Practical and Green Synthesis of Combretastatin A-4 and Its Prodrug CA4P Using Renewable Biomass-Based Starting Materials

Yu Chen,[a,b] Yong Zou,*[a] Hong-Yi Sun,[a,b] Xian-Ke Liu,[a,b] Chun-Fen Xiao,[a,b] Jie Sun,[a,b] Shu-Jie He,[a] Jun Li*[c]

[a] Guangzhou Institute of Chemistry, Chinese Academy of Sciences, Guangzhou 510650, P. R. of China
Fax +86(21)85231119; E-mail: zou_jinan@163.com

[b] Graduate School of Chinese Academy of Sciences, Beijing 100039, P. R. of China

[c] Second Affiliated Hospital, College of Medicine, Zhejiang University, Zhejiang 310009, P. R. of China

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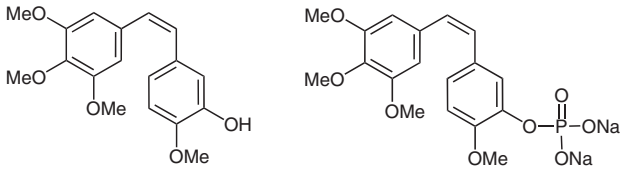
Abstract: A practical and green protocol for the synthesis of vascular disrupting agent combretastatin A-4 (CA4) and its water soluble prodrug CA4P is described. Starting from the biomass-based compound anethole, which is abundantly and sustainably available from Chinese star anise (*Illicium verum* Hook. f.), the key intermediate 3-hydroxy-4-methoxyphenylacetic acid can be obtained within five steps. Perkin condensation between this acid and another naturally derived compound 3,4,5-trimethoxybenzaldehyde, followed by decarboxylation gives combretastatin A-4 in good overall yield. The phosphate produrg CA4P can be prepared under simple and mild conditions in a sequential one-pot two-step reaction.

Key words: combretastatin A-4, combretastatin A-4 phosphate, renewable sources, green synthesis, Perkin reaction

Combretastatin A-4 (CA4, Figure 1) is a potent tubulin-binding agent first isolated from the bark of the South African bush willow *Combretum caffrum* by Pettit and coworkers in 1989.[1] Since then, it has received great and continuous attention owing to the strong inhibition of tubulin polymerization and selective targeting of tumor vascular systems, and has commonly been recognized to be the original lead compound for vascular disrupting agents (VDAs).[2] The water soluble prodrug of CA4,[3] namely combretastatin A-4 phosphate (CA4P, Figure 1), which is endowed with a much improved pharmacokinetic profile and being developed by Oxigene, has proved to be the first VDA candidate entering into phase III clinical trials for treatment of cancers as well as ophthalmological diseases (myopic and wet macular degeneration).[4]

The contradiction between encouraging biological results and the extreme scarcity in nature concerning about CA4/CA4P have stimulated the synthetic studies. As a polyphenolic stilbene with *cis*-configuration, the synthetic methodologies for CA4 are mainly based on Wittig reaction,[5] Perkin reaction,[6] Suzuki-type reaction,[7] Ramberg–Backlund reaction,[8] etc. Among them, the Perkin condensation developed by Gaukroger et al.[6] seems to be more convenient and *cis*-selective. Our group has also reported a concise route for CA4 and its dihydro analogue erianin utilizing the same portocol.[9] It could be noticed that the starting materials of the above mentioned methodologies were totally relying on petrochemicals, which were far from sustainable, thus the present synthetic protocols for CA4 and analogues have not fully met the concepts of modern 'green chemistry' and still remains to be improved.



combretastatin A-4 (**1**) combretastatin A-4 phosphate (**2**)

Figure 1 Chemical structures of CA4 and CA4P

Feedstocks derived from renewable sources play an increasingly important role in the modern chemical and pharmaceutical industry, the strategy of which is highlighted in the 'Twelve Principles of Green Chemistry' in which Article VII mentions that: a raw material or feedstock should be renewable rather than depleting whenever technically and economically practicable.[10] Consequently, in the course of our ongoing efforts to develop naturally occurring stilbenes and derivatives from renewable biomass-based materials endemic to China, we herein report a practical and green protocol using Perkin condensation for the synthesis of CA4/CA4P. The synthesis starts from the biomass-based and most importantly, the sustainable-supplied compound anethole (**6**, derived from Chinese star anise)[11] and 3,4,5-trimethoxybenzaldehyde (**5**, derived from Chinese gallnut),[12] providing a good example for the development of bioactive compounds in a green chemistry manner and revealing the potential in large-scale applications (Scheme 1).

