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An eight-step gram-scale synthesis of (−)-jiadifenolide

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Development of a biologically active secondary metabolite into a useful medicine requires continuous access to meaningful quantities of material. Although any chemical synthesis is broadly useful for its versatility, identification of a synthesis route that can be economically scaled represents a greater challenge. Here we report a concise synthesis of the neurotrophic trace metabolite (−)-jiadifenolide and its production on a gram-scale. The brevity of the route and the structural similarity of a key intermediate to many potent *Illicium* terpenes make chemical synthesis the unquestionable method for accessing and modifying these potential therapeutics.

A bioactive secondary metabolite is a double-edged sword. If its biological profile is promising, then its procurement must be addressed. For abundant metabolites from easily cultured or farmed organisms, procurement through isolation is straightforward[1]. For low-abundance metabolites from ecologically fragile or unculturable organisms[2], chemical synthesis becomes the ideal method of production. Of course, most syntheses are not ideal[3]: high material and labour costs and low yields of the target molecule can turn the cost–benefit analysis against chemical synthesis[4] and encourage a search for alternative means. The realization that methods such as genetic engineering and plant cell culture could outpace traditional synthesis as viable means to produce otherwise inaccessible secondary metabolites in bulk quantity has pressured chemists to invent better, non-traditional syntheses of complex molecules[5]. Here we report a concise and easily scaled route to access 1 g of (–)-jiadifenolide, a trace metabolite from the fruit of a southern China flowering plant that exhibits neurotrophic properties[6].

The *lllicium* genus of shrub is widely distributed and best known for the *verum* species, which produces the star anise fruit, an ingredient in Vietnamese phở and the five-spice powder of Chinese cuisine[7]. Other species, such as *I. anisatum* or *I. jiadifengpi*, are toxic[7] and should not be consumed. Substitution of the traditional medicinal herb *I. difengpi* with *I. jiadifengpi* led to accidental poisonings in the 1970s[8]. The constituents of *I. jiadifengpi* and other *Illicium* species exhibit pronounced biological effects at the organism level[7,8] and *in vitro*, especially **1**–**5** (Fig. 1), which enhance neurite outgrowth[6,9–11] in nerve growth factor (NGF)-stimulated cells[12]. The potentiating activity of **6** was not determined because, like **1**–**5**, it is produced in only trace amounts (0.00008% yield from the pericarps (fruit) of *I. jiadifengpi*). Although chemical synthesis has provided some material to verify[12,13] the neurotrophic activity disclosed in the original isolation reports, only basic structure–activity relationships (SAR) have been gleaned from these studies and the production of **1** through multistep syntheses (≥20 steps) has yielded quantities comparable to isolation (<9 mg)[13–16]. Validation of these neurotrophic terpenes for the treatment of neurodegenerative diseases is therefore restricted to cell culture-scale assays[17]. The mechanisms of action of **1**–**5** have not been reported. We disclose a concise synthesis **1** that allows its straightforward production on a gram scale. Given the structural similarity between **1** and many other neurotrophic *Illicium* terpenes, we expect the basic precepts of the synthesis to be broadly applicable across the family.

A structural feature of the *Illicium* terpenes that is conserved among almost all members is the directly joined cyclopentane–lactone ring system, highlighted in red in Fig. 1. Stereoselective formation of the connecting bond in **1** from two simple building blocks is challenging, because the bond flanks a chiral *tert*-alkyl ester and an all-carbon quaternary centre[18,19]. However, scission of this bond appeared straightforward if the dissonant oxygen appendages were removed first. The resultant intermediate (**7**) then contains two short consonant pathways between the three carbonyls that might be broken in concert[20]. The chemical reactions corresponding to this transform could rely on the reactivity of 2-oxyfurans, which

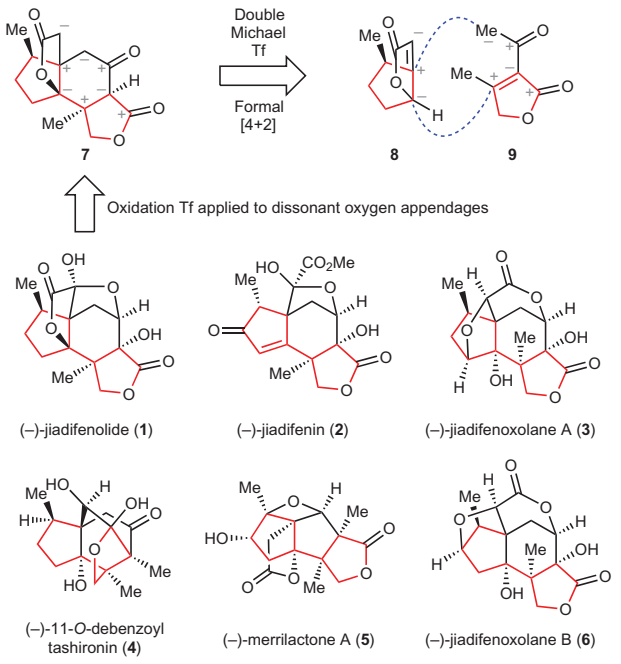

Figure 1 | Retrosynthetic analysis of the *Illicium* terpene family. All family members contain directly joined five-membered rings, which can be dissected easily if dissonant appended oxygens are first removed.

