMAP = 4-dimethylaminopyridine.

2.2. Migrastatin

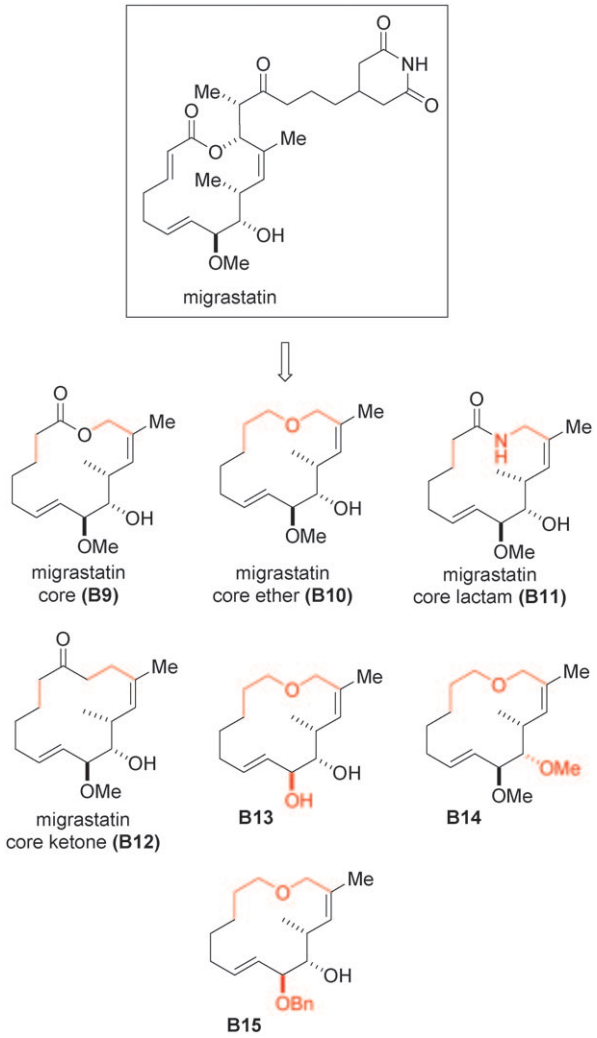
In the progression of cancer, tumor cell metastasis often marks the onset of the most devastating phase of the disease.[16] Given the central role of in vivo cellular motility in the phenomenon of tumor metastasis, small molecule therapeutic agents that prevent such cell migration would be highly desirable, in that they would help to simplify tumor resection and to minimize the number of organs impacted by the disease. Moreover, selective cell migration inhibitors might be expected to be significantly less toxic than traditional "cytotoxic" drugs. In this context, we took note of the disclosure, by Imoto and co-workers, of the isolation of a naturally occurring tumor cell migration inhibitor, migrastatin, from the *Streptomyces* sp. MKI-929-43F1.[17] As reported by Imoto, pre-treatment of a monolayer of human esophogeal cancer (EC17) cells with 30 mg mL^{-1} of migrastatin in the context of a wound healing assay, served to dramatically suppress the ability of the cells to re-infiltrate a defined cell-free area. Although the reported potency of migrastatin itself is rather modest ($IC_{50} = 29\ \mu M$), we were hopeful that the natural product might serve as a valuable lead compound, from which more active analogues could be developed.

With this objective in mind, we accomplished a concise, enantioselective total synthesis of migrastatin (Scheme 4).[18] Key features of this route include a diastereoselective Lewis-acid catalyzed diene–aldehyde cyclocondensation (LACDAC) reaction (of a type we had discovered 20 years earlier) to afford **B3**. An esterification–metathesis sequence served to provide the macrocyclic system. The in vitro reported activity was confirmed with our synthetic material.[19a]

We next proceeded to prepare, through DTS, a number of structurally simplified synthetic analogues (Scheme 5). Needless to say, these core structures cannot be readily accessed from the natural product itself, though they are easily obtainable from advanced intermediates in the migrastatin synthetic route. In our first-generation analogue study, we were very encouraged to find the "migrastatin core" (**B9**) to be three orders of magnitude more potent ($IC_{50} = 24$ nM) than the natural product itself in in vitro studies.[20] Unfortunately, however, despite this excellent in vitro activity, the migrastatin lactone based core did not perform well in mouse plasma stability studies, presumably due to the presence of the resident lactone functionality, which serves to render the molecule susceptible to the action of esterases. A second generation of analogues attempted to address this problem by "editing" out the lactone, and replacing it with a more stable functionality—such as lactam (**B11**), ether (**B10**), or ketone (**B12**).[20] Indeed, from these investigations there emerged a



Scheme 4. Synthesis of migrastatin. TFA = trifluoroacetic acid.



Scheme 5. Diverted total synthesis of the migrastatins.

very promising candidate, which we term "migrastatin core ether" (**B10**).[20]

Migrastatin core ether has demonstrated very promising activity, in both in vitro and in vivo settings. In preliminary studies, mice treated with this compound have exhibited decreased levels of cancer metastasis and prolonged survival times. In one promising in vivo study, conducted in collaboration with the laboratory of Malcolm Moore at MSKCC, NOD-SCID mice were injected with human breast cancer cells. Groups of mice were injected with low-dose **B10** (40 mg kg^{-1}, i.p., 3 × weekly), high-dose **B10** (200 mg kg^{-1}, i.p., 3 × weekly), or with placebo (control, PBS). After 4 weeks, the primary tumors were resected, and bioimaging for metastatic tumor was performed weekly. At 7 weeks, mice were sacrificed and the lung, liver, spleen, and thymus were removed and imaged. Though extensive metastatic tumor growth was observed in the lung and liver of the control mice, no growth was observed in either the low- or high-dose **B10**-treated mice. By 9 weeks, detectable metastatic tumor was observed in the lung and liver of the low-dose **B10**-treated mice, but the high-dose group had no detectable metastases at this time. These data clearly underscore the ability of the migrastatin core ether, **B10**, to inhibit tumor cell migration and to thus mitigate tumor metastasis in in vivo mouse settings.

In a separate study, the group of Joan Massagué at MSKCC conducted an in vivo experiment, in which immunocompromised mice were injected with a line of metastatic human breast cancer cells (LM2-4175), which specifically metastasize to the lung. After 27 days, mice were treated with low-dose **B10** (100 mg kg^{-1}), high-dose **B10** (200 mg kg^{-1}), or control (DMSO/PBS buffer). The mice treated with a high dose of migrastatin core ether (**B10**) exhibited a 4.5-fold reduction in lung metastasis, as compared with the control group (Figure 2).

These preliminary in vivo results serve to confirm the potential of the migrastatin framework as a lead platform for drug development. Migrastatin core ether (**B10**) clearly demonstrates enhanced in vivo stability and efficacy over the parent natural product. The migrastatin program lends

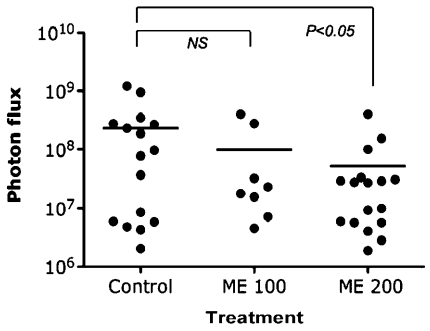


Figure 2. Analysis of mammary tumor growth and lung metastasis. Mammary tumor growth: Luciferase-transduced LM2 cells were injected bilaterally into the fourth mammary gland fat pad of NOD-SCID mice. The size of the mammary tumor was measured regularly using a caliper. On day 27 after injection, mice underwent treatment with **B10** (ME) 100 mg kg^{-1}, **B10** (ME) 200 mg kg^{-1}, or vehicle as control. The treatment was administered three times per week via intraperitoneal injection. Control: $n=16$; ME 100 mg kg^{-1}: $n=8$; ME 200 mg kg^{-1}: $n=17$. Lung metastasis at endpoint was measured by luminescence. At day 42, mice were analyzed for lung metastasis by ex vivo bioluminescence, quantifying luciferase activity in the lungs. ME 100: **B10** 100 mg kg^{-1}, ME 200: **B10** 200 mg kg^{-1}.

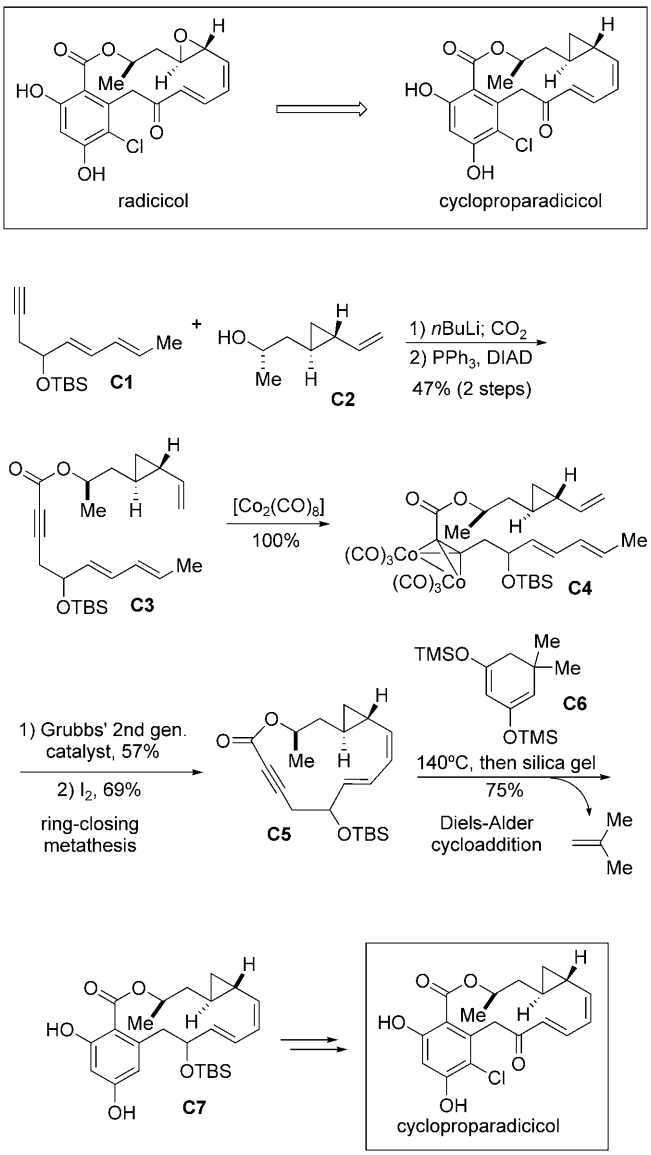
further evidence of the value of SMNPs and of diverted total synthesis in the search for lead agents of clinical value. Though at an early stage, this anti-metastasis synthesis program is a textbook case of DTS.

2.3. Radicicols

First isolated from *Monocillium bonorden* in 1953,[21] radicicol is reported to exhibit high binding affinity and inhibition of the heat shock protein 90 (Hsp90) molecular chaperone.[22] Hsp90 is considered an attractive target for inhibition by anticancer agents due to its central role in mediating the folding of several oncogenic proteins, such as *Raf1* and *Her2*. With other Hsp90 inhibitors, radicicol does not carry the burden of a quinone substructure, with its attendant cardiotoxicity issues. On the basis of these preliminary in vitro reports, we launched a program directed toward the total synthesis of optically active radicicol.

This goal was achieved in 2001 with our completion of the first asymmetric total synthesis of radicicol.[23] With synthetic material in hand, we did indeed confirm the inhibitory activity of the natural product against the Hsp90 chaperone. However, radicicol had been found to be ineffective in in vivo settings. We attributed the disappointing lack of in vivo activity to the presence of the epoxide moiety, which is presumed to cause in vivo instability as well as nonspecific cytotoxicity. In an effort to address this issue, we designed an analogue, termed cyclopropradicicol, in which the epoxide has been "edited" out and replaced with a more stable cyclopropyl unit.[24]

An interesting feature of the cyclopropradicicol synthesis, presented in Scheme 6, is the ring-closing metathesis sequence by which **C5** is constructed.[25b] Initial efforts to achieve RCM directly from the alkynoate ester **C3** were



Scheme 6. Synthesis of cyclopropradicicol. DIAD = diisopropyl azodicarboxylate.

unsuccessful. A variety of metathesis conditions led only to recovery of starting material. It was speculated that the steric constraint imposed by the linear acetylenic functionality was perhaps responsible for the failure of the substrate to cyclize. Presumably, the alkyne function could be further undermining the reaction by unproductively coordinating the catalyst. Fortunately, we found that it was possible to solve the problem by temporarily masking the acetylene functionality through engagement in a dicobalt carbonyl complex, **C4**. As hoped, this intermediate was more geometrically inclined to undergo ring closing metathesis, and upon exposure to the Grubbs' catalyst, **C4** readily underwent cyclization to provide the macrocyclic adduct in 57% yield. The alkyne was reconstituted upon exposure of the dicobalt complex to iodine. The "ynolide" functionality of **C5** proved to be a productive dienophile, and Diels–Alder cyclization with **C6**

served to install the aromatic sector of the resorcylilic system (**C7**) in good yield.

The synthetic cyclopropparadicicol was found to bind the Hsp90 molecular chaperone at approximately 160 nM levels. Importantly, unlike the parent natural product, this modified analogue does retain its efficacy in in vivo settings. In a preliminary in vivo study against mice implanted with human mammary carcinoma (MX-1), cyclopropparadicicol was found to effect tumor suppression (Figure 3). This finding lends

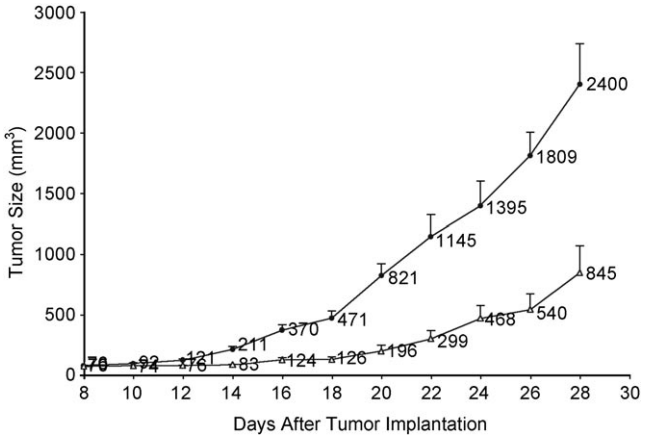


Figure 3. Therapeutic effect of cyclopropparadicicol in nude mice bearing human mammary carcinoma MX-1 xenograft. ● control; △ cyclopropparadicicol, 20 mg kg^{-1}, Q2Dx3; 50 mg kg^{-1}, Q2Dx1, 100 mg kg^{-1}, Q2Dx5, i.v. injection, $n=3$.

support to our original hypothesis regarding the liability of the epoxide moiety in an in vivo environment. The viability of cyclopropparadicicol as a lead candidate for further development is currently under investigation.

