Utrecht University

A Behemoth from the Deep: The Discovery and Synthesis of Maitotoxin.

A literature review

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III. List of Abbreviations

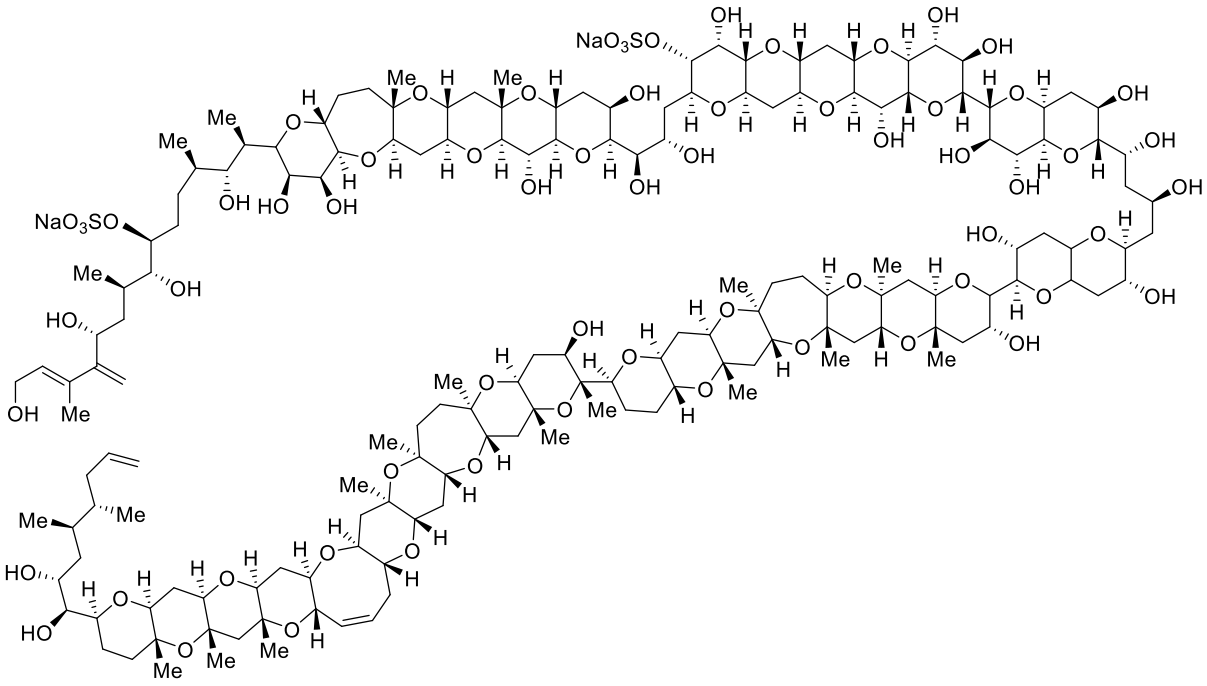
AFO	Algar-Flynn-Oyamada
CSA	Camphorsulfonic acid
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DMAP	4-Dimethylaminopyridine
DMP	Dess-Martin periodinane
HWE	Horner-Wadsworth-Emmons
KHMDS	Potassium bis(trimethylsilyl)amide
LD	Lethal dose
*m*CPBA	*meta*-Chloroperoxybenzoic acid
MS	Mass spectrometry
NBS	*N*-Bromosuccinimide
NMO	*N*-Methylmorpholine *N*-oxide
NMR	Nuclear magnetic resonance
RSA	Retrosynthetic analysis
TBAF	Tetra-*n*-butylammonium fluoride
TBAI	Tetra-*n*-butylammonium iodide
TEA	Triethylamine
TEMPO	2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
TES	Triethyl silyl
TMEDA	Tetramethylethylenediamine
TMS	Trimethyl silyl
TPP	Triphenylphosphine

IV. Abstract

Maitotoxin is a unique molecule that is not only the largest known secondary metabolite that is not a polymer or protein, but is also one of the most potent toxins known to date, with an LD_{50} of 50 ng/kg.

First isolated from *Gambierdiscus toxicus* in 1977 by *Yasumoto et al.*, its structure was determined in 1993 by *Yasumoto et al.,* and its stereochemistry in 1996 by *Kishi et al.* and *Tachibana et al.*. Although in 2006, it was called into doubt when *Gallimore & Spencer (2006)* argued that, based on biosynthetic theories, the stereochemistry was assigned incorrectly. Following this disagreement, *Nicolaou et al.* set out to prove that the originally-proposed structure was correct and undertake the total synthesis of Maitotoxin as a whole.

This paper discusses how *Nicolaou et al.* settled the structural dispute in 2007 and further looks at how the other fragments of Maitotoxin were synthesised during an eight-year-long synthesis project that was unfortunately cut short in the end due to a lack of funding. Finally, a plan for connecting the synthesised fragments is proposed that would allow for the completion of the Maitotoxin ring system.

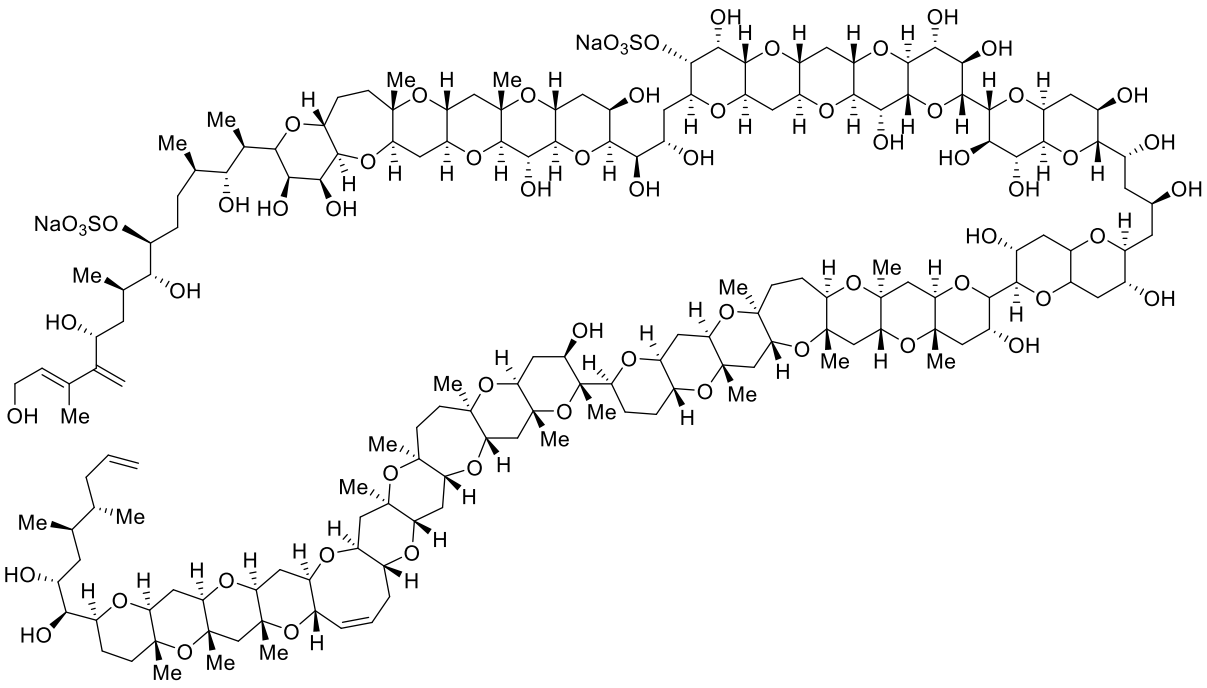


V. Layman Abstract

Maitotoxin is a very large molecule that was isolated from algae that can be found in subtropical waters. It is one of the strongest poisons ever discovered, and between 1993 and 1996, its entire complex structure was solved by two research groups in Japan.

However, in 2006, two American researchers reported doubts about whether the previously determined structure was correct. They based this on the way nature produces molecules with similar structures. The research group of K.C. Nicolaou set out to create large pieces of Maitotoxin in the laboratory to conduct experiments to prove that the original structure was as it was suggested. They did not stop there, though, as they continued to create most of the molecule in the form of four large fragments, which unfortunately were never connected because of a lack of research funding.

In this paper, the ways in which Nicolaou and colleagues synthesised these pieces are discussed, and at the end, a plan is proposed for putting all the pieces together to form the main body of Maitotoxin.



Introduction

A brief history of total synthesis.

When Friedrich Wöhler first, by chance, discovered a method for synthesising urea in 1828 without using processes in living organisms, the field of chemistry changed forever.[1] By disproving the Vital Force Theory, which was created by Jöns Jacob Berzelius in 1809 and stated that organic compounds could only be created by using the "vital force" in living creatures, scientists were now motivated to discover new methods for creating a wide variety of chemicals in a laboratory setting that were previously only found in nature.[2] One noteworthy example from the 19th century is the synthesis of acetic acid by Hermann Kolbe. The synthesis of this seemingly simple molecule from carbon disulfide marks an important moment in the history of synthetic chemistry. While urea is a simple, one-carbon-containing molecule, the creation of acetic acid first proved the viability of generating carbon-carbon bonds.[3] In the same century, the complexity of compounds that could be synthesised was further extended, most notably by Fischer's synthesis of glucose in 1890.[4]

It comes as no surprise that innovation did not slow down in the 20th century, with the complexity of both natural and unnatural molecules being synthesised continuously increasing. To name a few, the syntheses of acetylsalicylic acid (Aspirin, 1899)[5], penicillin V (1959)[6], fluoxetine (Prozac, 1974)[7], paclitaxel (Taxol, 1994)[8] and sildenafil (Viagra, 1989)[9] addressed the medical needs of patients worldwide. Even today, the discovery and total synthesis of natural compounds and their derivatives still occupies many research groups worldwide. *Llanos et al. (2019)* found that, since 1800, the number of new chemical compounds being produced has increased exponentially, even being unaffected by worldwide disasters such as the World Wars in the first half of the 20th century.[10]

Of course, with the increasing complexity of the molecules being synthesised in laboratories, the techniques used in these endeavours have also continuously developed. One of the most fundamental and widely used techniques that synthetic chemists use at the start of any synthesis is retrosynthetic analysis (RSA), described and utilised by E.J. Corey in the 1960s.[11] This method consists of hypothetically breaking a target molecule down into simpler precursors that can be reconnected via known chemical reactions. This process is repeated until the obtained precursors are commercially-available structures. However, while E.J. Corey contributed significantly to the field of RSA, earlier utilisations of this technique can be found in literature. Robert Robinson famously used this disconnection approach in 1917 when establishing a method for synthesising tropinone (**1**). By making symmetric disconnections in the structure of the target molecule, Robinson discovered that tropinone could be synthesised using the resulting three building blocks: succinaldehyde (**2**), methenamine (**3**) and acetone (**4**).[12] This early example of RSA is shown in figure 1.

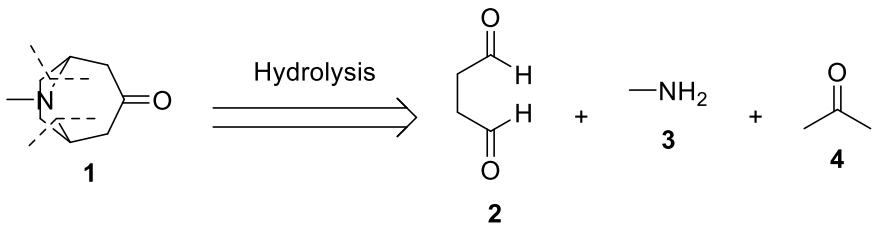


Figure 1. Robinson's use of RSA to establish a new method for synthesising tropinone (**1**) from succinaldehyde (**2**), methenamine (**3**) and acetone (**4**).

This disconnection approach is still widely taught to new chemists and remains an important technique in the synthetic chemist's toolbox.[13] Even today, this method is being further developed. In particular, several software programs are being developed that utilise AI to design retrosynthetic schemes. These computer programs aim to streamline the design of potential synthesis schemes for new target compounds with an optimal estimated yield, even considering the cost of potential starting materials. Some notable examples are Spaya, AiZynthFinder and Reaxys Predictive Retrosynthesis.[14–16]

Total synthesis – a technical process or a form of art?

> "***A composer combines notes to make a symphony***. **In a similar way, a synthetic chemist combines chemical maneuvers to make a molecule."**
> *– K.C. Nicolaou*

There is no denying that the field of total synthesis has furthered humanity in ways that were likely unimaginable in the 19th century prior to the disproval of the Vital Force Theory. While to an outsider, designing and synthesising novel compounds may appear as a complex, technical process, many chemists will consider this a form of art. K.C. Nicolaou, the head of the KCN group, strongly supports this viewpoint. Nicolaou has published over 1300 papers in the field of total synthesis, with some of his group's most noticeable achievements being the synthesis of the anticancer drug Taxol (**5**), Rapamycin (**6**), which prevents organ transplant rejections, and several antibiotics such as vancomycin (**7**).[17–19] These structures are shown in figure 2.

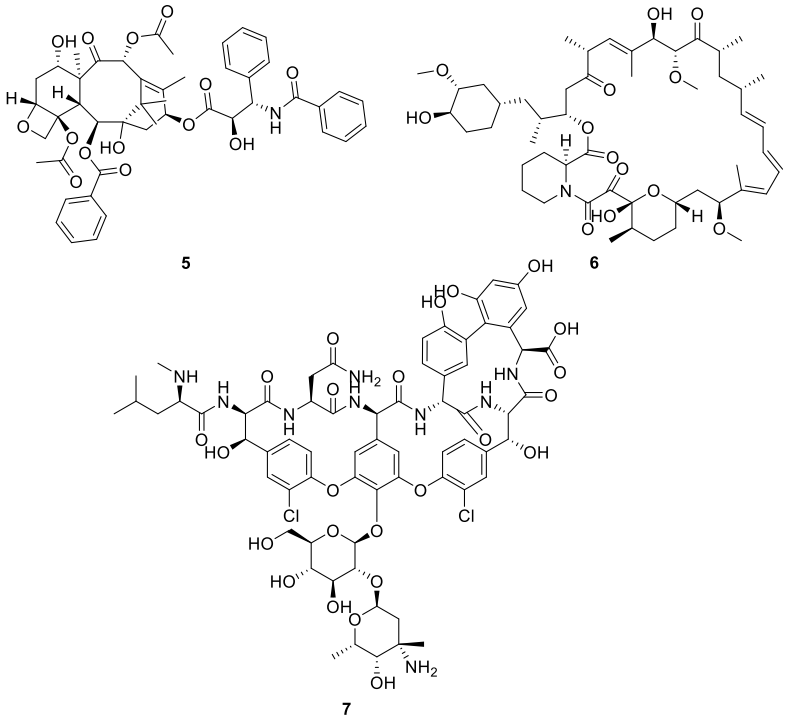


Figure 2. Three of the complex structures synthesised by *Nicolaou et al.*, including Taxol (**5**), Rapamycin (**6**) and Vancomycin (**7**).

