ABSTRACT

ZOU, YAN. Applications of [2+2+2] Cyclotrimerization Reactions and Light-Cleavable Groups in the Generation of Biologically Active Molecules. (Under the direction of Dr. Alexander Deiters).

[2+2+2] Cyclotrimerization reactions are a versatile tool for constructing the poly-substituted carbo- and hetero- cyclic ring systems. A number of transition metal catalysts, such as Co, Ru, Rh, Ni and Pd complexes, have been applied. However, there are still regio- and chemo-selectivity issues present in [2+2+2] cyclotrimerization reactions, and only few applications of cyclotrimerization reactions in the synthesis of natural products have been reported. We aimed to develop cyclotrimerization reactions for the assembly of benzene and pyridine core structures found in natural products and for applications in the synthesis of fluorophores.

Microwave irradiation, a more recent method used to assist organic reactions, was employed in various cyclotrimerization reactions. In order to obtain greater control over the chemoselectivity of these reactions, we developed a solid-supported method of cyclotrimerization reactions through the immobilization of an alkyne or diyne onto a polymer backbone. This methodology was applied to the assembly of poly-substituted pyridines. Furthermore, the microwave mediated cyclotrimerization methodology was utilized to synthesize various anthracene and azaanthracene fluorophores. Additionally, a cyclotrimerization reaction was used in key steps of the total synthesis of the natural products cryptoacetalide and the pyridine core of cyclothiazomycin. Currently, the cyclotrimerization reaction is being utilized in the ongoing synthesis of petrosasponglide L.

Photolabile protecting groups (caging groups) have attracted considerable attention in the field of chemical biology. These caging groups are installed on biomolecules of interest
to control their function with light. We applied the photocaging technology towards the development of a caged rapamycin, a biologically relevant natural product. Several caged rapamycin analogs were successfully synthesized and applied in the photo-regulation of enzyme activities. Additionally, a photocaged erythromycin was synthesized and used to control gene expression in *E. coli*.

Carbon-nitrogen (C-N) bond forming coupling reactions play an important role in organic chemistry. A Cu(I)-Re complex was synthesized and successfully used to catalyze C-N coupling reactions. Additionally, a “turn-off” fluorescence switch was developed using [3+2] cycloaddition reactions. Lastly, a set of Coenzyme A analogs were synthesized to study the reaction mechanisms of non-ribosomal peptide synthetases.
Applications of [2+2+2] Cyclotrimerization Reactions and Light-Cleavable Groups in the Generation of Biologically Active Molecules

by
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BIOGRAPHY

The author, Yan Zou, was born in Liancheng, Fujian Province, P. R. China. Yan attended University of Science and Technology of China (USTC) in 1999, where she got her BS of chemistry degree. Her undergraduate research focused on the transition metal-catalyzed C-N, C-S coupling reaction, under the supervision of Professor Qingxiang Guo. In 2005, Yan moved to the US and studied in Miami University of Ohio and studied the non-bonding interaction in the physical organic field under the supervision of Professor Benjamin W. Gung. In August of 2006, she got married to Haisheng Lin and decided to transfer to North Carolina. In 2007, she graduated from Miami University of Ohio with a MS in Chemistry degree and moved to North Carolina State University to pursue her Ph.D. study in organic chemistry. Her Ph.D. research was performed under the supervision of Professor Alexander Deiters and covered several fields, including the development and application of cyclotrimerization reactions, the synthesis of caged small molecules and CoA analogs, and transition metal catalyzed C-N coupling. After she got her Ph.D. degree in August of 2012, she began her postdoctoral research at Emory University.
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Ts \quad p\text{-toluenesulfonyl}
CHAPTER 1: Transition Metal-Catalyzed [2+2+2] Cyclotrimerization Reactions

Applications of [2+2+2] cyclotrimerization reactions have been reported in the synthesis of various carbo- and heterocyclic structures.\(^1\text{-}^6\) Recently, it has been discovered that microwave irradiation significantly affects the transition metal-catalyzed [2+2+2] cyclotrimerization reaction,\(^7\text{-}^{10}\) thereby enhancing the applicability of cyclotrimerization reactions in organic synthesis.

The [2+2+2] cyclotrimerization reaction was first discovered in 1866, by Berthelot et al.\(^11\) Acetylene was cyclized to benzene along with other byproducts at ~400 °C. The high temperature and the mixture of products limited the application in both research and industry laboratories. A major improvement came in 1948, when Reppe\(^12\) reported the first transition metal catalyzed [2+2+2] cyclotrimerization in which acetylene was cyclized to substituted benzenes in the presence of the transition metal catalyst (PPh\(_3\))\(_2\)Ni(CO)\(_2\). Following Reppe’s steps, other types of transition metals, such as Co,\(^13\)\(^,\)\(^14\) Ru,\(^15\) Rh,\(^16\) Pd,\(^17\) and Cu,\(^18\) have also been developed for cycloaddition reactions leading to substituted benzene derivatives. Furthermore, it was discovered that a nitrile can substitute one of the alkyne reaction partners to form substituted pyridine derivatives instead of benzene derivatives.\(^13\)\(^,\)\(^19\)\(^,\)\(^20\)

1.1 The [2+2+2] Cyclotrimerization Reaction Approach to Benzene Derivatives

1.1.1 Mechanism

In the different [2+2+2] cyclotrimerization reactions, the mechanisms are dependent on the catalyst. The most common mechanism for many of the metal catalysts (Co, Rh, Ni, and Pd) involves a metallacyclopentadiene intermediate.\(^21\) Two alkynes I replace two ligands
of the catalyst to give the metal complex 3, which then undergoes oxidative cyclization to form a metallacyclopentadiene intermediate 4. The third alkyne coordinates to the metal, delivering the complex metallacyclopentadiene complex 5, which can react in two ways to yield the same benzene-metal complex 8. The metallacyclopentadiene complex 5 undergoes an insertion where the alkyne is inserted into a metal-carbon bond to give a metallacycloheptatriene 6 (pathway a) that subsequently undergoes reductive elimination to produce a benzene-metal complex 8, or the metallacyclopentadiene complex 5 undergoes a Diels-Alder type of cyclization to the 7-metallanorbornadiene complex intermediate 7 (pathway c) followed by a reductive elimination resulting in the benzene-metal complex 8. The metal is eliminated to produce the benzene 9 and the metal catalyst is regenerated, which will coordinate the alkyne 1 in a new catalytic cycle (Scheme 1.1). \(^1\)

![Scheme 1.1. Possible cycloolimerization pathways with a metallacyclopentadiene intermediate.](image-url)
Another proposed mechanism is the cascade carbometallation route shown in Scheme 1.2. The cascade carbopalladation often occurs with Pd (II) complexes. The first step is the oxidative cis-addition of the alkyne 1 and PdX₂ to form the cis-halopalladation intermediate 10. This is followed by the consecutive cis-addition of two alkynes to give the halopalladation intermediate 12, which then cyclizes to generate the six-membered ring intermediate 13. The reductive elimination of this intermediate with the assistance of another oxidative reagent, CuCl₂ for example, generates the cyclized product benzene ring 14 and Pd (II), which could be reused in the cycle.

\[
\begin{align*}
\text{1} & \xrightarrow{\text{PdX}_2, \text{oxidative addition}} \text{10} & & \text{10} & \xrightarrow{\text{11}} \text{11} & & \text{11} & \xrightarrow{\text{12}} \text{12} & & \text{12} & \xrightarrow{\text{13}} \text{13} \\
\text{14} & & \xrightarrow{\text{reductive elimination}} & & \text{14} & & + & & \text{14} & & \text{PdX}_2
\end{align*}
\]

**Scheme 1.2.** Proposed mechanism for the Pd-catalyzed [2 + 2 + 2] cyclotrimerization reaction.

The third mechanism is the metathesis cascade route. The metathesis route uses the Grubbs’ ruthenium carbene complex as catalyst. Here, each alkyne is added in a series of [2+2] cycloaddition reactions and cycloreversions (Scheme 1.3) ultimately leading to the ring closing metathesis reaction that yields the benzene product 21 and regenerates the catalyst 16.
1.1.2 Regioselectivity of Cyclotrimerization Reactions

For the metallacyclopentadiene mechanism, the regiochemical outcomes of the intermolecular cyclotrimerization reaction of unsymmetrically substituted alkynes are either 1,2,4-trisubstituted or 1,3,5-trisubstituted patterns depending on steric interactions (Scheme 1.4). The intermediates 23 and 24 lead exclusively to the 1,2,4-trisubstituted pattern, while the intermediate 25 can lead to both the 1,2,4- and 1,3,5-trisubstituted pattern. Due to the steric effects of the two R groups, the intermediate 24 can be excluded. It has been observed that the metallacyclopentadiene intermediate 23 is the major intermediate, therefore it would be expected the 1,2,4-trisubstituted benzene to be the major product when only one unsymmetrical alkyne 26 is used in the intermolecular cyclotrimerization reaction. However, when two or three different unsymmetrical alkynes are employed, a complex mixture of regioisomeric products is obtained.

In order to prevent chemoselectivity problems in the intermolecular cyclotrimerization, one solution is to tether two alkynes together, as in the intermolecular cyclotrimerization between the diyne 29 and the alkyne 30. This will lead to fused benzene or heterocyclic rings. As shown by Yamamoto and McDonald, this type of reaction can be catalyzed by Ru$^{26}$ or Rh$^{27}$ complexes (Schemes 1.5 and 1.6). The symmetrical diyne 29 (R = H) cyclotrimerizes with the terminal alkyne, 1-hexyne 30, and produces only one fused benzene product in high yield. When the diyne is not symmetrical, for example, when R=Me, two products will be generated (31 and 32). Due to steric interaction between the R and Bu groups in 32, the meta-isomer 31 is the major product.$^{28}$
Scheme 1.5. Intermolecular cyclotrimerization between a diyne and an alkyne (E=CO$_2$Me).

The regioselectivity of the reaction and the reactivity of the substrate is highly dependent on the size of the substituents on the diyne and the alkyne (Scheme 1.6).

When using the diyne 33 and the alkyne 34, bulkier R$^3$ groups (entry 1 and 2) lead to a higher 35/36 ratio and the product is exclusively the meta-product 35 when R$^3$ is C(CH$_3$)$_2$OH. Bulky R$^1$ groups on diyne 33 also cause a higher 35/36 ratio (entry 1 and 4).

Scheme 1.6. Results of RhCl(PPh$_3$)$_3$-catalyzed cyclotrimerization reactions of diyne 33 and alkyne 34.
The intramolecular cyclotrimerization reaction of the triynes 37-41 provides the tricyclic products 42-46. Formation of 5,6-fused carbo- and heterocyclic ring systems is facile with most catalysts, including Co, Ni, Ru, and Pd complexes. To avoid an intermolecular cyclotrimerization reaction of the triynes 37-41, a low concentration is required. Tether length and substitution on the alkyne also affect the reaction rates (Scheme 1.7). The triyne 37 (n=1) was cyclotrimerized in a 0.1 M solution with 1 mol% of Cp*RuCl (COD) for 2 h to give the 5,6,5-membered fused product 42 in 82% yield (entry 1). A 6-membered ring (43) formation was successfully realized in 89% yield under a higher dilution condition (0.01 M), a higher loading of catalyst (5 mol%) and a longer reaction time (entry 2). In order to achieve a 7-membered ring formation, a solution of the triyne 39 was added slowly to the catalyst, but a much lower yield (53%) was obtained. The substitution of the alkyne also affected the reaction. With increasing steric demand of R1 or R2 (Me > H), the reaction required a longer reaction time (entry 4 and 5), especially when both R1 and R2 were Me groups. In that case the reaction had to be refluxed in chlorobenzene instead of stirring in DCM at room temperature.
Scheme 1.7. The intramolecular [2+2+2] cyclotrimerization reaction of the triynes 37-41. A solution of 44 in DCM was added by syringe pump for 19 h, and then the solution was stirred for 1 h. The reaction was carried out in refluxing PhCl.

When planning a [2+2+2] cyclotrimerization reaction, one must consider many factors including the nature of the metal catalyst and the steric and electronic properties of the three alkynes. In general, terminal alkynes are more reactive substrates and consequently, react at faster rates than di-substituted alkynes. Sometimes systems might require the assistance of the kinetic Thorpe-Ingold effect. As shown in Scheme 1.8, the diyne 47 failed to cyclize, but with two extra carboxyl groups, the diyne 48 was cyclized to the 6-6-fused product 49.

Scheme 1.8. Effect of the tether on cyclotrimerization reactions (E = CO₂Me).
1.2 [2+2+2] Cyclotrimerization Approach to Pyridine Derivatives

Derivatives of pyridines and its fused analogs have attracted considerable interest in organic synthesis.\textsuperscript{33} The pyridine structural unit can be found in a large number of natural products and pharmaceutical agents, as a pharmacophore of considerable historic importance and a valuable synthetic building block in drug discovery, heterocyclic chemistry, and natural product synthesis.\textsuperscript{34} Wide-ranging applications of pyridine derivatives have been discovered, including their use as reagents in organic synthesis and precursor for non-heterocycles, polymers, dyestuffs, additives, pharmaceuticals, agrochemicals, veterinary products and surfactants.\textsuperscript{4, 35-39}

Pyridine was originally isolated from coal tar. However, pyridine derivatives have been synthesized through a variety of approaches, such as the Hantzsch pyridine synthesis,\textsuperscript{40} the Krohnke pyridine synthesis\textsuperscript{41, 42} and the Guareschi-Thorpe condensation.\textsuperscript{43} Bohlmann and Rahtz first reported the two-step synthesis of trisubstituted pyridines through the reaction of enamines and ethynyl ketones or aldehydes back in 1957.\textsuperscript{34, 44} Then Bagley et al. developed faster and more facile one-pot methods for the cyclocondensation of enamines and alkynone by adding catalysts and modifying the conditions.\textsuperscript{34} Ever since Reppe \textsuperscript{12} et al. reported a \((\text{PPh}_3)_2\text{Ni(CO)}_2\) catalyzed [2+2+2] cyclotrimerization reaction in which acetylene was cyclized to substituted benzenes, the transition metal catalyzed cyclization has been used to produce pyridine and benzene derivatives.\textsuperscript{2, 4-6, 13}

Unlike alkyne cyclotrimerization reactions, a successful pyridine synthesis requires the alkyne and nitrile to combine in a 2:1 ratio. Fortunately, nitriles cyclotrimerize less readily than alkynes in the presence of a transition metal catalyst.\textsuperscript{45} Most of the [2+2+2]
Cyclotrimerization reactions of pyridines use a Co catalyst. However, other catalysts, such as Rh, Fe, Ru, and Ti, have also been used in the formation of pyridines. Most of the mechanisms for the [2+2+2] cyclotrimerization reaction toward pyridines are related to the Co catalyst. Similar to the mechanism of the [2+2+2] cyclotrimerization reaction for the synthesis of benzene derivatives, the mechanism of the pyridine formation also proceeds through a metallacyclopentadiene (Scheme 9). The Co catalyst coordinates to two alkynes to give the cobalt-complex. The oxidative cyclization of the two alkynes and the Co catalyst produces the cobaltacycle, which coordinates to the nitrogen of the nitrile forming the complex. The nitrile complex then reacts either to the metallacycloheptatriene, in which the nitrile has been inserted into the metallacycle with its nitrogen center bound to the metal, or to the cobaltacycle via a Diels-Alder-type reaction. In either case, reductive elimination will give the pyridine product and regenerate the CpCo catalyst, which will coordinate to two alkynes in a new catalytic cycle (Scheme 1.9).

The regiochemical outcome of the intermolecular [2+2+2] cyclotrimerization reaction towards pyridines depends on the alkynes. When ethylene (or other symmetrical alkynes) and a nitrile are used, 2-substituted pyridines are formed without any regiochemistry issues. However, when terminally substituted alkynes and nitriles are cyclotrimerized in the presence of a Co catalyst, the products are 2,3,6- (66) and 2,4,6- (67) trisubstituted pyridines in roughly equal yield. No 2,4,5-trisubstituted products are obtained due to a steric interaction in the metallacyclopentadiene 63 (Scheme 1.10).
Scheme 1.10. Co-catalyzed cyclotrimerization reactions between terminally substituted alkynes and nitriles.

When two different, unsymmetrical internal alkynes are used in the formation of pyridines without a connecting tether, the regiochemical outcome is quite complicated. This could be circumvented by tethering two or three components, which will generate a fused pyridine. When two alkynes are tethered, for example as in diyne 69, the regioselectivity is controlled by a steric effect with the nitrile 70 nitrogen undergoing bond formation with the higher substituted alkyne carbon center 71 (Scheme 1.11).

Scheme 1.11. Co-catalyzed cyclotrimerization reactions forming pyridines.
However, when long-chain diynes 72 were cyclotrimerized with a nitrile, 2,4,6-73 and 2,3,6-substituted pyridines 74 are formed, in approximately a 1:1 ratio, similar to non-tethered alkynes (Scheme 1.12).56


Another way to control the regiochemical outcome is to tether one alkyne with a nitrile. Five-, six-, seven-membered fused-ring systems can be obtained in low to moderate yields (Scheme 1.13). Lower yields were obtained when the terminal alkynes are used, due to the capability of self-trimerizing (entry 8 and 11). For the asymmetrical alkynes, when reacted with alky-nitriles, the bulkier substitutes are always placed next to the nitrogen (entry 3, 7, 8, 9, 11, 12 and 13). When the two substituents of the alkynes are equally bulky, an approximately 1:1 ratio of the two products were obtained (entry 10).57 Based on this observation the intermediate 79 (Figure 1.1) was proposed.
Scheme 1.13. The preparation of [b]annelated pyridines with different substituents.

The intramolecular cyclotrimerization reactions are capable of assembling tricyclic aromatic rings in one step from a triyne or nitrile-diyne. For example, the nitrilediyne 80 undergoes an intramolecular [2+2+2] cyclotrimerization reactions in the presence of Co(II) and Zinc powder as catalysts to give polycyclic pyridines 81 in moderate to excellent yields (Scheme 1.14)\textsuperscript{58} The catalytic reaction is likely be initiated by the reduction of the Co(II)
species to Co(I) by zinc powder. Then the Co is coordinated to the diyne to form the cobaltacyclopentadiene intermediate 82 which would undergo an intramolecular coordination of the nitrile group and subsequent insertion into a cobalt(III) carbon bond to form the cobaltacycloheptadiene intermediate 83 (Figure 1.2). The polycyclic pyridines along with the Co(I) are generated by reductive elimination of the intermediate 83.


Figure 1.2. Two possible intermediates of intramolecular cyclotrimerization reactions.

In conclusion, transition metals, such as Co, Rh, and Ru can be used as effective catalysts for [2+2+2] cyclotrimerization reactions of alkynes and nitriles to give substituted pyridines. The choices of catalyst depends on the electronic and steric nature of the reaction substrates.4, 50
1.3 The Use of Temporary Linkers in [2+2+2] Cyclotrimerization Reactions

As discussed in Chapter 1.2, the partially or completely intramolecular cyclotrimerization reaction produces polycyclic aromatic rings. This limits its application in the synthesis of mono-cyclic aromatic rings. Thus, it is necessary to install easily removable linkers in generating diynes or triynes.59

Recently, Yamamoto et al. used a boron temporary tether in the cyclotrimerization reaction.59 When the three alkynes (alkynylboronates 84, propargyl alcohol 95, terminal alkyne 86) were treated with a ruthenium catalyst, the bicyclic benzene 89 was generated via formation of boron-tethered diyne 87 intermediate and Ru-complex 88 intermediate in good yields and excellent regioselectivity. The bicyclic benzene ring 89 underwent a Suzuki-Miyaura coupling with aryl iodides, delivering tetra-substituted benzene 90 (Scheme 1.15). The boron-benzene ring 90 could also be converted to the phenyl ester 91, the ketone 92, and the lactone 93.
Scheme 1.15. Cyclotrimerization reaction of alkynylboronates.

Another temporary tether is the silyl linker. In 2003, Malacria et al. reported the preparation of unsymmetrical silaketals and their application in the synthesis of silyl tethered diynes and triynes. Later on, they applied the silyl tethered triyne in the cyclotrimerization reaction. The silyl tether temporarily links two or three individual alkynes to a diyne or triyne, thus converting an intermolecular cyclotrimerization reaction into a partially or completely intramolecular cyclotrimerization reaction. This greatly enhanced the reaction yield and solves the regio- and chemoselectivity problems of the intermolecular cyclotrimerization reaction. The silyl-tethered triyne underwent a smooth cyclotrimerization reaction, giving the tricyclic benzene ring. The silyl tethers of were removed using F, producing the tetra-substituted benzene ring (scheme 1.16).
Scheme 1.16. Cyclotrimerization reaction of a silyl-tethered triyne and removal of the silyl groups.

Our group has also applied the silyl tether in the cyclotrimerization reaction to pyridines. The silyl tethered diyne 99 was cyclotrimerized with nitriles using CpCo(CO)$_2$ as catalyst under microwave irradiation giving the bi-cyclic pyridines 100 in excellent yields. Upon removal of silyl tether with TBAF, the 2,4,6-trisubstituted pyridines 101 were obtained. Further transformations of the pyridine 101 delivered heterotaxin 102 (scheme 1.17).

Scheme 1.17. The synthesis of heterotaxin (102).

1.4 Natural Product Synthesis via [2+2+2] Cyclotrimerization Reactions

1.4.1 Benzene-Containing Natural Products

The [2+2+2] cyclotrimerization reaction can assemble a benzene ring from three components in one single step. However, due to the chemo- and regioselectivity issues of the
intermolecular cyclotrimerization reaction of three alkynes, the use of the intermolecular cyclotrimerization reaction is limited. In order to solve the selectivity problems, two alkynes or three alkynes are tethered together to form partially intramolecular or intramolecular cyclotrimerization reactions. As a result, fused benzene rings are formed. A number of benzene-containing natural products have been synthesized via [2+2+2] cyclotrimerization reactions.

The first report of a natural product synthesis using a cyclotrimerization reaction was from the Vollhardt lab. The tetracyclic core 107 of the natural product *dl*-estrone (108, scheme 1.18) was assembled in a single step via a [2+2+2] cyclotrimerization reaction of the diyne 103 with BTMSA (104), using CpCo(CO)$_2$ as a catalyst and further heating of the triene 106 as an intermediate with an overall 81% yield. The product 107 was converted to *dl*-estrone (108) in only three steps (scheme 1.18).

Scheme 1.18. Total synthesis of *dl*-estrone (108) using a cyclotrimerization key step.
In 2002, Witulski et al. reported the total synthesis of (R)-alcyopterosin E using Wilkinson’s catalyst (RhCl(PPh$_3$)$_3$). The author applied an intramolecular [2+2+2] cyclotrimerization reaction to assemble the tricyclic benzene ring 110 in one step from the triyne precursor 109. The benzene 110 was reacted with NaNO$_3$ and Bu$_4$NNO$_3$ in toluene, delivering (R)-alcyopterosin E (111) in one step (69% yield).

\[
\text{109} \xrightarrow{\text{RhCl(PPh$_3$)$_3$ (10 mol%)}} \text{110} \xrightarrow{\text{NaNO$_3$, Bu$_4$NNO$_3$, toluene}} \text{111}
\]

**Scheme 1.19.** Total synthesis of (R)-alcyopterosin E (111).

The 9H-carbazole rings have also been assembled via [2+2+2] cyclotrimerization reactions. Witulski et al. reported the synthesis of the natural products hyellazole (115), clausine C (119), and antiostatin A$_1$ (122) using the cyclotrimerization reaction of ynamides and alkynes under rhodium catalysis as the key step (Scheme 1.20). High yields and good regioselectivity were obtained with diynes 112, 116 and 120. Hyellazole (115), clausine C (119) and antiostatin A$_1$ (122) were synthesized in six, seven, and ten steps, respectively.
Scheme 1.20. The synthesis of hyellazole (115), clausine C (119), and antiostatin A₁ (122).

In 2004, Sorensen et al. published a paper on the total synthesis of viridin (127). An intramolecular cyclotrimerization reaction of the triyne 123 assembled the tricyclic benzene ring 124 in 88% yield. The alcohol 124 was transformed to the furan-containing product 125, which underwent a retro-[2+2] ring opening and a subsequent Diels-Alder type cyclization. A DDQ oxidation delivered the tetracyclic ring structure 126. Further transformations provided viridin (127) (scheme 1.21).
The assembly of tricyclic aromatic rings via intramolecular [2+2+2] cyclotrimerization reactions has been applied in the synthesis of alcyopterosin analogs.\textsuperscript{70} Alcyopterosin I (129) was synthesized in one step from the intramolecular cyclotrimerization reaction of the triyne 128 in 89\% yield. Similarly, the synthesis of alcyopterosin L (132) and alcyopterosin M (133) applied the intramolecular cyclotrimerization reaction of triyne 130 to construct the central tricyclic benzene ring (Scheme 1.22).
Scheme 1.22. Total synthesis of alcyopterosin analogs.

Also in 2004, the application of a cyclotrimerization reaction using the aryne 135 (formed in situ from 134) as a precursor in the synthesis of the natural products taiwanin C and taiwanin E was reported. The diyne 136 underwent a smooth cyclotrimerization reaction with the aryne precursor 135, producing the naphthalene derivative 137. Further treatments of 137 delivered the taiwanin C (138), and taiwanin E (139), respectively (scheme 1.23).
Bridged natural products, such as bruguierol A (144), have also been generated via cyclotrimerization reactions.\textsuperscript{72} A Ru-catalyzed cyclotrimerization reaction of the diyne 140 with propargyl alcohol (84) furnished the bridged benzene products 141 and 142 as a 1:1 regioisomeric mixture. The mixture was oxidized with MnO$_2$, treated with $m$-CPBA and then NaOH to generate the (−)-bruguierol A (143) (Scheme 1.24).
Nicolaou et al.\textsuperscript{73} exploited the cyclotrimerization reaction of halo-alkynes to construct the central halo-benzene ring \textsuperscript{146} of sporolide B (\textsuperscript{147}) with \textsuperscript{Cp*ClRu(COD)} as catalyst.\textsuperscript{73} The benzene \textsuperscript{146} was converted to sporolide B (\textsuperscript{147}) in 9 steps (Scheme 1.25).
Recently, our group successfully applied the cyclotrimerization reaction to the synthesis of natural products indanones (151), cannabinoids (155), and illudinine (159). Solid-supported diyne 148 underwent a cyclotrimerization reaction with propyne (149), generating the adduct 150 on a solid-support. The product was released from the polymer and oxidized to furnish indanones (151) in 73% over 3 steps. Ni catalysis was used for the cyclotrimerization reaction of the diyne 152 and the alkyne 153 to deliver the benzene product 154, which was further converted to illudinine (155). The synthesis of cannabinoids (159) utilized a Ru catalyst in the cyclotrimerization reaction of diyne 156 and alkyne 157 to assemble a benzene ring in the key step (Scheme 1.26).
1.4.2 Pyridine-Containing Natural Products

The [2+2+2] cyclotrimerization reaction has been used in quite a few syntheses towards natural products containing a benzene ring. However, limited examples have been reported for the use of the [2+2+2] cyclotrimerization reaction towards pyridine natural products.

The first pyridine natural product synthesis using a [2+2+2] cyclotrimerization reaction was accomplished by Vollhardt in 1985. The cyclotrimerization reaction of the

Scheme 1.26. Total syntheses of indanones (151), illudinine (155), and cannabinoids (159).
bis(trimethylstanny1)-diyne 161 and acetonitrile, using CpCo(CO)\(_2\) as the catalyst, assembled the pyridine 162. One of the trimethylstannyl groups was selectively removed by alumina, producing 163 in 76% over 2 steps. Further manipulation of 163 delivered vitamin B\(_6\) (164) (Scheme 1.27).

Scheme 1.27. Total synthesis of vitamin B\(_6\) via [2+2+2] cyclotrimerization reaction.

In 1994, Vollhardt reported the total synthesis of the ergot alkaloids (±)-lysergene and (±)-LSD via a [2+2+2] cyclotrimerization reaction.\(^7^6\) The alkyne-nitrile 166 underwent a cobalt-catalyzed cyclotrimerization reaction with the alkynes 166 and 167 under light irradiation to produce the tetracyclic products 168 and 169 in low yields. The pyridine 169 was converted to lysergene (171) using MeOTf followed by sodium borohydride. In a similar fashion, 169 was transformed to lysergic acid diethyl amide (LSD, 170) in 45% yield (Scheme 1.28).
Recently, the synthesis of the natural product complanadine A has been achieved using a double [2+2+2] cyclotrimerization reaction.\textsuperscript{77} The first cyclotrimerization reaction of the alkyne-nitrile 172 and 1,4-bis(trimethylsilyl)buta-1,3-diyne (173) was conducted under CpCo(CO)\textsubscript{2} catalysis at 140 °C, delivering the two cyclotrimerization products 174 and 175 with a 25:1 ratio. Both TMS groups of the major product 174 were removed followed by the addition of a single TMS group to the alkyne to give the internal alkyne 176, which underwent a second cyclotrimerization reaction with the alkyne-nitrile 177, furnishing the desired isomer 179 as the minor cyclotrimerization product together with 178 as the major product. It was found that the addition of excess PPh\textsubscript{3} switched the regioselectivity of the cyclotrimerization reaction of the alkyne-nitrile 176 and alkyne 177. In the presence of PPh\textsubscript{3}, the cyclotrimerization reaction of 176 and 177 delivered the desired 179 as the major

\textbf{Scheme 1.28.} Total synthesis of ergot alkaloids.
product. Further treatment of 179 provided the natural product complanadine A (180) (Scheme 1.29).

**Scheme 1.29.** Total synthesis of complanadine A (180).

The synthesis of the natural product lavendamycin (184) has also been accomplished via a cyclotrimerization reaction key step.\textsuperscript{78} Cp*RuCl(COD) efficiently catalyzed the cyclotrimerization reaction of the electron-deficient nitrile, methyl cyanoformate with the
diyne 181, furnishing the pyridine 182 as the only regioisomer in excellent yield. The natural product lavendamycin (183) was obtained in several steps from 182 (Scheme 1.30).

![Scheme 1.30. Total synthesis of lavendamycin.](image)

The application of the cyclotrimerization reaction towards the total synthesis of pyridine natural products has also been studied in our group. The alkaloids dehydrotylophorine (185) and tylophorine (186) were synthesized using a convergent cyclotrimerization reaction/cyclization strategy from the diyne 184.\(^{79}\) The diyne 184 was cyclotrimerized with 3-cyanopropyl methanesulfonate using CpCo(CO)\(_2\) as catalyst, delivering the polycyclic product 185 in 78% yield. The pyridinium salt 186 was reduced with NaBH\(_4\) to furnish the natural product tylophorine (Scheme 1.31).
Scheme 1.31. The total synthesis of dehydrotylophorine (185) and tylophorine (186).

1.5 Microwave-Assisted Cycloitetramerization Reactions

1.5.1 Microwave-Assisted Organic Synthesis

Recently, microwave assisted organic synthesis (MAOS) has attracted considerable amount of attention due to its ability to heat faster, reduce reaction time, minimize side reactions, and increase reaction yield and reproducibility. The electro-magnetic frequency range of microwave irradiation is from 0.3 to 300 GHz. The household microwave oven and microwave synthesis reactor operate at the frequency of 2.45 GHz and the energy is 0.0016 eV, which is too low to cleave C-C bonds (3.61 eV) or even hydrogen bonds (0.04-0.44 ev) (Table 1.1), thus unable to induce chemical reactions.

Table 1.1. Energy table of chemical bonds.

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<tr>
<td>7</td>
<td>Hydrogen bond</td>
<td>0.04-0.44</td>
<td>4-42</td>
</tr>
</tbody>
</table>

Microwave dielectric heating occurs by two mechanisms: dipolar polarization and ionic conduction.\cite{83,84} Upon microwave irradiation, the dipoles or ions in the reaction mixture align in the applied electric field and the heat, generated through molecular friction and dielectric loss, is absorbed by solvents or reagents. The heating properties of solvents or reagents are dependent on their dielectric properties. Solvents with high microwave absorbance (e.g., ethylene glycol and DMSO) can be heated to high temperatures in minutes, even in seconds. Some solvents, such as dioxane, benzene and carbon tetrachloride, which do not possess a permanent dipole moment, have low or no microwave absorbance. Under a closed vessel microwave irradiation, which uses a sealed microwave vessel, solvents can be heated to temperatures far above their boiling points in a very short period of time. What is the microwave effect? The answer is still under debate.\cite{83,84} Most researchers agree that the dramatic rate enhancements of microwave are mostly considered by thermal effects acceleration, similar to conventional heat acceleration. According to Arrhenius law \[ k = A \exp \left( -\frac{E_a}{RT} \right) \], the high temperature greatly enhances the reaction rates.\cite{80} Additional thermal
effects, so called specific microwave effects, which can not be reached by conventional heating, are also considered. These effects include the superheating effect, the heating selectivity effect of solvents and the minimizing of wall effect, etc.\textsuperscript{83, 84} The “non-thermal microwave effect” has also been addressed.\textsuperscript{85} It has been argued that the electric field can cause change of the orientation of dipole molecules, thus leading to activation energy change.\textsuperscript{80}

In contrast, the conductive heating with an external heating source is an inefficient way to transfer energy, which transfers energy from the vessel to the reaction, resulting in higher temperature of the reaction vessel than the reaction mixture.\textsuperscript{80} The microwave irradiation directly couples the microwave energy with reaction mixture, leading to an efficient internal heating, which induces an inverted temperature gradient (Figure 1.3). Moreover, the microwave vessels are usually made of microwave transparent material, which minimizes “wall effects”,\textsuperscript{80} this could lead to “specific microwave effect”. This effect can reduce diminishing of sensitive catalyst under conductive heating.\textsuperscript{80}
The first microwave assisted organic reaction was reported by Gedye and Giguere/Majetich in 1986 using a domestic microwave oven.\textsuperscript{86, 87} However, there are several disadvantages using domestic microwave irradiation: 1) It is hard to control and monitor the reaction temperature and pressure; 2) The heating is heterohomogeneous; 3) it is risky to use flammable solvents and reagents.\textsuperscript{80} The lack of controllability and reproducibility of domestic microwave oven prevented its wide application in organic synthesis.

It was not until the late 1990s, when dedicated microwave reactors for organic synthesis were designed, that microwave heating began to be used widely to assist organic synthesis. The microwave reactor contains built-in magnetic stirring, an IR sensor or fiber-optic probe that can monitor the reaction temperature, an auto cooling system that can help cool the reaction faster, and software to control the reaction temperature and pressure (Figure 1.3).
1.4). Several different modes, such as standard mode (the reaction is run at a set temperature) and power mode (fixed power throughout the whole reaction) are available. The reactions can be performed in both closed vessels (with high pressure) and open vessels (refluxing at ambient pressure). These advanced features of the microwave reactor enable easy controllability of the chemical reactions. Microwave irradiation has been used to assist a variety of organic reactions, including transition metal catalyzed C-C bond formations (such as Suzuki reaction,\textsuperscript{88, 89} Negishi cross-coupling reaction,\textsuperscript{90, 91} Heck reaction,\textsuperscript{92, 93} Stille reaction,\textsuperscript{94} Sonogashira reaction\textsuperscript{95} etc.), C-heteroatom bond formation (such as Buchwald-Hartwig reaction,\textsuperscript{96, 97} Ullmann reaction\textsuperscript{98, 99} etc.), ring-closing metathesis reaction,\textsuperscript{100, 101} heterocycle synthesis,\textsuperscript{102} as well as cycloaddition reactions,\textsuperscript{103, 104} and more.

![Microwave Reactor](image)

**Figure 1.4.** A microwave reactor from CEM.

1.5.2 Microwave-Assisted [2+2+2] Cyclotrimerization Reactions

The synthesis of pyridine derivatives via [2+2+2] cyclotrimerization reaction is usually performed under light-irradiation or with additives.\textsuperscript{13, 105, 106} To take the advantage of MAOS, our group and others have applied microwave irradiation to assist [2+2+2]
cyclotrimerization reactions. In 2005, Ley et al. reported a microwave-assisted non-metal-catalyzed intramolecular [2+2+2] cyclotrimerization reaction. When the triynes 187 were irradiated with microwaves using DMF as solvent, the intramolecular [2+2+2] cyclotrimerization reaction was promoted and the tricyclic products 188 were produced in good to excellent yield. Under similar conditions (200 °C, 1 h), the intramolecular cyclotrimerization reaction of the triyne 187 (X = O, n = 1) proceeded with 100% conversion under microwave irradiation, but only with 9% conversion when using conventional oil bath heating (Scheme 1.32). The mechanistic pathways might involve either a diradical species or a cyclobutadiene intermediate that may be generated at the high temperature.

Moreover, microwave irradiation has also been used to promote the transition-metal catalyzed [2+2+2] cyclotrimerization reactions. The first report of this reaction was published in 2006 by Hrdina et al. The bipyridines 190 were assembled via the
cycotrimerization reaction between diynes 189 with benzonitrile using CpCo(CO)\(_2\) as catalyst under microwave irradiation (Scheme 1.33).

\[
\begin{array}{ccc}
\text{N} & \text{PhCN} \\
\text{CpCo(CO)} \_2 & \text{MW 300 W} \\
& \text{20 min} \\
& \text{30-84%} \\
\end{array}
\]

\(X = \text{H, Me, CF}_3, \text{F, CN, OCH}_3\)

**Scheme 1.33.** The first microwave-assisted transition-metal-catalyzed cycotrimerization reaction.

After the first report by Hrdina et al., the microwave-assisted cycotrimerization reaction has been used in the synthesis of a variety of benzene and pyridine rings. In 2007, Zhou et al. applied both intermolecular and intramolecular cycotrimerization reactions with microwave irradiation to synthesize tetrahydro-napthyridines. The tetrahydro-1,6-napthyridines 193 were assemble from the intermolecular cycotrimerization reaction between alkyne-nitrile 191 and alyne 192 using Co catalyst under microwave irradiation. The intramolecular cycotrimerization reaction of 194 furnished tricyclic tetrahydro-2,5-napthyridine 195 in 80% yield (Scheme 1.34).
Scheme 1.34. The synthesis of tetrahydro-1,6-(or 2,5-naphthyridine via microwave-assisted cyclotrimerization reaction.

The Deiters lab has successfully applied microwave irradiation to mediate the [2+2+2] cyclotrimerization reaction to produce phenanthridines, triphenylenes and azatriphenylenes (Scheme 1.35). The diyne underwent a microwave-mediated cyclotrimerization reaction with the alkyne, delivering the tricyclic product, which was treated with CAN to furnish the phenanthridines. The synthesis of the triphenylene and azaphenylene analogs were achieved via the microwave-assisted cyclotrimerization reaction of the diyne and nitriles or alkyne.
Scheme 1.35. The synthesis of phenanthridines, triphenylenes, azatriphenylenes, and isoindolines.

Microwave irradiation has been used to assist solid-supported cyclotrimerization reaction towards the pyridines derivatives in our lab.\textsuperscript{10, 63} The microwave-assisted cyclotrimerization reaction of the immobilized diyne 203 with nitriles assembled the solid-supported pyridines 204 that were subsequently cleaved from the resin as 205a-c in 92-95% overall yield (Scheme 1.36). However, without microwave irradiation and under conventional heating, the cyclotrimerization only produced in less than 5% of 205a (Scheme 1.36). The immobilized alkyne-nitriles 206 underwent cyclotrimerization reactions with nitriles under microwave irradiation, followed by cleavage from the resin, affording the pyridines 208 in good to excellent yields. The pyridones 210 were also synthesized via a microwave-assisted solid-supported cyclotrimerization reaction of the immobilized diyne 203 (Scheme 1.36).\textsuperscript{10}
Scheme 1.36. Pyridine derivatives synthesis via solid-supported cyclotrimerization reactions

Microwave-assisted cyclotrimerization reactions have also been employed to assemble various pyridine derivatives by other groups.\textsuperscript{109, 110} Turek et al. reported the assembly of the 6-pyridylpurines 213 via a microwave-promoted cyclotrimerization reaction of diyne 211 and nitrile 212 (Scheme 1.37).\textsuperscript{109, 110} It was also noticed that this assembly was only accomplished under microwave irradiation.
Nicolaus et al.\textsuperscript{110} also used microwave irradiation to promote the intramolecular cyclotrimerization reaction of triynes and alkynylnitriles \textbf{214} to furnish 6-oxa-allocolchicinoids derivatives \textbf{215} (Scheme 1.38).

