Syntheses of Combretastatin A-4 and Related Stilbenes by Using Aqueous Conditions

Natalie G. Barnes, [a, b] Amjed A. Ahmed Mal Ullah, [a, c] Patricia A. Ragazzon, [a, d] Nadia Charafi, [a] and John A. Hadfield[d]

Combretastatin A-4 (CA4) is a potent anti-mitotic and vascular disrupting agent. Organic chemists have been working to optimize the synthesis of CA4 for the past 3 decades, with methods requiring hazardous solvents and harsh reaction conditions. Here, we report the synthesis of CA4 and a variety of stilbenes in an aqueous Wittig system.

Introduction

Until recent years, chemists have synthesised target molecules without realising the environmental impacts of the solvents and reagents required in these synthetic procedures.[1] In general, synthetic procedures can produce a huge amount of chemical and solvent waste. Green chemistry has a far and wide effect in both the chemical and pharmaceutical industry. Both synthetic and medicinal chemists are in a continuous search to find an eco-friendly route in the area of drug development.

There are a number of advances for the environmentally friendly synthesis of biologically active molecules such as microwave assisted synthesis,[2,3] solid phase synthesis,[4] ultrasound,[5] catalyst-free and one-pot syntheses.[6] Organic synthesis in water has attracted the attention of chemists for many years. Water is one of the most inexpensive and environmentally benign solvents.

Combretastatin A-4 (CA4, 1a, Figure 1), a natural product isolated from the South African tree Combretum caffrum, exhibits potent anti-mitotic effects as well as being a lead vascular disrupting agent. CA4 binds to the colchicine site of tubulin, disrupting microtubule polymerisation and eventually inducing apoptosis. In its water-soluble disodium phosphate derivative form (CA4P, 1b, Figure 1), it has undergone several clinical trials as an antitumour agent on its own, or in combination with other cytotoxic agents.[7] Owing to its antivascular properties CA4P has also been trialled to combat age-related macular degeneration.[8] A poly(L-glutamic acid) nanoparticle conjugate of combretastatin A-4, used for improved delivery, has shown the ability to suppress C26 colon carcinoma tumours in mice by 74% whilst CA4P’s tumour suppression rate was considerably lower (24%).[9] In combination with tirapazamine this conjugate caused complete tumour reduction in 4T1 xenograft mice.[10]

There have been various routes described for the synthesis of CA4 (1a). The original synthesis described by Pettit et al. dates back to 1995 and uses Wittig chemistry (Scheme 1).[11] Here flammable solvents and reagents (tetrahydrofuran/n-butyllithium) are used to form the silyl protected E- and Z-stilbenes (3a, 3b, Figure 1) as a mixture and the reaction requires an inert atmosphere to exclude moisture and oxygen.

Potassium carbonate or lithium hydroxide were used as base in this Wittig reaction to give excellent yields of mixtures of E- and Z-stilbenes. The synthesis of CA4 was achieved using tetrahydropyran (THP) or silyl protected phenolic aldehydes. The THP groups were removed using dilute acid whilst the silyl groups fortuitously fell off during work up.

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Separation using silica chromatography followed by desilylation provides the \( E \)- and \( Z \)-isomers of CA4 (1a, 1b). This Wittig method is not stereoselective and so substantially reduces the yield potential of the biologically active \( Z \)-isomer.

More recently, methods which produce solely the \( Z \)-isomer of CA4 (1a) have been described. One method uses a Suzuki coupling starting from isovanillin (6) (Scheme 2). Although this method produces solely \( Z \)-CA4 (1a) it requires protection and deprotection of the phenol to allow a good yield of the intermediate dibromo olefin (9) and the use of a toxic tin reagent and carbon tetrabromide to provide the \( Z \)-vinyl bromide (10). Coupling this bromide (10) with 3,4,5-trimethoxyphenyl boronic acid (11) yields \( Z \)-CA4 (1a).