Nicolaou is particularly interested in synthesising complex molecules found in nature to test the current state of the art of synthetic chemistry, and by pushing the limits, motivating chemists to develop novel methodologies to synthesise even more complex compounds. Like other research groups, he considers fungi and marine organisms to be unique treasure troves for discovering novel molecules of biological importance. [20–24] The KCN group has published several works on marine neurotoxins, including brevetoxins A (**8**) and B (**9**) and, most notably, several fragments of Maitotoxin (**10**), the structures of which can be seen in figure 3.[25–27]

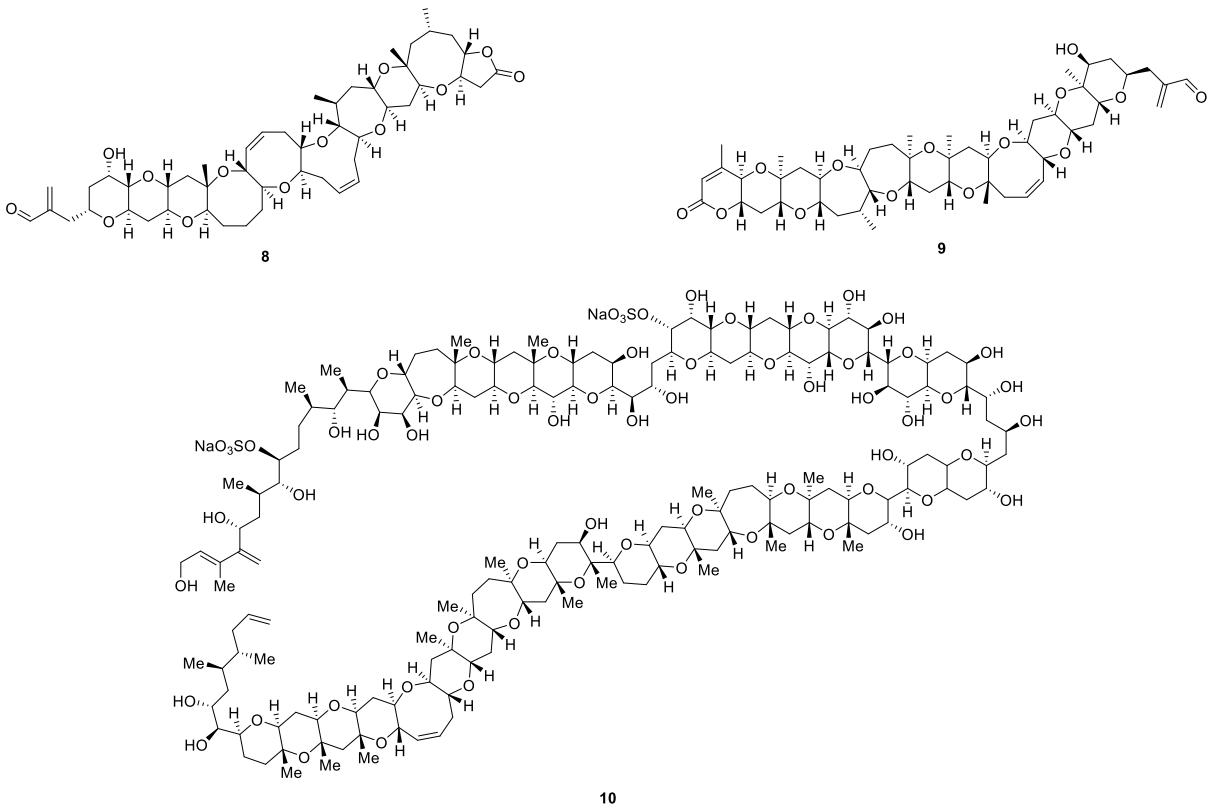


Figure 3. The structures of marine neurotoxins Brevetoxin A (**8**), Brevetoxin B (**9**) and Maixotoxin (**10**).

Maitotoxin: a behemoth from the depths of tropical waters.

As previously mentioned, the complexity of molecules synthesised by chemists in a laboratory has increased continuously since the 19th century, and Maitotoxin is an excellent example of this. This marine neurotoxin, first identified in surgeon fish by *Yasumoto et al. (1976)*, was first estimated to have a lethal dose of 15 to 20 mg/kg in mice, though this was disproved in later studies.[28] A later study by *Takahashi et al. (1982)* found an LD_{50} value of about 50 ng/kg in mice, making it, by far, the most potent marine toxin discovered to date, meaning that its toxicity is 50 times higher than that of tetrodotoxin.[29]

The work of three different research groups in Japan led to the determination of the full structure by 1996 using a combination of NMR, MS and different chemical methods.[30–32] It was determined that Maitotoxin comprises a total of 32 ether rings of varying sizes, 98 stereocenters and a variety of substituents, along with different chains at the beginning and end of the ring system. Its structure was annotated with a series of 32 ring fragments, as seen in Figure 4.

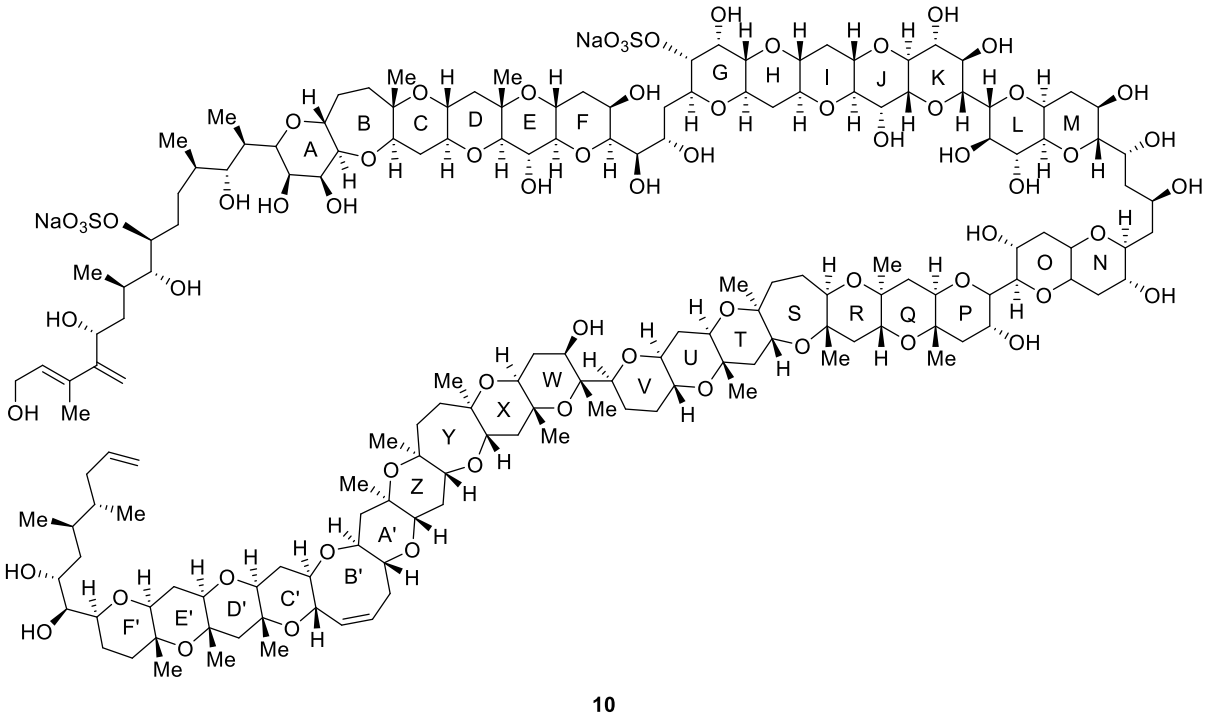


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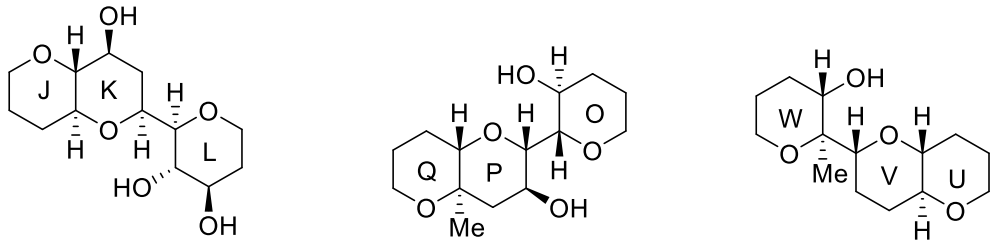
Figure 4. The structure of Maitotoxin (**10**) divided into 32 fragments.

Following the determination of Maitotoxin's structure, the KCN group was compelled to synthesise several of its fragments using methods they had previously discovered to be useful while synthesising similar, though smaller, structures, such as brevetoxin A and B.[25] In 1996, *Nicolaou et al.* synthesised fragments JKL, OPQ and UVW, as shown in figure 5.[33]



11 **12** **13**

Figure 5. Fragments JKL (**11**), OPQ (**12**) and UVW (**13**), as synthesised by *Nicolaou et al. (1996)*.

Alas, despite this initial success and claims in the paper that the methods used to synthesise these fragments may prove useful in assembling Maitotoxin in its entirety, it appeared as though the group had initially lost interest in the structure, publishing no further research on the synthesis of its fragments until a decade later.

Maitotoxin's (hypothesised) unique mode of action.

Even today, the reason for Maitotoxin's unique toxicity has not fully been determined. However, several studies have found that Maitotoxin expresses its effects in multiple ways via interaction with extracellular calcium channels, including, but not limited to, causing involuntary smooth- and skeletal muscle contractions and increased Ca^{2+}

concentrations in the cytosol, leading to cell death.[34–37] When comparing the values in Table 1, it becomes clear that fragments of Maitotoxin do not share the same extremely potent toxicity of the complete molecule, with some fragments not showing any toxicity towards its test subject at all (i.e. fragment ABCDEFG).

Table 1. IC_{50} comparison between Maitotoxin, several of its fragments and similar marine neurotoxins. It should be noted that the precise structure of the fragments may vary slightly from that of natural Maitotoxin due to the synthesis strategies employed by the researchers. Toxicity is given as IC_{50} or LD_{50}, depending on the available data. When a range is given, the toxicity depends on the substituents.

Compound	Toxicity	Test subject
Maitotoxin fragment ABCDEFG	Inactive[38]	Rat C6 glioma cells
Maitotoxin fragment ABCDE	IC_{50} > 30 µm[38]	
Maitotoxin fragment C'D'E'F'	IC_{50} = 2.3 µm to > 30 µm)[39,40]	
Maitotoxin fragment WXZYA'	IC_{50} > 30 µm[41]	
Maitotoxin	LD_{50} = 50 ng/kg[42]	Mice, intraperitoneal injection
Ciguatoxin	LD_{50} = 0.25 µg/kg[43]	Mice, intravenous injection
Brevetoxin A	LD_{50} = 180 µg/kg[44]	Mice, intravenous injection

These observations would be in line with the hypothesis of *Murata et al. (2008)*, who stated that the lower, more lipophilic part of Maitotoxin embeds itself in the membrane of neuron cells, while the upper half, which is more hydrophilic, remains outside the membrane, effectively providing a channel for Ca^{2+} ions to enter the neuron cells in an uncontrolled manner.[45]

The structural dispute that reignited the flame.

While the KCN group had, at the time, abandoned working on Maitotoxin, other researchers certainly did not. In particular, a study published by *Gallimore & Spencer (2006)* suggested that the structural assignment reported back in 1996 contained a flaw regarding the assignment of the stereochemistry at one part of the molecule.[46] This claim was based on their research into the biosynthesis of Maitotoxin, and they hypothesised that the stereochemistry at the site where the J and K fragments are connected should be in a *syn* relationship, which is not the case in the structure proposed back in 1996. In 2007, *Nicolaou & Frederick* published an article that revisits the original methods that were used to determine the structure of Maitotoxin (i.e. 2D- and 3D-NMR methods), arguing that the original structure is still most likely the correct one. However, due to a lack of the X-ray crystal structure of Maitotoxin, they suggested that the best approach to settle this dispute would be via chemical synthesis of the relevant fragments.[47]

This marked the start of a novel project in the KCN group that would continue for about seven years.

The synthesis of Maitotoxin: a chemical challenge.

> "**Adversity causes some men to break; others to break records.**"
> *– William Arthur Ward*

In total, 20 different chemists of varying experience levels would work on synthesising the fragments of Maitotoxin. Funding was requested from the National Institution of Health (NIH) via their marine neurotoxin grant. Nicolaou argued that the unique biological mode of action may lead to novel drugs to treat neurodegenerative diseases such as Alzheimer's and that the journey towards synthesising this large, complex molecule may lead to novel synthetic strategies for creating polycyclic ether systems. Further than that, proving that the synthesis of Maitotoxin was, indeed, possible via total-synthetic strategies was to serve as a source of inspiration for scientists in a multitude of fields in chemistry and biology.[25]

Even though funding was cut in 2012, leading to the project's abandonment in 2014, the work of the KCN group has led to the development of some unique approaches that required creative problem-solving and applications of retrosynthetic analysis. This literature review aims to provide an overview of some of these unique strategies applied in this project, not only to learn from what has been done, but also to take a closer look at this inspirational effort.

Exploring the vast synthesis of Maitotoxin.

The discussion of the individual fragments shall proceed in the order in which they were synthesised. Where possible, the yields obtained and methods applied shall be compared to the results of other scientists. The amount of research groups working on the synthesis of Maitotoxin is limited.

While the synthesis of every fragment would provide sufficient material for a literature review, this review paper aims to focus in particular on the synthesis of the GHIJK fragment to settle the dispute on the stereochemistry of the J/K domain (as done by *Nicolaou et al.*) and to highlight some key reactions and/or discoveries, whilst providing a more brief overview of the synthesis of the other fragments. As such, not every individual reaction shall be discussed, as this would far exceed the scope of this writing assignment.

Settling the dispute – The synthesis of the GHIJK ring system.

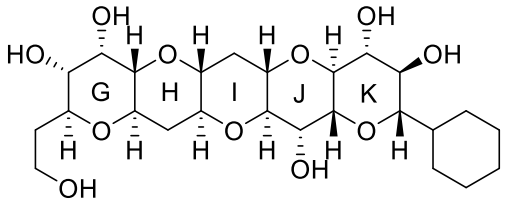


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Figure 6. Structure of the GHIJK fragment (**14**) of Maitotoxin (**10**).

As mentioned before, the GHIJK fragment was synthesised with the motivation of wanting to confirm the stereochemistry at the J/K intersection. *Nicolaou et al. (2007)* introduce some strategies and reactions used in forming many of the fragments throughout their previous synthetic endeavours.[48]

The synthesis of the first fragment (G) occurred in 17 steps, and immediately highlighted some of the systematic strategies used in the syntheses of other fragments as well. As previously discussed, Maitotoxin has 98 stereocenters, so an enantioselective synthesis strategy was crucial.

The reaction scheme of the synthesis of the G fragment is shown in Figure 7.

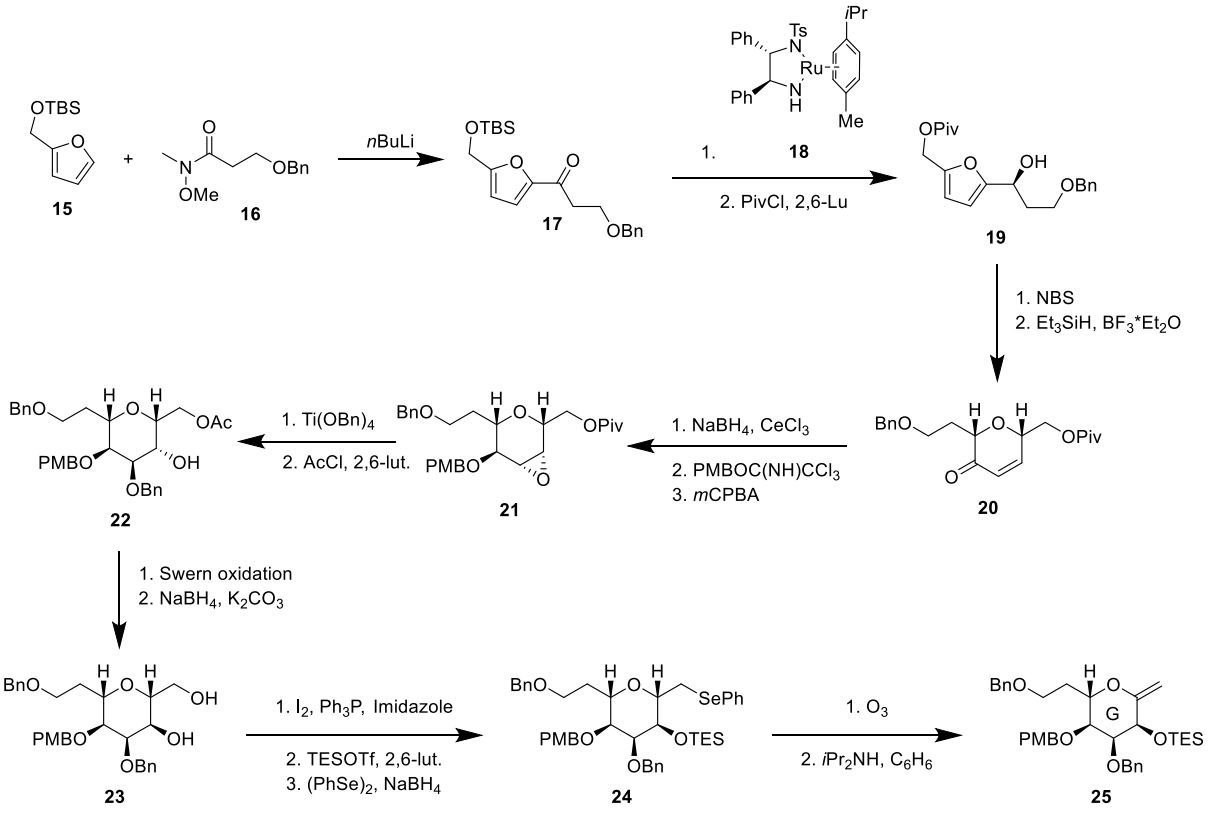


Figure 7. The synthesis scheme of fragment G (**25**).[48]

Nicolaou et al. achieved this by using a prochiral furan derivative (15) and applying stereoselective reactions such as the Noyori reduction.[49] Using this reaction, obtaining the correct stereochemistry in the molecule was possible via the chiral Ru(II) catalyst (**18**). Formally called "Noyori Asymmetric Hydrogenation", it is commonly used to hydrogenate carbonyl groups and imines stereoselectively.

