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Total Synthesis of (±)-Jiadifenin and Studies Directed to Understanding Its SAR: Probing Mechanistic and Stereochemical Issues in Palladium-Mediated Allylation of Enolate-Like Structures

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Abstract: The total synthesis of jiadifenin has been accomplished. The synthesis allows us to build an SAR profile which suggests that the jiadifenin skeleton may be less desirable from the standpoint of nominating a potential drug than that of its prerearrangement precursor. The key steps of the jiadifenin problem involve the construction of two 1,3-related quaternary carbons. The paper describes how the stereochemistry was managed in this context. The issue was studied in considerable detail at the level of a then new allyl transfer reaction arising from a palladium-mediated transfer process of an allyl carbonate. By use of externally deuterated diallyl carbonate, we could probe, for the first time, the stereochemical relationship between the inter- and intramolecular versions of this process. The existence of concurrent inter- and intramolecular allylation reactions was demonstrated by deuteration experiments. While in the particular case at hand, we find very little difference in stereochemical outcome as one partitions between the inter- and intramolecular pathways, the techniques employed are applicable to other systems.

Introduction

Naturally occurring polypeptidyl neurotrophic factors, such as NGF, BDNF, and GDNF, play an important role in mediating neuronal survival, differentiation, growth, and apoptosis.[1] Decreased neurotrophic support has been linked to the progression of a number of neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, and Huntington's disease. Given their potential therapeutic value, it is not surprising that neurotrophic factors have been the focus of a considerable amount of interdisciplinary research. Indeed, several neurotrophic factors have been evaluated in animal studies. However, due to issues of poor pharmacokinetics and bioavailability, the prospects for use of these polypeptides as clinical agents appears to be limited. Consequently, the development of small molecule nonpeptidyl CNS-permeable neurotrophins represents an area of growing interest. In this context, our laboratory has launched a research program directed to the total synthesis of small molecule natural products that mimic the effects of naturally occurring neurotrophic factors. To date, we have synthesized several such compounds, including tricycloillicinone,[2] merrilactone A,[3] scabronine G methyl ester,[4] and NGA0187.[5] Our involvement in this field led us to pursue the total synthesis of the structurally complex small molecule neurotrophin, jiadifenin. In this paper, we describe how this goal was accomplished.[6] This program led us to investigate some of the finer mechanistic and stereochemical issues associated with the creation of a C-allyl quaternary center by decarboxylative allylation, starting with an enol carbonate. Finally, the beginnings of the mapping of a preliminary SAR profile of neurotrophic activity are described.

Fukuyama's group recently reported the isolation and structural identification of a novel majucin-type prezizaane, jiadifenin (Scheme 1, **1**), from the *Illicium jiadifengpi* species of China.[7] The known (2*S*)-hydroxy-3,4-dehydronemajucin (**2**) had also

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(1) (a) Lodish, H.; Berk, A.; Zipursky, S. L.; Matsudaria, P.; Baltimore, D.; Darnell, J. *Molecullar Cell Biology*, 4th ed.; W. H. Freeman & Co.: New York, 1999; Section 23.8. (b) Kaneko, M.; Saito, Y.; Saito, H.; Matsumoto, T.; Matsuda, Y.; Vaught, J. L.; Dionne, C. A.; Angeles, T. S.; Glicksman, M. A.; Neff, N. T.; Rotella, D. P.; Kauer, J. C.; Mallamo, J. P.; Hudkins, R. L.; Murakata, C. *J. Med. Chem.* **1997**, *40*, 1863−1869. (c) Levi-Montalcini, R.; Hamburge, V. *J. Exp. Zool.* **1951**, *116*, 321−362. (d) Bennett, M. R.; Gibson, W. G.; Lemon, G. *Auton. Neurosci.* **2002**, *95*, 1−23. (e) Hefti, F. *Annu. Rev. Pharmacol. Toxicol.* **1997**, *37*, 255−257. (f) Dawbarn, D.; Allen, S. J. *Neuropathol. Appl. Neurobiol.* **2003**, *29*, 211−230.

(2) Pettus, T. R. R.; Chen, X.-T.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1998**, *120*, 12684.

(3) Birman, V.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2002**, *124*, 2080.

(4) Waters, S.; Tian, Y.; Li, Y.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2005**, *127*, 13514.

(5) Hua, Z.; Carcache, D. A.; Tian, Y.; Li, Y.-M.; Danishefsky, S. J. *J. Org. Chem.* **2005**, *70*, 9849.

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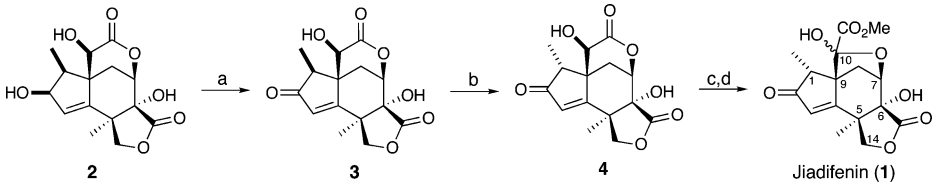
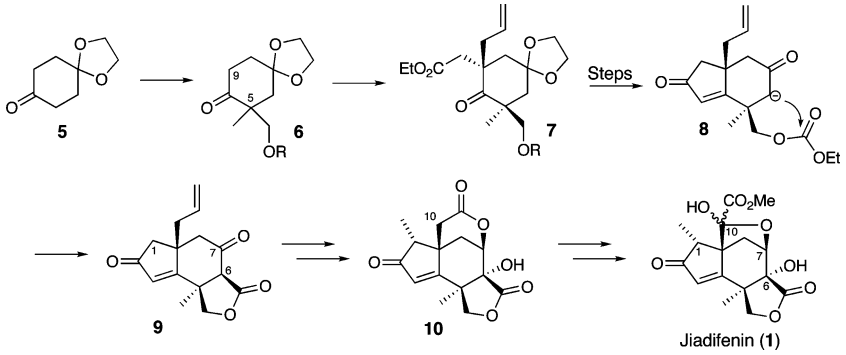
10.1021/ja056980a CCC: $33.50