Our strategy is based on Perkin reaction, which has commonly been recognized to be stereoselective (*cis*/*trans*: >95/5 after one crystallization), easy to handle, high-yielding, and atom economic. Retrosynthetic analysis that led to the Perkin-based strategy for the synthesis of CA4 is outlined in Scheme 1. The decarboxylation precursor **3** would be prepared through Perkin condensation between the key building blocks 3-hydroxy-4-methoxyphenylacetic acid (**4**) and 3,4,5-trimethoxybenzaldehyde (**5**), which could be further traced back to biomass-based raw materials anethole (**6**) and gallic acid (**7**), respectively.

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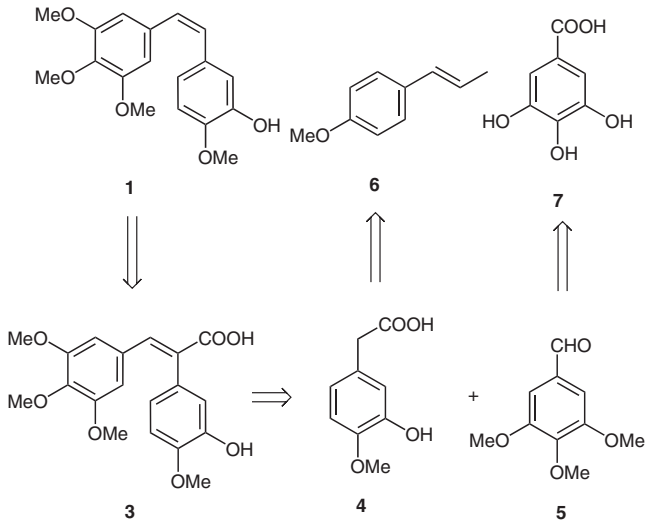


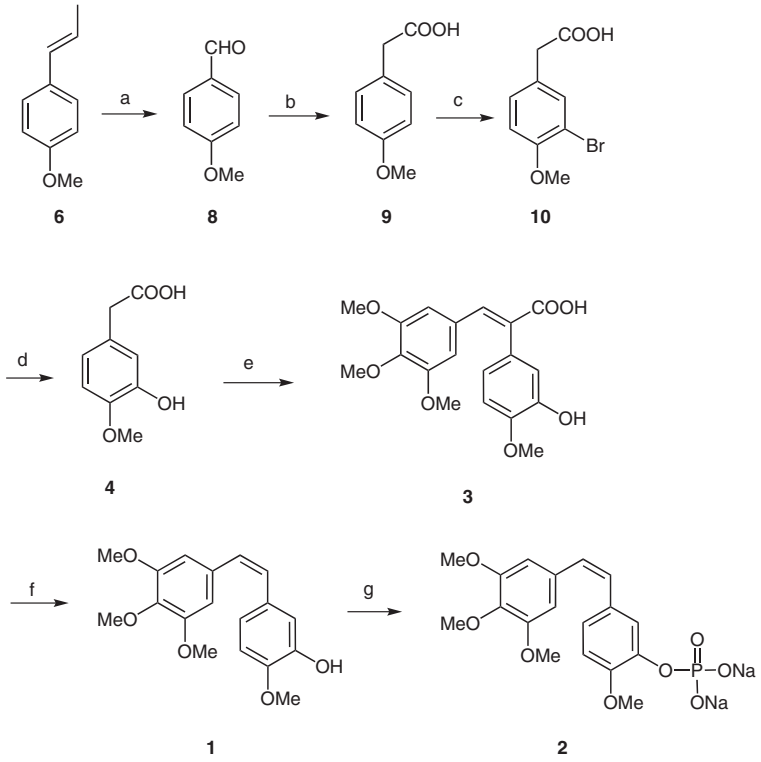
Scheme 1 Biomass-based retrosynthetic analysis of CA4

Anethole (4-propenylanisole, **6**) is the major volatile component of Chinese star anise (*Illicium verum* Hook. f.) and could be obtained sustainably in large quantity from fruits, branches, and leaves of the plant. The content of anethole could be as high as 8.88% in the fruit of *Illicium verum* and 92% in volatile essential oil.[11] It is worth mentioning that the plant of *Illicium verum* belongs to the category of non-corn biomass and that the anethole is not obtained from barks, stems, and roots of the plant, thereby no ethical problems are caused by competition with food, and the acquisition process of anethole brings no damage to the plant sources. Hence, the anethole is deserved to be an ideal renewable biofeedstock and served as a typical example for elaborating the concept of sustainable (green) chemistry.[13] Similarly, gallic acid (**7**) is also a sustainable biomass-based feedstock obtained in large amounts from Chinese Gallnuts and Tara (from Peru),[14] and has found wide applications as a starting material for the development of a series of fine chemicals.[12a] 3,4,5-Trimethoxybenzaldehyde (**5**) is, for example, a well-known pharmaceutical intermediate derived from gallic acid (**7**) and the synthetic approach has been well established.[12] As a matter of fact, from modern green chemistry point of view, the biomass-based feedstocks such as anethole, gallic acid, shikimic acid, pinene, etc., which are endowed with the unambiguous character of sustainability, deserve further research and applications as alternative resources in place of petroleum-based feedstocks for modern chemical and pharmaceutical industry.