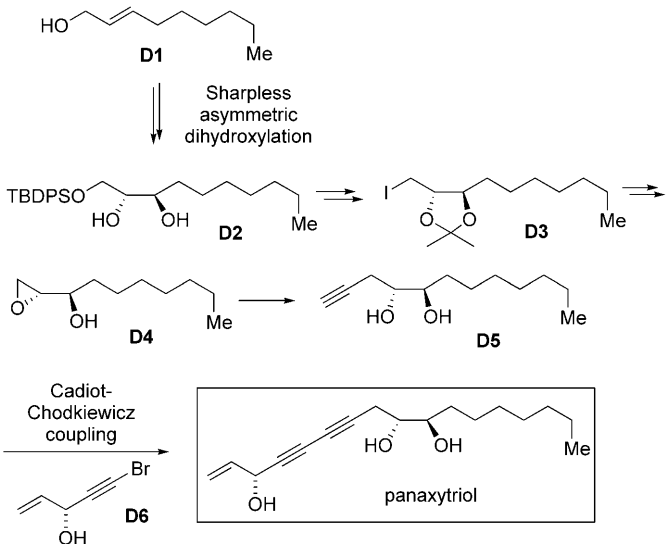
2.4. Panaxytriols

Red ginseng is considered to be a leading botanical nutraceutical—that is, a food extract that possesses demonstrated medicinal benefits.[25] This herbal root has been used throughout Asia as a folk medicine for the treatment of a variety of maladies for over 2000 years, and is believed to have possible applications in cardiovascular health, diabetes, and cancer. In 1983, panaxytriol was isolated as a characteristic constituent of Korean red ginseng.[26] This natural product was found to exhibit in vitro inhibitory activity against a range of tumor cells. Recent evidence suggests that panaxytriol exhibits cancer prevention activity, in part through the induction of phase 2 enzymes.[27] These chemoprotective phase 2 enzymes promote detoxification reactions through a variety of mechanisms, thus protecting cells against the toxicities of reactive electrophiles and oxygen species. The induction of phase 2 enzymes may serve to counteract carcinogenesis through neutralization of reactive electrophiles that might otherwise emerge as carbinogens or mutagens. Thus, panaxytriol stands out as a rare instance of an antitumor agent with documented anticancer properties that is found in a widely consumed food product. For this reason, even a moderate effect could be of value, since any side effects are clearly manageable.

With these considerations in mind, we completed a concise asymmetric total synthesis of panaxytriol.[28] As shown in Scheme 7, the reaction makes use of the Sharpless asymmetric dihydroxylation for the installation of the diol moiety (**D1**→**D2**). A late-stage Cadiot–Chodkiewicz coupling (**D5** + **D6**) serves to emplace the diyne functionality as well as the final hydroxy stereocenter of panaxytriol.

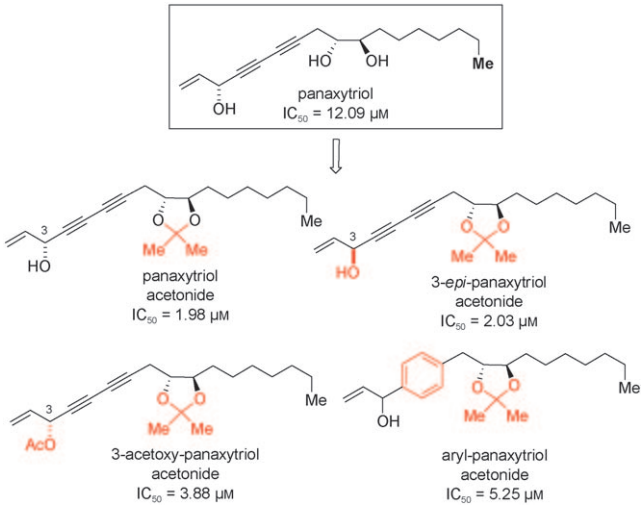
With an efficient route toward panaxytriol in hand, we next sought to prepare a range of related compounds through DTS. As shown in Scheme 8, panaxytriol and a series of synthetic analogues were evaluated for in vitro inhibitory activity against CCRF-CEM cancer cells.[29] In this regard, most of the synthetic analogues were in fact found to be more potent than the natural product itself. Thus, engagement of the diol as an acetonide moiety leads to enhanced in vitro activity (panaxytriol acetonide). The C3 hydroxy moiety does not appear to be essential for activity, as demonstrated by the fact that good levels of activity are observed with both 3-acetoxy-panaxytriol and with 3-*epi*-panaxytriol acetonide.

In an exciting finding, we recently demonstrated that in vivo mouse models, our synthetic panaxytriol-based com-

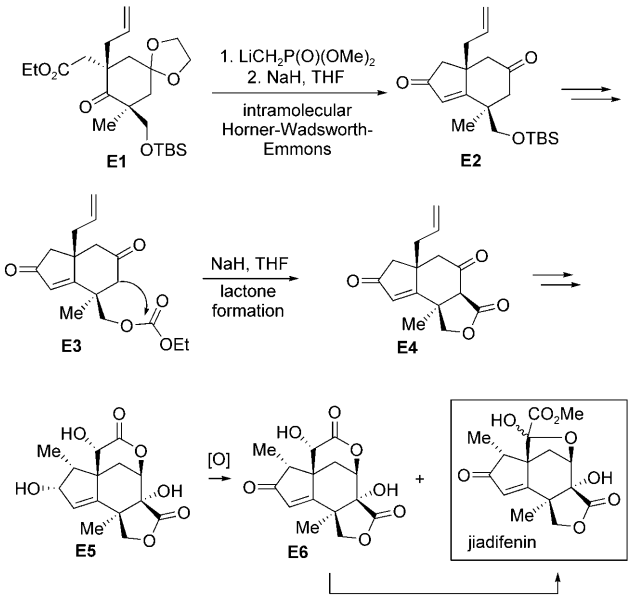


Scheme 7. Synthesis of panaxytriol.

pounds are able to mitigate the toxic side effects associated with exposure to cytotoxic chemotherapeutic agents. Thus, when panaxytriol analogues and cytotoxic anticancer agents (such as taxol and fludelone) were co-administered to tumor-bearing mice, we observed modest, but clear and reproducible synergism in the anti-profilerative effects of the cytotoxic agents, as well as increases in the maximum tolerated dose (MTD) and lethal dose (LD) of the cytotoxic agents, and attenuation of behavioral characteristics typically associated with peripheral neuropathy—a common side effect of treatment with such drugs. Furthermore, the panaxytriol compounds may exhibit mild anti-inflammatory properties,



Scheme 8. Diverted total synthesis of the panaxytriols. IC_{50} against CCRF-CEM in XXT assay following 72-hour inhibition.



Scheme 9. Synthesis of jiadifenin.

reduce immunosupression, and provide clear relief from radiation-induced peripheral tissue damage. We believe that these effects may be attributed to upregulation of the chemoprotective phase II enzymes by the panaxytriol-based agents.

2.5. Synthesis and Evaluation of Neurotrophically Active Compounds

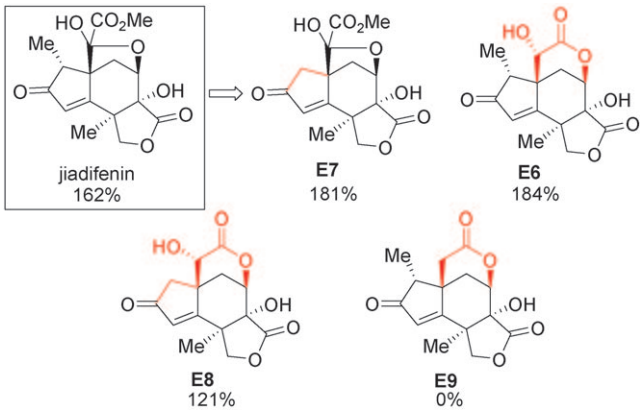
Naturally occurring, polypeptidyl neurotrophic factors are known to play a central role in promoting neuronal survival, differentiation, and outgrowth.[30] Importantly, the advancement of common neurodegenerative disorders—including Alzheimer's, Huntington's, and Parkinson's diseases—has been linked to decreased levels of neurotrophic support. Our laboratory has an active interest in the development of small molecule, central nervous system (CNS)-permeable neurotrophically active compounds which might be of use in the treatment of neurodegenerative disorders.[31] Toward this end, we have taken note of a growing class of SMNPs which are purported to demonstrate some type of potentially exploitable CNS activity. This section describes our efforts toward the total synthesis, diverted total synthesis, and biological evaluation of four structurally diverse, neurotrophically active natural products: 1) jiadifenin, 2) scabronine, 3) 11-*O*-debenzoyltashironin, and 4) merrilactone.

2.5.1. Jiadifenin

Isolated from the *Illicium jiadifengpi* species of China, jiadifenin was reported to promote neurite outgrowth in rat cortical neurons at concentration levels as low as 0.1 μM.[32] On the basis of these early in vitro findings, we undertook the total synthesis of this structurally interesting natural product, and completed the inaugural total synthesis of jiadifenin in 2004 (Scheme 9).[33] Key features of the synthesis included an intramolecular Horner–Wadsworth–Emmons reaction (**E1**→**E2**) as well as the installation of the lactone ring through the intermediacy of a mixed carbonate ester (**E3**→**E4**).

With synthetic material in hand, we were able to independently confirm the reported neurotrophic activity of jiadifenin. In the presence of the naturally occurring neurotrophic factor NGF (nerve growth factor), jiadifenin was found to enhance neurite lengths to 162% (relative to a control). However, in the absence of NGF, no neurite outgrowth was observed. This finding suggests that jiadifenin operates through the upregulation of the action of NGF, rather than through independent means.

We have prepared a range of jiadifenin analogues through diverted total synthesis and evaluated the NGF-dependent neurotrophic activity of each. As outlined in Scheme 10, two of these synthetic analogues—the normethyl version of jiadifenin (**E7**) and the unrearranged jiadifenin precursor (**E6**)—were found to be more active than the parent compound, jiadifenin. In the presence of NGF, compounds **E7** and **E6** enhance neurite lengths by 181% and 184%,

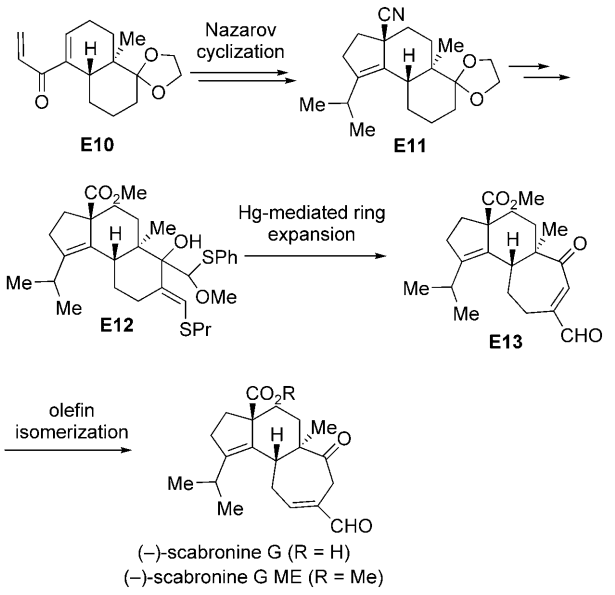


Scheme 10. Diverted total synthesis of the jiadifenins. Neurite length enhancement relative to DMSO-NGF control.

respectively. Interestingly, the unrearranged, normethyl analogue (**E8**) exhibits only modest activity (121% length enhancement), perhaps suggesting a complex SAR profile for this natural product. Clearly, however, the level of C10 oxidation is important, as the unoxidized congener **E9** exhibits no activity in this assay. Further studies are needed to better ascertain the therapeutic potential of this class of compounds.

2.5.2. Scabronine G

The scabronines are metabolites isolated from the bitter mushroom *Sarcodon scabrosus*.[34] The most active member of this family, scabronine G, was found to induce the production and secretion of NGF in human astroglial (1321N1) cell lines.[35] Interestingly, the methyl ester derivative of scabronine G has been reported to induce enhanced levels of NGF production, in comparison to the natural product. Moreover, scabronine G methyl ester was shown to enhance production of a second neurotrophin, interleukin-6 (IL-6). Presumably as a consequence of this neurotrophin induction, both compounds were reported to induce dramatic neuronal differentiation in rat pheochromocytoma (PC-12) cells. Our total synthesis of scabronine G, completed in 2005,[36] features a key Nazarov cyclization (**E10**→**E11**) and a late-stage Hg-mediated ring expansion to provide **E13** which, upon olefin isomerization, affords scabronine G methyl ester (Scheme 11). Subsequent acid hydrolysis yields the natural product itself.



Scheme 11. Synthesis of scabronine G and scabronine G methyl ester.

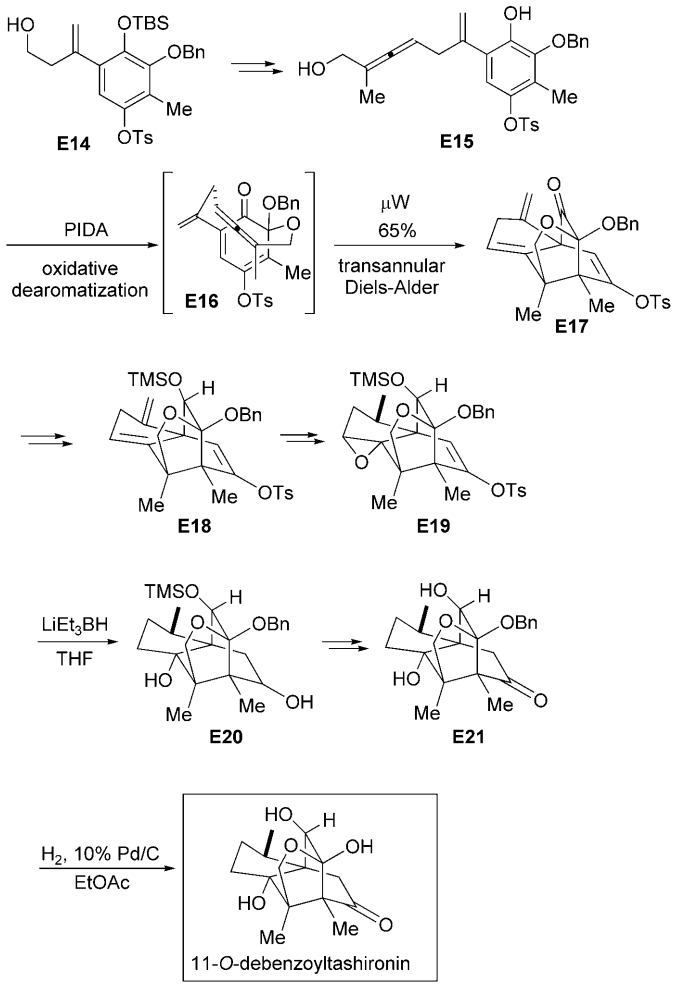
Upon completion of the synthesis, we were able to confirm the reported neurotrophic activity of scabronine G and scabronine G methyl ester. Both compounds did successfully enhance the production and secretion of neurotrophic factors in 1321N1 cells. Furthermore, significant neurite outgrowth was observed when scabronine G methyl ester was introduced to PC-12 cells. In a screen of analogues, we observed that the unisomerized precursor to the scabronine G methyl ester, **E13**, induced more neurite outgrowth than was observed with scabronine G methyl ester itself. In a subsequent study, the scabronine compounds were evaluated in a motor neuron assay, which examines the survival and axonal growth in mouse motor neurons derived from embryonic stem cells grown on an inhibitory setting (on a myelin protein, MAG, which inhibits axonal growth). In this setting, scabronine G methyl ester was found to effect a modest (10–20%) increase in axonal growth. Further studies which seek to examine the therapeutic potential of the scabronine compounds will be forthcoming.

