A two-directional approach to the anatoxin alkaloids: second synthesis of homoanatoxin and efficient synthesis of anatoxin-a†‡

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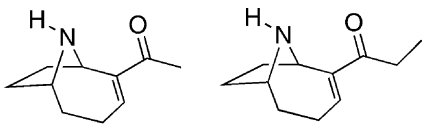
Total syntheses of the potent neurotoxins, environmental hazards, and biochemical probes anatoxin-a and homoanatoxin have been achieved from a common intermediate using a combined two-directional synthesis–tandem reaction strategy which includes key advances in oxidative desymmetrisation, tandem Michael–intramolecular Mannich cyclisations and a new method for deprotection of *N*-tosyl anatoxin-a.

Anatoxin-a (**1**) was first identified after investigations into the cause of incidents of fatal poisoning of wild and domestic animals by cyanobacterial blooms in North America and Europe,[1] leading it to be initially called VFDF (very fast death factor). Anatoxin-a was initially isolated from the blue-green algae *Anabaena flos aquae* in the 1970's,[2] and has subsequently been isolated from several other toxic strains of fresh water cyanobacteria.[3] Homoanatoxin (**2**) was first characterised as a synthetic analogue of anatoxin-a by Gallagher *et al.* in 1992,[4] and was subsequently isolated from strains of *Oscillatoria formosa*[5] and *Raphidiopsis mediterranea*.[3f]



Anatoxin-a (**1**) Homoanatoxin (**2**)

Both anatoxin-a (**1**) and homoanatoxin (**2**) display potent activity as agonists at nicotinic acetylcholine receptors (nAChR) with a high degree of selectivity over muscarinic acetylcholine receptors (mAChR), which has led to them being used as tools to investigate muscle and neuronal nAChR.[6] Due to their modulatory role in the brain, nAChRs have attracted attention as potential targets for therapeutic intervention in a range of disease states including neurodegenerative diseases, attention deficit disorder, pain, smoking cessation, schizophrenia, and epilepsy.[7] Thus, the intriguing bicyclic structure and enticing combination of potent and specific biological activity have prompted a high degree of interest in the synthetic community, resulting in numerous syntheses of anatoxin-a[8] and surprisingly just one synthesis of homoanatoxin[4] (from *N*-BOC anatoxin-a). In this paper, we present a 10 step total synthesis of anatoxin-a (27.1% overall yield) and the second total synthesis of homoanatoxin (10 steps, 14.8% overall yield) using a combined two-directional synthesis and tandem reaction strategy which allows both natural products to be accessed from a common intermediate. Our retrosynthetic analysis is shown in Fig. 1.

We envisaged that a Mannich and a Horner–Wadsworth–Emmons disconnective approach would allow the use of iminium synthon **3** as a precursor to both anatoxin-a and homoanatoxin by choice of keto-phosphonate coupling partner. Access to iminium **3** should be available from a symmetrical dialdehyde derived from oxidative cleavage of dialkene **5**, enabling the use of two-directional synthesis for the formation of our key common intermediate—iminium precursor **4**. The synthesis of key intermediate **4** is shown in Scheme 1.

Formation of the Grignard reagent of 3-butenyl bromide, followed by reaction with 0.5 equivalents of ethyl formate, resulted in the formation of symmetrical alcohol **6** in 76% yield. Mitsunobu coupling of alcohol **6** with *N*-tosyl-*tert*-butyl carbamate cleanly gave doubly protected amine **7**, which was then treated with TFA in order to remove the BOC protecting group. We then exploited Schreiber's desymmetrising cyclic alkene ozonolysis methodology[9] to access non-symmetrical dialdehyde derivative **4**: initial ring-closing metathesis was carried out using 4.6 mol% of Grubb's first generation catalyst[10] to convert dialkene **5** into cyclic alkene **8**, followed by the desymmetrising oxidative cleavage of **8** using ozone in methanol followed by a reductive work-up, giving a quantitative yield of aldehyde **4**. This constitutes the first example of a hemiaminal formation using Schreiber's strategy, and the proposed mechanism for this transformation is shown in

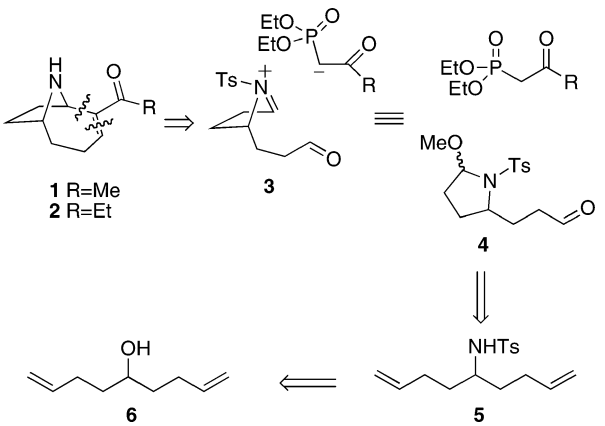


Fig. 1 Retrosynthetic analysis.

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† This paper is dedicated to Professor Philip Magnus, FRS, on the occasion of his 65th birthday.