Scheme 1. Structure of Jiadifenin[a]



[a] Key: (a) Dess−Martin reagent (ref 9), CH_2Cl_2, rt, 81%; (b) DBU, benzene, 80 °C, 74%; (c) Dess−Martin reagent, dioxane, rt; (d) MeOH, rt, 9%.

Scheme 2. Synthetic Strategy



been previously isolated from *I. jiadifengpi*.[8] Fukuyama's group demonstrated that **2**, the absolute configuration of which had already been determined, could be converted to jiadifenin (**1**), as outlined in Scheme 1. This interesting series of manipulations prompts consideration of **2** as a potential biosynthetic precursor to jiadifenin. Moreover, since the absolute configuration of **2** is known, the absolute configuration of jiadifenin is securely established.

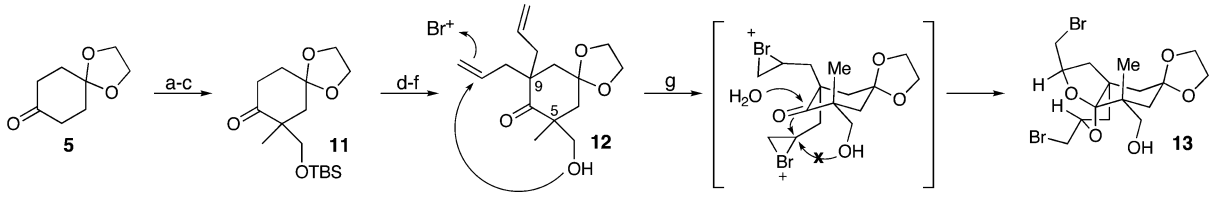
It is not uncommon that there are chemical teachings to be learned from a disciplined pursuit of this type of compact but functionality-laden target. Moreover, both jiadifenin and **2** have been shown by Fukuyama's group to promote neurite outgrowth in primary cultures of rat cortical neurons at levels as low as 0.1−10 μM.[7] While the field of pursuing the neurochemical foundations and implications of this type of finding is still in its infancy, it is not inconceivable that, in due course, a compound of neuronal enhancing characteristics could be used to advantage. Hence, from both biological and chemical perspectives, jiadifenin presented itself as a particularly appropriate target for chemical synthesis.

Our preliminary synthetic strategy is adumbrated in Scheme 2. It was assumed that compound **6** should be obtainable, albeit most likely in racemic form, from the commercially available 1,4-cyclohexanedione monoethylene ketal **5**. If this were indeed the case, we planned to install the C_9 quaternary carbon center through sequential alkylation reactions, recognizing, of course, that there could well be difficulties in achieving diastereocontrol in such a progression. From intermediate **7**, the requisite cyclopentenone functionality would be installed through an intramolecular Horner−Wadsworth−Emmons reaction and, following appropriate functional group management, intermediate **8** would be in hand. At this stage, we anticipated installation of the lactone through an intramolecular Claisen-type condensation with a pendant carbonate moiety (cf., **8** to **9**). We were optimistic that, with the tricyclic core structure in place, stereochemical biases could be exploited to achieve adequate control in the installation of the three remaining stereocenters at C_1, C_6, and C_7. All indications from Fukuyama's work suggested that the hemiacetal at C_{10} of jiadifenin cannot be obtained as a single anomer. Thus, a projectable series of transformations could allow access to **10**. On the basis of the earlier work of Fukuyama's group. (Scheme 1), it could be anticipated that, upon oxidation of C_{10}, intermediate **10** would undergo ring contraction to produce jiadifenin (**1**).[7]

Hoping to circumvent anticipated difficulties associated with controlled, selective dialkylation at C_9, starting with a compound of the type **6**, we first explored the possibility of bypassing this issue through desymmetrization of a 9,9-diallylcyclohexanone (cf., **12**). In the event, methylation of **5**, followed by hydroxymethylation[10] and protection of the resultant alcohol gave rise to **11**. Sequential diallylation with allyl bromide was followed by silyl deprotection to afford intermediate **12** (Scheme 3). At this point, the goal was to differentiate the two C_9 allyl substituents by oxidative etherification based on their differing stereochemical relation to the C_5 hydroxymethyl functionality. For instance, it was hoped that, upon treatment of **12** with *N*-bromosuccinimide, the hydroxyl group of the future C_5 would participate to produce a bromoether across the cyclohexyl ring system, thereby distinguishing the two allyl functionalities. In the event, however, treatment of **12** with NBS gave rise to **13**, in which the formal ketone hydrate had facilitated bromination of both olefin functionalities.[11] The failure of the alcohol to participate in the bromination event may be attributed, in part, to the configuration of the cyclohexanone ring system, which requires the hydroxymethyl group to be situated 1,3-syn diaxial. Having not been successful in achieving diastereocontrol through