The synthesis (Scheme 2) of the key intermediate 3-hydroxy-4-methoxyphenylacetic acid (**4**) starting from anethole (**6**) commenced with a simple and conventional oxidation reaction in the presence of natural pyrolusite (containing 60–70% MnO_2) in sulfuric acid to afford *p*-anisaldehyde (**8**) in good isolated yield (75%).[15] Our goal was, however, to find a way to minimize the disposal problems of large amount of acidic waste water and residue in this reaction and to recover the manganese element mostly.[16] As shown in Figure 2, when the oxidation reaction was complete, the crude oil of anisaldehyde (**8**) could be obtained by filtration and separation. The acidic aqueous phase and the excess slag of pyrolusite, which still contained approximately 25% manganese dioxide, were treated with oxalic acid at 80 °C for 30 minutes. A non-manganese, nonhazardous residue (mainly consisting of SiO_2 and $BaSO_4$) was then obtained and separated by filtration. The filtrate was treated by successive addition of H_2O_2 and aqueous ammonia to give a solution with a pH of 5.4–6.0, which resulted in the precipitation of $Al(OH)_3$ and $Fe(OH)_3$. After filtration, the filtrate was treated with 5% aq $(NH_4)_2S$, and most of the heavy metal ions except Mn^{2+} were precipitated as sulfide MS ($M = Pb^{2+}$, Cd^{2+}, Zn^{2+}, Cu^{2+}, Ni^{2+}, etc.). Then, a calculated amount of $(NH_4)_2SO_4$ was added to the filtrate to form an equivalent content of $(NH_4)_2SO_4$ and $MnSO_4$ in the solution. To our delight, when the solution was evaporated partially and repeatedly, a pale reddish, single crystal with high purity were steadily obtained. Indeed, the high purity of the inorganic compound enabled us to perform a single-crystal X-ray diffraction analysis to determine its chemical composition, and it was confirmed to be $(NH_4)_2SO_4{\cdot}MnSO_4{\cdot}6H_2O$.[17] It crystallizes in the monoclinic $P2_1/c$ space group with half a molecule in the asymmetric unit. The molecule possesses crystallographic inversion symmetry. The Mn(II) center is six-coordinated by six O donors from H_2O with Mn–O distances of 2.15–2.19 Å. Interestingly, the six H_2O molecules are connected with Mn(II) through coordination bonds rather than hydrogen bonds as we expected (Figure 3). The packing in the crystals is determined by rather complex intermolecular networks of hydrogen bonds (Figure 4). The strongest hydrogen bonds in the crystal structure are formed between the hydrogen of H_2O and oxygen of SO_4^{2-} (O–H···O: 1.87 Å, 177°), in which the coordinated H_2O molecule acts as H-bond acceptor. Moreover, the recovery of manganese in the form of $(NH_4)_2SO_4{\cdot}MnSO_4{\cdot}6H_2O$ can be as high as 90% of the total amount of manganese in form of MnO_2 existed in pyrolusite. In addition, $(NH_4)_2SO_4{\cdot}MnSO_4{\cdot}6H_2O$ can be further converted into high purity $MnCO_3$ by treatment with a stoichiometric amount of 10% of aqueous NH_4HCO_3.

p-Anisaldehyde (**8**) was converted into sodium 4-methoxymandelate by TEBA-catalyzed phase-transfer reaction with chloroform in aqueous NaOH, followed by a hydrogenolytic reduction by means of stannous chloride dihydrate in the presence of concentrated hydrochloric acid. It is noteworthy that, owing to the strong acidic conditions, the crude product of sodium 4-methoxymandelate could be directly used in hydrogenolytic step without being further transformed into its acidic form and purified, thus readily affording 4-methoxyphenylacetic acid (**9**) in 76% yield. Subsequently, **9** was readily brominated to give 3-bromo-4-methoxyphenylacetic acid (**10**) in 96% yield.[18]

In 1984, Weller et al.[18a] reported the transformation reaction of 3-bromo-4-methoxyphenylacetic acid (**10**) to 3-hydroxy-4-methoxyphenylacetic acid (**4**) under harsh