2.5.3. 11-O-Debenzoyltashironin

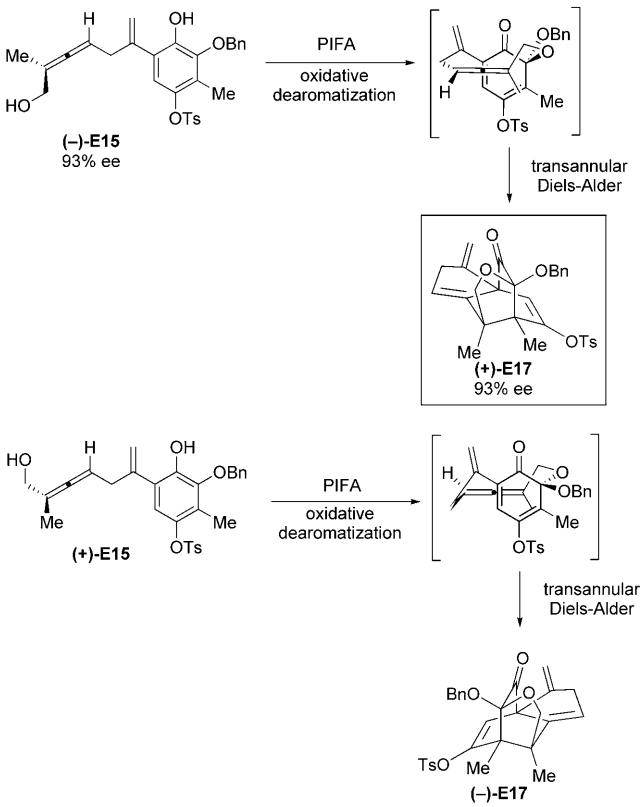
Isolated from the pericarps of the *Illicium merrillianum* tree of eastern Asia, 11-*O*-debenzoyltashironin was reported to promote neurite outgrowth in fetal rat cortical neurons at levels as low as 0.1 μM.[37] This densely functionalized, highly oxygenated tetracyclic natural product was targeted for total synthesis in our laboratories, and the inaugural synthesis was accomplished in 2006.[38] More recently, we reported an asymmetric route to either antipode of 11-*O*-debenzoyltashironin.[39] As outlined in Scheme 12, the synthesis is organized around a key cascade sequence, commencing with oxidative dearomatization of allene **E15**. The resultant intermediate, **E16**, undergoes microwave-induced transannular Diels–Alder cyclization to generate the tetracyclic adduct **E17**. With the molecular backbone in place, the natural product is accessed through a series of functional-group manipulations.

Our second-generation, asymmetric route to 11-*O*-debenzoyltashironin was facilitated by the development of a means by which to access enantiomerically enriched allene (**E15**) through asymmetric preparation of the precursor propargylic alcohol. With the optically active key intermediate in hand, we were able to accomplish the subsequent oxidative dearomatization without erosion of asymmetry, and to ultimately gain separate access to both enantiomers of the natural product. Thus, as illustrated in Scheme 13, the (−)-**E15** allene undergoes facially selective oxidative dearomatization to afford the **E16** intermediate, which subsequently suffers transannular Diels–Alder cyclization to yield (+)-**E17**. The latter is advanced to the natural enantiomer of 11-*O*-debenzoyltashironin. Similarly, the opposite allene enantiomer (+)-**E15** adds across the opposite face of the aromatic sector in the oxidative dearomatization step. Transannular Diels–Alder cyclization of this intermediate provides (−)-**E17**, which is ultimately advanced to the nonnatural enantiomer of the natural product. The mechanistic details of this fascinating transformation are beyond the scope of this Review—suffice it to say that the execution of the asymmetric route provided unique insight into the subtleties of the oxidative dearomatization which had not been apparent in our first-generation racemic synthesis.

The reported neurotrophic activity of the synthetic material has been corroborated. In an important finding, we recently determined that only the natural antipode promotes



Scheme 12. Synthesis of 11-*O*-debenzoyltashironin. PIDA = phenyliodine(III) diacetate.



Scheme 13. Asymmetric approach to 11-*O*-debenzoyltashironin. PIFA = phenyliododitrifluoroacetate.

neurite outgrowth. This discovery will, of course, have implications for our analogue studies, which will need to be accessed as single antipodes. Analogue synthesis is underway, and further SAR studies will be forthcoming.

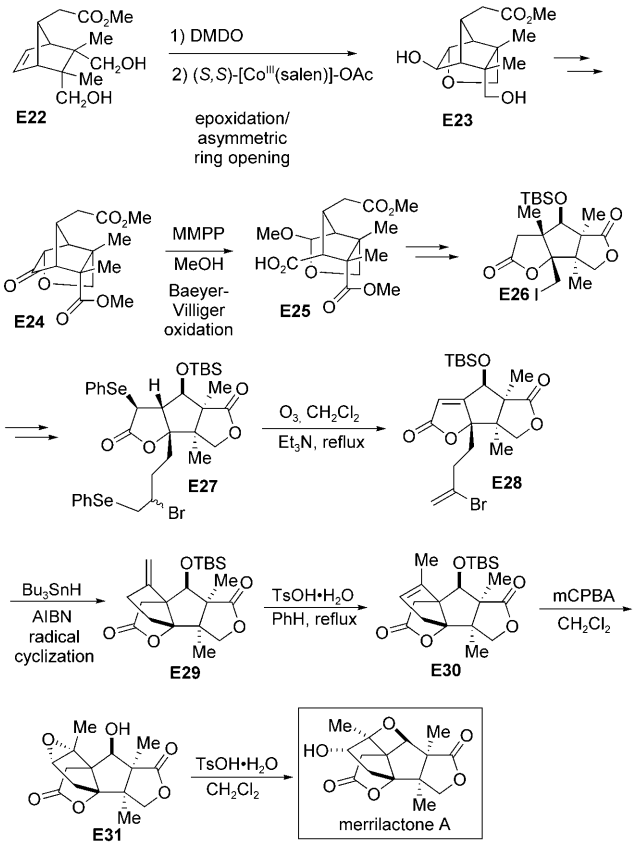
2.5.4. *Merrilactone*

Merrilactone was isolated from the *Illicium merrillianum* tree of east Asia and was purported to significantly promote neurite outgrowth in fetal rat cortical neurons at concentrations of 0.1 to 1 μM.[40] We first reported the racemic synthesis of this structurally fascinating natural product in 2002.[41] More recently, a second-generation asymmetric route to merrilactone was developed.[42] As shown in Scheme 14, the synthesis features a desymmetrizing epoxidation/asymmetric ring opening sequence (**E22**→**E23**) which serves to establish the absolute configuration at an early stage of the synthesis. The intermediate is elaborated to **E24**, at which point Baeyer–Villiger oxidation gives rise to **E25**. A second key transformation involves the free radical-induced cyclization of vinyl bromide **E28** to provide the tetracyclic **E29**, possessing the carbon backbone of the natural product itself. This adduct is advanced to merrilactone in a straightforward fashion, as shown.

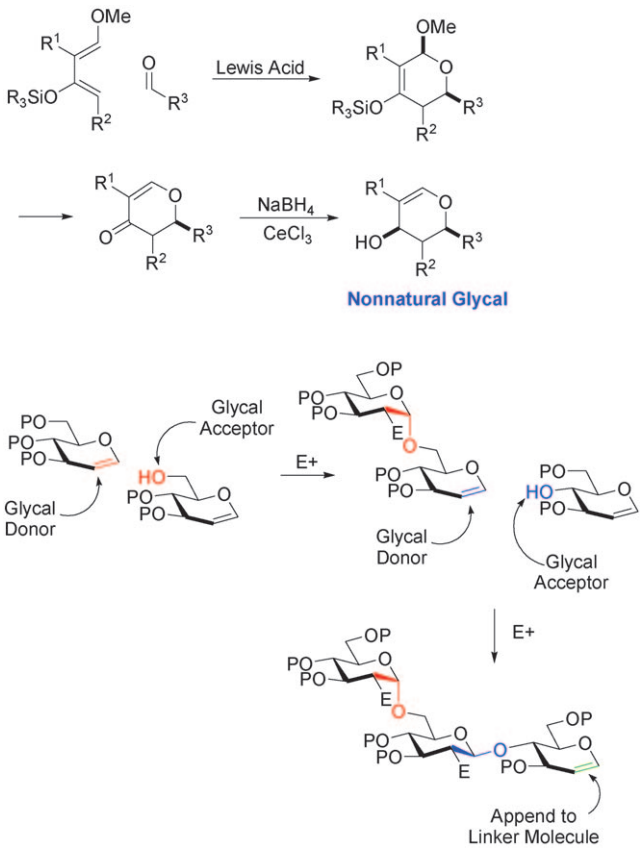
3. Possible Therapeutic Biologics through Chemical Synthesis

3.1. Carbohydrate Synthesis: Background

Our laboratory's entry into the field of oligosaccharide synthesis first arose from the development of a cycloaddition reaction which leads to the selective formation of dihydropyrones (Scheme 15).[43] We began to consider whether the adducts thus obtained could be employed as useful monomeric units in the broader context of carbohydrate synthesis. Toward this end, we postulated that the glycal motif, which can be derived from the dihydropyrone adduct, might serve as a convenient building block in the synthesis of oligosaccharides. Glycals, which contain only three hydroxy groups instead of five, possess an olefinic functional handle which may serve as a site of differentiation in the oligomerization process. Although others had previously recognized that glycals could serve as efficient *donors* (for example, through iodoglycosidation), our major advance was in the recognition that these building blocks could serve as valuable glycosyl *acceptors*.[44] Thus, we proposed that, through an appropriately conceived series of protecting group manipulations, glycals might be used exclusively to iteratively assemble complex oligosaccharides. These synthetic oligosaccharides would, of course,



Scheme 14. Synthesis of merrilactone A. DMDO = dimethyldioxirane, MMPP = magnesium monoperoxyphthalate, AIBN = 2,2′-azobisisobutyronitrile, Ts = 4-toluenesulfonyl, mCPBA = *meta*-chloroperoxybenzoic acid.



Scheme 15. Glycal assembly approach to carbohydrate synthesis.

terminate in glycal-type olefinic functionality, and the application of this method would require the development of efficient methods for their derivatization and appendage to appropriate linking motifs.

We have developed a number of methods for the stereoselective functionalization of the terminating glycal and appendage to the linker molecule. As outlined in Scheme 16, stereoselective functionalization may proceed through the intermediacy of either an α-epoxide (a, b) or an iodosulfonamide species (c, d). Direct nucleophilic addition to the α-epoxide produces the α-linked adduct, as shown in Scheme 16a. Alternatively, the iodosulfonamide may be formed from the glycal. Upon exposure to base, a sulfonylaziridine is generated, which then readily undergoes nucleophilic addition, as outlined in Scheme 16c.

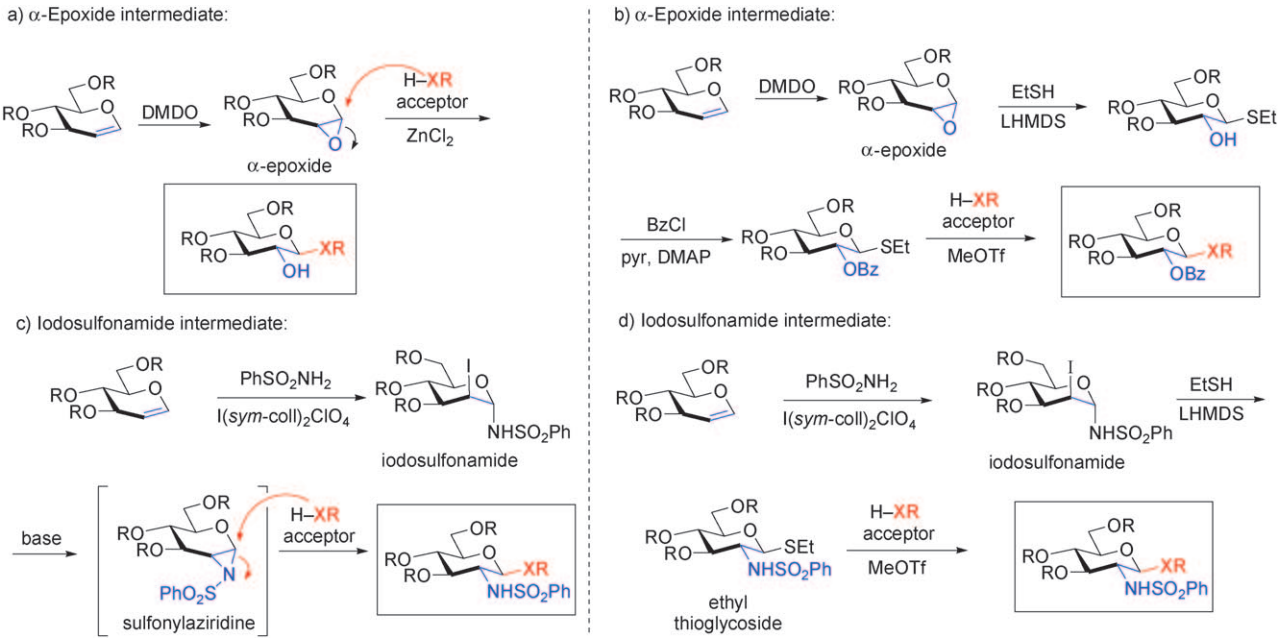
In certain complex cases, the steric constraints of the system are such that direct glycosylation of the α-epoxide or the iodosulfonamide intermediate is unsuccessful. In such instances, a two-stage process may be utilized, wherein an intermediate ethyl thioglycoside species is produced (cf. Scheme 16b and d). This species may subsequently be induced to undergo addition, as shown.

In short, over the course of the past 25 years, we and others have developed a broad menu of methods which allow for the selective and efficient assembly of very complex carbohydrate domains. These synthetic advances have been extensively reviewed in other settings.[45]

3.2. Carbohydrate-Based Anticancer Vaccines

Clinicians in the field of cancer immunology have long sought to develop effective means by which to incite the human immune system to recognize and eradicate tumor cells. Of course, in the design of an anticancer vaccine, one must first identify structural features unique to malignantly transformed cells, which might be exploited to prompt the immune system to recognize the cancer cells as "non-self". In this context, we and others have taken note of the finding that cancer cells tend to exhibit significant alterations in the nature and quantity of carbohydrates displayed on their cell surfaces, either as glycoproteins or as glycolipids.[45] Conceivably, if introduced properly to the immune system, a tumor-associated carbohydrate-based antigen could invoke an immune response, leading to the generation of antibodies that would selectively bind to and eliminate tumor cells over-expressing the carbohydrates in question.[46]

Although a number of tumor-associated cell surface carbohydrates have been identified, efforts to obtain significant quantities of these epitopes through isolation from natural sources have been complicated due to the heterogeneity of naturally occurring carbohydrates. Our group has long promoted the therapeutic potential of *fully synthetic*



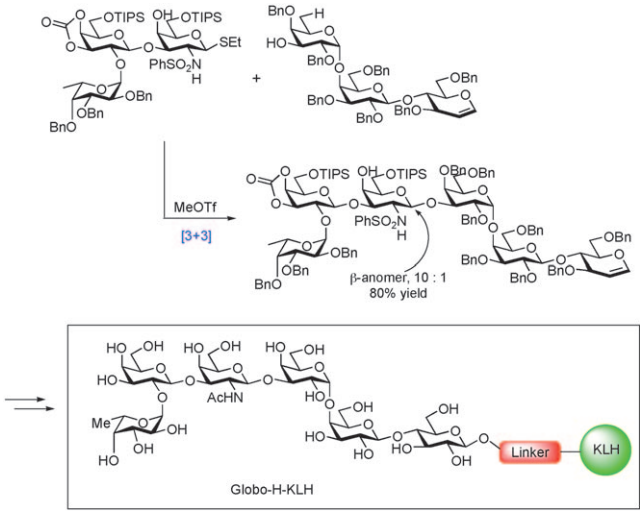
Scheme 16. Methods for functionalization of the terminal glycal.

carbohydrate-based antigens. Through synthesis, it is possible to ensure carbohydrate purity and homogeneity to an extent not possible through isolation. As described above, a long-standing program in our laboratory has been directed toward the development of technologies to enable carbohydrate assembly. We have utilized our continuously improving methodologies to prepare fully synthetic carbohydrate-based antitumor vaccines of increasing levels of complexity and, hopefully, therapeutic value.