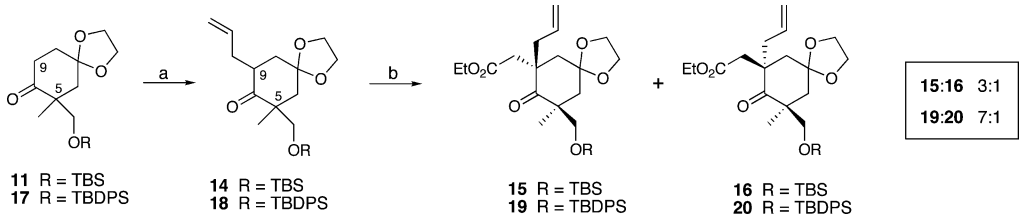
(7) Yokoyama, R.; Huang, J.-M.; Yang, C.-S.; Fukuyama, Y. *J. Nat. Prod.* **2002**, *65*, 527.
(8) Kuono, I.; Baba, N.; Hashimoto, M.; Kawano, N.; Takahashi, M.; Kaneto, H.; Yang, C.-S. *Chem. Pharm. Bull.* **1990**, *38*, 422.
(9) Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1982**, *104*, 902.
(10) Brieskorn, C. H.; Schwack, W. *Chem. Ber.* **1981**, *114*, 1993.
(11) Literature precedents of such a reaction: (a) Heusler, K.; Ueberwasser, H.; Wieland, P.; Wettstein, A. *Helv. Chim. Acta* **1957**, *40*, 787. (b) Ernst, L.; Gorlitzer, K.; Boventer, K. *Arch. Pharm. (Weinheim, Ger.)* **1990**, *323*, 361.

Scheme 3 [a]



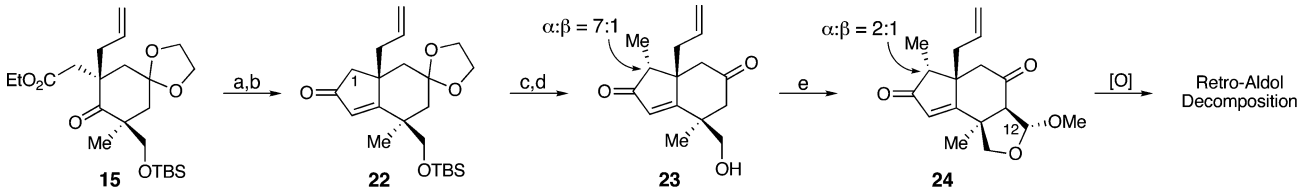
[a] Key: (a) LHMDS, THF, −78 °C, then MeI, −78 °C to room temperature; (b) 10% KOH, MeOH, HCHO(aq), 0 °C; (c) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 64% for three steps; (d) LHMDS, THF, −78 °C, then allyl bromide, −78 °C to room temperature, 73%; (e) LHMDS, THF, −78 °C, then allyl bromide, −78 °C to room temperature, 95%; (f) TBAF, THF, rt, 76%; (g) NBS, THF, −45 °C, 66%.

Scheme 4 [a]



[a] Key: (a) LHMDS, THF, −78 °C, then allyl bromide, −78 °C to room temperature, 73% (**14**) (99% based on recovered starting material), 51% (**18**); (b) LDA, THF, −78 °C to −20 °C, then $BrCH_2CO_2Et$, HMPA, −78 °C, 62% (**15** + **16**), 65% (**19** + **20**).

Scheme 5 [a]

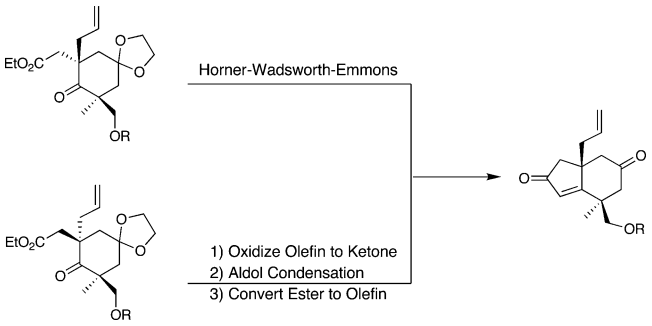


[a] Key: (a) $LiCH_2P(O)(OMe)_2$, THF, −78 °C, 81% (99% based on recovered starting material); (b) NaH, THF, reflux, 91%; (c) LDA, THF, −20 °C, then MeI, −30 °C; (d) 2 N HCl, THF, 85% for two steps; (e) $HC(OMe)_3$, PPA, 105 °C, 39% (**24**-C_1-α) and 19% (**24**-C_1-*β*).

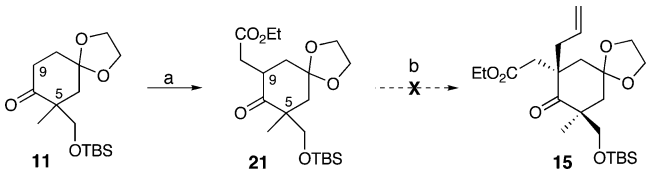
intramolecular differentiation, we next examined the selectivity levels attainable through direct sequential dialkylation.

Thus, intermediate **11** was subjected to C_9 allylation followed by C_9 carboethoxymethylation to afford compounds **15** and **16** in an approximately 3:1 ratio, as shown (Scheme 4). The observed product distribution suggests some bias for the second alkylation event to occur anti to the protected C_5 hydroxymethyl functionality. It was later found (vide infra) that the α,*β* ratio in the second alkylation event could be enhanced by increasing the steric bulk of the hydroxymethyl moiety. Thus, substitution of the −TBS protecting group with the bulkier −TBDPS moiety (cf., **17**) led to formation of products **19** and **20** in an approximately 7:1 ratio, as shown.