As shown above, microwave irradiation is another useful tool besides convection heat and light-irradiation to assist cyclotrimerization reactions towards the synthesis of complex carbo- and heterocyclic molecules. In addition, there are more reports on the synthesis of benzene and pyridine derivatives of interest, including natural products, via the microwave-assisted cyclotrimerization reaction.
CHAPTER 2: Pyridine Synthesis via Solid-Supported Cyclotrimerization Reaction

2.1 Introduction

Solid-supported reactions were first employed towards the synthesis of peptides by Merrifield in 1963.\textsuperscript{111} Since then, solid-supported chemistry has attracted considerable attention due to its rapid automatization, parallelization and ease of product purification.\textsuperscript{112-114} Moreover, solid-supported multicomponent reactions have been widely used in peptide synthesis.\textsuperscript{115, 116} Immobilization of a catalyst onto a polymer support is also attractive, since it allows the facile recovery of the catalyst for regeneration and reuse, enhancing the turnover of the catalyst, and thus affording a degree of “green chemistry”.\textsuperscript{117, 118}

Pyridine moieties are important in both the chemical and biological field.\textsuperscript{4} A variety of methods have been developed for the synthesis of pyridine rings,\textsuperscript{119, 120} however, there are limitations for these methods, especially when highly-substituted pyridine rings are required. One means of overcoming these limitations is by employing [2+2+2] cyclotrimerization reactions to construct highly-substituted benzene and pyridine rings.\textsuperscript{1, 2, 13, 121} This extremely efficient multicomponent reaction is also benefited from the wide range of the easily available alkyne and nitrile precursors. Overall, these features make the solid-supported [2+2+2] cyclotrimerization reaction a useful tool in the synthesis of benzene and pyridine libraries.\textsuperscript{9, 10, 122}

However, due to chemo- and regio-selectivity issues associated with the cyclotrimerization reactions,\textsuperscript{123} they have not been widely used. When two unique alkynes and one nitrile are employed in the cyclotrimerization reaction, a large number of possible products can be produced, including the desired pyridine ring products\textsuperscript{219} (and regioisomers...
the pyridine rings 223-226 (and regioisomers) generated from two equivalents of 216 (or two equivalents of 217) with the nitrile 218, benzene rings 227-230 and isomers generated from alkynes 216 and/or 217 (Scheme 2.1). This results in a large number of products that reduces the overall yield of the desired product and requires substantial purification. Moreover, the physical separation and identification of these products is problematic and prevents the reaction from being synthetically useful. One potential solution for the chemo- and region-selectivity issues is to apply solid-supported chemistry. One of the alkynes can be immobilized on a polystyrene resin, forming pseudo high dilution conditions. This prevents the formation of benzene or pyridine rings of the trimer or dimer of the immobilized alkyne.\textsuperscript{124} Moreover, the large size of the polymer linked to the alkyne may also direct the regioselectivity of the cyclotrimerization reaction due to steric effects.

\begin{Scheme}
\begin{center}
\includegraphics[width=\textwidth]{scheme2_1.png}
\end{center}
\end{Scheme}

\textbf{Scheme 2.1}. An example of intermolecular [2+2+2] cyclotrimerization reaction.
Previous studies on the solid-supported [2+2+2] cyclotrimerization reaction have been applied to the synthesis of 2,4,6-trisubstituted pyridines,\textsuperscript{124} fused pyridines\textsuperscript{10} and bicyclic benzene rings.\textsuperscript{9,122} The 2,4,6-trisubstituted pyridine was assembled using polymer-supported propargyl alcohol via a trityl linker. CpCo(CO)\textsubscript{2} was used as catalyst and the cyclotrimerization reaction was carried out in oil bath (80 °C) for 48 h. The bicyclic benzene and pyridine rings were furnished in a similar fashion via solid-supported cyclotrimerization reaction of immobilized diynes with nitriles or alkynes. The catalysts were switched to Wilkinson’s catalysts or Cp*ClRu(COD) to favor the benzene ring formation and the reactions were heated by either conventional heating (oil bath) or microwave irradiation. Both internal and terminal diynes were examined in the cyclotrimerization reaction to form benzene rings. However, only one report\textsuperscript{10} has shown such fused pyridine formation via solid-supported [2+2+2] cyclotrimerization reaction from immobilized diynes. Herein, the solid-supported cyclotrimerization reaction to assemble 2,4,6-trisubstituted pyridines and fused pyridines with the assistance of microwave irradiation are reported. Moreover, new strategies to address continuing issues associated with the regioselectivity of the reaction have been investigated.

2.2 Results and Discussion

Propargyl alcohol (84) was immobilized on a polystyrene resin (100-200 mesh 1% cross-linked), using an acid labile trityl linker following previous literature reports.\textsuperscript{124} Upon cleavage using 1% TFA in DCM followed by GC-MS analysis, the loading of the immobilized alcohol was determined to be 0.45 mmol/g. The solid-supported
cyclotrimerization reaction of immobilized propargyl alcohol was carried out with a set of alkynes 231 (10 eq.) and nitriles 232 (20 eq.) using CpCo(CO)$_2$ (Scheme 2.1) to assemble an array of pyridines 233. The reaction was conducted with microwave irradiation for 20 min and the resin was washed and dried. The pyridines were cleaved from the resin by stirring with 5% TFA/DCM at rt for 2 h.$^{124}$ As shown in the Scheme 2.2, 2,4,6-trisubstituted pyridines 233a-c were obtained together other regio-isomers. When both R$^1$ and R$^2$ were a phenyl group, a low yield of 53% was obtained (Scheme 2.2, entry 1), probably due to the self trimerization of phenylacetylene. When the R$^2$ was switched to a butyl group, a much better yield (86% yield when R$^1$ was Ph and 82% yield when R$^1$ was an ethyl group) was produced. While this serves as a route to 2,4,6-trisubstituted pyridines, issues still persist in the formation of undesirable regioisomers. The reaction yields were comparable to a literature report,$^{124}$ however, by using the microwave irradiation, the reaction time was greatly reduced, from 48 h to 20 min.

scheme 2.2. 2,4,6-Trisubstituted pyridine synthesis via cyclotrimerization reaction.
Tethering the two alkynes together, via a diyne greatly reduces the number of possible products formed in the cyclotrimerization reaction. However, it is still possible to form several undesired products in the solution-phase [2+2+2] cyclotrimerization reaction, including the byproducts from the dimerization or trimerization of the diynes. Immobilization represents a mechanism to resolve the chemoselectivity issues associated with the reaction. Diyne immobilization significantly suppresses benzene ring or pyridine ring formation from two diynes. Moreover, employing a less reactive internal diyne further prevents undesired dimer- and trimerization. Thus, the solid-supported cyclotrimerization reaction of an internal diyne immobilized with an acid labile trityl linker was investigated. The diyne was synthesized in two steps from commercially available tosylamine. The tosylamine was doubly alkylated with 1-bromo-2-butyne, delivering in 92% yield. Upon the removal of the tosyl group using sonication in the presence of magnesium turnings in MeOH, the diyne was obtained. The diyne was immobilized on the polystyrene resin, and the loading of was 1.40 mmol/g, as analyzed by weight and \(^1\)H NMR upon cleaving the diyne from the resin. The immobilized diyne was cyclotrimerized with a series of nitriles (25 eq) possessing a number of different functional groups, including alkene, aromatic, vinyl, ether as well as isocyanate groups. The cyclotrimerization reaction was carried out using CpCo(CO)\(_2\) as a catalyst and toluene as solvent with the assistant of microwave irradiation (300 W, 40 or 80 min). The formed pyridines were cleaved from the resin by stirring the resin with 2% TFA in DCM. As shown in Scheme 2.3, the solid-supported cyclotrimerization reaction of the diyne went smoothly; a 94-99% yield was obtained, when acetonitrile, propiononitrile, benzonitrile,
acronitrile, and 2-methoxyacetonitrile were used. Due to the symmetrical nature of the diyne, the regioselectivity of the reaction was not an issue. Excellent yields were observed in the solid-supported [2+2+2] cyclotrimerization reactions, even when internal diynes were used.

Scheme 2.3. The solid-supported cyclotrimerization reaction of the internal diyne 239.

Then the solid-supported cyclotrimerization reaction using an unsymmetrical diyne 246 was examined. The synthesis of the diyne 246 commenced with commercially available 242. The alkylation of 242 via a Mitsunobu reaction127 with but-3-yn-1-ol delivered 243 in 92% yield. Protecting group removal of 243 was achieved using TFA in DCM to give 244, which underwent a second Mitsunobu reaction with but-2-yn-1-ol, producing the tosylated diyne 245. The tosyl group was removed to yield the resulting diyne 246, which was then immobilized to the trityl resin in the presence of TFA in DCM. The cyclotrimerization reactions of the immobilized diyne 247 with nitriles were conducted with CpCo(CO)₂ under
microwave irradiation (300 W, 40 min). Upon stirring the resin with 2% TFA in DCM, the bicyclic pyridines 248 and 249 were cleaved from the resin and analyzed by $^1$H NMR and mass spectrometry. It was found that the solid-supported cyclotrimerization of the diyne 247 was successful, as almost quantitative yields were obtained when acetonitrile, propiononitrile, benzonitrile, acronitrile and 2-methoxyacetonitrile were used. Two different regioisomers were generated, favoring 248 with the nitrogen atom on the pyridine ring next to the more sterically hindered position, determined by $^1$H NMR. The greatest regioselectivity was observed with acetonitrile (92:8), while the lowest regioselectivity was detected for 2-methoxyacetonitrile (78:22) (Figure 2.4). Usually, when the unsymmetric diynes were used to form pyridine rings via cyclotrimerization reactions, the steric effect is one of the main effects to direct the selectivity, leading to the pyridine with the bulkier substitutions next to nitrogen atom.$^1$ Hence, the bulkier phenyl group shows better selectivity than vinyl group and methoxymethyl group. It is unclear why the methyl and ethyl have higher selectivity than more bulky phenyl group, an unproven possibility could be complexation of the metal center by the methoxy group. Thus, good regioselectivities could be obtained from the solid-supported cyclotrimerization reactions when unsymmetrical diynes were used. These results demonstrate the possibility to utilize substrate control to obtain chemo- and regioselectivity.
Scheme 2.4. The solid-supported cyclotrimerization reaction of the diyne 247.

2.3 Summary and Outlook

In summary, several 2,3,6-trisubstituted pyridines and a number of bicyclic pyridines were successfully synthesized by using solid-supported [2+2+2] cyclotrimerization reactions. Excellent reaction yields were accomplished even when internal diynes were used. The microwave irradiation was applied to assist the cyclotrimerization reactions to achieve good to excellent yields in a much shorter time. It was shown that immobilizing the alkynes or diynes on the polymer resin formed “pseudo high dilution concentration” greatly reduced the side reactions.
Future work will focus on the improvement of the regioselectivity of the solid-supported cyclotrimerization reaction. Some functional groups, such as silyl groups and boron groups, which can be removed or converted into other functional groups in a “traceless” fashion, can be used as a regio-directing groups in the solid-supported cyclotrimerization reactions.\textsuperscript{125, 128} The solid-supported cyclotrimerization reaction could be applied to the synthesis of a library of benzene and pyridine ring containing molecules with pharmaceutical and biological activities. Furthermore, the solid-supported cyclotrimerization reaction can be applied in the synthesis of natural products. While significant advances have been made, the utility of this reaction can be dramatically expanded by completely solving the associated chemo- and regioselectivity issues.

2.4 Experimental

All reactions were performed in flame-dried glassware under a nitrogen atmosphere and stirred magnetically unless indicated. Chemicals were used directly from commercial sources without further purification unless indicated. Solvents were distilled and stored with molecular sieves (3 Å for methanol and ethanol and 4 Å for all other solvents) prior to use. Toluene, xylene, dioxane were distilled from sodium/benzophenoneketyl. TEA, DIPEA, DMSO, DMF, DCE, CH$_3$CN and pyridine were distilled from calcium hydride. Methanol and ethanol were distilled from magnesium and iodole. CH$_2$Cl$_2$, THF and ether were dried by MB SPS Compact solvent purification system. All other reagent quality solvents were used without further purification. The microwave reactions were performed in a sealed microwave tube in a CEM Discover microwave synthesizer at 300 W. $^1$H and $^{13}$C NMR spectra were
performed using a Varian Mercury (300 MHz and 400 MHz). Mass spectrometry was performed by North Carolina State University facilities.

**N-(But-2-ynyl)-N-tosylbut-2-yn-1-amine (236).** Tosylamide (234, 65.0 mg, 0.38 mmol) was dissolved in DMF (2 mL) and the reaction mixture was cooled to 0 °C. NaH (60% in mineral oil, 45.6 mg, 1.14 mmol) and 1-bromo-2-butyn (100 µL, 1.14 mmol) were added slowly and the reaction mixture was stirred while warming to rt for 1 h. The reaction was quenched with H₂O (2 mL) and extracted with ether (3 x 2 mL). The combined organic layers were washed with brine (5 mL), dried over Na₂SO₄, filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc (3:1) to give 236 (96.0 mg, 92%) as a white solid. ¹H NMR data matched the literature report.¹²⁹

**General procedure for the Mitsunobu reaction.** Alcohol (1 eq) was dissolved in THF (8 mL/mmol). PPh₃ (1 eq) and tosylamide (1 eq) were added, followed by the addition of DIAD (1 eq). The reaction mixture was stirred at rt overnight and ether was added to dilute the reaction mixture. The mixture was washed with H₂O (8 ml/mmol), dried over Na₂SO₄, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with EtOAc/hexanes mixture to give the desired product.

**tert-Butyl N-(but-3-yn-1-yl)-N-(4-methylbenzenesulfonyl)carbamate (243).** The compound was eluted with hexanes/EtOAc (10:1) to give 243 as a white solid in 92% yield. ¹H NMR data matched the literature report.¹³⁰
**N-(But-2-ynyl)-N-tosylbut-3-yn-1-amine (246).** The compound was eluted with hexanes/EtOAc (8:1, 4:1) to give 246 as a white solid in 63% yield. $^1$H NMR (300 MHz, CDCl$_3$) δ 7.72 (d, $J = 8.3$ Hz, 2H), 7.27 (d, $J = 7.9$ Hz, 1H), 4.17 – 4.06 (m, 3H), 3.33 (dd, $J = 7.9$, 7.0 Hz, 2H), 2.49 (ddd, $J = 8.1$, 6.9, 2.7 Hz, 2H), 2.40 (s, 3H), 1.98 (t, $J = 2.7$ Hz, 1H), 1.56 (t, $J = 2.4$ Hz, 4H).

**N-Tosylbut-3-yn-1-amine (244).** The amide 243 (0.730 g, 2.26 mmol) was dissolved in DCM (22 mL) and TFA (0.86 mL, 11.30 mmol) was added dropwise. The reaction mixture was stirred at rt over night. EtOAc (50 mL) was added and the resulting mixture was washed with saturated NaHCO$_3$ (20 mL) and H$_2$O (20 mL). The organic layer was dried over Na$_2$SO$_4$, filtered and concentrated in vacuo to give 244 (0.475 g, 92%) as a white solid. $^1$H NMR data matched the literature report.$^{130}$

**General procedure for tosyl removal.** The tosyl compound (1 eq) was dissolved in MeOH (33 mL/mmol). Magnesium turnings (5 eq) was added and the reaction mixture was sonicated for 180 min (small scale required shorter time). The MeOH was removed under reduced pressure, 1 N HCl (20 mL/mmol) and DCM (20 mL/mmol) were added, and the two layers were separated. The aqueous layer was extracted with DCM (3 x 20 mL). 1 N NaOH solution was added to the aqueous layers till pH ~9 and then extracted with DCM. The combined organic layers were washed with brine, dried over Na$_2$SO$_4$, filtered and concentrated in vacuo to give the corresponding amines.

**Bis(but-2-ynyl)amine (237).** The compound was synthesized in 90% yield as a colorless liquid. $^1$H NMR (300 MHz, CDCl$_3$) δ 3.41 (q, $J = 2.4$ Hz, 4H), 1.79 (dd, $J = 2.7$, 2.1 Hz, 6H).
**N-(But-2-ynyl)but-3-yn-1-amine (246).** The compound was synthesized in 74% yield as a colorless liquid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 3.38 (q, $J = 2.4$ Hz, 2H), 2.82 (t, $J = 6.6$ Hz, 2H), 2.38 (td, $J = 6.6$, 2.6 Hz, 2H), 1.98 (t, $J = 2.6$ Hz, 1H), 1.80 (t, $J = 2.4$ Hz, 3H).

**General procedure for immobilizing an amine on a trityl resin.** Resin (1 eq) was placed in a flame-dried vial, DCM (3.7 mL/mmol) was added, and the vial was sealed and shaken for 15 min. The amine (~1 eq) and TEA (10 eq) were added and the resulting mixture was shaken at rt over night. The resin was filtered and washed with DCM (3.7 mL/mmol) followed by MeOH (3.7 ml/mmol) four times, with a final wash of DCM (3.7 mL/mmol). The resin was dried *in vacuo* and stored in a desiccator.

**General procedure for analyzing the loading of the immobilized amine.** The resin (40-100 mg scale) was placed in a dried vial and 2% TFA in DCM (40 ml/g) was added. The mixture was allowed to swell at rt for 1 h and was filtered and washed with DCM (10 mL/g). The filtrate was concentrated and dried *in vacuo* to give the amine-TFA salt. The loading of the immobilized amine was calculated based on the amount of used immobilized amine and the amount of obtained amine-TFA salt.

**General procedure for solid-supported [2+2+2] cyclotrimerization reactions.** The immobilized diyne (40-100 mg scale, 1 eq) and the nitrile (25 eq) were suspended in toluene (12.5 ml/mmol) in a flame-dried microwave vial. CpCo(CO)$_2$ (0.2 eq) was added and the microwave vial was sealed and heated in a CEM microwave reactor (300 W) for 40 (or 80) min. After cooling to rt, the resin was filtered and washed (with DCM (30 mL/g) then MeOH (30 mL/g)) four times and finally with DCM. The resin was dried *in vacuo* and was
transferred to a flame-dried vial and 2% TFA in DCM (~10 eq) was added, and the mixture was shaken for 1 h. The resin was filtered and washed with DCM (1 x 60 mL/g). The filtrate was concentrated in vacuo to give the desired pyridines.

2,3-Dihydro-4,7-dimethyl-6-methyl-1H-pyrrolo[3,4-c]pyridinium trifluoroacetic acid salt (241a). 99% yield. $^1$H NMR (300 MHz, CD$_3$OD) δ 4.87 (s, 2H), 4.80 (s, 2H), 2.68 (s, 3H), 2.63 (s, 3H), 2.34 (s, 3H). HRMS calcd. for M$^+$ C$_{10}$H$_{15}$N$_2^+$ 163.1230, found 163.1232.

2,3-Dihydro-4,7-dimethyl-6-ethyl-1H-pyrrolo[3,4-c]pyridinium trifluoroacetic acid salt (241b). 94% yield. $^1$H NMR (300 MHz, CD$_3$OD) δ 4.87 (s, 2H), 4.80 (s, 2H), 3.03 (q, J = 7.6 Hz, 2H), 2.64 (s, 3H), 2.38 (s, 3H), 1.29 (t, J = 7.6 Hz, 3H). HRMS calcd. for M$^+$ C$_{11}$H$_{17}$N$_2^+$ 177.1386, found 177.1393.

2,3-Dihydro-4,7-dimethyl-6-phenyl-1H-pyrrolo[3,4-c]pyridinium trifluoroacetic acid salt (241c). 99% yield. $^1$H NMR (300 MHz, CD$_3$OD) δ 7.84 – 7.36 (m, 5H), 4.93 (s, 2H), 4.89 (s, 2H), 2.68 (s, 3H), 2.28 (s, 3H). HRMS calcd. for M$^+$ C$_{15}$H$_{17}$N$_2^+$ 225.1386, found 225.1392.

2,3-Dihydro-4,7-dimethyl-6-vinyl-1H-pyrrolo[3,4-c]pyridinium trifluoroacetic acid salt (241d). 98% yield. $^1$H NMR (300 MHz, CD$_3$OD) δ 7.04 (dd, J = 17.5, 11.6 Hz, 1H), 6.23 (dd, J = 17.5, 1.9 Hz, 1H), 5.99 (d, J = 11.5 Hz, 1H), 4.84 (s, 2H), 4.79 (s, 2H), 3.92 (s, 1H), 2.64 (s, 3H), 2.36 (s, 3H). HRMS calcd. for M$^+$ C$_{11}$H$_{15}$N$_2^+$ 175.1230, found 175.1232.

2,3-Dihydro-6-(methoxymethyl)-4,7-dimethyl-1H-pyrrolo[3,4-c]pyridinium trifluoroacetic acid salt (241e). 98% yield. $^1$H NMR (400 MHz, CD$_3$OD) δ 4.87 (s, 2H), 4.81 (s, 2H), 4.79 – 4.73 (m, 2H), 3.52 (d, J = 1.4 Hz, 3H), 2.67 (d, J = 1.2 Hz, 3H), 2.35 (s, 3H). HRMS calcd. for M$^+$ C$_{11}$H$_{17}$N$_2$O$^+$ 193.1335, found 193.1338.
1,2,3,4-Tetrahydro-6,8-dimethyl-2,7-naphthyridinium trifluoroacetic acid salt (248a) and 1,2,3,4-tetrahydro-7,8-dimethyl-2,6-naphthyridinium trifluoroacetic acid salt (248a). HRMS calcd. for M$^+$ C$_{10}$H$_{15}$N$_2$ $^+$ 163.123, found 163.1226. The ratio of major/minor products was 92:8, determined by the NMR integration. Major product (248a): $^1$H NMR (300 MHz, CD$_3$OD) δ 7.53 (s, 1H), 4.37 (s, 2H), 3.48 (t, J = 6.2 Hz, 2H), 3.23 (t, J = 6.2 Hz, 2H), 2.62 (s, 3H), 2.57 (s, 3H).

Minor product (249a): $^1$H NMR (300 MHz, CD$_3$OD) δ 8.45 (s, 1H), 4.52 (s, 2H), 4.37 (s, 2H), 3.48 (t, J = 6.2 Hz, 2H), 3.23 (t, J = 6.2 Hz, 2H), 2.62 (s, 3H), 2.57 (s, 3H).

6-Ethyl-1,2,3,4-tetrahydro-8-methyl-2,7-naphthyridinium trifluoroacetic acid salt (248b) and 7-ethyl-1,2,3,4-tetrahydro-8-methyl-2,6-naphthyridinium trifluoroacetic acid salt (249b). HRMS calcd. for M$^+$ C$_{11}$H$_{17}$N$_2$ $^+$ 177.1386, found 177.1380. The ratio of major/minor products was 91:9, determined by the NMR integration. Major product (248b): $^1$H NMR (major product, 300 MHz, CD$_3$OD) δ 7.58 (s, 1H), 4.37 (s, 2H), 3.48 (t, J = 6.2 Hz, 2H), 2.91 (q, J = 8.0 Hz, 2H), 2.58 (s, 3H), 1.29 (t, J = 7.7 Hz, 3H).

Minor product (249b): $^1$H NMR (minor product, 300 MHz, CD$_3$OD) δ 8.46 (s, 1H), 4.51 (s, 2H), 3.48 (t, J = 6.2 Hz, 2H), 3.26 (d, J = 8.1 Hz, 2H), 3.00 (q, J = 7.5 Hz, 2H), 2.58 (s, 3H), 1.29 (t, J = 7.7 Hz, 3H).

1,2,3,4-Tetrahydro-8-methyl-6-vinyl-2,7-naphthyridinium trifluoroacetic acid salt (248c) 1,2,3,4-tetrahydro-8-methyl-7-vinyl-2,6-naphthyridinium trifluoroacetic acid salt (249c). HRMS calcd. for M$^+$ C$_{11}$H$_{15}$N$_2$ $^+$ 175.1230, found 175.1232. The ratio of major/minor products was 86:14, determined by the NMR integration. Major product (248c): $^1$H NMR (300 MHz, CD$_3$OD) δ 7.91 (s, 1H), 6.86 (ddd, J = 17.6, 11.2, 1.4 Hz, 1H), 6.44 (dd, J = 17.5,
1.5 Hz, 1H), 5.92 (dd, $J = 11.2, 1.5$ Hz, 1H), 4.41 (s, 2H), 3.52 (td, $J = 6.3, 1.4$ Hz, 2H), 3.29 (t, $J = 6.4$ Hz, 2H), 2.60 (s, 17H). Minor product (249c): $^1$H NMR (300 MHz, CD$_3$OD) δ 8.50 (s, 1H), 7.21 – 7.04 (m, 1H), 6.16 (dd, $J = 17.4, 1.5$ Hz, 1H), 5.78 (dd, $J = 18.7, 1.7$ Hz, 1H), 4.52 (s, 2H), 3.52 (td, $J = 6.3, 1.4$ Hz, 2H), 3.29 (d, $J = 6.4$ Hz, 2H), 2.33 (s, 3H).

1,2,3,4-Tetrahydro-8-methyl-6-phenyl-2,7-naphthyridinium trifluoroacetic acid salt (248d) and 1,2,3,4-tetrahydro-8-methyl-7-phenyl-2,6-naphthyridinium trifluoroacetic acid salt (249d). HRMS calcd. for M$^+$ C$_{15}$H$_{17}$N$_2$+ 225.1386, found 225.1388. The ratio of major/minor products was 82:18, determined by the NMR integration. Major product (248d): $^1$H NMR (300 MHz, CD$_3$OD) δ 7.86 (s, 1H), 7.85 – 7.76 (m, 2H), 7.62 – 7.52 (m, 3H), 4.43 (s, 2H), 3.52 (t, $J = 6.2$ Hz, 2H), 3.37 – 3.27 (m, 2H), 2.65 (s, 3H). Minor product (249d): $^1$H NMR (300 MHz, CD$_3$OD) δ 8.57 (s, 1H), 7.85 – 7.76 (m, 2H), 7.50 (ddd, $J = 8.6, 4.8, 1.7$ Hz, 3H), 4.55 (s, 2H), 3.52 (t, $J = 6.2$ Hz, 3H), 3.37 – 3.27 (m, 3H), 2.23 (s, 3H).

1,2,3,4-Tetrahydro-6-(methoxymethyl)-8-methyl-2,7-naphthyridinium trifluoroacetic acid salt (248e) and 1,2,3,4-tetrahydro-7-(methoxymethyl)-8-methyl-2,6-naphthyridinium trifluoroacetic acid salt (249e). HRMS calcd. for M$^+$ C$_{11}$H$_{17}$N$_2$O$^+$ 193.1335, found 193.1338. The ratio of major/minor products was 78:22, determined by the NMR integration. Major product (248e): $^1$H NMR (300 MHz, CD$_3$OD) δ 7.70 (s, 1H), 4.68 (s, 2H), 4.46 (s, 2H), 3.56 (dd, $J = 7.2, 5.4$ Hz, 2H), 3.49 (s, 3H), 3.39 – 3.24 (m, 2H), 2.66 (s, 3H). Minor product (249e): $^1$H NMR (300 MHz, CD$_3$OD) δ 8.53 (s, 1H), 4.85 (s, 2H), 4.60 (s, 2H), 3.56 (dd, $J = 7.2, 5.4$ Hz, 2H), 3.49 (s, 3H), 3.39 – 3.24 (m, 2H), 2.29 (s, 3H).
CHAPTER 3: Synthesis of Anthracene and Azaanthracene Fluorophores via [2+2+2] Cyclotrimerization Reactions

3.1 Introduction

In recent years, anthracene derivatives have attracted increasing attention due to their wide range of applications, making them an important class of polycyclic aromatic compounds. They have intrinsic fluorescence and play a key role in the design of luminescent supramolecular materials. Moreover, they have been used in a number of practical applications, such as optical devices, potential therapeutics, polymeric materials, and as imaging agents for cellular processes. Anthracene derivatives possess photochromic properties that are useful in the design of molecular switches and environmentally sensitive chemosensors. Substituted anthracenes have also been employed in the synthesis of molecular models for the study of bonding interactions.

A number of methods have been developed for the synthesis of anthracenes, such as the Elbs reaction, aromatic cyclodehydration, Lewis acid-catalyzed Bradsher-type reactions using o-diarylmethanes, Friedel-Crafts reactions, [4+2] cycloaddition reactions, and Lewis acid-catalyzed benzannulation. Moreover, cyclotrimerization reactions catalyzed by transition metals, such as Zr and Pd, have been reported as well. However, compared to the vast number of methods employed in the syntheses of anthracenes, only a limited number of methods for the assembly of azaanthracenes have been reported.
3.2 Results and Discussion

The application of the [2+2+2] cyclotrimerization reaction to the rapid assembly of fluorophores based on an anthracene and an azaanthracene scaffold is described. Although several synthetic routes to anthracenes have been reported, the synthesis of 2-azaanthracenes is an undeveloped field. Moreover, it was discovered that the synthesized 2-azaanthracenes have very unique fluorescent properties in contrast to regular anthracenes.

The synthesis of anthracene and azaanthracene commenced with the known 1,2-di(prop-2-ynyl)benzene (252). The 1,2-di(prop-2-ynyl)benzene (252) was synthesized in three steps from commercially available o-xylene dibromide (250) according to literature procedure. o-Xylene dibromide (250) was converted to o-xylene diiodide (251) via Finkelstain reaction in 98% yield. The diiodide 251 was subsequently reacted with [2-(bromomagnesio)ethynyl]trimethylsilane in the presence of CuCl, followed by the TMS deprotection, delivering the 1,2-di(prop-2-ynyl)benzene (252) in 74% yield. The diyne 252 was cyclotrimerized with a set of six alkynes containing alkyl chains, benzenes, hydroxy groups, nitriles, and imides. These reactions were conducted with 10 mol% (PPh$_3$)$_2$Ni(CO)$_2$ in toluene at 120 °C under microwave irradiation (300W) in 10 min, delivering the tricyclic compounds 253-258 in 66-86% yield (Scheme 3.1). Byproducts observed in some reactions resulted from the dimerization of 252, as well as cyclotrimerization of 252 with two mono-alkyne molecules. The tricyclic compounds 253-258 underwent a rapid microwave-assisted oxidation with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ), generating the anthracenes 259-264 in yields of 70-79% (Scheme 3.1). This two-step procedure provides a flexible and facile approach to the introduction of an anthracene moiety into a wide range of alkynes.
We subsequently investigated the feasibility of this route towards the synthesis of 2-azaanthracenes, a mostly unexplored class of compounds. The diyne 252 was reacted with nitriles bearing a variety of functional groups, including alkyl and alkyne chains, hydroxy groups, benzene, and pyridine rings. The reactions were conducted under CpCo(CO)$_2$ catalysis in toluene using microwave irradiation (300W) delivering the cyclotrimerization products 265-270 in 80-94%. The change in catalyst system was necessary in order to achieve cyclotrimerization reactions towards pyridines.$^{7e-k}$ The subsequent DDQ oxidation step proceeded smoothly and yielded the azaanthracenes 271-276 in 53-85% yield. In order to investigate the effects of a permanently positively charged nitrogen center on the fluorescent properties of azaanthracenes, and in order to increase their solubility in an

\[
\begin{align*}
\text{Scheme 3.1. The synthesis of anthracene } & \text{259-264.}
\end{align*}
\]
aqueous environment, we methylated the azaanthracenes 271-276 in neat methyl iodide at 60 °C to obtain the salts 277-282 in quantitative yields (Scheme 3.2).

![Scheme 3.2. Azanthracene synthesis via [2+2+2] cyclotrimerization.](image)

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<th>compd</th>
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<td>81%</td>
<td>282</td>
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*Pyr = pyridine.

A prominent feature of anthracenes is their intrinsic fluorescence,¹³¹,¹³² a property which is largely unexplored in case of the corresponding 2-azaanthracenes. The developed array approach to both compound classes prompted the investigation of the fluorescent properties of the synthesized molecules, since combinatorial approaches to fluorophores have been succesfully applied to biological imaging problems.¹⁷⁰-¹⁷² Their application as environmentally sensitive probes was very attractive, and therefore the dependence of their fluorescent spectra on a) the polarity of the solvent, b) the pH of the solvent, and c) the presence of different metal cations was investigated. These experiments were conducted in a 96-well format.
The absorbance spectra of the azaanthracenes and azatriphenylene were recorded (Figure 3.1). In general, the azaanthracenes and quaternized azaanthracenes show similar absorption patterns, even under different solvent conditions. Absorption maxima at 250 nm and 265 nm, and a weaker absorbance at 350 nm were observed. A wavelength of 350 nm was selected as the excitation wavelength as it provided the most reproducible fluorescence data across all fluorophores tested, and since it represents a common excitation wavelength in laboratory instruments.

![Absorption spectra of the representative azaanthracene 271.](image)

**Figure 3.1.** Absorption spectra of the representative azaanthracene 271.

Generally, adding electron-donating group substitution to aromatic rings induces an increase in the molar absorption coefficient. The lone pairs are involved directly in the π bonding with the π electrons of the aromatic system causing a significant intramolecular charge transfer character of the molecules. Thus, in order to increase the fluorescence emission of the 2-azaanthracenes, we introduced two methoxy groups (Scheme 3.3).
The synthesis of the azaanthracenes 296-307 started with the precursor diyne 289. The diyne 289 was synthesized from commercially available dimethoxybenzene (283) in 4 steps (Scheme 3.3). The dimethoxybenzene 283 was transformed to bis(bromomethyl)-4,5-dimethoxybenzene (285) according to the literature report.\textsuperscript{173} A Finkelstein reaction converted the bis(bromomethyl)-4,5-dimethoxybenzene (284) to the diiodomethylbenzene 285 in excellent yield. Compound 285 was treated with trimethylsilylethynylmagnesium bromide (286), providing the two products 287 and 288 in 38\% and 41\% yield, respectively. The phenol 287 was converted into 288 through methylation with MeI in the presence of Cs$_2$CO$_3$ in DMF. Both TMS groups of 288 were removed, yielding the cyclotrimerization precursor 289 in 86\% yield. The diyne 289 underwent efficient \([2+2+2]\) cyclotrimerization reactions with a series of nitriles bearing different functional groups such as alkyl and alkene chains, aromatic rings, and hydroxyl groups. The reactions used CpCo(CO)$_2$ as the catalyst and toluene as the solvent with the assistance of microwave irradiation (300 W), delivering the cyclotrimerization products 290-295 in 84-92\% yield. The cyclotrimerization products were immediately oxidized with DDQ in toluene under microwave irradiation, yielding the azaanthracenes 296-301 in 52-90\% yield. The nitrogen center of the azaanthracenes 296-301 was quaternized with methyl iodide, generating the salts 302-307, in order to investigate the effect of the positive charge on the fluorescent properties of the azaanthracenes.

While the anthracenes 259-264 did not show any significant changes in fluorescence under different conditions, many of the azaanthracenes 271-282 exhibited environmental sensitivity (Figure 3.2a). This can be explained by the ability of the nitrogen center to undergo coordination to the solvent, to protons, and to metal ions. Moreover, the azaanthracenes 271-282 exhibited generally higher fluorescence levels than the anthracenes 259-264 (Figure 3.2). A significant change in fluorescence intensity was observed for most of the azaanthracenes 271-276 between protic (H₂O at pH 4-10) and aprotic (DMSO)
solvents (Figure 3.2d). More drastic changes in fluorescence emission were visually observed in other non-polar solvents such as CH₂Cl₂ and toluene (Figure 3.2b and c); however, these solvents were incompatible with the 96-well microtiter plates employed in the fluorescence measurements. This phenomenon has previously been investigated for 2-azaanthracene 271, finding that a polar solvent causes a broadening of the $\pi \rightarrow \pi_1^*$ energy levels resulting in a bathochromic shift.¹⁴ A loss of solvent sensitivity was observed upon quaternization of the nitrogen center in 271-276, and a moderate broadening of the emission spectrum occurred when compared to the non-quaternized analogs (Figure 3.3a). Additionally, a visible bathochromic shift in emission could be detected upon quaternization from blue to green (see compounds 271-276 and 277-282 in Figure 3.4a). At low pH (<4), protonation of the azaanthracenes 271-276 lead to a general bathochromic shift (~480 to 520 nm) of their fluorescence emission spectra, similar to their quaternized analogs 277-282 (Figure 3.3b). No changes in fluorescence were observed between pH 7-10.
Figure 3.2. Fluorescence in different solvents of (a) anthracene 260, (b) azaanthracene 275 and (c) quaternized azaanthracene 281, (d) azaanthracenes 271-282 in a 96 well plate, upon excitation at 365 nm.

Fluorescence measurements were conducted in a 96-well format in order to investigate a wide range of conditions. Compounds 271-282 and 296-307 were excited at 350 nm and the representative fluorescence emission spectra are shown in Figure 3.3. The fluorescent properties of the azaanthracenes showed significant dependence on the polarity of the solvents, pH and cation ions. In general, compounds 271-276 and 296-301 share similar emission patterns, as do compounds 277-282 and 302-307 (Figure 3.3 a-d). The
azaanthracenes 271-276 and 296-301, display a bathochromic shift in their emission spectra when switched from an aprotic solvent (DMSO) to a protic solvent (H₂O) (Figure 3.4e). The bathochromic shift in polar solvents is probably due to the lowering of the π* energy levels caused by the polar solvents. However, for the quaternized azaanthracenes 277-282 and 302-307, the difference in fluorescence intensity and emission wavelength was not observed (Figure 3c and d). The loss of solvent sensitivity upon quaternization is most likely caused by the loss of hydrogen bonding ability to solvents by the nitrogen center.
Figure 3.3. Fluorescence emission spectra of a. representative azaanthracene 271; b. representative azaanthracene 296; c. representative quaternized azaanthracene 277; d. representative quaternized azaanthracene 302, in different solvent environments (pH 4, pH 7, pH 10, DMSO, MeOH, Cu$^{2+}$, Mg$^{2+}$, Zn$^{2+}$). All fluorophores were excited at 350 nm; and e. fluorescence spectra of 271 and 277 demonstrating the effect of quaterinization on the fluorescence properties in DMSO and H$_2$O.

By comparing the emission spectra of the azaanthracenes 271-282 and 296-307, it is found that at pH 4, a red shift in emission was observed and the intensity was much lower than at pH 7 or 10 for azaanthracenes 271-276 (Figure 3.4a). At pH 4, azaanthracenes 296-301 have similar bathochromic shifts in emission as 271-276 (Figure 3.4a). This red shift is presumably caused by the protonation of the nitrogen center of the azaanthracenes, similar to
the quaternized azaanthracenes 277-282. However, at pH 4, an increase (compared to pH 7 and pH 10) in the fluorescence intensity was observed in the emission spectra for compounds 271-276. At pH 4, the azaanthracenes 271-276 and 296-301 are positively charged. The two methoxy groups on the azaanthracenes 296-301 help stabilize the positive charge via resonance structure, thus increasing the fluorescence intensity. The difference in fluorescence intensity and emission wavelength between pH 7 and pH 10 is negligible (Figure 3.4a).

Generally, compounds 296-301 show higher fluorescence intensities than compounds 271-276, and compounds 302-307 have higher intensities than compounds 277-282. This indicates that the addition of the two electron-donating groups to the azaanthracene rings increases the fluorescence intensity. While compound 271-276 and 296-301 have similar emission maximum wavelengths, compounds 27-32 show a red shift compared to compounds 277-282 on the emission spectra (Figure 3.4b). As expected, the azaanthracene 271-276 and 296-301 show sensitivity to different cations (Figure 5c). A red shift (from 460 nm to 500 nm) and a lower fluorescence intensity are observed, indicating the binding ability of the nitrogen center of the azaanthracene to cations. The fluorescence intensity for compound 276 in the emission spectrum is pH 7 > Zn^{2+} ≈ Mg^{2+} > Cu^{2+} showing that the binding ability of cations to the nitrogen center is Cu^{2+} > Mg^{2+} ≈ Zn^{2+} (Figure 3.4c), since binding fluorophores to metal ions would induce fluorescence quenching.174
Figure 3.4. Fluorescence emission spectra (with excitation at 350 nm) of a. azaanthracenes 272 and 297 in pH 4, pH 7, and pH 10 solutions; b. azaanthracenes 272, 278, 297, and 303 in DMSO and MeOH; c. azaanthracene 276 in the presence of different metal ions.

Then the fluorescence of different compounds in the same solution was analyzed. The represent fluorescent spectra are shown in Figure 3.5. At pH 4, compounds 271-282 show emission wavelengths at ~500 nm (Figure 3.5a), indicating that protonation of azaanthracene at low pH has a similar effect as quaternizing azaanthracene. A higher fluorescence intensity is shown for the quaternized azaanthracenes 277-282 than the corresponding azaanthracenes 271-276. At pH 7, pH 10, Cu$^{2+}$, Mg$^{2+}$, and Zn$^{2+}$, a red shift is detected after quaternizing the azaanthracenes 271-276. However, such shift is not observed for azaanthracenes 296-301 (Figure 3.5b). This is probably caused by the electron-donating groups leading to more bathochromic shift of the azaanthracenes than that of quaternized azaanthracenes. In DMSO and MeOH, the red shift after quaternization exists in both azaanthracenes 271-276 and 296-
Interestingly, while for all other compounds in all other conditions there is only one fluorescence peak, compounds 296-301 show two fluorescence peaks (~450 and 520 nm) in MeOH in the emission spectra (Figure 3.5f). This is probably due to the large orientation polarity of the methanol.  

**Figure 3.5.** Fluorescence emission spectra (with excitation at 350 nm) of a. compounds 271-282 in pH 4; b. compounds 296-307 in pH 4; c. compounds 271-282 in DMSO; d. compounds 296-307 in DMSO; e. compounds 271-282 in MeOH; f. compound 296-307 in MeOH.
3.3 Conclusion and Outlook

The transition metal-catalyzed [2+2+2] cyclotrimerization reaction was successfully used to assemble azaanthracenes and azatriphenylene s in a rapid and efficient way. Utilizing microwave irradiation to accelerate the cyclotrimerization reaction, various azaanthracenes with distinct fluorescent properties can be rapidly accessed. The fluorescent properties of the azaanthracene derivatives are modulated in different environments exhibiting solvodynamic shifts in wavelength and intensity. Additionally, a change was observed in the emission spectra for the pyridine substituted azaanthracene, indicating the sensitivity of the azaanthracene to different metal ions. However, quaternization of azaanthracenes results in loss of sensitivity to the solvent environment. Addition of electron-donating groups to the azaanthracenes induces an increase in fluorescent intensity and a shift in emission wavelength. The azaanthracenes may have practical applications as sensors for pH and metals. The distinct fluorescent properties and ability to modulate these properties via the use of different substituents will have wide applications in fluorescent imaging, localization of protein probes, and potential therapeutics.

3.4 Experimental

All reactions were performed in flame-dried glassware under a nitrogen atmosphere and stirred magnetically unless indicated. Chemicals were used directly from commercial sources without further purification unless indicated. Solvents were distilled and stored with molecular sieves (3 Å for methanol and ethanol and 4 Å for all other solvents) prior to use. Toluene, xylene, dioxane were distilled from sodium/benzophenone ketyl. TEA, DIPEA,
DMSO, DMF, DCE, CH₃CN and pyridine were distilled from calcium hydride. Methanol and ethanol were distilled from Magnesium and iodole. CH₂Cl₂, THF and ether were dried by MB SPS Compact solvent purification system. All other reagent quality solvents were used without further purification. ¹H and ¹³C NMR spectra were performed using a Varian Mercury (300 MHz and 400 MHz). Mass spectra analysis was performed by North Carolina State University facilities.

