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Concise Synthesis of (+)-[$^{13}C_4$]-Anatoxin-a by a Dynamic Kinetic Resolution of a Cyclic Iminium Ion

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Abstract: An asymmetric total synthesis of [$^{13}C_4$]-anatoxin-a ([$^{13}C_4$]-**1**) has been developed from commercially available ethyl [$^{13}C_4$]-acetoacetate ([$^{13}C_4$]-**15**). The unique requirements associated with isotope incorporation inspired a new, robust, and highly scalable route, providing access to 0.110 g of this internal standard for use in the detection and precise quantification of anatoxin-a in freshwater. A highlight of the synthesis is a method that leverages a cyclic iminium ion racemization to achieve dynamic kinetic resolution in an enantioselective Morita-Baylis-Hillman (MBH) cyclization.

Anatoxin-a (**1**) is a cyanobacterial toxin produced by several genera of blue-green algae, including *Anabaena*, *Aphanizomenon*, and *Oscillatoria*. [1] This potent agonist of nicotinic acetylcholine receptors (nAChRs) causes prolonged excitation at the neuromuscular junction and a blockage of further electrical transmission, which can lead to muscular paralysis and death by asphyxiation.[2] It has been associated with the poisoning and deaths of livestock, domestic animals, and waterfowl after exposure to cyanobacteria-contaminated water.[3]

Cyanobacterial algal blooms have occurred with increasing frequency in recent years, likely caused by factors such as rising water temperatures and fertilizer runoff.[4] These blooms pose a significant threat to public safety due to the production of cyanobacterial toxins, such as anatoxin-a. As such, methods for the detection and quantification of anatoxin-a in fresh water are critically important to human health.[1b] The analytical method currently adopted by the Environmental Protection Agency for anatoxin-a quantification in finished drinking water involves a combination of liquid chromatography, electrospray ionization, and tandem mass spectrometry (LC/ESI-MS/MS) using deuterated phenylalanine (L-phenylalanine-d_5) as an internal standard.[5] While this method is useful, its accuracy hinges on the assumption that L-phenylalanine-d_5 and anatoxin-a will have identical behavior during sample preparation and analysis.[6] A more ideal standard, which would account for potential sample loss and ion suppression from other components present in the sample matrix, would be an isotopically labelled variant of anatoxin-a; however, no such standard is available.

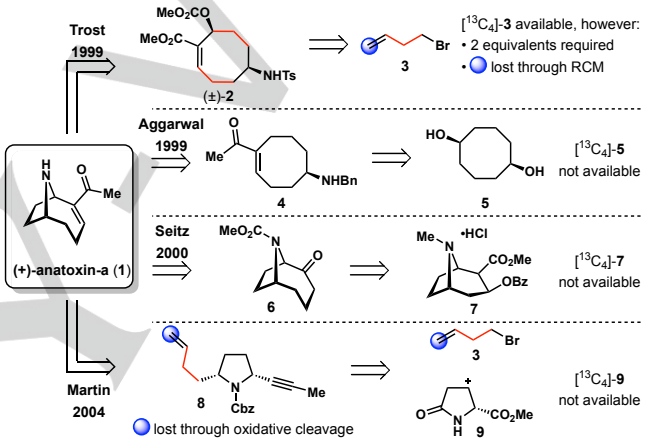
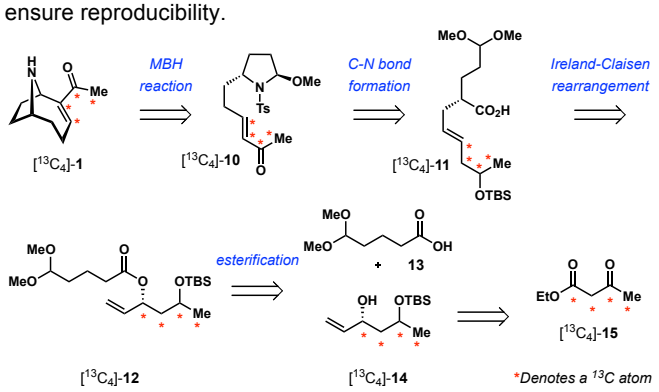


Figure 1. Previous synthetic approaches to (+)-anatoxin-a and consideration of their ability to produce an isotopically labelled internal standard.

Since its isolation, a plethora of synthetic approaches to the natural isotope of anatoxin-a have been developed and extensively reviewed.[7] Its popularity as a synthetic target has inspired several innovative routes (Figure 1). Elegant work has been described by the Trost,[8] Aggarwal,[9] Seitz,[10] and Martin[11] groups relying on a variety of creative strategies including asymmetric allylic amination, asymmetric deprotonation, tropanone ring expansion, and enyne metathesis. These impressive syntheses proceeded in as few as 8 steps (Seitz) and up to 27% overall yield (Martin), truly setting a high bar for future approaches to this important target.