Scheme 2 Synthesis of combretastatin A-4 and its prodrug CA4P. *Reagents and conditions*: (a) pyrolusite, 32% H_2SO_4, 50–100 °C, 75%; (b) i. 50% aq NaOH, $CHCl_3$, TBAB, 60 °C, 4.5 h, ii. concd HCl, $SnCl_2·2H_2O$, 80 °C, 2 h, 71%; (c) Br_2, AcOH, 0 °C to r.t., 96%; (d) bis(8-quinolinolate)copper(II) catalyst, 30% aq NaOH, 110 °C, 6 h, 98%; (e) 3,4,5-trimethoxybenzaldehyde (**5**), Et_3N, Ac_2O, 110 °C, 6 h, 67%; (f) Cu, quinoline, 200 °C, 3 h, 70%; (g) i. Et_3N, $POCl_3$, 25 °C, 5 h, ii. 1 M aq NaOH, r.t., 10 h, 86%.

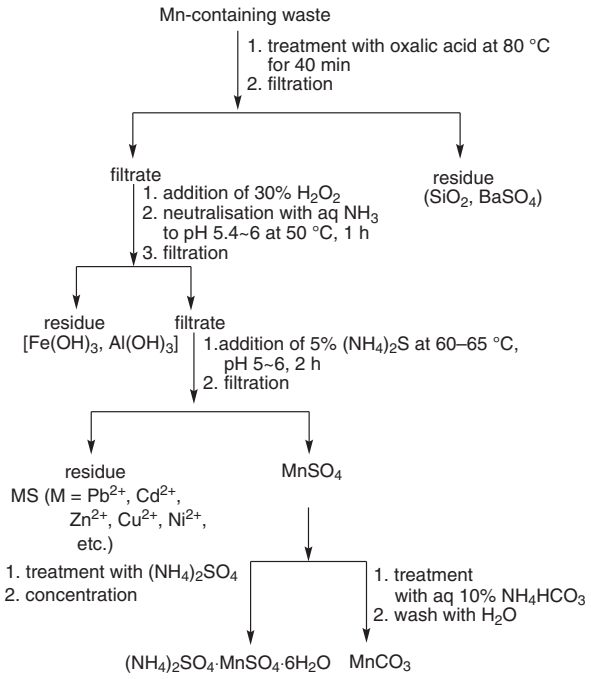


Figure 2 Protocol for waste-product treatment and manganese recovery in the oxidation of anethole

conditions by means of $CuSO_4$/NaOH at 150 °C in a sealed stainless steel bomb for 36 h. To simplify the procedure, we used bis(8-quinolinolate)copper(II) in 10% molar ratio instead of $CuSO_4$ as the catalyst and found that

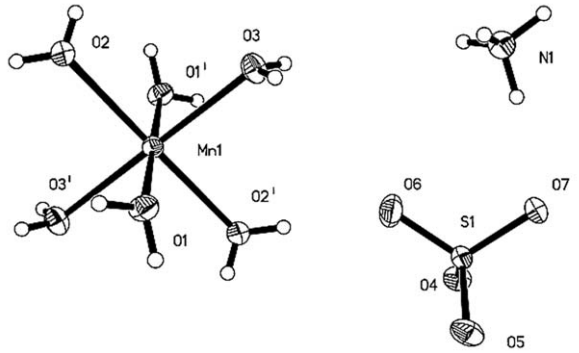


Figure 3 Metal coordination in the crystal structure of $(NH_4)_2SO_4·MnSO_4·6H_2O$

the transformation of bromo group to hydroxy group proceeded smoothly under atmospheric pressure at 110 °C within six hours, giving 3-hydroxy-4-methoxyphenylacetic acid (**4**) in 98% yield. The catalytic bis(8-quinolinolate)copper(II) complex (oxine-copper) can be readily obtained by reacting 8-quinolinol with a copper(II) salt such as $CuSO_4·5H_2O$, $CuCl_2·2H_2O$ and $Cu(NO_3)_2·3H_2O$ in water, methanol, or ethanol.[19] For practical applications, lifetime of catalyst and the levels of reusability are very important factors. We therefore conducted a set of experiments to investigate the reusability of the catalyst for the transformation reaction of compound **10** to **4** under similar optimized conditions. After completion of the first reaction in 98% yield, the catalyst was recovered by filtra-