**1,2-Dimethoxy-4,5-bis(3-(trimethylsilyl)prop-2-ynyl)benzene** (288).

Ethylmagnesium bromide (40% in ether, 1.72 mL, 5.17 mmol) was added dropwise to a solution of trimethylsilyl acetylene (0.74 mL, 5.17 mmol) in THF (5 ml). The resulting mixture was heated to 40 °C for 1 h. After cooling to rt, CuCl (64 mg, 0.65 mmol) and a solution of 1,2-bis(iodomethyl)-4,5-dimethoxybenzene (285) (0.540g, 1.29 mmol) in 5 mL of THF were added and the mixture was heated under reflux overnight. After cooling to rt, saturated NH₄Cl (10 mL) was added and the two layers were separated. The aqueous layer was extracted with ether (3 x 10 mL). The combined organic layers were washed with 10 mL of H₂O and 10 mL of brine, dried over anhydrous Na₂SO₄, filtered, concentrated in vacuo, and the product was purified by column chromatography, eluting with hexanes : ethyl acetate (10 :1) to give the desired product 1,2-dimethoxy-4,5-bis(3-(trimethylsilyl)prop-2-ynyl)benzene (288) (0.177 g, 38% yield) as a light yellow solid and 2-methoxy-4,5-bis(3-(trimethylsilyl)prop-2-ynyl)phenol (287) (0.177 g, 41% yield) as a yellow solid. The 2-methoxy-4,5-bis(3-(trimethylsilyl)prop-2-ynyl)phenol (287) was converted to 1,2-dimethoxy-4,5-bis(3-(trimethylsilyl)prop-2-ynyl)benzene (288) by treatment with MeI and Cs₂CO₃ in DMF. ¹H NMR (300 MHz, CDCl₃) δ 4.08 (s, 6H), 3.75 (s, 4H), 0.38 (s, 9H); ¹³C
NMR (75 MHz, CDCl$_3$) $\delta$ 147.7, 126.0, 112.2, 104.0, 87.6, 55.9, 23.5, 0.2; MS calcd for [M + H]$^+$ C$_{20}$H$_{30}$NaO$_2$Si$_2$ 381.2, found 381.2.

1,2-Dimethoxy-4,5-di(prop-2-ynyl)benzene (289). Under a nitrogen atmosphere, 1,2-dimethoxy-4,5-bis(3-((trimethylsilyl)prop-2-ynyl)benzene (288, 170.0 mg, 0.474 mmol) was dissolved in MeOH (2.5 mL) and ether (2.5 mL) and K$_2$CO$_3$ (262.0 mg, 1.896 mmol) was added. The mixture was stirred at rt for 4 h. H$_2$O (5 mL) was added and the mixture was stirred for 10 min. The mixture was extracted with ether (3 x 5 mL). The combined organic layers were washed with 5 mL of brine, dried over anhydrous Na$_2$SO$_4$, filtered, and the filtrate was concentrated in vacuo. The product was purified by column chromatography and eluted with hexanes/EtOAc (10:1, 6:1), yielding 85.0 mg (84% yield) of the desired product as a yellow solid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 6.97 (s, 2H), 3.87 (s, 6H), 3.54 (d, $J$ = 2.7 Hz, 4H), 2.17 (t, $J$ = 2.7 Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 148.14, 126.06, 112.56, 81.68, 70.97, 56.22, 22.26; MS calcd for [M + H]$^+$ C$_{14}$H$_{15}$O$_2$ 215.1, found 215.1.

Cyclotrimerization reactions toward 253-258. The diyne 252 (20 mg, 0.13 mmol), alkyne (1.3 mmol), dry toluene (4 ml) and (Ph$_3$P)$_2$Ni(CO)$_2$ (8.3 mg, 0.013 mmol) were added to a flame dried microwave vial equipped with a stir bar. The vial was flushed with nitrogen, capped with a microwave vial septum, and irradiated for 10 min in a CEM Discover microwave synthesizer at 300W. After cooling, the volatiles were evaporated, and the crude mixture was purified by silica gel chromatography, eluting with hexanes/EtOAc. Due to compound instability, the product was only characterized via NMR and directly subjected to the next reaction without HRMS measurement.
2-Butyl-9,10-dihydroanthracene (253). The compound was eluted with hexanes/EtOAc (50:1) to give 253 as a white solid in 75% yield. \( ^1H \) NMR (400 MHz, CDCl\(_3\)) \( \delta \) 7.28 (m, 2H), 7.16 (m, 3H), 7.11 (s, 1H), 7.00 (d, \( J = 7.2 \) Hz, 1H), 3.90 (s, 4H), 2.58 (t, \( J = 8.0 \) Hz, 2H), 1.58 (m, 2H), 1.34 (m, 2H), 0.91 (q, \( J = 8.0 \) Hz, 3H); \( ^{13}C \) NMR (75 MHz, CDCl\(_3\)) \( \delta \) 140.7, 136.9, 136.8, 136.5, 133.8, 127.4, 127.4, 127.2, 126.1, 126.0, 36.2, 35.8, 35.3, 33.9, 22.4, 14.0.

9,10-Dihydro-2,3-diphenylanthracene (254). The compound was eluted with hexanes/EtOAc (50:1) to give 254 as a white solid in 86% yield. \( ^1H \) NMR (400 MHz, CDCl\(_3\)) \( \delta \) 7.37 (s, 2H), 7.32 (m, 2H), 7.21 (m, 8H), 7.12 (m, 4H); \( ^{13}C \) NMR (75 MHz, CDCl\(_3\)) \( \delta \) 141.7, 138.7, 136.7, 136.2, 130.1, 129.8, 128.0, 127.7, 126.5, 126.4, 36.0.

9,10-Dihydro-2-(hydroxymethyl)anthracene (255). The compound was eluted with hexanes/EtOAc (4:1) to give 255 as a white solid in 66% yield. \( ^1H \) NMR (300 MHz, CDCl\(_3\)) \( \delta \) 7.29 (m, 4H), 7.17 (m, 3H), 4.66 (s, 2H), 3.93 (s, 3H), 1.60 (s, 1H); \( ^{13}C \) NMR (75 MHz, CDCl\(_3\)) \( \delta \) 142.8, 139.0, 137.3, 136.8, 136.5, 127.8, 127.6, 127.2, 126.9, 126.4, 125.1, 65.6, 36.4, 36.1.

4-(9,10-Dihydroanthracen-6-yl)butanenitrile (256). The compound was eluted with hexanes/EtOAc (6:1) to give 256 as a white solid in 74% yield. \( ^1H \) NMR (300 MHz, CDCl\(_3\)) \( \delta \) 7.28 (m, 2H), 7.20 (m, 3H), 7.11 (d, \( J = 7.8 \), 1H), 7.00 (m, 1H), 2.75 (t, \( J = 7.2 \) Hz, 2H), 2.29 (t, \( J = 7.2 \) Hz, 2H), 1.96 (p, \( J = 7.2 \) Hz, 2H); \( ^{13}C \) NMR (75 MHz, CDCl\(_3\)) \( \delta \) 151.6, 137.7, 137.3, 136.9, 136.7, 136.2, 127.9, 127.7, 127.6, 126.4, 126.4, 119.8, 36.4, 36.0, 34.2, 27.3, 16.6.
3-(9,10-Dihydroanthracen-6-yl)propan-1-ol (257). The compound was eluted with hexanes/EtOAc (4:1) to give 257 as a white solid in 78% yield. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.27 (m, 2H), 7.26 (m, 1H), 7.21 (m, 2H), 7.17 (m, 1H), 7.01 (dd, $J_1 = 7.6$ Hz, $J_2 = 1.4$ Hz, 1H), 3.90 (s, 4H), 3.65 (b, 2H), 2.68 (t, $J = 7.8$ Hz, 2H), 1.80 (m, 2H), 1.26 (s, 1H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 161.7, 160.7, 146.8, 146.0, 139.8, 137.1, 136.9, 134.4, 127.7, 127.6, 126.4, 126.3, 62.5, 36.4, 36.0, 34.6, 31.9.

2-(2-(9,10-Dihydroanthracen-6-yl)ethyl)isoindoline-1,3-dione (258). The compound was eluted with hexanes/EtOAc (5:1) to give 258 as a white solid in 86% yield. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.82 (d, $J = 3.3$ Hz, 1H), 7.81 (d, $J = 3.3$ Hz, 1H), 7.69 (d, $J = 3.0$ Hz, 1H), 7.67 (d, $J = 3.0$ Hz, 1H), 7.26 (m, 2H), 7.19 (m, 4H), 7.09 (dd, $J_1 = 7.8$ Hz, $J_2 = 1.5$ Hz, 1H), 3.89 (m, 6H), 2.96 (t, $J = 6.9$ Hz, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 203.0, 168.4, 137.1, 136.9, 136.8, 136.0, 135.2, 134.1, 132.3, 128.1, 127.8, 127.6, 127.6, 126.8, 126.3, 123.4, 39.6, 36.4, 36.0, 34.5.

Cyclotrimerization reactions toward 265-270 and 290-295. The diyne 252 or 289 (20 mg, 0.13 mmol), nitrile (1.3 mmol), dry toluene (4 ml) and CpCo(CO)$_2$ (1.56 µl, 0.013 mmol) were added to a flame dried microwave vial equipped with a stir bar. The vial was flushed with nitrogen, capped with a microwave vial septum and irradiated for 20 min in a CEM Discover microwave synthesizer at 300W. After cooling, the reaction was purified by silica gel chromatography, eluting with hexanes/EtOAc. Due to compound instability, the products were only characterized via NMR and directly subjected to the next reaction without HRMS measurement.
5,10-Dihydro-3-methylbenzo[g]isoquinoline (265). The product was eluted with hexanes/EtOAc (2:1) to deliver 265 as a white solid in 94% yield. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.57 (s, 1H), 7.42 (m, 4H), 7.24 (s, 1H), 4.05 (s, 4H), 2.69 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 155.8, 147.4, 146.0, 135.7, 134.8, 129.3, 127.7, 127.6, 126.5, 126.4, 121.8, 35.3, 32.3, 23.9.

5,10-Dihydro-3-propylbenzo[g]isoquinoline (266). The product was eluted with hexanes/EtOAc (3:1) to deliver 266 as a white solid in 87% yield. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.42 (s, 1H), 7.27 (m, 2H), 7.19(m, 2H), 7.06(s, 1H), 3.89 (s, 4H), 2.72 (t, $J = 7.5$ Hz, 2H), 1.73 (m, 2H), 0.95 (t, $J = 7.5$ Hz, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 160.1, 147.8, 146.0, 136.0, 135.2, 129.7, 128.0, 126.7, 126.6, 121.5, 40.2, 35.6, 32.6, 23.5, 14.1.

2-(5,10-Dihydrobenzo[g]isoquinolin-3-yl)ethanol (267). The product was eluted with hexanes/EtOAc (1:3) to deliver 267 as a white solid in 83%. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.38 (s, 1H), 7.36 (m, 4H), 7.08 (s, 1H), 4.00 (t, $J = 5.4$ Hz, 2H), 3.89 (s, 4H), 2.97 (t, $J = 5.7$ Hz, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 158.3, 147.3, 146.7, 135.6, 134.9, 130.5, 127.9, 127.9, 126.8, 126.7, 122.1, 62.2, 38.8, 35.6, 32.6.

5,10-Dihydro-3-vinylbenzo[g]isoquinoline (268). The product was eluted with hexanes/EtOAc (3:1) to deliver 268 as a white solid in 90% yield. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.42 (s, 1H), 7.25 (m, 2H), 7.20 (m, 3H), 6.77 (dd, $J_1 = 10.8$ Hz and $J_2 = 17.2$ Hz, 1H), 6.13 (d, $J = 17.2$ Hz, 1H), 5.40 (d, $J = 10.8$ Hz, 1H), 3.89 (s, 4H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 153.6, 147.9, 136.8, 135.3, 134.6, 131.3, 127.7, 127.6, 126.6, 126.5, 119.7, 117.3, 35.4, 32.5.
5,10-Dihydro-3-phenylbenzo[g]isoquinoline (269). The product was eluted with hexanes/EtOAc (6:1) to deliver 269 as a white solid in 87% yield. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.59 (s, 1H), 7.97 (d, $J = 8.0$ Hz, 2H), 7.63 (s, 1H), 7.46 (t, $J = 7.6$ Hz, 2H), 7.39 (d, $J = 6.4$ Hz, 1H), 7.30 (m, 2H), 7.23 (m, 2H), 3.97 (m, 4H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 155.6, 148.4, 146.5, 139.7, 135.6, 135.0, 131.1, 128.9, 128.8, 128.0, 127.9, 127.0, 126.8, 126.7, 119.4, 35.8, 32.7.

5,10-Dihydro-3-(pyridin-2-yl)benzo[g]isoquinoline (270). The product was eluted with hexanes/EtOAc (2:1, 1:1, 1:2) to deliver 270 as a white solid in 80% yield. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.66 (d, $J = 4.4$ Hz, 1H), 8.58 (s, 1H), 8.36 (d, $J = 8.0$ Hz, 1H), 8.32 (s, 1H), 7.79 (dt, $J_1 = 7.6$ Hz, $J_2 = 1.6$ Hz, 1H), 7.30 (m, 3H), 7.22 (m, 2H), 4.04 (s, 2H), 4.02 (s, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 156.3, 154.0, 149.0, 147.8, 146.5, 136.9, 135.2, 134.8, 132.6, 127.8, 126.6, 123.5, 121.0, 119.6, 35.5, 32.6.

5,10-Dihydro-7,8-dimethoxy-3-methylbenzo[g]isoquinoline (290). The product was eluted with hexanes/EtOAc (1:1) to deliver 290 as a white solid in 86% yield. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.43 (s, 1H), 7.04 (s, 1H), 6.79 (s, 1H), 6.75 (s, 1H), 3.86 (d, 10H), 2.49 (s, 1H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 155.54, 147.54, 145.54, 145.65, 129.08, 127.16, 126.25, 121.76, 110.91, 110.83, 55.97, 34.52, 31.54, 23.86.

5,10-Dihydro-7,8-dimethoxy-3-propylbenzo[g]isoquinoline (291). The product was eluted with hexanes/EtOAc (1:1) to deliver 291 as a white solid in 91% yield. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.38 (s, 1H), 7.02 (s, 1H), 6.79 (s, 1H), 6.76 (s, 1H), 3.85 (m, 10H), 2.71 (t, $J = 5.7$ Hz, 2H), 1.73 (m, 2H), 0.93 (t, $J = 5.4$ Hz, 3H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$
2-(5,10-Dihydro-7,8-dimethoxybenzo[g]isoquinolin-3-yl)ethanol (292). The product was eluted with EtOAc, 5% MeOH/EtOAc to deliver 292 as a white solid in 86% yield. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.36 (s, 1H), 7.07 (s, 1H), 6.79 (s, 1H), 6.76 (s, 1H), 3.98 (t, $J$ = 6.4 Hz, 2H), 3.80 (m, 10H) 2.97 (t, $J$ = 6.4 Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 157.89, 147.96, 147.92, 147.31, 146.64, 130.40, 127.16, 126.28, 122.23, 111.24, 111.16, 62.22, 56.26, 56.24, 38.74, 34.83, 31.83.

5,10-Dihydro-7,8-dimethoxy-3-vinylbenzo[g]isoquinoline (293). The product was eluted with hexanes/EtOAc (3:1) to deliver 293 as a white solid in 84% yield. $^1$H NMR (300 MHz, CDCl$_3$) δ 8.43 (s, 1H), 7.23 (s, 1H), 6.79 (dd, $J$ = 18 Hz and $J$ = 9 Hz, 3H), 6.13 (d, $J$ = 18 Hz, 1H), 5.41 (d, $J$ = 9 Hz, 1H), 3.86 (d, 10H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 153.42, 148.10, 147.68, 147.62, 145.69, 136.78, 131.08, 126.83, 126.07, 119.75, 117.19, 110.97, 110.88, 56.00, 55.98, 34.58, 31.76;

5,10-Dihydro-7,8-dimethoxy-3-phenylbenzo[g]isoquinoline (294). The product was eluted with hexanes/EtOAc (3:1) to give 294 as a white solid in 92% yield. $^1$H NMR (300 MHz, CDCl$_3$) δ 8.57 (s, 1H), 7.98 (s, 1H), 7.95 (s, 1H), 7.60 (s, 1H), 7.45 (m, 3H), 6.80 (d, $J$ = 7.8 Hz, 2H), 3.92 (s, 4H), 3.88 (s, 3H), 3.87 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 155.17, 148.27, 147.66, 147.60, 145.96, 139.45, 130.69, 128.62, 128.52, 126.90, 126.71, 126.14, 119.15, 110.96, 110.88, 55.99, 55.97, 34.80, 31.68.

5,10-Dihydro-7,8-dimethoxy-3-(4-methoxyphenyl)benzo[g]isoquinoline (295). The product was eluted with hexanes/EtOAc (3:1) to deliver 295 as a white solid in 86%
yield. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.54 (s, 1H), 8.94 (s, 1H), 7.92 (s, 1H), 7.56 (s, 1H), 6.99 (d, $J = 9.6$ Hz, 2H), 6.27 (d, $J = 9.6$ Hz, 2H), 3.89 (s, 4H), 3.88 (s, 6H), 3.84 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 160.42, 155.18, 148.40, 147.94, 147.89, 146.22, 132.38, 130.29, 128.25, 127.33, 126.53, 118.73, 114.31, 111.28, 111.20, 56.30, 56.27, 55.58, 35.13, 31.96.

Oxidation reactions toward 259-264, 271-276 and 296-301. The precursors 253-258, 265-270 or 290-295 (1 eq), DDQ (1.2 eq), and dry toluene (2 ml) were added to a flame dried microwave vial equipped with a stir bar. The vial was flushed with nitrogen, capped with a microwave vial septum and irradiated for 5 min in a CEM Discover microwave synthesizer at 300 W. After cooling, the crude mixture was purified by silica gel chromatography, eluting with hexanes/EtOAc to yield the anthracene products 259-264 and azaanthracene products 296-301. The azaanthracenes 271-276 were obtained in high purity by adding EtOAc (10 mL) to the reaction mixture which was subsequently washed with NaHCO$_3$ (3 × 5 mL), dried with MgSO$_4$, and concentrated under reduced pressure. The analytical data for 259-261 was identical with literature reports.$^{175-177}$

4-(Anthracen-6-yl)butanenitrile (262). The product was eluted with hexanes/EtOAc (4:1) to deliver 262 as a white solid in 74% yield. $^1$H NMR (300 MHz, CDCl$_3$) δ 8.38 (s, 1H), 8.35 (s, 1H), 7.98 (m, 3H), 7.46 (s, 1H), 7.45 (m, 2H), 7.27 (dd, $J_1 = 8.9$ Hz, $J_2 = 1.5$ Hz, 1H), 2.96 (t, $J = 7.2$ Hz, 2H), 2.36 (t, $J = 7.1$ Hz, 2H), 2.12 (p, $J = 7.2$ Hz, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 136.7, 132.2, 131.9, 131.7, 130.8, 129.0, 128.4, 128.3, 126.8, 126.3, 125.9, 125.7, 125.5, 119.9, 34.9, 26.6, 16.7; HRMS calcd for [M + H]$^+$ C$_{18}$H$_{16}$N 246.1283, found 246.1281.
3-(Anthracen-6-yl)propan-1-ol (263). The product was eluted with hexanes/EtOAc (3:1) to deliver 263 as a white solid in 70% yield. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.37 (s, 1H), 8.33 (s, 1H), 7.96 (m, 3H), 7.76 (s, 1H), 7.43 (m, 2H), 7.32 (d, $J$ = 8.8 Hz, 1H), 3.73 (t, $J$ = 6.4 Hz, 2H), 2.90 (t, $J$ = 7.6 Hz, 2H), 2.01 (m, 2H), 1.33 (s, 1H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 138.9, 132.1, 131.6, 130.8, 128.5, 128.4, 128.3, 127.5, 126.2, 125.7, 125.5, 125.2, 62.5, 33.9, 32.6; HRMS calcd for [M + H]$^+$ C$_{17}$H$_{17}$O 237.1280, found 237.1279.

2-(2-(Anthracen-6-yl)ethyl)isoindoline-1,3-dione (264). The product was eluted with hexanes/EtOAc (5:1) to deliver 264 as a white solid in 76% yield. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.36 (s, 1H), 8.31 (s, 1H), 7.94 (m, 3H), 7.81 (m, 3H), 7.66 (m, 2H), 4.05 (t, $J$ = 7.7 Hz, 2H), 3.18 (t, $J$ = 7.7 Hz, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 203.1, 168.5, 135.1, 134.1, 132.3, 132.0, 131.7, 130.9, 128.8, 128.4, 128.3, 127.3, 127.3, 126.3, 125.9, 125.6, 125.4, 123.5, 39.1, 35.2; HRMS calcd for [M + H]$^+$ C$_{24}$H$_{18}$NO$_2$ 352.1338, found 352.1340.

3-Methylbenzo[g]isoquinoline (271). The product was purified via washing with NaHCO$_3$ to give 271 in 75% yield. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 9.41 (s, 1H), 8.53 (s, 1H), 8.24 (s, 1H), 8.03 (d, $J$ = 8.4 Hz, 1H), 7.97 (d, $J$ = 8.4 Hz, 1H), 7.53 (s, 1H), 7.50 (m, 2H), 2.71 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 154.2, 149.4, 149.4, 134.6, 133.0, 132.0, 129.1, 128.2, 127.8, 127.5, 125.9, 125.5, 124.1, 117.5, 24.4; HRMS calcd for [M + H]$^+$ C$_{14}$H$_{12}$N 194.0970, found 194.0961.

3-Propylbenzo[g]isoquinoline (272). The product was purified via washing with NaHCO$_3$ giving 272 in 85% yield. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 9.43 (s, 1H), 8.53 (s, 1H), 8.27 (s, 1H), 8.03 (d, $J$ = 8.4, 1H), 7.97 (d, $J$ = 8.7 Hz, 1H), 7.53 (m, 3H), 2.93 (t, $J$ = 7.5 Hz,
2H), 1.87 (m, 2H), 1.02(t, J = 7.5 Hz, 3H); $^{13}$C NMR (75 MHz, CDCl3) δ 154.3, 153.3, 134.6, 133.0, 132.0, 129.1, 128.2, 127.7, 127.5, 125.9, 125.8, 124.3, 117.1, 40.3, 23.1, 14.1; HRMS calcd for [M + H]$^+$ C$_{16}$H$_{16}$N 222.1283, found 222.1281.

2-(Benzo[g]isoquinolin-3-yl)ethanol (273). The product was purified by column chromatography on silica gel, eluting with CH$_2$Cl$_2$, delivering 273 53% yield. $^1$H NMR (400 MHz, CD$_3$OD) δ 9.41 (s, 1H), 8.72 (s, 1H), 8.42 (s, 1H), 8.12 (d, J = 8.4 Hz, 1H), 8.06 (d, J = 8.4 Hz, 1H), 7.78 (s, 1H), 7.57 (m, 2H); 4.00 (t, J = 6.8 Hz, 2H), 3.15 (t, J = 6.4 Hz, 2H); $^{13}$C NMR (75 MHz, CDCl3) δ 155.4, 150.3, 136.5, 135.6, 134.1, 133.8, 130.2, 129.4, 129.3, 129.1, 127.4, 125.7, 120.4, 62.7, 47.9, 41.7; HRMS calcd for [M + H]$^+$ C$_{15}$H$_{14}$NO 224.1075, found 224.1072.

3-Vinylbenzo[g]isoquinoline (274). The product was purified via washing with NaHCO$_3$ delivering 274 in 80% yield. $^1$H NMR (300 MHz, CDCl$_3$) δ 9.45 (s, 1H), 8.56 (s, 1H), 8.32 (s, 1H), 8.05 (d, J = 8.4 Hz, 1H), 7.99 (d, J = 8.4 Hz, 1H), 7.62 (s, 1H), 7.56 (m, 2H), 6.95 (dd, J$_1$ = 17.3 Hz and J=9.9, 1H), 6.44 (dd, J=17.3 and J=1.8, 1H), 5.49 (dd, J=9.9 and J=1.8, 1H); $^{13}$C NMR (75 MHz, CDCl3) δ 154.77, 147.34, 136.76, 134.75, 132.72, 132.36, 129.18, 128.31, 128.06, 127.75, 126.51, 126.29, 125.31, 118.03, 117.29; HRMS calcd for [M + H]$^+$ C$_{15}$H$_{12}$N 206.0970, found 206.0960.

3-Phenylbenzo[g]isoquinoline (275). The compound was purified via washing with NaHCO$_3$, delivering 275 in 84% yield. $^1$H NMR (400 MHz, CDCl$_3$) δ 9.56 (s, 1H), 8.59 (s, 1H), 8.40 (s, 1H), 8.17 (s, 1H), 8.16 (d, J = 5.6 Hz, 2H), 8.06 (d, J = 8.4 Hz, 1H), 8.02 (d, J = 8.4 Hz, 1H), 7.55 (m, 4H), 7.43 (t, J = 7.2 Hz, 1H); $^{13}$C NMR (75 MHz, CDCl3) δ 154.6,
3-(Pyridin-2-yl)benzo[g]isoquinoline (276). The compound was purified via washing with NaHCO₃, delivering 276 in 81% yield. $^1$H NMR (300 MHz, CDCl₃) $\delta$ 9.56 (s, 1H), 8.90 (s, 1H), 8.73 (d, $J = 4.2$ Hz, 1H), 8.62 (s, 1H), 8.56 (m, 2H), 8.06 (t, $J = 8.7$ Hz, 2H), 7.85 (t, $J = 7.7$ Hz, 1H), 7.55 (m, 2H), 7.32 (t, $J = 4.8$ Hz, 1H); $^{13}$C NMR (75 MHz, CDCl₃) $\delta$ 156.7, 154.4, 149.7, 147.8, 137.3, 135.1, 134.8, 132.9, 129.2, 128.6, 127.9, 127.8, 127.5, 126.6, 125.2, 123.4, 121.5, 117.4; HRMS calcd for [M + H]$^+$ C$_{19}$H$_{14}$N 256.1126, found 256.1124.

7,8-Dimethoxy-3-methylbenzo[g]isoquinoline (296). The compound was eluted with DCM/EtOAc (1:1, 3:7, 0:1) to give 296 as a light orange-yellow solid in 82% yield. $^1$H NMR (300 MHz, CDCl₃) $\delta$ 9.29 (s, 1H), 8.30 (s, 1H), 8.03 (s, 1H), 7.49 (s, 1H), 7.20 (s, 1H), 7.15 (s, 1H), 4.02 (s, 3H), 4.03 (s, 3H), 2.68 (s, 3H); $^{13}$C NMR (100 MHz, CDCl₃) $\delta$ 153.37, 151.76, 150.43, 149.28, 148.87, 132.51, 132.03, 128.88, 125.13, 121.67, 117.32, 105.58, 104.82, 56.20, 56.17, 24.39; MS calcd for [M + H]$^+$ C$_{18}$H$_{13}$N$_2$ 257.1079, found 257.1067.

7,8-Dimethoxy-3-propylbenzo[g]isoquinoline (297). The compound was eluted with DCM/EtOAc (1:1, 3:7, 0:1) to give 297 as a yellow solid in 90% yield. $^1$H NMR (300 MHz, CDCl₃) $\delta$ 9.31 (s, 1H), 8.30 (s, 1H), 8.04 (s, 1H), 7.46 (s, 1H), 7.19 (s, 1H), 7.14 (s, 1H), 4.03 (s, 3H); 4.02 (s, 3H), 2.90 (t, $J = 7.2$ Hz, 2H), 1.85 (m, 2H), 1.00 (t, $J = 7.5$ Hz, 3H); $^{13}$C NMR (75 MHz, CDCl₃) $\delta$ 153.27, 152.71, 151.75, 150.45, 132.44, 132.00, 128.90, 125.06, 124.97, 121.84, 116.92, 105.58, 104.80, 56.16, 56.13, 40.23, 23.22, 14.10; MS calcd for [M + H]$^+$ C$_{18}$H$_{20}$NO₂ 282.1, found 282.1.
7,8-Dimethoxy-3-ethynolbenzo[g]isoquinoline (298). The compound was eluted with 8% MeOH/EtOAc To give 298 as a yellow solid in 52% yield. $^1$H NMR (300 MHz, CD$_3$OD) δ 9.37 (s, 1H), 8.56 (s, 1H), 8.26 (s, 1H), 7.82 (s, 1H), 7.42 (s, 1H), 7.37 (s, 1H), 4.03-3.94 (m, 8H), 3.16 (t, $J$ = 6.3 Hz, 2H); $^{13}$C NMR (75 MHz, CD$_3$OD) δ 154.4, 153.0, 152.7, 147.2, 135.2, 133.7, 131.5, 127.6, 125.5, 123.4, 121.4, 106.7, 106.0, 62.5, 56.7, 56.6, 40.4; MS calcd for [M + Na]$^+$ C$_{17}$H$_{17}$NNaO$_3$ 306.1, found 306.1

7,8-Dimethoxy-3-vinylbenzo[g]isoquinoline (299). The compound was eluted with hexanes/EtOAc (3:1, 1:1, 0:1) to give 299 as a light yellow solid in 80% yield. $^1$H NMR (300 MHz, CDCl$_3$) δ 9.33 (s, 1H), 8.32 (s, 1H), 8.10 (s, 1H), 7.56 (s, 1H), 7.24 (s, 1H), 7.17 (s, 1H), 6.93 (dd, $J$ = 18 Hz and $J$ = 9 Hz, 1H), 6.38 (d, $J$ = 18 Hz, 1H), 5.45 (d, $J$ = 9 Hz, 1H), 4.04 (s, 3H); 13C NMR (75 MHz, CDCl$_3$) δ 153.63, 151.65, 150.55, 146.79, 136.77, 131.91, 131.85, 129.10, 125.60, 125.18, 122.61, 117.61, 116.39, 105.50, 104.74, 56.00, 55.98; MS calcd for [M + H]$^+$ C$_{17}$H$_{16}$NO$_2$ 266.1, found 266.1.

7,8-Dimethoxy-3-phenylbenzo[g]isoquinoline (300). The compound was eluted with DCM/EtOAc (0:1, 1:1) to give 300 as a yellow solid in 82% yield. $^1$H NMR (300 MHz, CDCl$_3$) δ 9.43 (s, 1H), 8.31 (s, 1H), 8.12 (m, 3H), 8.06 (s, 1H), 7.50 (t, $J$ =7.5 Hz, 2H), 7.38 (t, $J$ =7.4 Hz, 1H), 7.17 (d, $J$ =7.2 Hz, 2H), 4.03 (s, 3H), 4.02 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 153.38, 151.56, 150.44, 148.41, 139.80, 132.10, 131.82, 129.14, 128.69, 128.11, 126.76, 125.18, 124.80, 122.66, 115.39, 105.36, 104.61, 55.94, 55.91; MS calcd for [M + H]$^+$ C$_{21}$H$_{18}$NO$_2$ 316.1, found 316.1.

7,8-Dimethoxy-3-(4-methoxyphenyl)benzo[g]isoquinoline (301). The compound was eluted with DCM/EtOAc (0:1, 1:1) to give 301 as a yellow solid in 83% yield. $^1$H NMR
(400 MHz, CDCl$_3$) $\delta$ 9.43 (s, 1H), 8.35 (s, 1H), 8.15 (s, 1H), 8.08 (d, $J = 8.4$ Hz, 2H), 8.01 (s, 1H), 7.23 (s, 1H), 7.02 (d, $J = 8.4$ Hz, 2H), 4.05 (s, 6H), 3.87 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 160.1, 153.6, 151.9, 150.7, 148.4, 132.6, 132.2, 129.3, 128.3, 125.2, 122.7, 114.6, 114.4, 105.7, 104.9, 56.3, 56.2, 55.6; MS calcd for [M + H]$^+$ C$_{22}$H$_{20}$NO$_3$ 346.1, found 346.2

Methylation of 259-264, 271-276 and 296-301 to 265-270, 277-282 and 302-307. The azaanthracenes 259-264, 271-276 and 296-301 (0.02 mmol) and MeI (100 µL) were added to a flame-dried vial under nitrogen atmosphere. The reaction mixture was heated at 60 °C overnight. After cooling to room temperature, the solvent was removed under reduced pressure to yield the desired products 265-270, 277-282 and 302-307 in quantitative yield.

2-Methyl-3-methylbenzo[g]isoquinolinium iodide (277). $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 10.31 (s, 1H), 9.24 (s, 1H), 8.83 (s, 1H), 8.49 (s, 1H), 8.42 (d, $J = 8.4$ Hz, 1H), 8.32 (d, $J = 8.8$ Hz, 1H), 7.89 (t, $J = 7.4$ Hz, 1H), 7.80 (t, $J = 7.4$ Hz, 1H), 4.43 (s, 3H), 2.84 (s, 3H); $^{13}$C NMR (75 MHz, DMSO-d$_6$) $\delta$ 154.6, 141.2, 137.0, 132.6, 132.4, 130.9, 129.6, 128.4, 128.1, 125.1, 124.5, 123.0, 45.8, 18.8; HRMS calcd for [M]$^+$ C$_{15}$H$_{14}$N 208.1126, found 208.1117.

2-Methyl-3-propylbenzo[g]isoquinolinium iodide (278). $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 10.36 (s, 1H), 9.24 (s, 1H), 8.88 (s, 1H), 8.46 (s, 1H), 8.43 (d, $J = 8.8$ Hz, 1H), 8.32 (d, $J = 9.2$ Hz, 1H), 7.89 (t, $J = 7.8$ Hz, 1H), 7.80 (t, $J = 7.4$ Hz, 1H), 4.46 (s, 3H), 3.13 (t, $J = 7.8$ Hz, 2H), 1.88 (q, $J = 7.6$ Hz, 2H), 1.11 (t, $J = 7.6$ Hz, 3H); $^{13}$C NMR (75 MHz, DMSO-d$_6$) $\delta$ 155.1, 144.1, 137.0, 132.7, 132.4, 131.5, 130.9, 129.6, 128.5, 128.1, 125.4, 123.4, 122.9, 45.5, 32.6, 20.5, 13.5; HRMS calcd for [M]$^+$ C$_{17}$H$_{18}$N 236.1439, found 236.1429.
2-(2-Methyl(benzo[g]isoquinolinum)-3-yl)ethanol iodide (279). $^1$H NMR (300 MHz, CD$_3$OD) $\delta$ 10.06 (s, 1H), 9.12 (s, 1H), 8.75 (s, 1H), 8.43 (s, 1H), 8.27 (d, $J = 8.4$ Hz, 1H), 8.22 (d, $J = 8.4$ Hz, 1H), 7.81 (td, $J_1 = 6.6$ Hz, $J_2 = 0.9$ Hz, 1H), 7.72 (t, $J = 6.6$ Hz, 1H), 4.52 (s, 3H), 4.05 (t, $J = 6.0$ Hz, 2H), 3.37 (t, $J = 6.0$ Hz, 2H); $^{13}$C NMR (75 MHz, CD$_3$OD) $\delta$ 154.8, 138.2, 134.6, 133.8, 132.5, 131.1, 129.5, 128.5, 128.4, 126.8, 125.7, 123.7, 120.6, 60.2, 45.8, 34.3; HRMS calcd for [M]$^+$ C$_{16}$H$_{16}$NO 238.1232, found 238.1230.

2-Methyl-3-vinylbenzo[g]isoquinolinum iodide (280). $^1$H NMR (300 MHz, CD$_3$OD) $\delta$ 10.12 (s, 1H), 9.19 (s, 1H), 8.86 (s, 1H), 8.71 (s, 1H), 8.32 (d, $J = 9.0$ Hz, 1H), 8.27 (d, $J = 8.4$ Hz, 1H), 7.89 (td, $J_1 = 6.6$ Hz, $J_2 = 1.2$ Hz, 1H), 7.78 (td, $J_1 = 6.6$ Hz, $J_2 = 1.2$ Hz, 1H), 7.27 (dd, $J_1 = 11.0$ Hz, $J_2 = 17.0$ Hz, 1H), 6.31 (dd, $J_1 = 17.0$ Hz, $J_2 = 0.6$ Hz, 1H), 5.96 (dd, $J_1 = 11.0$ and $J_2 = 0.6$, 1H), 4.52 (s, 3H); $^{13}$C NMR (75 MHz, DMSO-d$_6$) $\delta$ 155.6, 141.2, 137.6, 133.6, 133.4, 131.9, 131.8, 130.4, 129.3, 129.2, 128.7, 127.0, 125.6, 123.8, 123.1, 47.1; HRMS calcd for [M]$^+$ C$_{16}$H$_{14}$N 220.1126, found 208.1119.

2-Methyl-3-phenylbenzo[g]isoquinolinum iodide (281). $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 11.65 (s, 1H), 9.59 (s, 1H), 8.57 (s, 1H), 8.31 (d, $J = 8.4$ Hz, 1H), 8.14 (d, $J = 7.8$ Hz, 2H), 7.76 (t, $J = 8.4$ Hz, 1H), 7.68 (t, $J = 8.4$ Hz, 1H), 7.59 (m, 5H), 4.50 (s, 3H); $^{13}$C NMR (75 MHz, DMSO-d$_6$) $\delta$ 155.7, 142.9, 138.3, 134.7, 134.1, 131.9, 131.3, 130.5, 129.9, 129.8, 128.9, 128.5, 126.4, 125.9, 123.8, 47.5; HRMS calcd for [M]$^+$ C$_{20}$H$_{16}$N 270.1277, found 270.1275.

2-Methyl-3-(pyridin-2-yl)benzo[g]isoquinolinum iodide (282). $^1$H NMR and $^{13}$C NMR could not be obtained due to solubility issues. HRMS calcd for [M]$^+$ C$_{19}$H$_{15}$N$_2$ 271.1230, found 271.1226.
7,8-Dimethoxy-2-Methyl-3-methylbenzo[g]isoquinolinum iodide (302). $^1$H NMR (300 MHz, CDCl$_3$) 11.30 (s, 1H); 9.12 (s, 1H), 8.21 (s, 1H); 7.95 (s, 1H); 7.34 (s, 1H), 7.25 (s, 1H), 4.55 (s, 3H), 4.10 (s, 3H), 4.06 (s, 3H), 2.79 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-d$_6$) δ 154.74, 153.29, 152.23, 141.14, 136.28, 131.70, 131.09, 129.18, 124.43, 122.66, 122.48, 106.63, 105.82, 56.89, 56.72, 45.97, 19.37; MS calcd for M$^+$ C$_{17}$H$_{18}$NO$_2$ 268.1, found 268.1.

7,8-Dimethoxy-2-Methyl-3-propylbenzo[g]isoquinolinum iodide (303). $^1$H NMR (300 MHz, CDCl$_3$) 11.12 (s, 1H); 9.02 (s, 1H), 8.24 (s, 1H); 7.89 (s, 1H); 7.27 (s, 1H), 7.26 (s, 1H), 4.54 (s, 3H), 4.10 (s, 3H), 4.02 (s, 3H), 2.96 (t, $J = 7.5$ Hz, 2H), 1.85 (m, 2H) 1.13 (t, $J = 7.5$ Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 154.92, 153.35, 142.52, 136.48, 131.37, 131.02, 129.91, 123.21, 122.37, 122.05, 106.23, 105.01, 56.91, 56.74, 45.15, 33.92, 21.67, 13.94; MS calcd for M$^+$ C$_{19}$H$_{22}$NO$_2$ 296.2, found 296.1.

7,8-Dimethoxy-2-Methyl-3-vinylbenzo[g]isoquinolinum iodide (305). $^1$H NMR (300 MHz, CD$_3$OD) δ 9.85 (s, 1H), 8.84 (s, 1H), 8.56 (s, 1H), 8.54 (s, 1H), 7.56 (s, 1H), 7.55 (s, 1H), 7.23 (dd, $J = 17.1$ Hz and $J = 17.1$ Hz, 1H), 6.23 (d, $J = 17.1$ Hz, 1H), 5.90 (d, $J = 11.1$ Hz, 1H), 4.44 (s, 3H), 4.09 (s, 3H), 4.06 (s, 3H); $^{13}$C NMR (75 MHz, CD$_3$OD) δ 156.6, 154.2, 153.7, 141.9, 138.1, 133.1, 132.9, 130.3, 129.3, 125.4, 124.6, 124.2, 123.7, 107.0, 106.3, 57.1, 56.9, 46.8; MS calcd. for M$^+$ C$_{18}$H$_{18}$NO$_2$ 280.3, found 280.3.

7,8-Dimethoxy-2-Methyl-3-phenylbenzo[g]isoquinolinum iodide (306). $^1$H NMR (400 MHz, CD$_3$OD) δ 11.35 (s, 1H); 9.23 (s, 1H), 8.30 (s, 1H); 7.99 (s, 1H); 7.53 (m, 6H), 7.31 (d, $J = 14.4$ Hz, 2H), 4.37 (s, 3H), 4.12 (s, 3H), 4.03 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 155.16, 153.63, 152.33, 142.19, 136.68, 132.25, 131.53, 130.99, 130.59, 129.99,
129.65, 125.60, 122.91, 122.69, 106.50, 105.10, 56.92, 56.80, 47.05; MS calcd for M⁺
C_{22}H_{20}NO₂ 330.1, found 330.1.