Our first-generation fully synthetic carbohydrate-based vaccines were monovalent in nature—that is, each construct consisted of a single tumor-associated antigen conjugated to an immunogenic carrier molecule (such as the KLH protein) through a short linker. A number of these first-generation monovalent vaccines showed promise in immunological settings, and one, Globo-H–KLH[47] (Scheme 17) is scheduled to advance to phase II/III clinical trials against breast cancer in the near future.

As our anticancer carbohydrate vaccine program progressed, we gained valuable insight into the nature of the immunogenic response achieved with our synthetic constructs, and some of the shortcomings inherent in the first-generation monovalent approach became evident. With new information in hand, we began to design even more complex vaccine constructs. Fortunately, we were concurrently developing increasing powerful methods for carbohydrate synthesis. A full account of the progression of the carbohydrate-based vaccine program is beyond the scope of this Review, and may be found elsewhere.[48] We present herein some of our most recent efforts at the forefront of this program.

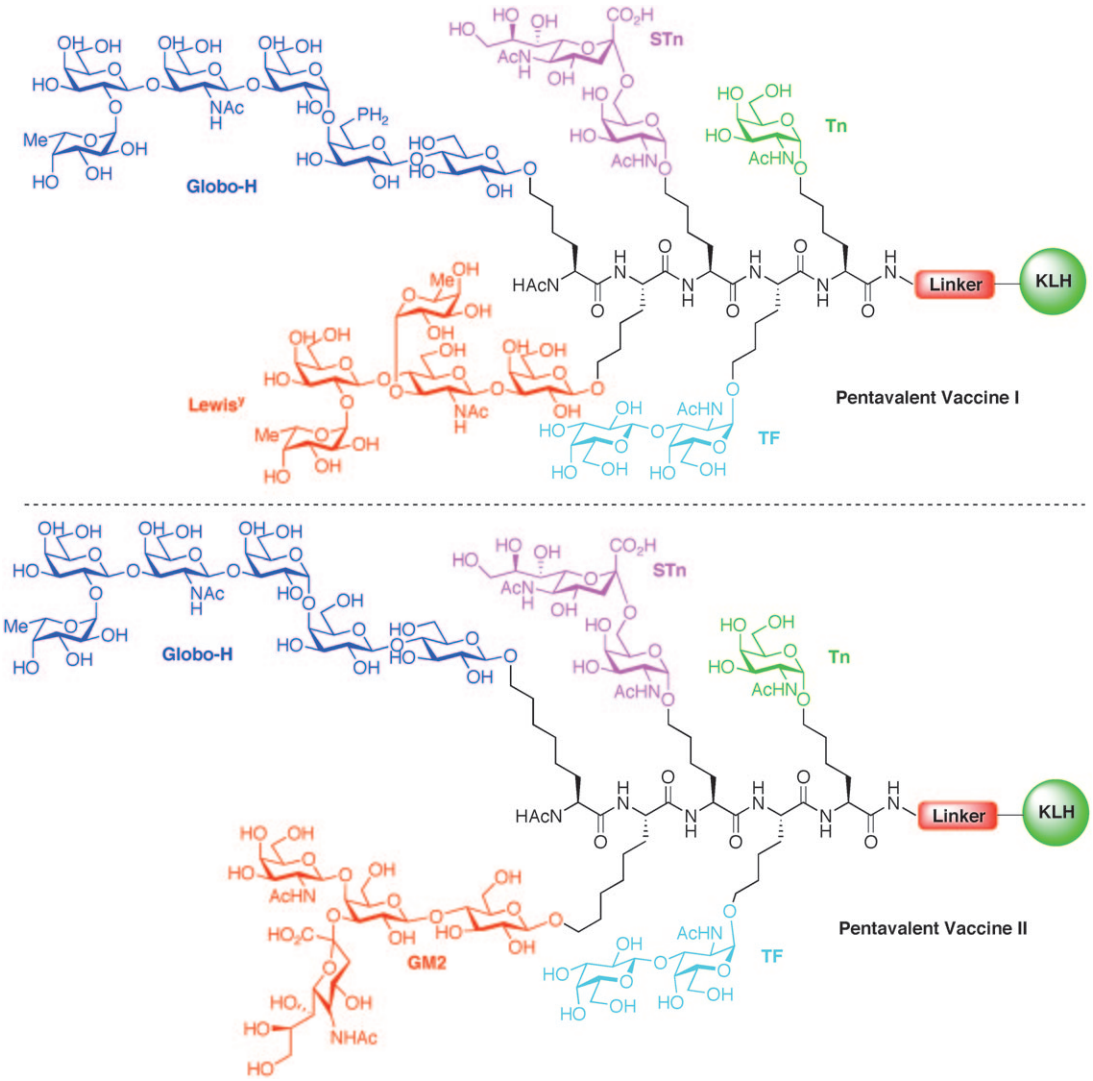
One significant constraint of the monovalent vaccine concept stems from the fact that there is actually substantial heterogeneity of antigen expression on tumor cell surfaces. Thus, varying degrees of heterogeneity are observed with regard to the type and distribution of antigens expressed on



Scheme 17. Globo-H–KLH conjugate.

the cell surfaces, with levels and distributions often fluctuating as a function of the stage of cellular development.[49] Indeed, even within a particular cancer type, there is often a great deal of antigen heterogeneity. In theory, a multiantigenic construct, incorporating multiple different carbohydrate-based antigens associated with a particular cancer type, could be employed to induce varied antibodies that would effectively target a greater proportion of tumor cells.

We have sought to address the issue of heterogeneity of cell surface carbohydrates through the synthesis of unimolecular, multiantigenic vaccine constructs. As shown in Scheme 18, we synthesized a highly complex pentavalent vaccine construct incorporating five antigens—Globo-H, Lewis^Y, STn, TF, and Tn—each of which is associated with both breast and prostate cancer.[50] Preliminary immunolog-

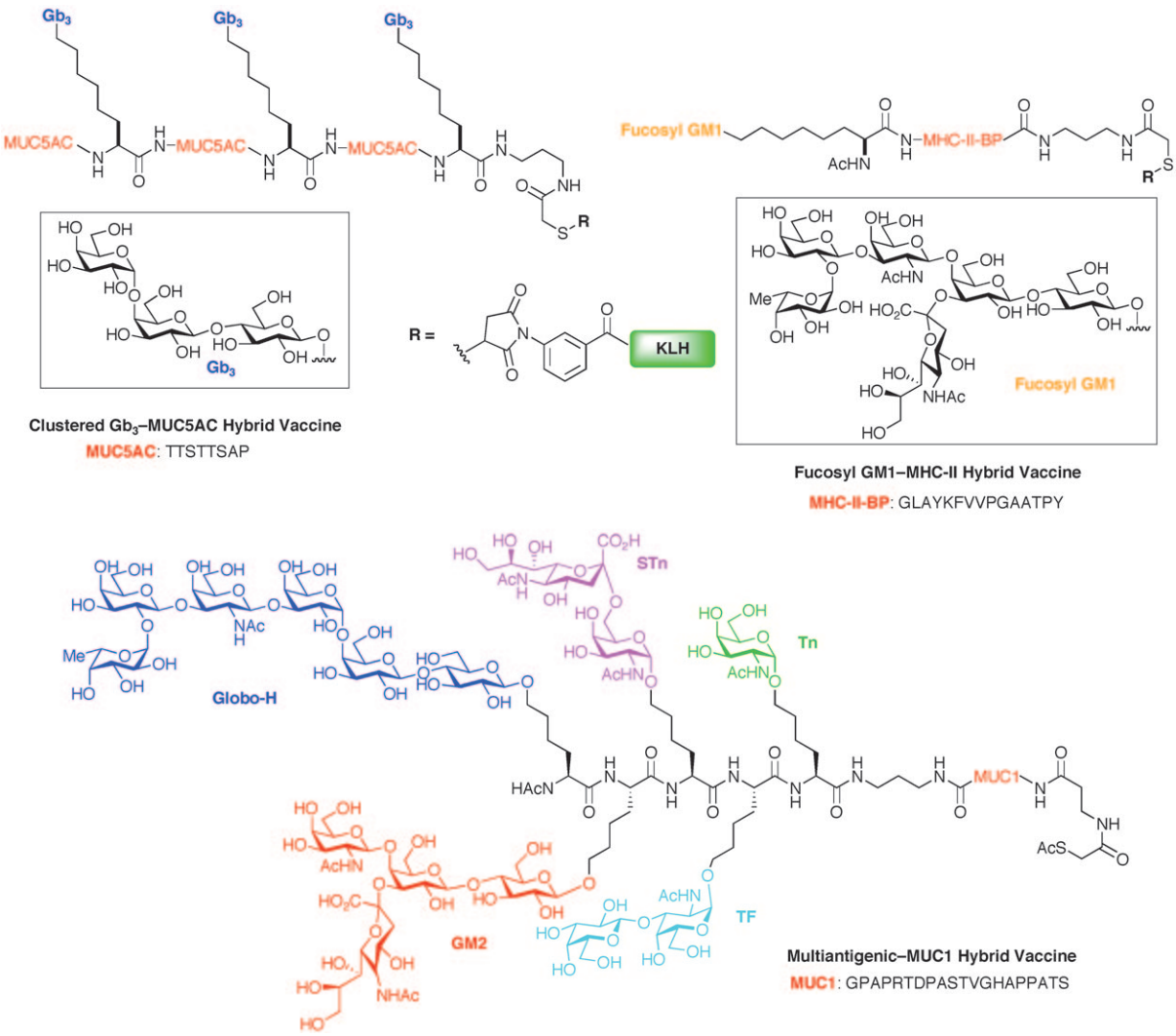


Scheme 18. Unimolecular multiantigenic vaccines.

ical studies yielded promising results.[51] Mice immunized with the KLH-conjugated pentavalent vaccine I produced IgM and/or IgG antibodies against four of the five antigens, as determined by ELISA assay. Only the Lewis^Y antigen evoked no measurable immune response, presumably due to the fact that it is endogenously expressed.

On the basis of these data, we prepared a second-generation pentavalent vaccine II, in which the Lewis^Y carbohydrate was replaced with GM2, which is also known to be overexpressed on breast and prostate cancer cell surfaces.[52] Happily, upon inoculation of mice with the KLH-conjugated pentavalent vaccine II, IgM and/or IgG antibodies were generated against each of the five antigens in the vaccine construct. This second-generation pentavalent vaccine II is currently being readied for phase I clinical trials.

Having identified what we believe to be a promising concept based on chemical synthesis for future anticancer vaccine development, we are simultaneously pursuing improved strategies for the presentation of the vaccine construct to the immune system. A particular challenge which we seek to address is that of evoking a robust T-cell response. Studies have indicated that carbohydrate-based antigens, on their own, do not necessarily induce strong T-cell responses. Several options are now being explored which seek to address this limitation. At the forefront of these studies are a number of newly synthesized bidomainal vaccine constructs which incorporate both the tumor associated carbohydrate sectors as well as peptide fragments that are expected to enhance the T-cell response. In this regard, we take particular note of the tumor-associated mucin peptides,[53] which are believed to trigger a T-cell response.[54] Their incorporation into the backbone of the vaccine construct could conceivably result in an enhanced cumulative antibody response. As shown in Scheme 19, we have now prepared a clustered Gb_3-MUC5AC hybrid vaccine conjugate, consisting of alternating repeats of the tumor-associated carbohydrate antigen, Gb_3, and the MUC5AC-based mucin peptide.[55] We have similarly made substantial progress toward the synthesis of a multi-



Scheme 19. Next generation bidomainal carbohydrate-based anticancer vaccines.

antigenic–MUC1 hybrid, in which the immunogenic MUC1-based peptide is incorporated on the pentavalent vaccine II.[56]

A slightly different strategy is pursued in the fucosyl GM1–MHC-II hybrid vaccine construct. Immunogenic carrier proteins, such as KLH, incorporate MHC-II binding peptides, which assist in presenting the carbohydrate epitopes of the vaccine to the T-cells for activation.[57] The immunogenicity of a vaccine can be enhanced by ensuring proximity of the MHC-II binding peptides to the carbohydrate epitopes. Although our constructs are already conjugated to KLH, proximity to an MHC-II sequence could be further ensured through incorporation of an MHC-II binding peptide onto the actual glycopeptide. We have thus synthesized a hybrid conjugate, incorporating the carbohydrate antigen, fucosyl GM1, appended to an MHC-II binding peptide.[58] Immunological evaluations of these vaccine constructs are now underway.

In short, the fully synthetic carbohydrate-based anticancer vaccine program underway in our laboratory has developed markedly over the past decade. Eight phase I trials with fully synthetic carbohydrate based antigens have been conducted. The strength and versatility of the program rests on de novo chemical syntheses of increasingly complex carbohydrate-based constructs. Through close interaction with immunological and clinical collaborators, we are able to identify the strengths and shortcomings of our synthesized constructs, and to develop ever more effective and broadly potent candidates for development. Although these type of large, complex molecules are traditionally classified in the realm of "biologicals", this program has greatly benefited from the logic of iterative chemical synthesis and biological evaluation which we also bring to our small-molecule endeavors. Needless to say, the execution of this type of complex carbohydrate synthesis program brings with it its own set of synthesis level issues, and the advancement of the program has necessitated the development of a range of enabling methodologies, which should be of value to the broader carbohydrate synthesis community.

3.3. Glycoprotein Synthesis

We close out this Review with a discussion of a more recent focus of our "biological" synthesis program: the de novo synthesis of naturally occurring, biologically active glycoproteins. The post-translational glycosylation of proteins is a common natural phenomenon, and the carbohydrate domains thus appended often play an important role in conferring protein stability and biological activity.[59] There is a growing appreciation of the potential therapeutic value to be derived from the glycoprotein estate. Among the most high profile glycoprotein therapeutics currently used in the clinic are the red blood cell stimulating agent, erythropoietin alpha (EPO),[60] and the fertility agent, human follicle stimulating hormone (hFSH).[61]

Despite widespread research in the glycoprotein field, a significant complicating factor remains: that is, the glycoprotein is typically biosynthesized as a mixture of glycoforms, which are not readily separable.[62] The question arises as to whether there are any advantages to having access to homogeneous versions of such systems. Recent advances in biosynthetic engineering have begun to successfully address the problem of biosynthesizing homogeneous glycoproteins. Notably, glycoengineering of the yeast *Pichia pastoris*,[63] has enabled the production of homogeneous sialylated glycoproteins. As an alternative strategy, de novo chemical synthesis should provide a viable means by which to gain access to single glycoforms of a glycoprotein, and to study in a systematic fashion the consequences of glycosylation on molecular structure and biological function.

