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A versatile synthesis of "tafuramycin A": a potent anticancer and parasite attenuating agent†

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An improved and versatile synthesis of tafuramycin A, a potent anticancer and parasite-attenuating agent, is reported. The three major improvements that optimized yield, simplified purification and allowed the synthesis of more versatile duocarmycin analogues are: a first-time reported regioselective bromination using DMAP as catalyst; the control of the aryl radical alkene cyclization step to prevent the dechlorination side reaction; and the design of a new protection/deprotection method to avoid furan double bond reduction during the classical *O*-benzyl deprotection in the final step. This alternative protection/deprotection strategy provides ready access to duocarmycin seco-analogues that carry labile functionalities under reducing reaction conditions. Tafuramycin A (**3**) was prepared in either 8 steps from intermediate **6** or 7 steps from intermediate **17** in 52% or 37% yield respectively. Our strategy provides a significant improvement on the original procedure (11% overall yield) and greater versatility for analogue development.

Introduction

Duocarmycins, exemplified by duocarmycin SA (DSA, **1**), are a group of highly potent anticancer alkaloids separated from *Streptomyces* sp. (Fig. 1).[1–3] They exert their cytotoxic effect through binding at the minor groove of AT-rich sequences in the DNA and by covalently alkylating adenine-N3.[2–4] In spite of their high potency, all natural duocarmycins failed to reach the market as anticancer drugs because of their severe toxicity, particularly to bone marrow and liver.[5–7] Over the last two decades, numerous synthetic analogues of duocarmycins have been developed that introduce functionalization at both its DNA-alkylating unit (part A) and DNA-binding unit (part B).[8–11] These analogues have enabled the development of a SAR profile of this compound class to facilitate the discovery of more selective and less toxic derivatives that may have clinical application (for detailed review see ref. 1–3).

A number of these reported modifications were successful in simplifying the structure of duocarmycins while maintaining high potency, for example centanamycin (**2**).[9,12] In addition to its highly potent anticancer activity, centanamycin also displayed a remarkable antiparasitic activity both *in vitro* and *in vivo* against a number of protozoan parasites, such as different *Plasmodium* species.[13,14] However, the potential *in vivo* toxicities of such compounds remain a limiting factor for their clinical use, either as an anticancer or as an antiparasitic agent. Nevertheless, a recent study on centanamycin opened the door to a potential use of the compound,

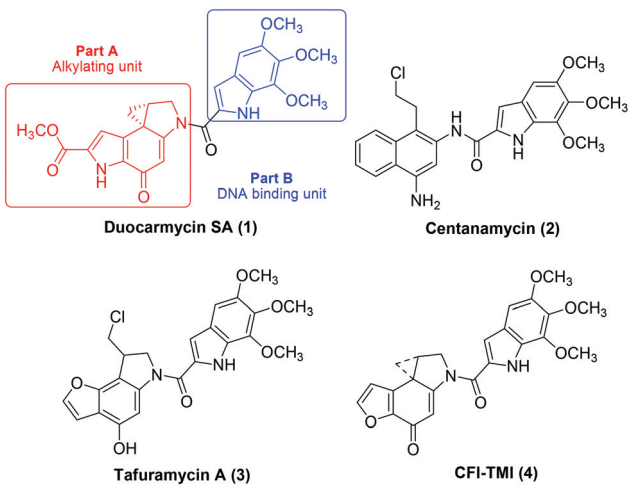

Fig. 1 Structures of duocarmycin SA (**1**), centanamycin (**2**), tafuramycin A (**3**) and CFI-TMI (**4**).