7,8-Dimethoxy-2-methyl-3-(4-methoxyphenyl)benzo[g]isoquinolinum iodide (307). ¹H NMR (400 MHz, CD₃OD) δ 9.96 (s, 1H), 8.92 (s, 1H), 8.57 (s, 1H), 8.23 (s, 1H),
7.62-7.53 (m, 4H), 7.19 (d, 2H), 4.24 (s, 3H), 4.09 (s, 3H), 4.07 (s, 3H), 3.92 (s, 3H); ¹³C
NMR (100 MHz, CD₃OD) δ 154.2, 138.3, 133.2, 132.9, 132.7, 132.6, 130.2, 130.0, 127.3,
126.2, 124.4, 115.8, 107.0, 106.3, 57.1, 56.9, 56.2, 47.7; MS calcd for M⁺ C_{23}H_{22}NO₃ 360.4,
found 360.4.
CHAPTER 4: Total Synthesis of Cryptoacetalide

4.1 Introduction

The diterpenoids, cryptoacetalide (308) and epicryptoacetalide (epi-308) were isolated in 1990\textsuperscript{178} from Dan-shen, the dried root of \textit{salvia miltiorrhiza} (Labiatae), which is commonly used as a Chinese medicine for the treatment of angina pectoris, irregular menstruation, amenorrhea, dysmenorrheal, injuries due to impact fractures, constitutions and strains, palpitation, insomnia, carbuncle and furuncle.\textsuperscript{179} The diterpenoids isolated from this plant include tanshinones I, IIA, and IIB, isotanshinones I and II, cryptotanshionone I, salviol, protocatechuic acid, \(\beta\)-(3,4-dihydroxyphenyl) lactic acid, vitamin E,\textsuperscript{179} cryptoacetalide, and epicryptoacetalide.\textsuperscript{178} The diterpenoids, cryptoacetalide and epicryptoacetalide were isolated as a 3:1 inseparable mixture; possible due to an epimerization under aqueous conditions. They both contain a lactone group, a benzene ring and also a spiroacetal moiety (Figure 4.1). To date, no synthesis of cryptoacetalide and epicryptoacetalide has been reported.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4_1.png}
\caption{Structure of cryptoacetalide and epicryptoacetalide.}
\end{figure}
4.2 Total Synthesis of Crytoacetalide

The [2+2+2] cyclotrimerization reaction was applied to the synthesis of cryptoacetalide and epicryptoacetalide. The synthesis of the model compound 309, which has protons instead of methyl groups on C-4 and C-15 of the cryptoacetalide 308 was first investigated (Scheme 4.1). It was envisioned that the key step in this synthesis is an intramolecular [2+2+2] cyclotrimerization reaction of the triyne 311 to assemble the fused tricyclic benzene ring. The retro-synthetic analysis of the cryptoacetalide model system is shown in Scheme 4.1. The compound 309 can be made via oxidation and cyclization from the alcohol 210, which is expected to be derived from the triyne 311 via a [2+2+2] intramolecular cyclotrimerization reaction. The triyne 311 could be synthesized by coupling the acid 312 with the secondary alcohol 313.

Scheme 4.1. Retro-synthetic route for the synthesis of a model system of cryptoacetalid 309.

First, the intramolecular cyclotrimerization reaction of different model triynes was investigated (Scheme 4.2). The triyne ether 314 was used as a model system, since it is the
simplest triyne based on our retrosynthetic analysis. The triyne 314 underwent a smooth cyclotrimerization reaction to 316 in the presence of 10 mol% Cp*RuCl(COD) and microwave irradiation in 88% yield (entry 1). A literature reported cyclotrimerization of the same compound furnished 314 in the absence of any catalyst in 80% yield, using microwave irradiation (Biotage AG Emrys microwave synthesizer) in DMF at 200 °C for 1 h. However, this result was not reproduced (entry 2) using 314 under identical reaction conditions (except a CEM Discover microwave synthesizer is used). A model triyne 315, more similar to the compound 308 due to its ester linkage, underwent an efficient cyclotrimerization reaction to 317 using the same catalyst (Cp*RuCl(COD), 10 mol%) and microwave irradiation (entry 3). Good yields were also obtained by employing (Ph₃P)₂Ni(CO)₂ (10 mol%) at room temperature (entry 4). No desired product 317 was obtained when no catalyst was added in the cyclotrimerization reaction of the triyne 315 (entry 5). The complexity of the triyne was increased to investigate the effects of additional substituents. The triyne 316 was transformed smoothly in a microwave-assisted [2+2+2] cyclotrimerization reaction under Ru or Ni catalysis in 78-81% yield (entry 6 and 7). The TBS protected propargyloxy triyne 317 underwent cyclotrimerization under microwave irradiation with Cp*RuCl(COD) in 84% yield (entry 8). Surprisingly, the triyne 317 underwent a cyclotrimerization reaction even in the absence of any catalysts (in contrast to 314 and 315) only using microwave irradiation (DMF, 200 °C, 1 h), yielding 321 in 81% yield (entry 9), this is possibly due to the Thorpe-Ingold effect induced by the sterically demanding OTBDMS group.32
Conditions: a) toluene, catalyst, MW 300 W, 130 °C, 20 min; b) DMF, MW 200 W, 200 °C, 1 h; c) reference reported an 80% yield; d) THF, rt, 17 h; see reference.

Scheme 4.2. Cyclotrimerization reactions of several model substrates 314-315.

The synthesis of the model compound 309 started with the known acid 312 and the alcohol 313. The alcohol 313 was synthesized from the known aldehyde 322 by treating 322 with ethynylmagnesium bromide in 96% yield according to literature report. The coupling of the acid 312 and the secondary alcohol 313 in the presence of DCC and DMAP provided the cyclotrimerization precursor in 64% yield. The triyne 311 underwent a smooth intramolecular [2+2+2] cyclotrimerization reaction using 10 mol% Cp*RuCl (COD) as the catalyst under microwave irradiation (300 W), delivering the tricyclic benzene ring 323 in 81% yield (Scheme 4.3).
Evidence in the literature,\textsuperscript{185} 310 was converted the spirolactone 309 in 65\% yield in the presence of I\(_2\) and PhI(OAc)\(_2\), upon light irradiation (Xe/Hg lamp, 250 W) (Scheme 4.4).

Based on the successful synthetic route of the model compound 309, it is envisioned that crytoacetalide (308) can be synthesized from the oxidative cyclization of 324. The gem-dimethyl group can be installed via a Reetz reaction\textsuperscript{186} of the ketone 325. The tricyclic benzene ring of 325 could be assembled via the intramolecular [2+2+2] cyclotrimerization reaction of the triyne 326, which could be made from the coupling reaction of acid 327 and alcohol 328 (Scheme 4.5).
Scheme 4.5. Retrosynthetic analysis of cryptoacetalide (308) via Reetz reaction.

The problematic step of the synthesis of cryptoacetalide was the Reetz reaction of the ketone 325.\textsuperscript{186} When the ketone 325 was treated with Me$_2$TiCl$_2$ (freshly prepared \textit{in situ} from Me$_2$Zn and TiCl$_4$)\textsuperscript{186} using several different conditions, no pure desired product was obtained. Due to the acidity of the TiCl$_4$, the trityl group was cleaved, preventing the reaction from completion. Instead, an inseparable mixture of the mono- and di-methylated products was obtained.

Since the installing the gem-dimethyl group on the ketone 325 was troublesome, the installation of the gem-dimethyl group prior to the cyclotrimerization reaction was explored (Scheme 4.6). The synthesis of cryptoacetalide (308) using an already-installed gem-dimethyl group triyne as the cyclotrimerization reaction precursor was carried out. It is envisioned that the cryptoacetalide (308) could be made from the oxidative cyclization of 329. The tricyclic benzene ring of 329 could be assembled from the intramolecular [2+2+2]...
cyclotrimerization reaction of the triyne 330, which could be synthesized from the coupling reaction with the acid 331 and the alcohol 332.

Scheme 4.6. Retro-synthetic analysis of cryptoacetalide.

The synthesis commenced with the TBDMS-protection of the known 6-chlorohex-2-yn-1-ol (333), followed by a Finkelstein reaction with NaI to provide the iodide 335 in excellent yield. The installation of the geminal dimethyl group was accomplished by deprotonation of isobutyronitrile with LDA followed by a nucleophilic substitution with 331, delivering the nitrile 336 in 86% yield. Reduction of the nitrile 336 with DIBAL-H at –78 °C afforded the aldehyde 337 in 93% yield. The second triple bond was then installed via a Corey-Fuchs reaction directly followed by a deprotection, providing the diyne 335. A Jones oxidation of the alcohol 335 then delivered the acid 327 in 97% yield. The secondary alcohol 328 was generated as a 1:1 diastereomeric mixture in 90% yield by the addition of ethynylmagnesium bromide into the known, enantiomerically pure aldehyde 336 (synthesized in 5 steps from methyl (S)-3-hydroxy-2-methylpropionate). The acid 327 was then coupled with the alcohol 328 to provide the cyclotrimerization precursor 326 in 79%
yield. The [2+2+2] cyclotrimerization reaction of the triyne 326 was conducted with Cp*RuCl(COD) in toluene under microwave irradiation (300 W) affording the tricyclic product 337 in 90% yield. Deprotection of the PMB ether with DDQ generated the corresponding alcohol 329, which set the stage for the spiroketalization reaction. The lacton 329 was irradiated with light (200 W Xe/Hg lamp) in the presence of iodine and iodobenzene diacetate for 1 h. The natural products cryptoacetalide (308) and epi-cryptoacetalide (epi-308) were isolated as a 2:1 mixture in an excellent yield of 84% (Scheme 4.7). As previously reported, 308 and epi-308 could not be separated by column chromatography, HPLC, or GC. The NMR spectrum of the mixture matches the NMR spectrum of the material isolated from nature.
Scheme 4.7. Total synthesis of cryptoacetalide.

4.3 Summary and Outlook

In summary, the first synthesis of the terpene natural product cryptoacetalide was accomplished in 12 steps in an overall yield of 26% from known, easily accessible starting materials. Key steps of the developed synthetic approach are a microwave-mediated
intramolecular [2+2+2] cyclo-trimerization reaction and a light-mediated spiro-ketalization. Moreover, the intramolecular cyclotrimerization reactions of different triynes were investigated under various conditions using different catalyst systems in the absence and presence of microwave irradiation. To understand the mechanism of the catalyst-free [2+2+2] cyclotrimerization reaction is important.

4.4 Experimental.

Unless otherwise stated, reactions were performed under nitrogen using flame-dried glassware. Some solvents used in reactions were dried by different ways. CH₂Cl₂, THF and ether were dried by MB SPS Compact solvent purification system. Toluene was distilled from sodium metal/benzophenone ketyl. DMF and acetone were distilled from CaCO₃. All other reagent quality solvents were used without further purification. The microwave used is a CEM Discover microwave synthesizer. All spectra were recorded in CDCl₃, and chemical shifts are reported relative to CHCl₃ (7.24 ppm for ¹H NMR) or CDCl₃ (77.23 ppm for ¹³C NMR).

**General procedure to synthesis ester from acid and alcohol with DCC as coupling reagent.** The alcohol (1 eq) and DMAP (0.6 eq) were dissolved in DCM (0.1 M) and the solution was cooled to 0 °C. To the solution was added the solution of the acid (1.2 eq) in DCM (0.1 M), followed by the solution of DCC (1.2 eq) in DCM (0.1 M). The mixture was stirred at room temperature overnight. The solution was concentrated and the resulting residue was purified by column chromatography on SiO₂, eluting with hexanes/EtOAc mixture.
6-[(tert-Butyldimethylsilyl)oxy]hex-1-yn-3-yl nona-2,8-diynoate (311). The reaction mixture was eluted with hexanes/ethyl acetate (20:1, 10:1, 5:1) to give 311 as a white solid in 64% yield. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 5.41 (td, $J = 6.6$, 2.2 Hz, 1H), 3.63 (t, $J = 6.2$, 2H), 2.47 (d, $J = 2.2$, 1H), 2.36 (t, $J = 6.8$, 2H), 2.21 (td, $J = 6.6$, 2.7 Hz, 2H), 1.98 – 1.81 (m, 3H), 1.81 – 1.60 (m, 6H), 1.00 – 0.75 (m, 9H), 0.03 (s, 6H).

6-(tert-Butyldimethylsiloxy)-1-yn-3-yl nona-2,8-diynoate (316). The reaction mixture was eluted with hexanes/ethyl acetate (20:1, 10:1, 5:1) to give 316 as a white solid in 75% yield. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 5.40 (dt, $J = 2.0$ Hz and $J = 6.8$, 1H), 3.63 (t, $J = 6.4$, 2H), 2.47 (d, $J = 2.0$, 1H), 2.36 (t, $J = 6.4$, 2H), 2.21 (dt, $J = 2.8$ Hz and $J = 6.8$, 2H), 1.94 (t, $J = 2.8$, 1H), 1.90-1.84 (m, 2H), 1.74-1.57 (m, 6H), 0.87 (s, 9H), 0.03 (s, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 152.8, 90.1, 83.8, 80.5, 74.5, 73.2, 69.0, 65.3, 62.5, 35.2, 31.3, 28.3, 27.6, 26.6, 26.2, 18.5, 18.1, -5.1; HRMS calcd for [M + Na] C$_{21}$H$_{32}$NaO$_3$Si 383.2018, found 383.2011.

5-(R)-Methyl-6-(trityloxy)hex-1-yn-3-yl 7-(tertbutyldimethylsiloxy)nona-2,8-diynoate (317). The reaction mixture was eluted with hexanes/ethyl acetate (20:1, 10:1 5:1) to give 317 as white solid in 64% yield. $^1$H NMR (300 MHz, CD$_3$OD) $\delta$ 7.47-7.44 (m, 6H), 7.33-7.21 (m, 9H), 5.49-5.35 (m, 1H), 4.43-4.39 (m, 1H), 3.00-2.97 (m, 2H), 2.50-2.48 (m, 1H), 2.43-2.38 (m, 3H), 2.09-1.09 (m, 2H), 1.80-1.67 (m, 5H), 1.02 (d, $J = 6.3$, 3H), 0.91 (s, 9H), 0.16 (s, 3H), 0.13 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 152.54, 152.50, 144.2, 129.7, 127.7, 126.9, 90.0, 86.3, 86.2, 84.9, 80.7, 80.3, 74.5, 74.2, 72.9, 72.5, 67.8, 67.7, 64.1, 63.7, 63.1, 38.8, 38.4, 37.3, 30.6, 30.4, 25.7, 23.0, 18.5, 18.1, 17.3, 17.1, -4.6, -5.1; HRMS calcd for [M + Na] C$_{41}$H$_{48}$NaO$_4$Si 655.3220, found 655.3211.
Spiro[furan-2(3H),3'(1'H)-4,5,6',7',8',9'-hexahydro-naphtho[1,2-c]furan]-1'-one (309). The lactone 310 (9.6 mg, 0.039 mmol) was dissolved in benzene (2 mL) in a vial and cooled to 0 °C. Iodine (20.0 mg, 0.078 mmol) and iodosobenzene diacetate (37.7 mg, 0.117 mmol) were added. The vial was sealed and irradiated with a 200 W Xe/Hg lamp (Newport) for 2 h. After cooling to rt, 10 ml of ether were added and the mixture was washed with H₂O (2 ml) and brine (2 ml), dried over anhydrous Na₂SO₄, filtered and the filtrate was concentrated in vacuo. The product was purified by column chromatography on SiO₂, eluting with hexanes:EtOAc = 1:1, delivering 6.2 mg (65% yield) of 309 as white solid. ¹H NMR (300 MHz, CDCl₃) δ 7.34 (d, J = 7.8 Hz, 1H), 7.18 (d, J = 7.9 Hz, 1H), 4.55 – 3.97 (m, 2H), 3.29 – 3.09 (m, 2H), 2.92 – 2.76 (m, 2H), 2.50 – 2.12 (m, 4H), 1.81 (dq, J = 6.6, 4.0, 3.1 Hz, 4H).

General procedure of Reetz reaction. TiCl₄ (6 eq) was dissolved in DCM (0.3 M) and the solution was cooled to −40 °C. Me₂Zn (1.2 M in toluene, 6 eq) was added quickly. The reaction mixture was stirred at −40 °C for 20 min. The solution of ketone (1 eq) in DCM (0.05 M) was added dropwise. The reaction mixture was stirred at −40 °C for 30 min and warmed to room temperature and stirred at room temperature for 1.5 h. The reaction mixture was poured to saturated NH₄Cl and extracted with ether. The combined organic layers were washed with brine, dried over Na₂SO₄, filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc to give dimethyl products.

General procedure for microwave-assisted cyclotrimerization reaction in the present of transition-metal catalysts. In a dried microwave vial, the triyne was dissolved in
toluene. The transition-metal catalyst (0.1 eq) was added and the vial was sealed and heated in a CEM Discover microwave synthesizer (power mode, 300 W, 130 °C) for 20 min. After cooling to rt, solids were removed through filtration and the filtrate was concentrated \textit{in vacuo}. The product was purified by column chromatography on SiO\textsubscript{2} (eluted with hexanes/ethyl acetate mixture), delivering the benzene products \textsuperscript{104}318\textsuperscript{104} and \textsuperscript{180}319\textsuperscript{180} (both literature-known), as well as 320 and 321.

**General procedure for the catalyst-free microwave-assisted cyclotrimerization reaction.** In a dried microwave vial, the triyne was dissolved in DMF. The vial was sealed and heated in a CEM Discover microwave synthesizer (standard mode, 200 W, 200 °C) for 1h. After cooling to rt, saturated NH\textsubscript{4}Cl was added and the mixture was extracted with ether 3 times. Combined organic layers were washed with brine, filtered and filtrate was concentrated under vacuum. The product was purified by column chromatography on SiO\textsubscript{2} (eluted with hexanes/ethyl acetate mixture), delivering the benzene derivative.

3-\[3-\textit{(t}er\textit{t}-\textit{Butyldimethylsiloxy)}propyl\]-6,7,8,9-tetrahydro-naphtho[2,1-c]furan-1(3H)-one (320). The product was eluted with hexanes/Ethyl acetate = 10:1. \textsuperscript{1}H NMR (400 MHz, CD\textsubscript{3}OD) \(\delta\) 7.30 (d, \(J = 8.0\) Hz, 1H), 7.09 (d, \(J = 8.0\) Hz, 1H), 5.39-5.36 (m, 1H), 3.67-3.58 (m, 2H), 3.20 (br, 2H), 2.81 (br, 2H), 2.12-2.06 (m, 1H), 1.83-1.78 (m, 4H), 1.76-1.72 (m, 1H), 1.71-1.62 (m, 2H), 0.89 (s, 9H), 0.02 (s, 3H), 0.01 (s, 3H); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}) \(\delta\) 171.2, 148.7, 138.7, 138.6, 135.3, 123.4, 118.5, 80.2, 62.6, 31.7, 29.6, 28.1, 26.1, 25.2, 22.8, 22.4, 18.5, -5.1; HRMS calcd for [M + H] C\textsubscript{23}H\textsubscript{33}O\textsubscript{3}Si 361.2199, found 361.2196.

3-\[(R)\-3\textit{Trityloxy}-2-methylpropyl\]-6,7,8,9-tetrahydro-6-\textit{(t}er\textit{t}-\textit{Butyldimethylsiloxy)}naphtho[2,1-c]furan-1(3H)-one (321). The product was eluted with
hexanes/ethyl acetate = 8:1. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 7.64 (dd, $J = 8.0$ Hz and $J = 4.0$ Hz, 1H), 7.48-7.41 (m, 6H), 7.32-7.26 (m, 6H), 7.25-7.18 (m, 3H), 7.15 (dd, $J = 8.0$ Hz and $J = 4.0$ Hz, 1H), 5.41-5.37 (m, 0.5 H), 5.20-5.16 (m, 0.5H), 4.81 (br, 1H), 3.22-3.11 (m, 3H), 3.03-2.94 (m, 1H), 2.28-2.15 (m, 1H), 2.05-2.02 (m, 3H), 1.82-1.77 (m, 3H), 1.45-1.25 (m, 1H), 1.11 (d, $J = 6.4$ Hz, 3H), 0.94 (s, 9H), 0.20-0.17 (m, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 170.7, 150.32, 150.30, 150.24, 144.13, 144.11, 141.3, 141.21, 141.17, 138.37, 138.35, 133.74, 138.72, 128.6, 127.72, 127.66, 126.9, 126.8, 122.7, 122.5, 118.70, 118.66, 86.20, 86.16, 78.9, 78.8, 78.12, 78.08, 68.86, 68.81, 68.2, 68.64, 66.59, 39.78, 39.73, 39.34, 39.27, 32.3, 31.0, 30.61, 30.56, 25.8, 24.85, 18.61, 18.58, 18.1, 16.85, 16.79, -4.1, -4.6; HRMS calcd for [M + Na] C$_{41}$H$_{48}$NaO$_4$Si 655.3220, found 655.3218.

**6-Iodohex-2-ynyloxy-(tert-butyl)dimethylsilane (335).** To a solution of the alcohol 333 (440 mg, 3.33 mmol) in DMF (1.7 ml) were added imidazole (272 mg, 4.00 mmol) and TBDMSCl (603 mg, 4.00 mmol). The reaction mixture was stirred at rt for 3 h. Saturated ammonium chloride solution (3 ml) was added and the mixture was extracted with ether (3 x 5 ml). The combined organic layers were washed with brine (5 ml), dried over anhydrous Na$_2$SO$_4$, filtered and the filtrate was concentrated under vacuum. The product was purified by column chromatography on SiO$_2$ (eluted with hexanes/ethyl acetate = 10:1) delivering 773 mg (94% yield) of 6-chlorohex-2-ynyloxy-(tert-butyl)dimethylsilane (334). $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 4.28 (t, $J = 2.4$ Hz, 2H), 3.27 (t, $J = 6.8$ Hz, 2H), 2.33 (dt, $J = 2.4$ Hz and $J = 6.8$ Hz, 2H), 1.98-1.89 (m, 2H), 0.89 (s, 9H), 0.10 (s, 6H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 83.5, 80.0, 52.1, 43.9, 31.5, 26.1, 18.6, 16.5, -4.9; HRMS calcd for [M + Na] C$_{12}$H$_{23}$ClNaOSi 269.1104, found 269.1104. To the solution of 6-chlorohex-2-ynyloxy-(tert-butyl)
dimethylsilane (772 mg, 3.14 mmol) in dry acetone (4 ml) was added NaI (1.176 g, 7.85 mmol). The mixture was refluxed overnight. After cooling to rt, water (3 ml) was added and the mixture was extracted with ether (3 x 4 ml). The combined organic layers were washed with brine (5 ml), dried over anhydrous Na$_2$SO$_4$, filtered and the filtrate was concentrated under vacuum. The product was purified by column chromatography on SiO$_2$ (eluted with hexanes/ethyl acetate = 10:1), delivering 335 1.040 g (98% yield) as light yellow liquid. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 4.27 (t, $J$ = 2.4 Hz, 2H), 3.27 (t, $J$ = 6.8 Hz, 2H), 2.33 (dt, $J$ = 2.4 Hz and $J$ = 6.8 Hz, 2H), 1.98-1.94 (m, 2H), 0.89 (s, 9H), 0.09 (s, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 83.2, 80.1, 52.1, 32.2, 26.1, 20.0, 18.6, 5.6, -4.9; HRMS calcd for [M + Na] C$_{12}$H$_{23}$INaOSi 361.0461, found 361.0471.

8-(tert-Butyldimethylsiloxy)-2,2-dimethylct-6-ynenitrile (336). At −78 °C, n-BuLi (1.34 ml, 2.82 mmol, 2.1 M in hexanes) was added dropwise to the solution of the isopropylamine (0.413 ml, 2.95 mmol) in dry THF (3 ml) under argon. Then the temperature was raised to 0 °C and stirred for 1 h. The temperature was cooled to −78 °C, the solution of the isobutyronitrile (0.23 ml, 2.56 mmol) in dry THF (3 ml) was added dropwise the reaction mixture and the temperature was raised to 0 °C and stirred for 1 h. The temperature was cooled to −78 °C, the solution of the 335 (1.040 g, 3.08 mmol) in dry THF (3 ml) was added dropwise to the reaction mixture and the mixture was stirred at −78 °C for 30 min and rt overnight, quenched with saturated NH$_4$Cl (aq. 5 ml). The mixture was extracted with ether (4 x 5 ml). The combined organic layers were dried over anhydrous Na$_2$SO$_4$, filtered and filtrate was concentrated under vacuum. The product was purified by column chromatography on SiO$_2$ (eluted with hexanes/ethyl acetate = 50:1, 20:1, 10:1), delivering
0.630 g (86% yield) 336 as colorless liquid. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 4.27 (t, $J$ = 2.0 Hz, 2H), 2.26 (dq, $J$ = 2.0 Hz and $J$ = 8.4 Hz, 2H), 1.69-1.60 (m, 4H), 1.32 (s, 6H), 0.89 (s, 9H), 0.90 (s, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 125.4, 84.5, 80.1, 52.4, 40.7, 32.7, 27.2, 26.4, 24.8, 19.3, 18.9, -4.6; HRMS calcd for [M + Na] C$_{16}$H$_{29}$NNaOSi 302.1916, found 302.1930.

8-(tert-Butyldimethylsiloxy)-2,2-dimethyl-octa-6-ynal (337). The nitrile 336 (560 mg, 2.00 mmol) was dissolved in dry DCM (15 ml) and the solution was cooled to $-78$ °C and DIBAL-H (4.21 ml, 4.21 mmol, 1 M in hexanes) was added slowly. The reaction mixture was stirred at $-78$ °C for 100 min, quenched with saturated aqueous NH$_4$Cl solution (5 ml), and warmed to rt for 1 h. The mixture was filtered through a pad of celite and washed with DCM. The filtrate was washed with brine (5 ml), dried over anhydrous Na$_2$SO$_4$, filtered and the filtrate was concentrated under vacuum. The product was purified by column chromatography on SiO$_2$ (eluted with hexanes/ethyl acetate = 10:1), delivering 522 mg (93% yield) of the aldehyde 337 as colorless liquid. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 4.27 (t, $J$ = 2.0 Hz, 2H), 2.21-2.16 (m, 2H), 1.57-1.52 (m, 2H), 1.44-1.38 (m, 2H), 1.03 (s, 6H), 0.89 (s, 9H), 0.09 (s, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 206.3, 84.7, 79.5, 52.2, 45.9, 36.5, 26.1, 23.6, 21.5, 19.5, 18.6, -4.9; HRMS calcd for [M + H] C$_{16}$H$_{31}$O$_2$Si 283.2093, found 283.2089.

7,7-Dimethylnona-2,8-diyn-1-ol (339). At 0 °C, dry DCM (5 ml) was added to a mixture of carbon tetrabromide (704 mg, 2.12 mmol) and triphenylphosphine (1115 mg, 4.25 mmol). The orange solution was stirred at 0 °C for 15 min. A solution of the aldehyde 337 (300 mg, 1.06 mmol) in dry DCM (3 ml) was added dropwise. The ice bath was removed and the reaction mixture was stirred at rt overnight. Hexane (30 ml) was added and the mixture
was filtered through a pad of celite and washed with hexanes. The filtrate was concentrated under vacuum. The product was purified by column chromatography on SiO$_2$ (eluted with hexane/ethyl acetate = 50:1) delivering 389 mg (86% yield) of (9,9-dibromo-7,7-dimethylnon-8-en-2-ynyloxy)(tert-butyl)dimethylsilane (338) as a colorless liquid. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 6.49 (s, 1H), 4.28 (t, $J = 2.0$ Hz, 2H), 2.19 (dt, $J = 2.0$ Hz and $J = 8.4$ Hz, 2H), 1.58-1.53 (m, 2H), 1.50-1.46 (m, 2H), 1.15 (s, 6H), 0.89 (s, 9H), 0.10 (s, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 146.3, 85.7, 85.2, 79.3, 52.2, 41.5, 4.14, 27.4, 26.1, 24.2, 19.6, 18.6, -4.8; HRMS calcd for [M + Na] C$_{17}$H$_{30}$Br$_2$NaOSi 459.0330, found 459.0334. At $-78^\circ$C, BuLi (0.48 ml, 1.01 mmol, 2.1 M in hexanes) was added dropwise to a solution of (9,9-dibromo-7,7-dimethylnon-8-en-2-ynyloxy)(tert-butyl)dimethylsilane (110 mg, 0.25 mmol) in dry THF (13 ml) and the reaction mixture was stirred at $-78^\circ$C for 2 h, quenched with saturated NH$_4$Cl (aq. 20 ml) and warmed to rt. The mixture was extracted with ether (3 x 20 ml). The combined organic layers were dried over anhydrous Na$_2$SO$_4$, filtered and the filtrate was concentrated under vacuum to yield a light yellow liquid as crude product. The product was dissolved in THF (3 ml) and cooled to 0 $^\circ$C. TBAF (0.38 ml, 0.38 mmol, 1 M in THF) was added and the mixture was stirred at 0 $^\circ$C for 1 h. Water (3 ml) was added and the mixture was extracted with ether (3 x 5 ml). The combined organic layers were dried over anhydrous Na$_2$SO$_4$, filtered and the filtrate was concentrated under vacuum. The product was purified by column chromatography on SiO$_2$, (eluted with hexanes/ethyl acetate = 2:1), delivering 33 mg (79% yield) of the diyne 339 as a colorless liquid. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 4.23 (t, $J = 2.8$ Hz, 2H), 2.24-2.22 (m, 2H), 2.06 (s, 1H), 1.70-1.60 (m, 3H), 1.47-
1.43 (m, 2H), 1.19 (s, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 91.8, 86.5, 78.8, 68.2, 51.6, 42.5, 31.0, 29.3, 24.8, 19.3; HRMS calcd for [M + Na] C$_{11}$H$_{16}$NaO 187.1099, found 187.1114.

**7,7-Dimethylnona-2,8-diynoic acid (331).** 7,7-Dimethylnona-2,8-diyn-1-ol (339) (50.0 mg, 0.31 mmol) was dissolved in acetone (4 ml) and the solution was cooled to 0 °C. Jones’ reagent (0.25 ml, 0.671 mmol, 2.74 M in H$_2$O) was added dropwise and the reaction mixture was stirred at 0 °C for 1 h. Water (1 mL) was added and the mixture was extracted with ether (4 x 4 ml). The combined organic layers were washed with brine until the yellow color disappeared. The ether layer was dried over anhydrous Na$_2$SO$_4$, filtered and the filtrate was concentrated under vacuum to deliver 53 mg (97% yield) of 7,7-dimethylnona-2,8-diynoic acid (331) as a colorless liquid. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 8.25 (br, 1H), 2.37 (t, $J$ = 7.2 Hz, 2H), 2.07 (s, 1H), 1.79-1.71 (m, 2H), 1.49-1.45 (m, 2H), 1.94 (s, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 158.2, 92.3, 91.3, 73.0, 68.5, 42.4, 31.0, 29.3, 23.7, 19.4; HRMS calcd for [M + H] C$_{11}$H$_{15}$O$_2$ 179.1072, found 179.1075.

**(R)-5-((4-Methoxybenzyloxy)methyl)hex-1-yn-3-ol (332).** A solution of the aldehyde 340 (136.0 mg, 0.612 mmol) in dry DCM (5 ml) was cooled to 0 °C, and ethynylmagnesium bromide (0.5 M solution in THF, 2.5 ml, 1.225 mmol) was added slowly. The reaction mixture was stirred at 0 °C for 1 h, water (5 ml) was added, and the mixture was warmed to rt. The aqueous layer was extracted with ether (4 x 5 ml). The combined organic layers were washed with brine (5 ml), dried over anhydrous Na$_2$SO$_4$, filtered and the filtrate was concentrated under vacuum. The product was purified by column chromatography on SiO$_2$ (eluted with hexanes/ethyl acetate = 3:1), delivering 136 mg (90% yield) of the alcohol 332 as a colorless oil. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 7.25 (d, $J$ = 8.4 Hz, 2H), 6.87 (d, $J$ =
8.4 Hz, 2H), 4.52-4.42 (m, 3H), 3.80 (s, 3H), 3.41-3.34 (m, 1H), 3.30-3.23 (m, 1H), 2.44-2.42 (m, 1H), 2.30-2.22 (m, 0.5H), 2.06-1.98 (m, 0.5H), 1.87-1.83 (m, 0.5H), 1.82-1.78 (m, 1H), 1.75-1.69 (m, 0.5H), 0.95 (d, J = 4.8 Hz, 1.5 Hz), 0.94 (d, J = 4.8 Hz, 1.5 Hz); 13C NMR (100 MHz, CDCl3) δ 159.52, 159.49, 130.1, 129.9, 129.7, 129.6, 114.09, 114.08, 85.7, 85.3, 75.80, 75.78, 73.1, 72.8, 72.3, 61.3, 60.8, 55.5, 44.0, 43.4, 31.6, 30.2, 18.2, 18.1; HRMS calcd for [M + Na] C15H20NaO3 271.1310, found 271.1324.

(5R)-6-(4-Methoxybenzyloxy)-5-methylhex-1-yn-3-yl 7,7-dimethylnona-2,8-diynoate (330) At 0°C, the solution of the acid 331 (19.1 mg, 0.107 mmol) in the DCM (0.7 ml) was added dropwise to the solution of the alcohol 332 (22.1 mg, 0.0891 mmol) and DMAP (6.5 mg, 0.053 mmol) in DCM (0.7 ml). The solution was stirred at 0 °C for 10 min and a solution of DCC (23.9 mg, 0.116 mmol) in DCM (0.7 ml) was added dropwise at 0 °C and the resulting mixture was stirred at rt overnight. The mixture was cooled to 0 °C and additional DCC (23.9 mg, 0.116 mmol) in DCM (0.7 ml) was added and the resulting mixture was stirred overnight. The mixture was concentrated and the crude was purified by column chromatography on SiO2 (eluted with hexanes/ethyl acetate = 50:1, 25:1, 10:1 and 5:1), delivering 28.7 mg (79% yield) of triyne 330 as colorless liquid. 1H NMR (400 MHz, CD3OD) δ 7.25-7.20 (m, 2H), 6.85 (d, J = 8.4 Hz, 2H), 5.52-5.44 (m, 1H), 4.41 (s, 2H), 3.79 (s, 3H), 3.28 (d, J = 7.8 Hz, 2H), 2.47 (t, J = 0.8 Hz, 1H), 2.35 (t, J = 7.2 Hz, 2H), 2.07 (s, 1H), 2.01-1.96 (m, 2H), 1.78-1.65 (m, 3H), 1.55-1.45 (m, 2H), 1.20 (s, 6H), 0.96 (d, J = 6.4 Hz, 3H); 13C NMR (100 MHz, CDCl3) δ 159.3, 152.9, 130.7, 129.4, 114.0, 91.4, 90.50, 90.48, 81.0, 80.6, 75.1, 74.9, 74.6, 74.4, 73.1, 72.92, 72.86, 68.5, 64.3, 63.9, 55.5, 42.4, 39.0,
38.6, 31.0, 30.4, 30.2, 29.3, 23.8, 19.3, 17.3, 17.2; MS calcd for [M + Na]⁺ C₂₆H₃₂NaO₄ 431.2198, found 431.2.

3-((R)-3-(4-Methoxybenzylxyloxy)-2-methylpropyl)-6,7,8,9-tetrahydro-6,6-dimethylnaphtho[2,1-c]furan-1(3H)-one (341). To a flame-dried microwave vial was added the triyne 330 (10.0 mg, 0.025 mmol) and toluene (1 ml) under argon. Cp*RuCl(COD) (0.9 mg, 0.0025 mmol) was added and the vial was sealed and heated in a CEM Discover microwave synthesizer (power mode, 300 W, 130 °C final temperature) for 20 min. An additional 0.1 eq (0.9 mg) of Cp*RuCl(COD) was added and the reaction was continued for 30 min. The solid was filtered off and solvent was removed in vacuo. The product was purified by column chromatography on SiO₂ (eluted with hexanes/ethyl acetate = 10:1), delivering the product 341 (9.0 mg, 90% yield) as a colorless liquid. ¹H NMR (400 MHz, CD₃OD) δ 7.61 (d, J = 7.6 Hz, 1H), 7.28-7.25 (m, 2H), 7.19-7.16 (m, 1H), 6.90-6.87 (m, 2H), 5.45-5.40 (m, 1H), 4.48 (s, 1H), 4.43 (s, 1H), 3.81 (s, 3H), 3.52-3.48 (m, 0.5H), 3.41-3.34 (m, 0.5H), 3.37-3.34 (m, 1H), 3.24 (t, J = 6.4 Hz, 2H), 2.25-2.21 (m, 1H), 1.86-1.84 (m, 0.5H), 1.86-1.78 (m, 3H), 1.72-1.69 (m, 2H), 1.52-1.47 (m, 0.5H), 1.32 (s, 6H), 1.10 (d, J = 6.0 Hz, 1H), 1.05 (d, J = 6.0 Hz, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 171.2, 159.4, 159.3, 149.1, 149.1, 147.3, 138.1, 132.8, 130.8, 129.43, 129.37, 123.0, 122.9, 119.03, 118.98, 114.02, 113.96, 79.1, 78.3, 75.5, 74.8, 73.0, 72.8, 55.5, 40.0, 39.7, 38.7, 34.2, 32.1, 32.0, 31.0, 30.6, 29.9, 26.1, 18.8; MS calcd for [M + Na]⁺ C₂₆H₃₂NaO₄ 431.2198, found 431.2.

3-((R)-3-Hydroxy-2-methylpropyl)-6,7,8,9-tetrahydro-6,6-dimethylnaphtho[2,1-c]furan-1(3H)-one (329). The PMB ether 341 (9.0 mg, 0.022 mmol) was dissolved in DCM (0.8 ml) and H₂O (0.03 ml), and the solution was cooled to 0 °C. DDQ (5.5 mg, 0.024 mmol)
was added and the reaction mixture was stirred at rt overnight. Saturated NH₄Cl (1 mL) was added to quench the reaction and the mixture was extracted with DCM (4 x 2 ml). The combined organic layers were washed with brine (2 ml), dried over anhydrous Na₂SO₄, filtered and the filtrate was concentrated in vacuo. The product was purified by column chromatography on silica gel (eluted with hexanes/ethyl acetate = 5:1-2:1), delivering the alcohol **329** (6.3 mg, 99% yield) as a white solid. ¹H NMR (400 MHz, CD₃OD) δ 7.59 (dd, J = 8.0 Hz and J = 1.2 Hz, 1H), 7.16 (dd, J = 8.0 Hz and J = 3.6 Hz, 1H), 5.46-5.38 (m, 1H), 3.68-3.57 (m, 1H), 3.55-3.53 (m, 1H), 3.21 (t, J = 6.0 Hz, 2H), 2.14-2.02 (m, 1.5H), 1.86-1.77 (m, 2.5H), 1.69-1.64 (m, 2H), 1.62-1.49 (m, 2H), 1.28 (s, 6H), 1.07 (d J = 6.8 Hz 1.5H), 1.02 (d J = 6.8 Hz 1.5H); ¹³C NMR (100 MHz, CDCl₃) δ 174.0, 148.9, 148.9, 147.5, 147.5, 138.2, 134.7, 132.95, 132.92, 122.8, 118.96, 118.88, 78.8, 78.5, 68.3, 67.5, 39.5, 39.3, 38.7, 34.2, 33.3, 33.1, 32.1, 32.0, 29.9, 26.1, 18.7; MS calcd for [M + Na]⁺ C₁₈H₂₄NaO₃ 311.1623, found 311.1.

**Cryptoacetalide (308).** The alcohol **329** (6.2 mg, 0.022 mmol) was dissolved in dry benzene (1.5 ml). Iodobenzene diacetate (20.8 mg, 0.065 mmol) and iodine (10.9 mg, 0.043 mmol) were added under nitrogen. The vial was sealed and irradiated with a 200 W Xe/Hg lamp (Newport) for 1 h. After cooling to rt, 10 ml of ether were added and the mixture was washed with H₂O (2 ml) and brine (2 ml), dried over anhydrous Na₂SO₄, filtered and the filtrate was concentrated in vacuo. The product was purified by column chromatography on silica gel (eluted with hexanes/ethyl acetate = 20:1, 10:1), delivering 5.2 mg (85% yield) of the natural product **308** as a yellow solid. ¹H NMR showed a 2:1 diastereomeric ratio of **308** to epi-**308**. ¹H NMR (300 MHz, CD₃OD) 7.62 (d, J = 8.1 Hz, 1H), 7.26-7.24 (m, 1H), 4.37
(t, J = 8.1 Hz, 0.66 H), 4.31 (t, J = 8.1 Hz, 0.33 H), 3.81 (t, J = 8.1 Hz, 0.33 H), 3.71 (t, J = 8.1 Hz, 0.66 H), 3.18 (t, J = 6.3 Hz, 2H), 2.88-2.80 (m, 0.66 H), 2.70-2.60 (m, 0.33 H), 2.58 (dd, J = 9.4 Hz and J = 13.3 Hz, 0.33 H), 2.42 (dd, J = 6.8 Hz and J = 13.3 Hz, 0.66 H), 2.10 (dd, J = 4.5 Hz and J = 13.3 Hz, 0.33 H), 2.00-1.90. (m, 0.66 H), 1.85-1.75 (m, 2H), 1.69-1.60 (m, 2H), 1.29 (s, 4H), 1.27 (s, 2H), 1.24 (d, J = 6.6 Hz, 1H), 1.18 (d, J = 6.6 Hz, 2H);

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 168.7, 149.2, 149.1, 145.7, 144.9, 138.0, 137.8, 133.4, 133.3, 124.4, 119.5, 119.3, 113.15, 113.11, 77.2, 45.7, 44.7, 38.6, 34.34, 33.6, 32.7, 32.03, 32.01, 29.9, 26.2, 24.9, 18.7, 18.4, 17.6. MS calcd for [M + Na]$^+$ $^{\text{C}_{18}{\text{H}_{22}}\text{NaO}_3}$ 309.1467, found 309.1.
5.1 Introduction

Thiopeptide antibiotics have attracted considerable attention due to their intriguing architecture and their inhibition of bacterial protein synthesis, preventing the growth of gram-positive bacteria, including methicillin-resistant *S. aureus*.\(^{194}\) Cyclothiazomycin (342) is one of 76 structurally distinct actinomycete thiopeptide antibiotics. It was isolated from the fermentation broth of *Streptomyces* sp. NR0516, which was obtained from a soil sample collected at Kanagawa, Japan. It exhibited activity against human plasma rennin IC\(_{50}\) being 1.7 \(\mu\text{M}.\(^{195}\)

![Structure of Cyclothiazomycin (342)](image)

**Figure 5.1.** Structure of Cyclothiazomycin (342), a representative thiopeptide antibiotic.

Cyclothiazomycin was determined to be a unique polythiazole-containing bicyclic peptide. The structure consists of a (1-amino-1-ethyl)pyriding heterocyclic domain embedded
in two macrocyclic peptide loops. Acid hydrolysate of cyclothiazomycin produced a heterocyclic amino acid as γ-lactam (344) and saramytic acid (343) (Scheme 5.1). 