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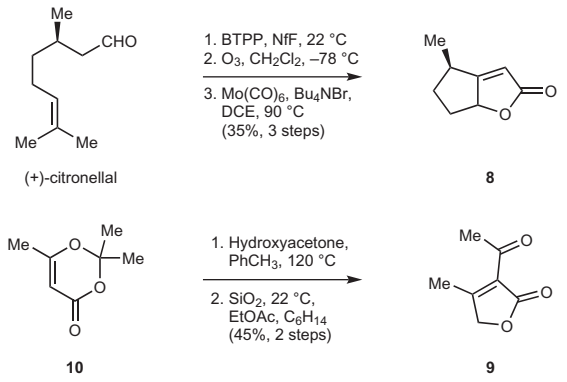

Figure 2 | Synthesis of butenolides 8 and 9. Three- and two-step routes, respectively, quickly access the key building blocks. DCE, 1,2-dichloroethane.

behave as γ-nucleophiles in Michael and aldol reactions[21]. The stereoselectivity in this process[22] was left to chance. Thus, division of **7** in half via two Michael transforms would deliver two butenolides, **8** and **9**.

Results

Chiral butenolide **8** was unknown, so we devised a short route for its synthesis from (+)-citronellal (Fig. 2). The dehydration of citronellal in one step and 78% yield is precedented using BTPP (*tert*-butylimino-tri(pyrrolidino)phosphorane) and NfF (nonafluorobutanesulfonyl fluoride)[23]. However, BTPP is prepared from *tert*-butyl azide, which slowly eliminates hydrazoic acid and is therefore a safety hazard. On a larger scale, a two-step sequence of dibromination and elimination proved more economical and safe (Supplementary Section 2.1). Ozonolysis of the alkene delivered its corresponding aldehyde, which underwent a hetero-Pauson–Khand (hPK)[24,25] reaction in the presence of molybdenum hexacarbonyl ($Mo(CO)_6$) and tetra-*n*-butylammonium bromide (TBAB), resulting in butenolide **8**. This in-house protocol for the hPK reaction provided the highest yield of **8** of the conditions screened (Supplementary Section 2.3) and avoided the air- and moisture-sensitive $Mo(CO)_3(DMF)_3$ complex commonly employed[26]. Furthermore, the procedure proved capable of producing multigram quantities of **9** at reasonable batch concentrations (0.1 M).

Achiral acetylbutenolide **9** has already been synthesized in a high-yielding, one-step sequence[27]. However, a modification of the experimental procedure was necessary to render this sequence efficient on a large (100 mmol) scale. Thermolysis of dioxinone **10** at 120 °C in the presence hydroxyacetone yielded an intermediate β-ketoester, which, upon treatment with silica, underwent condensation to yield **9**.

Inspired by Zhai's work on merrilactone[28], we explored the conversion of **8** into a siloxyfuran to effect conjugate addition to **9** according to Taguchi's protocol[29]. Unfortunately all the silyl ethers we explored proved unstable to isolation and were ineffective as nucleophiles prepared *in situ*. The enolate of **8** was therefore used directly. Treatment of **8** with lithium diisopropylamide (LDA) at −78 °C, followed by addition of **9** at −100 °C (Fig. 3a), led, almost exclusively, to one new spot by thin layer chromatography (TLC). Addition of aqueous ammonium chloride at a low temperature resulted in the isolation of cyclic enol ether **11** (Fig. 3a, 8:1 mixture of diastereomers), the product of a [4+2] cycloaddition between butenolide **9** and the enolate of **8**. The stereochemistry of