For the plans we had in mind (vide infra), either of the two series (**15**, **16** or **19**, **20**) could in principle be incorporated into our proposed progression.



Hence, we attempted to reverse the sequence of alkylation at C_9 (in the series carrying the TBS function; i.e., **11**). While compound **21** could be readily prepared by alkylation of **11**, attempted base-induced allylation failed (cf., **21** → **15**). On the basis of these and related studies, there was reason to believe that allylation adjacent to the ester was a complicating factor.



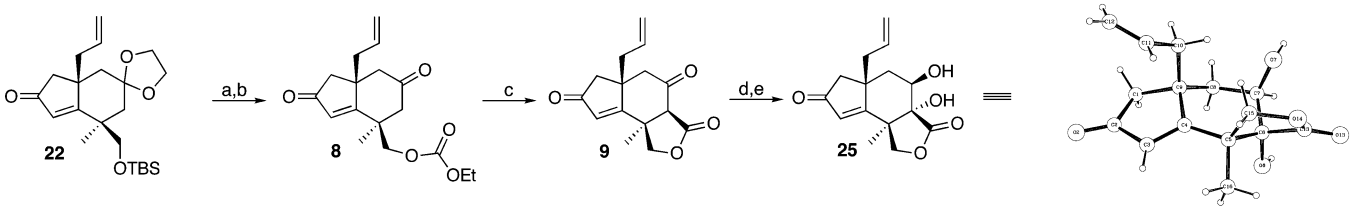
In the above graphic, the following conditions were used: (a) LDA, THF, −78 °C to −20 °C, then $BrCH_2CO_2Et$, −78 °C to room temperature, 68% (87% brsm); (b) KHMDS, THF, −78 °C, then allylbromide, HMPA, THF, −78 °C.

At that time we had, on hand, compound **15**, and subsequent initiatives started with this intermediate. Indeed, conversion of **15** to the corresponding *β*-ketophosphonate was readily accomplished. This was followed by intramolecular Horner−Wadsworth−Emmons reaction[12] to afford intermediate **22** (Scheme 5). At this stage, α-methylation at C_1 occurred primarily from the α-face, anti to the pendant allyl functionality to afford, upon deprotection, **23**, as a 7:1 mixture of isomers.

It was initially sought to interpolate carbon 12 through a mixed orthoformate ester formation−cyclization sequence. Indeed, this type of reaction had found excellent application in our synthesis of tazettine some years ago.[13] In the event, intermediate **23** was first heated with trimethylorthoformate.

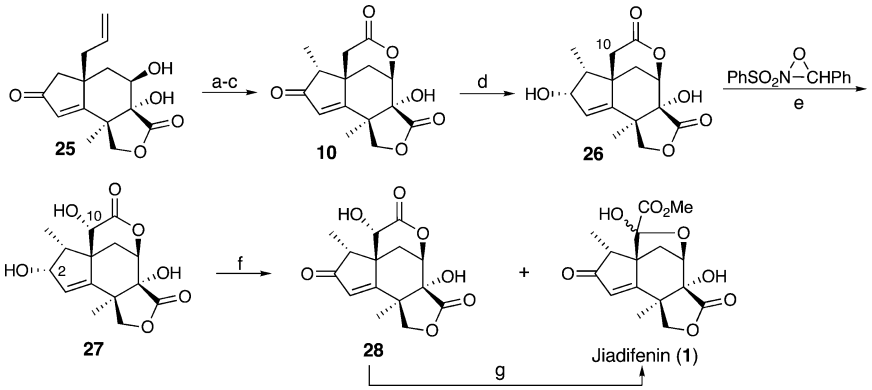
(12) Halterman, R. L.; Vollhardt, K. P. C. *Organometallics* **1988**, *7*, 883.

Scheme 6 [a]



[a] Key: (a) 2 N HCl, THF, 94%; (b) $ClCO_2Et$, py, DMAP, CH_2Cl_2, 0 °C to room temperature, 93%; (c) NaH, THF, reflux, 94%; (d) *m*-CPBA, CH_2Cl_2, 90%; (e) $NaBH_4$, THF−MeOH (1:1), −78 °C, 93%.

Scheme 7. Completion of the Synthesis of Jiadifenin[a]



[a] Key: (a) LDA, THF, −40 °C to −15 °C, then MeI, HMPA, −35 °C, 64% (77% based on recovered starting material); (b) O_3, sudan 7B red, CH_2Cl_2−EtOH (1:1), −78 °C; (c) Jones' reagent, acetone, 90% for two steps; (d) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, THF−MeOH (3:1), −65 °C, 88%; (e) NaHMDS, THF, −78 °C, then Davis' oxaziridine, THF, −78 °C, 42% after one recycle; (f) Jones' reagent, acetone, MeOH, **28** (29%) and **1** (40%); (g) Jones' reagent, acetone, MeOH, 0 °C, 46%.

When the alcohol had been consumed, the reaction was treated with polyphosphoric acid to afford, upon workup, **24** as only a 2:1 mixture of C_1 epimers. Unfortunately, various attempts to oxidize the glycoside-like linkage (at C_{12}) of **24** to the requisite lactone were unsuccessful, resulting only in retro-Aldol decomposition products.

