A Ramberg–Bäcklund route to the stilbenoid anti-cancer agents combretastatin A-4 and DMU-212†

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A concise route to combretastatin A-4, a potent inhibitor of tubulin polymerisation, using a Ramberg–Bäcklund reaction to form the key (*Z*)-stilbene unit has been developed; this Ramberg–Bäcklund approach has also been extended to prepare the (*E*)-stilbene DMU-212, which also possesses interesting growth inhibitory properties.

The combretastatins, isolated from the African tree *Combretum caffrum*, are a potent class of anti-mitotic and anti-vascular agents that have attracted considerable interest in recent times.[1] Although a large family are now known (*e.g.* **1–11**, Fig. 1) the most potent cancer cell growth inhibitor is the (*Z*)-stilbene combretastatin A-4 **3**,[2] which has been shown to act as an inhibitor of tubulin polymerisation by binding at the same site as colchicine. Combretastatin A-4 has been described as "the simplest natural product known with such potent antitubulin effects".[3]

The combination of potent biological properties and relatively straightforward molecular structures has resulted in the development of a number of synthetic routes to the natural products, and to a wide range of analogues.[1–3] Structure–activity relationship studies[4] strongly indicate that a (*Z*)-stilbene configuration is essential for the cytotoxicity of the combretastatins, as is the 3,4,5-trimethoxy substitution pattern on the A-ring.

We have an interest in the application of the Ramberg–Bäcklund reaction to the synthesis of biologically active compounds,[5] and have recently reported high levels of unexpected (*Z*)-stereoselectivity in the construction of novel stilbene systems.[6] These results prompted us to investigate the development of a flexible synthetic route to the combretastatins, as shown for combretastatin A-4 **3** in Scheme 1.

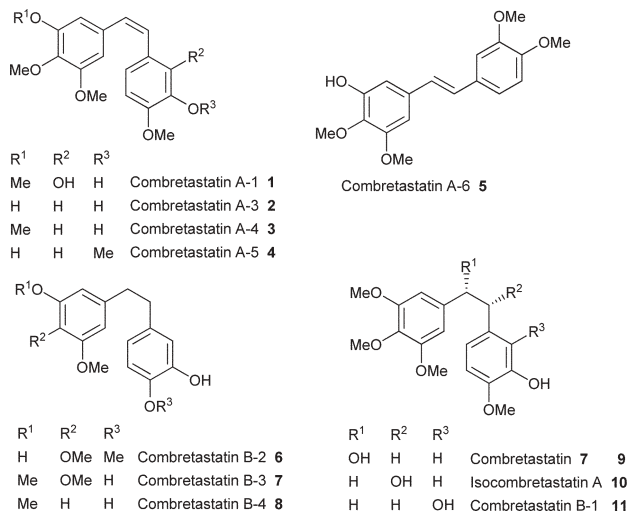

Fig. 1 Selected members of the combretastatin family.

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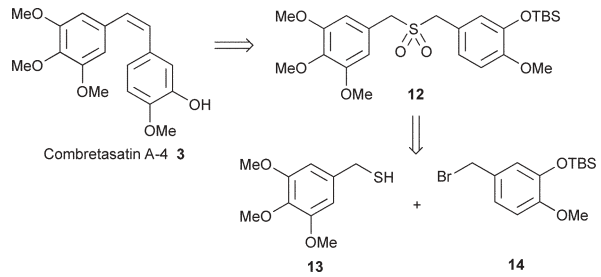
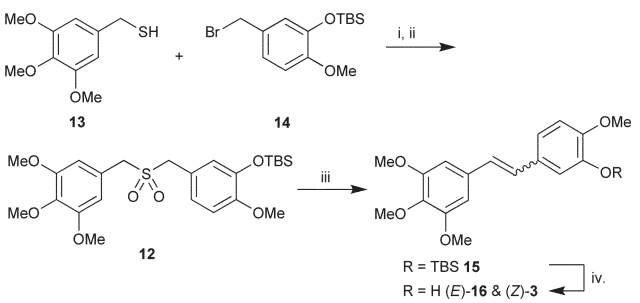


Scheme 1 Retrosynthesis of combretastatin A-4 **3**.