![Scheme 5.1. Cyclothiazomycin (342) and its hydrolysates.](image)

The central 2,3,6-trisubstituted pyridine motif 344 can also be found in a number of other thiopeptide antibiotics, including berninamycins, geninthiocin, promothiocins, radamycin, and thioxamycin. However, previous syntheses of thiopeptide antibiotics have often struggled with the assembly of this pyridine center. Several groups have developed sophisticated and specially designed approaches, including aza-Diels-Alder reactions and Bohlmann-Rahtz heteroannulations. To date, three syntheses of the pyridine motif present in cyclothiazomycin (342) have been reported, however, only one of the three approaches is a stereospecific synthesis. This synthesis utilized Bohlmann-Rahtz to assemble the trisubstituted pyridine ring from a (R)-β-ketoester and Ethyl 2-
(propynoyl)thiazole-4-carboxylate with good yield and good regioselectivities. No total synthesis of cyclothiazomycin is known.

5.2 Synthesis of the Pyridine Core of Cyclothiazomycin

It was envisioned that 344 could be assembled from the cyclotrimerization of the enantiopure alkynylnitrile 345 and the thiozaline alkyne 346 (Scheme 5.2). Although it is highly convergent, this approach potentially entails several pitfalls. 1) Alkynylnitriles are traditionally poor substrates in cyclotrimerization reactions due to the possibility of several undesired side reactions.  57, 105, 203 2) The cyclotrimerization precursor 345 can exist as two different amide isomers, of which only the shown one can undergo the desired reaction. This problem can potentially be suppressed through protection of the nitrogen center (with R$^1$). 3) The cyclotrimerization reaction might yield mixtures of regioisomers. 105 A solution to this problem could be the installation of a temporary regiodirecting group R$^2$ which is removable in a traceless fashion.

Scheme 5.2. Retrosynthetic analysis of 344 (R$^1$ = H, or protecting group (e.g. Ac or Bn); R$^2$ = H or regiodirecting group (e.g. TMS)).
However, the cyclotrimerization reaction of the alkynynitrile 345 \((R^1 = \text{Ac}, \ R^2 = \text{TMS})\) and the thiazole alkyne 346 did not proceed as expected (data not shown). Even though a wide range of conditions were explored, a number of catalysts were investigated at different temperatures, with and without microwave irradiation, the product yields were generally below 15%.

Based on our\textsuperscript{63} and others\textsuperscript{60-62, 207} recent successes in constructing highly substituted benzenes and pyridines via a silyl-tethered [2+2+2] cyclotrimerization, we applied a similar approach to the synthesis of the pyridine core 344 of cyclothiazomycin (342) (Scheme 5.3). The silyl tether transforms an otherwise intermolecular cyclotrimerization reaction into an intramolecular one, providing enhanced regio- and chemoselectivity. Here, the application of a silyl tether enabled switching of the synthetic approach from a notoriously difficult\textsuperscript{105, 203} to cyclotrimerize alkynynitrile to a regular diyne cyclotrimerization precursor. The linker can be easily and selectively removed in a traceless fashion, delivering the desired products.

It was envisioned that the pyridine core 345 could be obtained from the oxidation of the alcohol 348, which in turn could be synthesized via removal of the silyl group from 349. The pyridine ring 349 could be assembled by a [2+2+2] cyclotrimerization reaction of the enantiopure diyne 350 and the thiazolecarbonitrile 351. The diyne 351 could be synthesized from the known dibromoalkene 352. The dibromoalkene 351 could be synthesized from the commercially available \((R)-2\text{-aminopropan-1-ol} (353)\).
First, the ability to react various silylether diynes in [2+2+2] cyclotrimerization reactions was investigated. Two model substrates, silylether diynes 361 and 362, were synthesized (Scheme 5.4). The diyne 361 was prepared in one step from the commercially available alcohol 360 and freshly prepared alkynylsilyl bromide 359 (synthesized in two steps from chlorodiisopropylsilane (357)). The synthesis of the diyne 362 was accomplished in two steps via an SN2 reaction of N-benzyl-p-toluene sulfonamide with 4-hydroxybut-2-ynyl methanesulfonate, followed by reacting 356 with alkynylsilyl bromide to form 362 in good yields. The known thiazolecarbonitrile 350 was prepared in one step from ethyl 2-bromothiazole-4-carboxylate (354) in 59% yield.
Scheme 5.4. The synthesis of thiazole nitrile 350 and model diynes 361 and 362.

With the model diynes and nitriles in hand, we investigated the cyclotrimerization reactions under different reaction conditions (Table 5.1). The CpCo(CO)$_2$ catalyst was the first used due to its wide application in the formation of pyridines.$^{2, 5, 209, 210}$ However, no product was obtained from the diynes 361-362 and benzonitrile or thiazolecarbonitrile 350 under microwave irradiation (entry 1, 2 and 4). When the cyclotrimerization reaction between 361 and benzonitrile was thermally heated to 150 °C in the presence of CpCo(CO)$_2$, only 39% of the desired product 366 was obtained (entry 3). Yamamoto et al.$^{211}$ successfully used Cp*RuCl(COD) as the catalyst for cyclotrimerization reactions of electron-deficient nitriles. Gratifyingly, the diyne 363 underwent a smooth cyclotrimerization reaction with the electron-poor thiazolecarbonitrile 350 in the presence of Cp*RuCl(COD) at either room temperature or at 60 °C (entry 6 and 7), furnishing the desired pyridine 367 in excellent yield. The cyclotrimerization reaction between diyne 362 and thiazolecarbonitrile 350 was successful and the product 368 was obtained in good yield and with excellent
regioselectivity. This was the first time a thiazole nitrile was employed in a [2+2+2] cyclotrimerization reaction. The successful reaction between the silyl-tethered diyne solved potential regioselectivity problems and previously encountered reactivity problems of alkynylnitriles. These discovered results were subsequently applied to the synthesis of the pyridine core of cyclothiazomycin 345.

Table 5.1. Cyclotrimerization reactions of model diynes and nitriles.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Subs.</th>
<th>R1</th>
<th>X</th>
<th>Y</th>
<th>R2</th>
<th>cat.</th>
<th>Prod.</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>361</td>
<td>CH2NEt2</td>
<td>O</td>
<td>Si((Pr)2</td>
<td>Ph</td>
<td>a</td>
<td>364</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>361</td>
<td>CH2NEt2</td>
<td>O</td>
<td>Si((Pr)2</td>
<td>Ph</td>
<td>a</td>
<td>365</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>361</td>
<td>CH2NEt2</td>
<td>O</td>
<td>Si((Pr)2</td>
<td>Ph</td>
<td>b</td>
<td>366</td>
<td>39%</td>
</tr>
<tr>
<td>4</td>
<td>362</td>
<td>CH2NBrTs</td>
<td>O</td>
<td>Si((Pr)2</td>
<td>Ph</td>
<td>a</td>
<td>366</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>362</td>
<td>CH2NBrTs</td>
<td>O</td>
<td>Si((Pr)2</td>
<td>Ph</td>
<td>c</td>
<td>366</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>363</td>
<td>H</td>
<td>C(CO2Et)2</td>
<td>CH2</td>
<td>d</td>
<td>367</td>
<td>93%</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>363</td>
<td>H</td>
<td>C(CO2Et)2</td>
<td>CH2</td>
<td>e</td>
<td>367</td>
<td>95%</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>363</td>
<td>H</td>
<td>C(CO2Et)2</td>
<td>CH2</td>
<td>d</td>
<td>368</td>
<td>78%</td>
<td></td>
</tr>
</tbody>
</table>

*a*CpCo(CO)2, toluene, MW 300 W;  
*b*CpCo(CO)2, xylene, 150 °C, 16 h;  
*c*CpCo(COD), 150 °C, 16 h;  
*d*Cp*RuCl(COD), DCM, rt, 20 h;  
*e*Cp*RuCl(COD), DCE, 60 °C, 20 h.
After the best cyclotrimerization reaction condition was selected, the synthesis of the pyridine core of cyclothiazomycin \(344\) was carried out. \(^{60}\) An electron-deficient amino protecting group, Boc, was selected, since the cyclotrimerization reaction did not proceed when the amino group was protected with a dibenzyl group, an electron-rich protecting group. \((R)\)-2-Aminopropan-1-ol \(353\) was transformed to dibromoalkene \(352\) according to literature procedure via protection with tert-butyl dicarbonate (Boc) group, oxidation with Py-SO\(_3\), followed by Corey-Fuchs reaction.\(^{212}\) The dibromoalkene \(352\) was deprotonated with \(n\)-BuLi and reacted with DMF to the corresponding aldehyde which was directly reduced to the propargyl alcohol \(371\) (Scheme 5.5).\(^{213}\) The alcohol \(371\) was alkylated with freshly prepared alkynylsilyl bromide,\(^{60}\) producing the silylether-tethered cyclotrimerization precursor \(351\) in 84% yield. As expected from the model studies, the diyne \(351\) underwent an efficient [2+2+2] cyclotrimerization reaction with the thiazolecarbonitrile \(350\) in the presence of Cp*RuCl(COD), delivering the tetrasubstituted pyridine \(349\) in 82% yield. Importantly, the reaction proceeded with complete regio- and chemoselectivity, as no other cyclotrimerization products were observed. The silylether linkage was removed by treatment with TBAF, generating the alcohol \(348\) in 97% yield. The alcohol was subsequently oxidized to the corresponding carboxylic acid using Jones’ reagent and the Boc group was removed through treatment with TFA, which delivered the lactam \(345\) in 80% yield over both steps. Thus, the pyridine core of cyclothiazomycin was efficiently assembled in regio- and enantiomerically pure form. The stereochemical information was retained throughout the entire synthesis, as the optical rotation of \(345\) \([\alpha]^{20}_D +51.9\) (c 0.54, CHCl\(_3\)) is in excellent agreement with the literature reported value \([\alpha]^{24}_D +48.1\) (c 0.54, CHCl\(_3\)).\(^{202}\) The lactam
345 has previously been converted to the hydrolysate 344 of cyclothiazomycin. Even though our synthetic approach is slightly longer than Bagley’s published route, the overall efficiency of both synthesis is comparable, with our approach being more easily adaptable to the synthesis of structural analogs.

Scheme 5.5. The synthesis of the pyridine core of cyclothiazomycin 345.

5.3 Summary and Outlook

In summary, we have described an effective and regioselective approach to the pyridine core of cyclothiazomycin (345). The key step of the synthesis is a transition metal-catalyzed [2+2+2] cycloolimerization reaction of a diyne and a nitrile. The electron-deficient
nature of the thiazole-bearing nitrile enables ruthenium-catalysis under mild reaction conditions with excellent yields. Complete chemo- and regioselectivity in the construction of the trisubstituted pyridine core was achieved by applying a temporary silyl-tether. We believe that this approach, of tethering functionalized alkynes and cyclotrimerizing them with heterocyclic nitriles, will be applicable to a wide range of pyridine motifs found in natural products and pharmacologically relevant molecules.

5.4 Experimental

**General methods.** Unless otherwise stated, reactions were performed under nitrogen using flame-dried glassware. Some solvents used in reactions were dried by different ways. CH$_2$Cl$_2$, THF and diethyl ether were dried by MB SPS Compact solvent purification system. Toluene and xylenes were distilled from sodium metal/benzophenoneketyl. 1,2-Dichloroethane, CH$_3$CN and pyridine were distilled from CaH$_2$. DMF and acetone were distilled from CaCO$_3$. EtOH was distilled over Mg/I$_2$. All other reagent quality solvents were used without further purification. The microwave used is a CEM Discover microwave synthesizer. All spectra were recorded in CDCl$_3$ or CD$_3$OD, and chemical shifts are reported relative to CDCl$_3$ (7.24 ppm for $^1$H NMR) or CDCl$_3$ (77.23 ppm for $^{13}$C NMR), CD$_3$OD (3.30 ppm for $^1$H NMR) or CD$_3$OD (49.15 ppm for $^{13}$C NMR). Chiral compounds were analyzed using a Jasco P-1010 polarimeter, using the sodium D line at the indicated temperature, and are given in deg cm$^3$ g$^{-1}$ dm$^{-1}$ for and 10$^{-2}$ g cm$^{-3}$ for c.

**Ethyl 2-bromothiazole-4-carboxylate (354).** To a mixture of thiourea (2.810 g, 36.60 mmol) in 2 mL of ethanol was added dropwise a solution of ethyl bromopyruvate (5.0
mL, 35.80 mmol) in 2 mL of ethanol. The reaction mixture was heated slowly to 100 °C and kept at that temperature for 40 min to give a clear brown solution. Upon cooling to rt, a brown precipitate formed and the precipitate was dissolved in sulfuric acid (9 N, 168 mL). The solution was transferred into a 1 L three-necked bottom equipped with a mechanical stirrer and an additional funnel and a gas outlet with an inverted wide-mouth funnel suspended just above a NaOH solution. The solution was cooled in an ice-water bath and CuSO₄ (10.550 g, 42.24 mmol) and NaBr (12.90 g, 125.3 mmol) were added portionwise. A solution of NaNO₂ (3.800 g, 54.80 mmol) in 21 mL of H₂O was then added dropwise over 1 h. The reaction mixture was stirred at rt overnight. The mixture was diluted with 100 mL of H₂O and the mixture was extracted with ether (3 x 150 mL). The combined organic layers were washed with saturated NaHCO₃ (2 x 50 mL) and brine (50 mL), dried over anhydrous Na₂SO₄, filtered and the filtrate was concentrated in vacuo. The product was purified by column chromatography on SiO₂, eluting with hexanes:EtOAc = 8:1, delivering 4.90 g (58% yield) of 354 as white solid. ¹H NMR (300 MHz, CDCl₃) δ 8.10 (s, 1H), 4.41 (q, J = 7.1 Hz, 2H), 1.39 (t, J = 7.1 Hz, 3H).

Ethyl 2-cyanothiazole-4-carboxylate (350). Ethyl 2-bromothiazole-4-carboxylate (354) (900.0 mg, 3.81 mmol) was dissolved in 2.5 mL of dry pyridine in a pressure tube. CuCN (684 mg, 7.62 mmol) was added. The tube was sealed and heated at 150 °C for 2 h. Cooled to rt, 10 mL of saturated NH₄Cl was added and the mixture was extracted with EtOAc (4 x 20 mL). The combined organic layers were washed with brine (5 mL), dried over anhydrous MgSO₄, filtered and the filtrate was concentrated. The product was purified by column chromatography SiO₂, eluted with hexanes/ethyl acetate (2:1) to give 350 (412.0 mg,
59%) as a white solid. $^1$H NMR (300 MHz, CDCl$_3$) δ 8.45 (s, 1H), 4.47 (q, $J = 7.2$ Hz, 2H), 1.42 (t, $J = 7.2$Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 160.0, 149.5, 137.9, 112.0, 62.6, 14.5; MS calcd for [M + Na] C$_7$H$_6$N$_2$NaO$_2$S 205.0048, found 205.0113.

**tert-Butyl (R)-1-hydroxypropan-2-ylcarbamate (369).** (R)-(+-)2-amino-1-propanol (1.00 mL, 12.82 mmol) and TEA (2.05 mL, 14.74 mmol) were dissolved in MeOH (10 mL) and cooled to °C. Boc$_2$O (3.078g, 14.10 mmol) was added and the mixture was stirred at rt overnight. The solvent was removed under reduced pressure and the resulting residue was redissolved in 50 mL of DCM, washed with 20 mL of H$_2$O, dried over MgSO$_4$, concentrated and dried under high vacuum to deliver 1.935 g (88% yield) desired product as white solid. $^1$H NMR (300 MHz, CDCl$_3$) δ 4.62 (br, 1H), 3.74 (br, 1H), 3.62 (ddd, $J = 10.0$, 6.0, 3.7 Hz, 1H), 3.55 – 3.39 (m, 1H), 2.60 (br, 1H), 1.42 (s, 9H), 1.12 (d, $J = 6.7$ Hz, 3H).

**tert-Butyl (R)-1-formylethylcarbamate (370).** The alcohol 369 (1.558 g, 8.89 mmol) and TEA (3.16 mL, 22.67 mmol) were dissolved in 9 mL of DMSO and cooled to 0 °C and then the ice-water bath was removed. The solution of SO$_3$·Py (3.297 g, 20.72 mmol) in 12 mL of DMSO was added dropwise. The reaction mixture was stirred at rt for 2 h. 20 mL of H$_2$O was added and it was extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with 10 mL of brine, dried over anhydrous MgSO$_4$, filtered and the filtrate was concentrated under vacuum, delivering 1.477 g (96% yield) of 370 as light yellow solid. $^1$H NMR (300 MHz, CDCl$_3$) δ 9.54 (s, 1H), 5.08 (br, 1H), 4.21 (br, 1H), 1.3 (s , 9H), 1.31 (d, $J = 7.5$ Hz, 3H).

**tert-Butyl (R)-4,4-dibromobut-3-en-2-ylcarbamate (352).** PPh$_3$ (4.540 g, 17.32 mmol) was dissolved in 22 mL of DCM and cooled to 0 °C. CBr$_4$ (2.872 g, 8.66 mmol) was
added slowly. The mixture was stirred at 0 °C for 20 min and the aldehyde 370 (750.0 mg, 0.409 mmol) was added at 0 °C and the mixture was stirred at rt for 2 h. The reaction mixture was added dropwise to 300 mL hexanes while stirring and filtered. The filtrate was concentrated and the resulting residue was purified by column chromatography on SiO$_2$ (eluted with hexanes/EtOAc = 6:1), delivering 888.5 mg (63% yield) of 352 as white solid. 

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 6.34 (d, $J = 8.2$ Hz, 1H), 4.52 (s, 1H), 4.34 (d, $J = 8.7$ Hz, 1H), 1.45 (s, 9H), 1.25 (d, $J = 6.8$ Hz, 3H).

(R)-4-[N-(tert-butoxycarbonyl)amino]-2-pentyn-1-ol (371). The dibromoalkene 352 (200.0 mg, 0.61 mmol) was dissolved in THF (1.2 mL) under argon and cooled to −78 °C. $n$-BuLi (0.96 mL, 1.82 mmol, 2.45 M in hexanes) was added dropwise and stirred for 20 min. The solution of DMF (94 µl, 1.22 mmol) in 0.24 mL of THF was added dropwise. The temperature was warmed to 0 °C and stirred for 1 h. Saturated citric acid solution (0.35 mL) was added and warmed to room temperature and stirred for 10 min. Saturated NH$_4$Cl solution (2 mL) was then added and extracted with ether (3 x 2 ml). The combined organic layers were washed with brine (2 mL), dried over anhydrous MgSO$_4$, filtered and the filtrate was concentrated under high vacuum. The resulting solid was dissolved in EtOH (0.7 mL) and cooled to 0 °C. NaBH$_4$ (23.0 mg, 0.61 mmol) was added and the reaction mixture was stirred at 0 °C for 50 min. Saturated citric acid solution (1 mL) was added, stirred at 0 °C for 5 min. The reaction mixture was extracted with ether (3 x 2 mL). The combined organic layers were washed with brine (2 mL), dried over anhydrous MgSO$_4$, filtered and the filtrate was concentrated under vacuum. The product was purified by column chromatography on SiO$_2$ (eluted with hexanes/EtOAc = 3:1, 2:1), delivering 60.6 mg (50% yield) of 371 as colorless.
liquid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 4.75 (br, 1H), 4.49 (br, 1H), 4.23 (s, 2H), 2.23 (br, 1H), 1.42 (s, 9H), 1.35 (d, $J$ = 6.9 Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$155.0, 86.4, 80.5, 51.2, 38.6, 28.6, 22.8; MS calcd for [M + Na] C$_{10}$H$_{17}$NaO$_3$ 222.1106, found 222.1171. $[\alpha]^{20}_D$ +76.9 (c 1.00, CHCl$_3$).

4-(N-Benzyl-N-tosylamino)but-2-yn-1-ol (356). TsBnNH (50.0 mg, 0.18 mmol) and 4-hydroxybut-2-ynyl methanesulfonate (355) (36.3 mg, 0.22 mmol) were dissolved in dry CH$_3$CN (3 mL). To this stirring solution was added K$_2$CO$_3$ (31.0 mg, 0.22 mmol). The reaction mixture was reflux overnight, cooled to rt, filtered and the filtrate was concentrated under vacuum. The product was purified by column chromatography on SiO$_2$ (eluted with hexanes/EtOAc= 1:1), delivering 52.0 mg (86% yield) of 356 as colorless liquid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.77 (d, $J$ = 8.4 Hz, 2H), 7.33-7.24 (m, 7H), 4.31 (s, 2H), 3.94 (br, 2H), 3.91 (s, 2H), 2.42 (s, 2H), 1.66 (br, 1H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 143.9, 136.1, 135.1, 129.6, 128.9, 128.8, 128.3, 128.1, 84.4, 78.3, 50.8, 50.3, 36.1, 21.7; MS calcd for [M + Na] C$_{18}$H$_{19}$NaO$_3$S 352.0983, found 352.1.

**General procedure for diyne synthesis.** To the solution of the alcohol (1 equiv) in DCM (0.23 M) were added TEA (1 equiv) and DMAP (0.1 equiv), followed by the solution of freshly prepared alkynylsilyl bromide (359) (1 equiv) in DCM. The resulting mixture was stirred under N$_2$ at room temperature overnight, concentrated under vacuum. The product was purified by column chromatography on SiO$_2$ (eluted with hexanes/EtOAc mixture), delivering 356, 360 and 351.

{(R)-4-[((N-tert-Butoxycarbonyl)amino)pent-2-ynoxy](ethynyl)diisopropylsilane (351). Eluted with hexane/EtOAc = 10:1. 284.2 mg (84% yield) of 351 as colorless liquid was
obtained from 200.0 mg of alcohol. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 4.69 (br, 1H), 4.49 (br, 1H), 4.41 (s, 2H), 2.45 (d, $J = 0.6$ Hz, 1H), 1.42 (s, 9H), 1.35 (d, $J = 6.9$ Hz, 3H), 1.20-1.00 (m, 14H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 154.8, 95.8, 85.8, 84.0, 80.3, 53.1, 38.7, 28.6, 22.9, 17.1, 17.0, 13.0; MS calcd for [M + Na] C$_{18}$H$_{31}$NNaO$_3$Si 360.1971, found 360.2060. $[^{\alpha}]_{D}^{20} +53.54$ (c 0.54, CHCl$_3$).

(4-$N$-$N$-Diethylamino-but-2-nyloxy)(ethynyl)diisopropylsilane (361). Eluted with hexanes/EtOAc = 1:1, 1:2. 65.5 mg (70% yield) of 361 was obtained as colorless liquid from 54.4 mg of 360. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 4.44 (t, $J = 1.6$ Hz, 2H), 3.42 (t, $J = 1.6$ Hz, 2H), 2.52 (q, $J = 6.8$ Hz, 4H), 2.43 (s, 1H), 1.04-1.00 (m, 20H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 95.6, 84.0, 83.0, 80.0, 53.2, 47.4, 40.9, 18.0, 17.0, 16.9, 13.3, 13.0, 12.8; MS calcd for [M + H] C$_{16}$H$_{30}$NOSi 280.2097, found 280.2.

[4-(N-Benzyl-$N$-tosylamino)-but-2-nyloxy](ethynyl)diisopropylsilane (362). Eluted with hexanes/EtOAc = 3:1. 105.0 mg (81% yield) of 362 as white solid was obtained from 90.7 mg of 356. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.77 (d, $J = 7.6$ Hz, 1H), 7.35-7.24 (m, 7H), 4.31 (s, 2H), 4.14 (s, 2H), 3.95 (s, 2H), 2.44 (s, 1H), 2.43 (s, 3H), 1.09-0.94 (m, 14H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 143.6, 136.3, 135.2, 129.6, 129.0, 128.8, 128.3, 128.1, 95.8, 84.3, 83.9, 77.6, 52.8, 50.1, 36.2, 21.7, 17.3, 17.1, 13.0; MS calcd for [M + H] C$_{26}$H$_{34}$NO$_3$SSi 468.2029, found 468.0.

General procedure for cyclotramimerization reactions using CpCo(CO)$_2$. To the solution of diyne (1equiv) and nitrile (10 equiv) in dry xylenes (0.11 M for diyne) in a vial was added CpCo(CO)$_2$ (0.2 equiv). The vial was sealed and the reaction was heated to 150 °C overnight. After cooling to room temperature, the reaction mixture was concentrated
under vacuum. The product was purified by column chromatography on SiO$_2$ (eluted with EtOAc, EtOAc:MeOH = 95:5), delivering **366**.

$4\text{-}(N,N\text{-Diethylamino})\text{-}1,3\text{-dihydro-}1,1\text{-diisopropyl-}6\text{-}[4\text{-}(\text{ethoxycarbonyl})\text{thiazol-2-yl}]\text{-}[1,2\text{]oxasilolo[4,3-c]pyridine (18).}$ 17.9 mg (39% yield) of **366** as yellow solid was obtained from 33.2 mg of **10**. $^1$H NMR (400 MHz, CD$_3$OD) δ 8.01 (d, $J = 8.0$ Hz, 2H), 7.89 (s, 1H), 7.50-7.40 (m, 3H), 5.33 (s, 2H), 3.78 (s, 2H), 2.64 (q, $J = 6.8$ Hz, 4H), 1.34-1.24 (m, 2H), 1.08-1.01 (m, 18H); $^{13}$C NMR (100 MHz, CD$_3$OD) δ 159.1, 145.5, 145.1, 140.9, 130.0, 129.9, 129.7, 128.4, 123.4, 72.3, 59.0, 48.6, 17.4, 17.2, 14.1, 12.1; MS calcd for [M + Na] C$_{23}$H$_{35}$N$_2$OSi 383.2519, found 383.2.

**General procedure for cyclotrimerization reactions using Cp*RuCl(COD) in DCM.** The diyne (1 equiv) and nitrile (3 equiv) were dissolved in DCM and Cp*RuCl(COD) (0.05 equiv) was added. The resulting mixture was stirred at room temperature overnight, and concentrated under vacuum. The product was purified by column chromatography on SiO$_2$ (eluted with hexanes/ EtOAc mixture), delivering **367** and **368**.

**Diethyl 5-(4-(ethoxycarbonyl)thiazol-2-yl)-1H-indene-2,2(3H)-dicarboxylate (367).** The compound was eluted with hexanes/EtOAc = 2:1, 1:1 to deliver 22.9 mg (93% yield) of **367** as a white solid from 15.6 mg of **363**. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.40 (s, 1H), 8.19 (s, 2H), 4.41 (q, $J = 7.2$ Hz, 2H), 4.20 (q, $J = 7.2$ Hz, 4H), 3.62 (d, $J = 3.2$ Hz, 4H), 1.42 (t, $J = 7.2$ Hz, 3H), 1.23 (t, $J = 7.2$ Hz, 6H); 171.0, 170.2, 151.43, 149.4, 148.2, 145.3, 138.5, 129.4, 116.5, 62.3, 61.7, 60.4, 40.2, 38.4, 14.6, 14.2; MS calcd for [M + H] C$_{20}$H$_{23}$N$_2$O$_6$S 419.1277, found 419.
4-(N-Benzyl-N-tosylethanamine)-1,3-dihydro-1,1-diisopropyl-6-[4-(ethoxycarbonyl)thiazol-2-yl]-[1,2]oxasilolo[4,3-c]pyridine (368). The product was eluted with hexanes/EtOAc = 4:1, 2:1 to give 10.9 mg (78% yield) of 368 as a white solid from 10.0 mg of 362. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.22 (s, 1H), 8.18 (s, 1H), 7.52 (d, $J = 8.4$ Hz, 2H), 7.21-7.20 (m, 2H), 7.19-7.13 (m, 3H), 7.07 (d, $J = 8.4$ Hz, 2H), 5.10 (s, 2H), 4.65 (s, 2H), 4.44 (q, $J = 6.7$ Hz, 2H), 4.33 (s, 2H), 2.25 (s, 3H), 1.43 (t, $J = 6.7$ Hz, 3H), 1.23-1.17 (m, 2H), 0.97-0.94 (m, 12H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 170.5, 161.7, 149.2, 148.4, 147.5, 146.6, 145.4, 143.5, 136.0, 129.7, 129.3, 128.9, 128.7, 128.0, 127.3, 122.0, 70.6, 61.9, 53.1, 49.7, 21.7, 17.05, 17.02, 14.6, 12.9; MS calcd for [M + H] C$_{33}$H$_{40}$N$_3$O$_5$S$_2$Si 650.2179, found 650.0.

General procedure for cyclotrimerization reactions using Cp*RuCl(COD) in DCE. The solution of diyne (1equiv) in dry degrassed DCE (0.25 M for diyne) was added dropwise to the solution of nitrile (3 equiv) and Cp*RuCl(COD) (5 mol%) in dry degassed DCE (0.25M for nitrile). The reaction mixture was stirred at room temperature overnight and concentrated under vacuum. The product was purified by column chromatography on SiO$_2$ (eluted with hexanes/ EtOAc mixture), delivering 21 and 6.

(R)-2-[1-(N-tert-Butoxycarbonyl)aminoethyl]-1,3-dihydro-1,1-diisopropyl-6-[4-(ethoxycarbonyl)thiazol-2-yl]-[1,2]oxasilolo[4,3-c]pyridine (349). The product was eluted with hexanes/EtOAc = 5:1, 2:1 to give 24.4 mg (82% yield) of 349 as white solid from 19.2 mg of 351. $^1$H NMR (300 MHz, CDCl$_3$) δ 8.34 (s, 1H), 8.21 (s, 1H), 5.74 (d, $J = 8.4$ Hz, 1H), 5.26 (d, $J = 15.3$ Hz, 1H), 5.12 (d, $J = 15.3$ Hz, 1H), 4.76 (pentet, $J = 7.5$ Hz, 1H), 4.42 (q, $J = 7.2$ Hz, 2H), 1.53-1.21 (m, 15H), 1.19-1.17 (m, 2H), 0.99-0.93 (m, 12H); $^{13}$C NMR (100
MHz, CDCl$_3$) $\delta$ 170.5, 161.7, 155.3, 154.7, 148.3, 147.7, 145.7, 144.1, 129.4, 121.7, 79.7, 70.5, 61.8, 48.0, 28.6, 21.9, 16.94, 16.92, 12.9, 12.8; MS calcd for [M + Na] C$_{25}$H$_{37}$N$_{3}$NaO$_{5}$SSi 542.2121, found 542.2231. $[\alpha]^{20}_D$ +106.1 (c 0.98, CHCl$_3$).

$(R)$-2-[1-(N-tert-Butoxycarbonyl)aminoethyl]-3-(hydromethyl)-6-[4-(ethoxycarbonyl)thiazol-2-yl] pyridine (348). To a solution of 349 (110 mg, 0.21 mmol) in 5.1 mL of dry THF was added TBAF (1M solution in THF, 0.64 mL, 0.64 mmol) dropwise. The reaction mixture was stirred at room temperature until TLC showed completion of the reaction. The mixture was concentrated under vacuum and the product was purified by column chromatography on SiO$_2$ (eluted with hexanes/EtOAc= 1:2), delivering 83.5 mg (97% yield) of 5 as light yellow solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.21 (s, 1H), 8.15 (d, $J$ = 8.0 Hz, 1H), 7.81 (d, $J$ = 8.0 Hz, 1H), 5.54 (d, $J$ = 8.8 Hz, 1H), 5.11 (m, 1H), 4.92 (d, $J$ = 12.8 Hz, 1H), 4.62-4.57 (m, 1H), 4.44 (t, $J$ = 7.2 Hz, 2H), 4.1 (br s, 1H), 1.47 (d, $J$ = 6.0 Hz, 3H), 1.46 (t, $J$ = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 170.1, 161.7, 159.5, 156.0, 149.4, 148.4, 138.5, 134.8, 129.5, 119.1, 80.4, 61.7, 61.4, 46.7, 28.5, 21.7, 14.5; MS calcd for [M + Na] C$_{19}$H$_{25}$N$_{3}$NaO$_{5}$S 430.1413, found 430.1513. $[\alpha]^{20}_D$ +165.7 (c 0.62, CHCl$_3$).

$(R)$-2-[4-(Ethoxycarbonyl)thiazol-2-yl]-7-methyl-5-oxo-6,7-dihydro-5H-pyrrolo[3,4-b]pyridine (345). To a solution of the alcohol 348 (51.7 mg, 0.13 mmol) in 1.1 mL of acetone was added Jones’ reagent (2.7 M aqueous solution, 104 $\mu$M, 0.28 mmol) dropwise at 0 °C. The reaction mixture was stirred at room temperature for 1.5 h. 2 mL of H$_2$O was added and the mixture was extracted with DCM (3 x 2 mL). The combined organic layers were washed with brine (2 mL), dried over anhydrous MgSO$_4$, filtered and the filtrate
was concentrated and dried under high vacuum to yield crude solid. The solid was dissolved in 2 mL of dry DCM. TFA (211 µL, 27.6 mmol) was added and the reaction mixture was stirred at room temperature for 1 h until TLC show completion of reaction. Saturated NaHCO$_3$ (3 mL) was added and the mixture was extracted with DCM (3 x 3 mL). The combined organic layers were washed with brine (2 mL), dried over anhydrous MgSO$_4$, filtered and the filtrate was concentrated under vacuum. The product was purified by column chromatography on SiO$_2$ (eluted with EtOAc), delivering 30.8 mg (80% yield) of 2 as white solid.$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.45 (d, $J = 8.1$ Hz, 1H), 8.30 (s, 1H), 8.21 (d, $J = 8.1$ Hz, 1H), 6.76 (br s, 1H), 4.76 (q, $J = 6.9$ Hz, 1H), 4.47 (q, $J = 7.2$ Hz, 2H), 1.61 (d, $J = 6.9$ Hz, 3H), 1.43 (t, $J = 7.2$ Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 168.9, 168.5, 168.2, 161.5, 153.5, 148.8, 133.3, 130.5, 120.2, 61.9, 54.4, 18.9, 14.5; MS calcd for [M + Na] C$_{14}$H$_{13}$N$_3$NaO$_3$S 326.0575, found 326.0658. [$\alpha$]$^D_{20}$ +51.9 (c 0.54, CHCl$_3$).
CHAPTER 6: Progress Towards the Total Synthesis of Petrosaspongiolide L

6.1 Introduction

Petrosaspongiolide L (372) was isolated in 1997 by Paloma from the dichloromethane extract of the sponge *Petrosaspongia nigra* which was collected off the coast of New Caledonia.\(^{214}\) The biological activity of this natural product has not been studied. The petrosaspongiolide L (372) contains a pyridinium tetracyclic A, B, C and D ring structure. Other natural products, such as spongidine A (374), spongidine C (373), and spongidine D (375) also contain such pyridinium tetracyclic rings. The spongidine A, C, and D are marine metabolites phospholipase A\(_2\) inhibitors\(^{215, 216}\) and were isolated from a Vanuatu sponge of the genus *Spongia*.\(^{216}\) To our knowledge, there is no previous report on the synthesis of petrosaspongiolide L (372) and spongidine C (373). The spongidines A and D were synthesized by Basabe et al.\(^{215}\)

![Diagram of petrosaspongiolide L, spongidine C, spongidine A, spongidine D.]

**Figure 6.1.** Structure of petrosaspongiolide L, spongidine C, spongidine A, spongidine D.
6.2 Progress Towards the Total Synthesis of Petrosaspongiolide L

The [2+2+2] cyclotrimerization reaction will be used to install the pyridine moiety (D ring) as the key step of the synthesis of petrosaspingiolide L (372), spongidine A (374), spongidine C (373), and spondidine D (375) (Scheme 6.1). The A, B and C ring could be pre-installed from commercially available (3aR)-(+)−Sclareolide (380). Petrosaspongiolide L (372) could be obtained from the [2+2+2] cyclotrimerization reaction of the diyne 376 and the nitrile 377. The TMS group on the diyne 376 will induce complete regioselectivity of the cyclotrimerization reaction. The diyne 376 could be synthesized from the reaction of the bromide 378 and Grignard reagents in the presence of a Cu catalyst. The alkyne of 378 can be made from the aldehyde 379, which could be produced from commercially available substrate (3aR)-(+)−sclareolide (380) (Scheme 6.1). The spongidine A (374) and spondidine D (375) can also be assembled from the diyne 376 (Scheme 6.1).
Scheme 6.1. Retrosynthetic route to petrosaspongiolide L.

The synthesis commenced with the commercially available (3aR)-(+-)-Sclareolide (380), which was reduced to the diol 382, which was selectively protected with TBDPSCl to provide the alcohol 383 according to a literature procedure. With the tertiary alcohol 383 in hand, we attempted to convert it to the nitrile 384 in order to provide an aldehyde, which could subsequently be converted to an alkyne group. Several reaction conditions were explored, such as cyanur chloride/n-Bu₄NCN/CH₃CN, NaCN/ N-Tosylimidazole/TBAI/TEA/DMF or n-Bu₄NCN/PPh₃/DDQ/CH₃CN (Scheme 6.2), however, no desired nitrile 384 was obtained.
Scheme 6.2. Attempt of the synthesis of nitrile 384.

An attempt to obtain the tertiary alkyne from an aldehyde, which was made via oxidation of the alcohol, was carried out. The synthesis was started with the known tertiary alcohol 383. The alcohol 383 was treated with thionyl chloride in pyridine, providing a 4:1:1 mixture of the inseparable isomers 384, 385, and 386 in 91% yield. This mixture underwent a hydroboration reaction with BH₃·THF, delivering the primary alcohol 387 in 61% yield. This alcohol 387 was oxidized by PCC to give the corresponding aldehyde 388, which was α-alkylated to afford 389 in 44% yield. A number of reaction conditions were tested for the conversion of the aldehyde 389 to the tertiary alkyne 392, such as the Corey-Fuchs reaction, the Wittig reaction, or the Ohira-Bestmann reaction, however, these reactions did not proceed as desired (Scheme 6.3). It was assumed that the protecting group, TBDPS was so sterically demanding that it prevented further conversion of the tertiary aldehyde.
Thus, the TBDPS protecting group was replaced with the PMB group (Scheme 6.4). The primary alcohol of the diol 382 was selectively protected with PMB to deliver the tertiary alcohol 393, which underwent a smooth elimination reaction, generating an inseparable isomeric mixture of 394 (major product), 395, and 396. The mixture underwent a smooth hydroboration-oxidation reaction to deliver the alcohol 397, which was oxidized to the corresponding aldehyde 398. α-Alkylation of the aldehyde 398 produced the α-methyl
aldehyde 399. The Corey-Fuchs reaction of the aldehyde 399 was explored but this reaction did not proceed. Fortunately, when the aldehyde 399 was treated with the Ohira-Bestmann reagent,\textsuperscript{223} the desired alkyne 400 was generated in 57\% yield. The PMB group of 400 was removed\textsuperscript{224} and the resulting alcohol 401 was converted to a good leaving group via a tosylation reaction\textsuperscript{225} to give 402 or via an Appel reaction\textsuperscript{226} to give 378. However, when both 402 and 378 were reacted with (2-lithioethynyl)trimethylsilane, no desired product 376 was obtained. This was probably due to the deprotonation of the terminal alkyne of 378 or 402 by the lithium reagent. In order to avoid potential deprotonation of the terminal alkyne, the terminal alkyne was protected with a TMS group\textsuperscript{225} to give 403,\textsuperscript{225} which underwent an Appel reaction to produce the bromide 404. To our surprise, the reaction of converting the bromide to the alkyne failed to proceed as well (Scheme 6.4). In order to solve this problem, the internal alkyne would need to be installed before the terminal one.
Scheme 6.4. Attempted synthesis of $376$ and $405$.

Then an attempt to install the primary alkyne prior to the tertiary alkyne was carried out. The alcohol $383$ was protected with the PMB group to give $406$ in 57% yield. The TBDPS group was deprotected $^{217}$ and the resulting alcohol $407$ underwent an Appel reaction, delivering the bromide $408$ in 89% yield. The $S_N2$ reaction of the (2-
lithioethynyl)trimethylsilane in the presence of HMPA produced the alkyne 409 in 67% yield. The PMB group of 409 was removed to generate the alcohol 410, which was oxidized to the aldehyde 411. The aldehyde 412 was obtained via α-alkylation of the aldehyde 411. However, the reaction of the aldehyde 412 with the Ohira-Bestmann reagent failed to generate any product (Scheme 6.5). This is probably due to the low stability of the TMS group under basic condition (K₂CO₃ in methanol). In order to solve this problem, a more stable protecting group for the terminal alkyne of 376 should be selected.

Scheme 6.5. Attempted synthesis of 376.
6.3 Summary and Future Directions

In summary, three different approaches have been devised and investigated for the synthesis of the diyne 376. However, the synthesis of diyne 376 was troublesome, especially the installation of the tertiary alkyne. Future studies should focus on the assembly of the diyne 376.

Recently, Taylor et al. reported a stereo specific nickel-catalyzed crossing coupling reaction of alkyl ether (Scheme 6.6).\(^{227}\) The Ni catalyst converted the methyl ether of 413 into a methyl group of 414. In future work, this method to install the tertiary methyl group on the C ring can be applied (Scheme 6.6). Before the synthesis, a test reaction, which converts the ether 415 to 416 should be explored (Scheme 6.6).

![Scheme 6.6. Stereospecific nickel-catalyzed coupling reaction of alkyl ether.](image)

With the best coupling condition in hand, the synthesis of petrosaspongiolide L (372) will commence with the diol 382. The diol 382 can be treated with SOCl\(_2\) to give the alkene 417. Ozonolysis of the alkyne 417 will afford the aldehyde 418, which can be protected with ethylene glycol in the presence of acid to deliver 419. Finkelstein Reaction of 419 will
generate the iodide 420, which will be treated with (2-lithioethynyl)trimethylsilane to give the alkyne 421. The protecting group of 421 can be removed with PPTS and the resulting aldehyde will undergo the reaction with ethynylmagnesium bromide followed by treating with MeI to furnish the diyne 423. With diyne 423 in hand, the cyclotrimerization reaction of the diyne 423 and nitrile 377 (or 381) will be carried out and the pyridine ring 424 or 425 will be assembled. After the TMS group is removed, 426 or 427 can be converted to 428 or 429 respectively using the best condition from the model study. Upon hydrolysis of 428, the petrosaspongiolide L (372) will be produced. And when 372 is treated with bromoacetic acid, spongidine C (373) can be obtained. The pyridine 429 can also be respectively converted to spongidine A (473) and spongidine D (474) (Scheme 6.7).
Scheme 6.7. The proposed synthesis of petrosaspongiolide L (372).
The Reetz reaction can convert a tertiary alcohol into a gem-dimethyl compound using $\text{Cl}_2\text{TiMe}_2$. However, there is no report for the conversion of a tertiary alcohol to methyl compound. As a result, a test reaction (from 430 to 416, Scheme 6.8) should be conducted before the synthesis of petrosaspongiolide L via the Reetz reaction.