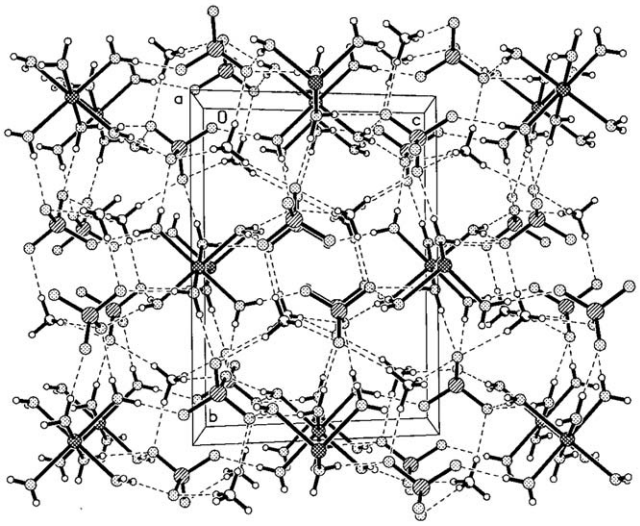


Figure 4 Packing diagram of $(NH_4)_2SO_4 \cdot MnSO_4 \cdot 6H_2O$ showing the hydrogen-bond interactions

tion, washed with water, and dried. Then the used catalyst was employed in another reaction run with fresh reactants under the similar conditions. To our delight, oxine-copper showed excellent recoverability and reusability over six successive runs, which confirmed the strong coordination and stability of copper in the catalyst.

(*E*)-2-(3′-Hydroxy-4′-methoxyphenyl)-3-(3′,4′,5′-trimethoxyphenyl)acrylic acid (**3**) was synthesized by means of Perkin reaction between 3-hydroxy-4-methoxyphenylacetic acid (**4**) (1.1 equiv) and 3,4,5-trimethoxybenzaldehyde (**5**) (1.0 equiv) in the presence of acetic anhydride and triethylamine at 110 °C, which runs as a much improved counterpart to the method previously reported by Gaukroger et al.[6] After the reaction, the mixture was acidified with ice-cold dilute hydrochloric acid, deacetylated, and discolored in basic conditions, and then acidified to give **3**. The *cis*-stilbene configuration of this compound was unambiguously established by ^{1}H NMR spectrum, on the basis of typical chemical shifts observed for the ethylenic proton (=CH) signal. Due to the carboxy group field effect in the *cis*-stilbene, a remarkable downfield shift of the acrylic alkene proton (=CH) at $\delta = 7.78$ is resulted. The decarboxylation of **3** was carried out in the presence of quinoline and copper powder at 220 °C under nitrogen, to afford the combretastatin A-4 in 70% yield. Combretastatin A-4 (**1**) was purified by solvent extraction with petroleum ether and subsequent recrystallization from ethyl acetate and petroleum ether, which avoided the use of flash column chromatography and thus be amenable to large-scale preparation. The geometrical configuration of combretastatin A-4 was assigned by the characteristic ^{1}H NMR coupling constants of the olefinic protons ($J_{CH=CH} = 12.4$ Hz for *cis*-stilbene).

The clinically applicable form of combretastatin A-4, namely CA4P (**2**) is designed as the phosphate prodrug with excellent water solubility and pharmacokinetic profile. At present, the most effective route was accomplished by phosphorylation with in situ formation of benzylchlorophosphite followed by cleavage of the benzyl ester protecting group with trimethyliodosilane. The phosphoric acid intermediate was treated with sodium methoxide to complete a practical route to the sodium phosphate prodrug.[3] In this study, phosphorylation of combretastatin A-4 by means of phosphorus oxychloride followed by hydrolysis, gave combretastatin A-4 dihydrogen phosphate, which is readily alkalized by means of sodium hydroxide in ethanol to give CA4P (**2**) in 86% yield in a more cost-competitive way, compared with Pettit's method. Particularly noteworthy was that these reaction conditions were apparently quite sensitive to temperature and pH value. When the reaction temperature was above 35 °C or hydrolysis of the phosphoryl chloride had gone to completion in the dilute acid solution, isomerization of CA4P was observed and less active *trans*-isomer of CA4P was obtained. The *cis* or *trans* geometries were determined by their characteristic ^{1}H NMR coupling constants for the olefinic protons in conformity with this class of compounds of approximately 12 Hz for the *cis* and 16–17 Hz for the *trans* isomers.[3,5]

In conclusion, starting from the renewable biomass-based compounds anethole (**6**) and 3,4,5-trimethoxybenzaldehyde (**5**), a practical and green protocol for the synthesis of CA4 and CA4P in satisfactory overall yields has been achieved. Our work has not only provided an alternative route for the synthesis of natural compounds with medical interests, but also provided a typical example for the sustainable development of valuable compounds in green chemistry manner without using petroleum-based starting materials. In addition, the minimization of disposal problems of pyrolusite/sulfuric acid promoted oxidation of anethole (**6**) and the successful recovery of manganese element in form of $(NH_4)_2SO_4 \cdot MnSO_4 \cdot 6H_2O$, the formation of the hydroxy intermediate **4** using oxine-copper as catalyst under mild conditions and the convenient phosphorylation process to form CA4P have also introduced aspects of green chemistry into the synthesis.