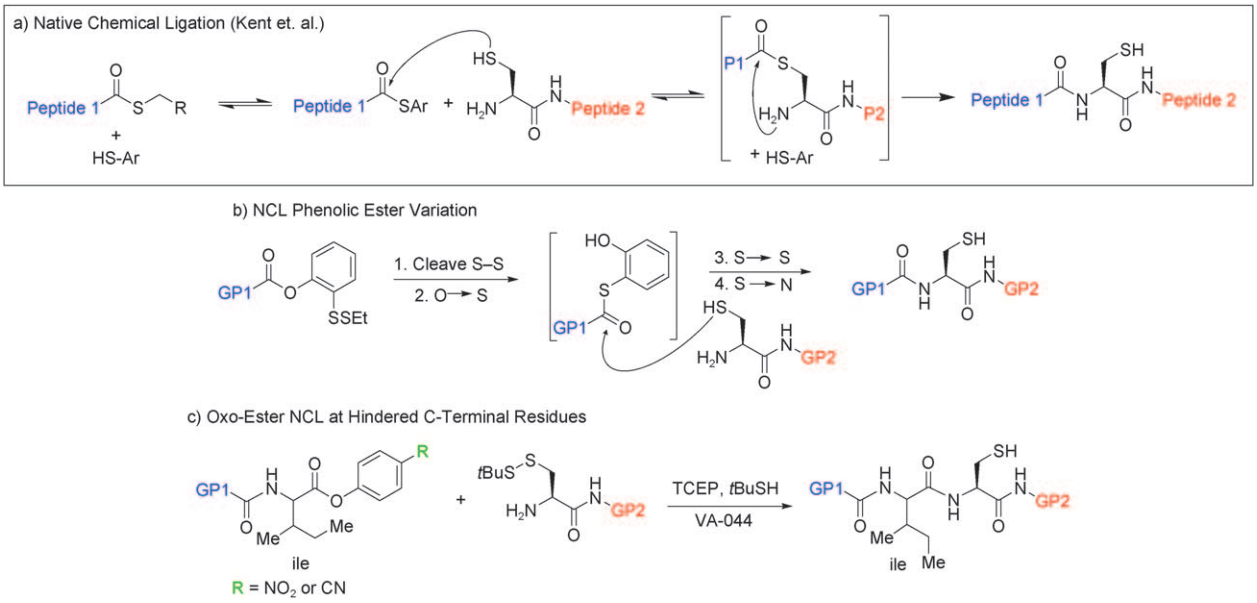
As described below, we have launched a broad based glycoprotein synthesis program, the ultimate goals of which are the homogeneous syntheses of two therapeutically relevant, multiply glycosylated proteins: EPO and hFSH. Along the way, we have developed a number of enabling methods for the efficient ligation of complex peptide and glycopeptide fragments.

3.3.1. Development of Methods for the Ligation of Glycopeptides

The glycoprotein total synthesis effort has provided myriad opportunities for the development of novel methodologies which we anticipate will accrue to the benefit of the synthetic community as a whole. The de novo synthesis of a large, multiply glycosylated protein is no straightforward task, and at the outset of this effort, there was a clear deficit in terms of the methodologies available to effect all aspects of the process, from the synthesis of the oligosaccharide domain, to the merger of the carbohydrate to the peptide domain, and finally to the ligation of two glycopeptide fragments. Although a full treatment of the methodologies developed in the course of this project are outside of the scope of this discussion,[64] it is perhaps useful to highlight several advances in the ligation area, which we have found to be particularly critical to the advancement of our glycoprotein total synthesis program.

In 1994, Kent and co-workers disclosed a major breakthrough in the field of peptide synthesis—native chemical ligation (NCL).[65] NCL is a broadly useful technique that enables the coupling of large peptide fragments. As shown in Scheme 20a, NCL involves the merger of two peptides, of which one is equipped with a C-terminal thioester, while the other presents an N-terminal cysteine residue.

In the context of our strategy toward multiply glycosylated proteins, we sought to extend the reach of NCL to encompass the merger to two glycopeptide fragments. A direct extension of the Kent NCL methodology was not considered to be a practical solution to this problem, as there was concern about the difficulty posed in the synthesis of a pre-formed glycopeptide thioester. Rather, we developed a



Scheme 20. Cysteine-based glycopeptide–glycopeptide ligation methods. TCEP = tris(2-carboxyethyl)phosphine, VA-044 = 2,2′-azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride.

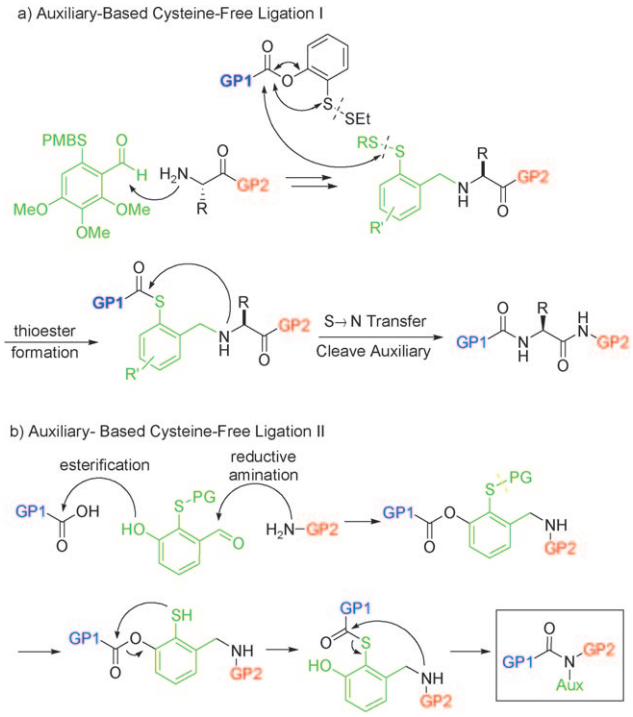
modified solution involving the installation of a relatively inert C-terminal *ortho*-thiophenolic ester on one of the glycopeptide fragments and a protected N-terminal cysteine residue on the other glycopeptide fragment (Scheme 20b).[66] Upon simultaneous reduction of the two disulfides, the phenol moiety undergoes intramolecular O→S migration to provide an intermediate thioester, which is sufficiently activated to undergo intermolecular thioester exchange with the free cysteine residue of the second glycopeptide. The resultant unimolecular intermediate spontaneously suffers intramolecular acyl transfer to yield the bidomainal glycopeptide adduct, incorporating two differential sites of glycosylation.

More recently, we have developed a direct oxo-ester variant, in which the phenolic ester, equipped with *p*-NO_2 or *p*-CN substitution, is sufficiently activated to undergo cysteine ligation (Scheme 20c).[67] These activated oxo-esters have been found to be particularly well suited to ligation at hindered C-terminal residues, such as isoleucine (ile).

With a viable solution to the general glycopeptide–glycopeptide ligation problem in hand, we began to consider a further complication posed by glycoprotein total synthesis endeavors. Thus, current glycopeptide ligation protocols, as depicted in Scheme 20, had required the presence of a cysteine residue at the ligation site. However, cysteine residues are actually quite rare in naturally occurring proteins and glycoproteins. Our global EPO synthesis strategy, for instance, calls for the assembly of four individual peptide fragments, each bearing a single carbohydrate domain, which will then be merged according to our glycopeptide ligation protocol; however, the EPO peptide backbone does not incorporate cysteine residues at logical disconnection points. Fortunately, we have devised several solutions to this problem.

Two auxiliary-based cysteine free ligation protocols have been developed in our laboratory. Both borrow heavily from the logic employed in our original cysteine-based ligation method (see Scheme 20b). The first protocol, as outlined in Scheme 21a, involves the installation, on the N-terminal glycopeptide fragment, of a thiobenzene auxiliary.[68] This auxiliary serves the role of a surrogate cysteine residue, temporarily engaging the two glycopeptide fragments in order to bring the reactants into sufficient proximity to undergo S→N acyl transfer. Following a simple two-step removal of the auxiliary, the glycopeptide is in hand. This cysteine-free ligation sequence has been found to be effective even in complex settings. A practical limitation of this ligation arises from the fact that the reaction is most efficient when at least one of the terminal amino acid residues is either a glycine or an alanine. In cases where both amino acids at the ligation site are highly branched, the reaction yield is drastically compromised.

A second cysteine-free ligation protocol developed in our laboratory is shown in Scheme 21b.[69] This ligation features a substrate, wherein the two glycopeptide fragments are positioned in a *meta* arrangement on the benzylic framework, with a protected thiol residing between the two fragments. Thiol deprotection then sets into motion an O→S acyl transfer, and the resulting thioester is then positioned to undergo intramolecular S→N acyl transfer with the amine of the second glycopeptide. Although this elegant reaction is quite efficient, a major practical issue currently remains in that the auxiliary is not readily removed through standard methods.

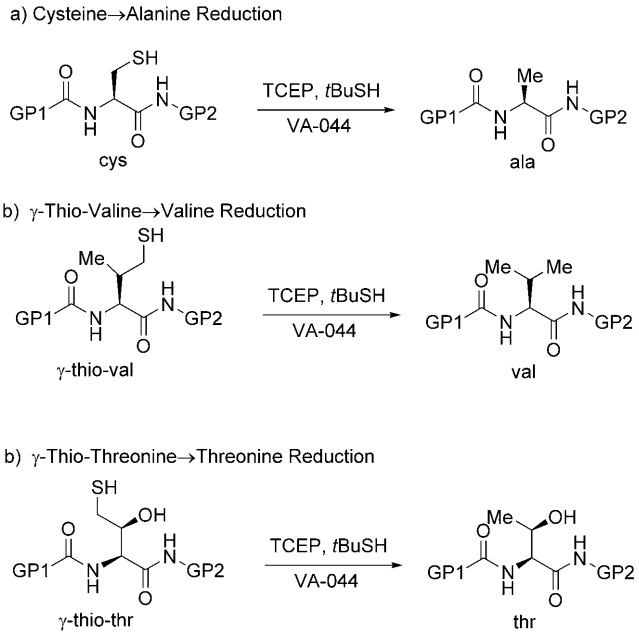


Scheme 21. Cysteine-free glycopeptide–glycopeptide ligation methods.

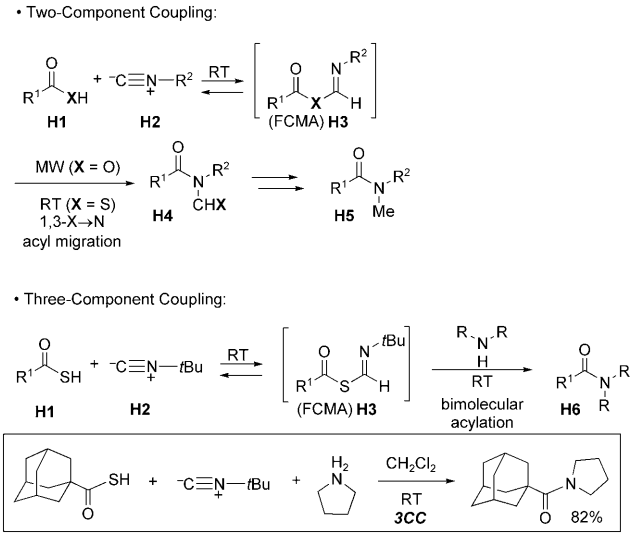
Finally, in a highly useful and practical methodological advance, we have developed an efficient, free radical-based desulfurization protocol,[70] which allows for the selective conversion of a cysteine to an alanine,[70] a γ-thiovaline to a valine,[71] and a γ-thiothreonine to a threonine,[72] each in the context of a complex glycopeptide (Scheme 22). Importantly, alanine, valine, and threonine residues are significantly more abundant in natural proteins and glycoproteins than are cysteine residues. Thus, through a simple two-step glycopeptide ligation/reduction strategy, we are now able to formally achieve ligation at alanine, valine, and threonine sites.

3.3.2. *Isonitriles*

Most recently, our explorations in the field of isonitrile chemistry have led to the development of novel methods for the formation of complex peptide bonds under neutral reaction conditions.[73] As outlined in Scheme 23, we have developed a "two-component coupling" (2CC) strategy, wherein acid and isonitrile substrates react to form *N*-formyl amide adducts. We have demonstrated that this reaction proceeds through the intermediacy of a formimidate carboxylate mixed anhydride (FCMA or thio-FCMA) species (cf. **H3**, X = S or O), which undergoes spontaneous 1,3-X→N acyl transfer to deliver the *N*-formyl amide adduct. Importantly, the *N*-formyl functionality is highly versatile and may be readily converted to the corresponding *N*-methyl group.[74a]



Scheme 22. Radical desulfurization-based cysteine-free glycopeptide–glycopeptide ligation methods.



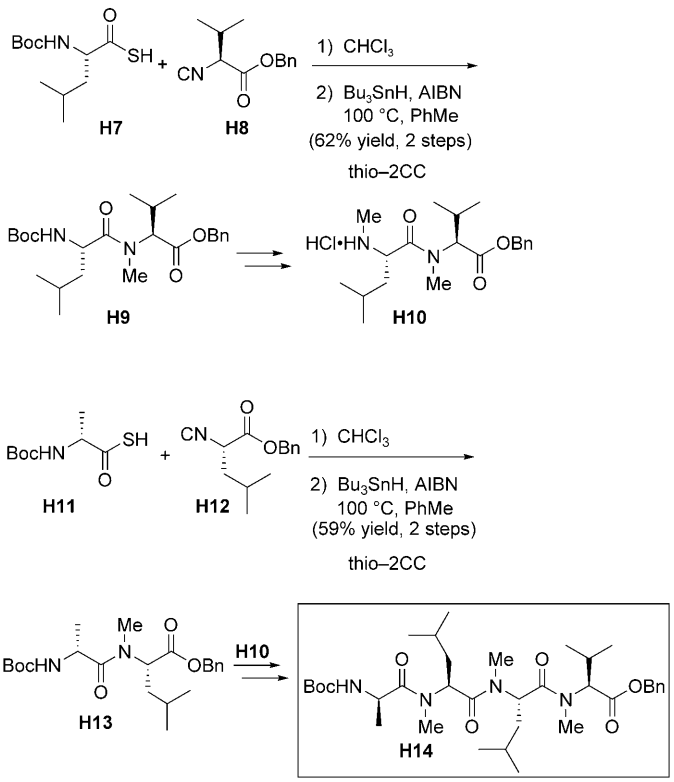
Scheme 23. Isonitrile-based amide coupling reactions: two-component coupling (2CC) and three-component coupling (3CC).

This transformation thus allows access to valuable and synthetically challenging tertiary amide motifs. We have observed thioacids to be significantly more reactive than the corresponding acid substrates, and, while coupling of carboxylic acid substrates requires the use of microwave conditions, 2CC reactions performed with thioacid coupling partners may be conducted at ambient temperatures.

In an important extension of this method, we next sought to investigate whether the presumed FCMA intermediates (**H3**) might also serve as viable bimolecular acylating agents.[74] Along these lines, we hoped to identify a set of reaction conditions wherein the FCMA species would be intercepted by an appropriate external nucleophile prior to undergoing intramolecular 1,3-X→N acyl migration. Under this scenario, compound **H2** would represent a simple, "throwaway" isonitrile species, and would not be incorporated into the final reaction product. The benefit of such a transformation would be that the amine coupling partner could be employed directly in the coupling step, thereby obviating the need to pre-form a "high value" isonitrile motif. The viability of this approach has indeed been demonstrated (Scheme 23). Thus, in the presence of *tert*-butylisonitrile, a range of thioesters and primary or secondary amine substrates undergo coupling at room temperature to provide amide adducts. Mechanistic studies have provided strong evidence for the role of the thio-FCMA intermediate as the active acyl donor in this transformation.