With the best condition in hand, the synthesis of 372 can be started with the ketone 422. The ketone 422 will be reacted with ethynylmagnesium bromide (431) to give the diyne 432, which will undergo the cyclotrimerization reaction with nitrile 377 to provide the pyridine ring 433. After the TMS group is removed, 434 can be converted to 428 using the best condition from the model study. After a hydrolysis, the petrosaspongiolide L (372) will be produced (Scheme 6.8).

Scheme 6.8. The proposed synthesis of petrosaspongiolide L (372) via a Reetz reaction.
Since the spongidine A, C and D are marine metabolites phospholipase A\textsubscript{2} inhibitors\textsuperscript{215, 216}, a set of the spongidine analogs can be easily assembled from the cyclotrimerization reaction.

6.4 Experimental

All reactions were performed in flame-dried glassware under a nitrogen atmosphere and stirred magnetically unless indicated. Chemicals were used directly from commercial sources without further purification unless indicated. Solvents were distilled and stored with molecular sieves (3 Å for methanol and ethanol and 4 Å for all other solvents) prior to use. Toluene, xylene, dioxane were distilled from sodium/benzophenone ketyl. TEA, DIPEA, DMSO, DMF, DCE, CH\textsubscript{3}CN and pyridine were distilled from calcium hydride. Methanol and ethanol were distilled from Magnesium and iodide. CH\textsubscript{2}Cl\textsubscript{2}, THF, and ether were dried with a MB SPS Compact solvent purification system. All other reagent quality solvents were used without further purification. \textsuperscript{1}H and \textsuperscript{13}C NMR spectra were recorded using a Varian Mercury (300 MHz and 400 MHz). Mass spectra analysis was performed by North Carolina State University facilities.

**General procedure for protecting an alcohol with a PMB protecting group.**

Alcohol 382 (1 eq) was dissolved in DMF (0.4 M) and cooled to 0 °C. NaH (60% in mineral oil, 2.1 eq) was added and the reaction was stirred at 0 °C for 10 min. PMBCl (1.5 eq) and TBAI (0.1 eq) were added. The reaction mixture was stirred at 0 °C for 1 h and then room temperature for 16 h. The reaction was quenched with ice-cold H\textsubscript{2}O and extracted with ether. The combined organic layers were washed with brine, dried over Na\textsubscript{2}SO\textsubscript{4}, filtered and
concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc mixture to give the desired PMB protected alcohols.

\((1R,2R,8aS)-1\{-2-[(4-Methoxyphenyl) methoxy]ethyl\}-2,5,5,8a-tetramethyl-decahydronaphthalen-2-ol (393)\). The compound was eluted with hexanes/EtOAc (5:1, 4:1) to give 393 in 90% yield as a white glassy solid. \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.21 (d, \(J = 8.6\) Hz, 2H), 6.85 (d, \(J = 8.6\) Hz, 2H), 4.44 (s, 2H), 3.78 (s, 3H), 3.59 (ddd, \(J = 9.0, 5.1, 4.0\) Hz, 1H), 3.30 (ddd, \(J = 10.1, 8.7, 4.2\) Hz, 1H), 1.88 (dt, \(J = 12.2, 3.1\) Hz, 1H), 1.80 – 1.46 (m, 5H), 1.46 – 1.30 (m, 3H), 1.29 – 1.14 (m, 2H), 0.94 – 0.81 (m, 4H), 0.76 (s, 3H), 0.76 (s, 3H).

\(\{2-[(1S,8aR)-2-\{[(4-Methoxyphenyl) methoxy]methyl\}-5,5,8a-trimethyl-decahydronaphthalen-1-yl\}ethoxy\}(\textit{tert}-butyl) diphenylsilane (406)\). The compound was eluted with hexanes/EtOAc (10:1) to give 406 in 57% yield as a white solid. \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.65 (dt, \(J = 7.9, 2.0\) Hz, 4H), 7.44 – 7.30 (m, 6H), 7.21 – 7.14 (m, 2H), 6.89 – 6.77 (m, 2H), 4.40 – 4.22 (m, 2H), 3.77 (s, 3H), 3.74 – 3.54 (m, 3H), 3.35 (d, \(J = 6.1\) Hz, 2H), 1.92 (d, \(J = 36.0\) Hz, 1H), 1.90 – 1.81 (m, 1H), 1.80 – 1.69 (m, 2H), 1.48 – 1.29 (m, 10H), 1.03 (s, 9H), 0.82 (s, 3H), 0.75 (s, 3H), 0.62 (s, 3H).

**General procedure for the elimination of the tertiary alcohol.** The alcohol 383 or 393 (1 eq) was dissolved in pyridine (0.18 M). DMAP (1 eq) and added and the reaction mixture was cooled to −45 °C. SOCl\(_2\) (2.5 eq) was added dropwise and the reaction mixture was stirred at −45 °C for 1.5 h. The reaction mixture was warmed to 0 °C, quenched with ice-H\(_2\)O, and extracted with ether. The combined organic layers were washed with brine, dried over Na\(_2\)SO\(_4\), filtered, and concentrated *in vacuo*. The residue was purified by flash
chromatography on silica gel, eluting with hexanes/EtOAc (8:1) to give isomers (4:1:1 ratio of 384, 385 and 386) (90%) as a white glassy solid or isomers (4:1:1 ratio of 394, 395 and 396) (91%) as a white glassy solid. The analytic data was not shown, due to products being a complex mixture.

General procedure of hydroboration of alkenes. The alkene isomers (1 eq) was dissolved in THF (0.3 M) and cooled to 0 °C. BH$_3$-THF (1M solution in THF, 1.33 eq) was added dropwise. The reaction mixture was stirred at 0 °C for 3 h. NaOH (3 N aqueous solution, 6.7 eq) and H$_2$O$_2$ (30%, 1.8 ml/mmol) were added and the reaction was stirred at 0 °C for 1 h and then rt for 1 h. The reaction was quenched with H$_2$O and extracted with H$_2$O. The combined organic layers were washed with 10% NaHSO$_3$, saturated NaHCO$_3$ and brine, dried over Na$_2$SO$_4$, filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc mixture to give the corresponding alcohols.

[(1S,8aR)-1-{2-[(4-Methoxyphenyl)methoxy]ethyl}-5,5,8a-trimethyl-decahydronaphthalen-2-yl]methanol (397). The compound was eluted with hexanes/EtOAc (15:1, 10:1, 5:1, 2:1) to give 397 in 64% yield as a colorless liquid. $^1$H NMR (300 MHz, CDCl$_3$) δ 7.23 (d, $J = 8.7$ Hz, 1H), 6.86 (d, $J = 8.7$ Hz, 1H), 4.45 – 4.34 (m, 2H), 3.78 (s, 3H), 3.68 (d, $J = 10.3$ Hz, 1H), 3.47 (dd, $J = 7.1$, 6.4 Hz, 3H), 2.00 – 1.89 (m, 1H), 1.75 (dtt, $J = 10.9$, 7.1, 3.9 Hz, 2H), 1.66 – 1.42 (m, 5H), 1.42 – 1.28 (m, 5H), 1.11 (td, $J = 13.4$, 4.3 Hz, 1H), 0.92 – 0.78 (m, 4H), 0.77 (s, 3H), 0.68 (d, $J = 0.8$ Hz, 3H).

[(1S,8aR)-1-{2-[(tert-Butyldiphenylsilyl)oxy]ethyl}-5,5,8a-trimethyl-decahydronaphthalen-2-yl]methanol (387). The compound was eluted with hexane/EtOAc
(10:1) to give 387 in 61% yield as a white glassy solid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.70 – 7.62 (m, 4H), 7.46 – 7.32 (m, 6H), 3.74 – 3.59 (m, 2H), 3.52 – 3.39 (m, 2H), 1.98 – 1.84 (m, 1H), 1.68 (ddd, $J = 14.9$, 6.6, 2.9 Hz, 1H), 1.62 – 1.38 (m, 5H), 1.37 – 1.18 (m, 6H), 1.17 – 1.07 (m, 1H), 1.04 (d, $J = 0.8$ Hz, 9H), 0.82 (s, 3H), 0.78-0.70 (s, 4H), 0.63 (s, 3H).

**General procedure for oxidizing an alcohol to the aldehyde.** The alcohol (1 eq) was dissolved in DCM (0.1 M). PCC (2 eq) was added and the reaction mixture was stirred at room temperature for 20 h. The reaction mixture was concentrated *in vacuo*, the residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc mixture to give the corresponding aldehydes.

(1S,8aR)-1-{2-[([tert-Butyldiphenylsilyl)oxy]ethyl}-5,5,8a-trimethyl-decahydronaphthalene-2-carbaldehyde (388). The compound was eluted with hexanes/EtOAc (8:1) to give 388 in 90% yield as a colorless sticky liquid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 9.86 (s, 1H), 7.64 (ddt, $J = 6.3$, 2.8, 1.6 Hz, 4H), 7.38 (ddddd, $J = 11.5$, 7.3, 6.0, 2.7 Hz, 7H), 3.77 (t, $J = 6.1$ Hz, 2H), 2.31 (dddd, $J = 12.7$, 8.0, 2.8 Hz, 2H), 1.82 (dq, $J = 8.2$, 5.8, 5.0 Hz, 1H), 1.71 – 1.55 (m, 2H), 1.51 – 1.28 (m, 2H), 1.28 – 1.08 (m, 2H), 1.04 (d, $J = 2.7$ Hz, 9H), 0.89 – 0.77 (m, 5H), 0.74 (s, 3H), 0.66 (s, 3H).

(1S,8aR)-1-{2-[(4-Methoxyphenyl)methoxy]ethyl}-5,5,8a-trimethyl-decahydronaphthalene-2-carbaldehyde (398). The compound was eluted with hexanes/EtOAc (10:1, 5:1, 4:1) to give 398 in 80% yield as a white solid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 9.97 (s, 1H), 7.22 (d, $J = 8.5$ Hz, 2H), 6.86 (d, $J = 8.5$ Hz, 2H), 4.51 – 4.34 (m, 2H), 3.78 (s, 3H), 3.56 (t, $J = 6.5$ Hz, 2H), 2.52 – 2.23 (m, 2H), 1.99 – 1.77 (m, 2H), 1.65
(dq, J = 8.8, 4.4, 3.6 Hz, 2H), 1.58 – 1.47 (m, 1H), 1.47 – 1.20 (m, 5H), 1.12 (td, J = 13.4, 4.5 Hz, 1H), 0.99 – 0.84 (m, 2H), 0.83 (s, 3H), 0.75 (s, 3H), 0.70 (s, 3H).

\( (1S,8aR)-5,5,8a\text{-Trimethyl}-1-[4-(\text{trimethylsilyl})\text{but-3-yn-1-yl}]\text{-decahydronaphthalene-2-carbaldehyde} \ (411) \). The compound was eluted with hexanes/EtOAc (7:1) to give 411 in 90% yield as a white solid. \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 9.97 (s, 1H), 2.55 – 2.20 (m, 4H), 1.81 (q, \( J = 6.9 \) Hz, 2H), 1.67 (dd, \( J = 11.9, 4.8 \) Hz, 2H), 1.42 – 1.22 (m, 4H), 1.14 (td, \( J = 13.6, 4.6 \) Hz, 1H), 1.01 – 0.86 (m, 2H), 0.84 (s, 5H), 0.76 (s, 3H), 0.71 (s, 3H), 0.13 (d, \( J = 1.1 \) Hz, 9H).

**General procedure for \( \alpha \)-alkylation of the aldehyde.** KOT-Bu (7 eq) was dissolved in glyme (0.02 M) and cooled to 0 \( ^\circ \)C and a solution of the aldehyde (1 eq) in MeI (50 eq) was slowly added. The reaction was stirred at rt for 2 h and another 3 eq of KOT-Bu was added in three portions over 30 min. Ether was added to dilute the reaction mixture and washed with ice-cold H\(_2\)O. The organic layers were washed with brine, dried over Na\(_2\)SO\(_4\), filtered, and concentrated \( \text{in vacuo} \). The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc mixture to give the corresponding products.

\( (1R,8aS)-1\{-2\{-((\text{tert}-\text{Butyldiphenylsilyl})\text{oxy})\text{ethyl}\}-2,5,5,8a\text{-tetramethyl-decahydronaphthalene-2-carbaldehyde} \ (389) \). The compound was eluted with hexanes/EtOAc (20:1) to give 389 in 44% yield as a white solid. \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 9.71 (d, \( J = 1.4 \) Hz, 1H), 7.67 (ddt, \( J = 6.5, 1.9, 0.9 \) Hz, 4H), 7.51 – 7.27 (m, 6H), 3.67 – 3.49 (m, 2H), 2.15 (dt, \( J = 13.4, 3.2 \) Hz, 1H), 1.73 – 1.56 (m, 1H), 1.51 – 1.37 (m, 2H), 1.30 (dd, \( J = 14.2, 3.8 \) Hz, 3H), 1.04 (s, 9H), 0.98 – 0.85 (m, 1H), 0.86 – 0.74 (m, 10H), 0.75 – 0.67 (m, 4H), 0.62 (s, 4H).
(1R,8aS)-1-[(4-Methoxyphenyl)methoxy]ethyl]-2,5,5,8a-tetramethyl-decahydronaphthalene-2-carbaldehyde (399). The compound was eluted with hexanes/EtOAc (10:1) to give 399 in 63% yield as a colorless liquid. $^1$H NMR (300 MHz, CDCl$_3$) δ 9.85 (s, 1H), 7.28 – 7.20 (m, 2H), 6.92 – 6.80 (m, 2H), 4.43 (s, 2H), 3.79 (s, 3H), 3.54 – 3.29 (m, 2H), 2.21 (dt, $J$ = 13.3, 3.2 Hz, 1H), 2.10 – 1.92 (m, 2H), 1.73 (ddt, $J$ = 14.7, 10.0, 5.4 Hz, 1H), 1.66 – 1.58 (m, 1H), 1.51 – 1.31 (m, 3H), 1.31 – 1.20 (m, 1H), 1.18 – 1.01 (m, 2H), 1.01 – 0.91 (m, 4H), 0.89 – 0.76 (m, 5H), 0.73 (s, 3H), 0.69 (s, 3H).

(1R,8aS)-2,5,5,8a-Tetramethyl-1-[4-(trimethylsilyl)but-3-yn-1-yl]-decahydronaphthalene-2-carbaldehyde (412). The compound was eluted with hexanes/EtOAc (1:0, 50:1) to give 412 in 34% yield as a white solid. $^1$H NMR (300 MHz, CDCl$_3$) δ 9.76 (d, $J$ = 1.5 Hz, 1H), 3.63 (s, 2H), 3.54 (d, $J$ = 2.8 Hz, 2H), 2.37 – 2.14 (m, 2H), 2.13 – 1.86 (m, 1H), 1.64 (d, $J$ = 12.6 Hz, 2H), 1.52 – 1.07 (m, 4H), 1.01 (d, $J$ = 2.6 Hz, 1H), 0.96 – 0.79 (m, 5H), 0.79 – 0.62 (m, 10H).

**General procedure of converting an aldehyde to the alkyne with the Bestmann reagent.** The aldehyde (1 eq) was dissolved in MeOH (0.14 M) and K$_2$CO$_3$ (3.0 eq) and the Bestmann reagent (2.5 eq) were added. The reaction mixture was stirred at rt for 24 h and another 3.0 eq of K$_2$CO$_3$ and 2.5 eq of Bestmann reagent were added. The reaction mixture was stirred for an additional 20 h and ether (20 mL/mmol) was added to dilute the reaction mixture and washed with H$_2$O (10 mL/mmol), saturated NaHCO$_3$ (10 mL/mmol) and H$_2$O (10 mL/mmol). The organic layer was dried over Na$_2$SO$_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc mixture to give the corresponding aldehyde.
(4aS,5R)-6-Ethynyl-5-{2-[(4-methoxyphenyl)methoxy]ethyl}-1,1,4a,6-tetramethyl-decahydronaphthalene (400). The compound was eluted with hexanes/EtOAc (4:1) to give 400 in 57% yield as a white solid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.25 (d, $J$ = 8.5 Hz, 2H), 6.87 (d, $J$ = 8.7 Hz, 2H), 4.43 (s, 2H), 3.79 (s, 3H), 3.52 – 3.28 (m, 2H), 2.04 (s, 1H), 1.94 (dt, $J$ = 13.0, 3.2 Hz, 1H), 1.83 – 1.73 (m, 2H), 1.71 – 1.59 (m, 2H), 1.59 – 1.49 (m, 2H), 1.37 (dq, $J$ = 12.4, 2.9 Hz, 2H), 1.32 – 1.20 (m, 1H), 1.15 (s, 3H), 1.15 – 1.07 (m, 1H), 1.04 (s, 3H), 0.84 (s, 3H), 0.82 (s, 3H), 0.80 – 0.67 (m, 2H), 0.59 (t, $J$ = 3.8 Hz, 1H).

General procedure for removing the PMB protecting group. PMB ether (1 eq) was dissolved in DCM/H$_2$O (10:1, 0.13 M) and cooled to 0 °C. DDQ (1.5 eq) was added and the reaction was stirred at 0 °C for 1.5 h. The reaction was quenched with NaHCO$_3$ and extracted with DCM. The combined organic layers were washed with brine, dried over Na$_2$SO$_4$, filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc mixture to give the corresponding alcohol.

2-[(1R,8aS)-2-Ethynyl-2,5,5,8a-tetramethyl-decahydronaphthalen-1-yl]ethan-1-ol (401). The compound was eluted with hexanes/EtOAc (8:1) to give 401 in 93% yield as a white solid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 3.68 – 3.47 (m, 2H), 2.04 (d, $J$ = 0.8 Hz, 1H), 1.94 (dt, $J$ = 13.0, 3.2 Hz, 1H), 1.71 (td, $J$ = 8.1, 3.8 Hz, 2H), 1.67 – 1.58 (m, 1H), 1.58 – 1.48 (m, 2H), 1.45 – 1.32 (m, 3H), 1.32 – 1.21 (m, 1H), 1.17 (s, 3H), 1.11 (dd, $J$ = 14.0, 4.4 Hz, 1H), 1.03 (s, 3H), 0.83 (s, 3H), 0.81 (s, 3H), 0.80 – 0.69 (m, 3H), 0.61 (t, $J$ = 3.8 Hz, 1H).
[([1S,8aR]-5,5,8a-Trimethyl-1-[4-(trimethylsilyl)but-3-yn-1-yl]-
decahydonaphthalen-2-yl]methanol (410). The compound was eluted with
hexanes/EtOAc (4:1) to give 410 as a colorless liquid. \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 3.76 –
3.44 (m, 2H), 2.41 – 2.07 (m, 2H), 2.00 (dt, \(J = 10.7, 3.1\) Hz, 1H), 1.89 – 1.76 (m, 1H), 1.67
(tdd, \(J = 14.1, 8.8, 4.7\) Hz, 2H), 1.57 – 1.20 (m, 8H), 1.12 (td, \(J = 13.4, 4.3\) Hz, 1H), 0.92-
0.78 (m, 6H), 0.77 (s, 3H), 0.67 (s, 3H), 0.13 (d, \(J = 0.7\) Hz, 9H).

2-[(1S,8aR)-2-[(4-Methoxyphenyl)methoxy]methyl]-5,5,8a-trimethyl-
decahydonaphthalen-1-yl]ethan-1-ol (407). The silyl ether 406 (174 mg, 0.31 mmol) was
dissolved in THF (2 mL) and cooled to 0 °C. TBAF (1 M solution in THF, 0.93 mL, 0.93
mmol) was added dropwise and the reaction mixture was stirred at 0 °C for 2 h and at rt for
18 h. H\(_2\)O (3 mL) was added to the reaction mixture and it was extracted with ether (3 x 4
mL). The combined organic layers were washed with brine (3 mL), dried over Na\(_2\)SO\(_4\),
filtered, and concentrated in vacuo. The residue was purified by flash chromatography on
silica gel, eluting with hexanes/EtOAc (4:1, 2:1) to give 407 (91.0 mg, 86% yield) as a white
solid. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.23 (d, \(J = 8.6\) Hz, 2H), 6.86 (d, \(J = 8.6\) Hz, 2H), 4.40
(s, 2H), 3.79 (s, 3H), 3.76 – 3.56 (br, 2H), 3.51 (d, \(J = 9.1\) Hz, 1H), 3.38 (t, \(J = 9.1\) Hz, 1H),
1.96 (d, \(J = 12.9\) Hz, 2H), 1.78 – 1.56 (m, 2H), 1.54 – 1.27 (m, 5H), 1.22 – 1.05 (m, 3H),
0.93 – 0.80 (m, 5H), 0.77 (d, \(J = 6.6\) Hz, 4H), 0.68 (s, 3H).

**General procedure for an Appel Reaction.** The alcohol (1 eq) and CBr\(_4\) (1.2 eq)
were dissolved in DCM (0.27 M) and cooled to 0 °C. A solution of PPh\(_3\) (1.5 eq) in DCM
(0.27 M) was added and the reaction mixture was stirred at rt for 2 h. The mixture was
concentrated in vacuo and the residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc mixture to give the corresponding bromide.

(4aS,5R,6R)-5-(2-Bromoethyl)-6-ethynyl-1,1,4a,6-tetramethyl-decahydronaphthalene (378). The compound was eluted with hexanes/EtOAc (8:1) to give 378 in 94% yield as a white solid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 3.44 – 3.24 (m, 2H), 2.06 (s, 1H), 2.06 – 1.95 (m, 3H), 1.92 (t, $J$ = 3.2 Hz, 1H), 1.65 (ddd, $J$ = 13.3, 4.4, 2.6 Hz, 1H), 1.56 (ddq, $J$ = 10.9, 9.5, 3.1 Hz, 3H), 1.47 – 1.32 (m, 2H), 1.32 – 1.21 (m, 1H), 1.19 (s, 3H), 1.12 (dd, $J$ = 13.8, 4.4 Hz, 1H), 1.02 (s, 3H), 0.84 (s, 3H), 0.83 – 0.73 (m, 5H), 0.64 (t, $J$ = 3.8 Hz, 1H).

(4aR,5S)-5-(2-Bromoethyl)-6-[(4-methoxyphenyl)methoxy]methyl]-1,1,4a-trimethyl-decahydronaphthalene (408). $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.29 – 7.18 (m, 2H), 6.95 – 6.77 (m, 2H), 4.40 (s, 2H), 3.79 (s, 3H), 3.56 – 3.24 (m, 4H), 2.01 – 1.68 (m, 4H), 1.66 – 1.30 (m, 6H), 1.21 – 1.07 (m, 2H), 0.98 – 0.80 (m, 5H), 0.78 (d, $J$ = 5.8 Hz, 4H), 0.68 (s, 3H).

{2-[(1R,2R,8aS)-1-(2-Bromoethyl)-2,5,5,8a-tetramethyl-decahydronaphthalen-2-yl]ethynyl}trimethylsilane (404). The compound was eluted with hexanes/EtOAc (20:1) to give 404 in 97% yield as a white solid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 3.50 – 3.19 (m, 2H), 2.01 (ddt, $J$ = 10.3, 7.0, 3.8 Hz, 2H), 1.91 (dt, $J$ = 12.8, 3.1 Hz, 1H), 1.72 – 1.59 (m, 2H), 1.59 – 1.47 (m, 2H), 1.39 (dddt, $J$ = 15.8, 7.5, 4.4, 2.7 Hz, 2H), 1.24 (tt, $J$ = 8.0, 5.5 Hz, 1H), 1.15 (s, 3H), 1.07 (dd, $J$ = 13.3, 4.4 Hz, 1H), 1.01 (s, 3H), 0.84 (s, 3H), 0.83 – 0.71 (m, 4H), 0.63 (t, $J$ = 3.8 Hz, 1H), 0.10 (d, $J$ = 0.8 Hz, 9H).
2-[(1R,2R,8aS)-2-Ethynyl-2,5,5,8a-tetramethyl-decahydronaphthalen-1-yl]ethyl 4-methylbenzene-1-sulfonate (402). The alcohol 401 (8.8 mg, 0.034 mmol) was dissolved in pyridine (0.3 mL). TsCl (12.8 mg, 0.067 mmol) was added and the reaction was stirred at rt for 18 h. The reaction was quenched with ice water (2 mL) and extracted with ether (3 x 2 mL). The combined organic layers were washed with 1 N HCl (2 mL), saturated NaHCO$_3$ (2 mL), and brine (2 mL), dried over Na$_2$SO$_4$, filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc (4:1) to give 402 (10.5 mg, 70% yield) as a white solid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.77 (d, $J = 8.1$ Hz, 2H), 7.33 (d, $J = 8.1$ Hz, 2H), 3.97 (td, $J = 7.8$, 1.9 Hz, 2H), 2.43 (s, 3H), 2.02 (s, 1H), 1.90 (dt, $J = 13.0$, 3.2 Hz, 1H), 1.76 (tdd, $J = 7.5$, 3.9, 1.9 Hz, 2H), 1.65 – 1.42 (m, 4H), 1.39 – 1.29 (m, 2H), 1.20 (dtd, $J = 11.4$, 9.0, 5.5 Hz, 2H), 1.05 (s, 3H), 0.96 (s, 3H), 0.82 (s, 3H), 0.78 (s, 3H), 0.72 – 0.59 (m, 2H), 0.55 (q, $J = 3.9$, 3.5 Hz, 1H).

2-[(1R,2R,8aS)-2,5,5,8a-Tetramethyl-2-[2-(trimethylsilyl)ethynyl]-decahydronaphthalen-1-yl]ethan-1-ol (403). The alkyne 401 (16.3 mg, 0.0621 mmol) was dissolved in THF and the solution was cooled to 0 °C. $n$-BuLi (2.5 M in hexanes, 60 µL, 0.149 mmol) was added over 30 min and the reaction was stirred at 0 °C for 1.5 h. TMSCl (18.8 µL, 0.149 mmol) was added and the reaction was stirred at rt for 19 h. The reaction was quenched with 1 N HCl (300 µL) and the reaction mixture was stirred for 20 min. H$_2$O (1 mL) and ether (2 mL) were added and the aqueous was extracted with ether (3 x 2 mL). The combined organic layers were washed with H$_2$O (2 x 2 mL) and brine (2 mL), dried over Na$_2$SO$_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc (5:1) to give 403 (15.3 mg, 74%
yield) as a white solid. $^1$H NMR (300 MHz, CDCl₃) δ 3.68 – 3.47 (m, 2H), 1.91 (dt, $J = 12.9$, 3.2 Hz, 1H), 1.81 – 1.44 (m, 6H), 1.44 – 1.28 (m, 2H), 1.28 – 1.16 (m, 2H), 1.13 (s, 3H), 1.09 – 0.99 (m, 4H), 0.83 (s, 3H), 0.81 (s, 3H), 0.74 (ddd, $J = 13.3$, 9.8, 3.7 Hz, 2H), 0.59 (t, $J = 3.8$ Hz, 1H), 0.10 (s, 9H).

{4-[(1S,8aR)-2-[[4-Methoxyphenyl)methoxy]methyl]-5,5,8a-trimethyl-decahydronaphthalen-1-yl]but-1-yn-1-yl}trimethylsilane (409). Ethynyltrimethylsilane (0.62 mL, 4.334 mmol) was dissolved in THF (10 mL) and cooled to −78 °C. n-BuLi (2.5 M in hexanes, 1.36 mL, 3.252 mmol) was added dropwise. The reaction mixture was stirred at -5 °C for 2 h and then cooled to −78 °C and the solution of the bromide 408 (473.0 mg, 1.084 mmol) in THF (3 mL) was added dropwise, followed by HMPA (1.5 mL). The reaction was warmed to rt and stirred at rt for 3 d. H₂O (5 mL) was added and the mixture was extracted with ether (3 x 5 mL). The combined organic layers were washed with brine (5 mL), dried over Na₂SO₄, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc (40:1) to give 409 (331.0 mg, 67% yield) as a colorless liquid. $^1$H NMR (300 MHz, CDCl₃) δ 7.23 (d, $J = 8.6$ Hz, 2H), 6.86 (d, $J = 8.6$ Hz, 2H), 4.55 – 4.30 (m, 2H), 3.79 (s, 3H), 3.50 – 3.29 (m, 2H), 2.28 (ddd, $J = 16.8$, 8.8, 4.9 Hz, 1H), 2.16 – 1.87 (m, 4H), 1.62 (d, $J = 14.5$ Hz, 2H), 1.52 – 1.03 (m, 7H), 0.92 – 0.80 (m, 5H), 0.77 (d, $J = 5.1$ Hz, 4H), 0.66 (s, 3H), 0.18 – 0.11 (m, 9H).
CHAPTER 7: Cu(I)-Catalyzed C-N Coupling

7.1 Introduction

C-N coupling reactions are important and established chemical processes for the formation of many significant products, including drugs, organic materials, and optical devices.\textsuperscript{229-233} Therefore, the development of efficient catalytic systems for this reaction has attracted considerable attention in the past few years. A recent focus has been on the use of copper catalysts in place of the more toxic and expensive palladium catalysts.\textsuperscript{232, 234-236} Additional progress has been made to widen the reaction scope and to develop mild reaction conditions through the optimization of copper-ligand systems,\textsuperscript{237-242} and the application of other types of copper-containing catalysts, such as Cu\textsubscript{2}O-coated, soluble copper nanoparticles,\textsuperscript{243} copper-exchanged apatites,\textsuperscript{244, 245} and copper-containing perovskites.\textsuperscript{246} More recently, mechanistic investigations of Cu-catalytic reactions have shown that the formation of defined copper-ligand complexes has substantial effects on the rate of C-N coupling reactions, although the isolation and full characterization of the solid-state and solution-phase structures of these copper-ligand intermediate complexes are still underdeveloped.\textsuperscript{247}

In search for new homogeneous and efficient catalyst systems for C-N coupling reactions, we became interested in the investigation and synthesis of new multi-metallic copper complexes in which the copper atoms are held at specific distances to each other in order to provide the opportunity to investigate the catalytic effects of multimetal-centered catalysis.\textsuperscript{248-250} The catalytic properties of trimeric Cu(I)-carbene complexes in C-N coupling reactions have been investigated previously, confirming that trinuclear Cu(I) complexes
catalyze highly efficient Ullman-type arylation reactions.\textsuperscript{251} However, the mechanism and comparative efficacy of polynuclear Cu(I) complexes as catalysts for coupling reaction is still unclear and has not been reported yet. On the other hand, our current research involves the syntheses of new oxide/organic hybrids containing a combination of d\textsuperscript{10} (i.e. Cu\textsuperscript{+}) and d\textsuperscript{0} (i.e. Re\textsuperscript{7+})transition metals, which have been explored for potential applications in areas such as small-molecule absorption, magnetism, and catalysis.\textsuperscript{[17]} For example, strong visible-light absorptions have been observed in Cu(pyz)ReO\textsubscript{4} and Cu\textsubscript{3}(q6c)\textsubscript{2}ReO\textsubscript{4}, both containing Cu(I) dimeric centers.\textsuperscript{[17b]} Hybrid solids in these systems can also undergo subsequent ligand-mediated structural transformations, such as found for Cu(bpy)ReO\textsubscript{4} and Cu(bpy)\textsubscript{2}ReO\textsubscript{4}\cdot1/2H\textsubscript{2}O. As part of this work, our continued interest in the coordination chemistry of pyridazine ligands led us to investigate the synthesis and characterization of a new tetranuclear copper(I)-pyridazine (pda)/rhenate hybrid and explore its catalytic activity in C-N coupling reactions.

7.2 Results and Discussion

The crystalline compound Cu\textsubscript{2}(pda)\textsubscript{3}(ReO\textsubscript{4})\textsubscript{2} (435) was synthesized by our collaborator Haisheng Lin (Maggard lab, North Carolina State University) using a hydrothermal reaction of pda with Cu\textsubscript{2}O and Re\textsubscript{2}O\textsubscript{7} (Figure 7.1).
Figure 7.1. Molecular structure of Cu$_2$(pda)$_2$(ReO$_4$)$_2$ (435) using 80% probability thermal ellipsoids, showing the planar tetranuclear Cu(I) unit with symmetry unique atoms labelled, where red ellipses are O, blue are N, white are C, yellow are Re and light-blue are Cu; H atoms have been omitted for clarity. Selected bond lengths (Å), angles and torsion angles (deg.): Cu1–N1 2.002(5), Cu1–N3 1.994(6), Cu1–O8 2.306(5), Cu2–N2 1.998(5), Cu2–N5 1.953(6), Cu2–N6 2.024(6), Cu2–O1 2.251(5), Re1–O1 1.757(5), Re1–O2 1.656(5), Re1–O3 1.686(8), Re1–O4 1.714(7), Re2–O5 1.804(10), Re2–O6 1.629(10), Re2–O7 1.691(9), Re2–O8 1.667(6), Cu1–Cu2 3.179(1), Cu1–Cu2’ 3.237(1); N1–Cu1–N3 116.8(2), N1–Cu1–N4 118.0(2), N1–Cu1–O8 94.8(2), N3–Cu1–O8 90.9(2), N3–Cu1–N4 122.6(2), N4–Cu1–O8 100.5(2), N2–Cu2–N5 129.5(2), N2–Cu2–N6 103.3(2), N2–Cu2–O1 93.6(2), N5–Cu2–N6 122.5(2), N5–Cu2–O1 106.4(2), N6–Cu2–O1 88.7(2), Cu1–Cu2–Cu1’–Cu2’ 0, N1–N3–N4–Cu1 11.3(1), N2–N5–N6–Cu2 11.4(1).

In order to first test the use of 435 as a precursor reagent leading possibly to either catalytic mononuclear or polynuclear Cu complexes in C-N arylation reactions, para-iodoacetophenone (436) and indole (437) were selected as coupling partners in our initial screen for optimal reaction conditions (Table 7.1). These two molecules were selected based on their known coupling efficiency. Initial reaction conditions used 1 mol% of 435 in pyridine with Cs$_2$CO$_3$ as a base at 170 °C for 3 h, delivering the product 438 in 90% yield (Table 7.1, entry 1). Reduced reaction times, lower temperatures, and different solvents, including DMSO, THF, toluene, xylene, glyme, and dioxane, led to greatly diminished yields (Table 7.1, entries 2-7). However, reducing the amount of catalyst to 0.5 mol% still led to
similar yields. Therefore, the turnover number of the reaction can reach up to 45 which is larger than that of previously reported Cu(I)/ligand catalysts (~10-20).\textsuperscript{232-234, 237, 238} Omitting Cs$_2$CO$_3$ (entry 8), or using the amine bases TEA and DBU (Table 7.1, entries 9-10), provided 438 in low yields. On the other hand, the application of K$_3$PO$_4$ as a base produced 438 in an excellent yield of 92\% (Table 7.1, entry 11). The high yields obtained from the application of inorganic bases may be due to their high thermodynamic strength in aprotic solvents coupled with their low solubility and thus low concentration.\textsuperscript{237} The 90-92\% yield of 438 compares favourably with previous reports in the literature.\textsuperscript{232-234, 237-242, 259} In addition, the coupling reactions display substantially reduced reaction times (3 h in contrast to 15-24 h) compared to literature reports.\textsuperscript{232-234, 237-242, 248}

**Table 7.1.** Optimization of the coupling conditions for the reaction between para-iodoacetophenone (436) and indole (437) catalyzed by 435. Optimal conditions are Cs$_2$CO$_3$ in pyridine at 170 °C for 3 h.

<table>
<thead>
<tr>
<th>entry</th>
<th>base</th>
<th>solvent</th>
<th>Yield / %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cs$_2$CO$_3$</td>
<td>pyridine</td>
<td>90</td>
</tr>
<tr>
<td>2</td>
<td>Cs$_2$CO$_3$</td>
<td>DMSO</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Cs$_2$CO$_3$</td>
<td>THF</td>
<td>48</td>
</tr>
<tr>
<td>4</td>
<td>Cs$_2$CO$_3$</td>
<td>toluene</td>
<td>32</td>
</tr>
<tr>
<td>5</td>
<td>Cs$_2$CO$_3$</td>
<td>xylene</td>
<td>13</td>
</tr>
</tbody>
</table>
Directed by the optimized reaction conditions above, we then investigated the
generality of the developed C-N coupling reaction. As shown in Table 7.2 and 7.3, a range of
differently substituted aryl halides and different functional groups on the nitrogen
heterocycles are tolerated, and fast and efficient catalytic C-N coupling reactions with a
broad substrate scope were conducted. First, we analyzed the reaction of several aryl iodides
with various electron-withdrawing and -donating substituents and three different N-
nucleophilic substrates (Table 7.2) under the optimized reaction conditions. Previous reports
have demonstrated that the electronic properties of the substituent on the aryl halide plays a
very important role in determining its reactivity in C-N reactions.\(^{232, 259}\) As expected, the
coupling of electron-withdrawing benzenes \(436, 441,\) and \(442,\) containing 4-acetyl or nitro
groups in the 2- or 4-position, proceeds in excellent yields of 85-97\% (Table 7.2, entries 1-3,
6-8, 11). An exception represents the coupling of \(441\) and \(442\) containing 2- and 4-NO\(_2\)
groups with caprolactam (440) due to the generation of undesired side products (Table 7.2, entries 12, 13). Simple iodobenzene (443) underwent coupling with indole (437) or caprolactam (440) in 93-94% yield (Table 7.2, entries 4 and 14). In the case of the electron-donating iododobenzene 444, the yield of the reaction with 437 and 439 was lowered to 45-58% (entries 5, 10). However, for 440 the reactions were tolerated for aryl iodides with both electron-withdrawing and electron-donating substituents. As shown in Table 7.2 (entries 11, 14, 15), electronic effects on the reactions were limited, and yields did not vary significantly between 4-acetyl and 4-methoxy iodobenzenes.

### Table 7.2. Coupling of differently substituted iodobenzenes 436, 441-444 with different N-nucleophilic substrates 437, 439, 440.

<table>
<thead>
<tr>
<th>entry</th>
<th>R&lt;sub&gt;2&lt;/sub&gt;NH</th>
<th>R&lt;sup&gt;1&lt;/sup&gt;</th>
<th>product</th>
<th>yield / %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4-C(O)CH&lt;sub&gt;3&lt;/sub&gt; (436)</td>
<td>437, 439-440</td>
<td>438</td>
<td>90</td>
</tr>
<tr>
<td>2</td>
<td>4-NO&lt;sub&gt;2&lt;/sub&gt; (441)</td>
<td>437, 439-440</td>
<td>445</td>
<td>97</td>
</tr>
<tr>
<td>3</td>
<td>2-NO&lt;sub&gt;2&lt;/sub&gt; (442)</td>
<td>437, 439-440</td>
<td>446</td>
<td>92</td>
</tr>
<tr>
<td>4</td>
<td>H (443)</td>
<td>437, 439-440</td>
<td>447</td>
<td>94</td>
</tr>
<tr>
<td>5</td>
<td>4-OCH&lt;sub&gt;3&lt;/sub&gt; (444)</td>
<td>437, 439-440</td>
<td>448</td>
<td>58</td>
</tr>
</tbody>
</table>
### Table 7.2 Continued

<table>
<thead>
<tr>
<th>entry</th>
<th>R$_2$NH</th>
<th>R$^1$</th>
<th>product</th>
<th>yield / %</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td></td>
<td>4-C(O)CH$_3$ (436)</td>
<td>449</td>
<td>84</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>4-NO$_2$ (441)</td>
<td>450</td>
<td>94</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>2-NO$_2$ (442)</td>
<td>451</td>
<td>93</td>
</tr>
<tr>
<td>9</td>
<td>439</td>
<td>H (443)</td>
<td>452</td>
<td>48</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>4-OCH$_3$ (444)</td>
<td>453</td>
<td>45</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>4-C(O)CH$_3$ (436)</td>
<td>454</td>
<td>85</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>4-NO$_2$ (441)</td>
<td>455</td>
<td>56</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>2-NO$_2$ (442)</td>
<td>456</td>
<td>59</td>
</tr>
<tr>
<td>14</td>
<td>440</td>
<td>H (443)</td>
<td>457</td>
<td>93</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>4-OCH$_3$ (444)</td>
<td>458</td>
<td>87</td>
</tr>
</tbody>
</table>

Next, we investigated the reactivity of different nitrogen heterocycles in C-N coupling reactions catalyzed. As shown in Table 3, we discovered that the coupling reactions of para-iodoacetophenone (436) proceed in excellent yields (81-90%, Table 7.3, entries 1, 5-7) with various aromatic amines, including indole (437), pyrazole (439), and imidazole (462), as well as the lactam 440. However, the coupling of the aryl iodide 436 with 1H-indazole (459) and benzimidazole (460) resulted in mediocre yields of 54-64% (Table 7.3, entries 2-3), due to the generation of unidentified side-products. Also, only a trace of the product was observed.
when \textit{para}-iodoacetophenone was coupled with tetrahydroquinoline (461), probably because of its lower acidity and the subsequently more difficult ionization.\textsuperscript{238, 260}

\textbf{Table 7.3.} Coupling between \textit{para}-iodoacetophenone (436) and the heterocycles 437, 439-440, 459-462.

\[
\begin{array}{ccc}
\text{entry} & \text{R}_2\text{NH} & \text{product} & \text{yield / %} \\
1 & \text{N} & 438 & 90 \\
2 & \text{N} & 463 & 64 \\
3 & \text{N} & 464 & 54 \\
4 & \text{N} & 465 & \text{trace} \\
5 & \text{N} & 466 & 81 \\
6 & \text{N} & 449 & 84 \\
7 & \text{N} & 454 & 85 \\
\end{array}
\]
Although a precise mechanistic proposal of copper-catalyzed cross-coupling reactions has not been established yet, several literature reports have indicated Cu I and Cu III intermediates.\(^{261-263}\) As determined by NMR, the tetranuclear complex \(435\) is broken up into mononuclear Cu I atoms stabilized by pyridine ligands. To understand the importance of this finding for the coupling reactions, several control experiments were carried out as shown in Table 7.4. Firstly, the coupling reaction between \(\text{para-iodoacetophenone} (436)\) and indole (437) catalyzed by 435 in pyridazine as the solvent delivered the product 438 in only 37% yield (Table 7.4, entry 1), indicating that maintaining the tetranuclear nature of the catalyst is a disadvantage for efficient catalysis. However, under the same condition using either pyridine or pyrazine ligands as the solvent, the yields were dramatically increased to 90% (Table 7.4, entry 2 and 3), which further indicates the importance of converting the tetranuclear species 435 to a mononuclear Cu I center. This is also supported by the observation that using 1 mol% of 435 in THF in conjunction with 16 mol% of pyridine, led to an increase the yield from 48% (Table 7.4, entry 3) to 84% (Table 7.4, entry 2). In order to investigate the importance of the \(\text{ReO}_4^-\) counter ion, simple CuI was selected as the copper source and was employed in the coupling reaction in the presence of different ligands (solvents). As shown in Table 7.4 (entries 5-8), the trends of the reaction yields are the same for both catalysts, 435 and CuI, and the yields are substantially higher in pyridine and pyrazine than that in pyridazine, because the latter will presumably form or maintain the tetranuclear nature of the catalyst as shown in Figure 7.1.
Table 7.4. Investigation of the Cu(I)-catalyzed coupling reaction between para-iodoacetophenone (436) and indole (437) catalyzed by 435 (1 mol%) or CuI (4 mol%).