Figure 8 shows how a carbonyl structure is stereoselectively reduced. During the synthesis of fragment G, formic acid was used to activate the Ru(II)-catalyst by acting as a hydrogen donor, which then bound itself to the carbonyl functionality. Finally, the catalyst is regenerated via a base, here triethylamine (TEA).

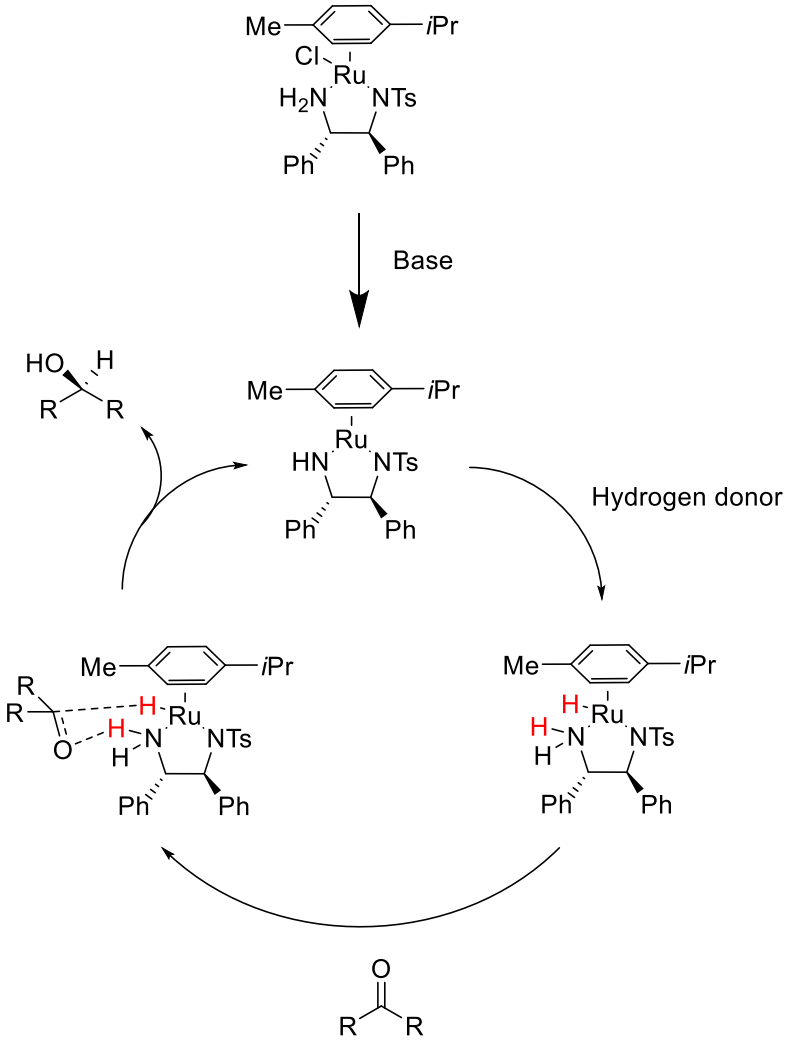


Figure 8. Mechanism of a Noyori Asymmetric Hydrogenation.[49,50]

Another prominent reaction frequently recurring throughout the synthesis of various fragments is the Achmatowicz reaction.[51] This reaction is generally used to convert furans into dihydropyrans, and in this case, it was used to form an unstable hemiacetal, which was immediately reduced to create **20**.

Figure 9 shows how the Achmatowicz reaction was used to expand the 5-membered ring to the desired 6-membered cyclic ether. After generation of the hemiacetal, the

ring was briefly opened during a transition state, after which quenching led to the formation of the desired pyran ring (**20**).[52] It should be noted that, while Br_2 is shown in Figure 9 to form the bromonium transition state, *Nicolaou et al.* use *N*-Bromosuccinimide (NBS), likely due to its lower toxicity.[53]

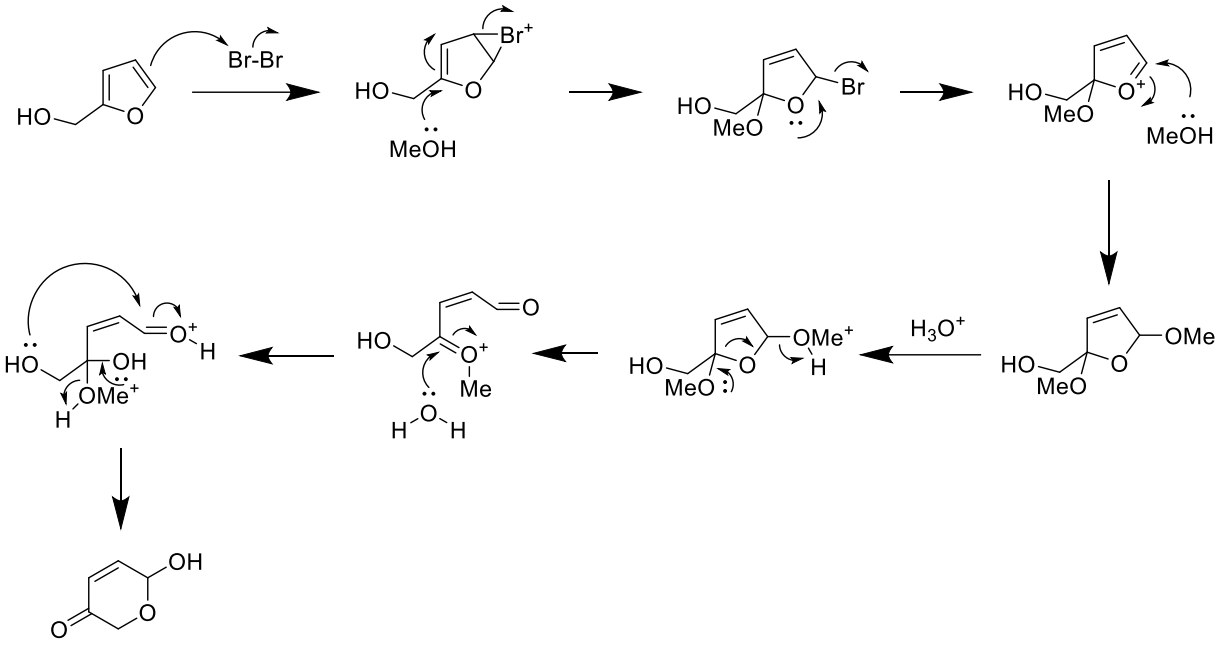


Figure 9. Mechanism of the Achmatowicz rearrangement.[54]

Following the Achamtowicz rearrangement, **20** underwent several further transformations to obtain the desired G precursor. To obtain the correct stereoisomer, the enone in **20** was reduced using a Luche reduction, and the resulting hydroxyl group was protected with a PMB ether.[55] Then, the remaining alkene functionality was converted into an epoxide with *m*CPBA.

The use of an epoxide to install hydroxyl groups and their protected derivatives stereoselectively is a strategy that was used by *Nicolaou et al.* throughout this eight-year synthesis project, and its usefulness is evident by the fact that the biosynthesis of this molecule (and other similar marine toxins), too, appears to utilise this three-membered ring. As such, the epoxide ring was opened at high temperatures in the presence of $Ti(OBn)_4$, generating a vicinal *trans*-diol, one of which was benzylated.[56] Finally, the pivaloyl protection group was cleaved and the resulting primary hydroxyl group was selectively acetylated to form **22**.

The hydroxyl group's stereochemistry formed upon the epoxide's opening proved to be the wrong one. So, it was inverted via oxidation of the alcohol under Swern conditions, followed by subsequent reduction whilst cleaving the previously installed acetyl group to form **23**.

After ensuring the correct stereochemistry throughout this fragment G precursor, the primary alcohol was iodinated with iodine, imidazole and triphenylphosphine (TPP), and the secondary alcohol was protected with a triethyl silyl (TES) ether. The iodine

was then converted into a selenide compound using diphenyl diselenide to form **24**, which then reacted with ozone and diisopropylamine to generate a terminal alkene, which led to the desired G precursor **25** with a total yield of 15.2% across 17 steps.

It should be noted that fragment G was also synthesised via an alternative route, starting from a glucose derivative. This method does not appear to have any unique benefits over the method previously described in this review, and due to the lack of description of the methods used to synthesise fragment G in its entirety from the carbohydrate precursor methyl-α-D-glucopyranoside, it shall not be discussed further at this point. However, the attempted usage of carbohydrate precursors will be a recurring theme throughout the synthesis of Maitotoxin. This could be attributed to Nicolaou's fascination with carbohydrate chemistry, or simply because using carbohydrate precursors appears to be common practice in synthesising marine toxins.[57,58]

"Among the most exciting aspects of organic chemistry in the last few decades has been the interplay between the specialized subdisciplines of carbohydrate chemistry and total synthesis, each enabling and advancing the other in new directions and towards greater heights."

– K.C. Nicolaou

The synthesis of the J fragment commenced similar to that of the G fragment. An overview of the complete reaction scheme is presented in Figure 10. The synthesis started with synthesising a prochiral furan derivative (**28**) from regular furan (**26**). Again, chirality was introduced into the molecule via the aforementioned Noyori reduction reaction, followed by another Achmatowicz reaction to form **30**.

Similar to the synthesis of the G fragment, the resulting enone was selectively reduced under Luche conditions, though this time, the resulting hydroxy group was protected with a pivaloyl group to form **31**. The remaining double bond was dihydroxylated using osmium tetroxide and *N*-Methylmorpholine *N*-oxide (NMO).[59]

At this point, *Nicolaou et al.* wanted to introduce two different protection groups on the resulting vicinal diol. Despite their clear similarity, they succeeded in selectively monobenzylating one of the hydroxy groups based on it being equatorial using dibutyltin oxide, benzyl bromide and catalytic TBAI via the intermediate tin-ketal. The axial hydroxy group, on the other hand, was simply acetylated to yield **32**.

To later connect the J fragment with other rings, *Nicolaou et al.* wanted to utilise an aldehyde, which was implemented by first replacing the C1 pivaloyl group with an allyl moiety (**33**). In three consequent steps, the terminal alkene was shifted towards the

ring (**34**) and was ultimately converted to the desired aldehyde with ozone and TPP (**35**).

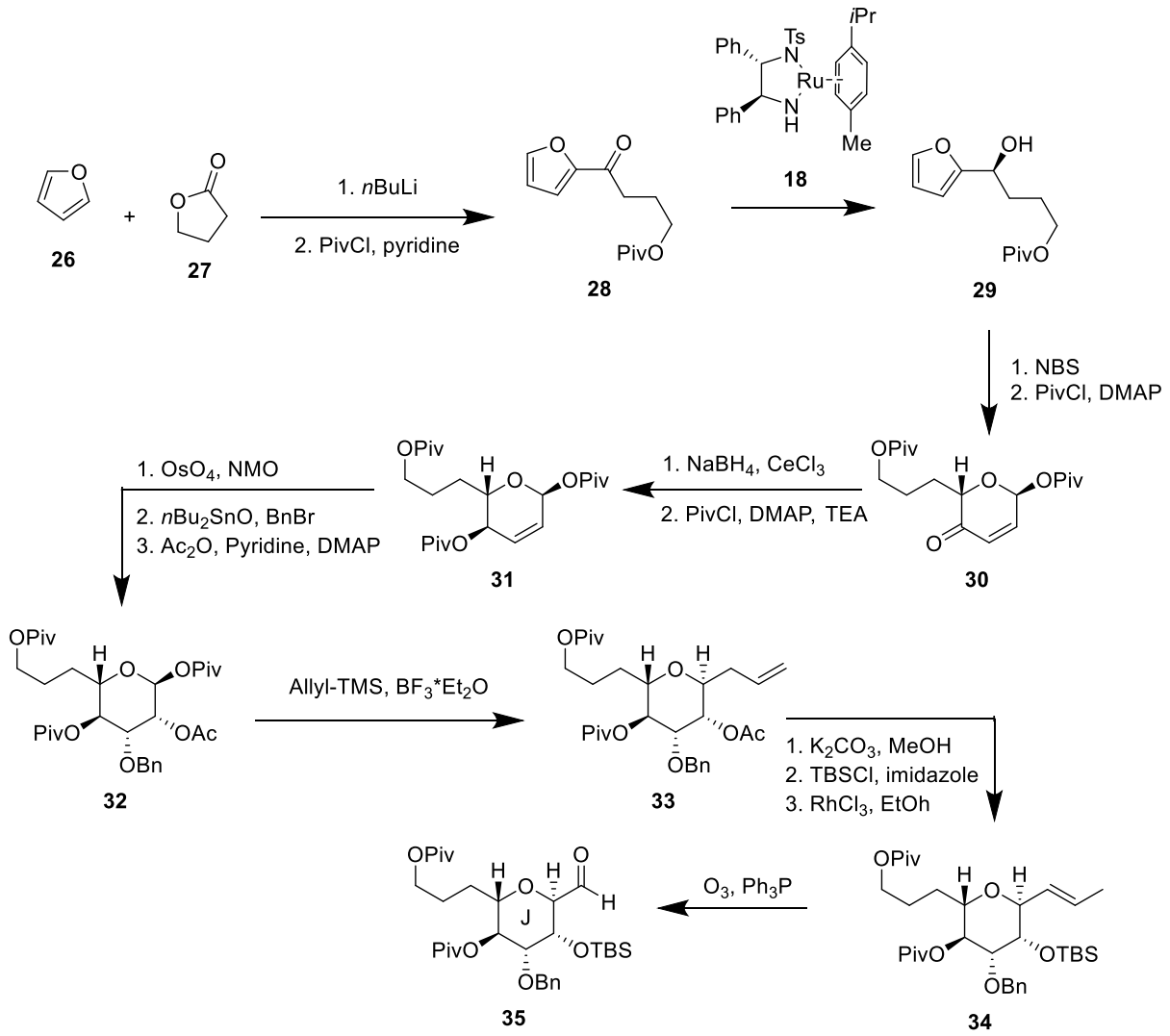


Figure 10. The synthesis scheme of fragment J (**35**).[48]

As previously mentioned, a key reaction in the synthesis of the J fragment (and in future fragments) was the introduction of an aldehyde, as this electrophilic group was used in a following step to extend the ring system to create, in this case, the K fragment. This introduction was achieved via the ozonolysis of the double bond using ozone and triphenylphosphine as an *in-situ* reductant to push the reaction towards completion, obtaining a respectable yield of 96% in this final step.