Our goal was to provide practical synthetic access to an isotopically labelled anatoxin-a that could be used as an internal standard for highly sensitive and precise freshwater quantification. In the initial stages of synthesis planning, there were several important criteria that had to be taken into consideration.[12] First, isotope incorporation at non-exchangeable positions throughout the synthesis is essential to preventing isotope loss and maintaining a uniform molecular mass in the final product. Second, to prevent any signal overlap during mass spectral analysis, we sought to prepare a standard with a mass difference of 4 atomic units compared to the natural anatoxin-a. Finally, given the high cost of isotopically labelled starting materials, the synthesis itself must be as concise as possible,

highly scalable, and rely on robust chemical transformations to ensure reproducibility.



Scheme 1. Synthesis plan for [$^{13}C_4$]-anatoxin-a.

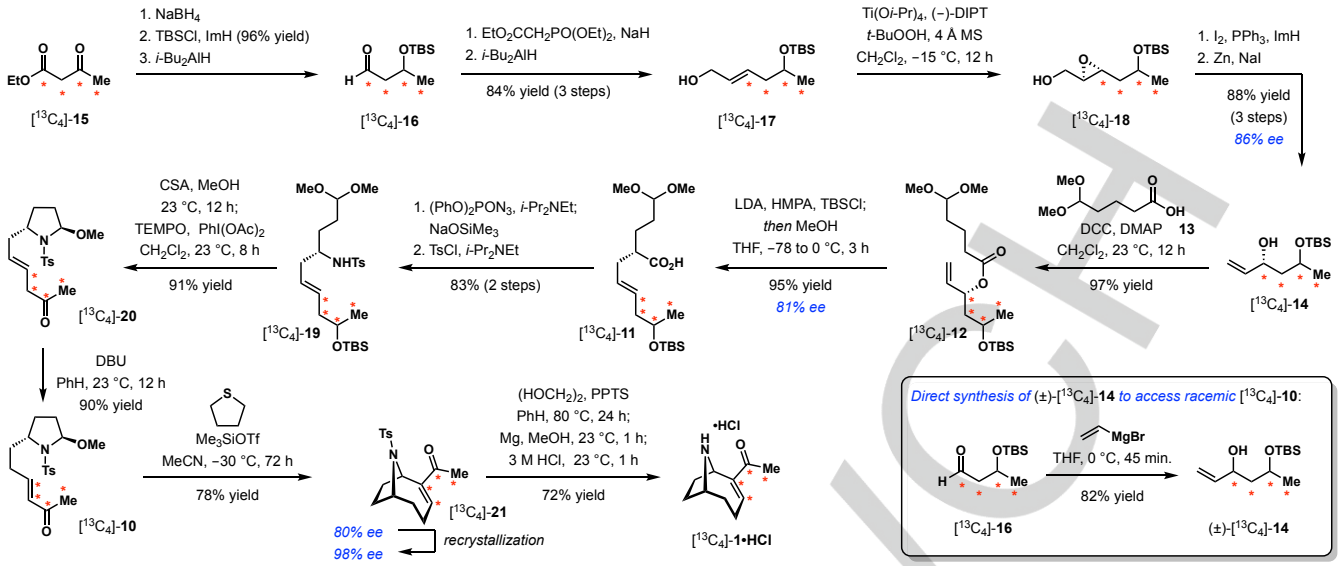
Inspired by the past approaches described above, we attempted to identity a suitable route to isotopically labelled anatoxin-a that would satisfy these criteria. Previous syntheses, while succinct and high yielding, appeared to be unfeasible in this regard due to a lack of commercially available isotopically labelled starting materials or synthetic transformations that would result in isotope loss (Figure 1). We therefore decided to develop a *de novo* synthesis of [$^{13}C_4$]-anatoxin-a ([$^{13}C_4$]-**1**) from a suitable source of heavy isotopes. We envisioned a synthetic route in which the final C-C bond of anatoxin-a would be constructed from an iminium Morita-Baylis-Hillman (MBH) cyclization of enone [$^{13}C_4$]-**10** (Scheme 1).[13,14] The cyclization precursor [$^{13}C_4$]-**10** would be derived from acid [$^{13}C_4$]-**11**, which in turn would arise from an Ireland-Claisen rearrangement of allylic ester [$^{13}C_4$]-**12**.[15] Disconnection of the ester C-O bond identified chiral alcohol **14** and carboxylic acid **15** as potential materials for the introduction of isotopes into the synthesis. After a thorough survey of commercially available isotopically labelled compounds, ethyl [$^{13}C_4$]-acetoacetate ([$^{13}C_4$]-**15**) was determined to be an excellent precursor to alcohol [$^{13}C_4$]-**14**. This material serves as the sole source of all heavy isotopes in the synthesis. More importantly, these isotopes are placed at non-exchangeable positions throughout the designed route. Herein, we describe our successful approach to [$^{13}C_2$,$^{13}C_3$,$^{13}C_{10}$,$^{13}C_{11}$]-anatoxin-a ([$^{13}C_4$]-**1**) from [$^{13}C_4$]-**15** and the development of a dynamic kinetic resolution process through enantioselective MBH cyclization.