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†Electronic supplementary information (ESI) available: Complete experimental procedures, characterization data, ^{1}H and ^{13}C NMR spectra of new intermediates and final compounds and CIF with details of X-ray data collection and refinement for compound **14**. CCDC 992249. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c4ob00842a

regardless of its *in vivo* toxicity, where it was successfully used as a chemical attenuating agent for the production of a candidate malaria vaccine.[13] It has been shown that centanamycin is effective in attenuating *Plasmodium berghei* both *in vitro* and *in vivo*, and mice vaccinated with *in vitro* treated sporozoites were protected against infection with wild type sporozoites.[13]

Following the discovery of centanamycin and its successful use as a parasite attenuating agent in vaccine development, tafuramycin A (**3**)[15] displayed superior effect compared with centanamycin. This discovery is now the subject of a patent in which **3** is claimed as a parasite attenuating agent in the production of a whole parasite malaria vaccine.[16] A single dose of tafuramycin A-attenuated parasite was found to be sufficient to protect the animals against subsequent malaria infection by the same isolate, strain or species of *Plasmodium* used in the immunogenic composition, or by one or more heterologous isolates, strains or species of *Plasmodium*.[17]

Tafuramycin A (**3**)[15] is a seco-prodrug that dehydrochlorinates inside the cell to generate the active cyclopropane-containing drug that subsequently alkylates DNA. It's also an isoform of the previously reported[18,19] furanoindoline analogue of duocarmycin SA (CFI-TMI, **4**). Herein, we describe a versatile synthesis of tafuramycin A that overcomes a number of difficulties observed in the original strategy. Furthermore, our approach allows for a high yielding, large-scale production of **3** and more versatility that enables the introduction of new functionality.

Results and discussion

Our approach towards the synthesis of tafuramycin A (Scheme 1) utilized readily synthesized intermediates **5–7**,[15] with **7** further purified by crystallization from hexanes. The first synthetic challenge was encountered during the regioselective bromination of intermediate **7** to provide the monobromo derivative **8**. The reported[15] reaction conditions for bromination, *i.e.* NBS in anhydrous THF–MeOH and *p*-toluenesulfonic acid (TsOH) at −78 °C, provides **8** in only 55% yield. Despite several attempts, no improvement in yield was observed by following similar conditions. Furthermore, the isolated product was made up of an inseparable mixture of the mono- and dibromo derivatives.

Similar reported regioselective brominations have employed various reaction conditions at extremely low reaction temperatures, ranging from −60 °C[20] to −78 °C,[21–24] in the absence of light and all used acid catalysis.[20–24] Alternatively, the more expensive iodinating reagent NIS has also been used in place of NBS.[25–27] Even with NIS, acid catalysis was required, and the reaction temperatures ranged typically between −20 °C[27] and −40 °C.[25] Based on these reports, a number of different reaction conditions, including varied solvent composition, catalyst and reaction temperature employed were evaluated. Both acid and base catalysts were tested and DMAP was shown to provide **8** in excellent yields at a reaction temperature of 25 °C (81%) or 0 °C (92%), in relatively short reaction time (1 h).

The allylation of intermediate **8** with (*E*/*Z*)-1,3-dichloropropene, following standard reaction conditions,[15] provided the key intermediate **9** in an excellent yield (96% of a mixture of *Z* : *E* isomer ≡ 6 : 4 *cf.* 80% previously reported[15]). This improved yield is most likely due to the greater purity of the starting material as a consequence of our optimized bromination conditions that yield selectively and exclusively the monobromo product **8**.

