Applications of Total Synthesis to Problems in Neurodegeneration: Fascinating Chemistry along the Way

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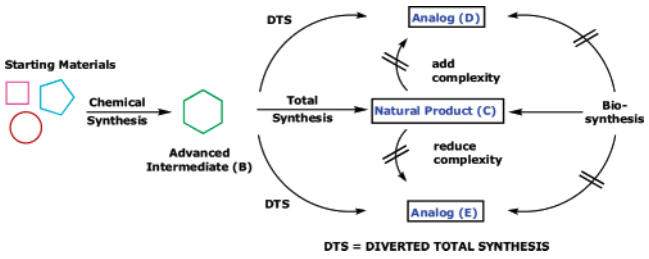
ABSTRACT

The possibility for the application of organic synthesis to the discovery of new agents in combating neurodegenerative disorders is described. Our focus has been on agents derived from natural-product leads and natural products themselves prepared through total synthesis. Herein, we describe some of the chemistry as well as interesting observations made along the way.

Introduction

Historically, the natural product estate has proven itself to be an invaluable resource in the quest to identify novel lead agents of potential medicinal import. Given the challenges associated with the isolation and purification of compounds from natural sources, it is often prohibitively difficult to gain access to sufficient quantities of material for extensive biological investigations. Total synthesis represents an attractive alternative, because a well-designed synthetic route can allow access to ample amounts of natural product for in vitro and in vivo evaluations.

Furthermore, this approach provides a unique opportunity to explore structure−activity relationships (SARs) in otherwise inaccessible areas of molecular space, through a process that we term diverted total synthesis.[1] Under this paradigm, the standard synthetic route is diverted at the stage of an advanced intermediate (cf. **B**) to provide natural-product analogues of varying degrees of structural complexity (cf. **D** and **E**). Notably, the compounds obtained in this manner would not necessarily be accessible in any other way (i.e., through derivatization of the natural product or modification of the biosynthetic pathway). The process of diverted total synthesis allows us to chemically "edit" the parent compound, by removing suspected sites of toxicity or by introducing additional structural features that would be expected to confer enhanced potency or stability.



Our laboratory has a longstanding tradition in the synthesis and evaluation of biologically relevant natural products. We have recently launched a total synthesis-based initiative directed toward the development of lead compounds of potential value in the treatment of neurodegenerative disorders, such as Alzheimer's, Huntington's, and Parkinson's diseases. Neurodegenerative disorders afflict a growing segment of the population. The development of clinically useful therapeutic options for those affected has thus emerged as a pressing public health concern. However, the development of broadly useful, demonstrably effective antineurodegenerative agents remains an elusive goal. Our research group has identified a number of structurally interesting natural products that possess some demonstrated central nervous system (CNS) activity. At this stage, we do not yet discriminate with regard to their mechanism of biological action or require that a specific molecular target for the drug be identified. Rather, our objectives are to accomplish the total synthesis of our chosen target, hopefully in a noteworthy fashion, and to validate the reported biological activity with synthetic material. Next, through the logic of diverted total synthesis,[2] as well as the derivatization of the synthetic natural product, we chart an SAR profile through the preparation and evaluation of structurally modified ana-

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Samuel J. Danishefsky received his B.S. degree at Yeshiva University. He did his graduate research under the late Professor Peter Yates. He then joined the laboratory of Professor Gilbert Stork at Columbia University as an NIH Postdoctoral Associate. His first academic position was at the University of Pittsburgh, where he joined as Assistant Professor in 1963. He was promoted to Associate Professor, Professor, and University Professor. In January 1980, he moved to Yale University and was named Eugene Higgins Professor in 1981. Appointed by President A. Bartlett Giamatti as Chairman of the Department of Chemistry, he served until 1987. He became Sterling Professor at Yale in 1990. In 1993, Professor Danishefsky moved back to New York as Professor of Chemistry at Columbia University and the Eugene Kettering Chair and Head of the Laboratory of Bioorganic Chemistry at Memorial Sloan-Kettering Cancer Center. In 1996, he shared the Wolf Prize in Chemistry with Professor 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.

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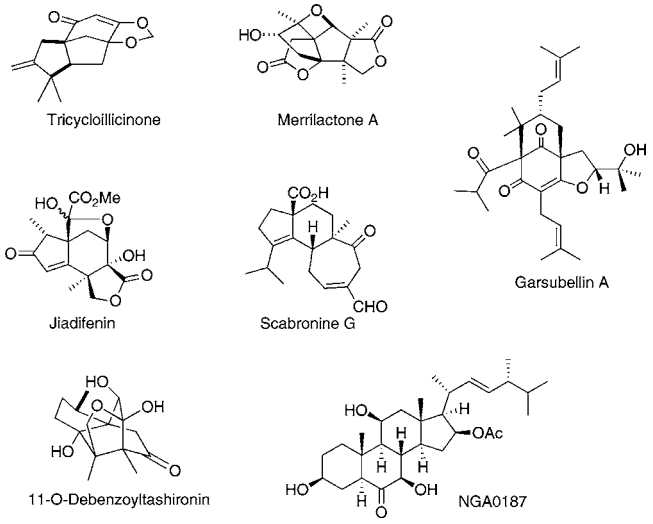
logues. The most promising lead agents are then subjected to structural iterations and more exhaustive biological studies, including attempted target identification.

In our forays into the total synthesis of naturally occurring CNS-active agents, the molecules that we have encountered thus far typically possess one of two types of activity. First, we have identified a number of small molecule, nonpeptidyl neurotrophically active agents. Naturally occurring neurotrophins are a class of polypeptidyl agents, which include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and glial-derived neurotrophic factor (GDNF), that mediate neuronal survival and outgrowth.[3] Notably, decreased neurotrophic support has been linked to the progression of neurodegenerative disease. One therapeutic option that has been explored in some depth has been the treatment of patients with naturally occurring polypeptidyl neurotrophic factors, in the hopes of promoting neurite outgrowth and survival. This general approach, while appealing in theory, suffers serious drawbacks in practice, because of the unfavorable pharmacokinetic properties of the peptidic agents, necessitating in their wake impractical drug-delivery techniques (such as direct microinjection to the brain). Consequently, a small molecule nonpeptidyl neurotrophic agent, which would presumably be better disposed to cross the blood-brain barrier, constitutes an attractive alternative. In this context, we have undertaken the total synthesis of a number of nonpeptidyl small molecules with demonstrated ability to enhance neurite outgrowth. This class of molecules includes merrilactone A, jiadifenin, scabronine G, 11-*O*-debenzoyltashironin, and NGA0187.

A second general class of CNS-related therapeutic candidates includes those that are responsible for the maintenance of cerebral acetylcholine levels. Importantly, the progression of Alzheimer's disease is typically marked by a significant decline in activity of the choline acetyltransferase (ChAT) enzyme, which is responsible for the synthesis of acetylcholine.[4] The associated decrease in acetylcholine levels is believed to play a central role in the observed symptoms of Alzheimer's disease. Most "anti-Alzheimer's" drugs in use today seek to maintain acetylcholine levels through the inhibition of the acetylcholine esterase (AChE) enzyme, which degrades acetylcholine.[5] Given the relative lack of convincing efficacy of such AChE inhibitors, we have adopted a slightly modified approach and have sought out, for synthesis and evaluation, natural-product agents that have demonstrated the ability to enhance the activity of the ChAT enzyme. Conceivably, when at least some of the activity of the ChAT enzyme is restored, it might be possible to realize an increase in cerebral acetylcholine levels in a clinically relevant setting. Two ChAT-enhancing natural products of note include tricycloillicinone and garsubellin A.