The route hinges on the use of a Ramberg–Bäcklund reaction for the formation of the required stilbene from the sulfone precursor **12**. It was envisaged that the requisite sulfone **12** would in turn be available *via* the coupling of benzylic thiol **13** and bromide **14**.

The synthesis of combretastatin A-4 **3** therefore commenced with the coupling of thiol **13**, prepared *via* treatment of commercially available 3,4,5-trimethoxybenzyl alcohol with Lawesson's reagent, and the known bromide **14**[7] using potassium hydroxide in ethanol (Scheme 2). Oxidation of the resultant sulfide[8] with *m*-chloroperoxybenzoic acid afforded the corresponding sulfone **12** in 49% yield over 2 steps. With sulfone **12** in hand, the tandem halogenation–Ramberg–Bäcklund reaction was carried out, initially using the conditions devised by Chan *et al.*[9] (CF_2Br_2, ^{t}BuOH, KOH–Al_2O_3) as shown in Scheme 2. We were delighted

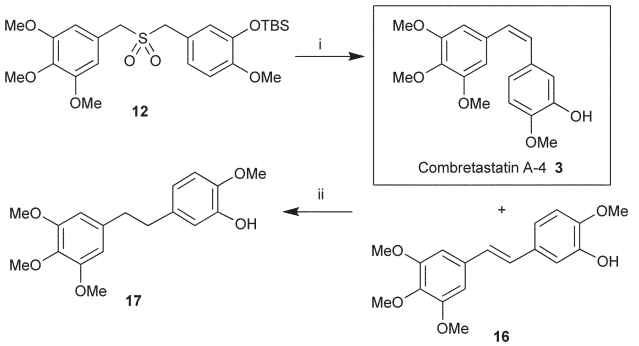


Scheme 2 *Reagents and conditions*: i. KOH, EtOH, 0 °C to rt, 12 h; ii. *m*-CPBA, $NaHCO_3$, CH_2Cl_2, 0 °C to rt, 12 h, 49% over 2 steps; iii. **Chan conditions**: CF_2Br_2, ^{t}BuOH, KOH–Al_2O_3, 0 °C to rt, 12 h, **15** 81% (*E* : *Z* = 90 : 10); **Franck conditions**: $C_2F_4Br_2$, ^{t}BuOH, KOH–Al_2O_3, Δ, 12 h, **16** 72% (*E* : *Z* = 85 : 15); iv. TBAF–SiO_2, THF, 0 °C, 12 h, 72%.

to observe that the one-pot halogenation–Ramberg–Bäcklund reaction proceeded extremely efficiently to give the *O*-silylated stilbene **15**[3] ((*E*)-**15**: J = 16.2 Hz, lit.[3] J = 16.3 Hz; (*Z*)-**15**: J = 12.2 Hz, lit.[3] J = 12.2 Hz) as a 90 : 10 mixture of (*E*)- and (*Z*)-isomers in 81% yield. Desilylation of **15** using tetrabutylammonium fluoride on silica provided the corresponding phenols **16** and **3** in 72% yield. Both combretastatin A-4 **3** and the (*E*)-combretastatin analogue **16** have previously been prepared by a Wittig approach.[3] Conducting the reaction under the conditions reported by Franck *et al.*[10] similarly provided **16** and **3** as an 85 : 15 mixture of (*E*)- and (*Z*)-isomers in 72% yield.

We next investigated the original halogenation–Ramberg–Bäcklund conditions (CCl_4, ^{t}BuOH, KOH, H_2O) reported by Meyers *et al.*[11] Under these conditions *in situ* deprotection of the *tert*-butyldimethylsilyl ether occurred but, more importantly, the required stilbene was produced in 69% yield as a 47 : 53 mixture of inseparable (*E*)- and (*Z*)-isomers **16** and **3**[2,3] (J = 12.2 Hz, lit.[3] J = 12.2 Hz) (Scheme 3). The ^{1}H and ^{13}C NMR data for the mixture of **16** and **3** was in agreement with that reported in the literature.[2,3] Hydrogenation of either pure (*E*)-stilbene **16** or a mixture of the (*E*)- and (*Z*)-isomers **16** and **3** proceeded in quantitative yield to furnish the known[3] dihydrostilbene **17**.