Anethole (**6**) was kindly supplied by Guangxi Wanshan Spice Co. Ltd. as a natural product with chromatography grade. 3,4,5-Trimethoxybenzaldehyde (**5**) was purchased from Zhangjiajie Maoyuan Chemical Industry Co., Ltd. and proved to be a biomass-based product derived from natural gallic acid. Other reagents and chromatography grade solvents were obtained from commercial sources and used without further purification, unless otherwise stated. Petroleum ether (PE) used refers to the boiling fraction 60–90 °C. Reactions were monitored by TLC using silica gel (0.2 mm, GF254) percolated, glass-backed plates. The purity of products was analyzed by Shimadzu GC-9A gas chromatograph. The melting points were determined on Thiele apparatus and are uncorrected. IR spectra were recorded on an Analect RFX-65A IR spectrometer. ^{1}H NMR was obtained from a Bruker DRX-400 MHz spectrometer. Chemical shifts (δ) are given in ppm downfield from TMS as internal standard and coupling constants (*J* values) are in hertz (Hz). EI/MS analyses were carried out using a Shimadzu GCMS-QP5050A mass spectrometer. Elemental analyses were carried out by Elementar Vario EL element analyzer. The single X-ray diffraction measurement was performed on a Bruker SMART 1000 CCD X-ray diffractometer.

4-Methoxybenzaldehyde (8)

To a stirred mixture of pyrolusite (containing 56.44% of MnO_2, 160 g, 1.04 mol) in H_2O (360 g) was added anethole (**6**; 60 g, 0.41 mol) at 50 °C. The resulting mixture was heated to 65 °C, and 32% aq H_2SO_4 (600 g, 1.96 mol) was added dropwise over 30 min. After stirring at 100 °C for 1 h, the separated pyrolusite was filtered off and washed with $CHCl_3$ (3 × 30 mL). The organic layer was separated from the filtrate and neutralized to pH 8–9 with sat aq Na_2CO_3, and allowed to stay overnight. The organic layer was separated, washed neutral with H_2O, and concentrated under reduced pressure to afford a crude oily product, which was purified by fractional distillation under reduced pressure to give **8** as a colorless viscous liquid (41.2 g, 75%) (GC assay 98.9%).

Removal of the excess slag of pyrolusite and recycling of these salts back to $MnCO_3$ began with combining the filtered pyrolusite with the aqueous layer after removal of anisaldehyde (**8**) by separation. This mixture was treated with oxalic acid (25 g, 0.198 mol) for 0.5 h at 80 °C followed by a filtration to remove the residue (mainly consisting of SiO_2 and $BaSO_4$). The filtrate was treated by the successive addition of 30% aq H_2O_2 (10 mL) and 25% aq NH_3 (142 mL) to give a solution of pH 5.4–6.0, which resulted in the precipitation of $Al(OH)_3$ and $Fe(OH)_3$. After filtration, the filtrate was treated with 5% aq $(NH_4)_2S$ (4 mL), and the heavy metal ion were precipitated as sulfide MS (M = Pb^{2+}, Cd^{2+}, Zn^{2+}, Cu^{2+}, Ni^{2+}, etc.). After filtration, the filtrate was treated by the addition of calculated amount of $(NH_4)_2SO_4$ over 0.5 h, giving a homogeneous solution, which after concentration by heating, afforded $(NH_4)_2SO_4{\cdot}MnSO_4{\cdot}6H_2O$ (365.3 g, 90%) as pale reddish single crystal with high purity. Furthermore, $(NH_4)_2SO_4{\cdot}MnSO_4{\cdot}6H_2O$ can be converted into high quality $MnCO_3$ by treatment with stoichiometric amount of 10% aq NH_4HCO_3.

4-Methoxyphenylacetic Acid (9)

To a mixture of compound **8** (12.3 mL, 0.1 mol) and Bu_4NBr (TBAB, 1.6 g, 0.005 mol) in $CHCl_3$ (16 mL) was added dropwise 50% aq NaOH (18 mL, 0.5 mol) at 60 °C under N_2 over 1.5 h. The resulting mixture was stirred at 60 °C for 3 h. Upon cooling, the reaction mixture was filtered and the precipitate was collected, washed with $CHCl_3$ (3 × 10 mL), and dried. The obtained white solid, which was a mixture of sodium 4-methoxymandelate and NaCl (ratio ~1:3) could be used in the next step without further purification. To a solution of the above crude sodium 4-methoxymandelate in concd HCl (100 mL) was added $SnCl_2{\cdot}2H_2O$ (33.9 g, 0.15mol). The reaction mixture was stirred vigorously for 2 h at 80 °C. After stirring further for 1 h, the mixture was poured into ice-water and the precipitate was collected, washed with ice-cold H_2O (3 × 10 mL), and dried. The crude product was recrystallized from H_2O to afford **9** as light white crystals (14.2 g, 71% from **8**); mp 86–87 °C.