Happily (for organic chemistry), the family of nonpeptidyl neurotrophic factors is endowed with interesting and challenging structures. Quite aside from the motivations in the area of CNS drug discovery, which provides particular urgency to the enterprise, there is a great deal



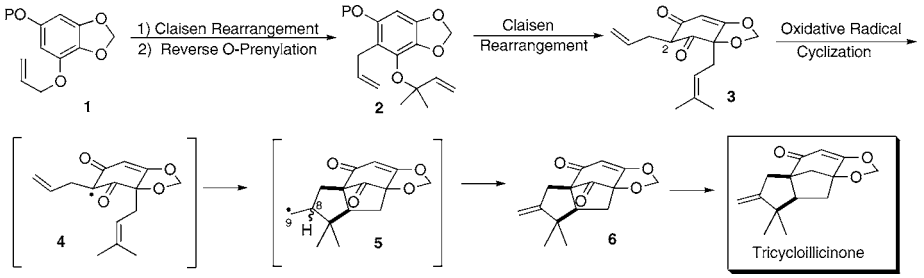
of chemistry to be learned from working with challenging structures. Such is particularly the case if one attempts to accomplish the synthesis in somewhat speculative ways, which was our intention in undertaking these projects. In this Account, we share with our readers some findings that we considered to be particularly pleasing from a purely chemical perspective. Our hope is that this Account, however fragmentary, will serve to underscore again the extraordinary vitality and intellectual excitement at the intersection of structure, isolation and discovery, chemical synthesis, and neurobiological follow-up. We view this whole area as compellingly exciting scientific terrain.

More specifically, we describe herein the highlights of the syntheses of seven different CNS-active natural products and their structurally related analogues. In the context of this Account, we do not seek to provide the exhaustive details of every individual transformation. Rather, we select only what we deem to be the most interesting and novel portions of the syntheses and leave the curious and industrious reader to peruse the original disclosures for more in depth treatments. It goes without saying that the pleasing outcomes that we are able to describe herein, in many instances, followed numerous setbacks. Success often belongs to the rugged as well as nimble.

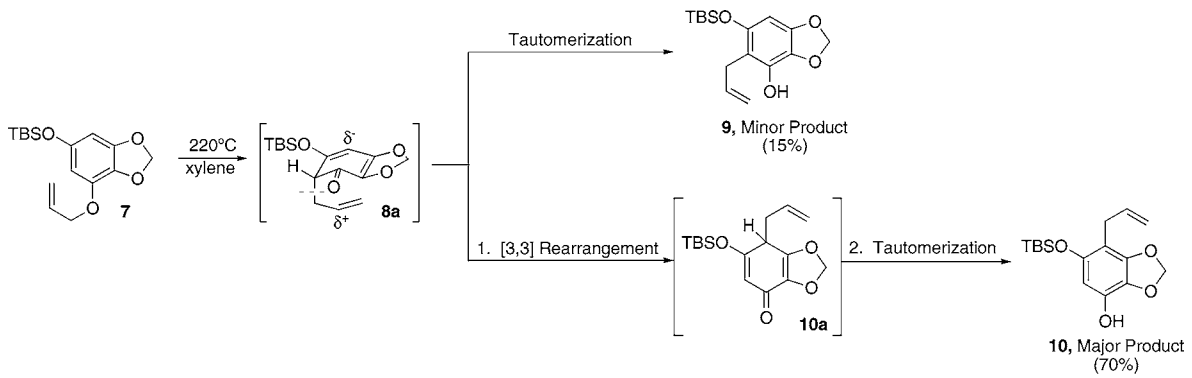
Tricycloillicinone

Isolated from *Illicium tashiroi* by Fukuyama and coworkers, tricycloillicinone had been found to induce ChAT activity at levels as low as 30 μM.[6] Under our synthetic plan, a key transformation would involve the oxidative radical cyclization of compound **3**, to provide the skeleton of tricycloillicinone (cf. **3** to **6**). Thus, on the basis of precedents of Corey[7] and Snider,[8] we anticipated that, upon exposure to Mn^{III}, the oxidative loss of the C_2 hydrogen would provide radical intermediate **4**, which would then undergo sequential cyclizations. Oxidation of the resultant C_9 radical (cf. **5**) through elimination of the C_8 hydrogen would furnish **6**, which we expected would be readily converted to tricycloillicinone and other analogues of potential interest. We envisioned gaining access

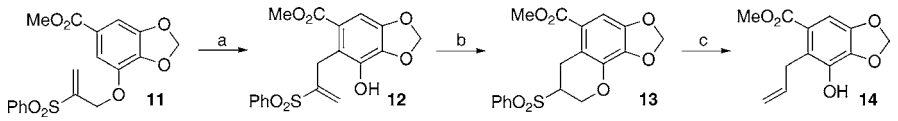
Scheme 1



Scheme 2



Scheme 3[a]



[a] Key: (a) neat, 165 °C, 12 h, 94%; (b) K_2CO_3, acetone, Δ, >95%; (c) 10% Na−Hg, MeOH−EtOAc, −20 °C, 87%.

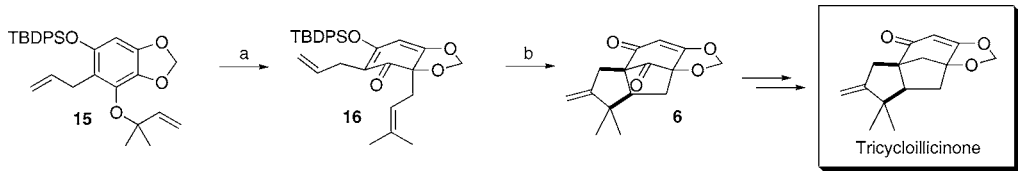
to system **3** through sequential aromatic Claisen rearrangements starting with a compound of the type **1** (Scheme 1).[9]

In the event, allyl ether **7** was prepared and subjected to thermolytic conditions (Scheme 2). However, the desired adduct (**9**), arising directly from tautomerization of the *ortho* Claisen intermediate (cf. **8a**), was obtained in only 15% yield. Instead, the major observed product was the *para* adduct, **10**, presumably resulting from a subsequent Cope rearrangement of **8a**. An experiment involving resubjection of the isolated *ortho* adduct (**9**) to thermolytic conditions revealed no interconversion between the *ortho* and *para* products, thus suggesting the system to be under kinetic control. Given that the second sigmatropic rearrangement (cf. **8a** to **10a**) appeared to proceed more rapidly than the conversion of the former to **9**, we were obliged to consider modifications of the allyl moiety. The hope was to find a disposable *substituent* on the allyl group that would suppress the sigmatropic rearrangement (of **8a** → **10a**) relative to tautomerization (**8a** → **9**).

Toward that end, we prepared **11**, incorporating a phenylsulfonyl function at C_2 of the allyl group (Scheme 3). We reasoned that in the Cope-like rearrangement of the initial cyclohexadienyl isomer, **8a**, to its immediate [3,3] rearrangement product (**10a**) en route to **10**, the allylic group migrates in a somewhat biased cationoid sense across a phenoxide-like surface (dotted line, **8a**). Were this the case, an electron-withdrawing group on the allylic moiety would raise the activation energy of the putative rearrangement.[10] Upon thermolysis, **11** smoothly underwent sigmatropic rearrangement to provide the *ortho* Claisen adduct, **12**, as the only observed regioisomer. There still remained the issue of disposal of the now extraneous sulfone. An elegant possibility suggested itself. Upon treatment with potassium carbonate, **12** underwent base-induced Michael-type cyclization to generate **13**. The latter was readily converted to **14** through exposure to sodium mercury amalgam. Thus, through temporary appendage of the phenylsulfonyl moiety, we were able to obtain the requisite *ortho* Claisen adduct **14** in good overall yield.

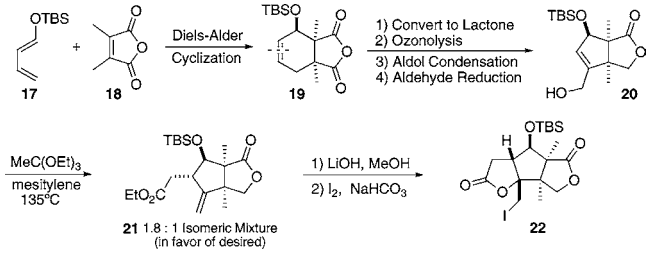
We next directed our efforts toward the second Claisen rearrangement. Thus, upon exposure to thermolytic conditions, allyl ether **15** underwent sigmatropic rearrangement to produce **16** in nearly quantitative yield (Scheme 4). The stage was set for the key projected radical cyclization sequence. Indeed, upon treatment with $Mn(OAc)_3$ and $Cu(OAc)_2$, **16** readily cyclized to form **6** in 80% yield. Presumably, the particularly "high-energy" silyl enol ether linkage in **16** had been cleaved en route to **6**. Tricycloillicinone was reached in three steps from **6**.