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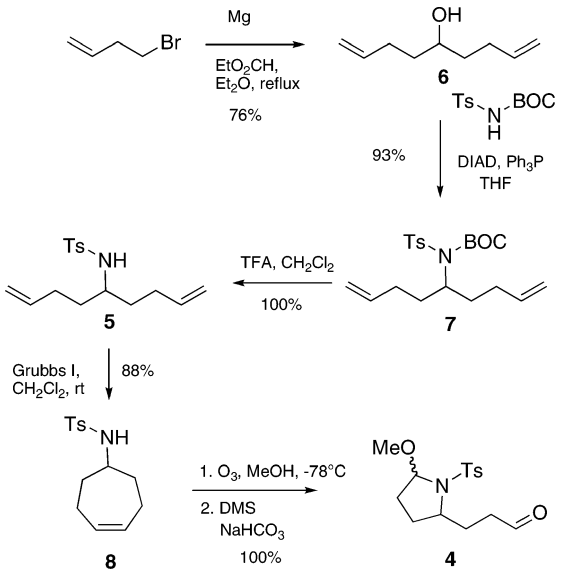
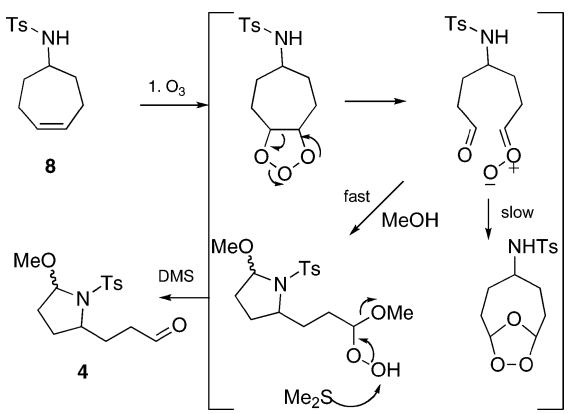


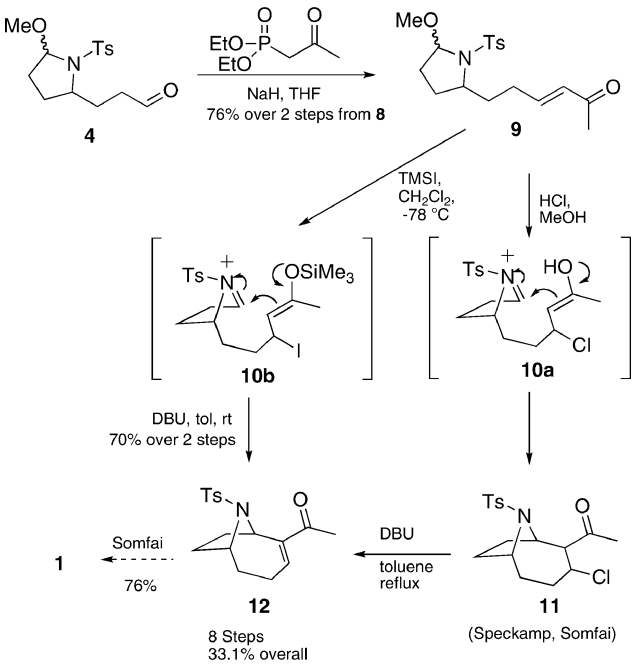
Scheme 1 Synthesis of key intermediate **4**.

Scheme 2. Thus the primary ozonide ring-opens, but due to the tether, the formation of the secondary ozonide is slow, and the carbonyl oxide is instead trapped as a hydroperoxy hemiacetal by the methanol solvent. This allows hemiaminal formation between the nitrogen on the chain and the aldehyde. Finally, addition of dimethyl sulfide reduces the hydroperoxy hemiacetal and produces the desymmetrised aldehyde **4**.

Scheme 3 describes our formal synthesis of anatoxin-a from key intermediate **4**. Treatment of crude aldehyde **4** (which was found to be unstable to chromatography and was thus used unpurified) with diethylphosphono-acetone and sodium hydride in THF gave enone **9** as a single alkene isomer but a mixture of diastereomers across the pyrrolidine ring in 76% yield over two steps from **8**. Somfai and Åhman had already shown that *trans-E*-**9** was able to undergo a tandem iminium ion formation–Baylis–Hillman type reaction *via* an intermediate of the type **10a** to form a mixture of chlorides **11**, which in turn could be converted to *N*-tosyl anatoxin-a **12** by elimination of HCl with DBU in toluene at reflux in 67% overall yield.[11,12] In our hands, and using a mixture of diastereomers of **9** as substrates, we were only able to attain moderate yields of **12** at best using these conditions.[13] Due to the capricious



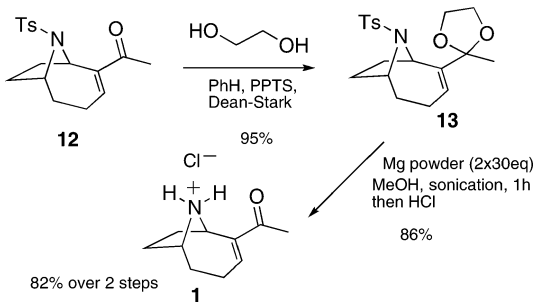
Scheme 2 Mechanism of desymmetrising ozonolysis.