This selective Suzuki coupling has recently been improved to yield \( Z \)-CA4 (1a) in an overall yield of 56% [13]. In this method, the \( Z \)-vinyl iodide (14) was readily prepared using Stork-Zhao olefination methodology [14] from phosphonium iodide (13) and 3,4,5-trimethoxybenzaldehyde (12) in the presence of Sodium bis(trimethylsilyl)amide (NaHMDS, scheme 3). Suzuki-coupling of this \( Z \)-iodostyrene (14) compound with 3-hydroxy-4-methoxyphenyl boronic acid (15) produced solely \( Z \)-CA-4 (1a) in 78% yield, avoiding the need for protection and deprotection steps, and more importantly avoiding the use of highly toxic carbon tetrabromide and tin.

Another method that produces solely the \( Z \)-isomer is the Perkin condensation reaction reported by Gaukroger et al. in 2001. The Perkin-type condensation of 3,4,5-trimethoxyphenyl
acetic acid (16) with isovanillin (6) yields the cinnamic acid (17) in good yield. Decarboxylation then yields Z-CA4 (1a) in 70% yield (Scheme 4). However, this decarboxylation step requires strong heating and the use of toxic quinoline.\[12\]

Although the later syntheses of CA4 have improved substantially; both in yield and the safety of materials used since the first synthesis was reported in 1995, these methods still require the use of somewhat hazardous solvents and harsh reaction conditions.\[13\] There are other methods described in the literature for carrying out the Wittig reaction in water alone (scheme 5) and using a base (scheme 6). El-Batta and co-workers reported that heating (methoxycarbonylmethylene) triphenylphosphorane (18) with o-anisaldehyde (19) at room temperature for 1 hour afforded the ester (20) as a mixture of E- and Z- alkenes in 81% yield with an E/Z ratio of 76/24. No base was required for this Wittig reaction in water.\[16\]

The E-pterostilbene (23) in scheme 6 has been prepared by reacting tosylated phosphonium salt (21) with aldehyde (22) in

Scheme 3. Improved Suzuki cross coupling synthesis of Z-CA-4.\[13\] Reagents and conditions: a) NaHMDS, THF, −20 to −78 °C, 2 h, 72% (14); b) tetrakis(triphenylphosphine)palladium(0), sodium carbonate (Na₂CO₃, 2 M), DME, 80 °C, 20 h, 78% (1 a).

Scheme 4. Perkin reaction.\[13\] Reagents and conditions: a) triethylamine, acetic anhydride, reflux, 60% (17); b: Cu, quinoline, 200 °C, 72% (1 a).

Scheme 5. Example Wittig reaction in water.\[16\] Reagents and conditions: a) water, 20 °C, 81% (20) E/Z ratio of 76/24.

Scheme 6. Aqueous stilbene synthesis. Reagents and conditions: a) LiOH (3 equiv), H₂O (1 M), 90 °C, 12 h, 1 M HCl, 91% (23).
water using lithium hydroxide as base for this Wittig reaction. Acid hydrolysis afforded stilbene (23) predominantly as the E-isomer (<5% Z) (Scheme 6).

**Results and Discussion**

With the above aqueous reactions, and their individual difficulties in mind, it was prudent to develop a fresh approach for the synthesis of combretastatin A-4 (1a) and related stilbenes. For these Wittig reactions both lithium hydroxide and potassium carbonate were used as a base. Potassium carbonate has been previously used in Wittig-Horner reactions with aliphatic aldehydes to generate E-alkenes. The phosphonium bromide (4) was heated with a range of aldehydes (Scheme 7) in the presence of three equiv. lithium hydroxide or three equiv. potassium carbonate in one millilitre of water. In all cases, except when isovanillin (6) was used to try to prepare combretastatin A-4 (1a, 1b), a mixture of E- and Z-stilbenes was isolated. Also, no stilbene was formed when using 3-hydroxybenzaldehyde or 4-hydroxybenzaldehyde under these aqueous Wittig methodologies. With the failure of this methodology to yield stilbenes from phenolic substrates, alternative methods were sought to generate combretastatin A-4 (1a) using aqueous conditions. The tetrahydropropyranol ethers of combretastatin A-4 (24I, 25I) have already been described in the literature and were synthesised from phosphonium salt (4) and THP protected isovanillin using a solid phase column containing alumina-KF and tetrahydrofuran. Using our aqueous methodology these two stilbenes (24I, 25I) were synthesised in excellent yields using both LiOH (69%) and K$_2$CO$_3$ (82%) in water, as shown in Table 1. Separation followed by treatment with dilute HCl to cleave off the protecting groups yielded Z- and E-combretastatin A-4 (1a, 1b) quantitatively. The silyl ethers of both combretastatin A-4 (3a, 3b) have also already been synthesised in excellent yields.