Ultimately, the J fragment was synthesised with a total yield of 12.8% across 15 steps.

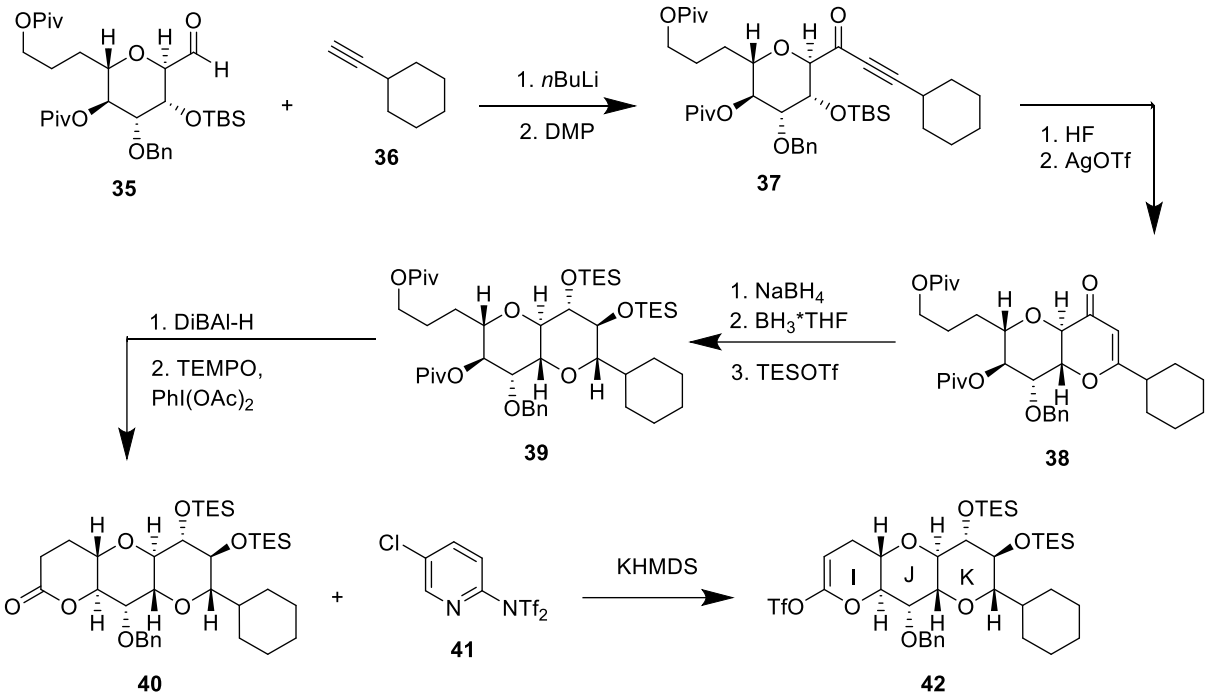


Figure 11. The synthesis scheme of the IJK ring system.[48]

From structure **35**, the ring system was expanded from both sides of the J fragment. As seen in Figure 11, the formation of the adjacent K fragment commenced with an organolithium reagent that was formed through the combination of *n*BuLi and cyclohexylacetylene (**36**). A nucleophilic attack of the resulting reagent on the previously formed aldehyde of the J fragment led to the consequent reduction of the alcohol, which was immediately re-oxidised using a Dess-Martin reaction.[60] This reaction is unique in the sense that, contrary to many other oxidation methods, it can be done at room temperature, is water-compatible, does not require any toxic oxidation reagents and is easy to work up. One common drawback of the reaction may be the higher price tag of the required oxidant compared to more classic oxidants.[61]

The mechanism of this oxidation is presented in Figure 12. It commences when the to-be-oxidised alcohol group replaces one of the acetate groups attached to the (λ5) hypervalent iodine of the Dess-Martin periodinane (DMP). The acetate ion then deprotonates the resulting compound, breaking the bond to the DMP, which completes the oxidation reaction.[62]

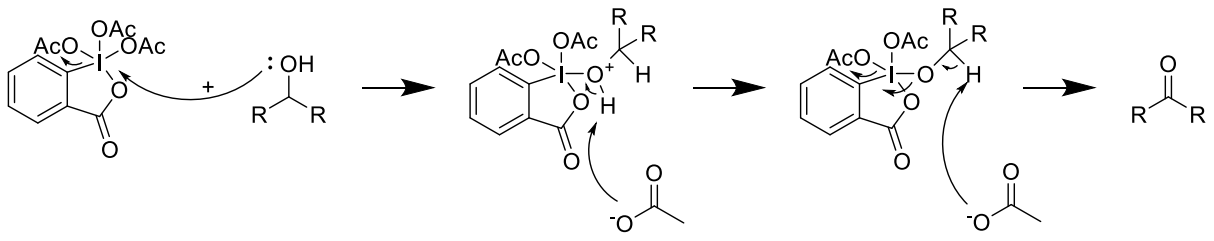


Figure 12. Mechanism of the Dess-Martin oxidation.[60,63]

The subsequent removal of the *tert*-Butyldimethylsilyl protection group enabled a ring-closing reaction between the resulting alcohol group and the alkyne moiety. Though not described in-depth in the paper, some complications were encountered with finding the right conditions for this ring-closing reaction. Some other methods for facilitating a reaction between a hydroxyl group and an alkyne are the use of triphenylphosphine at high temperatures[64], potassium carbonate in water at room temperature[65] and nitrogen bases such as 4-methyl-morpholine and TEA.[66,67] Why these methods were not chosen/did not work is not described in this work.[48]

Ultimately, the group found that silver triflate (AgOTf) could be used to catalyse the reaction between these two moieties. Whilst an explanation for choosing this Lewis-Acid to facilitate the cyclisation was not further commented upon, it is a rather interesting choice, considering silver triflate was not primarily used for this purpose. Even six years after publishing this work, researchers were surprised by its ability to catalyse cyclisation reactions.[68–70]

Liu et al. (2013) proposed an explanation for this reactivity. According to their hypothesis, silver triflate activates the alkyne triple bond, which allows the hydroxy group to perform a nucleophilic attack, leading to the desired cyclic ether.[69]

Finally, the carbonyl group was reduced stereoselectively via a Luche reduction to obtain the hydroxy group with the correct stereochemistry. Two following steps then led to fragment J/K (**39**).

From the resulting structure (**39**), three additional steps led to the formation of the IJK fragment (**42**), as seen in Figure 11. In the final step of the aforementioned three, a vinyl triflate moiety was implemented in the fragment to enable the later connection of other fragments to the ring system. This was done via Comins vinyl triflate synthesis, which converts ketones and (thio)esters into vinyl triflates.[71] Initially, a strong base (here KHMDS) deprotonates the α-position of the carbonyl functionality. Then, the resulting enolate reacts with *N*-(5-chloro-2-pyridyl)triflimide (also known as Comin's reagent) to form the desired vinyl triflate. The mechanism is shown in Figure 13.

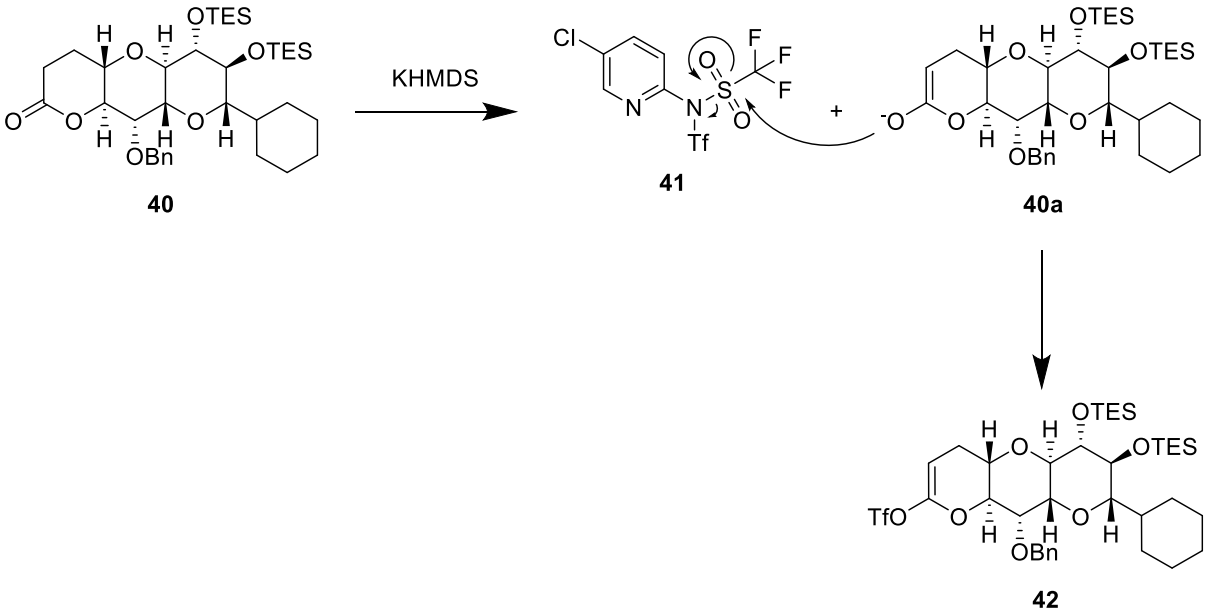


Figure 13. Mechanism of Comin's vinyl triflate synthesis.[72,73]

In the previously described steps, additional fragments were introduced via the extension of the ring system. The next step, joining the G fragment with the IJK fragment (which also leads to the formation of the H fragment), was the first instance in which two separate fragments are connected. This is not only important for understanding how fragments may be joined in consequent syntheses, but also for the hypothesis presented in this research paper on how Maitotoxin may have ultimately been completed, had funding not been cut (as described in a later chapter).

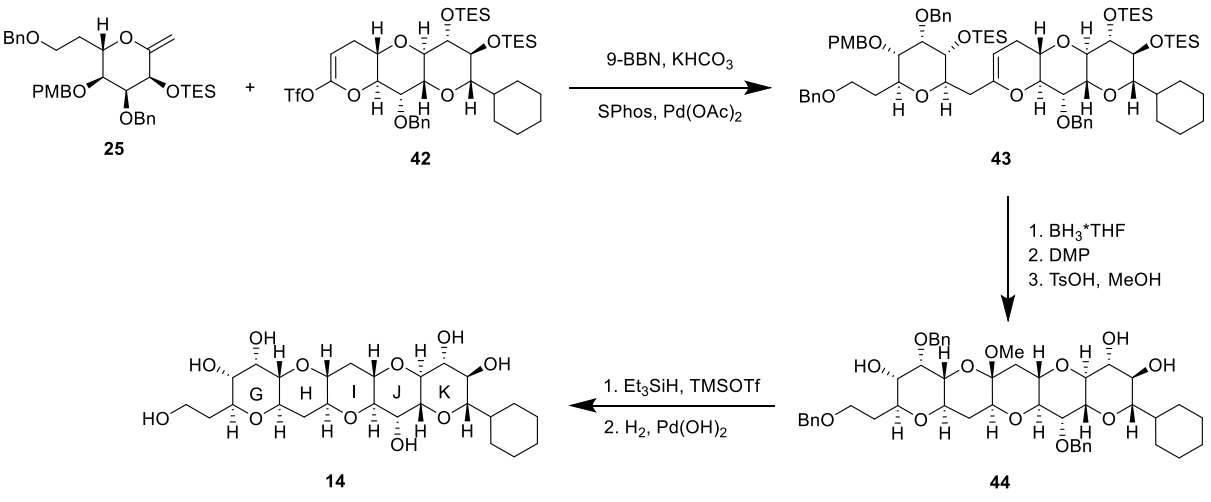


Figure 14. The synthesis scheme of the GHIJK fragment (**14**).[48]

An overview of the reaction scheme is shown in Figure 14. The previously synthesised fragment G (**25**) first underwent hydroboration at the alkene functionality. The resulting structure then underwent a Suzuki coupling reaction to facilitate the connection between the G fragment and IJK fragment via reaction with the previously added vinyl

triflate moiety of the IJK fragment. This initial connection also laid the groundwork for the formation of the H fragment.

As the Suzuki coupling will be one of the recurring reactions in this large synthesis project, it is worth elaborating on it. This cross-coupling reaction (also known as the Suzuki-Miyaura Cross-Coupling) utilises a palladium(0)-catalyst (here, a catalytic system of $Pd(OAc)_2$ and SPhos is used) to facilitate the formation of C-C bonds between, in this case, the vinyl triflate functionality and the alkylborane, formed via the reaction between **25** and 9-BBN, which is not only highly regioselective towards alkenes, but also does not lead to the di-substituted by-product when simply using borane due to steric hindrance.[74]

The H ring was formed via additional hydroboration at the last remaining double-bond to form a hydroxy-group, which was consequently oxidised to the corresponding ketone under Dess-Martin conditions. In the final step, the resulting intermediate was heated in methanol in the presence of TsOH for two days. This led to the removal of all PMB and TES protecting groups in the molecule and facilitated the ring closure under the formation of a methyl acetal (**44**). Finally, to finish the desired GHIJK fragment, the methyl acetal was removed using triethylsilane and TMSOTf, and the remaining benzyl protection groups were cleaved via hydrogenation with a Pd/C catalyst, which led to the desired GHIJK ring system. These last steps could be achieved with an overall yield of 30.7% across 6 steps.

Comparing the data.

To prove (or disprove) the initially hypothesised structure of Maitotoxin, *Nicolaou et al.* intended to gather NMR data of the synthesised GHIJK fragment and compare it to that of the data collected from natural Maitotoxin.

Table 2 presents a comparison of the ^{13}C-NMR values of 1) the original paper that hypothesised the Maitotoxin structure in 1995, and 2) the experimentally determined values of the fragment that was synthesised as described above.[30,48] Additionally, Figure 15 shows the numbering of the carbon atoms. presents a comparison of the ^{13}C-NMR values of 1) the original paper that hypothesised the Maitotoxin structure in 1995, and 2) the experimentally determined values of the fragment that was synthesised as described above.[30,48] Additionally, Figure 15 shows the numbering of the carbon atoms.

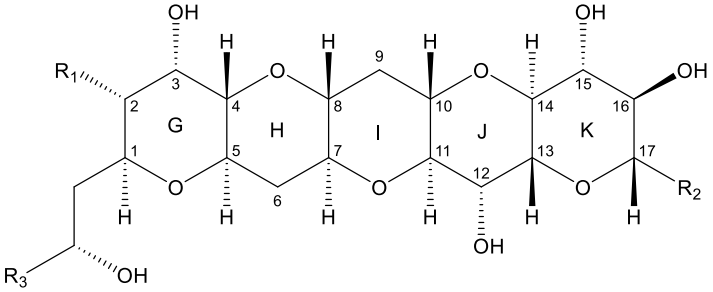


Figure 15. GHIJK ring system with numbered C-atoms. R_1 = -OH (*Nicolaou et al.)* or $-OSO_3Na$ (*Satake et al.*), R_2 = $-C_6H_{11}$ (*Nicolaou et al.)* or fragment L (*Satake et al.*), R_3 = -H (*Nicolaou et al.*) or fragment F (*Satake et al.*).

Table 2. ^{13}C-NMR value comparison. 1) Experimental values found by *Satake et al. (1995)*, 2) Experimental values found by *Nicolaou et al. (2007)*. Solvent: C_5D_5N/CD_3OD (1:1), Experiment: 3D NOESY-HMQC (*Nicolaou et al.*: 150 MHz, *Satake et al.*: 600 MHz)

Carbon number	δ [ppm] (*Nicolaou et al., 2007)*	δ [ppm] (*Satake et al., 1995*)	Δδ [ppm]
1	74.5	72.3	2.2
2	73.2	78.9	-5.7
3	70.3	68.5	1.8
4	81.2	80.6	0.6
5	69.8	69.6	0.2
6	36.3	36.3	0
7	77.8	77.7	0.1
8	77.4	77.5	-0.1
9	37.7	37.4	0.3
10	67.7	67.8	-0.1
11	85.8	85.8	0
12	70.1	70.1	0
13	74.9	74.9	0
14	72.1	72.1	0
15	79.3	79.4	-0.1
16	71.3	69.8	1.5
17	85.0	78.2	6.8
	Average Δδ [ppm]		1.15

Based on experimental findings,

Table 2 shows that the recorded ^{13}C-NMR data of the synthesised fragment by *Nicolaou et al.* closely mimics that of the data recorded by *Satake et al.* of natural Maitotoxin, with an average difference of merely 1.15 ppm. In particular, there appears to be no divergence in the area disputed by *Gallimore & Spencer* (C13 and C14).