Scheme 4[a]



[a] Key: (a) toluene, 100 °C, 2 h, >95%; (b) 2 equiv of $Mn(OAc)_3 \cdot H_2O$, 1 equiv of $Cu(OAc)_2 \cdot H_2O$, HOAc, 50 °C, 3 h, 70−80%.

Scheme 5



Merrilactone A

Merrilactone A, isolated from *Illicium merrillianum*, significantly promotes neurite outgrowth in fetal rat cortical neurons at concentrations as low as 0.1−10 μM.[11] This report of neurotrophic activity combined with the densely functionalized structure of merrilactone A prompted us to undertake its total synthesis.[12]

Our first-generation route to merrilactone A, while fascinating in its own right, was nonenantiodiscriminating. Thus, intermediate **19**, the product of a cycloaddition of **17** and **18**, was converted to the lactone, **20** (Scheme 5). At this stage, Claisen rearrangement, as set forth by Johnson,[13] provided **21** as a 1.8/1 mixture of diastereomers, in favor of the desired product. Surprisingly, a series of attempts to improve upon the observed isomeric ratio proved fruitless. In any event, the correct diastereomer (**21**) was readily advanced to the key iodolactone, **22**.

The diselenide, **23**, was reached via **22** following several steps. Concurrent oxidative deselenylation of **23** provided **24** (Scheme 6). The stage was now set for the key cyclization reaction. In the event, upon treatment with tributyltin hydride and AIBN (azobisisobutyronitrile), intermediate **24** underwent anticipated free-radical-induced cyclization to provide the backbone of merrilactone A in 90% yield. The exocyclic olefin was then isomerized to the internal position, with concomitant removal of the *tert*-butyldimethylsilyl (TBS)-protecting group. The resultant olefin was epoxidized to afford **27**, and finally, acid-mediated intramolecular epoxide opening provided merrilactone A.

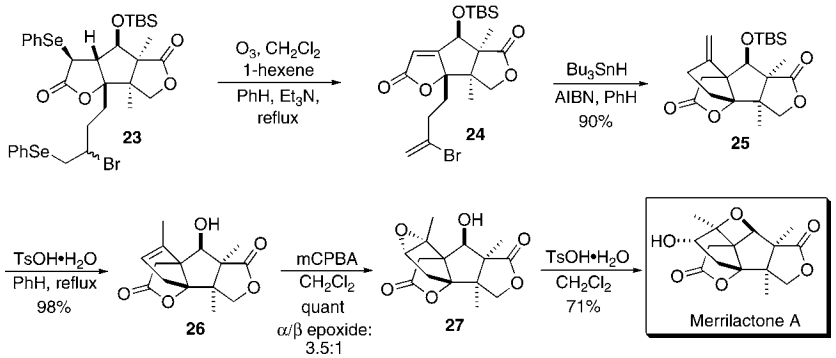
It was our intention to explore a second-generation synthesis that might be responsive to the lack of specificity, which compromised the elegance of the first-generation route. The most apparent drawback of the original synthesis is that it produces racemic merrilactone A. Given the high temperatures required for the Diels−Alder cycloaddition, we deemed it unlikely that the Diels−Alder route would be amenable to enantioinduction. A second shortcoming of the original route was that it was nonregioselective in the opening phase of the conversion of the anhydride to the lactone (cf. **19** to **20**), although the two regioisomers arising from this step could each be rehabilitated in the context of the earlier synthesis. Finally, the Johnson *ortho* ester Claisen rearrangement proceeded with a disappointing level of diastereoselectivity, which we were unable to improve upon, notwithstanding considerable effort. In contrast to these troublesome issues inherent in the early part of this synthesis, we did feel that the route from **22** to merrilactone A was eminently acceptable.

We thus set, as a target for our modified asymmetric route, the goal of reaching both enantiomeric versions of iodolactone **22**.[14] We came to favor a perhaps nonobvious degradative approach to the problem. If successful, the plan that we formulated would alleviate some of the awkwardnesses of the first-generation route. Our modified protocol would commence with a *meso* compound **28** (Scheme 7). In an overall sense, the conversion of **28** → **22** would require the differentiation of the carbon atoms of the *meso* epoxide (*a* and *b*) such that one (*a*) would carry an alkyl iodide and the other (*b*) would become a secondary alcohol. Similarly, the two primary alcohols of **28** would become a lactone, wherein one carbon (*c*) would remain in the alcohol oxidation state and the other (*d*) would emerge in oxidized form. Of course, the sense of the differentiation must be accomplished regioselectively, such that the lactone carbonyl (*d*) would be "*ortho*" to the secondary alcohol (*b*) in **22**.

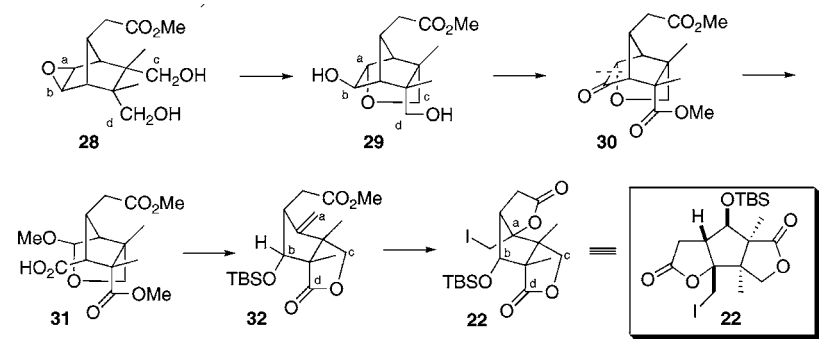
It was our expectation that enantioselectivity would be achieved through an asymmetric ring opening of the *meso* epoxide **28** following the appealing precedent of Jacobsen.[15] Oxidation of the resultant diol, **29**, followed by regioselective Baeyer−Villiger reaction, would allow access to a compound of the type **31**, which we envisioned could be readily converted to **22**, as shown.

In the event, following dimethyldioxirane (DMDO)-mediated epoxidation of *meso* intermediate, **33**, exposure to Jacobsen asymmetric ring-opening conditions afforded the diol **29** in 86% enantiomeric excess (Scheme 8). This compound was converted to **30,** and as expected, Baeyer−Villiger oxidation with magnesium monoperoxyphthalate hexahydrate (MMPP) proceeded with complete regioselectivity to provide hemiacetal **31**. The latter was readily converted to **22** following a degradative route in which the aldehyde-like carbon or the acetal became a terminal methylene group and the carboxyl function was degraded via carboxy inversion[16] to give rise to a secondary alcohol, thus establishing an efficient route to either antipode of merrilactone A. We now have these antipodes in hand.