3.3.3. *Cyclosporine A*

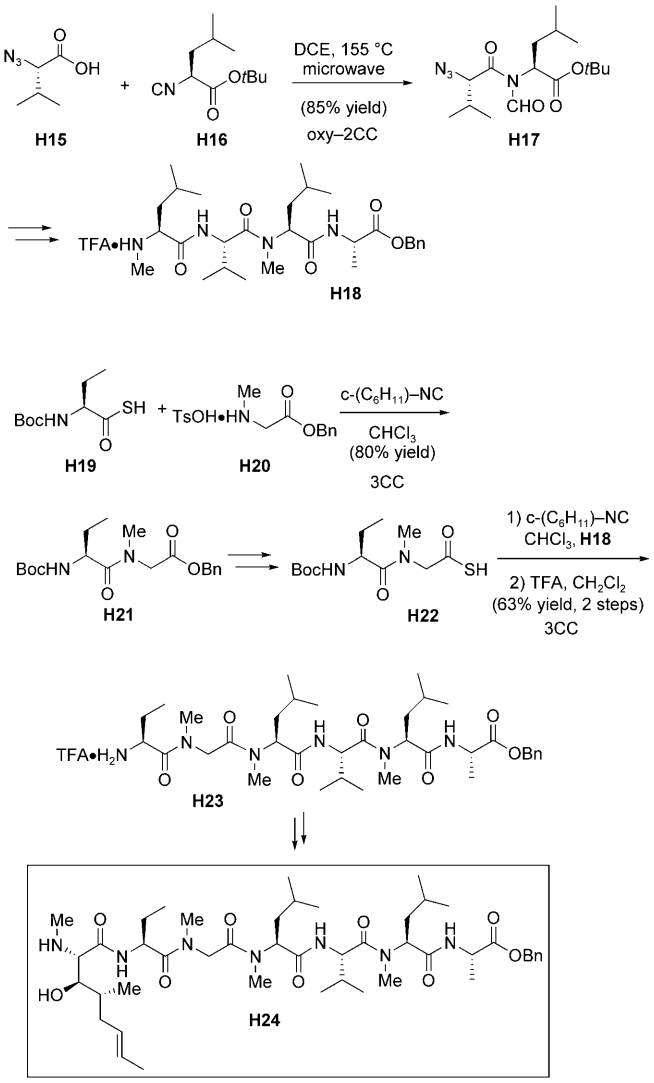
The power and versatility of the isonitrile-based amide formation approach, described above, has now been demonstrated in the context of a rapid synthesis of the cytokine inhibitor, cyclosporine A (Schemes 24–26).[75] This high-profile cyclic peptide natural product[76] possesses seven sites of N-methylation, which are critical to its observed biological activity. We anticipated that our novel isonitrile-based methods would be ideally suited to deliver the cyclic peptide backbone presenting the requisite N-methylation pattern. Our synthetic approach toward cyclosporine envisioned the assembly of two peptide fragments—**H14** and **H24**. As outlined in Scheme 24, **H14** was prepared in short order



Scheme 24. Synthesis of **H14** en route to cyclosporine A.

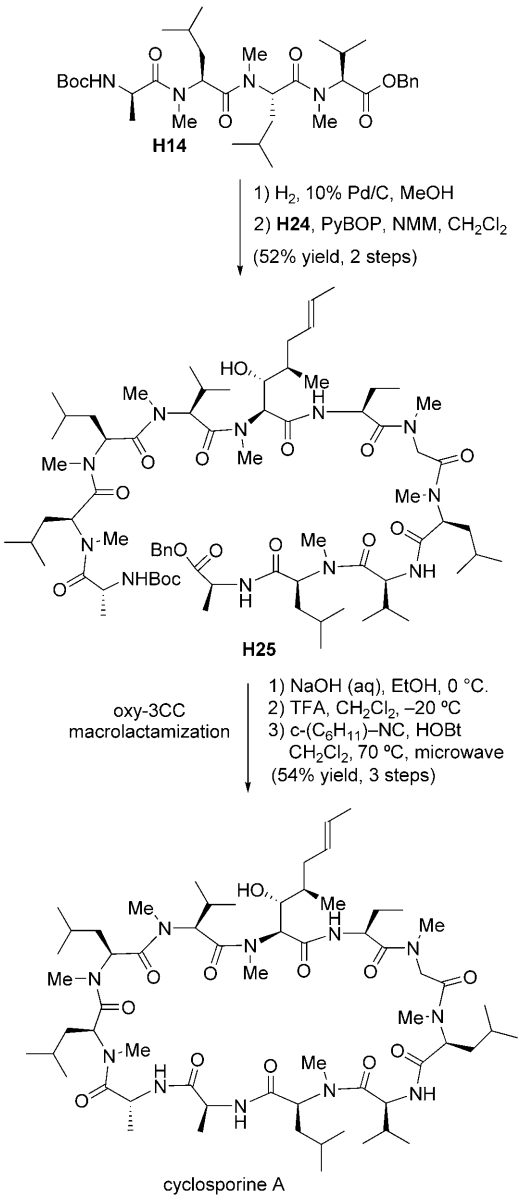
through a sequence featuring two-component coupling reactions between thioacid and isonitrile substrates.

The synthesis of dipeptide **H17**, en route to **H24**, was accomplished through a microwave-mediated 2CC between carboxylic acid **H15** and isonitrile **H16** (Scheme 25). The synthesis of **H24** also featured two direct 3CC reactions between thioacid and amine substrates in the presence of "sacrificial" cyclohexylisonitrile.



Scheme 25. Synthesis of **H24** en route to cyclosporine A. DCE = 1,2-dichloroethane.

As outlined in Scheme 26, peptides **H14** and **H24** were joined to afford intermediate **H25**. At this stage, we were able to successfully extend the logic of our 3CC methodology to the context of the key macrolactamization event. The success of this lactam forming reaction was particularly noteworthy in light of the fact that our previous success with 3CC reactions had been limited to the more reactive thioacid substrates. However, in the case at hand, the substrate is preorganized as



Scheme 26. Synthesis of cyclosporine A. PyBOP = (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate, HOBt = Hydroxybenzotriazole.

a result of intrastrand hydrogen bonding. Thus, in the presence of HOBt and cyclohexylisonitrile, the Boc-deprotected carboxylic acid derived from **H25** underwent macrolactamization to furnish the natural product, cyclosporine A in good overall yield (54% from **H25**). In short, the rapid and efficient synthesis of cyclosporine A, outlined herein, serves to illustrate the complexity-building potential of our newly developed isonitrile-based amide formation approach.

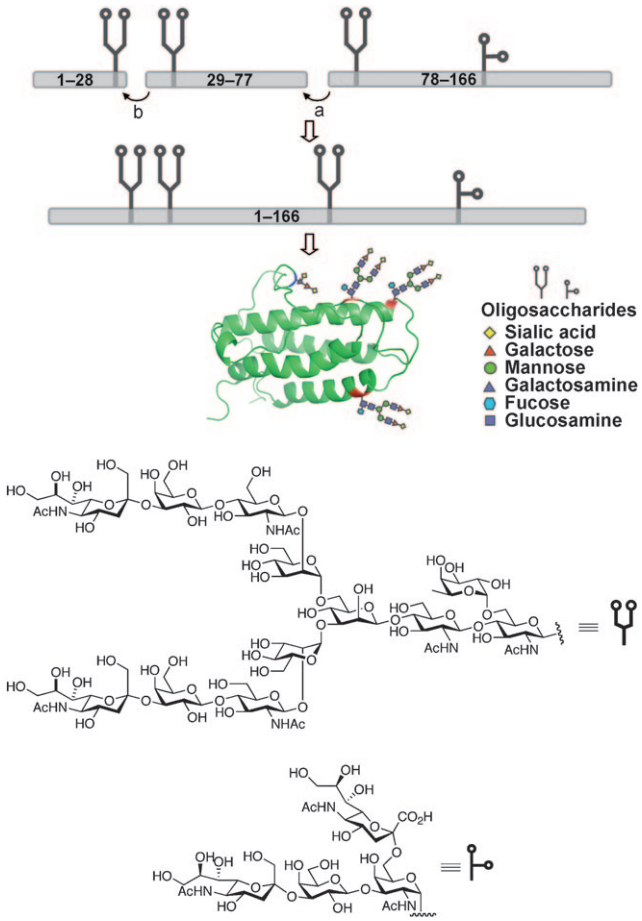
3.3.4. Erythropoietin

Erythropoietin (EPO), a naturally occurring glycoprotein that stimulates the body to produce red blood cells, is commonly used in the clinic for the treatment of cancer-

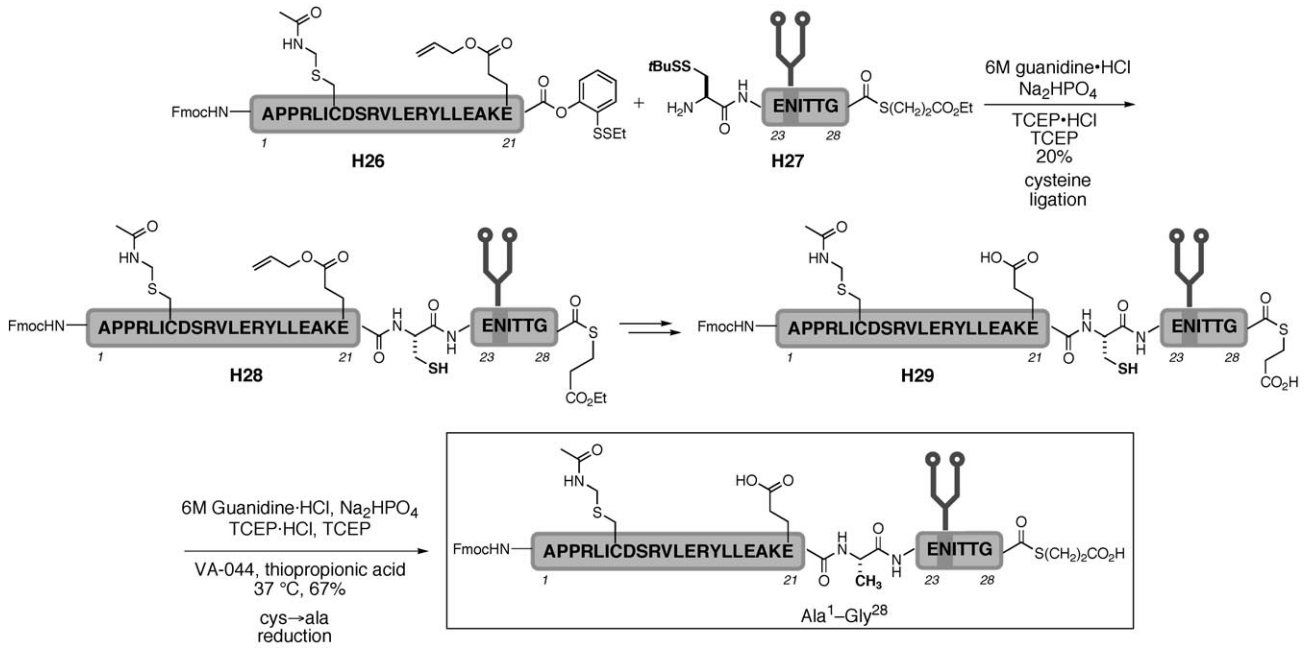
related and chronic anemia. Cancer patients may develop anemia during chemotherapy, and erythropoietin is often required in order to allow for the continuation of such treatment. However, erythropoietin, a 166-residue conserved protein possessing four sites of glycosidation, is currently obtained from natural sources or through recombinant methods only as a mixture of glycoforms. It is understood that the various erythropoietin glycoforms presumably exhibit differential levels of biological activity; however, lacking the ability to access structurally homogeneous erythropoietin, it becomes very difficult to rigorously evaluate the relative value of individual glycoforms of the glycoprotein, and to thus attempt to develop improved erythropoiesis agents.

Our laboratory is pursuing the convergent total synthesis of homogeneous erythropoietin alpha. A long-term objective will be the assembly of a small collection of synthetic, homogeneous erythropoietin glycoforms, which will be evaluated in the hopes of establishing an SAR profile of erythropoietin and, perhaps, identifying improved erythropoiesis agents. As outlined in Scheme 27, our governing strategy toward EPO involves the assembly of three glycopeptide units: EPO(1–28), EPO(29–77), and EPO(78-166). These large fragments will then be iteratively joined through sequential fragment condensations to provide the homogeneous glycoprotein.

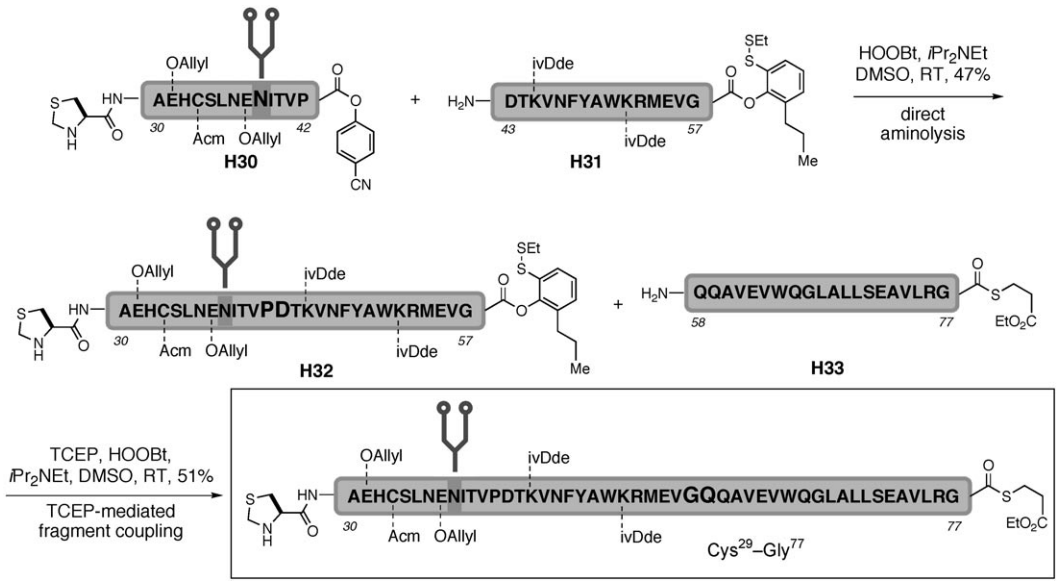
Through reliance on the glycoprotein ligation methods described above, we recently synthesized the three large glycopeptide fragments that together constitute the entire peptide backbone as well as the four oligosaccharide domains of erythropoietin. Thus, as outlined in Scheme 28, the synthesis of the Ala[1]–Gly[28] fragment featured a cysteine ligation between **H26** and **H27** to provide glycopeptide **H28**.[77] At the



Scheme 27. Synthetic strategy toward erythropoietin alpha (EPO).



Scheme 28. Synthesis of Ala[1]–Gly[28] fragment of EPO.



Scheme 29. Synthesis of Cys^{29}–Gly^{77} fragment of EPO. HOOBt = Hydroxy-3,4-dihydro-4-oxo-1,2,3-benzotriazine.

stage of intermediate **H29**, the sulfur reduction methodology developed in our laboratory (see Scheme 22) was used to effect the requisite cys→ala conversion at the ligation site, delivering the Ala^{1}–Gly^{28} fragment of EPO.

In our synthesis of the Cys^{29}–Gly^{57} unit, we employed a rapid and convergent reiterative fragment coupling strategy.[78] As shown in Scheme 29, we first prepared glycopeptide **H30**, presenting a C-terminal *para*-cyanophenyl ester, and peptide **H31**, equipped with a C-terminal masked thioester possessing both an *ortho*-disulfide group and an *ortho*-propyl functionality. We had found the *ortho*-propyl group to be crucial in ensuring suppression of hydrolysis during the first coupling reaction. In the event, **H30** and **H31** smoothly underwent direct aminolysis to provide **H32**. Next, intermediate **H32** participated in a TCEP-mediated fragment coupling with peptide **H33**, to generate the Cys^{29}–Gly^{77} glycopeptide domain. Thus, the masked C-terminal thioester, which had been inert in the first fragment coupling, subsequently served as an effective acyl donor under modified coupling conditions. We note that the Cys^{29}–Gly^{77} fragment possesses a C-terminal alkyl thioester, which is expected to serve as a useful functional handle in the eventual merger of the three EPO domains.

Finally, the Gln^{78}–Arg^{166} fragment, possessing two differentiated sites of glycosylation, was assembled through sequential TCEP/AgCl fragment couplings.[79] As outlined in Scheme 30, TCEP-mediated fragment coupling between glycopeptide **H34** and peptide **H35** provided intermediate **H36**, possessing a C-terminal alkyl thioester. While unreactive under TCEP conditions, this alkyl thioester functionality is susceptible to AgCl activation. Thus, under AgCl coupling conditions, **H36** readily underwent fragment coupling with glycopeptide **H37** to afford the Gln^{78}–Arg^{166} domain. Efforts are currently underway to merge these three large units and thus to complete the first total synthesis of the complex, multiply glycosylated protein, EPO.