The synthesis commenced with a sodium borohydride reduction of [$^{13}C_4$]-**15** (Scheme 2). Silylation of the nascent alcohol with TBSCl (96% yield over 2 steps) followed by partial reduction of the ethyl ester gave aldehyde [$^{13}C_4$]-**16**. Homologation with triethyl phosphonoacetate followed by reduction of the intermediate α,β-unsaturated ester furnished allylic alcohol [$^{13}C_4$]-**17** in 84% yield over 2 steps. Sharpless asymmetric epoxidation of [$^{13}C_4$]-**17** gave epoxide [$^{13}C_4$]-**18**, which was then immediately subjected to iodo-dehydroxylation conditions.[16] Reduction with zinc occurred with concomitant epoxide opening to deliver chiral alcohol [$^{13}C_4$]-**14** in 88% yield and 86% ee over 3 steps. It is worth mentioning that we did attempt to access [$^{13}C_4$]-**14** directly through enantioselective addition of divinyl zinc to aldehyde [$^{13}C_4$]-**16**.[17] However, both the yield and enantioselectivity for this one-step transformation were lower compared to the route outlined in Scheme 2 (see Supporting Information for more details).

Coupling of alcohol [$^{13}C_4$]-**14** with **13** in the presence of DCC gave ester [$^{13}C_4$]-**12** in 97% yield. Deprotonation with LDA followed by addition of TBSCl and Ireland-Claisen rearrangement of the resulting silyl ketene acetal delivered [$^{13}C_4$]-**11** in 95% yield and 81% ee. Acid [$^{13}C_4$]-**11** was transformed into the corresponding acyl azide with $(PhO)_2PON_3$, which then underwent Curtius rearrangement upon heating. Hydrolysis of the intermediate isocyanate with $NaOSiMe_3$ gave a primary amine, which was tosylated to give [$^{13}C_4$]-**19** in 83% yield over 2 steps.[18] Treatment with CSA resulted in pyrrolidine formation and simultaneous removal of the TBS group. In the same pot, addition of TEMPO and $PhI(OAc)_2$ gave ketone [$^{13}C_4$]-**20**, and subsequent treatment with DBU led to migration of the double bond into the α,β-position, delivering [$^{13}C_4$]-**10** in 83% yield over 2 steps. Enone [$^{13}C_4$]-**10** served as a precursor for the penultimate MBH cyclization. After extensive experimentation, it was found that the use of tetrahydrothiophene as the nucleophile in the presence of Me_3SiOTf in MeCN at –30 °C gave [$^{13}C_4$]-**21** in 78% yield and 80% ee, which could be enriched to 98% ee following recrystallization.[19] Heating [$^{13}C_4$]-**21** with ethylene diol and PPTS generated an acetal, tosyl group removal was successfully accomplished through reduction with magnesium, and *in situ* hydrolysis of the acetal gave [$^{13}C_4$]-anatoxin-a in 72% yield as its hydrochloride salt. High resolution mass spectral analysis revealed the final product has 99.1% ^{13}C incorporation at all four positions.

During optimization of the iminium MBH cyclization, we noticed an unexpected relationship between reaction temperature and enantiopurity of the cyclized product. At temperatures above –30 °C, significant racemization was observed. This caught us by surprise, given the apparent lack of an epimerizable carbon in the molecule. Tanner and co-workers observed the same phenomenon in their synthesis of anatoxin-a, in which they used an MBH cyclization on a similar precursor.[11] The authors propose that racemization occurs through a hydride shift of the initially formed cyclic iminium ion. However, this mechanism seems improbable given that [1,3]-hydride shifts are symmetry-forbidden.[20] It is conceivable that this racemization involves a simple trace acid-catalyzed isomerization of the initially-formed C-monosubstituted imine to the more stable C-disubstituted imine (**i** to **ii**, Scheme 3a). However, at present the exact origin of epimerization remains unknown.