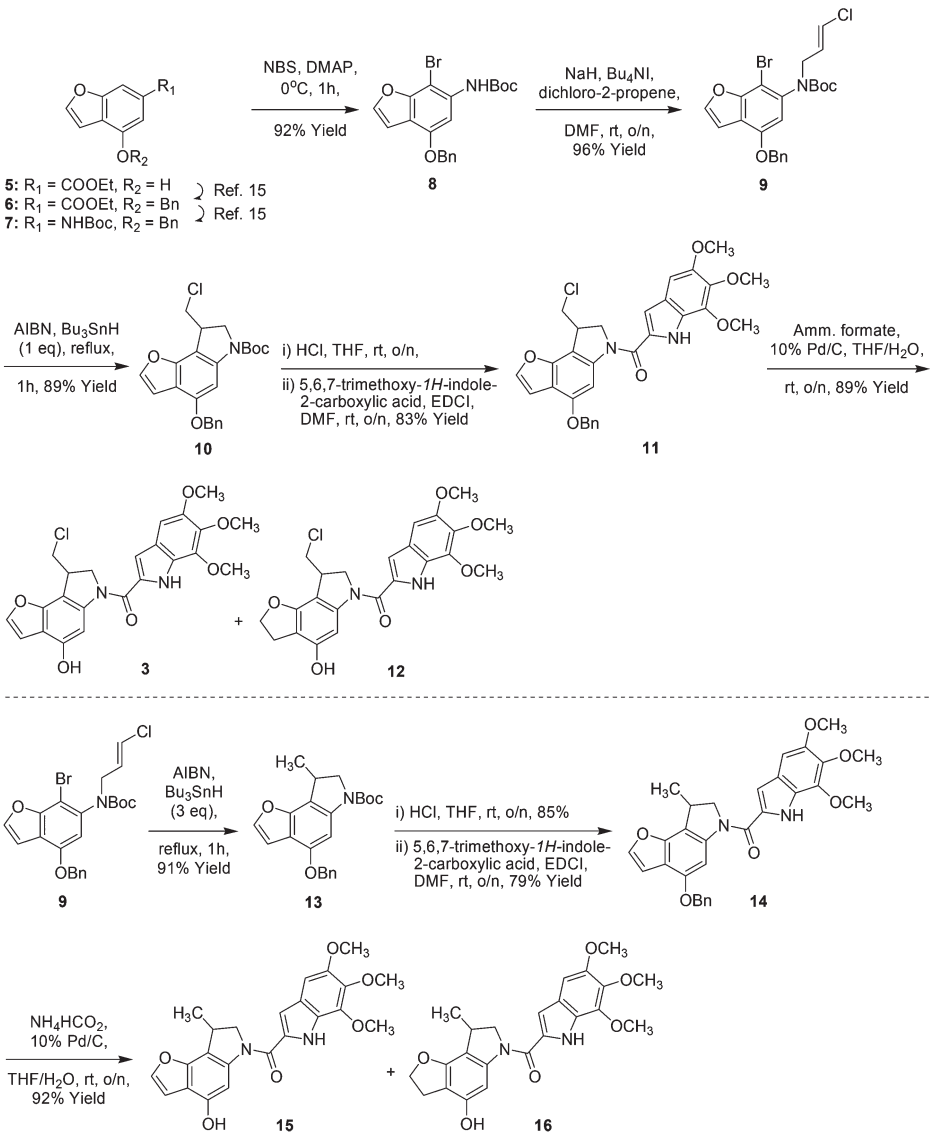
Cyclization of **9** in toluene, in the presence of AIBN and Bu_3SnH, has been reported to yield a mixture of two compounds; the required product **10** from a 5-*exo*-trig cyclization (obtained in 40% yield) and a seco-cyclopropyl-tetrahydrofurano[2,3-*f*]quinoline (seco-CFQ, Fig. 2) that resulted from 6-*endo*-trig cyclization (obtained in 48% yield).[15,28] A limited number of reports on a similar cyclization reaction that provides the thermodynamically less favored 6-membered quinoline have been published.[29–32] In these reports, the formation of the less favored 6-membered quinoline structure is the result of rearrangement of the 5-membered indoline as a consequence of nucleophilic attack by anions such as Cl^-, NC^- and MeO^-.[31]

Accordingly, the second challenge in this synthesis was to control the conditions of the cyclization step so as to exclusively obtain the required product **10**, or at least to maximize its yield. In our hands; the typical reaction conditions reported for this cyclization,[15] led to a mixture of two products. Characterization of each of the mixture's components by spectroscopic analysis confirmed one of the products as the intended chloromethyl furanoindole derivative **10**, while the other was determined to be its dechlorination product (methyl furanoindole derivative, **13**).

Surprisingly, no 6-membered furanoquinoline isomer was observed in several trials, under various conditions. Further support for the structure of the dechloro-derivative **13** was provided by X-ray crystal structure determination of its subsequent coupled product **14** as shown in Fig. 3 (CCDC 992249).