3.3.5. FSH

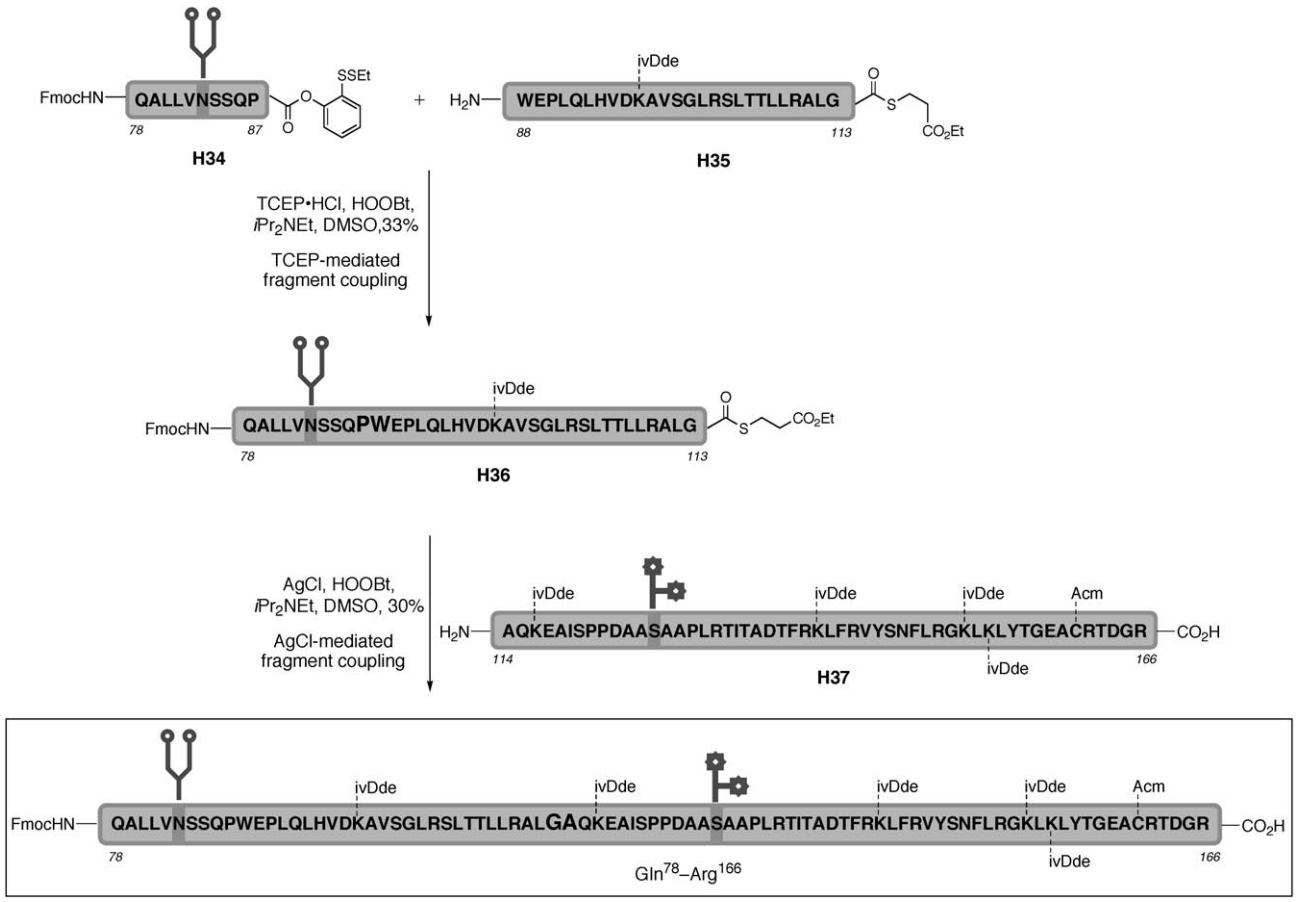
Human follicle stimulating hormone (hFSH, Scheme 31) is a biologically relevant glycoprotein that plays a role in the treatment of anovulatory disorders and in assisted reproductive technologies, such as intrauterine insemination (IUI). hFSH exists as a heterodimer, possessing two sites of glycosylation on each domain (α and β). In the context of our program directed toward the total synthesis of therapeutically important glycoproteins, we have been engaged in the total synthesis of hFSH.

At present, the syntheses of both the α- and β-FSH subunits are concurrently being pursued.[80] From a retrosynthetic standpoint, each domain is split into four subunits of roughly equal size. These will be merged through standard methodologies developed in our laboratory and elsewhere. Thus far, we have synthesized all four peptide portions of the α-domain, as well as three of the four peptide fragments of the β-domain. In addition, the complex glycan that is common to subunits 1 and 2 of the β-domain has been synthesized. Efforts are currently underway to append the glycans to the peptide fragments.

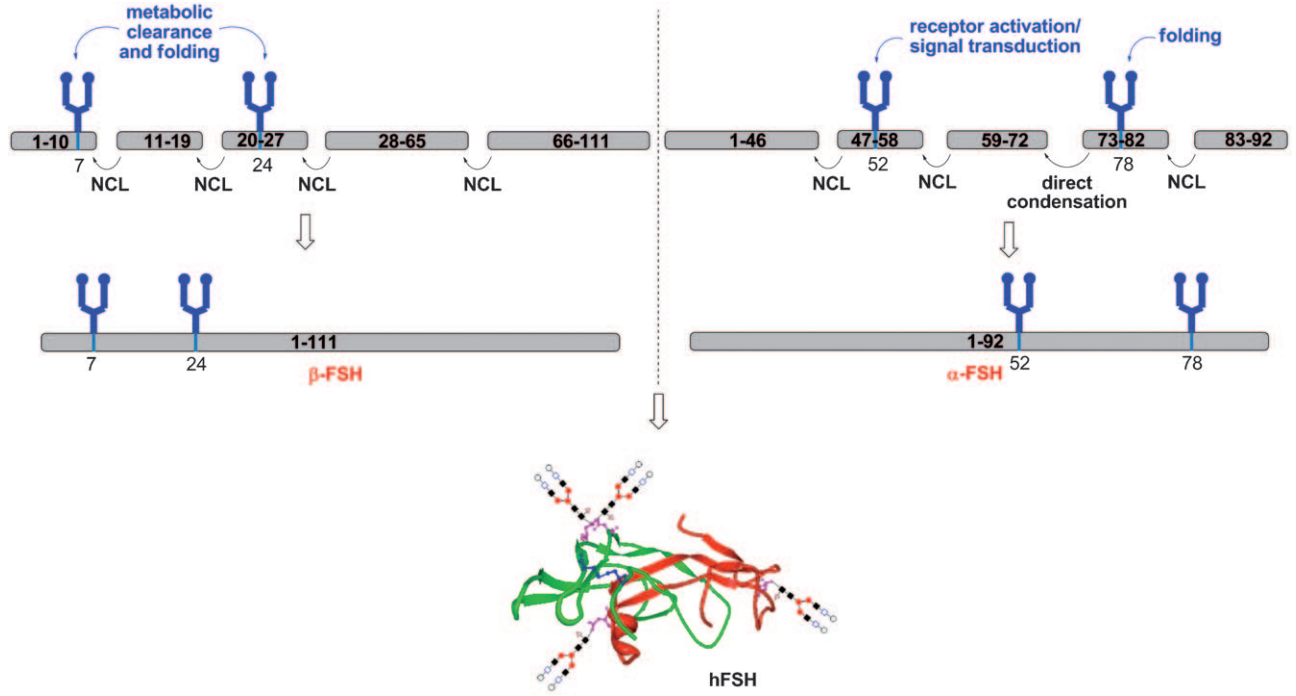
4. Conclusions

Traditionally, there are many reasons that are advanced on behalf of the field of organic synthesis. One of them, of course, is that synthesis has conventionally been the bedrock of the pharmaceutical industry in creating and optimizing lead compounds. Moreover, there is the problem-solving dimension which complex target synthesis provides. Without these sorts of frontier seeking challenges, the field of synthesis is not likely to realize the quantum jump advances that it can in the context of responding to these exciting callings.

Perhaps in a broader sense, the work described above indicates that there need not be a tension between these various pursuits. Even work in complex systems can have ramifications for drug discovery as well as optimization. Needless to say, our laboratory is not a pharmaceutical company and lacks the resources to go from discovery, through DTS, to actual state-of-the-art diligence. For that, including advancements to human clinical trials, to happen, the concepts we provide here would have to form the basis of



Scheme 30. Synthesis of Gln^{78}–Arg^{166} fragment of EPO.



Scheme 31. Synthetic strategy toward human follicle stimulating hormone (hFSH).

collaborations which are capable of moving from discovery to implementation. We hope that this will be the case.

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[1] a) A. M. Rouhi, *Chem. Eng. News* **2003**, *78*(October 13), 77; b) G. A. Cordell, *Phytochem. Rev.* **2002**, *1*, 261; c) W. R. Strohl, *Drug Discovery Today* **2000**, *5*, 39.

[2] For lead references on the role of natural products in drug development, see: a) D. J. Newman, G. M. Cragg, K. M. Snader, *J. Nat. Prod.* **2003**, *66*, 1022; b) M. S. Butler, *J. Nat. Prod.* **2004**, *67*, 2141; c) Y.-Z. Shu, *J. Nat. Prod.* **1998**, *61*, 1053; d) D. J. Newman, G. M. Cragg, K. M. Snader, *Nat. Prod. Rep.* **2000**, *17*, 215.

[3] For a review, see: K. C. Nicolaou, W.-M. Dai, R. K. Guy, *Angew. Chem.* **1994**, *106*, 38; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 15.

[4] Isolation: a) C. Vézina, A. Kudelski, S. N. Sehgal, *J. Antibiot.* **1975**, *28*, 721; b) S. N. Sehgal, H. Baker, C. Vézina, *J. Antibiot.* **1975**, *28*, 727; Structure elucidation: c) D. C. N. Swindells, P. S. White, J. A. Findlay, *Can. J. Chem.* **1978**, *56*, 2491; Synthesis: d) K. C. Nicolaou, T. K. Chakraborty, A. D. Piscopio, N. Minowa, P. Bertinato, *J. Am. Chem. Soc.* **1993**, *115*, 4419; e) D. Romo, S. D. Meyer, D. D. Johnson, S. L. Schreiber, *J. Am. Chem. Soc.* **1993**, *115*, 7906; f) C. M. Hayward, D. Yohannes, S. J. Danishefsky, *J. Am. Chem. Soc.* **1993**, *115*, 9345; g) A. B. Smith III, S. M. Condon, J. A. McCauley, J. L. Leazer, Jr., J. W. Leahy, R. E. Maleczka, Jr., *J. Am. Chem. Soc.* **1995**, *117*, 5407.

[5] a) M. Wainwright, *Miracle Cure: The Story of Penicillin and the Golden Age of Antibiotics*, Blackwell, Oxford, **1990**; b) D. J. Newman, G. M. Cragg, K. M. Snader, *Nat. Prod. Rep.* **2000**, *17*, 215; c) W. Sneader, *Drug Protoypes and their Exploitation*, Wiley, Chichester, **1996**; d) J. Mann, *The Elusive Magic Bullet: The Search for the Perfect Drug*, Oxford University Press, Oxford, **1999**, p. 39.

[6] For a lead review, see: P. A. Todd, K. L. Goa, *Drugs* **1990**, *40*, 583.

[7] For a lead review, see: A. P. Lea, D. McTavish, *Drugs* **1997**, *53*, 828.

[8] For a recent review on the synthesis (and DTS) of biologically relevant natural product inspired compound libraries, see: K. Kumar, H. Waldmann, *Angew. Chem.* **2009**, *121*, 3272; *Angew. Chem. Int. Ed.* **2009**, *48*, 3224.

[9] R. M. Wilson, S. J. Danishefsky, *J. Org. Chem.* **2006**, *71*, 8329.

[10] a) R. M. Wilson, S. J. Danishefsky, *J. Org. Chem.* **2007**, *72*, 4293; b) R. M. Wilson, S. J. Danishefsky, *Chem. Soc. Rev.* **2007**, *36*, 1207.

[11] G. Höfle, N. Bedorf, K. Gerth, H. Reichenbach, (GBF), DE-B 4138042, **1993** [*Chem. Abstr.* **1993**, *120*, 52841].

[12] D. M. Bollag, P. A. McQueney, J. Zhu, O. Hensens, L. Koupal, J. Liesch, M. Goetz, E. Lazarides, C. M. Woods, *Cancer Res.* **1995**, *55*, 2325.

[13] D.-S. Su, D. Meng, P. Bertinato, A. Balog, E. J. Sorensen, S. J. Danishefsky, Y.-H. Zheng, T.-C. Chou, L. He, S. B. Horwitz, *Angew. Chem.* **1997**, *109*, 775; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 757.

[14] A. Balog, D. Meng, T. Kamenecka, P. Bertinato, D.-S. Su, E. J. Sorensen, S. J. Danishefsky, *Angew. Chem.* **1996**, *108*, 2976; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2801.

[15] a) A. Rivkin, F. Yoshimura, A. E. Gabarda, Y. S. Cho, T.-C. Chou, H. Dong, S. J. Danishefsky, *J. Am. Chem. Soc.* **2004**, *126*, 10913; b) A. Rivkin, T.-C. Chou, S. J. Danishefsky, *Angew. Chem.* **2005**, *117*, 2898; *Angew. Chem. Int. Ed.* **2005**, *44*, 2838; c) Y. S. Cho, K.-D. Wu, M. A. S. Moore, T.-C. Chou, S. J. Danishefsky, *Drugs Future* **2005**, *30*, 737; d) T.-C. Chou, X. Zhang, Z.-Y. Zhong, Y. Li, L. Feng, S. Eng, D. R. Myles, R. Johnson, N. Wu, Y. I. Yin, R. M. Wilson, S. J. Danishefsky, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 13157.

[16] a) B. D. Hedley, E. Winquist, A. F. Chambers, *Expert Opin. Ther. Targets* **2004**, *8*, 527; b) L. Pérez, S. J. Danishefsky, *ACS Chem. Biol.* **2007**, *2*, 159.

[17] a) K. Nakae, Y. Yoshimoto, T. Sawa, Y. Homma, M. Hamada, T. Takeuchi, M. Imoto, *J. Antibiot.* **2000**, *53*, 1130; b) K. Nakae, Y. Yoshimoto, M. Ueda, T. Sawa, Y. Takahashi, H. Naganawa, T. Takeuchi, M. Imoto, *J. Antibiot.* **2000**, *53*, 1228; c) Y. Takemoto, K. Nakae, M. Kawatani, Y. Tahahashi, H. Naganawa, M. Imoto, *J. Antibiot.* **2001**, *54*, 1104; d) H. Nakamura, Y. Takahashi, H. Naganawa, K. Nakae, M. Imoto, M. Shiro, K. Matsumura, H. Watanabe, T. Kitahara, *J. Antibiot.* **2002**, *55*, 442.

[18] C. Gaul, J. T. Njardarson, S. J. Danishefsky, *J. Am. Chem. Soc.* **2003**, *125*, 6042.

[19] a) J. T. Njardarson, C. Gaul, D. Shan, X.-Y. Huang, S. J. Danishefsky, *J. Am. Chem. Soc.* **2004**, *126*, 1038; b) C. Gaul, J. T. Njardarson, D. Shan, D. C. Dorn, K.-D. Wu, W. P. Tong, X.-Y. Huang, M. A. S. Moore, S. J. Danishefsky, *J. Am. Chem. Soc.* **2004**, *126*, 11326; c) D. Shan, L. Chen, J. T. Njardarson, C. Gaul, X. Ma, S. J. Danishefsky, X.-Y. Huang, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 3772.