^{1}H NMR (400 MHz, acetone-d_6): δ = 10.66 (s, 1 H, CO_2H), 7.22 (d, J = 8.0 Hz, 2 H, 2,6-ArH), 6.85 (d, J = 8.0 Hz, 2 H, 3,5-ArH), 3.76 (s, 3 H, 4-OCH_3), 3.54 (s, 2 H, CH_2).

MS (EI): m/z = 166 $[M]^+$, 121, 106, 91, 77.

3-Bromo-4-methoxyphenylacetic Acid (10)

To a stirred solution of 4-methoxyphenylacetic acid **9** (9.6 g, 57.8 mmol) in AcOH (25 mL) was added Br_2 (3.1 mL, 60 mmol) dropwise over 1 h. After stirring for 3 h at r.t., the reaction mixture was quenched with an excess of 5% aq Na_2SO_3 and cooled at 0 °C. The precipitate was collected, washed with ice-cold H_2O (3 × 10 mL), and dried to obtain the crude product **10** (13.6 g, 96%), which could be used directly in the next step. The crude product was recrystallized from EtOH–H_2O to afford **10** as white plates; mp 113–114 °C (Lit.[18] mp 113–115 °C).

^{1}H NMR (400 MHz, acetone-d_6): δ = 7.52 (d, J = 2 Hz, 1 H, 2-ArH), 7.27 (dd, J = 8.4, 2.0 Hz, 1 H, 6-ArH), 7.02 (d, J = 8.4 Hz, 1 H, 5-ArH), 3.87 (s, 3 H, 4-OCH_3), 3.58 (s, 2 H, CH_2).

MS (EI): m/z (%) = 244 (50, $[M]^+$), 246 (48, $[M + 2]^+$), 199 (100), 201 (98), 121 (10), 105 (30), 77 (70), 51 (45).

3-Hydroxy-4-methoxyphenylacetic Acid (4)

Compound **10** (24.5 g, 0.1 mol), 30% aq NaOH (90 mL), and bis(8-quinolinolate)copper (II) (3.52 g, 0.01 mol) were loaded into a stainless steel bomb. The resulting mixture was refluxed under N_2 at atmospheric pressure for 6 h. After completion of the reaction (monitored by TLC), the reaction mixture was cooled to r.t. and neutralized by the addition of concd HCl. The precipitated bis(8-quinolinolate)copper(II) was separated by filtration and was recycled. The resulting filtrate was acidified and extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with brine (2 × 10 mL), dried ($MgSO_4$). and evaporated under reduced pressure to afford a yellow solid, which was recrystallized with EtOAc–PE to afford **4** as white crystals (17.93 g, 98%); mp 127.5–129 °C (Lit.[18a] mp 128–130 °C).

^{1}H NMR (400 MHz, acetone-d_6): δ = 7.54 (s, 1 H, OH), 6.86 (d, J = 8.0 Hz, 1 H, 5-ArH), 6.81 (d, J = 2.0 Hz, 1 H, 2-ArH), 6.72 (dd, 1 H, J = 8.0, 2.0 Hz, 6-ArH), 3.80 (s, 3 H, 4-OCH_3), 3.55 (s, 2 H, CH_2).

MS (EI): m/z = 182 $[M]^+$, 183 $[M + 1]^+$, 137, 122, 94, 77.

(*E*)-2-(3′-Hydroxy-4′-methoxyphenyl)-3-(3′,4′,5′-trimethoxyphenyl)acrylic Acid (3)

To a solution of compounds **4** (4.01 g, 0.022 mol) and **5** (3.92 g, 0.02 mol) in Ac_2O (12 mL) was added Et_3N (12 mL) under N_2. The mixture was stirred at 110 °C for 6 h. The mixture was poured into ice-cold H_2O (20 mL) and acidified with dil. HCl with stirring. A yellow solid was obtained, which was collected by filtration. It was dissolved in 10% aq NaOH (100 mL) and decolored by extracting with EtOAc (2 × 15 mL). The organic layers were separated and the aqueous layer was acidified with concd HCl to pH 2–3. The precipitated crude product was collected by filtration and recrystallized from EtOH to afford **3** as pale yellow crystals (4.82 g, 67%); mp 233–234 °C (Lit.[9] mp 233–234 °C).