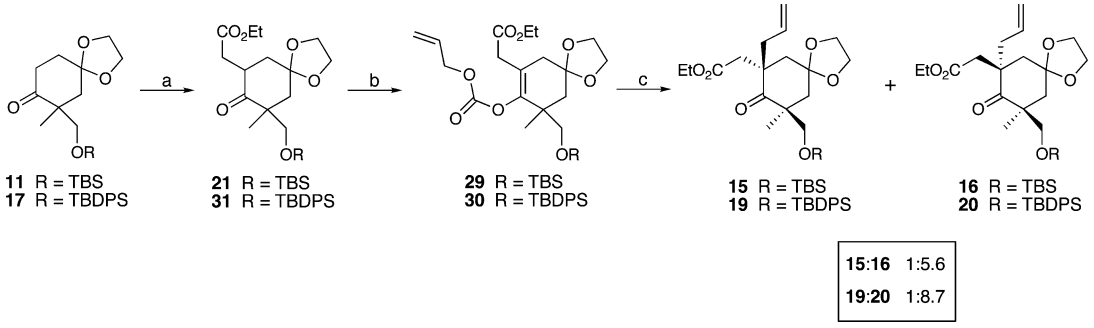
In light of the difficulties encountered in reaching the required lactone via **24** followed by oxidation, we attempted the one carbon interpolation with a carbon dioxide rather than orthoformate acid equivalent, hoping that this might allow direct access to the tricyclic system in the required C_{12} oxidation state. In addition, it seemed prudent to delay the installation of the epimerizable C_1 methyl group until later in the synthesis. Accordingly, intermediate **22** was converted to mixed carbonate ester **8**, as shown (Scheme 6). Happily, cyclization proceeded smoothly to afford the tricyclic core system **9**. As expected, hydroxylation of **9** with *m*CPBA proceeded exclusively from the α-face and, following stereoselective reduction, trans diol **25** was in hand. The assignments of the relative configurations of the newly installed stereocenters were confirmed through X-ray crystallographic analysis of **25**.

Stereoselective methylation of **25** at C_1, followed by a two-step oxidative cyclization sequence, furnished intermediate **10** (Scheme 7). Upon exposure to Luche reduction conditions, **10** was stereoselectively reduced to **26**. Incorporation of the C_{10} hydroxyl group was achieved with the Davis oxaziridine[14] to afford α-hydroxylactone **27** as a 6:1 diastereomeric mixture. With **27** in hand, we hoped to accomplish concurrent oxidation of the C_2 and C_{10} hydroxyl groups, thereby prompting rearrangement of the α-ketolactone moiety to the hydroxytetrahydrofuran carboxylate acetal of jiadifenin. Indeed, upon exposure of **27** to Jones' oxidation conditions, we isolated jiadifenin (**1**, 40%) along with intermediate **28**, in which only C_2 had been oxidized (29%). Following separation, the latter could be converted to jiadifenin upon resubmission to Jones' oxidation conditions. The overall yield for the conversion of **27** to jiadifenin (**1**) was 53%. The spectral data of the synthetic material are in complete accord with the data published for the isolated material.[7] We were able to further confirm the identity of the synthesized material through comparison with the NMR spectrum of the isolated material, kindly provided by Professor Fukuyama. As is the case with the naturally derived jiadifenin, the fully synthetic material exists as an anomeric mixture at C_{10}. Two separate peaks, corresponding to the jiadifenin anomers, were observed by HPLC analysis. Each peak was isolated; however, NMR spectroscopy once more revealed an anomeric mixture similar in ratio to that observed for the preseparated material, suggesting rapid C_{10} equilibration.

Having successfully accomplished the total synthesis of racemic jiadifenin (**1**), it was now appropriate to revisit the issue of the stereoselective installation of the C_9 quaternary carbon center. Our direct dialkylation route had been only moderately selective, providing, at that time, a 3:1 product ratio of the desired **15** and the undesired **16** (although we subsequently learned that this ratio was enhanced when the hydroxymethyl functionality was protected with the bulkier −TBDPS moiety (cf., **19**:**20**, 7:1)). Aside from the local jiadifenin priorities, we were hoping to bring forth a method to install a quaternary carbon center through allylation of a γ-ketoester substrate. As noted above, attempts to directly allylate compounds of the type **21** through conventional enolate chemistry had failed to produce