Two clear outliers in the data are C2 and C17, which has a difference of 5.7 ppm and 6.8 ppm, respectively. However, this can easily be justified and explained by the fact that the ring system synthesised by Nicolaou et al. has simplified substituents in certain regions (see Figure 15). In natural Maitotoxin, C2 has a sodium sulfate group (R_1); at C17, there is a dihydroxypyran (fragment L).

With the dispute seemingly settled and using the methods from this initial synthesis project within 38 steps, *Nicolaou et al.* went on to synthesise further fragments of Maitotoxin. While no direct comments on the results of this initial work could be found from *Gallimore & Spencer*, A.R. Gallimore wrote in a review that, while the

experimental results from *Nicolaou et al.* appear to point towards the initial hypothesised structure being correct, he remains sceptical until the recording of the crystal structure.[75] At the date of writing this paper, no such data appears to have been published.

Extending the ring system: All roads lead to furan.

While using furan precursors proved to be an effective method when synthesising Maitotoxin fragments, using carbohydrate precursors remains a common practice when synthesising marine neurotoxins. It is perhaps for this reason that *Nicolaou et al.*, in their attempt to further extend the initially synthesised GHIJK fragment, explored two different synthesis routes for the LMNO domain: a carbohydrate approach, using methyl-α-D-glucopyranose as a starting point, and a furan approach, similar to the one used in the synthesis of the GHIJK fragment.

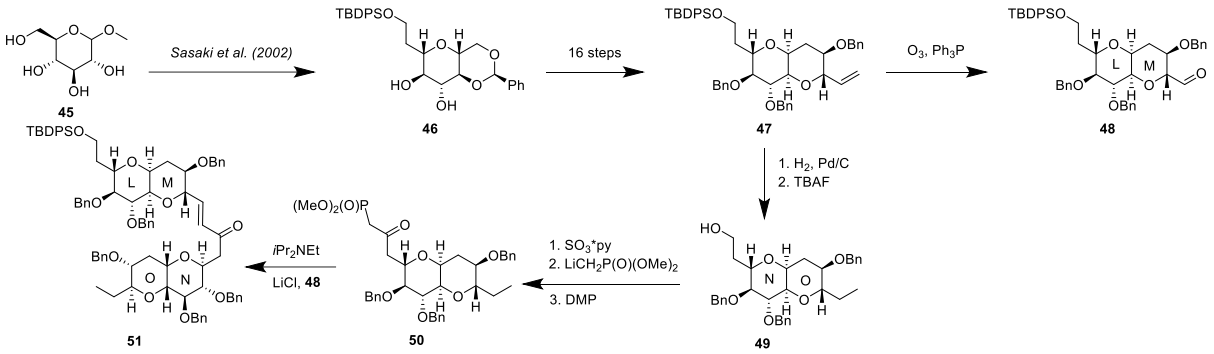
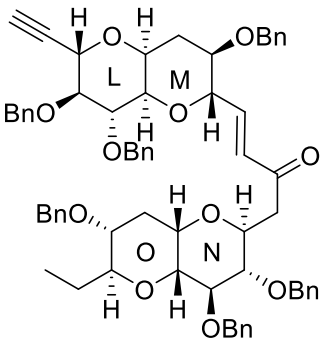


Figure 16. The synthesis scheme of LMNO precursor **51** (carbohydrate route).

Initially, the carbohydrate approach was explored to determine whether it may be superior to the furan method for synthesising the LMNO fragment (as seen in Figure 16). Starting from the aforementioned methyl-α-D-glucopyranose (**45**), the initial precursor for the L and N fragment (**46**) was synthesised following a protocol by *Sasaki et al. (2002)*.[76] Then, **47** was synthesised in 16 steps, which acted both as a precursor for the LM and NO domain. From here, **47** underwent ozonolysis to form LM fragment **48**, and underwent four additional steps to create the NO ketophosphonate **50**. Finally, **50** and **48** were coupled via a Horner-Wadsworth-Emmons (HWE) olefination to generate LMNO precursor **51**.

The desired precursor **52** for the ultimate GHIJKLMNO fragment (as shown in Figure 17) required the implementation of an alkyne to facilitate a reaction similar to the one shown in Figure 11. It was deemed that this would be more complex than if the furan route were to be followed. Ultimately, this route was abandoned by the authors.

The furan route to synthesise the LMNO fragment followed a similar reaction pathway to that of the synthesis of the GHIJK fragment. Again, a prochiral furan derivative was formed, and the Noyori reaction was used to implement the desired chirality into the reaction, followed by an Achmatowicz rearrangement to generate the initial cyclic ether.

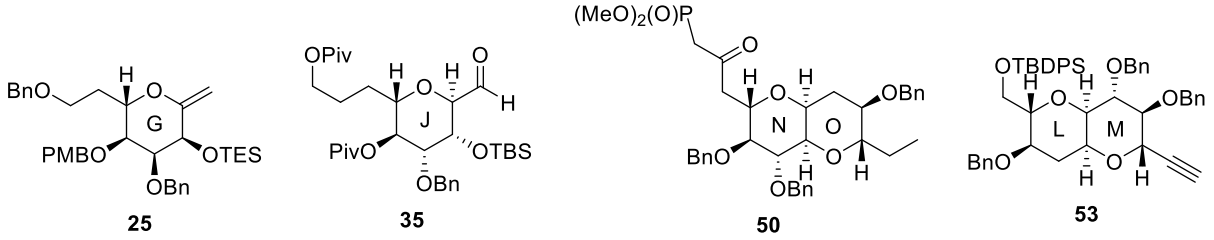


52

Figure 17. The LMNO fragment targeted by *Nicolaou et al. (2008)*.

Ultimately, fragment LM was synthesised within 29 steps with a yield of 1.94%, while the NO fragment was synthesised across 30 steps with a yield of 1.90%.

At this point, *Nicolaou et al.* attempted to synthesise the GHIJKLMNO fragment from four building blocks, as shown in Figure 18. The authors noted that the order in which the building blocks are connected (and the other rings are synthesised) is important, as the rigidity of the ring system may cause complications.



25 **35** **50** **53**

Figure 18. Four building blocks of the GHIJKLMNO fragment: fragment G (**25**), fragment J (**35**), fragment NO (**50**) and fragment LM (**53**).

First, **53** and **35** were connected by generating an organolithium compound from **53** with *n*BuLi, which then attacked the aldehyde of **35**. Consequent oxidation of the resulting hydroxy group led to intermediate **54**. Again, the K ring was synthesised via cyclisation with AgOTf. The substituent of the J ring then underwent several transformations to facilitate the formation of the adjacent I ring (intermediate (**55**)). Again, a vinyl triflate moiety was introduced into the I ring, which allowed for the connection of the G ring to the rest of the framework via a Suzuki cross-coupling reaction (See Figure 19).

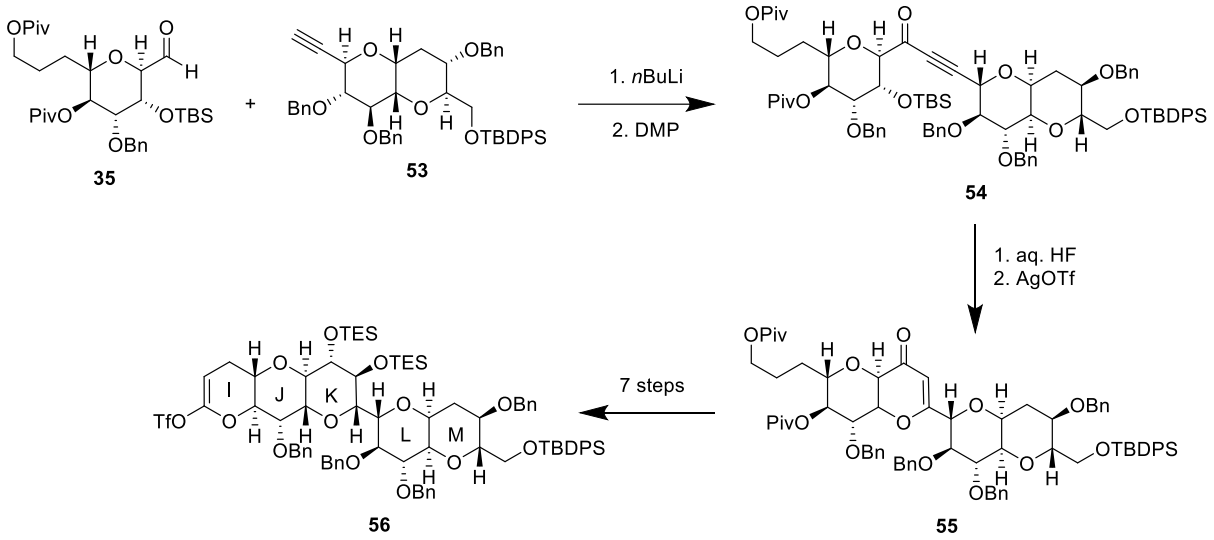


Figure 19. Combining the J (**35**) and LM (**53**) fragments to form the IJKLM (**56**) ring system.

Ring H was ultimately formed using the same method as previously used in the synthesis of the GHIJK fragment via the formation of the methyl acetal and consequent removal of said acetal (see Figure 20).

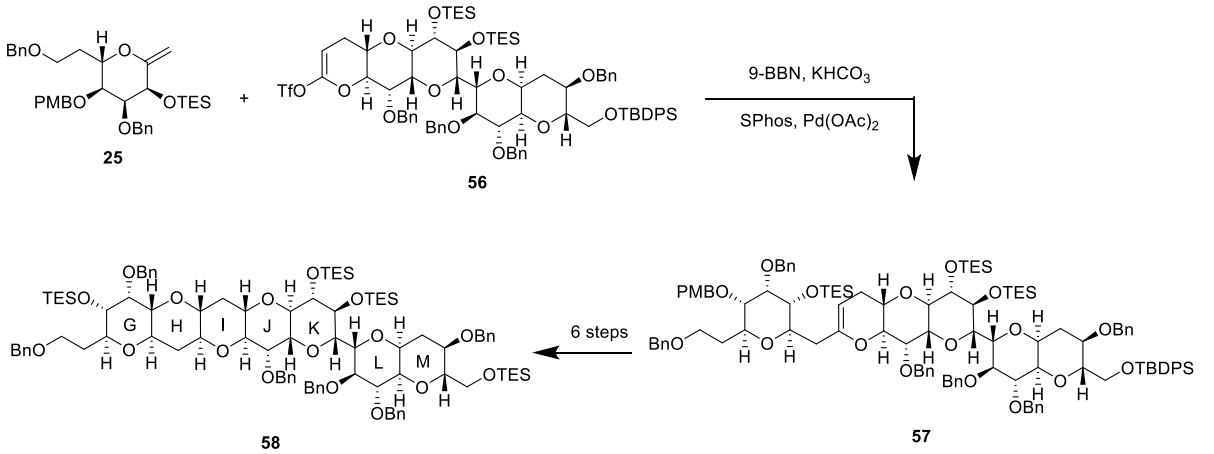


Figure 20. Connection of the G (**25**) and IJKLM (**56**) fragments to form the GHIJKLM (**58**) ring system.

Finally, the GHIJKLMNO ring system was completed via the selective deprotection of the OTES group at the M ring and consequent oxidation under Swern conditions, which then underwent an HWE olefination with the NO fragment. After the addition of various hydroxyl groups via epoxide precursors and the cleavage of the remaining protection groups, the desired fragment was completed (see Figure 21).

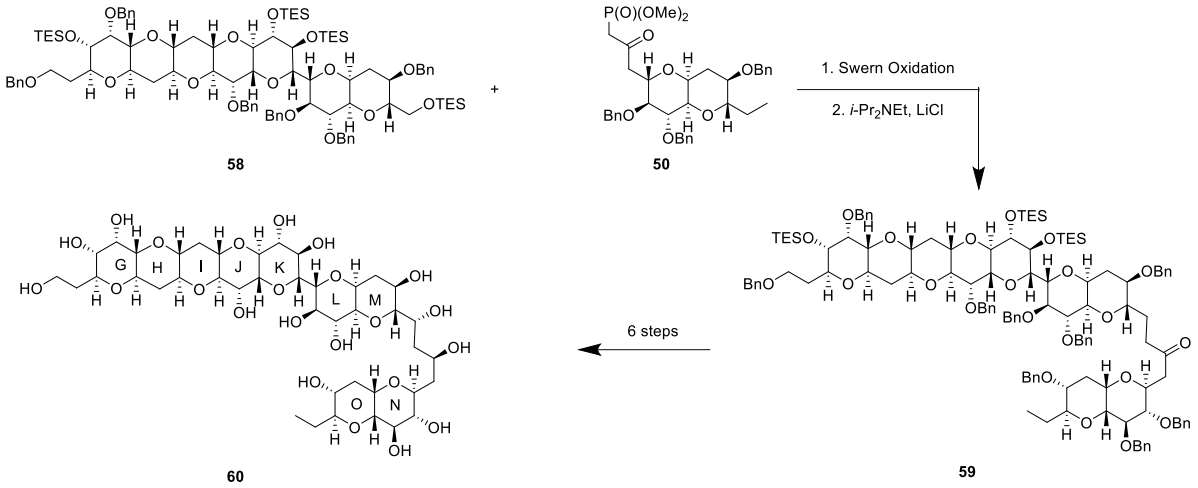


Figure 21. Completion of the GHIJKLMNO (**60**) fragment.

Synthesising the remaining three ring fragments.

The remaining ring systems synthesised by *Nicolaou et al.* are the C'D'E'F'-fragment (**61**), the ABCDEFG-fragment (**62**) and the QRSTUVWXYZA'-fragment (**63**), as shown in Figure 22. While discussing their syntheses in-depth would far exceed the scope of this literature review, a brief summary of each is therefore provided below.

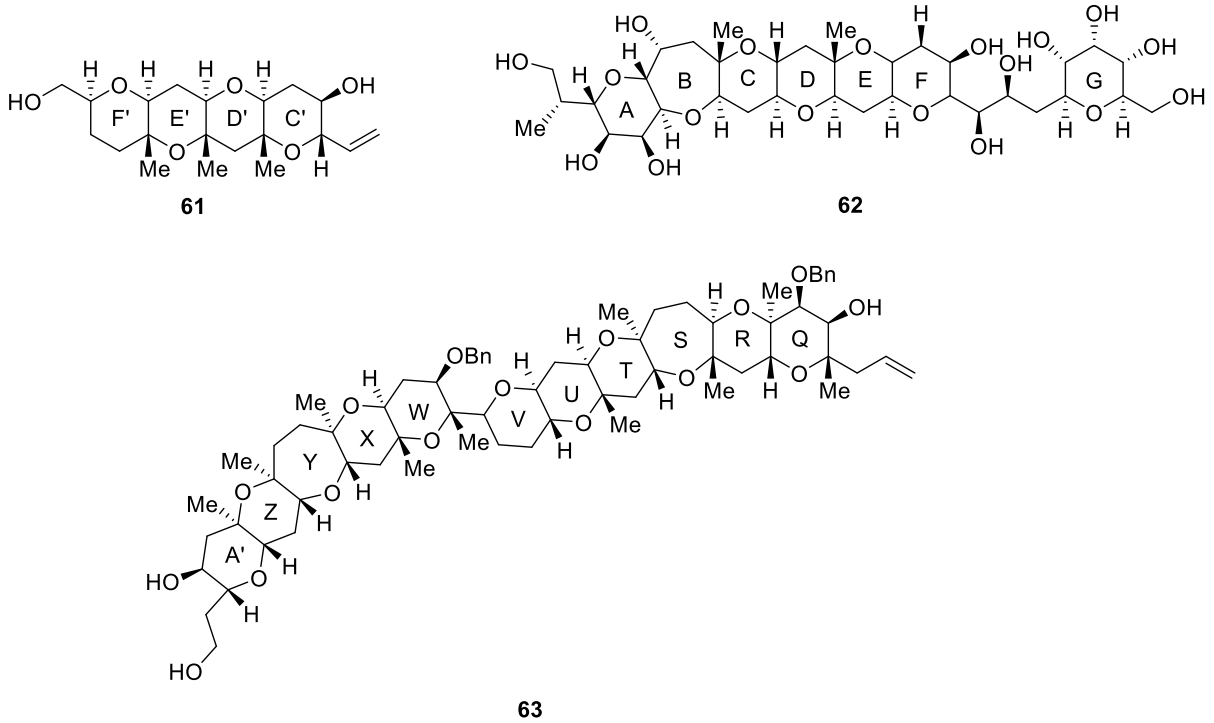


Figure 22. The remaining ring systems: C'D'E'F' (**61**), ABCDEFG (**62**) and QRSTUVWXYZA' (**63**).