IR (KBr): 3401, 2998, 2944, 2838–2518, 1679, 1581, 1508, 1459, 1378, 1334, 1184, 998, 946 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.78 (s, 1 H, CH=), 6.89 (d, J = 8.4 Hz, 1 H, 5′-ArH), 6.85 (d, J = 2.0 Hz, 1 H, 2′-ArH), 6.75 (dd, J = 8.4, 2.0 Hz, 1 H, 6′-ArH), 6.39 (s, 2 H, 2′,6′-ArH), 5.76 (br, 2 H, CO_2H, OH), 3.89 (s, 3 H, 4′-OCH_3), 3.81 (s, 3 H, 4′-OCH_3), 3.57 (s, 6 H, 3′,5′-OCH_3).

MS (EI): m/z (%) = 360 (100, $[M]^+$), 345 (20, $[M - OCH_3]^+$), 327 (5), 285 (15).

Anal. Calcd for $C_{19}H_{20}O_7$: C, 63.3; H, 5.6. Found: C, 63.3; H, 5.6.

Combretastatin A-4 (1)

To a suspension of Cu powder (1.5 g, 23.3 mmol) in quinoline (11.0 g, 85 mmol) was added compound **3** (1.0 g, 2.8 mmol) and the resulting mixture was stirred under N_2 at 200 °C for 3 h. Upon cooling, the mixture was diluted with EtOAc (50 mL), and the Cu was filtered off. The filtrate was washed with aq 1 M HCl (3 × 20 mL), and the aqueous layer was separated and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (2 × 10 mL), dried ($MgSO_4$), and concentrated under reduced pressure to afford a brown viscous solid. This was purified by extraction with PE and subsequent recrystallization from EtOAc–PE to give **1** as colorless crystals (0.62 g, 70%); mp 116–117 °C (Lit.[5a] mp 116 °C).

IR (KBr): 3424, 3002, 2938, 2836, 1610, 1579, 1508, 1459, 1419, 1328, 1182, 1025, 1004, 944, 881, 854, 796 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.91 (d, J = 2.0 Hz, 1 H, 2′-ArH), 6.79 (dd, J = 8.0, 2.0 Hz, 1 H, 6′-ArH), 6.71 (d, J = 8.0 Hz, 1 H, 5′-

ArH), 6.51 (s, 2 H, 2′,6′-ArH), 6.45 (d, J = 12.4 Hz, 1 H, CH=), 6.42 (d, J = 12.4 Hz, 1 H, CH=), 5.49 (s, 1 H, OH), 3.89 (s, 3 H, 4′-OCH_3), 3.84 (s, 3 H, 4′-OCH_3), 3.68 (s, 6 H, 3′,5′-OCH_3).

MS (EI): m/z (%) = 316 (100, $[M]^+$), 301 (75), 241 (8), 226 (6), 211 (5), 141 (12), 115 (8), 93 (5), 57 (8).

Anal. Calcd for $C_{18}H_{20}O_5$: C, 68.4; H, 6.3. Found: C, 68.3; H, 6.3.

Combretastatin A-4 Disodium Phosphate (2)

A solution of compound **1** (3.16 g, 10 mmol) in CH_2Cl_2 (15 mL) was added dropwise to a stirred solution of $POCl_3$ (5.5 mL, 60 mmol) in CH_2Cl_2 (15 mL) at r.t. over 1 h. A solution of Et_3N (3.5 mL, 24 mmol) in CH_2Cl_2 (5 mL) was then added dropwise over 2 h. The resulting mixture was stirred at r.t. for 3 h until completion of the reaction, then quenched by the addition of H_2O (20 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL) and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo to afford a colorless oil. The crude combretastatin A-4 dihydrogen phosphate (10 mmol) obtained was dissolved in EtOH (20 mL) at r.t. and 1 M aq NaOH (12 mL, 25 mmol) was added dropwise. The resulting mixture was stirred at r.t. for 10 h, then filtered, concentrated, and the residue was recrystallized from MeOH–acetone to afford **2** as a white solid (1.2 g, 86%); mp 194 °C (dec.) (Lit.[3b] mp 190–195 °C).

IR (KBr): 3386, 3002, 2937, 2836, 1579, 1509, 1458, 1428, 1411, 1329, 1267, 1236, 1126, 997, 952, 856, 829, 792 cm^{-1}.

^1H NMR (400 MHz, D_2O): δ = 7.27 (s, 1 H, 2′-ArH), 6.77 (d, J = 8.4 Hz, 1 H, 6′-ArH), 6.73 (d, J = 8.4 Hz, 1 H, 5′-ArH), 6.55 (d, J = 12.0 Hz, 1 H, CH=), 6.54 (s, 2 H, 2′,6′-ArH), 6.40 (d, J = 12.0 Hz, 1 H, CH=), 3.764 (s, 3 H, OCH_3), 3.64 (s, 3 H, OCH_3), 3.59 (s, 6 H, 2 × OCH_3).

FAB-MS: m/z = 441 $[M]^+$.

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