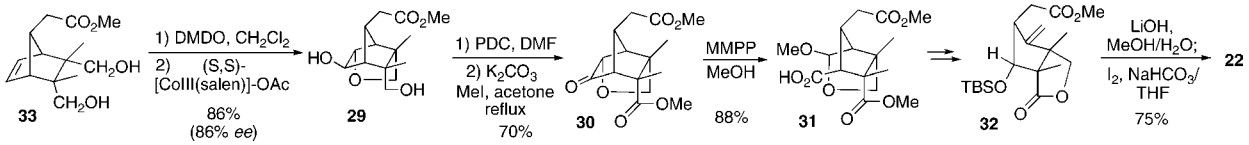
Scheme 6



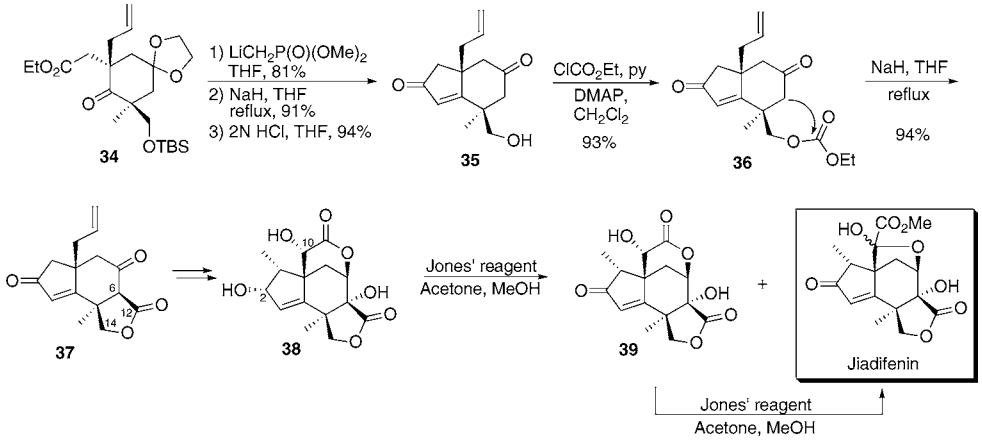
Scheme 7



Scheme 8



Scheme 9



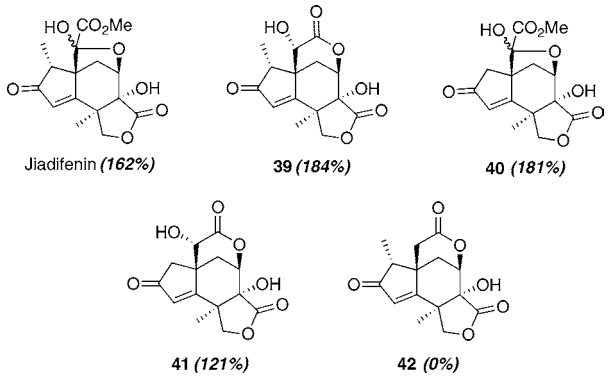
Jiadifenin

Jiadifenin, isolated from *Illicium jiadifengpi*, has been shown to promote neurite outgrowth in rat cortical neurons at concentrations as low as 0.1 μM.[17] This promising feature, not to mention the chemically challenging core structure, prompted us to undertake its total synthesis.[18] The tetrasubstituted cyclohexanone **34** was thus converted to **35** through an intramolecular Horner–Wadsworth–Emmons reaction followed by standard functional group manipulations (Scheme 9). At this point, the task would be that of forming the lactone ring. To accomplish this, we took recourse to a modified version of a cyclization strategy that had been previously employed in our synthesis of tazettine some years earlier.[19] As shown, the overall conversion of **35** → **37** would require the interpolation of the C_{12} carbonyl moiety between C_6

and C_{14}. In the event, we were able to effect this transformation through the intermediacy of the mixed carbonate ester, **36**. Upon exposure to base, the latter underwent cyclization to provide the lactone ring of jiadifenin. This tricyclic system was advanced to **38** through a series of unexceptional operations. At this point, it was our hope that, upon exposure to Jones' oxidation conditions, **38** would undergo concurrent oxidation of both of its secondary alcohols (at C_2 and C_{10}). We further anticipated that the α-ketolactone moiety derived from C_{10} oxidation would spontaneously suffer rearrangement to the hydroxytetrahydrofuran carboxylate of jiadifenin. In the event, treatment of **38** with Jones' reagent led to jiadifenin (40%), as well as an intermediate (**39**), in which only the C_2 alcohol had been oxidized. After isolation, **39** was readily converted to jiadifenin upon re-exposure to Jones' oxidation conditions. The overall process provided jiadifenin in 53% yield from **38**.

With synthetic jiadifenin in hand, we were able to corroborate the reported in vitro activity. Indeed, although no neurite outgrowth was observed in cells treated with jiadifenin in the *absence* of NGF, significant outgrowth was noted in those cells that were treated with both jiadifenin and NGF. These important results indicate that the observed neurotrophic activity of jiadifenin is attributable to the upregulation of the action of NGF. The potential importance of this finding is being pursued.

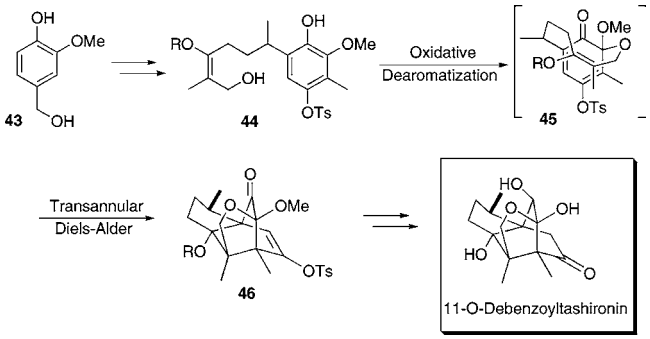
A small library of jiadifenin-related compounds has been prepared. To date, in vitro SAR studies have led to the identification of at least two compounds possessing enhanced neurite outgrowth activity relative to the natural product (cf. **39** and **40**). Although these data are surely preliminary, they do serve to underscore once again the potential value that natural-product synthesis may play in the identification of promising new drug candidates. In short, the jiadifenin story, while still a work in progress, looks to be quite interesting from several standpoints.



11-*O*-Debenzoyltashironin

Isolated from *illicium tashiroi*, 11-*O*-debenzoyltashironin promotes neurite outgrowth at concentrations as low as 0.1 μM.[20] Not surprisingly, we were initially drawn to 11-*O*-debenzoyltashironin on the basis of its neurotrophic activity. The potency level, not to speak of the unique challenges presented by the highly compact tetracyclic framework of 11-*O*-debenzoyltashironin virtually compelled a program directed toward its total synthesis.[21]

Scheme 10

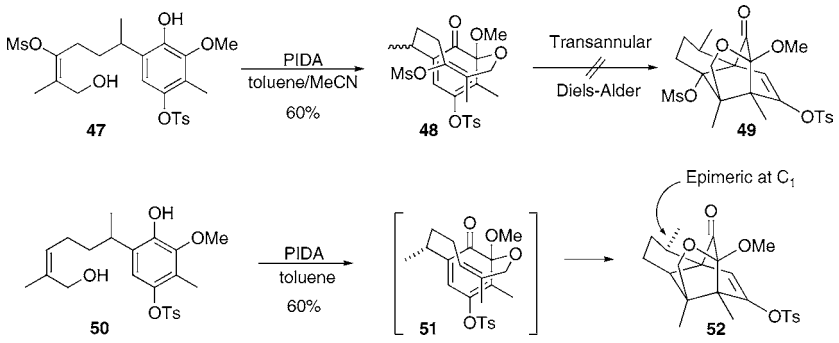


Upon examination of its compact structure, we envisioned a synthetic plan built around a highly concise, complexity-building sequence. The initial thought was that a compound of the type **44** would undergo oxidative dearomatization to afford intermediate **45** (Scheme 10). It might further be anticipated that **45** would undergo a spontaneous transannular Diels–Alder reaction to afford the advanced tetracyclic intermediate **46**. Although our proposal calls for the oxidative dearomatization to occur *meta* to the alkyl (as shown), we were not unmindful of the possibility of competition from an *ipso* cyclization pathway, which would produce an unproductive spirocyclic intermediate. In the context of our proposed total synthesis, spirocyclization would have been an unsalvageable setback. Nonetheless, the possibility of obtaining a highly complex tetracyclic intermediate (**46**) via **45** from a relatively simple aromatic precursor (**44**) *in one transformation* impelled us to explore its feasibility.