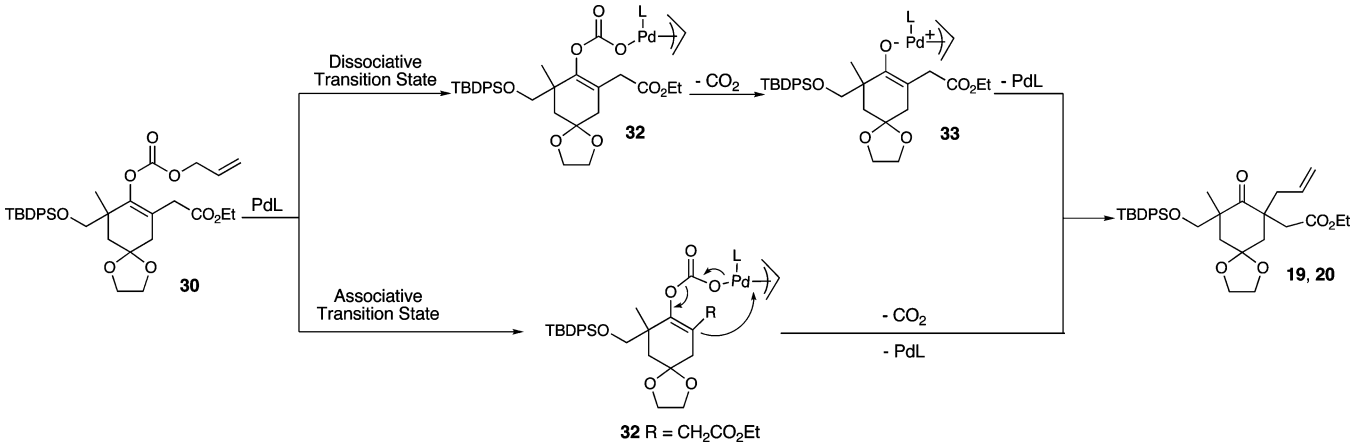
(13) Danishefsky, S. J.; Morris, J.; Mullen, G.; Gammill, R. *J. Am. Chem. Soc.* **1980**, *102*, 2838.
(14) Davis, F. A.; Chen, B. C. *Chem. Rev.* **1992**, *92*, 919.

Scheme 8. Palladium-Mediated Intramolecular Allylation[a]



[a] Key: (a) LDA, $BrCH_2CO_2Et$, THF, −78 °C to room temperature, 64−81%; (b) *t*-BuOK, allylchloroformate, THF, −78 °C, 80% to quantitative; (c) $Pd(PPh_3)_4$, THF, rt (**29**) or −40 °C (**30**), 62−65%.

Scheme 9



the desired product, leading instead to a complex mixture of alkylation products.

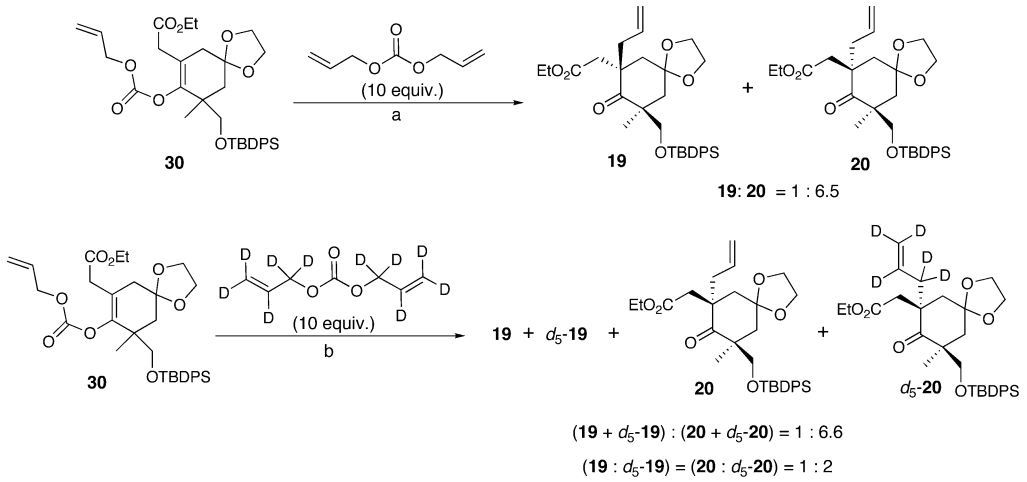
In particular, we hoped to address both issues of selectivity and reactivity through an alternative process involving intramolecular transfer of the required allyl group. To this end, we turned to a methodology suggested in a different context by Tsuji and co-workers.[15] While our work was in progress, two more closely related papers appeared from Trost and Xu[16] and Behenna and Stoltz,[17] which anticipated some important elements of our independent findings. However, as will be seen, some of the questions asked go to some issues which had not been explored even in these more recent disclosures. At the time, we proposed to install the allyl group at the quaternary carbon (C_9 in jiadifenin numbering) by palladium-mediated transfer from an enol carbonate moiety with concurrent exclusion of carbon dioxide (see, for instance, transformations of **29** to **15** and **30** to **19**, Scheme 8). Prior to the Trost and Stoltz disclosures,[16,17] no such reaction had been used to create a quaternary center. In our case, we asked another question; i.e., would the diastereoselectivity of such a reaction constructing two 1,3-related neopentyl groups approximate that of the more conventional metalloenolate alkylations.

In practice, **11** (R = TBS) and **17** (R = TBDPS) were alkylated individually, to afford intermediates **21** and **31**. These, upon treatment with *t*BuOK and allylchloroformate as shown, gave rise to the precursors **29** (R = TBS) and **30** (R = TBDPS). Following exposure to the conditions known to mediate π-allyl formation ($Pd(PPh_3)_4$ and THF), substrate **29** gave rise to **15** and **16** in a ratio of 1:5.6. Perhaps not surprisingly, the major product of this transformation was of the opposite relative stereochemistry to that obtained in the direct dialkylation sequence. This product distribution can be attributed to the preference for the allylation event to occur from the α-face of the molecule, anti to the protected hydroxymethyl functionality. Similarly, allylation of the TBDPS-protected analogue, **30**, led to formation of **19** and **20** in a more satisfactory ratio of 1:8.7. The levels of stereoselectivity observed in the allylations of both **29** and **30** were somewhat enhanced in comparison with those obtained from the analogous dialkylation sequences (vide supra).