First in line: Synthesis of the ABCDEFG fragment (61).

One interesting aspect of the ABCDEFG fragment is the obvious overlap between this fragment and the previously synthesised GHIJKLMNO fragment. In the paper, *Nicolaou et al.* described the addition of the G ring to this fragment to investigate potential connection points between fragments to create larger ring systems (in the case, the ABCDEFGHIJKLMNO fragment, which was, ultimately, never synthesised).[38]

Retrosynthetic analysis quickly showed that a compelling method for joining these two larger fragments would be through a Horner-Wadsworth-Emmons olefination, previously also used for generating the LMNO fragment. To assess the viability of this method, the group first synthesised the ABCDEF-fragment.

This commenced with synthesising the A ring (**65**), which was done in seven steps with a total yield of 41.6%. In the following step, the first seven-membered ring of the target compound was introduced via lactonisation under oxidative conditions, which led to the formation of the AB fragment (**66**).

Next, the DE fragment (**69**) was established, starting from synthesising the D ring (68), which was achieved in 11 steps with a yield of 25.0%. This synthesis, too, largely relied on methods discussed and established previously. The ring system was then extended in 13 steps to obtain the DE fragment (**69**), yielding 19.3%.

Finally, the two fragments were joined via the previously-discussed Suzuki Coupling using 9-BBN. The C ring was then established across 8 further steps to obtain the ABCDE fragment (**70**) with a 16.8% yield. An overview of the synthesis scheme is shown in Figure 23.

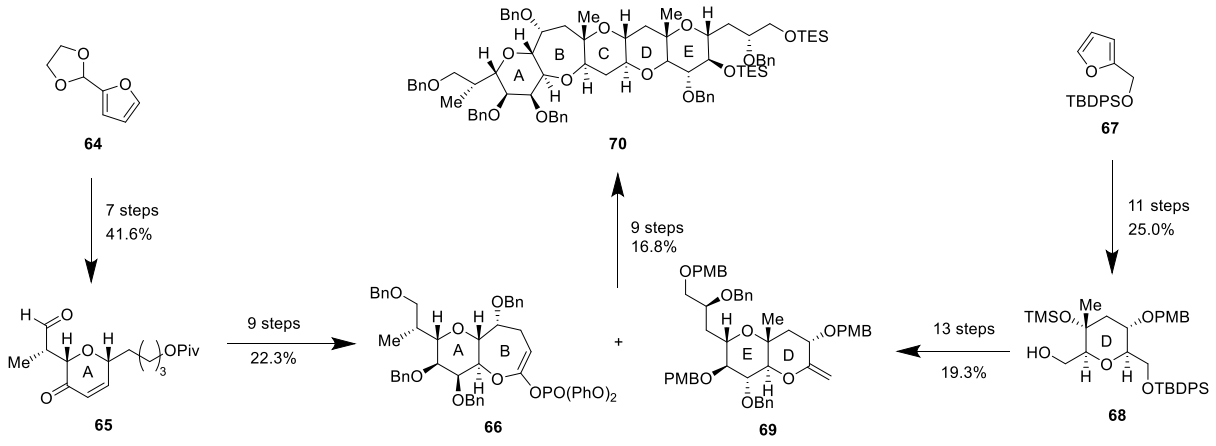


Figure 23. The synthesis scheme of the ABCDE fragment (**70**).

Within the scope of synthesising the ABCDEFG fragment, *Nicolaou et al.* decided to use a different variant of the G fragment approach based on the works of several other researchers.[77–83] No clear reason was given for the use of this alternative fragment, though its structure varies strongly from the G fragment synthesised by the group in

the year before. This G ring, being an almost fully protected hemiacetal with only a free aldehyde as a reactive species, was synthesised in 15 steps with a total yield of 18.2%.

As previously mentioned, the chosen method for connecting the G ring to the ABCDE fragment was an HWE olefination. To this end, **70** required a ketophosphonate as a coupling partner. To this end, the terminal triethylsilane group was converted into an aldehyde in two steps, which then reacted with an organolithium derivative of dimethyl methyl phosphonate, followed by the oxidation of the consequently formed secondary alcohol to form the ketophosphonate functionality (**71**). The authors described the following step – the HWE olefination – to proceed smoothly under "Masamune-Roush" conditions, which led to the formation of **72**, as seen in Figure 24.

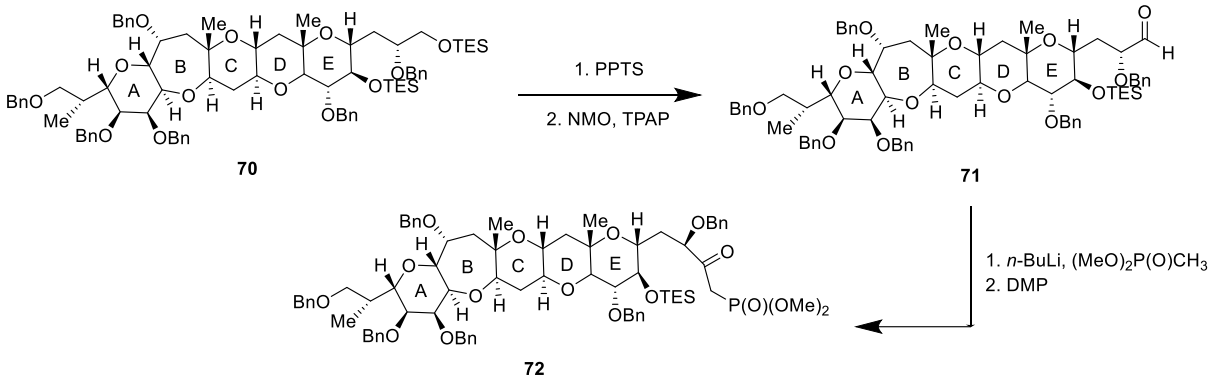


Figure 24. Implementation of the ketophosphonate functionality to form **72**.

Under regular HWE conditions, NaH is used as a base. This strong base, however, is not compatible with many base-sensitive compounds, which is why *Masamune et al. (1984)* developed a milder alternative using lithium chloride and DBU.[84] Mechanistically speaking, the lithium-ion interacts with the oxygen atoms of the carbonyl group and the phosphonate of the ketophosphonate, increasing the acidity of the central proton. This then allows it to be deprotonated by milder bases (here DBU), allowing the HWE olefination to proceed.[85] This mechanism is shown in Figure 25.

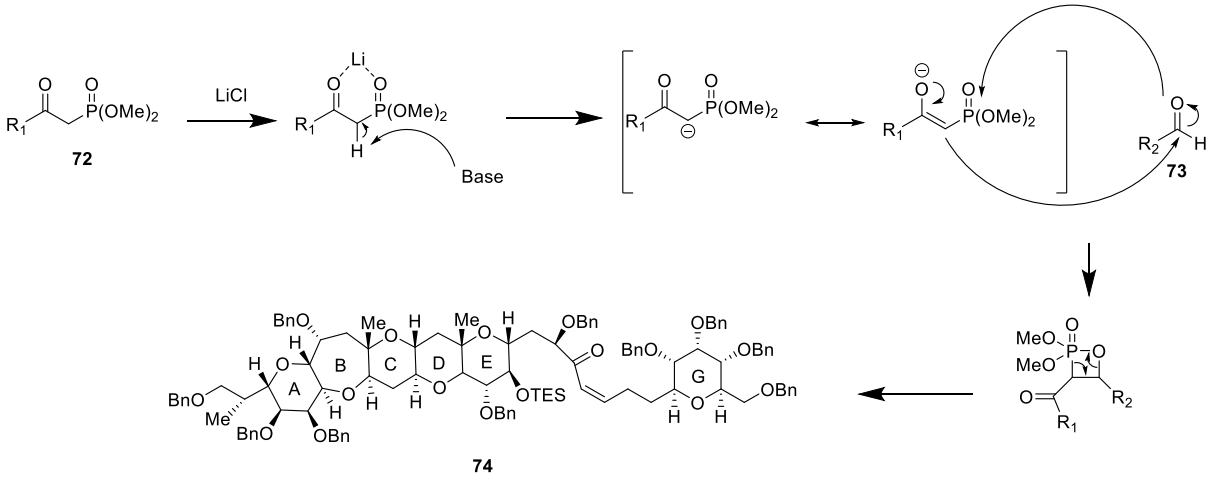


Figure 25. HWE olefination between ketophosphonate **72** and aldehyde **73** to form ABCDEG ring system **74**. R_1 = ABCDE fragment; R_2 = G fragment.

In four additional steps, the F ring was formed via intramolecular cyclisation, and all remaining protection groups were removed to form ABCDEFG fragment **62**. Again, *Nicolaou et al.* noted the exceptional efficiency of generation polycyclic ether compounds via the methods described above and recommended their use in future endeavours for synthesising similarly structured marine neurotoxins.

A lengthy middle: Synthesis of the QRSTUVWXYZA' fragment.

Out of all fragments synthesised by *Nicolaou et al.*, the QRSTUVWXYZA' fragment is the longest one synthesised, and though it re-uses many of the previously established methods, it also introduces a new one: the use of the Takai-Utimoto olefination.[86]

This method was, in particular, used to synthesise the Y ring, and required significant optimisation.[41,87] *Nicolaou* had previously optimised this reaction in 1996 for a similar ring system, though in this case, the chosen conditions ($TiCl_4$, TMEDA, Zn, $PbCl_2$, CH_3CHBr_2) did not lead to the desired product.[88]

When considering the starting point, which was the WZA' ring system (**75**), the authors argued that the bulkiness of the protecting group on the W ring might lead to the lack of reactivity (and, in some cases, decomposition). Four alternatives were tested: the use of the original TBS group and the TES group, neither leading to product formation; the use of TMS, which led to some product formation, but predominantly to the formation of side product (**76**), and simply leaving the hydroxy group deprotected. This, in addition to optimising the used solvent (THF), proved to be the correct choice, leading to a satisfactory yield of 67%. A brief overview is shown in Figure 26.

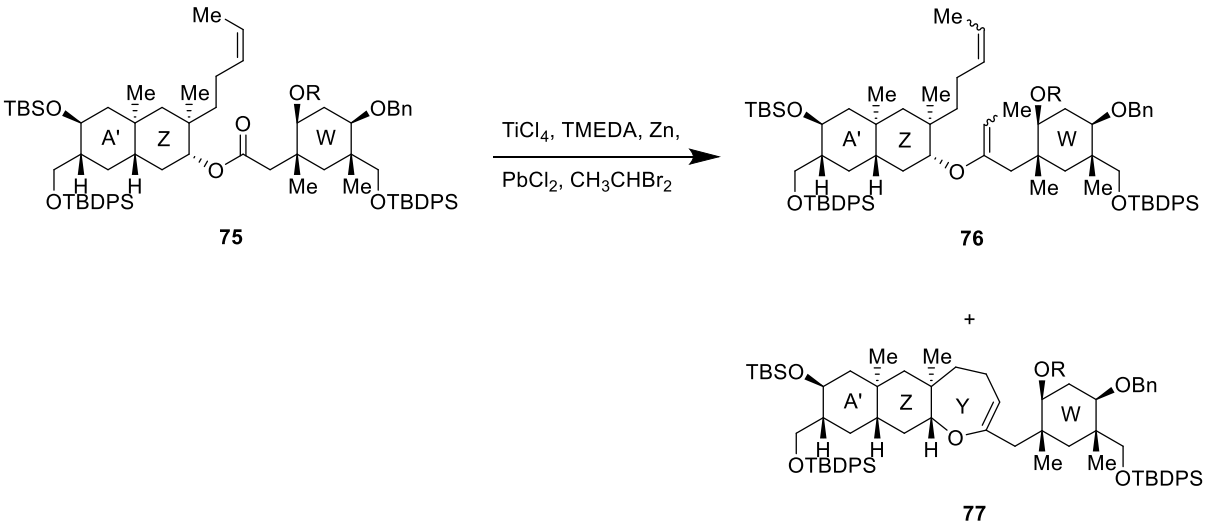


Figure 26. Formation of the Y ring under optimised Takai-Utimoto conditions. R = TBS/TES/TMS/H.

Several attempts were made at generating the desired seven-membered ring from **76** via ring-closing metathesis. However, neither Grubbs II catalyst, Schrock's catalyst, nor Hoveyda-Grubbs II catalyst proved effective. The authors explained that this was likely due to the steric hindrance at the compound's reactive sites, which made using the unprotected substrate the ideal choice for this reaction.

The synthesis of the rest of the QRSTUVWXYZA' ring system largely relied on methods previously discussed. The final ring system was formed from two smaller fragments, QRSTU (**78**) and WXYZA' (**79**), which were again connected via an HWE olefination. The V ring was ultimately formed via intramolecular cyclisation reactions, with the complete deprotection of the molecule leading to target compound **63** (see Figure 27).

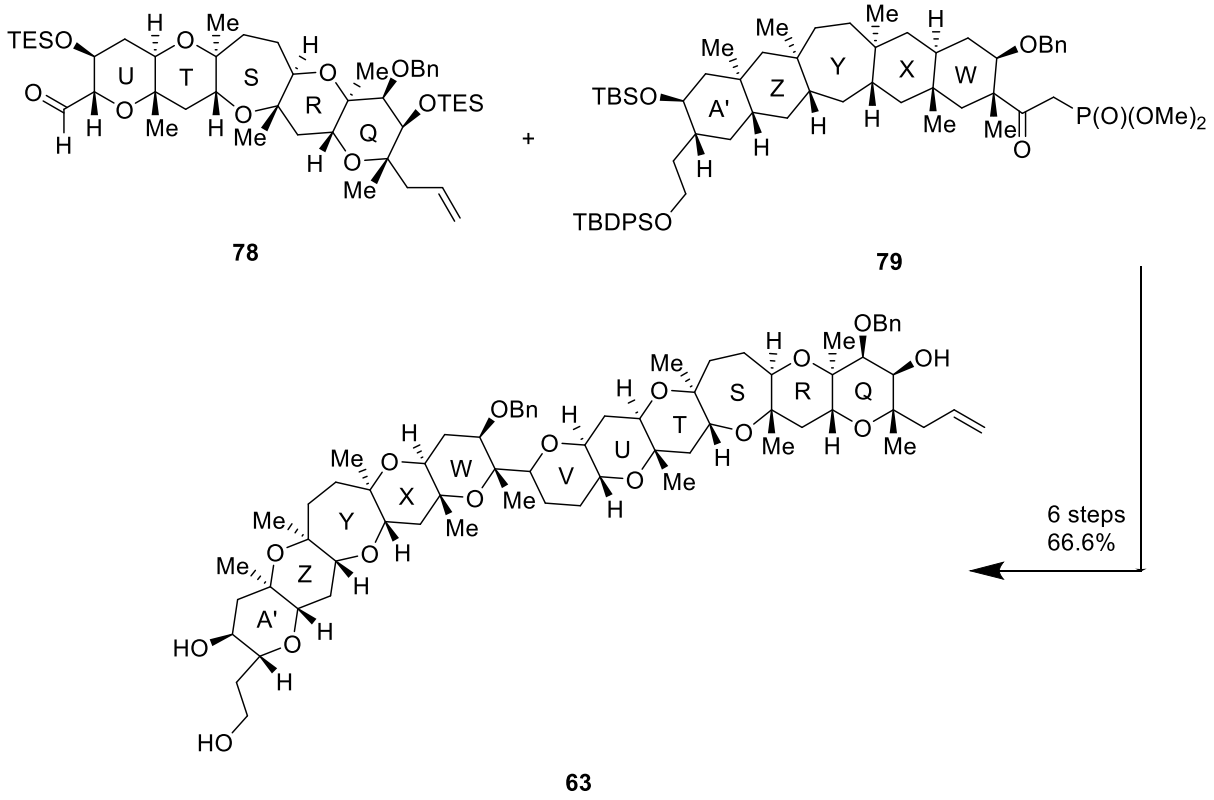


Figure 27. Connecting the QRSTU (**78**) and WXYZA' (79) fragments to obtain target compound **63**.