<table>
<thead>
<tr>
<th>entry</th>
<th>catalyst</th>
<th>solvent</th>
<th>Yield / %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>435</td>
<td>pyridazine</td>
<td>37</td>
</tr>
<tr>
<td>2</td>
<td>435</td>
<td>pyridine</td>
<td>90</td>
</tr>
<tr>
<td>3</td>
<td>435</td>
<td>pyrazine</td>
<td>90</td>
</tr>
<tr>
<td>4</td>
<td>435</td>
<td>THF + pyridine (16 mol%)</td>
<td>84</td>
</tr>
<tr>
<td>5</td>
<td>CuI</td>
<td>pyridine</td>
<td>92</td>
</tr>
<tr>
<td>6</td>
<td>CuI</td>
<td>pyrazine</td>
<td>85</td>
</tr>
<tr>
<td>7</td>
<td>CuI</td>
<td>pyridazine</td>
<td>22</td>
</tr>
<tr>
<td>8</td>
<td>CuI</td>
<td>pyridine + pyridazine (6 mol%)</td>
<td>95</td>
</tr>
</tbody>
</table>

Based on these results, we propose that the mechanism of the C-N coupling reaction catalyzed by 435 involves 4 steps (Scheme 7.1). Pyridine breaks up the tetranuclear complex 435 into the mono-nuclear Cu-pyridine complex 467 as the first step. The second step involves the oxidative addition of the aryl halide to CuI-pyridine to form a six-coordinated copperIII species 468. The third step consists of the N-nucleophilic substitution of ReO4− by indole anion. Finally, the CuIII complex undergoes reductive elimination to give the C-N cross coupled product. Interestingly, the PXRD patterns of complex 435 before and after
coupling reactions match well, showing that by evaporating the solvents, the tetra-nuclear complex 467 can be reformed.

Scheme 7.1. Proposed mechanism of the N-arylation reaction promoted by the copper(I) complex (435) in pyridine.

7.3 Conclusion and Outlook

In summary, a novel, air-stable, tetranuclear Cu\(^I\) complex that is broken up into mononuclear species in pyridine solution and displays excellent catalytic efficiency in homogeneous C-N coupling reactions was developed. Under the discovered optimal reaction
conditions we were able to couple a broad range of aryl iodides and various nitrogen heterocycles to the corresponding products in a short reaction time. Based on our mechanistic investigations it appears that the tetranuclear Cu-species needs to be converted into mononuclear units for efficient catalysis. It was also discovered that the CuI can catalyze the C-N coupling between \( p \)-iodoacetophenone (436) and indole (437) using pyridine as solvent without any addition of ligand. This discovery inspired us to investigate the “ligand-free” C-N coupling reaction using CuI as the catalyst in pyridine.

Nowadays, microwave irradiation has been widely used to assist organic reactions (discussed in Chapter 1) and moreover, microwave heating has been used to assist in the C-N coupling reaction.\(^{96, 264-267}\) Future work could involve a “ligand-free” C-N coupling reaction, which uses the CuI as the catalyst and needs no additional ligand, under microwave irradiation. The \( p \)-iodoacetophenone (436) and indole (437) could be the representative reagents for the C-N coupling reaction. CuI (1 - 5 mol%) will be used as the catalyst and dry pyridine will be used as the solvent (Scheme 7.2). Different bases and reaction time and temperature will be explored. The microwave irradiation can also be applied to assist the reaction. The selected best reaction condition can be used in the coupling reaction between varieties of amines (aromatic amine, aliphatic amine, amino acid etc) and arene halides (arene iodide, arene bromide and arene chloride).
Scheme 7.2. Future work on the C-N coupling reaction.

7.4 Experimental

**General methods.** Unless otherwise stated, all the reagents were used without further purification. Pyridine was distilled from CaH. The C-N coupling reactions were performed under N\(_2\) atmosphere using flame-dried sealed vial. All products were purified by column chromatography and the yields were determined by weight. All spectra were recorded in CDCl\(_3\), and chemical shifts are reported relative to CHCl\(_3\) (7.24 for \(^1\)H NMR) and CHCl\(_3\) (77.24 for \(^{13}\)C NMR).

**General procedure for the C-N coupling reaction.** The NH-containing substrate (0.15 mmol), Cs\(_2\)CO\(_3\) (0.2 mmol) and the Cu complex 435 (1 mol %) were added to a solution of the aryl halide (0.1 mmol) in dry pyridine (1 ml), under a nitrogen atmosphere. The vial was sealed and heated to 170 °C for 3 h in an oil bath, cooled to room temperature, filtered, and the filtrate was concentrated under vacuum. The product was purified by column chromatography on SiO\(_2\), eluted with hexanes/ethyl acetate, delivering 1-(4-(1H-Indol-1-yl)phenyl)-ethanone (438),\(^{252}\) 1-(4-nitrophenyl)-1\(^H\)-indole (445),\(^{268}\) 1-(2-nitrophenyl)-1\(^H\)-indole (446),\(^{268}\) 1-phenyl-1\(^H\)-indole (447),\(^{269}\) 1-(4-methoxyphenyl)-1\(^H\)-indole (448),\(^{270}\) 1-(4-pyrazol-1-yl-phenyl)-ethanone (449),\(^{241}\) 1-(4-nitrophenyl)-1\(^H\)-pyrazole (450),\(^{241}\) 1-(2-nitrophenyl)-1\(^H\)-pyrazole (451),\(^{271}\) 1-phenyl-1\(^H\)-pyrazole (452),\(^{269}\) 1-(4-methoxyphenyl)-1\(^H\)-
pyrazole (453),\(^{269}\) N-(4-acetylphenyl)caprolactam (454),\(^{272}\) N-(4-nitrophenyl)caprolactam (455),\(^{273}\) N-Phenylcaprolactam (457),\(^{272}\) N-(4-methoxyphenyl)caprolactam (458),\(^{272}\) 1-[4-(1H-benzimidazol-1-yl)phenyl]ethanone (464),\(^{274}\) 4-(Imidazol-1-yl)acetophenone (466)\(^{275}\) (All literature-known), as well as N-(2-nitrophenyl)caprolactam (456) and 1-(4-(1H-indazol-1-yl)phenyl)ethanone (463).

N-(2-nitrophenyl)caprolactam (456). Eluted with hexanes/ethyl acetate = 2:1, 1:1, 1:2. Yield 56%. \(^1H\) NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.91 (d, \(J = 8.0\) Hz, 1H), 7.59 (t, \(J = 8.0\) Hz, 1H), 7.38 (t, \(J = 8.0\) Hz, 1H), 7.27 (d, \(J = 8.0\) Hz, 1H), 3.87 (b, 1H), 3.69 (b, 1H), 2.64 (b, 1H), 2.74-1.65 (m, 6H); \(^{13}C\) NMR (100 MHz, CDCl\(_3\)) \(\delta\) 175.8, 138.1, 134.1, 128.97, 127.8, 125.2, 53.5, 37.6, 30.2, 28.8, 23.0; MS calcd for [M + Na] C\(_{12}\)H\(_{14}\)N\(_2\)NaO\(_3\) 257.0902, found 257.1.

1-(4-(1H-indazol-1-yl)phenyl)ethanone (463). Eluted with hexanes/ethyl acetate = 6:1. Yield 64%. \(^1H\) NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.23 (s, 1H), 8.12 (d, \(J = 8.4\) Hz, 2H), 7.87 (d, \(J = 8.4\) Hz, 2H), 7.84-7.80 (m, 2H), 7.47 (t, \(J = 8.0\) Hz, 1H), 7.26 (t, \(J = 8.0\) Hz, 1H), 2.64 (s, 3H); \(^{13}C\) NMR (100 MHz, CDCl\(_3\)) \(\delta\) 197.1, 144.2, 138.8, 136.9, 134.8, 130.1, 128.0, 126.1, 122.4, 121.8, 121.7, 110.8, 26.8; MS calcd for [M + H] C\(_{15}\)H\(_{13}\)N\(_2\)O 237.1, found 237.1.
CHAPTER 8: Fluorescence Activation and Quenching via a [3+2] Cycloaddition Reaction

8.1 Introduction

The Cu(I)-catalyzed [3+2] cycloaddition of terminal alkynes with azides (the CuAAC “click” reaction), introduced by Sharpless and Meldal, has become popular in the chemical biology field due to its mild reaction conditions, high yields, and high regioselectivity (exclusive 1,4-disubstituted-1,2,3-triazole products). Further advantages of the Cu(I)-catalyzed Click reaction are that the organic azides are nontoxic and the reaction can be performed in an aqueous environment at 37 °C. In addition, the Cu(I)-catalyzed click reaction is a nearly bioorthogonal chemical reaction and is compatible with many functional groups and biomolecules, such as sugars, proteins, and nucleic acids. However, one disadvantage of this reaction is that the Cu(I) is cytotoxic, therefore, reducing the cytotoxicity or increasing the reactivity of the [3+2] cycloaddition reaction is necessary in order to perform the reaction in living cells. The metal-free click cycloadditions of cyclooctynes with azides, also called “strain-promoted alkyne-azide cycloadditions (SPAAC)” have been reported to solve the cytotoxicity of the Cu(I) catalyst and have been widely used in biological systems. In 2005, Sharpless reported Ru-catalyzed cycloadditions of alkynes and azides forming 1,5-disubstituted-1,2,3-triazole products, fulfilling the need for different regioisomers of the triazole products.

Recently, a number of applications of the Click reaction for fluorescent labeling have been reported. Fluorescent labeling of protein, RNA, and DNA is an important tool in the study of biological processes in cells and animals. However, fluorescent labeling can
have high background fluorescence due to the starting material and the labeled product having similar fluorescent properties.\textsuperscript{286} One general solution to reduce the background is to wash away the unreacted starting materials, which would not only require additional methods to purify the products but also increase the cost. An alternate way to reduce the background is to explore new click reactions where the starting materials are non-fluorescent or have low fluorescence prior to the reaction, but after the click reaction is performed the resulting triazole product is fluorescent.

Fahrni and Krishnamoorthy reported the first fluorogenic CuAAC reaction based on coumarin derivatives.\textsuperscript{285, 287} Anthracene\textsuperscript{288} and naphthalimide\textsuperscript{289, 290} fluorophores have also been used. Recently, a benzothiazole derivative was also reported.\textsuperscript{291, 292} Here, we report a new derivative, the dimethylaminobenzalkyne, for fluorescent labeling. The dimethylaminobenzalkyne is fluorescent and the fluorescence can either be quenched or increased upon a [3+2] cycloaddition reaction. Dimethylaminobenzalkyne is a structurally simple compound that is commercially available. This allows for easier use than other fluorescent labeling reagents, since no synthetic preparation is required. 4-Azido-\textit{N},\textit{N}-dimethylbenzenamine contains an azido group that can be used for alkyn-containing molecules. Dimethylaminobenzalkyne was used and it was found that the dimethylaminobenzalkyne can “turn on” and “turn off” fluorescence upon a [3+2] cycloaddition reaction. 6-Ethynyl-\textit{N},\textit{N}-dimethynaphthalen-2-amine was also synthesized in order to investigate the effect of an additional conjugated \textit{\pi}-system. Additionally, the 4-azido-\textit{N},\textit{N}-dimethylbenzenamine was used for the alkyne containing system and it was found that it can “turn on” fluorescence upon a click reaction.
8.2 Results and Discussion

The alkynes 476-482 were commercially available or were synthesized via literature procedures.\textsuperscript{293-297} The \textit{N,N}-dimethylamino was placed on different benzene ring position (\textit{ortho-}, \textit{meta-}, and \textit{para-}) to the etynyl group to investigate the effect of the electron-donating group (EDG) at different benzene ring positions, since conjugation system changes when the EDGs are at different positions. In order to increase the excitation wavelength by increasing the number of conjugated \( \pi \)-bonds, 6-ethynyl-\textit{N,N}-dimethylnaphthalen-2-amine (475), 6-ethynyl-hydroxy-2-amine (480), and 6-ethynyl-methoxy-2-amine (481) were also explored. 6-Ethynyl-\textit{N,N}-dimethylnaphthalen-2-amine (475) synthesized in 5 steps from commercially available 6-aminonaphthalene-2-carboxylic acid (470). The amine 470 underwent a reductive amination to give 471,\textsuperscript{298} which was reduced to the alcohol 472 by BH\textsubscript{3}·THF.\textsuperscript{299} Several oxidation conditions were explored, and MnO\textsubscript{2}\textsuperscript{300} was found to be the best reagent to oxidize the alcohol 472 to the aldehyde 473. The aldehyde 473 was converted to the alkyne 474 via a Corey-Fuchs reaction (Scheme 8.1).\textsuperscript{290}
Scheme 8.1. The synthesis of the alkyne 475.

The alkynes 475-481 underwent a [3+2] cycloaddition reaction with the azide 483 in the presence of CuSO$_4$ and sodium ascorbate in THF/H$_2$O, generating the 1,4-disubstituted-1,2-3-triazole products 485-491 in good yields (80-95%), except 2-ethynyl-6-methoxynaphthalene (481), only 58% yield, probably due to the low solubility of 481 in this solvent system. The 1,4-disubstituted triazole 492 was obtained in 77% yield from the cycloaddition reaction of 4-azido-N,N-dimethylbenzenamine (482) and pent-4-yn-1-ol (484) (Scheme 8.2).
Scheme 8.2. Synthesis of 1,4-disubstituted triazole 485-492.

Compared to the numerous literatures reports on the formation of 1,4-disubstituted-1,2,3-triazole products, the reports on the formation of 1,5-disubstituted-1,2,3-triazole products are limited. However, the 1,5-disubstituted-1,2,3-triazoles have stronger conjugation effect with aromatic rings at 1- (or 5-) position than the 1,4-disubstituted-1,2,3-triazoles. Here in order to investigate the conjugation effect of placing the substitution onto a different nitrogen atom in the tetrazole ring, the 1,5-disubstituted-1,2,3-triazoles were also explored. The 1,5-disubstituted-1,2,3-triazole products 493-499 were produced using Cp*ClRu(PPh$_3$)$_2$ as catalyst.$^{283}$ The alkynes 475-481 and the azide 483 were heated in dioxane at 60 °C in the presence of Cp*ClRu(PPh$_3$)$_2$, delivering the triazoles 493-494 and 496-499 in 76-93% yield. Only trace amount of triazole 495 was obtained when o-ethynyl-N,N-dimethylbenzenamine (478) was used. This is probably due to steric hindrance in the
resulting triazole 495 (Scheme 8.3). No 1,5-disubstituted-1,2,3-triazole products were produced when the azide 482 was reacted with the alkyne 484 in the presence of Cp*ClRu(PPh₃)₂, due to the decomposition of the product under these reaction condition.

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\begin{align*}
R &= R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R'' \quad R"}
\end{align*}

Scheme 8.3. Synthesis of 1,5-disubstituted triazole 493-499.

One of the major applications of the 1,3-cycloaddition reaction is fluorescence labeling. With the trazoles in hand, the absorption spectra of the alkynes 475-481, the azide 482, and the corresponding triazoles 485-499 were recorded, in order to find the absorption \( \lambda_{\text{max}} \) as the exitation wavelength. The absorption \( \lambda_{\text{max}} \) was found to be at 285±2 nm and 295±2 nm for all benzene derivatives 476-479, 485-488 and 492-496, while the naphthalene derivatives 475, 480-481, 489-491, and 497-499 exhibited a \( \lambda_{\text{max}} \) at 317±3 nm and 330±3 nm. All naphthalene derivatives 475, 480-481, 489-491, and 497-499 (excited at 317 nm and 330 nm) showed strong fluorescence at the close wavelength with similar fluorescence intensities (data not shown), indicating these naphthalene derivatives can’t be used for fluorescent
labeling. Nevertheless, the compounds 476-479, 485-488 and 492-496 were excited at 285 nm and 295 nm and the fluorescence spectra were recorded (Figure 8.1). As shown in Figure 8.1, upon excitation at 285 nm, among the alkynes 476-479 and azide 482, only the alkyne 476 (p-ethynyl-N,N-dimethylbenzenamine) exhibits fluorescent properties in methanol, with an emission maximum at 350 nm. Other alkynes or azides showed no fluorescence. No fluorescent emission was observed for the “clicked” 1,4-disubstituted triazole product 485-488 and 492. This indicates that the fluorescence of the alkyne 476 was quenched upon the “click” reaction with the azide 483. This result was opposite from a literature report,286 where 10-(azidomethyl)anthracene was used and its fluorescence is “turned-on” or enhanced after a click reaction of alkynes. This is probably due to the enhancement in resonance structure of 476 by the N,N-dimethylamino (electron-donating group) at para-position of the alkyne, thus thus inducing the fluorescence of 476. After the click reaction, the alkyne of 476 forms a π-system in the triazole ring, instead of the benzene ring, thus quenching the fluorescence. Interestingly, the 1,5-trisubstituted triazole 493, formed via Ru-catalyzed [3+2] cyclolization reaction of the alkyne 476 and the azide 484, exhibited a stronger (4-fold) fluorescence intensity than the alkyne 476. Additionally, a red shift (from 350 to 430 nm) was observed in the emission spectra of the 1,5-trisubstituted triazole 493 compared to the alkyne 476 (Figure 8.1). This can probably explained by the ability of forming a larger π-system resonance structure in 1,5-disubstituted 1,2,3-triazole product 484.

Interestingly, when the triple bond of compound 476 was replaced with an azido group, an opposite result was obtained. In contrast to the p-ethynyl-N,N-dimethylbenzenamine (476), the p-azido-N,N-dimethylbenzenamine (482) was non-
fluorescent. The Cu-catalyzed [3+2] cycloaddition reaction of \( p \)-azido-\( N,N \)-dimethylbenzenamine (492) exhibited “turn-on” fluorescence and the triazole 492 showed an emission maximum at 420 nm (Figure 1). The azide 482 is structurely similar to the literature reported azidoanthracene derivatives and the result is similar as for literature reported azidoanthracene derivatives. It was conceived that the high electron-rich azido group of the 10-(azidomethyl)anthracene would induce electron transfer from the azido to the excited phenyl group, leading to the fluorescent quenching. However, after the [3+2] cycloaddition reaction, the lone pair of the nitrogen participates in the conjugation of the aromatic ring, inducing fluorescence. The consumption can also be applied to \( p \)-azido-\( N,N \)-dimethylbenzenamine (482).

Figure 8.1. Emission spectra of compounds 476-479, 482, 485-488 and 492-496 (20 \( \mu \)M in methanol solution), excited at 285 nm.
8.3 Conclusion

In summary, we investigated two [3+2] cycloaddition reactions of two derivatives, \(N,N\)-dimethylamino-naphthalene-alkyne and \(N,N\)-dimethylaminobenzalkyne, for fluorescence labeling. The naphthalene derivatives were found to be not useful in fluorescence labeling, since all their fluorescence spectra displayed close fluorescence wavelength with the similar intensity to their click products with azide, owing to their strong \(\pi\)-conjugations. In contrast, no fluorescence was observed for \(N,N\)-dimethylaminobenzalkynes, except for the \(p\)-\(N,N\)-dimethylamino ethynylbenzene, which exhibited fluorescence at 350 nm in MeOH. The fluorescence was quenched upon a Cu-catalyzed cycloaddition reaction with an azide, but was enhanced upon a Ru-catalyzed [3+2] cycloaddition reaction with an azide with a red shift from 350 nm to 430 nm. We hypothesized the fluorescence quenching and enhancing were related to the conjugation effect of \(\pi\)-systems. Furthermore, \(p\)-azido-\(N\), \(N\)-dimethylbenzenamine, a compound by replacing the alkyne group of \(p\)-\(N\), \(N\)-dimethylamino ethynylbenzene with an azide, was non-fluorescent and can be used to “turn on” fluorescence upon the Cu-catalyzed [3+2] cycloaddition with alkyne. Its triazole product exhibited strong fluorescence at 420 nm. These results can potentially be used in fluorescence labeling in the bioconjugation field.

8.4 Experimental

All reactions were performed in flame-dried glassware under a nitrogen atmosphere and stirred magnetically unless indicated. Chemicals were used directly from commercial sources without further purification unless indicated. Solvents were distilled and stored with
molecular sieves (3 Å for methanol and ethanol and 4 Å for all other solvents) prior to use. Dioxane was distilled from sodium/benzophenone ketyl. TEA, DIPEA, DMF, DCE, CH$_3$CN and pyridine were distilled from calcium hydride. Methanol was distilled from magnesium and iodine. CH$_2$Cl$_2$, THF, and ether were dried by MB SPS Compact solvent purification system. All other reagent quality solvents were used without further purification. $^1$H and $^{13}$C NMR spectra were measured using a Varian Mercury (300 MHz and 400 MHz). Mass spectrometry was performed by North Carolina State University facilities.

6-(Dimethylamino)naphthalene-2-carboxylic acid (471). 6-Aminonaphthalene-2-carboxylic acid (470, 2 g, 10.68 mmol) was dissolved in MeOH (80 mL). NaCNBH$_3$ (2.48 g, 39.5 mmol), HCHO (37% aqueous solution, 16 mL) were added and the reaction mixture was stirred at rt for 2 h. The reaction mixture was concentrated and H$_2$O (50 mL) and NaOH (1 N, 5 mL) were added. The mixture was extracted with EtOAc (2 x 20 mL) and the aqueous layer was acidified with 1 N HCl until pH ~3. The formed precipitation was filtered, washed with cold H$_2$O, and dried in vacuo to give 471 (2.34 g, quantitive yield) as a yellow solid. The $^1$H NMR and MS matched the literature report.$^{298}$

(2-(Dimethylamino)naphthalen-6-yl)methanol (472). The acid 471 (870 mg, 4.04 mmol) was dissolved in THF (40 mL) and the reaction mixture was cooled to 0 °C. BH$_3$·THF (1 M in THF solution, 12.5 mL, 12.5 mmol) was added dropwise. The reaction mixture was stirred at rt over night and then cooled to 0 °C. 1 N HCl (10 mL) was added carefully, the mixture was warmed to rt, and 1 N NaOH was added until a pH of ~8 was reached. The reaction mixture was extracted with DCM (3 x 40 mL) and the combined organic layers were washed with brine (10 mL), dried over Na$_2$SO$_4$, filtered, and concentrated in vacuo. The
residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc (1:1, 1:2) to give 472 (551 mg, 68% yield) as a light yellow solid. The $^1$H NMR and MS matched the literature report.\textsuperscript{298}

**6-(Dimethylamino)naphthalene-2-carbaldehyde (473).** The alcohol 472 (150 mg, 0.74 mmol) was dissolved in ether (12 mL). MnO$_2$ (648 mg, 7.45 mmol) was added and the reaction mixture was stirred at rt overnight. The reaction was filtered and the solid was washed with DCM. The filtrate was concentrated \textit{in vacuo} and the residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc (3:1) to give 473 (99 mg, 66% yield) as a yellow solid. The $^1$H NMR and MS matched the literature report.\textsuperscript{298}

**6-Ethynyl-N,N-dimethylnaphthalen-2-amine (475).** PPh$_3$ (511 mg, 1.95 mmol) and CBr$_4$ (323 mg, 0.97 mmol) were mixed and cooled to 0 °C. DCM (3 mL) was added slowly and the reaction mixture was stirred at 0 °C for 20 min. The solution of the aldehyde 473 (97 mg, 0.49 mmol) in DCM (3 mL) was added dropwise at 0 °C. The reaction mixture was stirred at rt overnight. The reaction mixture was concentrated \textit{in vacuo} and the residue was purified by flash chromatography on silica gel, eluting with TEA/DCM (1:100) to give 474 (133 mg, 77% yield) as a pale yellow solid. The dibromide 474 (130 mg, 0.36 mmol) was dissolved in THF (10 mL) and the reaction mixture was cooled to −78 °C. $n$-BuLi (0.59 mL, 1.464 mmol) was added dropwise and the reaction mixture was stirred at −78 °C for 2 h. Saturated NH$_4$Cl (5 mL) was added and the aqueous layer was extracted with ether (3 x 5 mL). The combined organic layers were washed with brine (10 mL), dried over Na$_2$SO$_4$, filtered and concentrated \textit{in vacuo}. The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc (5:1, 4:1) to give 475 (58 mg, 82% yield) as a light
yellow solid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.85 (s, 1H), 7.63 (d, $J = 9.0$ Hz, 1H), 7.55 (d, $J = 8.6$ Hz, 1H), 7.38 (d, $J = 8.8$ Hz, 1H), 7.13 (d, $J = 9.1$ Hz, 1H), 6.84 (s, 1H), 3.48 – 2.67 (m, 1H). $^{13}$C NMR (400 MHz, CDCl$_3$) $\delta$ 137.8, 135.0, 132.3, 129.2, 128.9, 126.3, 116.8, 106.1, 85.1, 76.2, 40.8. MS calcd. [M+H]$^+$ C$_{14}$H$_{14}$N 196.1, found 196.1.

**General procedure for the synthesis of 1,4-disubstituted triazoles.** The alkyne (20 mg, 1 eq) and the azide (2 eq) were dissolved in THF/H$_2$O (1:1). CuSO$_4$ (0.13 eq) and sodium ascorbate (0.4 eq) were added and the reaction mixture was heated at 40 °C overnight. The mixture was cooled to room temperature and concentrated *in vacuo*. The mixture was purified by column chromatograph, eluting with 5% MeOH in DCM, delivering the corresponding 1,4-disubstituted triazoles.

**3-(4-(2-(Dimethylamino)phenyl)-1H-1,2,3-triazol-1-yl)propan-1-ol (487).** The compound was synthesized in 80% yield as a white solid. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 8.43 (s, 1H), 8.11 (dd, $J = 7.2$ Hz and 2.0 Hz, 1H), 7.25 (dd, $J = 7.2$ Hz and 2.0 Hz, 1H), 7.14-7.08 (m, 2H), 4.55 (t, $J = 6.2$ Hz, 2H), 3.663 (t, $J = 6.2$ Hz, 2H), 3.25 (s, 1H), 2.61 (s, 6H), 2.18-1.13 (m, 2H); $^{13}$C NMR (100 MHz, CD$_3$OD) $\delta$ 153.2, 146.6, 130.3, 129.9, 126.2, 125.2, 124.3, 120.6, 59.4, 44.8, 34.3. MS calcd. for [M+H]$^+$ C$_{13}$H$_{19}$N$_4$O 247.16, found 247.15.

**3-(4-(3-(Dimethylamino)phenyl)-1H-1,2,3-triazol-1-yl)propan-1-ol (486).** The compound was synthesized in 95% yield as a white solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.75 (s, 1H), 7.27-7.22 (m, 2H), 7.03 (d, $J = 7.6$ Hz, 1H), 6.69 (dd, $J = 7.6$ Hz and $J = 2.0$ Hz, 1H), 4.52 (t, $J = 7.2$ Hz, 2H), 3.64 (t, $J = 5.6$ Hz, 2H), 2.97 (s, 6H), 2.83 (br, 1H), 2.17-2.10 (m, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) 151.15, 148.59, 131.24, 129.69, 120.50, 114.23,
112.65, 109.78, 58.86, 47.15, 40.83, 32.83. MS calcd. for [M+H]^+ C_{13}H_{19}N_4O 247.16, found 247.15.

3-(4-(4-(Dimethylamino)phenyl)-1H-1,2,3-triazol-1-yl)propan-1-ol (485). The compound was synthesized in 83% yield as a white solid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.69 (s, 1H), 7.66 (d, $J = 8.7$ Hz, 2H), 6.75 (d, $J = 8.7$ Hz, 2H), 4.52 (t, $J = 6.6$ Hz, 2H), 3.66 (q, $J = 5.4$ Hz, 2H), 2.97 (s, 6H), 2.17-2.09 (m, 2H), 1.94 (br, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$) 150.6, 148.3, 126.8, 118.97, 118.8, 112.65, 58.8, 47.1, 40.6, 32.9. MS calcd. for [M+H]^+ C_{13}H_{19}N_4O 247.16, found 247.15.

3-(4-(2-(Dimethylamino)naphthalen-6-yl)-1H-1,2,3-triazol-1-yl)propan-1-ol (489). The compound was synthesized in 83% yield as a light yellow solid. $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 8.56 (s, 1H), 8.17 (d, $J = 1.6$ Hz, 1H), 7.81 (dd, $J = 8.5$, 1.8 Hz, 1H), 7.77 (d, $J = 9.1$ Hz, 1H), 7.71 (d, $J = 8.6$ Hz, 1H), 4.72 (t, $J = 5.0$ Hz, 1H), 4.46 (t, $J = 7.1$ Hz, 3H), 3.44 (q, $J = 5.8$ Hz, 2H), 3.00 (s, 6H), 2.02 (p, $J = 6.6$ Hz, 2H); $^{13}$C NMR (101 MHz, DMSO-d$_6$) $\delta$ 148.56, 146.82, 134.26, 128.72, 126.53, 126.12, 124.16, 123.88, 123.19, 120.87, 116.80, 105.68, 57.47, 46.78, 32.90.

3-(4-(2-Methoxynaphthalen-6-yl)-1H-1,2,3-triazol-1-yl)propan-1-ol (491). The compound was synthesized in 58% yield as a white solid. $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 8.63 (d, $J = 1.0$ Hz, 1H), 8.31 (d, $J = 1.3$ Hz, 1H), 7.93 (dd, $J = 8.5$, 1.6 Hz, 1H), 7.90 – 7.83 (m, 2H), 7.33 (d, $J = 2.5$ Hz, 1H), 7.18 (dd, $J = 9.0$, 2.6 Hz, 1H), 4.72 (td, $J = 5.1$, 1.0 Hz, 1H), 4.47 (t, $J = 7.1$ Hz, 2H), 3.88 (d, $J = 0.9$ Hz, 3H), 3.49 – 3.40 (m, 2H), 2.03 (p, $J = 6.6$ Hz, 2H). $^{13}$C NMR (101 MHz, DMSO-d$_6$) $\delta$ 157.42, 146.45, 133.85, 129.52, 128.55, 127.35,
6-(1-(3-Hydroxypropyl)-1H-1,2,3-triazol-4-yl)naphthalen-2-ol (490). The compound was synthesized in 84% yield as a white solid. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 8.24 (d, $J = 1.0$ Hz, 1H), 8.15 (s, 1H), 7.81 – 7.76 (m, 1H), 7.74 (d, $J = 8.6$ Hz, 1H), 7.68 (d, $J = 8.6$ Hz, 1H), 4.54 (t, $J = 7.0$ Hz, 2H), 3.60 (t, $J = 6.0$ Hz, 2H), 2.15 (p, $J = 6.6$ Hz, 2H). $^{13}$C NMR (101 MHz, CD$_3$OD) $\delta$ 157.19, 149.27, 136.40, 130.92, 129.92, 128.92, 128.12, 126.28, 125.43, 125.17, 122.36, 120.14, 110.08, 59.46, 48.53, 34.16. MS calcd. [M+H]$^+$ C$_{16}$H$_{18}$N$_3$O$_2$ 284.1, found 284.1.

3-(1-(4-(Dimethylamino)phenyl)-1H-1,2,3-triazol-4-yl)propan-1-ol (492). The compound was synthesized in 77% yield as a white solid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.49 (d, $J = 9.0$ Hz, 2H), 6.72 (d, $J = 9.0$ Hz, 2H), 3.72 (t, $J = 6.2$ Hz, 2H), 2.98 (s, 6H), 2.87 (t, $J = 7.2$ Hz, 2H), 2.00-1.93 (m, 2H), 1.94 (br, 1H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 150.80, 148.11, 122.27, 119.71, 112.88, 62.34, 41.00, 32.42, 30.14, 22.59. MS calcd. [M+H]$^+$ C$_{13}$H$_{19}$N$_4$O 247.2, found 247.2.

**General procedure for the synthesis of 1,5-disubstituted triazoles.** Under N$_2$, a solution of the alkyne (20 mg, 1 eq) and the azide (2 eq) in degassed dioxane (2 mL/mmol) was added to a solution of Cp*ClRu(PPh$_3$)$_2$ (5 mol%) in degassed dioxane (2 mL/mmol). The reaction mixture was heated at 60 °C overnight. The mixture was then cooled to room temperature and concentrated under vacuum, followed by column chromatograph, eluting with 5% MeOH in DCM, delivering the desired 1,5-disubstituted triazoles.
3-(5-(3-(Dimethylamino)phenyl)-1H-1,2,3-triazol-1-yl)propan-1-ol (494). The compound was synthesized in 76% yield as a yellow solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.66, 7.28 (t, $J$ = 8.0 Hz, 1H), 6.80 (dd, $J$ =7.2 Hz and 0.8 Hz , 1H), 6.69 (dd, $J$ = 7.2 Hz and 0.8 Hz, 1H), 6.63 (d, $J$ = 2.0 Hz, 1H), 4.50 (t, $J$ = 6.8 Hz, 2H), 3.61 (br, 2H), 2.98 (s, 6H), 2.24 (s, 1H), 2.07-2.02 (m, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 150.9, 139.1, 133.1, 130.0, 127.8, 116.6, 113.4, 112.4, 59.3, 45.2, 40.6, 32.8. MS calcd. [M+H]$^+$ C$_{13}$H$_{19}$N$_4$O 247.16, found 247.15.

3-(5-(4-(Dimethylamino)phenyl)-1H-1,2,3-triazol-1-yl)propan-1-ol (493). The compound was synthesized in 87% yield as a light yellow solid. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 7.68 (br, 1H), 7.32 (d, $J$ = 7.2 Hz, 2H), 6.83 (d, $J$ = 7.2 Hz 2H), 4.48 (t, $J$ = 7.2 Hz, 2H), 3.52 (t, $J$ = 6.4 Hz, 2H), 2.99 (s, 6H), 2.04-1.98 (m, 2H); $^{13}$C NMR (100 MHz, CD$_3$OD) $\delta$ 152.8, 130.8, 114.8, 113.6, 59.7, 46.7, 40.5, 33.9. MS calcd. [M+H]$^+$ C$_{13}$H$_{19}$N$_4$O 247.16, found 247.15.

3-(5-(2-(Dimethylamino)naphthalen-6-yl)-1H-1,2,3-triazol-1-yl)propan-1-ol (497). The compound was synthesized in 85% yield as a yellow solid. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 7.85 – 7.80 (m, 1H), 7.78 (s, 1H), 7.77 (d, $J$ = 2.9 Hz, 1H), 7.75 (d, $J$ = 2.3 Hz, 1H), 7.40 (dd, $J$ = 8.5, 1.9 Hz, 1H), 7.26 (dd, $J$ = 9.1, 2.6 Hz, 1H), 6.98 (d, $J$ = 2.6 Hz, 1H), 4.61 – 4.51 (m, 2H), 3.52 (t, $J$ = 6.1 Hz, 2H), 3.06 (s, 6H), 2.11 – 1.98 (m, 2H). $^{13}$C NMR (101 MHz, CD$_3$OD) $\delta$ 151.21, 136.86, 133.62, 130.33, 129.30, 128.36, 127.83, 127.05, 120.80, 118.36, 106.91, 59.66, 46.85, 40.95, 33.89.

6-(3-(3-Hydroxypropyl)-3H-1,2,3-triazol-4-yl)naphthalen-2-ol (498). The compound was synthesized in 64% yield as a yellow solid. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$
7.92 (s, 1H), 7.85 – 7.74 (m, 3H), 7.47 (dd, \( J = 8.5, 1.8 \) Hz, 1H), 7.20 – 7.10 (m, 2H), 4.57 (q, \( J = 6.8 \) Hz, 2H), 3.52 (q, \( J = 6.2 \) Hz, 2H), 2.05 (dq, \( J = 8.0, 6.2 \) Hz, 2H). \(^{13}\)C NMR (101 MHz, CD\(_3\)OD) \( \delta \) 158.11, 136.77, 131.23, 131.21, 129.63, 129.54, 128.47, 128.45, 127.22, 127.21, 122.20, 120.68, 109.98, 109.96, 59.62, 46.89, 33.87. \([\text{M+H}]^+\) C\(_{15}\)H\(_{16}\)N\(_3\)O\(_2\) 270.1, found 270.1.

3-(5-(2-Methoxynaphthalen-6-yl)-1H-1,2,3-triazol-1-yl)propan-1-ol (499). The compound was synthesized in 93% yield as a light yellow solid. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 7.87 – 7.79 (m, 2H), 7.78 – 7.73 (m, 2H), 7.42 (dd, \( J = 8.6, 1.4 \) Hz, 1H), 7.21 (dd, \( J = 9.0, 2.6 \) Hz, 1H), 7.16 (d, \( J = 2.5 \) Hz, 1H), 4.60 – 4.51 (m, 2H), 3.93 (d, \( J = 1.0 \) Hz, 3H), 3.69 – 3.55 (m, 3H), 2.18 (s, 1H), 2.12 – 2.02 (m, 2H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \( \delta \) 158.90, 138.38, 134.89, 133.42, 129.91, 128.72, 128.35, 127.97, 126.39, 121.99, 120.27, 105.83, 59.25, 55.62, 45.30, 32.62. MS calcd. \([\text{M+H}]^+\) C\(_{16}\)H\(_{18}\)N\(_3\)O\(_2\) 284.1, found 284.1.
Photolabile protecting groups have attracted considerable attention in dynamic studies of biological systems. Photolabile protecting groups, also termed “caging groups”, are placed on a molecule of interest to mask its activity through steric blocking. The process of incorporating a photocleavable protecting group onto a molecule is called “caging” and the resulting inactive molecule is known as a “caged” compound. The photolysis of a caged substrate to release the molecule of interest and restore its activity is known as “decaging” (Figure 9.1). In general, decaging is induced by irradiation with non-damaging UV light of 365 nm. Since light irradiation is orthogonal to most cellular systems and can be controlled in a high spatio-temporal fashion, light is an ideal trigger to control biological processes.

In 1978, Kaplan et al. reported $o$-nitrobenzyl caged ATP as the first caged compound for biological studies. A free ATP molecule was generated by irradiation of the photocaged substrate. Additionally the side product $o$-nitrosobenzaldehyde ($R = H$) or 2-
nitrosobenzylketone (R = Me) was also generated. The photolysis reaction proceeded quickly: after 6-9 seconds 50% free ATP was detected (Scheme 9.1).

![Chemical structure](image)

**Scheme 9.1.** Photolysis of caged ATP. R = H, Me.

The requirements for caged substrates to be applicable for the biological studies are: 1) to be soluble in aqueous environments, 2) yield benign by-products, 3) decage with non-damaging irradiation, and 4) perform fast photolysis with high efficiency. A number of photolabile protecting groups with different photophysical properties have been designed in order to employ single-photon (UV) or two-photon (IR) excitation techniques for biological studies. The o-nitrobenzyl group and its analogs are commonly used caging groups for the application of single-photon excitation.\textsuperscript{309-311} Coumarin and quinoline derivatives have been used as two-photon caging groups.\textsuperscript{312-314}

Unlike single-photon excitation, two-photon excitation is a non linear optical (NLO) process. In 1931, Maria Goeppert-Mayer proposed the two-photon absorption (TPA) theory.\textsuperscript{315} This theory predicted that a molecule can be excited from a lower energy state to a higher energy state by absorbing two photons (of the same or different frequencies).
simultaneously. The sum of the energies of the two photons must be equal to the energy gap between ground state and the first excited state (Figure 9.2).

