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# Total synthesis of (+)-pentamethylsalvianolic acid C†

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The total synthesis of a methylated analogue of (+)-Salvianolic acid C has been achieved. Key aspects of the synthetic route include an economical Cu(I) acetylide coupling, unique carboxyl activation conditions *via* microwave irradiation and a novel lipase catalysed kinetic resolution of a racemic mixture of secondary alcohol Danshensu. The preparation of this methylated analogue will not only improve the bioavailability, but also enable access to new and wider bioactivity applications for (+)-Salvianolic acid C.

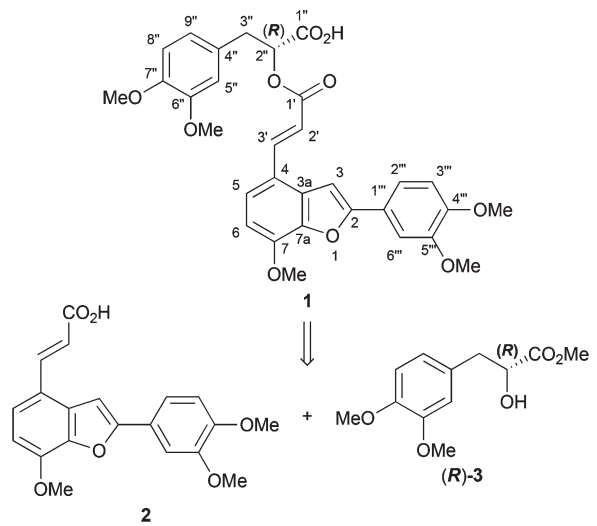


## Introduction

Danshen, the dried root and rhizome of *Salvia miltiorrhiza* Bunge, in the Lamiacea family (formerly Labiacea), is one of the most popular traditional Chinese medicines (TCM) that has been used globally to promote circulation and improve blood flow. With a history of at least 2000 years, Danshen has been used in China for decades (since 1970) to provide therapeutic relief from stroke and angina pectoris. Other medicinal properties of this herb include antiviral, antioxidant and antitumour activities.[1–3] Aqueous Danshen decoctions (tea) have been used for the prevention of the major causes of stroke including thrombosis, atherosclerosis, oxidative damage and cerebral ischemia–reperfusion.[4,5] Investigations into the chemical constituents of Danshen revealed two predominant classes of secondary metabolites. A family of lipid soluble, hydrophobic diterpenoids deemed Tanshinones and a hydrophilic, polyphenolic combination of compounds consisting mainly of caffeic acid dimers, trimers or tetramers (depsides): the Salvianolic acids. The polyphenolic acids are unique to the *Salvia* genus.[3] The water soluble compounds were found to be relatively more abundant (7% (w/w) for (+)-Salvianolic acid B in *Salvia bowleyana*[4]) and many are available.[6,7]

While the polyphenolic nature of Danshen water soluble components allows access to a huge array of bioactivities including antioxidant and anticancer activity, they are not without their limitations. Pharmacokinetic studies on the most abundant polyphenol, Salvianolic acid B, revealed that the natural product has very poor bioavailability and is metabolised to four methylated derivatives.[8–11] It is currently unknown whether these metabolites are pharmacologically active but as they have the ability to pass through cell walls, due to their lower polarity making them more lipophilic, specific assays for their biological function are required.[12] We report here a short, efficient total synthesis of (+)-pentamethylsalvianolic acid C **1**. The chemical synthesis and structural variation of Danshen secondary metabolites could lead to new drug candidates, thereby adding to the wide variety of natural products and their analogues already established as potent pharmaceuticals.