The final piece: Synthesis of the C'D'E'F' fragment.

Whilst this final fragment is certainly the shortest, *Nicolaou et al.* utilised some interesting strategies for its synthesis, which are worth a closer look. Contrary to the previous fragments, the group opted to attempt a biomimetic synthesis strategy, though the reason for this was not specified.

It has long been hypothesised that these polycyclic ether structures are, in nature, formed via a polyepoxide precursor. Via a cascade, the epoxide rings open, leading to the formation of the polycyclic structure.[89–91] An overview is shown in Figure 28.

The desired C'D'E'F' fragment was broken down into two main building blocks: Ring F' (**85**) with an allylic acetate functionality, and a vinyl stannane with an epoxide (**90**).

The F' fragment synthesis followed a similar reaction pathway to previously synthesised ring systems. Starting from the prochiral furan-derivative (**80**), the framework for the F' ring (**81**) was established via a previously developed synthesis strategy.[38,48,92]

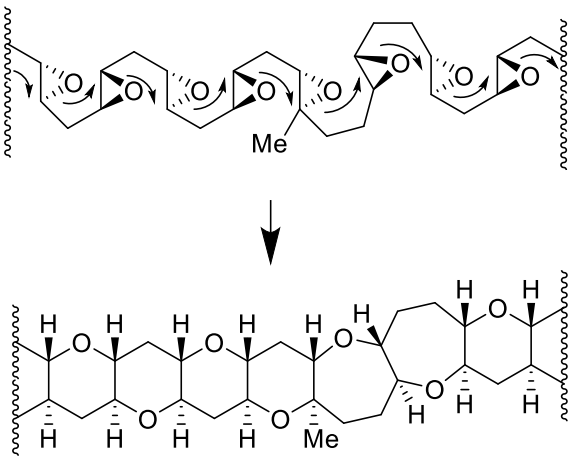


Figure 28. Graphical depiction of the polyepoxide hypothesis.

Following the formation of the F' ring, the enone carbonyl was methylated via a Grignard reaction, and the benzyl group was cleaved via hydrogenation, forming **82**. The primary alcohol was then oxidised using $PhI(OAc)_2$ and TEMPO, and, using a phosphorane (**83**), a vinyl ester was introduced into the molecule (**84**). Finally, to generate the desired vinyl acetate, the ester was reduced to an alcohol with DiBAl-H, which was then acetylated to form the desired precursor (**85**). An overview is presented in Figure 29.

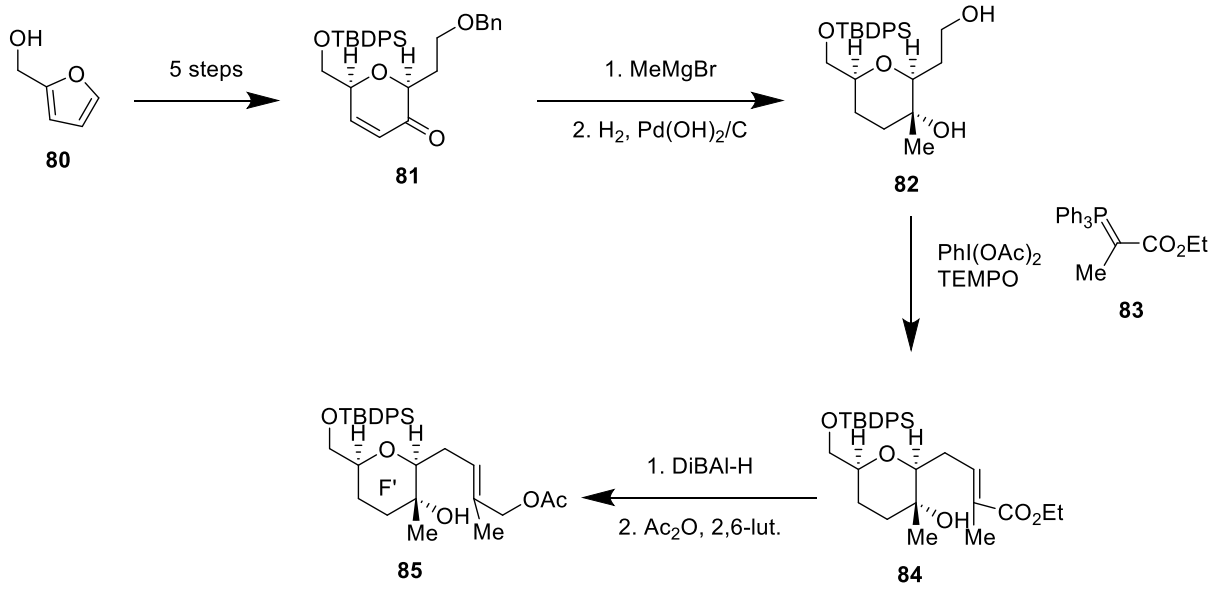


Figure 29. Synthesis of F' ring vinyl acetate **85**.

The desired stannane was generated from 1,2-dihydrofuran (**86**), with the first intermediate (**87**) being synthesised via a protocol by *Radosevich et al. (2008)* in just two steps.[93] The resulting compound was then oxidised with DMP, and a vinyl alcohol moiety replaced the resulting carbonyl group via a Wittig olefination, followed by a reduction with DiBAl-H. The epoxide was then implemented with the correct stereochemistry via Sharpless asymmetric epoxidation, followed by the protection of the remaining free hydroxy group. Finally, the stannane functionality was implemented

under palladium catalysis to achieve the desired building block (**90**). An overview is shown in Figure 30.

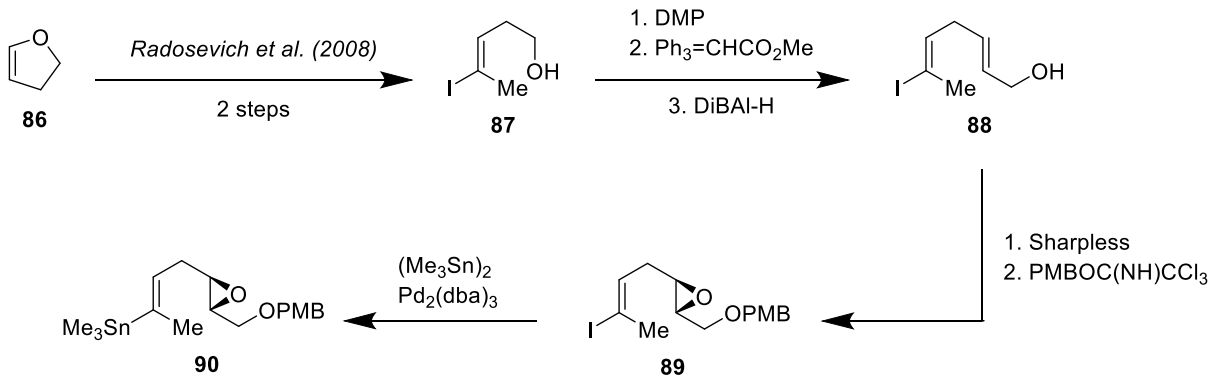


Figure 30. Synthesis of vinyl stannane **90**.

Connecting the building blocks then went as follows: **90** and **85** were initially coupled via a Stille coupling reaction, occurring between the stannane functionality and the acetate under catalysis by a palladium compound. Next, the two double bonds in the molecule were epoxidised via Shi epoxidation. Then, the terminal hydroxy group was deprotected, and the resulting alcohol was oxidised and finally replaced by a double bond via a Wittig reaction.

At this point, the group intended to attempt triggering the cascade ring opening via several different ways, including keeping the compound in water at 90 °C for seven days, microwave radiation, and Lewis acids, among others. Regardless, none of the attempted methods provided the desired target compound (**61**), with side reactions such as H_2O nucleophilic attacks dominating the cause for side product formation. The group assumed that the lack of reactivity was due to unfavourable interactions between the methyl groups.

Nicolaou et al. abandoned the cascade synthesis strategy and opted for a more closely resembling their previous work, extending the ring system from the F' fragment (**84**). The final C' ring was ultimately implemented via a singular intramolecular epoxide ring opening using CSA, as shown in Figure 31, leading to the desired fragment **61**.

The synthesis of the C'D'E'F' ring system marked the end of the KCN group's attempt at creating Maitotoxin via total synthesis, but with four large fragments available, the question remains: how could *Nicolaou et al.* have completed the ring system this exotic molecule?

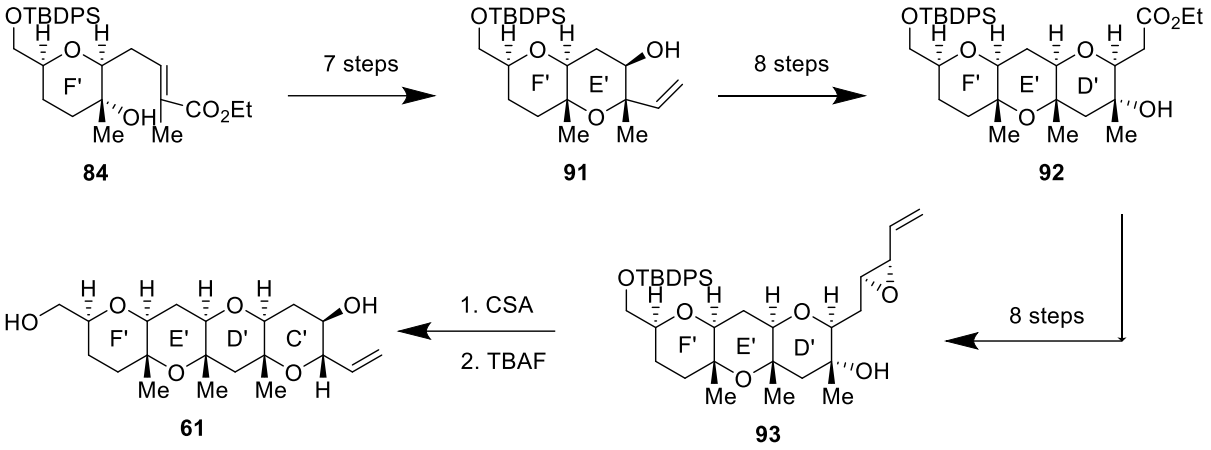


Figure 31. Synthesis of the C'D'E'F' fragment (**61**) via a linear method.

A possible path forward.

Although the four main fragments are synthesised by *Nicolaou et al.*, it is unlikely that they can be efficiently connected to complete the ring system of Maitotoxin. In particular, this is due to one problematic group: the ethyl group attached to the O ring of the GHIJKLMNO fragment is unreactive and cannot be used for connecting, so a path forward would be to replace it with a more reactive group. A possible (theoretical) method for completing the main framework of Maitotoxin is described below.

1. Changing the NO fragment.

As seen in Figure 16, **47**, which acts as a precursor to both the LM and NO fragments, contains a terminal alkene which is hydrogenated for the NO fragment, leading to the undesired ethyl group. However, to generate the LM fragment, the double bond is instead converted into an aldehyde (**48**). To maintain a reactive group in this key position, the aldehyde could, in this case, be protected, for example, via a dimethyl acetal (**94**). However, at this point, the new NO fragment should not yet be connected to the GHIJKLM fragment. (see Figure 32).

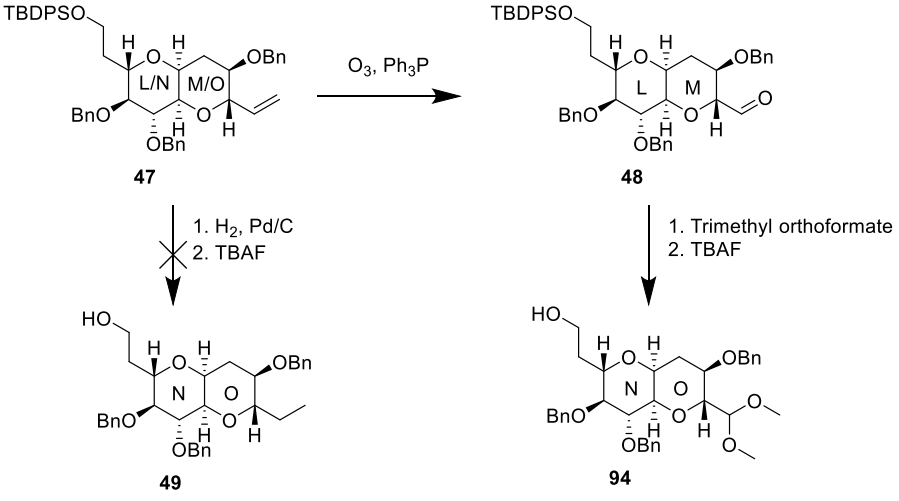


Figure 32. Alternative synthesis of the GHIJKLMNO fragment (**95**).

2. Preparing the G ring for docking.

When looking at the GHIJKLM fragment, the G ring contains a unique functional group: the only primary alcohol in the molecule. As previously described, *Nicolaou et al.* established a method for connecting the ABCDEF fragment to the G ring, though the problem here is that the G ring of the previously synthesised GHIJKLMNO fragment (**60**) slightly differs for the G ring used in the ABCDEFG fragment.

To this end, the benzyl groups in the fragment should first be selectively cleaved without removing the TES groups, i.e., via hydrogenation with a Pd/C catalyst.[94,95] The primary alcohol at the G ring can be selectively oxidised to an aldehyde via TEMPO oxidation, which is compatible with secondary alcohols, as shown by *Cai and Panek (2020)*.[96] Then, the formed aldehyde can be protected with trimethyl orthoformate. This then permits the reprotection of all secondary alcohols in the molecule with benzyl groups to minimise side product formation in later reactions required for the coupling. Finally, the dimethyl acetate is removed to reveal the previously formed aldehyde.

An overview is shown in Figure 33.