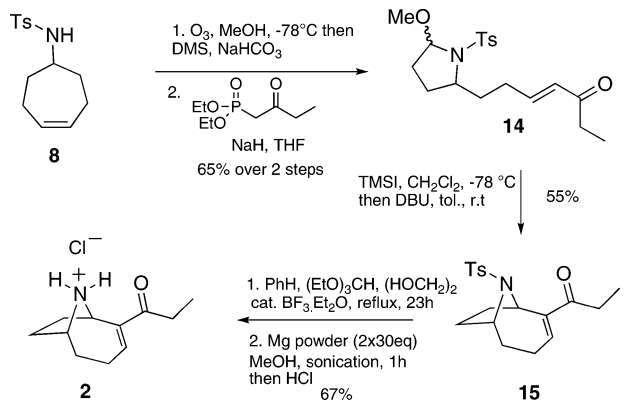
Scheme 3 Formal synthesis of anatoxin-a.

nature of the HCl–MeOH conditions, which have thus far been the only reported protocol which has proven successful,[13] we set about investigating alternative conditions for this key transformation. It transpired that TMSI in dichloromethane at −78 °C was able to promote a similar transformation (presumably *via* iodo silyl enol ether **10b**). The outcome of this reaction was a good crude yield of *N*-tosyl anatoxin-a accompanied by significant quantities of diastereomers of the non-eliminated hydroiodide precursor. It was found that by treating the crude mixture with DBU in toluene and stirring at room temperature, any hydroiodide was transformed into tosyl anatoxin-a (**12**), giving a 70% yield of **12** from **9** over the two-step operation.

Somfai,[11] and Trost[14] have previously deprotected *N*-tosyl anatoxin-a (**12**) using sodium amalgam in methanol at −40 °C in the presence of a phosphate buffer, and thus at this stage our synthesis of **12** in 8 steps and 33% overall yield from ethyl formate represented a formal synthesis. However, we wished to detail a scalable and potentially viable route for the production of anatoxin-a on a multigram scale, and thus we wished to deprotect our sample of **12**. Unfortunately all of our attempts to achieve the deprotection of our sample of (±)-**12** using the procedures reported by both Somfai and Trost (on enantiomerically enriched **12**) failed, as we found our racemic material to be insoluble under the conditions reported of methanol at −40 °C (we have considered the possibility that the racemic material has a higher lattice energy than the enantioenriched material, and may be less soluble). Addition of THF co-solvent led to complete solvation, but unfortunately reduction of the enone was observed rather than removal of the sulfonamide. We also tried a very wide range of other reducing conditions for this transformation, but were again unsuccessful, noting that if any reduction was seen, it was of the enone functionality in preference to the sulfonamide. Unable to resolve this issue, we decided to protect the



Scheme 4 Deprotection of *N*-tosyl anatoxin-a.



Scheme 5 Synthesis of homoanatoxin.

enone, such that more severe reducing conditions could be utilised to remove the sulfonamide (Scheme 4).

Reaction of **12** with ethylene glycol under Dean–Stark conditions gave the dioxolane **13** in 95% yield. In an analogous reaction to the final step of Bäckvall and co-workers's ferruginine synthesis,[15] sulfonamide **13** was treated with an initial portion of magnesium powder (30 equivalents) in methanol with sonication. After 40 minutes an additional 30 equivalents of magnesium powder were added and the sonication continued for a further hour before the addition of aqueous HCl. It was found that the dioxolane protecting group was removed and anatoxin-a was recovered as the hydrochloride salt **1** in 82% yield over two steps from **12**. Overall, this constitutes a synthesis of anatoxin-a in 10 steps and 27.1% overall yield from readily available starting materials.

With the synthesis of anatoxin-a (**1**) complete, our attention turned to the adaption of this strategy for the synthesis of homoanatoxin (**2**), which had previously been synthesised by Gallagher in two steps and 19.2% yield from *N*-BOC anatoxin-a.[4] Scheme 5 shows our synthesis.

Accordingly, alkene **8** was subjected to the desymmetrising ozonolysis reaction to give aldehyde **4** which underwent olefination with 1-(diethylphosphono)butan-2-one to give enone **14**. The TMSI-promoted tandem iminium formation–enol ether formation–intramolecular Mannich reaction and subsequent elimination worked as on our previous substrate, giving a 55% yield of **15** for this key step. Removal of the sulfonamide protecting group of **15** was accomplished using the same tactic of enone protection followed by reduction to give homoanatoxin as the hydrochloride salt **2** in 10 steps and 14.8% overall yield from readily available starting materials.‡

In conclusion, we have described a unified approach to the synthesis of anatoxin-a (**1**) and homoanatoxin (**2**) from a common precursor. Work is on-going in these laboratories to extend this approach to the synthesis of a range of unnatural anatoxin-a derivatives and for the multigram syntheses of these important biological tools. Our reports on these developments will be disclosed in due course.

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