Our retrosynthesis is outlined in Scheme 1. The key disconnection between carbons C1′ and C2″ gives the two main fragments **2** and **(*R*)-3**. The benzo[*b*]furan core (**2**) was prepared *via* cuprous acetylide coupling with bromoisovanillin and the (***R***)-Danshensu component **(*R*)-3** obtained from the racemic precursor *via* lipase catalysed kinetic resolution. The two elements of the natural product were joined *via* esterification. In an attempt to access the natural polyphenol, demethylation of the five methyl ethers will be attempted given the success of O'Malley *et al.*[13] in the final stages of their total synthesis of




**Scheme 1** Retrosynthesis of (+)-pentamethylsalvianolic acid C.

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(+)-Lithospermic acid. Even though this retrosynthesis is similar to that of Shen *et al.*[14] in their recent total synthesis of (+)-Salvianolic acid C, this approach offers several practical alternatives.

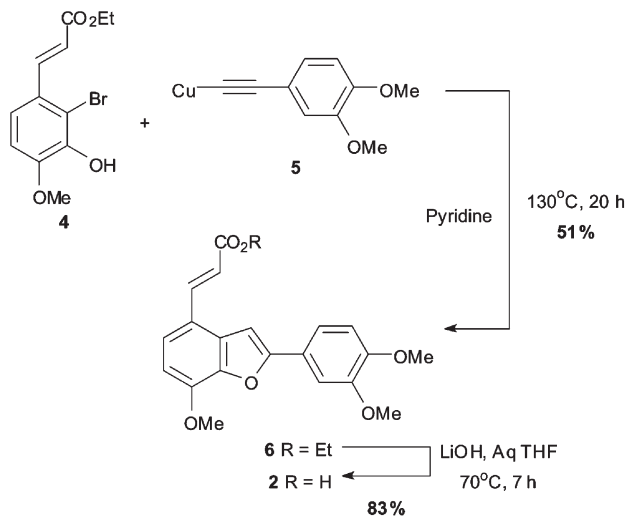
## Results and discussion

### Synthesis of the benzo[*b*]furan moiety of 1 *via* cuprous acetylide coupling

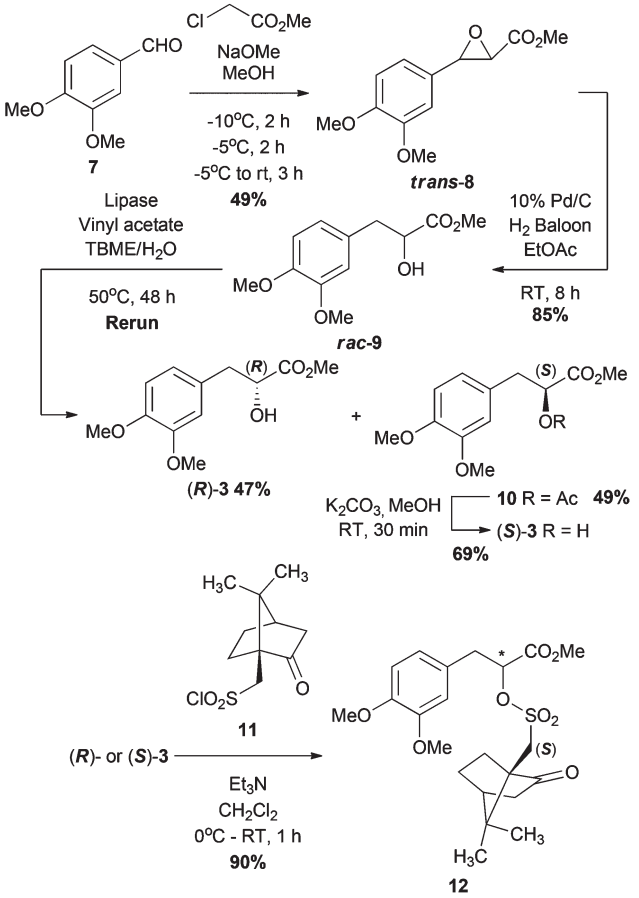
Ethyl bromocinnamate **4** was prepared in 74% yield from bromoisovanillin by Knoevenagel condensation with ethyl hydrogen malonate. As a model reaction, the Knoevenagel condensation was attempted with 3-iodovanillin but only trace amounts of product were observed. This is thought to be due to the generation of HI followed by decomposition and therefore bromine was used as the activator in further transformations. Cu(I) acetylide **5** was synthesised from aromatic aldehyde **7** in two steps *via* the Bestmann–Ohira homologation[15–17] (see ESI†) followed by deprotonation in aqueous ammonia in the presence of $Cu(II)SO_4$ according to Wong and co-workers.[18] Coupling of **5** with **4** was then performed using the optimised conditions of Scammells *et al.*[19] to form the 2-arylbenzo[*b*]furan core **6** in 51% yield after recrystallization from EtOAc (Scheme 2). The diyne by-product (12% yield) from the homocoupling of **5** was removed by FCC. The ethyl ester of **6** was then hydrolysed with LiOH in aqueous THF (83% yield) to form the benzo[*b*]furan scaffold **2** in a total of four linear steps with an overall yield of 22% from isovanillin.