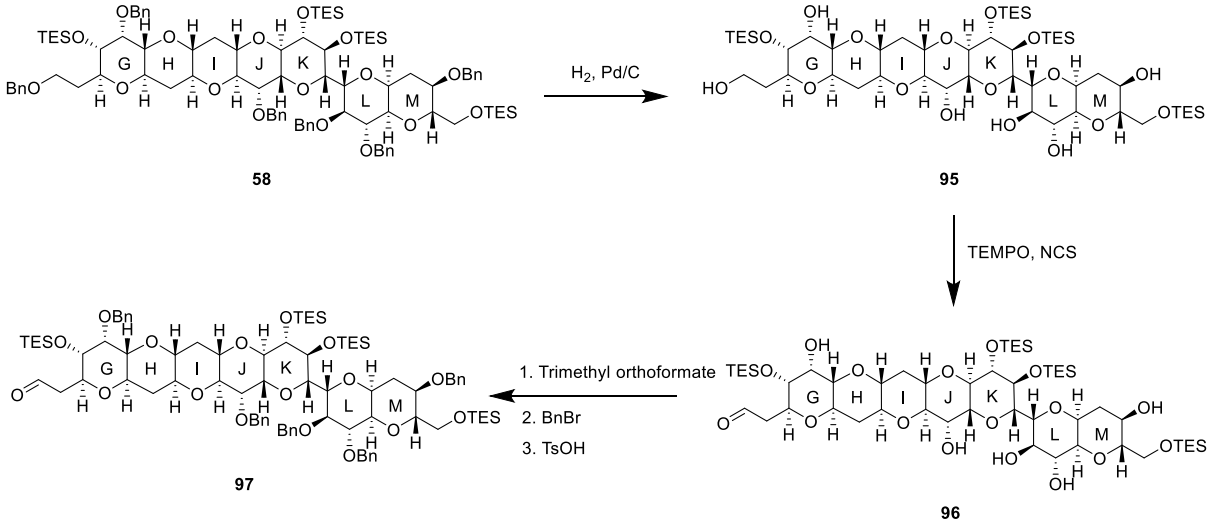


Figure 33. Aldehyde implementation on the G ring.

3. Connecting the ABCDE fragment (72) with the GHIJKLM fragment (58).

As the ABCDE fragment (**72**) already contains a ketophosphonate, the two fragments can now be connected via HWE olefination, followed by the previously established method for finishing the F ring via intramolecular cyclisation to generate the ABCDEFGHIJKLM fragment (**98**), as can be seen in Figure 34.

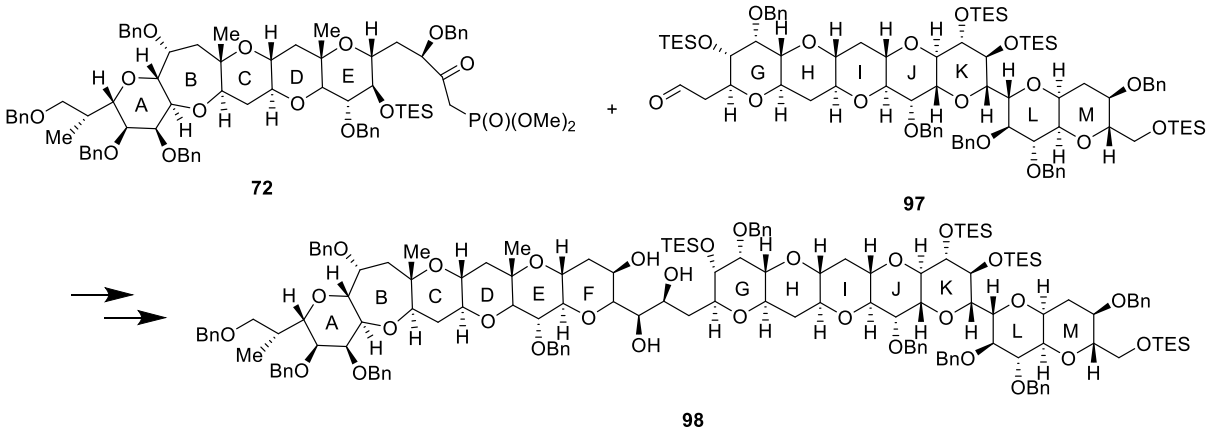


Figure 34. Connecting the ABCDE fragment (**72**) to the modified GHIJKLM fragment (**97**).

4. Connecting the NO fragment.

The connection of the new NO fragment is rather straightforward and would follow the same process as previously established for synthesising the GHIJKLMNO fragment, as shown in Figure 21. This would create the ABCDEFGHIJKLMNO fragment (**99**), as shown in Figure 35.

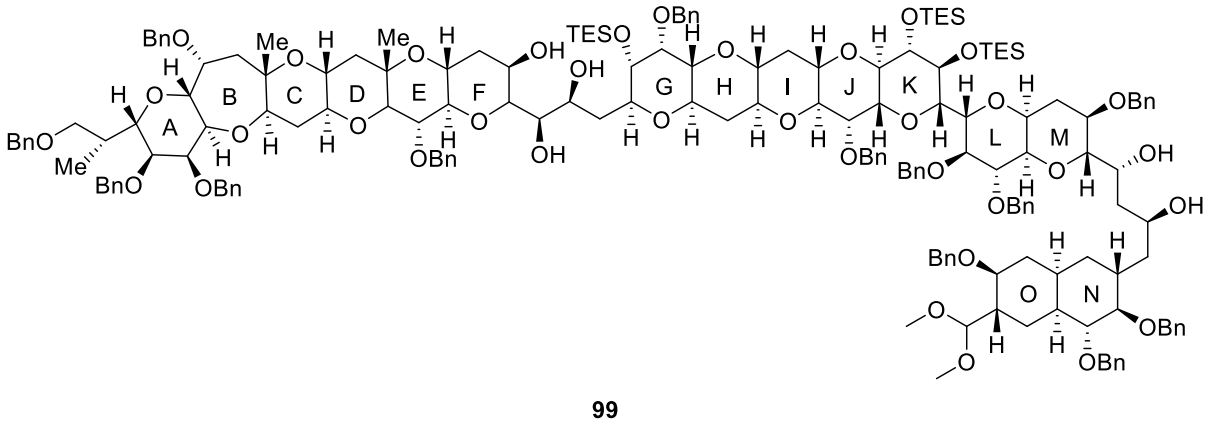


Figure 35. Structure of the ABCDEFGHIJKLMNO fragment (**99**).

At this point, two challenges remain: Connecting **99** with the QRSTUVWXYZA' fragment while forming the missing P ring and connecting the C'D'E'F' fragment while forming the eight-membered B' ring.

5. Connection 99 to the QRSTUVWXYZA' fragment under the formation of the P ring.

As a first step, the P ring can already be formed by utilising the terminal alkene's convenient positioning towards the Q ring's hydroxy group. An Algar-Flynn-Oyamada (AFO) reaction allows for the direct formation of the P ring with a conveniently placed double bond.[97] Coincidentally, this also places the required hydroxy group of the P ring in the right position. Then, the free hydroxy groups of **99** are benzylated to prevent side

reactions in later steps. By cleaving the dimethyl acetal attached to the O ring, the formed aldehyde can then undergo an Aldol condensation with the enol functionality of the P ring, connecting the two ring systems.[98–100] The formed carbonyl functionality can be reduced to the desired alcohol at a later stage. Finally, the remaining double bond can be hydrogenated later as well. However, the formed ketone of the P ring should be selectively protected here via acetalisation to prevent potential side reactions during the final step. An overview of the reaction is shown in Figure 36 (located in the appendix).[1]

6. Connecting the C'D'E'F' fragment to the QRSTUVWXYZA' fragment under the formation of the B' ring.

Considering that the B' ring is the only eight-membered ring in Maitotoxin, it presents a unique challenge not touched upon by *Nicolaou et al.* in their work. Considering the required double bond of the B' ring, one possible approach would be to first establish the ether of the B' ring, which can be done in multiple ways, both using methods that were previously used by *Nicolaou et al.* in the synthesis of the fragments, as well as several other methods described in literature.[101,102] Experimental optimisation would likely be required to investigate the optimal technique for this reaction. This would be followed by a ring-closing metathesis (e.g., Grubbs) to ensure the implementation of the required double bond in the right position. Of course, this would initially require the terminal primary alcohol of the A' ring to be converted into an alkene, which can be done via its oxidation, which would require the oxidation of the primary alcohol, followed by a Tebbe reaction. An overview of this scheme is presented in Figure 37 (located in the appendix), showing the complete ring system framework of Maitotoxin (**106**). While a side reaction with the double bond between the O and P ring may be possible, its occurrence is expected to be unlikely due to steric hindrance. In case it does cause competing reactions, it may be wise to either hydrogenate this double bond prior to this reaction or, alternatively, mask the double bond in the location, e.g. via epoxidation.[103]

Of course, it should be noted that to finish the total synthesis of Maitotoxin, the terminal side chains would need to be added. However, testing the toxicity of merely the complete framework may provide valuable information on the toxicity of this complex ring system on its own, as well as the function of said side chains in the biological mode of action of this extraordinary molecule. It should be noted, though, that, even without the side chains, the "upper half" of Maitotoxin is still more hydrophilic than the "bottom half", considering the far higher degree of methylation of the bottom half, which would be in line with Murata's hypothesis of Maitotoxin's mode of action (as discussed in a previous chapter).[45]

[1] At this point, due to the ever-growing size of the fragments, the remaining synthesis schemes are shown in the appendix.

Conclusion.

The synthesis of Maitotoxin: a testimonial to the greatness of modern total synthesis.

Even though the KCN group never completed the total synthesis of Maitotoxin, it still provided the scientific community with various techniques that can be added to the chemical toolbox of any avid marine neurotoxin chemist (as well as other scientists working with polycyclic ether structures). Further than that, by synthesising the fragments of this large secondary metabolite, they have proved that today's chemists have come a long way from Friedrich Wöhler's time, and it will be exciting to see what we will be able to achieve in the future.

Some final thoughts.

Undoubtedly, what Nicolaou and colleagues have achieved in these eight years of research is remarkable, though this does not mean that their work goes entirely without criticism.

One aspect that immediately stands out is the way yields are reported throughout the project. It is not rare to find the group's reported reactions to have proceeded with 100% (or the ever-elusive "quantitative") yield. Whilst theoretically not impossible, it is unlikely that considering the complexity of the molecules synthesised in the project, no side reactions occurred, and no product was lost during purification or product transfer.

Another issue is that certain quoted publications (i.e. "A. R.; Jamison, T. F. *Angew. Chem., Int. Ed.* **2009**, *48*, 4330.") cannot be found in literature. It should be noted, though, that the actual publication found in Angewandte Chemie, 2009 vol. 48 is by Phil Baran, a (previous) member of the KCN group.[104] It is thus entirely possible that this is simply a quotation error.

Whilst the two aspects mentioned above may slightly inconvenience any scientist wanting to reproduce their work, they do not take away from the vast amount of information that can be gathered from this project.

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Appendix

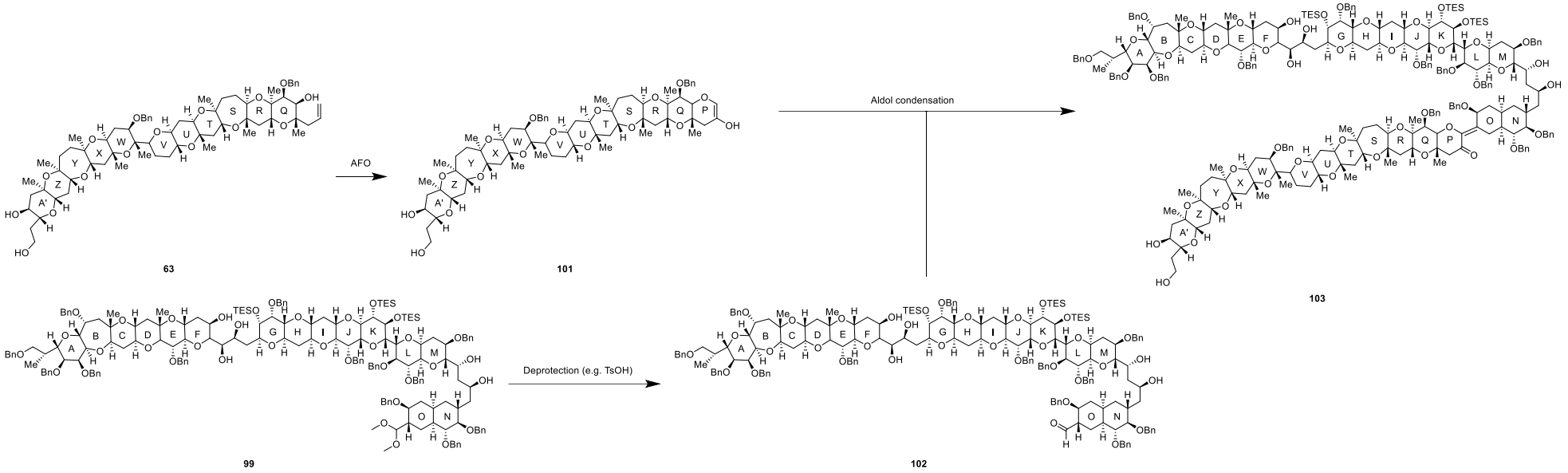

Figure 36. Synthesis of the ABCDEFGHIJKLMNOPQRSTUVWXYZA' fragment (**103**).

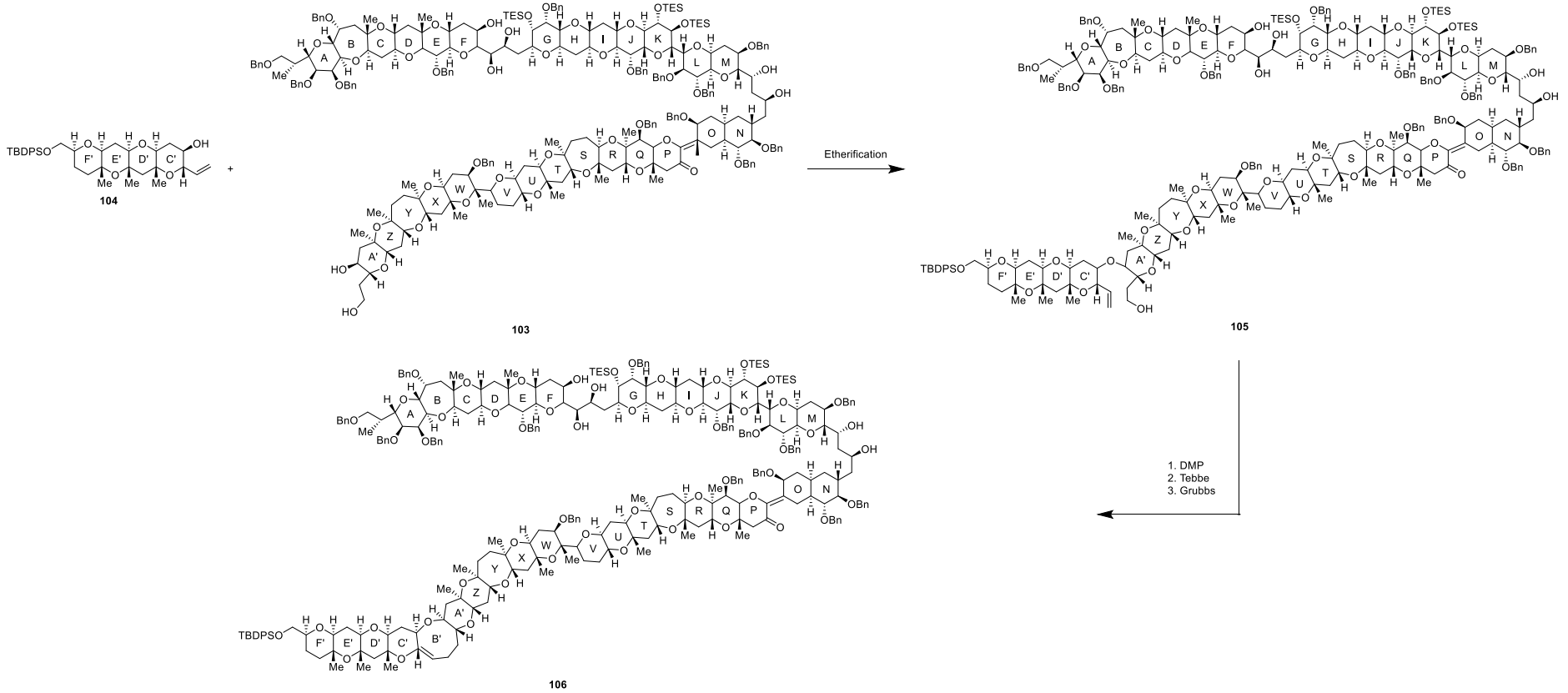

Figure 37. Finishing the Maitotoxin ring system framework (**106**).