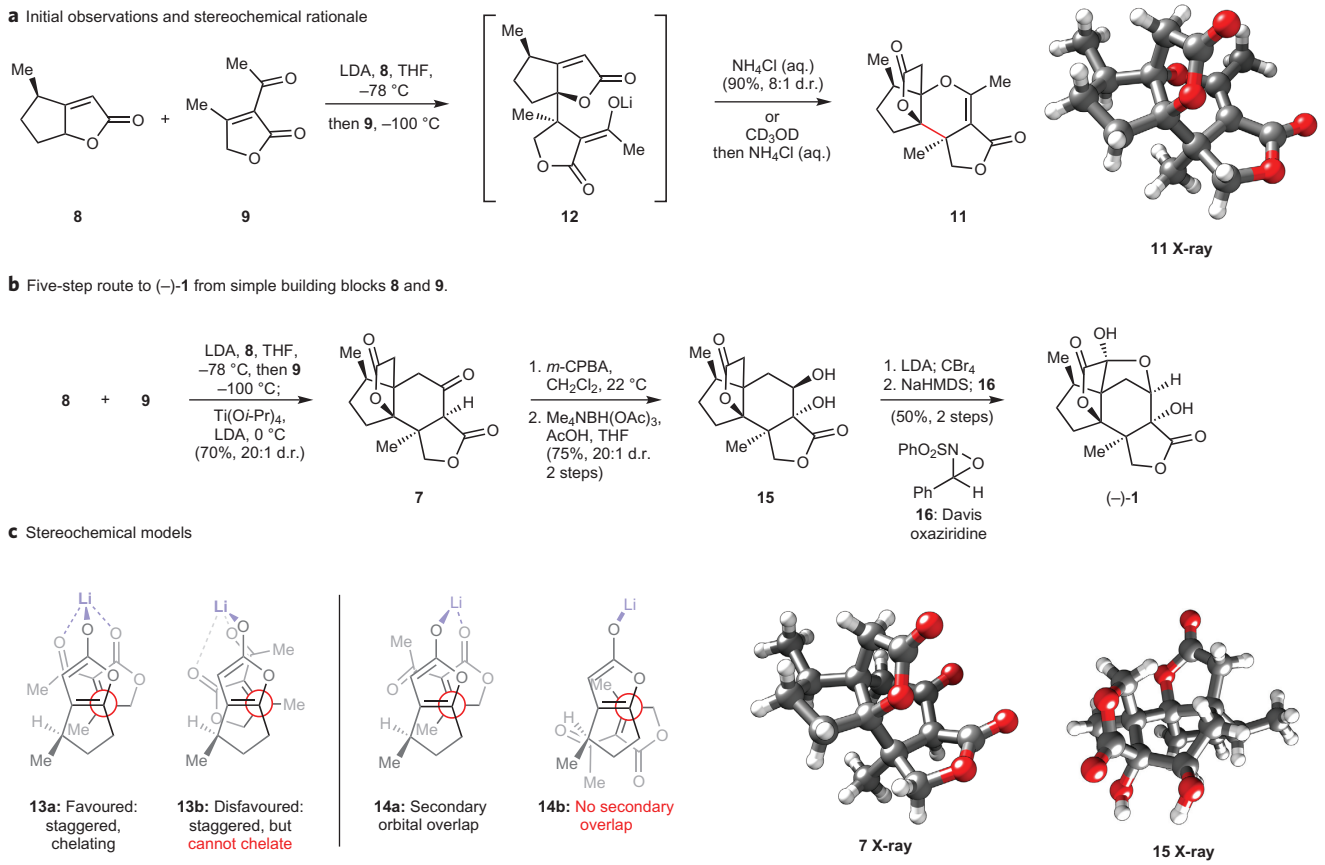

Figure 3 | Concise synthesis of (−)-jiadifenolide (1). a, Formal [4+2] cycloaddition leads to dihydropyran **11** via a kinetically stable enolate **12**. **b**, Stereoselective double Michael reaction allows one-step construction of the jiadifenolide scaffold **7**. **c**, Stereochemistry might be explained by chelated transition state **13a** or Diels–Alder-like transition state **14a**. Stereochemistries were proven by X-ray analysis.

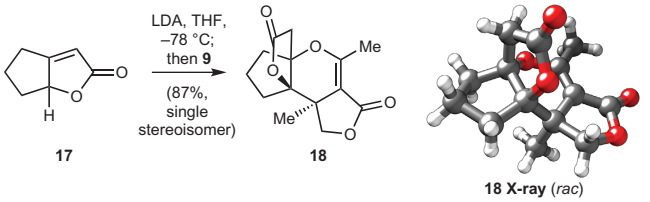

Figure 4 | Proof of bias. Relative stereocontrol is very high without a stereogenic methyl, highlighting the specificity of this process.