### Preparation of (*S*) and (*R*)-Danshensu *via* Darzens glycidic ester synthesis followed by kinetic resolution

Epoxide ***trans*-8** was prepared by Darzens condensation of aldehyde **7** with methyl chloroacetate.[20,21] When the reaction was performed with NaOMe as the base, epoxide ***trans*-8** was obtained in 49% yield after recrystallisation from EtOAc–hexane (Scheme 3). This was then hydrogenated at atmospheric pressure in the presence of 10% Pd/C to form α-hydroxy ester ***rac*-9** with an excellent yield of 85% after FCC.[22] The racemic mixture was then separated by kinetic lipase catalysed resolution. Using the lipase from *Burkholderia cepacia*, as recommended by Adam *et al.*[23] the optimum conditions were 10 equivalents of an acyl donor (vinyl acetate), up to 1 weight equivalent of lipase, performing the operation at 50 °C for 48 hours. After removing the acetylated product (**10**) from the crude mixture by FCC and repeating the resolution, **(*R*)-3** was obtained in 47% yield. Both fractions of **10** were then deacetylated under methanolysis conditions ($K_2CO_3$, MeOH) to afford **(*S*)-3** in 69% yield. For the determination of enantiomeric excess (ee) the two optically active α-hydroxy esters were esterified with (1*S*)-(+)-10-camphorsulfonyl chloride **11** (Scheme 3) and the resulting diastereomers (**12**) were analysed *via* $^1H$ NMR ($CDCl_3$) spectroscopy.[24] The ee for both Danshensu enantiomers was determined to be 96% (see ESI†). This is an improvement over the resolution performed by Eicher *et al.*[25] as the ee for the Danshensu motif has been increased by more than 10%. The identity of (*R*,*S*) and (*S*,*S*) diastereomic $CH_3$ singlets was confirmed by preparing a (+)-Danshensu standard from commercial (+)-Rosmarinic acid (according to O'Malley *et al.*[13]) and comparing the $^1H$ NMR spectra obtained for the diastereomer formed between this and **11** and ***rac*-9** and **11**.