[20] T. Oskarsson, P. Nagorny, I. Krauss, L. Perez, M. Mandal, G. Yang, O. Ouerfelli, D. Xiao, M. A. S. Moore, J. Massagué, S. J. Danishefsky, *J. Am. Chem. Soc.* **2010**, *132*, 3224.

[21] P. Delmotte, J. Delmotte-Plaquée, *Nature* **1953**, *171*, 344.

[22] S. M. Roe, C. Prodromou, R. O'Brien, J. E. Ladbury, P. W. Piper, L. H. Pearl, *J. Med. Chem.* **1999**, *42*, 260.

[23] R. M. Garbaccio, S. J. Stachel, D. K. Baeschlin, S. J. Danishefsky, *J. Am. Chem. Soc.* **2001**, *123*, 10903.

[24] a) K. Yamamoto, R. M. Garbaccio, S. J. Stachel, D. B. Solit, G. Chiosis, N. Rosen, S. J. Danishefsky, *Angew. Chem.* **2003**, *115*, 1318; *Angew. Chem. Int. Ed.* **2003**, *42*, 1280; b) Z.-Q. Yang, S. J. Danishefsky, *J. Am. Chem. Soc.* **2003**, *125*, 9602; c) Z.-Q. Yang, X. Geng, D. Solit, C. A. Pratilas, N. Rose, S. J. Danishefsky, *J. Am. Chem. Soc.* **2004**, *126*, 7881.

[25] T. Saito, H. Matsunaga, H. Yamamoto, F. Nagumo, H. Fujito, M. Mori, M. Katano, *Biol. Pharm. Bull.* **1994**, *17*, 798.

[26] I. Kitagawa, M. Yoshikawa, M. Yoshihara, T. Hayashi, T. Taniyama, *Yakugaku Zasshi* **1983**, *103*, 612.

[27] a) F. Ng, H. Yun, X. Lei, S. J. Danishefsky, J. Fahey, K. Stephenson, C. Flexner, L. Lee, *Tetrahedron Lett.* **2008**, *49*, 7178; b) M. Halim, D. J. Yee, D. Sames, *J. Am. Chem. Soc.* **2008**, *130*, 14123.

[28] H. Yun, S. J. Danishefsky, *J. Org. Chem.* **2003**, *68*, 4519.

[29] H. Yun, T.-C. Chou, H. Dong, Y. Tian, Y.-M. Li, S. J. Danishefsky, *J. Org. Chem.* **2005**, *70*, 10375.

[30] a) M. R. Bennett, W. G. Gibson, G. Lemon, *Auton. Neurosci.* **2002**, *95*, 1; b) D. Dawbarn, S. J. Allen, *Neuropathol. Appl. Neurobiol.* **2003**, *29*, 211.

[31] R. M. Wilson, S. J. Danishefsky, *Acc. Chem. Res.* **2006**, *39*, 539.

[32] R. Yokoyama, J.-M. Huang, C.-S. Yang, Y. Fukuyama, *J. Nat. Prod.* **2002**, *65*, 527.

[33] a) Y. S. Cho, D. A. Carcache, Y. Tian, Y.-M. Li, S. J. Danishefsky, *J. Am. Chem. Soc.* **2004**, *126*, 14358; b) D. A. Carcache, Y. S. Cho, Z. Hua, Y. Tian, Y.-M. Li, S. J. Danishefsky, *J. Am. Chem. Soc.* **2006**, *128*, 1016.

[34] T. Kita, Y. Takaya, Y. Oshima, T. Ohta, K. Aizawa, T. Hirano, T. Inakuma, *Tetrahedron* **1998**, *54*, 11877.

[35] Y. Obara, H. Kobayashi, T. Ohta, Y. Ohizumi, N. Nakahata, *Mol. Pharmacol.* **2001**, *59*, 1287.

[36] S. P. Waters, Y. Tian, Y.-M. Li, S. J. Danishefsky, *J. Am. Chem. Soc.* **2005**, *127*, 13514.

[37] J. M. Huang, R. Yokoyama, C. S. Yang, Y. Fukuyama, *J. Nat. Prod.* **2001**, *64*, 428.

[38] S. P. Cook, A. Polara, S. J. Danishefsky, *J. Am. Chem. Soc.* **2006**, *128*, 16440.

[39] A. Polara, S. P. Cook, S. J. Danishefsky, *Tetrahedron Lett.* **2008**, *49*, 5906.

[40] J.-M. Huang, R. Yokoyama, C.-S. Yang, Y. Fukuyama, *Tetrahedron Lett.* **2000**, *41*, 6111.

[41] V. B. Birman, S. J. Danishefsky, *J. Am. Chem. Soc.* **2002**, *124*, 2080.

[42] Z. Y. Meng, S. J. Danishefsky, *Angew. Chem.* **2005**, *117*, 1535; *Angew. Chem. Int. Ed.* **2005**, *44*, 1511.

[43] a) S. J. Danishefsky, J. F. Kerwin, S. Kobayashi, *J. Am. Chem. Soc.* **1982**, *104*, 358; b) S. J. Danishefsky, N. Kato, D. Askin, J. F. Kerwin, *J. Am. Chem. Soc.* **1982**, *104*, 360; c) S. J. Danishefsky, T. Kitahara, *J. Am. Chem. Soc.* **1974**, *96*, 7807.

[44] For a review, see: S. J. Danishefsky, M. T. Bilodeau, *Angew. Chem.* **1996**, *108*, 1482; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1380.

[45] I. C. Le Poole, M. A. Gerberi, W. M. Kast, *Curr. Opin. Oncol.* **2002**, *14*, 641.

[46] a) F. Helling, A. Shang, M. Calves, S. L. Zhang, S. L. Ren, R. K. Yu, H. F. Oettgen, P. O. Livingston, *Cancer Res.* **1994**, *54*, 197; b) F. Helling, S. Zhang, A. Shang, S. Adluri, M. Calves, R. Koganty, B. M. Longenecker, T. J. Yao, H. F. Oettgen, P. O. Livingston, *Cancer Res.* **1995**, *55*, 2783.

[47] a) M. T. Bilodeau, T. K. Park, S. Hu, J. T. Randolph, S. J. Danishefsky, P. O. Livingston, S. Zhang, *J. Am. Chem. Soc.* **1995**, *117*, 7840; b) T. K. Park, I. J. Kim, S. H. Hu, M. T. Bilodeau, J. T. Randolph, O. Kwon, S. J. Danishefsky, *J. Am. Chem. Soc.* **1996**, *118*, 11488; c) J. R. Allen, J. G. Allen, X. F. Zhang, L. J. Williams, A. Zatorski, G. Ragupathi, P. O. Livingston, S. J. Danishefsky, *Chem. Eur. J.* **2000**, *6*, 1366.

[48] For recent accounts of our carbohydrate-based anticancer vaccine program, see: a) O. Ouerfelli, J. D. Warren, R. M. Wilson, S. J. Danishefsky, *Expert Rev. Vaccines* **2005**, *4*, 677; b) R. M. Wilson, S. J. Danishefsky, *Pure Appl. Chem.* **2007**, *79*, 2189; c) J. Zhu, J. D. Warren, S. J. Danishefsky, *Expert Rev. Vaccines* **2009**, *8*, 1399.

[49] a) S. Zhang, C. Cordon-Cardo, H. S. Zhang, V. E. Reuter, S. Adluri, W. B. Hamilton, K. O. Lloyd, P. O. Livingston, *Int. J. Cancer* **1997**, *73*, 42; b) S. Zhang, H. S. Zhang, C. Cordon-Cardo, V. E. Reuter, A. K. Singhal, K. O. Lloyd, P. O. Livingston, *Int. J. Cancer* **1997**, *73*, 50.

[50] S. J. Keding, S. J. Danishefsky, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 11937.

[51] G. Ragupathi, F. Koide, P. O. Livingston, Y. S. Cho, A. Endo, Q. Wan, M. K. Spassova, S. J. Keding, J. Allen, O. Ouerfelli, R. M. Wilson, S. J. Danishefsky, *J. Am. Chem. Soc.* **2006**, *128*, 2715.

[52] J. Zhu, Q. Wan, D. Lee, G. Yang, M. K. Spassova, O. Ouerfelli, G. Ragupathi, P. Damani, P. O. Livingston, S. J. Danishefsky, *J. Am. Chem. Soc.* **2009**, *131*, 9298.

[53] a) P. Van den Steen, P. M. Rudd, R. A. Dwek, G. Opdenakker, *Crit. Rev. Biochem. Mol. Biol.* **1998**, *33*, 151; b) I. Brockhausen in *Glycoproteins* (Eds.: J. Montreuil, J. F. G. Vliegenthart, H. Schachter), Elsevier, New York, **1995**, p. 201; c) S. Zhang, H. S. Zhang, C. Cordon-Cardo, G. Ragupathi, P. O. Livingston, *Clin. Cancer Res.* **1998**, *4*, 2669.

[54] a) S. M. Barratt-Boyes, A. Vlad, O. J. Finn, *Clin. Cancer Res.* **1999**, *5*, 1918; b) E. M. Hiltbold, P. Ciborowski, O. J. Finn, *Cancer Res.* **1998**, *58*, 5066; c) B. Kocer, J. McKolanis, A. Soran, *BMC Gastroenterol.* **2006**, *6*, 4; d) K. L. Bondurant, M. D. Crew, A. D. Santin, T. J. O'Brien, M. J. Cannon, *Clin. Cancer Res.* **2005**, *11*, 3446; e) M. J. Cannon, T. J. O'Brien, L. J. Underwood, M. D. Crew, K. L. Bondurant, A. D. Santin, *Expert Rev. Anticancer Ther.* **2002**, *2*, 97.

[55] J. Zhu, Q. Wan, G. Ragupathi, C. M. George, P. O. Livingston, S. J. Danishefsky, *J. Am. Chem. Soc.* **2009**, *131*, 4151.

[56] D. Lee, S. J. Danishefsky, *Tetrahedron Lett.* **2009**, *50*, 2167.

[57] a) A. Y. Rudensky, P. Preston-Hurlbort, S.-Ch. Hong, A. Barlow, C. A. Janeway, Jr., *Nature* **1991**, *353*, 622; b) C. A. Bona, S. Casares, T. D. Brumeanu, *Immunol. Today* **1998**, *19*, 126; c) C. Musselli, P. O. Livingston, G. Ragupathi, *J. Cancer Res. Clin. Oncol.* **2001**, *127*, R20.

[58] P. Nagorny, W. H. Kim, Q. Wan, D. Lee, S. J. Danishefsky, *J. Org. Chem.* **2009**, *74*, 5157.

[59] For reviews, see: a) R. A. Dwek, *Chem. Rev.* **1996**, *96*, 683; b) F. Wold, *Annu. Rev. Biochem.* **1981**, *50*, 783; c) J. Roth, *Chem. Rev.* **2002**, *102*, 285; d) K. Ohtsubo, J. D. Marth, *Cell* **2006**, *126*, 855; e) R. J. Solá, J. A. Rodríguez-Martínez, K. Griebenow, *Cell. Mol. Life Sci.* **2007**, *64*, 2133; f) N. Mitra, S. Sinha, T. N. C. Ramya, A. Surolia, *Trends Biochem. Sci.* **2006**, *31*, 156.

[60] For general reviews on EPO, see: a) A. J. Sytkowski, *Erythropoietin*, Wiley-VCH, Weinheim, **2004**; b) W. Jelkmann, *Intern. Med.* **2004**, *43*, 649.

[61] a) S. C. Pang, *Women's Health* **2005**, *1*, 87; b) D. C. Herbert, *Am. J. Anat.* **1975**, *144*, 379; c) M. O. Dada, G. T. Campbell, C. A. Blake, *Endocrinology* **1983**, *113*, 970; d) P. Rathnam, B. B. Saxena, *J. Biol. Chem.* **1975**, *250*, 6735; e) B. B. Saxena, P. Rathnam, *J. Biol. Chem.* **1976**, *251*, 993.

[62] a) R. G. Spiro, *Glycobiology* **2002**, *12*, 43R; b) P. M. Rudd, R. A. Dwek, *Crit. Rev. Biochem. Mol. Biol.* **1997**, *32*, 1; c) R. Kornfeld, S. Kornfeld, *Annu. Rev. Biochem.* **1985**, *54*, 631.

[63] For a review, see: S. R. Hamilton, T. U. Gerngross, *Curr. Opin. Biotechnol.* **2007**, *18*, 387.

[64] For recent reviews, see: a) C. Kan, S. J. Danishefsky, *Tetrahedron* **2009**, *65*, 9047; b) Y. Yuan, J. Chen, Q. Wan, R. M. Wilson, S. J. Danishefsky, *Biopolymers: Peptide Science* **2010**, *94*, 373.

[65] P. E. Dawson, T. W. Muir, I. Clark-Lewis, S. B. H. Kent, *Science* **1994**, *266*, 776.

[66] J. D. Warren, J. S. Miller, S. J. Keding, S. J. Danishefsky, *J. Am. Chem. Soc.* **2004**, *126*, 6576.

[67] Q. Wan, J. Chen, Y. Yuan, S. J. Danishefsky, *J. Am. Chem. Soc.* **2008**, *130*, 15814.

[68] B. Wu, J. Chen, J. D. Warren, G. Chen, Z. Hua, S. J. Danishefsky, *Angew. Chem.* **2006**, *118*, 4222; *Angew. Chem. Int. Ed.* **2006**, *45*, 4116.

[69] G. Chen, J. D. Warren, J. Chen, B. Wu, Q. Wan, S. J. Danishefsky, *J. Am. Chem. Soc.* **2006**, *128*, 7460.

[70] Q. Wan, S. J. Danishefsky, *Angew. Chem.* **2007**, *119*, 9408; *Angew. Chem. Int. Ed.* **2007**, *46*, 9248.

[71] J. Chen, Q. Wan, Y. Yuan, J. L. Zhu, S. J. Danishefsky, *Angew. Chem.* **2008**, *120*, 8649; *Angew. Chem. Int. Ed.* **2008**, *47*, 8521.

[72] J. Chen, P. Wang, J. Zhu, Q. Wan, S. J. Danishefsky, *Tetrahedron* **2010**, *66*, 2277.

[73] a) X. Li, S. J. Danishefsky, *J. Am. Chem. Soc.* **2008**, *130*, 5446; b) X. Li, Y. Yuan, W. F. Berkowitz, L. J. Todaro, S. J. Danishefsky, *J. Am. Chem. Soc.* **2008**, *130*, 13222; c) X. Li, Y. Yuan, C. Kan, S. J. Danishefsky, *J. Am. Chem. Soc.* **2008**, *130*, 13225.

[74] Y. Rao, X. Li, S. J. Danishefsky, *J. Am. Chem. Soc.* **2009**, *131*, 12924.

[75] X. Wu, J. Stockdill, P. Wang, S. J. Danishefsky, *J. Am. Chem. Soc.* **2010**, *132*, 4098.

[76] D. Faulds, K. L. Goa, P. Benfield, *Drugs* **1993**, *45*, 953.

[77] C. Kan, J. D. Trzupek, B. Wu, Q. Wan, G. Chen, Z. Tan, Y. Yuan, S. J. Danishefsky, *J. Am. Chem. Soc.* **2009**, *131*, 5438.

[78] Y. Yuan, J. Chen, Q. Wan, Z. Tan, G. Chen, C. Kan, S. J. Danishefsky, *J. Am. Chem. Soc.* **2009**, *131*, 5432.

[79] Z. Tan, S. Shang, T. Halkina, Y. Yuan, S. J. Danishefsky, *J. Am. Chem. Soc.* **2009**, *131*, 5424.

[80] P. Nagorny, B. Fasching, X. Li, G. Chen, B. Fasching, B. Aussedat, S. J. Danishefsky, *J. Am. Chem. Soc.* **2009**, *131*, 5792.