The dechlorination product **13** was presumably the consequence of the excess (2.7 eq.) Bu_3SnH used in the reaction. In the presence of only 1.0 eq. of Bu_3SnH we found that **10** was the only reaction product observed. The product yield was however unsatisfactory (~20%), with the majority of starting material **9** unreacted. This is most likely why 2.7 eq. of Bu_3SnH has been used in published procedures[15] as it forces the reaction to completion, despite the formation of the unwanted side product **13**.

In our approach to **10** we sought to minimize the formation of **13** and to this end we explored a variety of conditions including varying the amount of AIBN and total solvent volume. Increasing the amount of AIBN had little influence on reaction rate and product yield, while remarkable effects were observed by varying the solvent volume. Thus, reduction of the solvent volume led to an increase in reaction rate and improved product yield. Further optimization through solvent



Scheme 1 Improved synthesis of tafuramycin A (**3**) and its methyl furanoindole analogue (**15**).

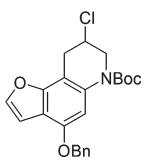


Fig. 2 Structure of the 6-membered furanoquinoline derivative (seco-CFQ).

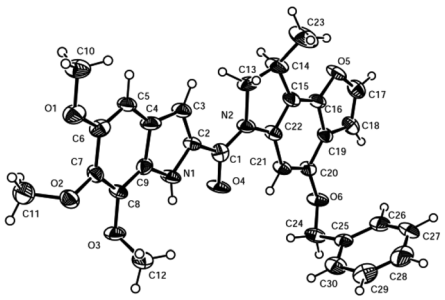
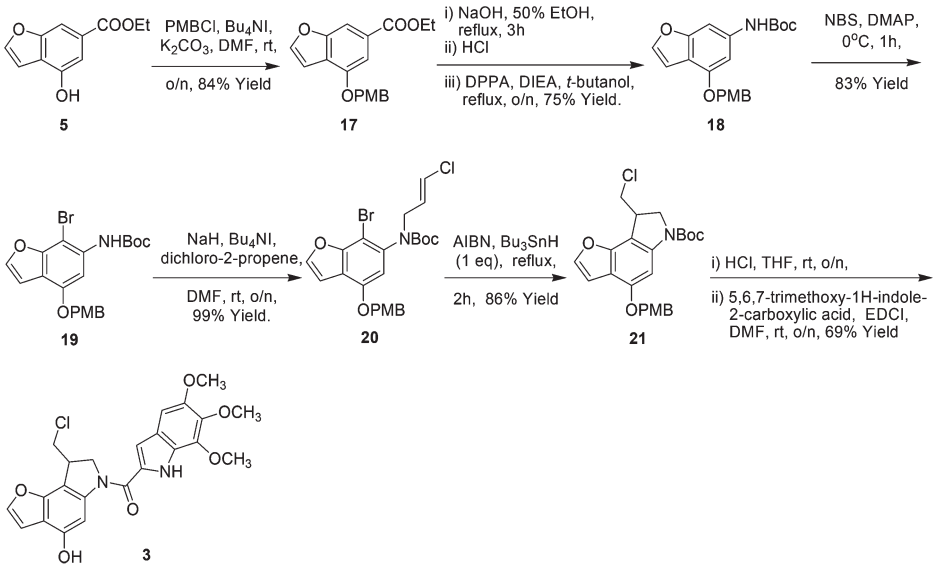


Fig. 3 X-ray crystal structure of **14**.

(toluene) volume adjustment to give **9** in a concentration 0.2 mmol mL^{-1}, in the presence of Bu_3SnH (1 eq.), resulted in formation of the desired product **10** in 89% isolated yield in 1 h. Interestingly, and as shown in Scheme 1, refluxing a similar molar concentration of **9** in toluene with excess Bu_3SnH (3.0 eq.) for 1 h yielded the dechloro-product **13** (91%).