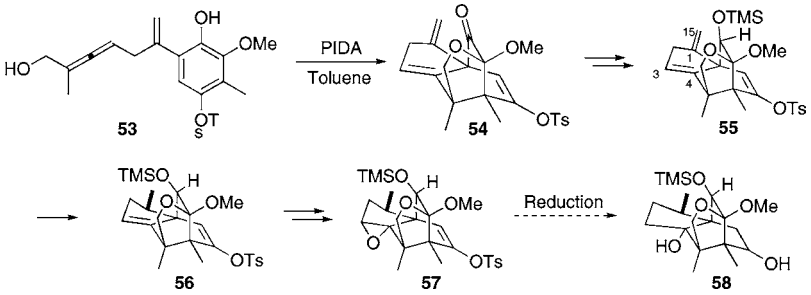
Our initial attempts to implement the tandem approach commenced with compound **47** (Scheme 11). In the event, **47** was exposed to oxidative dearomatization conditions with phenyliodine(III) diacetate (PIDA).[22] The product isolated from this reaction was determined to be **48**, the result of the desired *meta* mode of oxidative dearomatization. To our disappointment and surprise, all attempts to effect transannular Diels–Alder of **48** were unsuccessful. To further complicate matters, **48** was found to be quite unstable and prone to decomposition. We postulated that the failure of **48** to undergo cycloaddition may be attributed to the steric bulk of the mesyloxy group at the double bond of the putative dienophile. To evaluate this interpretation, we prepared the trisubstituted substrate **50**, lacking the mesyl substituent on the dienophile. We were pleased to find that, upon exposure to PIDA, **50** did indeed undergo tandem oxidative dearomatization and transannular Diels–Alder to provide **52** as a single diastereomer. Although the observed diastereomer was epimeric at C_1 to that required for 11-*O*-debenzoyltashironin, this result served to establish the feasibility of our tandem strategy. We thus set out to identify an appropriately functionalized substrate, which, upon cyclization, would afford a tetracyclic intermediate possessing the functional handles necessary to reach 11-*O*-debenzoyltashironin itself in diastereomerically controlled fashion.

In this context, we hoped to gain access to a product of the type **54**, which would be obtained from an allene

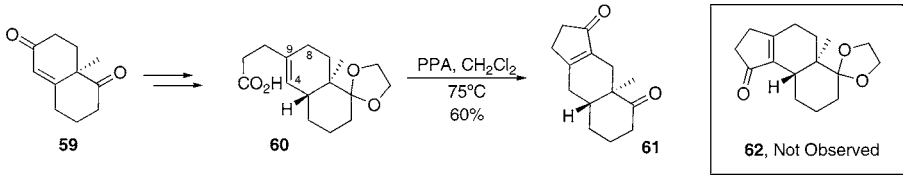
Scheme 11



Scheme 12



Scheme 13



precursor (**53**). It will be noted that this tetracyclic intermediate (**54**) should allow for diastereoselective emplacement of the requisite C_4 hydroxyl functionality through manipulation of the C_3-C_4 olefin. Furthermore, we expected that the exocyclic C_1-C_{15} olefin would be susceptible to face-selective reduction to provide the C_1 methyl in the requisite diastereomeric form.

In the event, we were pleased to find that **53** underwent our projected oxidative dearomatization–transannular Diels–Alder to provide **54**, which we viewed as a potentially viable precursor to 11-*O*-debenzoyltashironin (Scheme 12). Indeed, compound **54** was advanced to **55**. As expected, hydrogenation of the *exo* olefin in **55** occurred exclusively from the α face to furnish **56**, bearing the *syn*-like relationship of the C_1 methyl group with the fused tetrahydrofuran ring. Next, epoxidation of the C_3-C_4 olefin, again from the α face, yielded intermediate **57**.[23] The latter is, hopefully, a viable precursor to mount a final assault directed to the natural-product target.

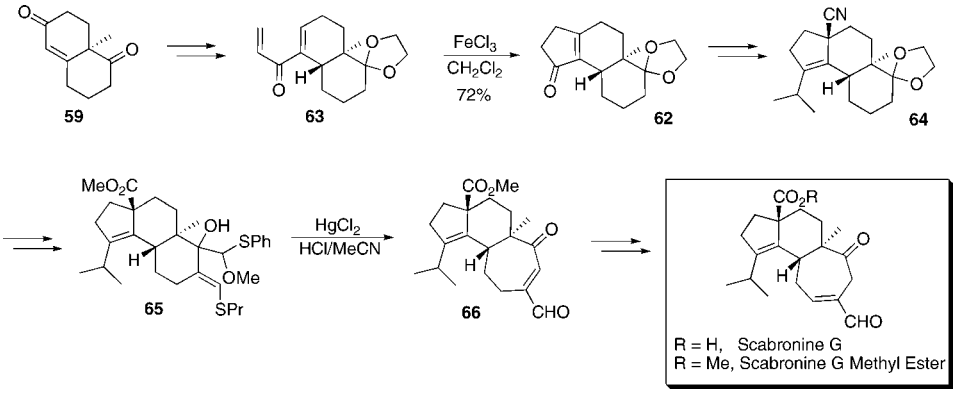
Thus, through recourse to the allene dienophile **53**, we were able to overcome the otherwise unmanageable reactivity as well as diastereoselectivity patterns encountered in the olefinic substrates (**47** and **50**). The synthesis of 11-*O*-debenzoyltashironin is in late stages.

Scabronine G

The scabronines are metabolites isolated from the bitter mushroom *Sarcodon scabrosus*.[24] Scabronine G, in particular, has been reported to induce production and secretion of NGF in human astroglial (1321N1) cell lines.[25] Moreover, the methyl ester derivative of scabronine G exhibits enhanced production of NGF compared to the natural product and has been shown to enhance production of a second neurotrophin, interleukin-6 (IL-6). On the basis of these observations, it is perhaps not surprising that both compounds induce dramatic neuronal differentiation when introduced to rat pheochromocytoma (PC-12) cells.

In formulating a strategy toward scabronine G, we came to view the tricyclic backbone as an annulated (A ring), one-carbon homologated (C ring) derivative of the Wieland–Miescher ketone (**59**).[26] We thus considered the first goal to be that of installing the A ring through appendage of a 3-carbon unit to **59**, followed by some type of ring-closing strategy. Accordingly, **59** was advanced to intermediate **60**, in which the 3-carbon unit has been appended at C_9 (Scheme 13). Interestingly, Friedel–Crafts acylation led exclusively to the cyclopentenone intermediate **61**, in which cyclization had occurred at C_8. None of

Scheme 14



the required regioisomer, **62**, was observed. Perhaps the failure of **60** to cyclize in the desired direction reflects the hindrance surrounding the C_4 center, which suffers a 1,3-diaxial interaction with the angular methyl group and is *ortho* to the B−C ring junction.[27]

Forewarned by the finding described above, the 3-carbon moiety would now be installed at C_4, thereby forcing cyclization to occur at C_9. In this context, intermediate **63** was prepared from **59** (Scheme 14). Predictably, Lewis-acid-mediated Nazarov cyclization[28] provided **62**. Conjugate addition to the cyclopentenone using Nagata's reagent,[29] followed by enolate triflylation and Negishi cross-coupling[30] gave rise to **64**.

Intermediate **64** was advanced to **65** through a series of anticipatable transformations. The stage was now set to attempt the one-carbon ring expansion of the C ring. In the event, upon treatment with $HgCl_2$ under acidic conditions, **65** underwent rearrangement to provide the cross-conjugated cycloheptenone **66**.[31] Thermodynamically driven olefin isomerization afforded scabronine G-methyl ester, which, upon hydrolysis, yielded the natural metabolite, scabronine G.

In keeping with our research plan, we sought to confirm the reported neurotrophic activity of scabronine G−methyl ester. We were pleased to find that our fully synthetic material successfully enhanced the production and secretion of neurotrophic factors in 1321N1 cells. Significant neurite outgrowth was observed in PC-12 cells treated with scabronine G−methyl ester. Furthermore, we were very excited to find that the unisomerized scabronine G−methyl ester analogue (**66**) induced even more pronounced neurite outgrowth than was observed with scabronine G−methyl ester itself. Once again, this encouraging finding serves to underscore, to all but the most recalcitrant, the potentialities of total synthesis and diverted total synthesis in the pursuit of promising therapeutic agents. Through diverted total synthesis, one gains access to natural-product-like structures, drawing upon the wisdom of nature, yet unrestrained by the need to be biosynthesizable by a "sanctioned" pathway.