The formation of a significant quantity of (*Z*)-stilbene **3** in this Ramberg–Bäcklund sequence under Meyers' conditions deserves further comment. Until the recent publications[6] from our laboratory, it was assumed that the use of benzyl benzyl sulfones in the Ramberg–Bäcklund reaction would always produce the (*E*)-stilbene, if not exclusively, then as the major isomeric product.[5] These recent examples, leading to an unexpectedly high ratio in



Scheme 3 *Reagents and conditions*: i. **Meyers conditions**: CCl_4, ^{t}BuOH, KOH, H_2O, Δ, 12 h, 69% (**16** : **3** = 47 : 53); ii. Pd/C, H_2, EtOAc, 99%.

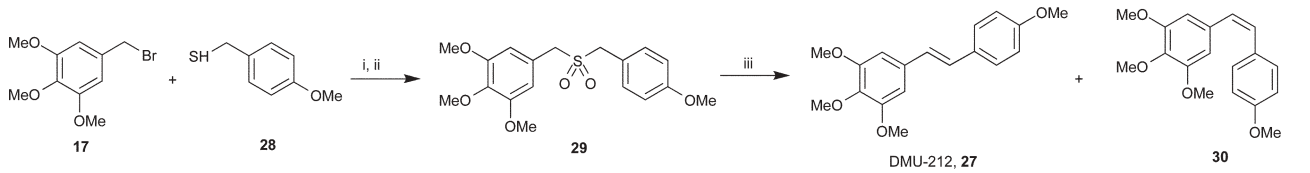
favour of the (*Z*)-isomer, all proceed with electron rich aromatic systems under Meyers' conditions. Further study is needed to fully understand this observation but it is of obvious applicability in terms of (*Z*)-stilbene targets such as the combretastatins.

We therefore went on to apply this Ramberg–Bäcklund route to the synthesis of other combretastatin analogues (Table 1, entries 2–4). First, we investigated the preparation of the methylated analogues (*E*)- and (*Z*)-**20**[12] (entry 2). Thus, the α-methylated sulfone **19** was prepared from the known[13] bromide **17** and the novel thiol **18** (readily prepared from the corresponding alcohol using Lawesson's reagent) following the earlier sequence. Moving on to the halogenation–Ramberg–Bäcklund sequence, Meyers' conditions again gave *in situ* desilylation to produce the expected stilbene **20** (70%) as a separable mixture of the novel (*E*)-isomer and known[12] (*Z*)-isomer (65 : 35). Franck conditions also

Table 1 Preparation of and Ramberg–Bäcklund reaction of sulfones **12**, **19**, **23**, **25**

Entry	Coupling partners	Sulfone (% over 2 steps)	Stilbene	Reaction conditions (%, E : Z)[b]
1	**13** + **14**	**12** (49)		Meyers (69, 47 : 53) (R = H **3** and **16**) Chan (81, 90 : 10) (R = TBS **15**) Franck (72, 85 : 15) (R = H **3** and **16**)
2	**17** + **18**	**19** (38)		Meyers (70, 65 : 35) (R = H **20**) Chan (59, 69 : 31) (R = TBS **21**) Franck (59, 90 : 10) (R = H **20**)
3	**17** + **22**	**23** (29)[a]	**24**	Meyers (40, 59 : 41) Chan (49, 76 : 24) Franck (74, 68 : 32)
4	**14** + **22**	**25** (25)[a]	**26**	Meyers No reaction Chan (24, 92 : 8) Franck (53, 85 : 15)

[a] Yield over 3 steps as desilylation was effected prior to conducting the Ramberg–Bäcklund reactions. [b] (*E*) : (*Z*) Ratios quoted as a percentage composition of total yield as estimated from ^{1}H-NMR spectra.