the major diastereomer was initially elucidated by nuclear Overhauser effect (NOE) studies and finally confirmed by X-ray diffraction of crystalline **11** (Fig. 3a, **11 X-ray**). As it was unclear at what temperature or under what conditions the C–O bond formed (anionic or acidic upon quench), we added CD_3OD to the reaction mixture before acidic work-up. However, we saw no incorporation of deuterium, suggesting that the enol-lactone adds conjugatively only after acidification and warming. This result implied that dihydropyran formation is not a kinetic trap and that a stable intermediate (**12**) could be manipulated to form the final skeletal C–C bond. Such an intramolecular Michael addition was accomplished by the addition of titanium(IV) isopropoxide ($Ti(O\textit{i}\text{-}Pr)_4$) and six more equivalents of LDA (Fig. 3b). Thus, in a single step, the entire skeleton of jiadifenolide, ketolactone **7**, was constructed from **8** and **9** (Fig. 3c, **7 X-ray**). We hypothesized that the stereochemistry of this process derives from either a chelated, staggered transition state **13a**, which would be lower in energy than the non-chelated, staggered approach **13b**, or from an alternative Diels–Alder-like transition state that benefits from ketone-furan secondary orbital overlap (**14a**, versus no secondary overlap in **14b**). The chelating transition state is tentatively excluded based on the geometry of the silyl enol ether derived from **12** (Supplementary Section 2.6).

Hydroxylation of the acidic α-position of β-ketolactone **7** was accomplished via the enol tautomer by oxidation with *m*-CPBA[12]. This tertiary alcohol then directed reduction of the proximal ketone by trimethylammonium triacetoxyborohydride[15], to yield diol **15**, the structure and stereochemistry of which were assigned by X-ray crystallography (Fig. 3b, **15 X-ray**). Direct conversion of this diol to jiadifenolide proved challenging without protection of the oxidation-prone secondary alcohol. Eventually, a two-step sequence was developed in which the trianion of **15** was treated with carbon tetrabromide to yield an α-bromolactone. Bromination must occur on the enolate face proximal to the diol, because the X-ray of **15** shows this approach to be open, and the absence of any etherification suggests displacement of the bromide is geometrically impossible. NOE studies also support this stereochemical assignment (Supplementary Section 2.9). Treatment of the intermediate bromolactone with sodium bis(trimethylsilyl)amide (NaHMDS) followed by Davis' racemic oxaziridine (**16**) yielded (−)-jiadifenolide **1**. Although the overall process has not been fully optimized, its brevity, generally good yields and high stereocontrol enabled gram-scale production of **1**. At this point, 1 g has been produced from a single pass of scale-up, and samples are available for assay.

Discussion

The route is not without flaw. For instance, the synthesis of butenolide **8** relies on the less available and more expensive enantiomer of citronellal ($1.4/mmol, abcr) and uses a metal carbonyl complex in stoichiometric quantities (1.2 equiv. relative to substrate). Though not a flaw in the context of (−)-**1**, the stereoselectivity in the combination of **8** and **9** is so high that reversal of the selectivity to access the alternative relative configurations of stereocentres found in merrilactone A (**5**) may be a significant challenge. We have found that reaction of des-methyl **8** (that is, **17**, Fig. 4)[26] in the Michael cascade with **9** generates only one diastereomer of **18** (by ^{1}H NMR). On the one hand, these data suggest that chelation or secondary orbital interaction between the two butenolides must be significantly altered to access the relevant transition states for the alternative stereochemistry (**13b**,**14b**), but on the other hand, it also suggests that lithium 2-alkoxyfurans could be generally useful species for obtaining extremely hindered stereodiads with very high stereocontrol, as directly joined rings (for example, **12**) or linearized to complex polyols.

Conclusion

We have reported a concise (eight-step), gram-scale chemical synthesis of (−)-jiadifenolide (**1**), a trace metabolite that potentiates the action of NGF towards cultured neurons. Isolation of 1 g of **1** would require 117 kilograms of *I. jiadifengpi* pericarps (dry weight), one 226 kilogram silica gel column, two more silica gel columns of unspecified relative size, and preparative HPLC separation[6]. The ability to easily produce 1 g of **1** through chemical synthesis will aid its validation (or invalidation) as a small-molecule neurotrophin used for the treatment of neurodegenerative diseases[30]. Our route relies on a stereoselective coupling of two simple butenolides to build the entire skeleton of **1** in one step. Laterally-productive functional group interconversions like protecting group operations, stereochemical inversions or zero-sum redox manipulations are minimized. We anticipate that this strategy might be applicable to all the *Illicium* family members by elaborating the coupled butenolides along probable biosynthetic pathways. For now, plenty of (−)-jiadifenolide (**1**) is available for any interested collaborators.

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Author contributions

All authors conceived and designed the experiments and analysed the data. H-H.L. and M.D.M. performed the experiments. R.A.S. wrote the paper.

Additional information

Supplementary information and chemical compound information are available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to R.A.S.

Competing financial interests

The authors declare no competing financial interests.