NGA0187

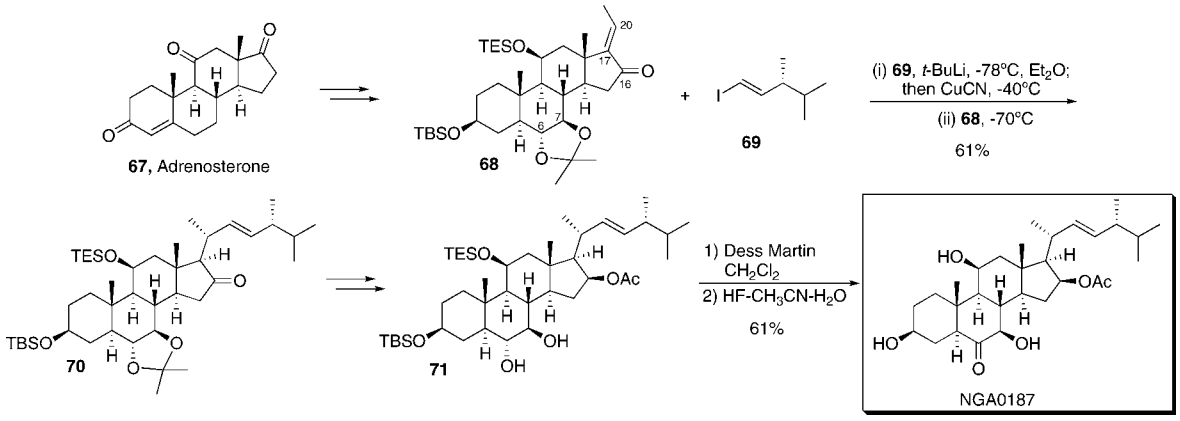
Isolated by Nazawa et al. from the microorganism *Acremonium* sp. TF-0356, NGA0187 is, to our knowledge, the first steroid that has been demonstrated to exhibit neurotrophic activity.[32] When introduced to rat cerebral cortical neuronal cells, NGA0187 induced neurite outgrowth at doses as low as 30 μg/mL. In light of our ongoing program directed toward the synthesis of natural products possessing neurotrophic activity, we set our sights on accomplishing the total synthesis of NGA0187.[32b] It should be noted that, in the context of its uniquely steroidal framework, NGA0187 does share with our other neurotrophin targets dense oxygenation presented on the β face of the tetracyclic system.

We elected to commence our synthesis with the commercially available steroid adrenosterone (**67**) (Scheme 15). Our rationale for choosing this particular steroid as a starting point was 2-fold. First, compound **67** appeared to incorporate the functional implements necessary to allow access to NGA0187 in a selective manner. Furthermore, although other commercially available steroidal starting materials could conceivably allow entry to the NGA0187 series in fewer overall transformations, we were mindful of the importance of synthetic flexibility if, eventually, we were to prepare structurally related analogues through diverted total synthesis. Compound **67** seemed to be ideally disposed to provide a means for a straightforward yet synthetically flexible route to NGA0187. A collateral feature of the program directed to NGA0187 is that it obliged us to revisit problems in the now dormant but still fascinating important steroidal class of natural products.

In the event, **67** was transformed to **68**, which incorporates, most notably, a suitably protected C_6-α, C_7-β vicinal diol, as well as an exocyclic α,β-unsaturated ketone encompassing carbons 16, 17, and 20. The installation of the C_{17} side chain was achieved through treatment of **68** with the vinyl cuprate derived from **69**. Happily, this transformation proceeded smoothly to provide **70** as a single diastereomeric product. The latter was subsequently converted to the key diol intermediate **71**.

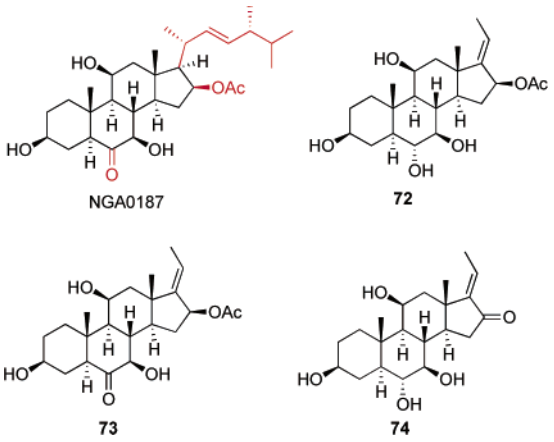
At this stage, we were required to devise a crucial selective oxidation of the C_6 alcohol. We took encouragement from the precedent of Krafft et al., who, in a similar setting, were able to selectively benzylate at the C_6-α-OH in the presence of a C_7-β-OH group.[33] In the event, upon exposure to Dess−Martin periodinane, **71** underwent

Scheme 15



exclusive oxidation of the C_6-α-OH group. Global deprotection provided NGA0187.

The neurite outgrowth activity of NGA0187 was confirmed with our synthetic material. In keeping with our overriding natural-product-fueled preference, we prepared and evaluated a number of structurally simplified synthetic analogues. Interestingly, each analogue, which lacks the C_{17} side chain, was essentially inactive in our assay. These results indicate that the presence of this side chain is critical to the observed activity of the compound perhaps by providing an important hydrophobic contact. Further SAR studies are currently underway and will be reported upon in due course.

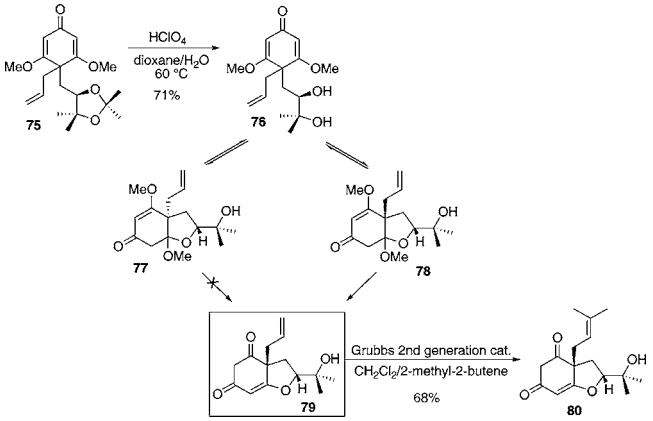


Garsubellin A

Garsubellin A was isolated by Fukuyama et al. from *Garcinia subelliptica* and has since been shown to enhance ChAT activity in P10 rat septal neurons (154% enhancement relative to the control).[34] Upon examination of the garsubellin A structure, we were struck by the synthetic challenges posed by this fascinating molecule, and accordingly, an effort directed to its total synthesis was launched.[34b]

As the plan evolved, it was envisioned that an intermediate such as **80**, which constitutes the core bicyclic segment of garsubellin A, would serve as a productive launching point. The first challenge would be that of preparing intermediate **80**. To this end, we synthesized acetonide **75** (Scheme 16). Presumably, exposure to perchloric acid first led to the formation of diol **76**, although this compound was not isolated. Indeed, the observed intermediates of this reaction were compounds **77** and **78**, the result of Michael-like cyclization of the free hydroxyl. Over time, the diastereomeric intermediates, which equilibrate under reaction conditions, were found to coalesce to a single product, **79**. Presumably, **78**, in which the allyl and isopropanol moieties are situated on opposite faces of the ring, is preferentially disposed to undergo elimination of methanol. This step was followed by cleavage of the vinylogous methyl carbonate, to provide **79**. From **79**, olefin cross-metathesis provided the prenyl functionality of **80**.[35]