Intermediate **10** was then smoothly converted to **3** using published conditions.[15] Thus, deprotection of **10** and coupling with 5,6,7-trimethoxy-1*H*-indole-2-carboxylic acid (TMI),[33] yielded the *O*-benzyl protected amide **11**. Finally **11** was



Scheme 2 Alternative synthesis of tafuramycin A (**3**).

deprotected by 10% Pd/C catalysed reduction to yield the target compound **3**. Unfortunately, under these catalytic reduction conditions, overreduction of **3** was observed and afforded the dihydro compound **12** as a byproduct. Numerous significant variations of reduction conditions (*e.g.* changing the catalyst, solvent, reaction time or temperature) failed to improve this outcome. Furthermore, the ratio of the two products (**3** and **12**) was variable and uncontrollable), and the resultant mixture could be only resolved using HPLC (see Experimental section). Similarly, Boc-deprotection of **13**, coupling of the product with TMI to yield **14** and benzyl deprotection by catalytic reduction yielded a HPLC-separable mixture of **15** and its dihydro-derivative **16** (Scheme 1).

In order to avoid overreduction of **3** during benzyl deprotection, an alternative protection/deprotection strategy was investigated. The use of acetate or other ester-protecting group was not possible, because of the basic reaction conditions (NaH) employed in one of the intermediate steps (preparation of **9**). Consequently, the use of a *p*-methoxybenzyl (PMB) ether-protecting group was explored (Scheme 2). This alternative PMB-ether protection strategy was only possible after the new bromination conditions, described for the synthesis of intermediate **8**, were adopted. The previously employed acid-catalysed bromination conditions[20–24] prevent the use of PMB as a protecting group as these ethers are known to be labile to oxidative (NBS) and acidic (TsOH or H_2SO_4) conditions.[34] Adoption of our optimized bromination conditions allows the reaction to proceed under mild basic (DMAP) conditions rather than acidic conditions. Moreover, our conditions shorten the bromination time to only one hour, leaving the PMB-ether installed to provide the desired bromo PMB-ether in good yield.

The new synthetic pathway started with the treatment of **5** with PMB-chloride in the presence of potassium carbonate and TBAI to yield intermediate **17** (84%). Subsequent ester hydrolysis and Curtius rearrangement provided the Boc-protected amine **18** in 75% yield. The intermediates **19** and **20** were prepared by analogous reaction conditions (Scheme 1) employed for the synthesis of compounds **8** and **9** respectively. Employing our optimized cyclization conditions (Scheme 1) the key intermediate **21** was isolated (~35%), leaving the majority of starting material unreacted. Increasing AIBN equivalents from 0.1 to 1.0 improved the reaction yield (86%) within 2 h. Finally, removal of the Boc and PMB groups by treatment with HCl in THF afforded an intermediate with free hydroxyl and amino groups that was coupled with TMI to afford tafuramycin A (**3**) in 69% yield.

Conclusions

In summary, an improved synthesis of the potent anticancer and parasite attenuating agent (tafuramycin A, **3**) is described. The first improvement described is the regioselective bromination, employing DMAP as a catalyst. This bromination step is a common key step in the synthesis of several duocarmycin analogues, and therefore, the modified regioselective bromination conditions will be applicable in many other related synthetic steps. The second improvement is the control of the aryl radical alkene cyclization step to yield the intended furanoindole intermediate **10** exclusively in an excellent yield.

The structure of the side product **13** produced in the cyclization step upon use of excess Bu_3SnH was also identified. X-ray crystal structure determination (CCDC 992249) of the subsequent coupling product **14** confirmed it as the dechloro-product of **10**.

These two major improvements (Scheme 1) provided the final product **3** starting from intermediate **6** in an overall yield of 52%. This represents a significant improvement over the original procedure (11% overall yield). The final improvement

that employed an alternative protection/deprotection strategy (Scheme 2) overcame the problem of overreduction in the final step. The success of the new protection method is ascribed to the newly adopted DMAP-catalysed bromination conditions that overcome the limitations of the classic acid-catalysed conditions preventing use of a PMB ether-protecting group. This strategy gave exclusively the desired product **3** in an overall yield of 37% from intermediate **17**. Importantly, this protection/deprotection strategy allows a more versatile synthesis of duocarmycin seco-analogues that carry labile groups under reducing reaction conditions. Such analogues were previously inaccessible by previously published methods.

Notes

The authors declare no competing financial interest.

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