Scheme 4 *Reagents and conditions*: i. KOH, EtOH, 0 °C to rt, 12 h; ii. *m*-CPBA, $NaHCO_3$, CH_2Cl_2, 0 °C to rt, 12 h, 49% over 2 steps; iii. **Meyers conditions**: CCl_4, ^{t}BuOH, KOH, H_2O, Δ, 12 h, 38% (*E* : *Z* = 42 : 58); **Chan conditions**: CF_2Br_2, ^{t}BuOH, KOH–Al_2O_3, 0 °C to rt, 12 h, 47% (*E* : *Z* = 91 : 9); **Franck conditions**: $C_2F_4Br_2$, ^{t}BuOH, KOH–Al_2O_3, Δ, 12 h, 89% (*E* : *Z* = 97 : 3) then recrystallisation (EtOH), 87% (*E* : *Z* = 100 : 0).

provided an (*E*)- and (*Z*)-isomeric mixture (90 : 10) of **20** in 59% yield. With substrate **19**, however, the Chan conditions produced a mixture of alkene isomers of stilbene **21** (59%, (*E*) : (*Z*) = 69 : 31). Desilylation of **21** proceeded smoothly in 72% yield to afford **20**, which was hydrogenated to the corresponding novel dihydrostilbene in quantitative yield.

In a similar manner, the novel sulfone **23** was prepared by coupling of thiol **22** with bromide **17** followed by oxidation of the resultant sulfide and removal of the *tert*-butyldimethylsilyl ether to furnish sulfone **23** in 29% yield over 3 steps. Thiol **22** was obtained from 2-acetylbenzoic acid by an efficient four step sequence (56%; reduction–selective protection–Mitsunobu coupling with thiolacetic acid–thioacetate reduction). However, the Ramberg–Bäcklund reaction of sulfone **23** proceeded with little selectivity (Table 1, entry 3); Meyers conditions gave stilbene **24** in 40% yield as a 59 : 41 mixture of (*E*)- and (*Z*)-isomers, and using the Chan conditions stilbene **24** was isolated in 49% yield as a 76 : 24 mixture of (*E*)- and (*Z*)-isomers. Franck conditions, while providing stilbene **24** in 74% yield, gave a similar mixture of (*E*)- and (*Z*)-isomers (68 : 32). The (*Z*)-stereoselectivity was even worse when the Ramberg–Backlund reaction of sulfone **25** was carried out (Table 1, entry 4); under Chan conditions stilbene **26** was obtained in low yield predominantly as the (*E*)-isomer, as was **26** under Franck conditions (53%, (*E*) : (*Z*) = 85 : 15). Notably, no reaction was observed for sulfone **25** under Meyers conditions.

The ease with which several of these stilbene systems were obtained with high (*E*)-stereoselectivity prompted an investigation concerning the use of the Ramberg–Backlund reaction to prepare the (*E*)-stilbene **27**,[14] known as DMU-212. DMU-212 is a synthetic analogue of resveratrol, a naturally occurring phytoalexin with cancer chemoprotective activity.[15] However, DMU-212 has been reported to possess chemoprotective activity superior to that of resveratrol, and as such has shown excellent promise as an anti-cancer agent.[16] This activity contrasts with the low activity of the (*E*)-combretastatin analogues compared to their (*Z*)-counterparts.[4]

Accordingly, 4-methoxybenzyl mercaptan **28**, readily available from treatment of 4-methoxybenzyl bromide with thiolacetic acid and potassium hydrogen carbonate, was reacted with bromide **17** (Scheme 4). Oxidation of the resultant sulfide furnished the desired sulfone **29**, the Ramberg–Bäcklund reaction of which using Meyers conditions gave DMU-212 **27** in 38% yield as a 42 : 58 mixture of (*E*)- and (*Z*)-isomers. Gratifyingly, Chan conditions afforded a 91 : 9 mixture of (*E*)- and (*Z*)-isomers in 47% yield. Conducting the reaction using the conditions of Franck provided DMU-212 **27** in 89% yield and enhanced the (*E*) : (*Z*) ratio to 97 : 3. Recrystallisation from ethanol gave colourless crystals of only (*E*)-**27** (87%, m.p. 157–158 °C, lit.[17] m.p. 160–161 °C).

In summary, the Ramberg–Bäcklund reaction has been utilised as part of a short and flexible route to the anti-cancer stilbenes combretastatin A-4 **3** and DMU-212 **27**, as well as to several novel analogues. During the course of these investigations further insight has been gained into the scope and limitations of the Ramberg–Bäcklund reaction for stilbene synthesis, particularly with respect to issues concerning stereoselectivity.

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