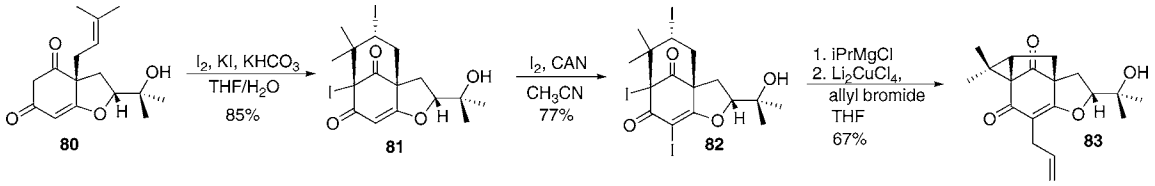
Scheme 16



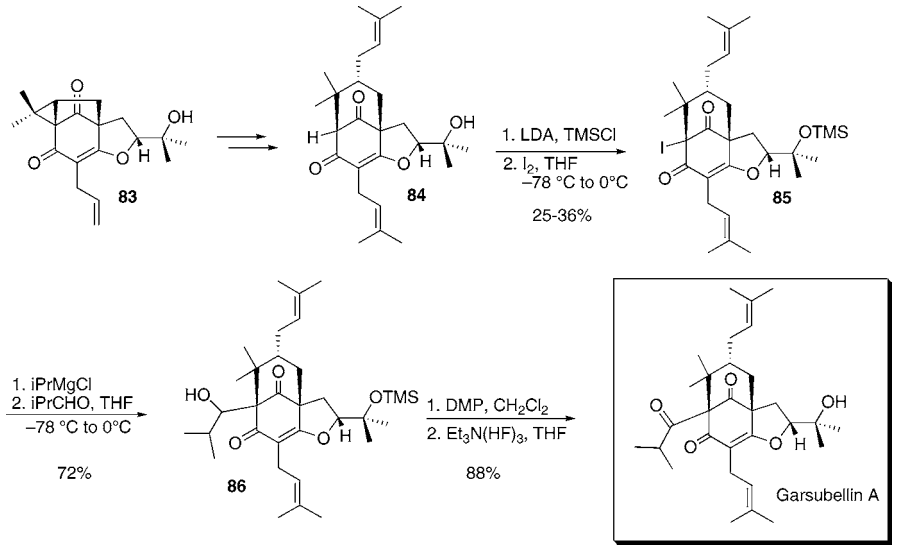
Compound **80** was then subjected to iodocarbocyclization, following the helpful precedent of Nicolaou et al. (Scheme 17).[36] Installation of the vinyl iodide was achieved through exposure to iodine and ammonium cerium(IV) nitrate (CAN).[37] At this stage, treatment of **82** with excess isopropylmagnesium chloride precipitated two sequential events.[38] The first, a transannular Wurtz cyclopropanation reaction, proceeded at −78 °C. Upon warming to 0 °C, the vinyl iodide underwent iodine−magnesium exchange to provide the vinyl Grignard intermediate, which, upon allylation, generated intermediate **83**.

Compound **83** was readily advanced to **84** (Scheme 18). At this point, the task would be that of installing the second isobutyryl moiety. Thus, treatment of **84** with lithium diisopropylamide (LDA) and trimethylsilyl chloride

Scheme 17



Scheme 18



(TMSCl) followed by trapping with iodine provided **85**. Magnesium−iodine exchange furnished the bridgehead nucleophile, which, upon exposure to isobutyrylaldehyde, provided aldol adduct **86** in good yield. The natural product, garsubellin A, was reached in two transformations. Gaining access to enantiopure garsubellin is an urgent but not yet realized goal.

Conclusions

In summary, we have presented several happy experiences in the course of our program directed toward bringing to bear nature's treasures of small molecule natural products on the momentous challenge of human neurodegenerative diseases. While biological results are now being accumulated for systematic disclosure, it is already clear that there is considerable potential in compounds obtained through plowing in the landscape of natural products. Particularly impressive are those compounds that are obtained through diverted total synthesis, i.e., through methodology, which was redirected from the original (and realized) goal of total synthesis, to encompass otherwise unavailable congeners. We are confident that the program will lead, minimally, to compounds that are deserving of serious preclinical follow-up. At the broader level, we note that this program will confirm once again (if further confirmation is, indeed, necessary) the extraordinary advantages of small molecule natural products as sources of agents, which interject themselves in a helpful way in various physiological processes.

We close with the hope and expectation that enterprising and hearty organic chemists will not pass up the unique head start that natural products provide in the quest for new agents and new directions in medicinal discovery. We would chance to predict that even as the currently fashionable "telephone directory" mode of research is subjected to much overdue scrutiny and performance-based assessment, organic chemists in concert with biologists and even clinicians will be enjoying as well as exploiting the rich troves provided by nature's small molecules.

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References

(1) Njardarson, J. T.; Gaul, C.; Shan, D.; Huang, X.-Y.; Danishefsky, S. J. Discovery of Potent Cell Migration Inhibitors through Total Synthesis: Lessons from Structure−Activity Studies of (+)-Migrastatin. *J. Am. Chem. Soc.* **2004**, *126*, 1038−1040.

(2) Reference deleted in proof.

(3) (a) Bennett, M. R.; Gibson, W. G.; Lemon, G. Neuronal Cell Death, Nerve Growth Factor and Neurotrophic Models: 50 Years On. *Auton. Neurosci.* **2002**, *95*, 1−23. (b) Dawbarn, D.; Allen, S. J. Neurotrophins and Neurodegeneration. *Neuropathol. Appl. Neurobiol.* **2003**, *29*, 211−230.

(4) Hefti, F. Pharmacology of Neurotrophic Factors. *Annu. Rev. Pharmacol. Toxicol.* **1997**, *37*, 239−267.

(5) Giacobini, E. Cholinesterase Inhibitors for Alzheimer's Disease Therapy: From Tacrine to Future Applications. *Neurochem. Int.* **1998**, *32*, 413−419.

(6) Fukuyama, Y.; Shida, N.; Kodama, M.; Chaki, H.; Yugami, T. Tricycloillicinone, a Novel Prenylated C-6−C-3 Compound Increasing Choline Acetyltransferase (ChAT) Activity, Isolated from *Illicium tashiroi*. *Chem. Pharm. Bull.* **1995**, *43*, 2270−2272.

(7) Corey, E. J.; Kang, M.-C. New and General Synthesis of Polycyclic γ-Lactones by Double Annulation. *J. Am. Chem. Soc.* **1984**, *106*, 5384−5385.

(8) (a) Kates, S. A.; Dombroski, M. A.; Snider, B. B. Manganese(III)-Based Oxidative Free-Radical Cyclization of Unsaturated β-Keto-Esters, 1,3-Diketones, and Malonate Diesters. *J. Org. Chem.* **1990**, *55*, 2427−2436; For a review, see (b) Snider, B. B. Manganese-(III)-Based Oxidative Free-Radical Cyclizations. *Chem. Rev.* **1996**, *96*, 339−363.

(9) (a) Pettus, T. R. R.; Chen, X.-T.; Danishefsky, S. J. A Fully Synthetic Route to the Neurotrophic Illicinones by Sequential Aromatic Claisen Rearrangements. *J. Am. Chem. Soc.* **1998**, *120*, 12684−12685. (b) Pettus, T. R. R.; Inoue, M.; Chen, X.-T.; Danishefsky, S. J. A Fully Synthetic Route to the Neurotrophic Illicinones: Syntheses of Tricycloillicinone and Bicycloillicinone Aldehyde. *J. Am. Chem. Soc.* **2000**, *122*, 6160−6168.

(10) For a more thorough analysis of this sort of problem, see Carpenter, B. K. Simple Model for Predicting Effect of Substituents on Rates of Thermal Pericyclic Reactions. *Tetrahedron* **1978**, *34*, 1877−1884.

(11) Huang, J.-M.; Yokoyama, R.; Yang, C.-S.; Fukuyama, Y. Merrilactone A, a Novel Neurotrophic Sesquiterpene Dilactone from *Illicium merrillianum*. *Tetrahedron Lett.* **2000**, *41*, 6111−6114.

(12) Birman, V. B.; Danishefsky, S. J. The Total Synthesis of (±)-Merrilactone A. *J. Am. Chem. Soc.* **2002**, *124*, 2080−2081.

(13) Johnson, W. S.; Werthemann, L.; Bartlett, W. R.; Brocksom, T. J.; Li, T.-T.; Faulkner, D. J.; Petersen, M. R. A Simple Stereoselective Version of Claisen Rearrangement Leading to Trans-Trisubstituted Olefinic Bonds. Synthesis of Squalene. *J. Am. Chem. Soc.* **1970**, *92*, 741−743.