We became intrigued by the practical implications of the cyclic imine undergoing facile racemization. At temperatures above –30 °C, the rate of imine isomerization begins to outcompete the rate of cyclization. We hypothesized that this phenomenon could be leveraged to achieve dynamic kinetic resolution through the use of a chiral nucleophile to induce enantioselectivity. Treatment of racemic cyclization precursor (±)-**10** with Me_3SiOTf produces a series of rapidly equilibrating cyclic imines (**i**, **ii**, and **iii**, Scheme 3a). If reaction parameters are adjusted such that equilibration between iminium ions **i** and **iii** occurs rapidly relative to cyclization, the product distribution should reflect the difference in energy between the two diastereomeric transition

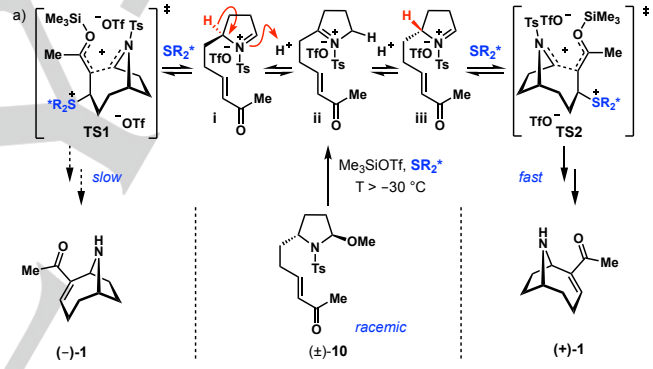


Scheme 2. Total synthesis of [$^{13}C_4$]-anatoxin-a.

states **TS1** and **TS2**, in accordance with the Curtin-Hammett principle.[21] The ability to use racemic (±)-**10** would obviate the need to introduce chirality into alcohol **14**. Racemic (±)-[$^{13}C_4$]-**14** can be accessed directly through addition of vinylmagnesium bromide to (±)-[$^{13}C_4$]-**16** in 82% yield (Scheme 2 inset), reducing the synthesis by 4 steps.

Our success using tetrahydrothiophene as a nucleophile in our original route inspired us to explore a variety of chiral sulfur-containing nucleophiles for enantioselective iminium MBH cyclization. We began our experiments by employing commercially available chiral sulfide **22** (Scheme 3b, entry 1).[22] In this case, product **21** was isolated in high yield, but virtually no enantioselectivity was achieved. We then screened the camphor-derived sulfide **23** developed by Aggarwal and co-workers.[23] We were excited by the potential of this sulfide to induce selectivity given its previous application in enantioselective MBH reactions.[24] Unfortunately, while the cyclized product was isolated in excellent yield, no enantioselectivity was observed (entry 2). Our attention was then turned toward a series of C2-symmetric *trans*-2,5-disubstituted thiolanes. The bis-primary alkyl substituted thiolane **24** gave the product in 68% yield with an unimpressive ee of –6% (entry 3). Diphenyl thiolane **25** gave **21** in 22% ee with a drop in yield to 53%.[25] In this case, the recovered sulfide had partially epimerized to a mixture of *cis*- and *trans*-2,5-diphenylthiolane (dr 14:86; 89% ee for the recovered *trans* isomer), likely due to formation of a benzylic carbocation under the reaction conditions. To prevent epimerization, we sought to install suitable electron withdrawing groups on the phenyl rings.

To this end, sulfide **26** was synthesized and applied in our optimization. The *para*-CF_3 groups did indeed prevent racemization; however, the yield and purity of the product declined drastically compared to **26** (entry 5). The use of dicyclopentyl sulfide **27** gave the product in reasonable yield with an improved ee of 44%. We then tested dicyclohexyl and ditetrahydropyranyl thiolanes **28** and **29**, postulating that increasing the steric bulk of the thiolane substituents could potentially lead to greater selectivity. In both cases, improved enantioselectivity was observed, with the cyclized products being isolated in 40% yield, 62% ee (entry 7) and 29% yield,



entry	solvent	sulfide	temp.	time	yield	ee
1	MeCN	**23**	0 °C	2 h	85%	–4%
2	MeCN	**24**	0 °C	3 h	85%	0%
3	MeCN	**25**	0 °C	2 h	68%	–6%
4[a]	MeCN	**26**	–20 °C	22 h	53%	–21%
5[b]	MeCN-CH_2Cl_2	**27**	–15 °C	24 h	19%	–
6	MeCN-CH_2Cl_2	**28**	0 °C	3 h	59%	44%
7	MeCN-PhMe	**29**	0 °C	3 h	40%	62%
8	MeCN-CH_2Cl_2	**30**	0 °C	3 h	29%	82%
9[c]	MeCN-CH_2Cl_2	**30**	0 °C	2 h	50%	78%
10	MeCN-PhMe	**29**	–40 °C	24 h	42%	70%
11	MeCN-CH_2Cl_2	**30**	–78 °C	4 h	28%	93%

[a]Epimerization of the sulfide was observed. [b]TBSOTf was used instead of Me_3SiOTf. [c]Me_3SiOTf was added at –78 °C, stirred for 1 h, then warmed to 0 °C and stirred 1 h.