Having developed the means to install, with selectivity, the C_9 quaternary carbon center through the palladium-mediated allylation reaction, and not then aware of prior findings in other laboratories,[16,17] we sought to learn more about the mechanism of this transformation. At the extreme, the allylation reaction could proceed either through a concerted, associative transition state or through a dissociative transition state involving in essence allylation of a pallado enolate. Even in the nonconcerted case, there would still be a question of whether the allyl group is held at some level in association with the enolate or is fully dissociated (Scheme 9). To evaluate whether the allyl group of the allyl carbonate starting material is ever released from the reacting sphere of the enolate, we sought to conduct the allylation reaction in the presence of an appropriately labeled external π-allyl source. Incorporation of external allyl group

(15) Tsuji, J.; Minami, I. *Acc. Chem. Res.* **1987**, *20*, 140. (b) Tsuji, J.; Yamada, T.; Minami, I.; Yuhara, M.; Nisar, M.; Shimizu, I. *J. Org. Chem.* **1987**, *52*, 2988. (c) Tsuji, J.; Ohashi, Y.; Minami, I. *Tetrahedron Lett.* **1987**, *28*, 2397.
(16) Trost, B. M.; Xu, J. *J. Am. Chem. Soc.* **2005**, *127*, 2846.
(17) Behenna, D. C.; Stoltz, B. M. *J. Am. Chem. Soc.* **2004**, *126*, 15044.

Scheme 10. Mechanistic Evaluation[a]



[a] Key: (a) diallyl carbonate (10 equiv), $Pd(PPh_3)_4$, THF, rt, 77%; (b) diallyl-d_5 carbonate (10 equiv), $Pd(PPh_3)_4$, THF, rt, 90%.

into the product would reflect a dissociative pathway. We first performed a control study in order to determine whether the reaction could, in fact, be conducted in the presence of an external π-allyl source. Thus, **30** was exposed to the reaction conditions shown in the presence of 10 equiv of diallyl carbonate at room temperature. The product distribution of **19**:**20** was observed, as shown in Scheme 10. Of course, this enabling experiment did not go to the question of inter- or intramolecularity in the allylation event.

Secure in the knowledge that the C-allylation reaction could indeed be performed in the presence of potential external allyl source, we exposed compound **30** to the same set of conditions, using diallyl-d_{10} carbonate, as shown. The distribution of products **19** and **20** and the level of deuterium incorporation were as shown. We found the ratio of **19** to **20** to be essentially unchanged from the control experiment (1:6.6). Interestingly, however, both the minor (**19**) and the major isomer (**20**) exhibited approximately 67% allyl-d_5 incorporation. This result is particularly suggestive because the deuterated products (**19**-d_5 and **20**-d_5) presumably arise from an intermolecular allyl transfer reaction with diallyl carbonate, while the nondeuterated products (**19** and **20**) arise from the intramolecular allyl transfer reaction. If the nature of the transition state of the intermolecular transfer (with diallyl-d_5 carbonate) were markedly different from that of the intramolecular transfer, it would be expected that the selectivity for the major isomer would be different in the deutero series. Were this the case, the ratio of **20**-d_5 to **19**-d_5 would be expected to be lower than the ratio of **20** to **19**. The fact that there is no apparent change in the distribution of stereoisomeric products when the allyl source is external (diallyl-d_5 carbonate) could be taken to suggest a similar transition state for both pathways. We conclude that, at least in this particular case, the decarboxylative allylation actually occurs through a tight but dissociative transition state even when actual crossover had not occurred. Certainly, our study provides no support for the notion of a concerted decarboxylation−allylation motif.

Having indeed accomplished an improved method for the stereoselective formation of the C9 quaternary carbon center, we turned our attention to demonstrating the feasibility of our proposal that **20** could indeed be merged with a validated intermediate in our first generation synthesis. Thus, **20** was subjected to Wacker oxidation to afford methyl ketone **34**. Intramolecular aldol condensation of **34** with *t*BuOK in THF provided the bicyclic acid **36**, presumably via intermediate **35**, with concomitant participation of the ethyl ester functionality in the aldol cyclization. Intermediate **36** was converted to terminal olefin **37** through a straightforward series of reactions involving thioester formation, Fukuyama reduction to the aldehyde, and Wittig reaction. Compound **37** corresponds to the −TBDPS-protected analogue of intermediate **22** of the original synthesis. Thus, we had realized our original expectation that either diastereomer relating C_5 and C_9 could be redeemable in our synthesis (Scheme 11).

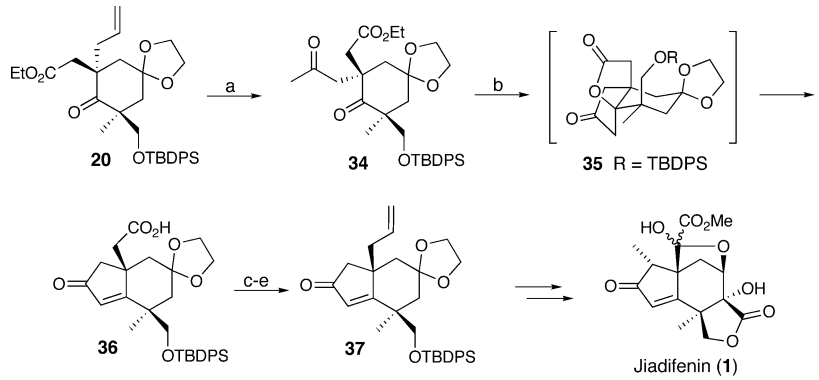
Biological Investigations

At this point, we were in a position to corroborate the claimed neurotrophic activity of jiadifenin (**1**) and to begin to establish an SAR profile. The ability of jiadifenin to promote neurite outgrowth was measured in both the presence and absence of nerve growth factor (NGF). In the presence of NGF, jiadifenin enhanced neurite lengths by 162% ($P < 0.05$). However, in the absence of NGF, no neurite outgrowth was observed, indicating that jiadifenin operates by upregulating the action of NGF rather than functioning independently.