(14) Meng, Z. Y.; Danishefsky, S. J. A Synthetic Pathway to Either Enantiomer of Merrilactone A. *Angew. Chem., Int. Ed.* **2005**, *44*, 1511−1513.

(15) Wu, M. H.; Hansen, K. B.; Jacobsen, E. N. Regio- and Enantioselective Cyclization of Epoxy Alcohols Catalyzed by a [Co^{III}(salen)] Complex. *Angew. Chem., Int. Ed.* **1999**, *38*, 2012−2014.

(16) Denney, D. B.; Sherman, N. Degradation of Acids to Alcohols by Carboxy-Inversion Reaction. *J. Org. Chem.* **1965**, *30*, 3760−3761. (b) Danishefsky, S.; Tsuzuki, K. Control of Remote Relative Chiralities—A Stereospecific Total Synthesis of D,L-Widdrol. *J. Am. Chem. Soc.* **1980**, *102*, 6891−6893.

(17) Yokoyama, R.; Huang, J.-M.; Yang, C.-S.; Fukuyama, Y. New Seco-Prezizaane-Type Sesquiterpenes, Jiadifenin with Neurotrophic Activity and 1,2-Dehydroneomajucin from *Illicium jiadifengpi*. *J. Nat. Prod.* **2002**, *65*, 527−531.

(18) Cho, Y. S.; Carcache, D. A.; Tian, Y.; Li, Y.-M.; Danishefsky, S. J. Total Synthesis of (±)-Jiadifenin, a Non-Peptidyl Neurotrophic Modulator. *J. Am. Chem. Soc.* **2004**, *126*, 14358−14359.

(19) Danishefsky, S.; Morris, J.; Mullen, G.; Gammill, R. Total Synthesis of D,L-Tazettine. *J. Am. Chem. Soc.* **1980**, *102*, 2838−2840.

(20) Huang, J. M.; Yokoyama, R.; Yang, C. S.; Fukuyama, Y. Structure and Neurotrophic Activity of Seco-Prezizaane-type Sesquiterpenes from *Illicium merrillianum*. *J. Nat. Prod.* **2001**, *64*, 428−431.

(21) Cook, S. P.; Gaul, C.; Danishefsky, S. J. En Route to the Total Synthesis of Tashironin: On the Exercise of Stereochemical Control by a Methyl Group in Mediating Remote Cyclization Reactions. *Tetrahedron Lett.* **2005**, *46*, 843−847.

(22) (a) Pelter, A.; Elgendy, S. Phenolic Oxidation with (Diacetoxyiodo)-benzene. *Tetrahedron Lett.* **1988**, *29*, 677−680. (b) Tamura, Y.; Yakura, T.; Haruta, J.; Kita, Y. Hypervalent Iodine Oxidation of *para*-Alkoxyphenols and Related Compounds—A General Route to *para*-Benzoquinone Monoacetals and Spirolactones. *J. Org. Chem.* **1987**, *52*, 3927−3930.

(23) Cook, S. P. Ph.D. Thesis, Columbia University, 2006.

(24) Kita, T.; Takaya, Y.; Oshima, Y.; Ohta, T.; Aizawa, K.; Hirano, T.; Inakuma, T. Scabronines B, C, D, E and F, Novel Diterpenoids Showing Stimulating Activity of Nerve Growth Factor Synthesis, from the Mushroom *Sarcodon scabrosus*. *Tetrahedron* **1998**, *54*, 11877−11886.

(25) Obara, Y.; Kobayashi, H.; Ohta, T.; Ohizumi, Y.; Nakahata, N. Scabronine G-Methylester Enhances Secretion of Neurotrophic Factors Mediated by an Activation of Protein Kinase C-ζ. *Mol. Pharmacol.* **2001**, *59*, 1287−1297.

(26) Wieland, P.; Miescher, K. Uber Die Herstellung Mehrkerniger Ketone. *Helv. Chim. Acta* **1950**, *33*, 2215−2228.

(27) Waters, S. P.; Tian, Y.; Li, Y.-M.; Danishefsky, S. J. Total Synthesis of (−)-Scabronine G, an Inducer of Neurotrophic Factor Production. *J. Am. Chem. Soc.* **2005**, *127*, 13514−13515.

(28) For a review, see Pellissier, H. Recent Developments in the Nazarov Process. *Tetrahedron* **2005**, *61*, 6479−6517.

(29) Yu, W. S.; Jin, Z. D. A Facile Generation of Enolates from Silyl Enol Ethers by Potassium Ethoxide. *Tetrahedron Lett.* **2001**, *42*, 369−372.

(30) For a review, see Negishi, E.-i. In *Metal-Catalyzed Cross-Coupling Reactions*; Dierdrich, F., Stang, P. J., Eds.; Wiley-VCH: New York, 1998.

(31) Guerrero, A.; Parrilla, A.; Camps, F. A New Ring Expansion Approach to 3-Formyl-2-Cyclohepten-1-ones. *Tetrahedron Lett.* **1990**, *31*, 1873−1876.

(32) Isolation: (a) Nozawa, Y.; Sakai, N.; Matsumoto, K.; Mizoue, K. A Novel Neuritogenic Compound, NGA0187. *J. Antibiot.* **2002**, *55*, 629−634. Total Synthesis: (b) Hua, Z.; Carcache, D. A.; Tian, Y.; Li, Y.-L.; Danishefsky, S. J. The Synthesis and Preliminary Biological Evaluation of a Novel Steroid with Neurotrophic Activity: NGA0187. *J. Org. Chem.* **2005**, *70*, 9849−9856.

(33) Krafft, M. E.; Dasse, O. A.; Fu, Z. Synthesis of the C/D/E and A/B Rings of Xestobergsterol A. *J. Org. Chem.* **1999**, *64*, 2475−2485.

(34) Isolation: (a) Fukuyama, Y.; Kuwayama, A.; Minami, H. Garsubellin A, a Novel Polyprenylated Phloroglucin Derivative, Increasing Choline Acetyltransferase (ChAT) Activity in Postnatal Rat Septal Neuron Cultures. *Chem. Pharm. Bull.* **1997**, *45*, 947−949. Total Synthesis: (b) Siegel, D. R.; Danishefsky, S. J. Total Synthesis of Garsubellin A. *J. Am. Chem. Soc.* **2006**, *128*, 1048−1049.

(35) (a) Spessard, S. J.; Stoltz, B. M. Progress toward the Synthesis of Garsubellin A and Related Phloroglucins: The Direct Diastereoselective Synthesis of the Bicyclo[3.3.1]nonane Core. *Org. Lett.* **2002**, *4*, 1943−1946. (b) Chatterjee, A. K.; Sanders, D. P.; Grubbs, R. H. Synthesis of Symmetrical Trisubstituted Olefins by Cross Metathesis. *Org. Lett.* **2002**, *4*, 1939−1942.

(36) (a) Nicolaou, K. C.; Pfefferkorn, J. A.; Kim, S.; Wei, H. X. Synthesis of the Fully Functionalized Bicyclic Core of Garsubellin A. *J. Am. Chem. Soc.* **1999**, *121*, 4724−4725. (b) Nicolaou, K. C.; Pfefferkorn, J. A.; Cao, G. Q.; Kim, S.; Kessabi, J. A Facile Method for the Solution and Solid-Phase Synthesis of Substituted [3.3.1] Bicycles. *Org. Lett.* **1999**, *1*, 807−810.

(37) Kuramochi, A.; Usuda, H.; Yamatsugu, K.; Kanai, M.; Shibasaki, M. Total Synthesis of (±)-Garsubellin A. *J. Am. Chem. Soc.* **2005**, *127*, 14200−14201.

(38) Knochel, P.; Dohle, W.; Gommermann, N.; Kneisel, F. F.; Kopp, F.; Korn, T.; Sapountzis, I.; Vu, V. A. Highly Functionalized Organomagnesium Reagents Prepared through Halogen−Metal Exchange. *Angew. Chem., Int. Ed.* **2003**, *42*, 4302−4320.

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