Scheme 3. a) Proposed dynamic kinetic resolution process through an enantioselective aza-MBH reaction. b) Optimization of the chiral sulfide used for enantioselective cyclization.

82% ee (entry 8), respectively. For sulfide **29**, adding Me_3SiOTf at –78 °C, stirring for 1 h, and then allowing the mixture to stir at 0 °C for 1 h led to a substantial increase in the yield (50%) with a similar ee (78%, entry 9). Finally, we also explored conditions in which the reaction temperature was kept low enough to prevent imine rearrangement (below –30 °C) in an attempt to achieve simple kinetic resolution. Performing the cyclization at –40 °C using sulfide **28** gave the cyclized product in a slightly improved yield of 42% and 70% ee (entry 10). Using sulfide **29** and performing the reaction at –78 °C, the cyclized product was isolated in 28% yield, this time with a remarkable enantioselectivity of 93% ee. These observations indicate that increased steric encumbrance surrounding sulfur leads to higher selectivity. On the other hand, this comes at the expense of decreased yield, perhaps due to a lower rate of conjugate addition for more hindered tetrahydrothiophenes, resulting in a greater prevalence of side reactions. In entries 1–9 in Scheme 3b, the remaining mass balance appears to be a complex mixture of products caused by decomposition or oligomerization. No spirocyclic cyclization products arising from cyclization of iminium ion **ii** were isolated under any of the surveyed conditions, likely owing to its decreased reactivity.

Our approach to [$^{13}C_4$]-anatoxin-a was framed by a need to develop a route which commenced from a commercially feasible isotopically-labeled starting material and utilized transformations that would retain all heavy isotopes. These constraints inspired the first synthesis of [$^{13}C_4$]-**1**, relying on a Sharpless asymmetric epoxidation as the enantiodetermining step, and an MBH cyclization to forge the final bicyclic scaffold of the anatoxin-a skeleton. The synthesis was completed in 12 steps from [$^{13}C_4$]-**15** and 14% overall yield through the kinetic resolution strategy. As a testament to the scalability of this route, 0.110 g of [$^{13}C_4$]-**1** have been prepared to date. The ability to access substantial quantities of [$^{13}C_4$]-**1** will enable the development of a new analytical method for its precise and highly accurate quantification in samples of fresh water. Finally, an observation made during the penultimate MBH cyclization in our original route prompted an investigation into a unique dynamic kinetic resolution process that leveraged a cyclic iminium ion rearrangement. Highly enantioselective cyclization was achieved starting from racemic precursor (±)-**10** through the use of chiral thiolanes **28** and **29**. Our results suggest that iminium ions derived from 2-alkoxy-5-alkylpyrrolidines undergo facile rearrangement under acidic conditions, and that this process can be taken advantage of to achieve dynamic kinetic resolution. This finding has potential application in future enantioselective syntheses of 2,5-disubstituted pyrrolidines.

Acknowledgements

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Keywords: anatoxin-a • isotopic total synthesis • enantioselective aza-Morita-Baylis-Hillman • chiral sulfide • iminium rearrangement

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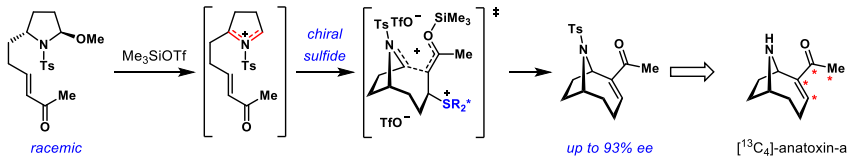

We report an asymmetric total synthesis of the isotopically labelled cyanotoxin [$^{13}C_4$]-anatoxin-a. A critical feature of the synthesis was the development of a unique enantioselective Morita-Baylis-Hillman cyclization that leverages a cyclic iminium ion racemization to achieve dynamic kinetic resolution. The synthesis produced substantial quantities of this internal standard with 99.1% isotope incorporation to enable the development of an analytical method for the precise quantification of anatoxin-a in freshwater.