In addition to jiadifenin itself, we evaluated the in vitro neurotrophic activity of several synthetic analogues of the natural product in order to establish an SAR profile (Figure 1). The most active compound was found to be intermediate **28**, which is the direct precursor to the natural product. This analogue was found to enhance neurite lengths by 184%. The normethyl jiadifenin analogue, **38**, also exhibits superior activity in comparison with jiadifenin, enhancing neurite lengths by 181%. On the basis of the activity observed with **28** and **38**, it might be expected that the unrearranged, normethyl analogue, **39**, would be particularly active. Surprisingly, however, **39** displays only moderate activity in this assay, suggesting a somewhat complex SAR profile for this natural product. Interestingly, intermediate **10**, in which C_{10} is unoxidized, exhibits no neurite length enhancement. Clearly, additional entries will be necessary to establish a definitive SAR profile.

In summary, at the chemical level, the total synthesis of jiadifenin has been accomplished. Among the important chemical steps are the creation of two 1,3-related quaternary centers (see transformation of **14** → **15**), eventually with reasonable

Scheme 11 [a]



[a] Key: (a) $PdCl_2$, $Cu(OAc)_2$, O_2, DMA/H_2O, rt, 69%; (b) *t*-BuOK, THF, 0 °C, 61%; (c) EtSH, DMAP, EDCI·HCl, CH_2Cl_2, 0 °C to room temperature, 95%; (d) Et_3SiH, Pd/C, CH_2Cl_2, rt, 67%; (e) $MePPh_3Br$, NaHMDS, THF, rt, 72%.

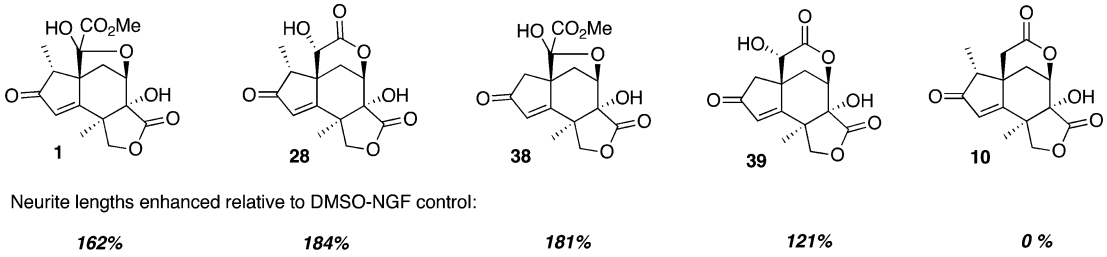


Figure 1.

stereoselection (see ratio **19**:**20**). Another important step was the creation of the γ-lactone ring by intramolecular acylation of a proximal carbonate ester (see transformation **8** → **9**). This having been accomplished, the synthesis could take advantage of some of the findings registered by the discoverers of the compound. Thus, hydroxylation of lactone **26** gave rise to **27**, which, upon oxidation followed by rearrangement, leads to jiadifenin itself. All indications which we have been able to garner suggest that it will not be possible to separate jiadifenin into its C_{10} anomers in that the equilibration of the two anomeric series is very rapid.

During the course of these studies we also had occasion to study applicability of enol allyl carbonates as a means of allylation with improvement of diastereofacial selectivity at C_9. Some improvement was in fact noted; however, the maximal stereoselectivity that we realized in the allyl carbonate allylation reaction was not very much greater than that which could be realized by optimization of the corresponding lithium enolate. A more careful study of this enol carbonate allylation route defined that, to a considerable extent, allyl residues from the initial cyclocarbonate exchange with a pool of external diallyl carbonate under the alkylation conditions. Interestingly, the diastereofacial selectivity is unaffected by whether the reaction had occurred via the initial, unexchanged allyl function or through the external allyl group. Thus, allyl which is delivered from the reacting sphere of the starting material and that which arises from bulk medium provide the same diastereofacial ratio. Apparently there is nothing in our data which supports the notion of an associative allyl transfer pathway (see **30** → **32** → **19** or **20**).

At the biological level, we note that the neurotrophic activity claimed by the discoverers of the compound has been well validated and perhaps more tightly focused. The drawing of an SAR map has begun, although at a very preliminary and still confusing level. Already it has been found that C_{10} must appear in oxidized form. For instance, compound **10** is inactive. It does seem that analogue synthesis could well lead to improved compounds in that products **28** and **38** already seem to exceed jiadifenin in their neurite-promoting activity. However, the large question in this area still remains to be addressed: can a nonpeptidyl small molecule cell-permeable compound of the type described here find application in clinical settings? This and other related biological questions are receiving continuing attention with a view toward identifying candidate structures for advancement into preclinical and, eventually, clinical evaluations. These goals tend to be among the central missions of our laboratory.

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Supporting Information Available: Experimental procedures and characterization data for compounds **8**−**22**, **25**−**31**, **34**, and **36**−**39** and CIF file for compound **25**. This material is available free of charge via the Internet at http://pubs.acs.org.

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