**Scheme 7.** Aqueous synthesis of combretastatin library. Reagents and conditions: a) LiOH or K$_2$CO$_3$ (3 equiv), water, reflux, 16 h.

| Aldehyde | LiOH | K$_2$CO$_3$ |
|----------|------|-------------|
|          | Z    | E           | Total   | Z    | E           | Total   |
| 24, 25 a | 4-Chloro | 26.90% | 23.60% | 50.50% | 33.70% | 27.00% | 60.70% |
| 24, 25 b | 4-Fluoro | 26.10% | 36.50% | 52.00% | 22.70% | 35.40% | 58.10% |
| 24, 25 c | 2-NO$_2$ | 38.80% | 38% | 76.80% | 25.70% | 25.50% | 51.20% |
| 24, 25 d | 3-NO$_2$ | 29.40% | 36.50% | 65.90% | 32.40% | 24.60% | 57.00% |
| 24, 25 e | 4-NO$_2$ | 28.38% | 33.40% | 61.78% | 26.80% | 22.80% | 49.60% |
| 24, 25 f | 4-Methoxy | 17.90% | 28.40% | 46.30% | 24.60% | 13.30% | 37.90% |
| 24, 25 g | 3,4,5-Trimesoxy | 30.70% | 25.30% | 56.00% | 25.20% | 20.60% | 45.80% |
| 24, 25 h | 3-Fluoro-4-methoxy | 27.50% | 31.20% | 58.70% | 34.20% | 30.00% | 64.20% |
| 24, 25 i | Benzaldehyde | 35.80% | 23.80% | 59.60% | 29.40% | 25.20% | 54.60% |
| 24, 25 j | 4-Trifluoromethyl | 31.60% | 26.30% | 57.90% | 30.60% | 21.50% | 52.10% |
| 24, 25 k | 4-Cyano | 32% | 37% | 69% | 27.20% | 24.90% | 52.10% |
| 24, 25 l | 4-Trihydroxybenzylaldehyde | 32% | 37% | 69% | 37% | 45% | 82% |
| 3a, 3b | [a] | 31% | 42% | 73% | 41% | 48% | 89% |

[a] Yields from aldehydes I and b refer to the yields of the deprotected Z- and E-combretastatin.
been described in the literature.\textsuperscript{[11]} These were synthesised using our LiOH and K$_2$CO$_3$ protocols in water. After work up, the silyl ethers (3a, 3b) were not isolated and solely Z- and E-combretastatin A-4 (1a, 1b) were produced in excellent yields (LiOH, 73\%)(K$_2$CO$_3$, 89\%, Table 1).

Conclusion

The methodology described herein has shown that the Wittig reaction using both potassium carbonate and lithium hydroxide in water can be used to synthesise the antivascular agent combretastatin A-4 (1a, 1b) in good yield. The synthetic method does not work when the benzaldehydes in the reaction possess a phenolic group. Therefore, THP and silyl protected phenols were used in the Wittig reaction to generate the required phenolic stilbenes (1a, 1b). The THP protected stilbenes were readily cleaved with dilute HCl whilst the silyl groups fortuitously fell off during work up to provide Z- and E-combretastatin A-4. This methodology was also successful for preparing a variety of other trimethoxyphenyl substituted stilbenes.

Supporting Information Summary

Full experimental details and NMR spectra for this article are available in the supporting information.

Acknowledgements

This work was supported by the EPSRC, UK (grant 1817912) and Kidscan Children’s Cancer Research, UK (grant KGR14). The authors thank Kirit Amin (Salford Analytical Services, UK) for technical assistance.

Conflict of Interest

The authors declare no conflict of interest.

Keywords: Combretastatins · Green Chemistry · Stilbenes · Water Chemistry · Wittig Chemistry

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Submitted: June 2, 2021
Accepted: July 19, 2021