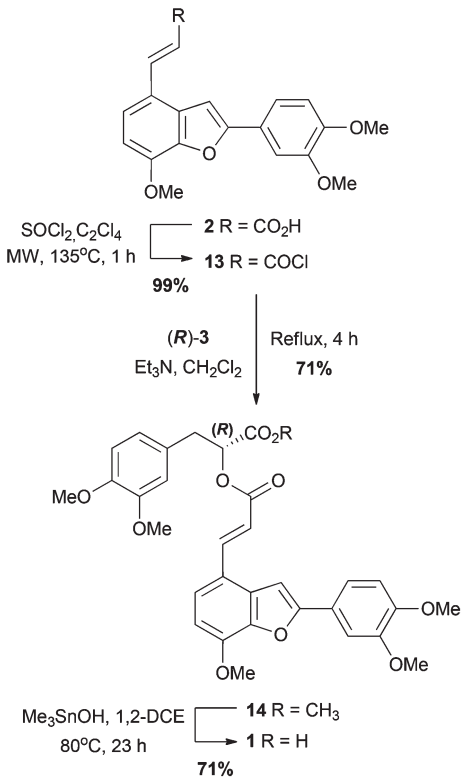

**Scheme 2** Synthesis of 2-arylbenzo[*b*]furan **2**.




**Scheme 3** Preparation of **(*R*)-3** and determination of ee.

### Esterification of the benzo[*b*]furan scaffold with (*R*)-Danshensu

At first the Steglich protocol was employed to esterify the two building blocks as has been done in related syntheses.[13,26,27] However, when this approach was attempted only the activated carboxylic acid was formed and a large amount of unreacted alcohol remained. When DIC (*N*,*N*′-diisopropylcarbodiimide) was used trace amounts of desired ester **14** were observed with the major product being the activated carboxylic acid intermediate and unreacted alcohol.[28] When acid chloride **13** was prepared the esterification with (***R***)-**3** proceeded readily in refluxing $CH_2Cl_2$. This approach was similar to that done by Bogucki and Charlton[29] in their total synthesis of (***S***)-(−)-Rosmarinic acid. To achieve quantitative yields of **13** the activation was performed using microwave irradiation with tetrachloroethylene ($C_2Cl_4$) as the solvent. Freshly prepared **13** was then treated with (***R***)-**3** (2 : 1) in refluxing $CH_2Cl_2$ in the presence of $Et_3N$ to afford methyl (+)-pentamethylsalvianolate C **14** in 71% yield (Scheme 4). Surprisingly during the reaction an anhydride by-product (see ESI†) was formed and isolated from the $CH_2Cl_2$ solution by precipitation from the reaction mixture in 20% yield. It is known that this by-product did not form during the microwave activation as the entire sample of the acid chloride was soluble in $CH_2Cl_2$ at room temperature. The structure of **14** was confirmed by comparison of the $^1H$ NMR data obtained for methyl (+)-pentamethylsalvianolate C from the original isolation (see ESI†).[30]




**Scheme 4** Synthesis of (+)-pentamethylsalvianolic acid C (**1**).

### Deprotection of methyl (+)-pentamethylsalvianolate C (14)

The methyl ester in **14** could be selectively hydrolysed with $Me_3SnOH$ to form carboxylic acid **1** in 71% yield (Scheme 4). Other ester hydrolysis techniques including LiOH in aqueous THF and $Ba(OH)_2$ in MeOH were not selective for the methyl ester as has been reported by Nicolaou *et al.*[31] An attempt was made to demethylate the remaining methyl ethers using the conditions described by O'Malley *et al.*[13] Unfortunately only complete decomposition was observed and an alternative procedure was required. Using the $BBr_3$ demethylation conditions of Lingham *et al.*[32] with the knowledge of McOmie *et al.*[33] four out of five methyl ethers could be removed. Attempts were made to isolate the mono-methylated product *via* prep-TLC but due to the incredibly low yield, complete characterisation could not be carried out. In future work, the use of alternative protective groups is recommended.

## Conclusions

(+)-Pentamethylsalvianolic acid C (**1**) has been prepared in a total of six linear steps with an overall yield of 11% from isovanillin. Highlights of the synthetic route include an economical Cu(I)-acetylide coupling with ethyl bromocinnamate **4** to form the 2-arylbenzo[*b*]furan skeleton **6**, lipase mediated resolution of ***rac*-9**, novel acid chloride formation and selective hydrolysis of the methyl ester in **14** with $Me_3SnOH$. The use of inexpensive, stable reagents has made this synthesis and potentially related syntheses scalable. Biological testing of analogue **1** is currently underway and will be presented in due course.

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