![Figure 9.2. Diagram of one-photon excitation and two-photon excitation of a chromophore.](image)

9.1 Single-Photon Caging Groups

An o-nitrobenzyl-caged substrate 503 absorbs light in the near UV region, between 300 nm and 400 nm.\textsuperscript{316} Photolysis of the caged substrate occurs via single-photon excitation in which one photon is used to excite the molecule from a lower excited state to a higher excited state. Single-photon excitation is a single-step process in which the probability of excitation is linearly related to the power of the incident optical irradiation.\textsuperscript{312, 316} The decaging reaction is based on a Norrish type II mechanism (Scheme 9.2) and results in an o-nitroso by-product. The rate of photolysis depends upon the nature of the caged substrate, pH
of the surrounding environment, and the composition of the buffer and/or dielectric constant of the medium.\textsuperscript{305,317-319}

The \textit{o}-nitrobenzyl group (ONB) and its derivatives are the most commonly used caging groups, due to their ease of synthesis and high photolysis yield.\textsuperscript{320} The mechanism of photolysis of ONB is shown in Scheme 9.2. Upon light irradiation, the caged substrate 503 is excited, resulting the corresponding diradical compound 504. The radical at the benzylic position of intermediate 504 delocalizes to one oxygen atom to generate an intermediate 505. From there the radical rearranges to deliver intermediate 506. Cyclization of intermediate 506 furnishes the cyclic 1,3-dihydrobenzo[c]isoxazole ring 507. Finally, the isoxazole intermediate 507 releases the molecule 508 along with the nitrosobenzaldehyde (509) as a byproduct.

\begin{scheme}
\begin{equation}
\text{Scheme 9.2. Mechanism of photolysis of } \textit{o}\text{-nitrobenzyl caged substrate 503.}
\end{equation}
\end{scheme}
It has been reported that the nitrosobenzaldehyde byproduct (509) can be reactive and harmful in a biological context.\textsuperscript{31, 32} Modification on the $\alpha$-methylene group is needed to minimize the reactivity of the byproduct. Modifications with of some functional groups, such as methyl 510\textsuperscript{31} carboxyl 511,\textsuperscript{310} $o$-nitrophenyl 513,\textsuperscript{323} and trifluoromethyl substitutes 512\textsuperscript{311} to $\alpha$-methylene position have been accomplished. One disadvantage of this modification is that it introduces a stereogenic center, bringing in difficulties in separation and identification.\textsuperscript{303} However, there are several benefits that can be obtained from the modification. The addition of the methyl group enhances the photolysis kinetics, making the photolysis of $o$-nitrophenylethyl (NPE) caged substrate 510 faster and producing a much less reactive byproduct, $o$-nitrosoacetophenone 516.\textsuperscript{303, 318} The addition of an aromatic ring or the addition of a second photoreactive nitro group 513,\textsuperscript{323} or a carboxylic acid 511,\textsuperscript{310} greatly enhances the photolysis rate compared to that of the ONB-caged substrate and produces less toxic byproducts. The strong electron-withdrawing trifluoromethyl group 513 greatly increases the acidity of hydroxyl group, enabling the formation of $o$-nitrobenzyl caged compounds 519 using mild Mitsunobu coupling condition instead of strongly basic Williamson ether synthesis condition (Scheme 9.3).\textsuperscript{311} This allows caging of base sensitive substrates with $o$-nitrobenzyl groups.
Scheme 9.3. $\alpha$-Nitrobenzyl protecting group derivatives and their photolysis.

The efficiency of the photolysis of the $\alpha$-nitrobenzyl derivatives highly depends on the substituents on the benzene ring. Addition of electron donating groups on the benzene ring induces an increase of absorbance of caging groups at longer wavelength, enabling the use of longer wavelength UV light during photolysis. The 3,4-dimethoxyl-6-nitrobenzyl (DMNB) and numerous other electron-donating group containing caging groups, such as etc (Figure 9.3), have been developed and applied. The 2-nitropiperonyl caging group is one of the most used caging groups because of its higher decaging efficiency compared to that of 2-nitro-4-methoxybenzyl and 2-nitro-4,5-dimethoxybenzyl analogs.
The way of installation of a caging group onto a small molecule depends on the structure of the molecule and the nature of caging group. Caging of alcohols usually is achieved through the formation of an ether or a carbonate. The formation of an ether is mostly accomplished through a Williamson ether synthesis.\textsuperscript{327, 328} For example, the adenosine \textsuperscript{524} reacted with \(\alpha\)-nitrobenzyl bromide \textsuperscript{525} under an \(S_N2\) reaction to obtain the caged adenosine \textsuperscript{526}, which was converted to 2'-caged adenosine phosphoramidite \textsuperscript{527} in several steps (Scheme 9.4). When the adenosine was ionized by the treatment of NaH, the vicinal 2’- and 3’- hydroxyl groups were preferentially dissociated due to the formation of hydrogen bond.\textsuperscript{329} However, this protocol may not be useful for base sensitive compounds.\textsuperscript{330} This can be overcome by employing the caging group \textsuperscript{528},\textsuperscript{311} allowing the ether formation via a Mitsunobu reaction, which provides milder reaction conditions than a Williamson ether synthesis.\textsuperscript{311} Another common way to install caging groups on alcohols is by forming a carbonate linkage. The carbonate linkage can be installed through the activation of a hydroxyl moiety of the caging group using coupling reagents such as CDI,\textsuperscript{331} DSC,\textsuperscript{332} \(p\)-nitrophenyl chloroformate,\textsuperscript{33} or phosgene (diphosgene)\textsuperscript{334} followed by reacting the activated
caging  group with an alcohol to produce the caged alcohol. A widely used caging group, \( \alpha \)-methyl-6-nitropiperonyl carbonate (MeNPOC), is applied to cage numerous alcohols.

**Scheme 9.4.** Examples of caging converting alcohols to caged ether compounds via Williamson ether synthesis.

Caging groups can be introduced onto amines by employing coupling reagents leading to carbamate linkages.\(^{326, 335-338}\) The formation of a carbamate linker is similar as that of a carbonate (Scheme 9.5). Another way to generate the carbamate linker is by reacting the hydroxyl moiety of the caging group with an isocyanate.\(^{319, 339}\)

Photocaged esters or amides can be synthesized using the caging group \( 529 \) and an acid \( 528 \) in the presence of coupling reagent, such as DCC,\(^{340, 341}\) EDCI,\(^{342}\) or PPh\(_3\)/DEAD.\(^{331, 343}\) Moreover, sterically less hindered ester or amide linkages can be prepared using the corresponding acid halide and an alcohol or amine \( 529 \)\(^{344, 345}\) (Scheme 9.5).
To minimize the drawbacks caused from the toxicity of nitrosocarbonyl compounds, several single-photon caging groups have been developed including the 2-(2-nitrophenyl)ethyl group 531, the pentadienynitrobenzyl group 532, the styrylsilyl protecting group 533, the $p$-hydroxyphenacyl group 534 and the (hydroxystyryl)dimethylsilyl analogs (Figure 9.4).

The caging group 2-(2-nitrophenyl)ethyl 531 is derived from 1-methyl-2-nitrobenzene by the insertion of a methylene group at the $\alpha$-methylene position.$^{323, 346}$ Photolysis of the caged substrate 531 releases the corresponding alcohol along with the less
toxic o-nitrostyrene as a byproduct (537). The application of the 2-(2-nitrophenyl)propyloxycarbonyl (NPPOC) caging group 535 occurred in the photolithographic synthesis of DNA, in peptide chemistry, and the synthesis of glycopyranosides. The photolysis of NPPOC caged substrates at 365 nm can easily be performed in both a neutral or a basic environment, is fast, efficient and produces 1-nitro-2-(prop-1-en-2-yl)benzene (536) and carbon dioxide as byproducts (Scheme 9.6).

\[
\begin{align*}
\text{R'} & = \text{H} \\
\text{R'} & = \text{Me (NPPOC)}
\end{align*}
\]

Scheme 9.6. Photolysis of the 2-(2-nitrophenyl)ethyl groups (531 and 532).

9.2 Two-Photon Caging Groups.

Photolysis of single-photon caged molecules uses UV or near-UV light irradiations (250-400 nm), which can be damaging to cells. Also, the penetration into tissues at this wavelength is limited. This limits the application of the single-photon caging groups for in vivo use. In order to alleviate the damaging effects of UV light, a caging group that could be removed with IR light is required.

Single-photon excitation is a linear process. In a single-photon excitation process, all the molecules exposed to the UV light are excited, and therefore, decaged. Two-photon excitation is a non-linear process. Two IR photons, generated from a tightly focused beam,
are absorbed by the chromophore simultaneously. These two photons provide the same energy as that of a single-photon of UV light, but at longer wavelengths. Unlike the single-photon decaging, the two-photon decaging only takes place at the focus of the laser beam (Figure 9.5) minimizing photodamage to the surrounding tissue.\textsuperscript{312, 348}

Since the energy of a photon inversely proportional to its wavelength, to obtain equal energy of single-photon of wavelength \textasciitilde 350 nm, two-photons of wavelength \textasciitilde 700 nm (near IR) must be absorbed.

![Image](image_url)

**Figure 9.5.** Difference of the excitation volume between single and multiphoton excitation. Adapted from \textsuperscript{312}

The key to evaluate two-photon caging groups is their two-photon decaging action cross-section, \( \delta_u \).\textsuperscript{349} The unit of \( \delta_u \) is a Goppert-Mayer (GM) \( (10^{-50} \text{ cm}^4 \text{s photon}^{-1}) \). The \( \delta_u \) of a caging group should be greater than 0.1 GM in order to be useful in a biological system.\textsuperscript{348} A
number of two-photon caging groups with desirable $\delta_u$ have been developed such as 6-bromo-7-hydroxycoumarin (Bhc)\textsuperscript{348} and 8-bromo-7-hydroxyquinoline (BHQ).\textsuperscript{312,313}

Furuta et al.\textsuperscript{348} reported the 6-bromo-7-hydroxycoumarin (Bhc) protecting group as an excellent caging group. The Bhc group undergoes two-photon decaging with IR irradiation at 780 nm. The cross section of two-photon decaging is approximately 1. Additionally, the single-photon photolysis efficiency of Bhc at 365 nm or longer wavelengths is much higher than that of most $o$-nitrobenzyl derivatives.\textsuperscript{348} The Bhc caging group was synthesized in two steps from 4-bromoresorcinol \textsuperscript{538}.\textsuperscript{348} The coumarin ring was assembled via the cyclization of 4-bromoresorcinol \textsuperscript{538} and ethyl 4-chloroacetoacetate (\textsuperscript{539}) in the presence of $\text{H}_2\text{SO}_4$. The chloride \textsuperscript{540} was converted to corresponding alcohol \textsuperscript{541} by heating at reflux temperature in water as a solvent (Scheme 9.7). The Bhc group has been used for caging alcohol, acid, and amine functionalities.

8-Bromo-7-hydroxyquinoline (BHQ) is another commonly used two-photon caging group developed by the Dore lab.\textsuperscript{312} The BHQ caging group has relatively good solubility in aqueous buffers, which benefits its application in biological systems. In addition, BHQ has a lower level of fluorescence background than Bhc when excited at 365 nm. This greatly
reduces the background when conjugating it with fluorescent indicators. Another advantage of BHQ is that the one-photon photolysis of BHQ is faster than that of Bhc at 365 nm. The BHQ caging group can be efficiently photolyzed by two-photon excitation in aqueous buffer at 740 nm ($\delta_u = 0.59$ GM) with high quantum efficiency. The synthesis of the BHQ caged substrates can be achieved from $m$-aminophenol (542) and crotonaldehyde in 7 steps (Scheme 9.8). The quinoline ring 543 was assembled via a Skraup reaction of the $m$-aminophenol (542) and crotonaldehyde, followed by the phenol alcohol protection. The 2-methyl group of 543 was oxidized by SeO$_2$ to give the aldehyde 544, which was reduced to the alcohol 545. The substrate was reacted with the alcohol 545, delivering the BHQ-caged 546. The BHQ ring was brominated to product the bromoquinoline 547. After the silyl protecting group was removed, the BHQ-caged substrate 548 was furnished. The BHQ caging group has been used for the caging of carboxylic acids, aldehydes, and phosphates. Photolysis of the BHQ-caged substrate 548 release the substrate 550 at wavelength at both 365 nm and 740 nm.

The \( o \)-hydroxycinnamic platform was investigated for two-photon caging applications.\(^{352}\) The mechanism of decaging involves a \textit{trans-cis} isomerization followed by an intramolecular cyclization to release the active molecule (Scheme 9.9). A number of \( o \)-hydroxycinnamic derivatives (555-559) have been studied and several of them can be efficiently photolyzed by one-photon or two-photon excitation (Figure 9.6). Compound 557, has one of the highest \( \delta_u \) value of 4.7 GM at 750 nm, which is much higher than Bhc (0.72 GM at 740 nm), BHQ (0.59 GM at 740 nm) and NDBF (0.6 GM at 710 nm).\(^{353}\) The \( o \)-hydroxycinnamic acid derivatives are good caging groups for alcohols and amines.\(^{354}\)
Scheme 9.9. Mechanism of photolysis of \( \omega \)-hydroxycinnamic derivatives.

Figure 9.6. \( \omega \)-Hydroxycinnamic derivatives with good sensitivity to 2 PE.

3-Nitro-2-ethyldibenzofuran (NDBF), a 1 PE or 2 PE caging group, has a high extinction coefficient and quantum yield. The photolysis of NDBF caging group is 16-160 times more efficient compared to that of \( \omega \)-nitrobenzyl analogs such as ONB and 4,5-dimethoxy-2-nitrobenzyl (DMNB) groups.\(^{353, 355}\) Several synthetic approaches to NDBF caging group derivatives have been reported.\(^{353, 355, 356}\) The most recent synthesis of (3-nitrodibenzofuran-2-yl)-ethanol was performed in 3 steps starting from 4-fluorobenzaldehyde (560) and 2-iodophenol (561). An Ullman reaction of 560 and 561 delivered the aldehyde 562, which was treated with trimethyl aluminum to give the alcohol 563. The alcohol underwent an intramolecular Heck reaction to assemble the dibenzofuran ring, producing the NDBF alcohol 564 (Scheme 9.10).\(^{353}\) The NDBF caging group has been used to cage
thymidine phosphoramidites for DNA synthesis,\textsuperscript{353} EGTA,\textsuperscript{355} and FerriCast (a macrocyclic cage for Fe\textsuperscript{3+}).\textsuperscript{357}

Scheme 9.10. The synthesis of NDBF (564).

Pirrung et al. discovered that NPPOC caged substrates (535, Scheme 9.6) can also be photolyzed with two-photon excitation using IR light ($\delta_u = 0.86$ GM at 766 nm).\textsuperscript{349, 358, 359}
CHAPTER 10: Caged Rapamycin

10.1 Introduction

Rapamycin (Rap, 565), also known as sirolimus, is a complex macrolide natural product isolated from the bacterium Streptomyces hygroscopicus, found in a soil sample on Easter Island in 1975. In recent years, rapamycin has been widely used as an immunosuppressant drug in organ transplantation to prevent rejection. It has received substantial attention due to immunosuppressant activity. Rapamycin mediates heterodimerization of the proteins FKBP12 (FK506 binding protein 12) and FRB (FKBP12 rapamycin binding domain). Due to rapamycin’s excellent physiological properties, including good pharmacokinetics, permeability across the blood-brain barrier, and oral bioavailability, it has been used as a small molecule dimerizer for a wide range of applications in mammalian cells and organisms. Furthermore, the FRB-FKBP12 interaction has proven valuable in a broad range of basic research applications, where it has been engineered to control gene function through rapamycin-induced transcription, protein localization, protein degradation, and DNA recombination. Recently, it has been shown that rapamycin can control kinase activity when an engineered version of FKBP is inserted at a conserved position in the kinase active site. Thus, a photo-activatable analog of rapamycin represents a unique and important biological research tool, enabling the regulation of heterodimerization, thus kinase activity, transcription, localization, protein degradation and DNA recombination using light as a non-invasive regulatory element that can be controlled with high spatial and temporal resolution.
Figure 10.1. Structure of rapamycin 565.

An analysis of the chemically accessible sites of rapamycin (565) revealed that the methoxy group on C-16 can undergo nucleophilic substitution\textsuperscript{375-379} and β-elimination.\textsuperscript{375} The hydroxyl groups at C-28 and C-40 can be protected with silyl groups,\textsuperscript{380,381} and a trifluoromethylsulfonyl group.\textsuperscript{382} The lactone at C-34 can be hydrolyzed and eliminated,\textsuperscript{383-385} and, importantly, the hydroxyl group at C-40 can be converted into a carbonate group\textsuperscript{382} and be esterified.\textsuperscript{377, 381, 386} Thus, C-40 represents the most suitable site for chemical modification with a carbonate-linked caging group that can provide facile installation and quick photolysis. Importantly, based on the crystal structure of the ternary complex of rapamycin, FRB, and FKBP12, the hydroxyl group at C-40 undergoes hydrogen bond formation with glutamine 53 of FKBP12 (Figure 10.2).\textsuperscript{387, 388}
To date, three caged rapamycin analogs have been reported.\textsuperscript{389-391} In 1997, Borchardt et al. described the first caged rapamycin \textsuperscript{566} (Figure 10.3). Rapamycin was immobilized on amino-modified polystyrene beads using a photocleavable linker in three steps.\textsuperscript{389} Irradiation of caged rapamycin \textsuperscript{566} at 365 nm released rapamycin (\textsuperscript{565}), which can either inhibit growth in wild-type \textit{Saccharomyces cerevisiae} yeast or induce growth in the engineered yeast strain Y153. In a growth inhibition assay, inhibition of TOR1p/TOR2p by rapamycin-FKBP 12 caused G1 cell cycle arrest, resulting in 75\% inhibition of growth upon irradiation for 15 seconds and complete inhibition of growth upon irradiation for 30 seconds or longer. In a growth induction assay, dimerization of FKBP and FRB by rapamycin initiated transcription of the \textit{HIS 3} reporter gene, allowing for cell growth in histidine media.\textsuperscript{389}
Figure 10.3. Structure of immobilized caged rapamycin 566.

The DMNB-caged rapamycin 567 (Figure 10.4) was developed by Sadovski et al. in 2010.\textsuperscript{391} It was synthesized in one step through an acid-catalyzed carbenium ion formation at C-16 with low yield (10%). This caged rapamycin 567 was inactive in the inhibition of mTORC1, while the activity was restored to near native levels upon decaging, by probing for changes in S6 and S6k phosphorylation levels via Western blot. Decaging at 365 nm provides 16-hydroxy rapamycin, instead of rapamycin, which has been reported to be active in treating or preventing fungal infections in mammals.\textsuperscript{392}
Also in 2010, Umeda et al. reported a caged rapamycin-biotin conjugate 568 (Figure 10.5), that was synthesized in 2 steps. An additional linker was inserted due to the author’s unsuccessful direct caging of the C40 hydroxyl group. Biotin was linked to rapamycin via click reaction with the caging group. Biotin was selected as a macromolecule to cage rapamycin, due to its extreme stability with a dissociation constant on the order of femtomolar. UV irradiation of 568 did not provide rapamycin, but instead delivered a C-40 2-hydroxyethylether which induced rapid dimerization of YFP-FKBP and membrane-targeted FRB, thus promoting translocation of YFP-FKBP from the cytoplasm to the plasma membrane and also induced formation of localized ruffle.390
10.2 Single-Photon Caged Rapamycin

10.2.1 Caged Rapamycin with Caging Groups that Photolyze at 365 nm

Inspired by the literature reports on the modification on rapamycin, we hypothesized that disruption of the C40-OH bond through installation of a sterically demanding group would prevent protein dimerization. Hence, we attempted to install a caging group on the C40-OH. The $\alpha$-methyl nitro-piperonyloxycarbonyl (MeNPOC) group 570 was selected due to its ease of synthesis and high photolysis efficiency. The caging group 1-(5-methoxy-2-nitro-4-(prop-2-ynyloxy)phenyl)ethanol (575) was also used. The structure of 575 is similar to 570, but 575 possesses an extra alkyne group attached to the phenol oxygen. This alkyne can potentially link additional functional groups or molecules to the caged substrates. The MeNPOC (570) was synthesized in two step from commercially available 6-nitropiperonal (569). 6-Nitropiperonal (569) was converted to $\alpha$-methyl-2-nitropiperonylalcohol (570) by reacting with trimethylaluminum according to a literature procedure. Then the hydroxyl...
group of α-methyl-2-nitropiperonylalcohol (570) was activated with DSC, generating α-
methyl-2-nitropiperonylol succinimidyl carbonate (NPOC-NHS, 571). The 1-(3,4-
(methylenedioxy)-6-nitrophenyl)ethanol (575) was assembled in three steps from
commercially available 4-hydroxy-3-methoxybenzaldehyde (572). The phenol group of 572
was alkylated with propargyl bromide, delivering 573 in 89%. Nitration of 573 afforded the
5-methoxy-2-nitro-4-(prop-2-ynyloxy)benzaldehyde 574, which was treated with Me₃Al to
generate 575 in 90% yield (Scheme 10.1).

\[
\begin{align*}
569 & \xrightarrow{\text{Me₃Al, DCM}} 570 \\
570 & \xrightarrow{\text{DSC, TEA, CH₃CN}} 571 \\
572 & \xrightarrow{\text{propargyl bromide, K₂CO₃, DMF}} 573 \\
573 & \xrightarrow{\text{HNO₃}} 574 \\
574 & \xrightarrow{\text{Me₃Al, DCM, 0°C-rt}} 575
\end{align*}
\]

**Scheme 10.1.** The synthesis of caging groups 570 and 575.

With the caging substrates 570 and 575 in hand, the condition for caging the
secondary alcohol. Cyclohexanol (577) was used as a model compound for rapamycin. We
carried out a set of reaction conditions. The hydroxyl group of 577 was activated with DSC,
generating succinimidyl carbonate 576. Then, the conditions for reacting the cyclohexanol
(577) and 576 were explored and the results are shown on Scheme 10.2. When TEA was
used as the base and THF was used as the solvent, the reaction generated only starting
material (Scheme 10.2, entry 1). When the solvent was switched to DCM, with addition of TEA and DMAP, byproducts 579 and 575 were produced together with only traces amount of the desired product 578 (Scheme 10.2, entry 2). If DIPEA was used as the base, no desired product 578 was obtained (Scheme 10.2, entry 3 and 4). We then changed the base to DMAP and the solvent to DCM. The reaction only gave 576 and 575 when the reaction was carried out at low concentration (0.01 M) (Scheme 10.2, entry 5). However, we were delighted to find out that the desired product 578 was obtained at a moderate yield 61% with increasing the reaction concentration to 0.05 M (Scheme 10.2, entry 6). This is probably due to reaction kinetic effect that the higher concentration promoted the reaction of 576 and 575 to the desired product 578, although some byproduct 579 were delivered too.

Scheme 10.2. Reaction conditions for caging cyclohexanol with 576.
With the best condition for caging the secondary alcohol in hand, we applied this reaction to cage rapamycin. Rapamycin (565) was reacted with both activated caging groups, MeNPOC-NHS (571) and 1-(5-methoxy-2-nitro-4-(prop-2-ynyloxy)phenyl)ethanol succinimidyl carbonate (MPNB-NHS) (576) in the presence of DMAP and DCM (Scheme 10.3). Both caging groups were able to react with rapamycin and produce desired caged rapamycin pRap (580) and MPNB-Rap 581. However, relatively low yields (36% for 580 and 45% for 581) were obtained. Increased reaction time didn’t improve the reaction yield (Scheme 10.3). This was probably due to the instability of rapamycin and caged rapamycin at room temperature.
We also examined several other conditions to cage rapamycin. The hydroxyl group of α-methyl-2-nitropiperonylalcohol 570 was activated with CDI, 4-nitrophenyl chloroformate and diphosgene, delivering the active carbonate of the caging groups 582, 583 and 584, respectively (Scheme 10.4). However, no or trace pRap was obtained when the caging group 582, 583 and 584 were used to cage rapamycin (Scheme 10.4).
Scheme 10.4. Activation of 570 and reaction with rapamycin.

In collaboration with Andrei V. Karginov (UNC Chapel Hill), the light-mediated regulation of protein dimerization was studied. He first tested whether pRap could induce dimerization of FKBP12 and FRB. He created a GFP-FRB protein fusion and wild-type FKBP12 fused to the N-terminus of focal adhesion kinase (FAK). FAK localizes prominently to focal adhesions in living cells, allowing for testing of dimerization in vivo by observing rapamycin-mediated translocation of GFP-FRB into focal adhesions. Prior to live cell co-localization studies, the constructs were tested in pull-down assays, comparing the ability of pRap (580) and Rap (565) to mediate the intracellular dimerization of FAK-FKBP and FRB. Cells expressing both FAK-FKBP and FRB were treated with rapamycin or pRap (580) for 1 hour, with or without irradiation. Complex formation was assayed by pulling down myc-FKBP-FAK from cell lysates and blotting for GFP-FRB. Surprisingly, both small molecules generated dimerization with similar effectiveness, with or without UV irradiation. This data
showed that the FKBP12-rapamycin-FRB complex was not sufficiently sensitive to rapamycin MeNPOC-modification for a successful light-activation approach through photocaging.

Then he tested a recently developed modified FKBP, named iFKBP, that is proposed to have increased structural mobility of the Lys52-Glu54 loop positioned next to the C-40 hydroxyl group of rapamycin (Figure 10.6 a).\(^{374}\) Using both an N-terminal iFKBP-FAK fusion and a fusion of iFKBP internally, at position 413 of FAK (Figure 10.6 b), he examined whether pRap (580) could mediate heterodimerization of iFKBP and FRB in a light dependent manner. Indeed, pRap (at concentrations of up to 20 µM) failed to mediate interaction between iFKBP-FAK and GFP-FRB, while irradiation of pRap-treated cells with 365 nm UV light successfully removed the caging group and induced iFKBP-FRB dimerization (Figure 10.6 c, d and e). Decaging kinetics were dependent on both light dosage and pRap (580) concentration. Importantly, in the presence of pRap (580), translocation of FRB into focal adhesions was observed only upon decaging, indicating successful protein dimerization between FAK-iFKBP and FRB in live cells (Figure 10.6 f and g). These studies demonstrated that pRap (580) can effectively mediate light-dependent protein heterodimerization when used with iFKBP rather than FKBP12.
Figure 10.6. Crystal structure and light activation of pRap in mammalian cells. (a). Crystal structure of the ternary complex between rapamycin, FKBP12 (green), and FRB (blue). The 2.65 Å hydrogen bond (possibly mediated through a water molecule) between Gln53 of FKBP12 and the C-40 hydroxyl group of rapamycin is indicated. PDB 2FAP Light-regulated dimerization of iFKBP and FAK. (b) Positions of iFKBP insertions into FAK. (c-e) HEK293T cells co-transfected with GFP-FRB and either myc-iFKBP-FAK (c,d) or myc-FAK-iFKBP413 (e) were treated with either Rap (0.5 µM) or the indicated concentrations of pRap. Ten minutes after addition of pRap or Rap, cells were irradiated with 365 nm UV light for 1 min (c,e) or 5 min (d) and incubated for 1 h. Control cells were not irradiated. Myc-iFKBP-FAK was immunoprecipitated from cell lysates using an anti-myc antibody, and co-immunoprecipitation of GFP-FRB was detected by Western Blot using an anti-GFP antibody. (f) HeLa cells co-transfected with GFP-FAK-iFKBP413 and mCherry-FRB were treated with pRap (20 µM) for 30 min, followed by UV irradiation (365 nm, 2 min). TIRF images were taken before and after irradiation. (g) HeLa cells co-transfected with GFP-RapR-FAK and mCherry-FRB were treated with pRap (5 µM) for 30 min, followed by UV irradiation (365 nm, 2 min). TIRF images were taken before and after irradiation.
From Andrei V. Karginov’s results, it is found that the caging group of pRap was not large enough to deactivate rapamycin, and an engineered version of FKBP, so called iFKBP had to be applied. In order to solve this problem, a larger caging group is needed to cage rapamycin.

The PEG group has been used widely in biological and pharmaceutical applications to improve the pharmacokinetic properties of biomolecules.\textsuperscript{395, 396} It has been found to be nontoxic and nonimmunogenic.\textsuperscript{395, 396} PEGs are relatively inexpensive polymers and a range of sizes are available.\textsuperscript{397} The bulk of the PEG polymer can sterically block the activities of small molecular as well as increase the solubility in water.\textsuperscript{398} The PEG polymer was attached to the caged rapamycin via a click reaction between the PEG-N\textsubscript{3} and the alkyne of MPNB-Rap (581). The 5000 Da PEG-azide (585) was reacted with MPNB-Rap (581) via a standard click reaction condition (Cu\textsubscript{2}SO\textsubscript{4}-5H\textsubscript{2}O, TBTA, Na ascorbate in \textit{t}-BuOH, H\textsubscript{2}O and DCM),\textsuperscript{390} to provide the PEGylate caged rapamycin 587. However, due to identification difficulty (using MS or NMR), the purity of the 5000 Da PEGylated caged rapamycin 587 was not determined. We then purchased a pure 500 Da PEG-N\textsubscript{3} from Quanta Biodesign. In a similar manner, the 500 Da PEG-N\textsubscript{3} was reacted with MPNB-Rap (581) to afford the 500 Da PEGylated rapamycin 588 in 38\% yield (Scheme 10.5). The identification and purity were confirmed by \textsuperscript{1}H NMR and MS. In collaboration with Jie Zhang (Deiters lab), the light-mediated regulation of DNA recombination of the 500 Da PEGylated rapamycin 588 was studied using a split Cre system.\textsuperscript{399}
Cre recombinase is an enzyme that recognizes two palindromic sequences of DNA, known as *loxP* sites. Cre recombinase has the ability to delete, insert, or invert any DNA sequence that is located between the *loxP* sites, depending on the orientation of the sites.\(^{399}\) The split Cre system, developed by the Herman lab, divides Cre into two inactive fragments, one composed of amino acids 19-59 and the other containing amino acids 60-343.\(^{400}\) The

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**Scheme 10.5.** Synthesis of PEGylated caged rapamycin \(587\) and \(588\).
two fragments were then fused to FKBP12 and FRB, respectively, allowing for the control of Cre-catalyzed DNA recombination by rapamycin. In the presence of rapamycin, FKBP12 and FRB can dimerize and bring the two fragments of split Cre recombinase into close proximity, resulting in enzyme activation and DNA recombination. To quantify the activity of Cre recombinase, the Cre Stoplight system was used. DsRed protein is expressed when Cre recombinase is absent or non-functional. In the presence of active Cre, the gene coding for dsRed is excised from the plasmid due to flanking \textit{loxP} sites and GFP is expressed. Jie examined the ability of the 500 Da PEGylate rapamycin 588 to control Cre catalyzed DNA recombination. She found that the 500 Da PEGylate rapamycin 588 was still active towards inducing the dimerization of FKBP and FRB. This indicated that the size of the caging group was not big enough to mask its activity.

Another way to increase the size of the caging group could be by using rapamycin itself as a sterically demanding structure. This could be accomplished by synthesizing a photocleavable rapamycin dimer, dRap (590). The dRap 590 was synthesized from the already prepared caged rapamycin MPNB-Rap (581) via a double [3+2] bioconjugation reaction with 1,4-diazidobutane (589) in an 81% yield. Photolysis (365 nm) of 590 releases two equivalents of native rapamycin (565) (Scheme 10.6). In collaboration with Jie Zhang (Deiters lab) and Kalyn Brown (Deiters lab), the light-mediated regulation using dRap (590) was examined.
Jie used the dRap (590) to control Cre catalyzed DNA recombination. HEK293T cells were transfected with plasmids encoding the split Cre system and the Cre stoplight. The cells were treated with 10 nM rapamycin and 5 nM dRap. It was found that the absence of rapamycin results in only dsRed expression. With rapamycin (565) in the cell media, active Cre enzyme is reconstituted through formation of the ternary FKBP12-rapamycin-FRB complex leading to GFP expression. In the presence of 5 nM dRap (590) before UV irradiation, only dsRed was expressed, indicating that dRap (590) was not active to heterodimerize FKBP12 and FRB, thus Cre recombinase is inactive. Upon 5 minutes of irradiation at 365 nm, Cre activity was restored, as seen by the GFP expression (Figure 10.7).
Activity is restored by the native rapamycin molecules, released from irradiation, inducing the FKBP12-rapamycin-FRB ternary complex and thus recombining of Cre fragments.

**Figure 10.7.** Light-activated Cre DNA recombination in HEK293T cells. Cells not treated with rapamycin were only able to express dsRed. In the presence of 10 nM rapamycin, active Cre was present leading to GFP expression. dRap (5 nM) can not heterodimerize FKBP12 and FRB, thus dsRed is expressed. Decaging of dRap allows excision of the dsRed gene by reconstituted Cre and GFP expression.

Kalyn applied the dRap to the photochemically control tobacco etching virus N1a protease (TEVp) activity. TEVp is a widely used protease that recognizes a specific seven amino acid sequence and cleaves proteins between the glutamine and glycine residues. In a split TEVp system, TEVp was divided into an N- (amino acids 1-118) and a C- (amino acids 119-241) terminal fragments and each fragment was fused to FRB and FKBP12, respectively. Co-expression of both fusion proteins did not display any protease activity until the addition of rapamycin, which induces dimerization and restores activity of TEVp (Figure 10.8). She used a circularly permuted luciferase reporter, GloSensor, to measure the activity of TEVp. The active TEVp will cleave TEV recognition site of GloSensor, causing a conformational change and active luciferase will produce a luminescence signal. When dRap (50 nM) was added, in the absence of UV light, only background luminescence was
observed. However, upon UV irradiation (5 min), a luminescence signal was detected (Figure 10.8), indicating that the dRap was decaged upon UV irradiation and released natural rapamycin, which induced the dimerization of FKBP and FRB, thus causing the TEVp active. The signal intensity for 50 nM of dRap after irradiation was comparable to the signal for 100 nM of native rapamycin (Figure 10.8), illustrating that photolysis of dRap releases two equivalents of rapamycin.

![Figure 10.8](image_url).

**Figure 10.8.** Luminescence data from split TEVp/GloSensor assay using dRap. HEK293T cells transfected with FKBP12-TEVct, FRB-TEVnt, and GloSensor were treated with 100 nM wildtype rapamycin or 50 nM dRap and irradiated for 5 minutes at 365 nm. Treatment with 100 nM rapamycin shows luminescence signal in the absence or presence of UV light. In comparison, cells without rapamycin showed very little GloSensor background. Cells treated with 50 nM dRap before irradiation causes only luminescence comparable to cells without rapamycin. Photolysis of dRap yields rapamycin as shown by the increase in luminescence.
10.2.2 Caged Rapamycin with Caging Groups that Photolyse at 405 nm

As discussed in Chapter 9, coumarin analogs are good caging groups with high one-photon and two-photon decaging efficiency. Electron-donating substitutions (such as OH, OMe, NMe₂ groups) on C6 and C7 position of coumarin greatly enhance the release rate and hydrolytic stability. However, it also perturbs the quantum yield. It was discovered that the caging group with a dimethylamino substituent has a higher quantum yield. In addition, these substitutions affect the absorption $\lambda_{\text{max}}$. Among all the known coumarin analogs, the (7-diethylamino-coumarin-4-yl)methyl (DEACM, 593) shows the highest absorption $\lambda_{\text{max}}$ (around 390 nm). Photolysis efficiency of DEACM-caged molecules is high. It is proposed that DEACM-caged rapamycin can be photolyzed with light at a near UV region (wavelength at 405 nm).

We synthesized the caging group DEACM (593) in two steps from commercially available 7-(diethylamino)-4-methyl-coumarin (591) according to literature report. The alcohol of 593 was activated with DSC, delivering the DEACM-NHS (594) in 49% yield. Due to the poor stability of the DEACM-NHS (594), longer reaction time led to a lower yield. The DEACM-NHS (594) was reacted with rapamycin in the similar way as that of pRap, leading to 9% of DEACM-caged rapamycin 595 (Scheme 10.7).
Scheme 10.7. Synthesis of DEACM-caged rapamycin 595.

In a similar concept as the rapamycin dimer, we would like to synthesize a coumarin-caged rapamycin dimer (602). The synthesis of 602 commenced with 7-(diethylamino)-4-carbaldehyde coumarin (592). Alkylation of 7-(diethylamino)-4-carbaldehyde coumarin (592) with allyltributyltin delivered the allyl alcohol 596, which underwent a hydroboration reaction to generate the diol 597. The primary alcohol of diol 597 was selectively mesylated to 598 in the presence of secondary alcohol. The mesylate group of 598 was replaced by an azido group via a S_N2 reaction, producing the azido compound 599. The secondary alcohol of 599 was then activated with NHS, and the resulting NHS carbonate 600 was reacted with rapamycin (565) to deliver the caged rapamycin 601 in 33% yield. However, the [3+2] cycloaddition reaction of caged rapamycin 601 and 1,7-octadiyne did not provide any desired
product 602 under different conditions. This was probably due to the limited space between the two rapamycins in the molecule 602.
Scheme 10.8. Attempt to synthesize the coumarin rapamycin dimer 602.
In order to increase the distance between the two rapamycin units in the coumarin rapamycin dimer, an additional linker was inserted between the coumarin ring and rapamycin. Initially, a propargyl group was used to replace the azide group to increase the chain length of the linker. The alcohol 596 was protected with a TBDMS group, delivering 603. Hydroboration of the double bond of 603 produced the primary alcohol 604. However, we encountered the problem of the ether formation of alcohol 605 under several conditions (Scheme 10.9).

![Scheme 10.9. Attempted synthesis of 605.](image)

Since the formation of ether bond for the alcohol 604 was problematic, we tried to connect the alkyne chain to the alcohol 604 via a carbonate or carbamate linker. Three different alkynes, 3-butyn-1-ol, propargyl amine, and N-benzylprop-2-yn-1-amine were used to provide an additional alkyne group to the coumarin caging group. The alcohols 604 were
activated with DSC and the resulting NHS carbamate 606 was reacted with 3-butyn-1-ol (with DMAP in DCM), propargyl amine (in DMF), or N-benzylprop-2-yn-1-amine (in DMF), delivering the carbonate 607, and the carbomates 608 and 609 in excellent yields, respectively. The removal of the TBDMS protecting group of 607, 608, and 609 went smoothly, delivering the free alcohols 610, 611, and 612. The alcohols 610, 611, and 612 were activated with DSC to give the NHS carbomates 613, 614, and 615. However, when rapamycin was treated with the coumarin-NHS 613, 614, or 615 were using the same conditions as for synthesizing 580, no desired caged rapamycin 616, 617 or 618 was obtained (Scheme 10.10).
Scheme 10.10 Attempted synthesis of 616-618.

The unsuccessful synthesis of the coumarin caged rapamycin dimers could be caused by the steric issues from the rapamycin moiety and alkyne/azide moiety both being connected on the C4 position of the coumarin ring. To overcome this issue, a new caging group 629 based on the DEACM caging group was designed. One of the ethyl groups on the amino group in DEACM was replaced with a propargyl group, which allows attaching
additional functional molecules, e.g. peptides. The photolysis properties of 629 under 405 nm UV light was explored by using a coumarin-caged acetic acid 629 (Scheme 10.11).

The synthesis of 629 commenced with 3-aminophenol (619). The amino group was protected with ethyl chloroformate to generate ethyl 3-hydroxyphenylcarbamate (620), which was reacted with ethyl 4-chloroacetoacetate (621) in methanesulfonic acid, assembling the coumarin ring 622 in excellent yield. The protecting group on the amino substrate was removed with acetic acid and sulfuric acid, delivering the 7-amino-4-chloromethylcoumarin 623. The chloride was converted to an OAc group under S\textsubscript{N}2 reaction condition, generating 624. Alkylation of the amino group with iodoethane produced 625, which underwent another alkylation with propargyl bromide to furnish 626. The acetate 626 underwent hydrolysis, delivering the caging group 629. The alkyne group of 626 was reacted with 3-azidopropan-1-ol under click reaction condition, producing triazole compound 627 (Scheme 10.11). As expected, upon irradiation at 405 nm, 627 (50 µM in pH 7.4 PBS buffer) decaged and released 628, as indicated by TLC.
Scheme 10.11. The synthesis and decaging of 627.

The synthesis of the coumarin caged rapamycin dimer 632 was accomplished using the similar way towards 590. The alcohol 629 was activated with DSC, delivering the NHS succinamide 630 in 58% yield. The NHS succinamide was reacted with rapamycin in the presence of the DMAP in DCM, producing the caged rapamycin 631. The caged rapamycin 632 underwent a double click reaction with 1,4-diazidobutane, furnishing the coumarin caged rapamycin dimer 632 in 83% yield (Scheme 10.12). The identity of purified 632 was
confirmed by $^1$H NMR. The coumarin caged rapamycin dimer 632 will be sent to collaborator to explore the photochemical control of protein functions.

10.3 Two-Photon Caged Rapamycin

As discussed in Chapter 9, single-photon caged molecules are photolyzed by UV light, usually at 365 nm. After successfully synthesizing 580 (caged by a single-photon caging group), we would like to apply the same approach to synthesize two-photon caged rapamycin analogs.

We first used a 6-bromo-7-hydroxycoumarin (Bhc) caging group. Bhc is an excellent two-photon caging group with a cross-section of $\delta_u \approx 1$. We started the synthesis of Bhc caged-rapamycin from 6-bromo-7-hydroxy-4-(hydroxymethyl)-2H-chromen-2-one (541, provided by Andrew McIver). The phenol group of Bhc (541) was protected with a TBS group, delivering protected Bhc 633 in 75% yield. However, the reaction of 633 with DSC was not successful. This was probably due to the poor solubility of 633 in most solvents.

![Scheme 10.13. Attempt of the synthesis of Bhc-caged rapamycin.](image)

The 8-bromo-7-hydroxyquinolinyl (BHQ) group is a caging group that can be efficiently photolyzed by one photon excitation (1 PE) at 365 nm or two-photon excitation (2 PE) at 740 nm. The synthesis of BHQ-caged rapamycin (640) commenced with 7-(triisopropylsilyloxy)quinoline-2-carbaldehyde (635, provided by Andrew McIver). The quinoline 635 was brominated with Br$_2$ to afford 8-bromo-7-(triisopropylsilyloxy)quinoline-
2-carbaldehyde (636), which was reduced to the alcohol 637. Activation of 637 with DSC produced the BHQ-NHS 638, which was reacted with rapamycin, delivering 639 in 32% yield. Upon removal of the TIPS group with KF, the BHQ-caged rapamycin 640 was obtained in 69% yield (Scheme 10.14).


The NDBF caging group (564) is also a good two-photon caging group, with a high extinction coefficient and quantum yield. The synthesis of NDBF-caged rapamycin 642 was accomplished in two steps from rapamycin (565). NDBF-OH (564, provided by Qingyang Liu) was reacted with DSC, delivering NDBF-DSC. Chemoselective acylation at
C-40 with NDBF-NHS (641) produced the NDBF-caged rapamycin 642 in 18% yield (Scheme 10.15). The photochemically control experiments for the BHQ-caged rapamycin 640 and the NDBF-caged rapamycin 642 are being performed by our collaborator Andrei V. Karginov in the Hahn lab at UNC.

Since the C16-methoxy group of rapamycin is readily undergoing nucleophilic substitution,\textsuperscript{379, 406} The synthesis C16-NDBF caged rapamycin 650 was carried out. The synthesis commenced with 4-fluoro-1-methyl-2-nitrobenzene (643). The compound 643 was
converted to 4-(2-iodophenoxy)-2-nitrobenzaldehyde (646) in two steps according to a literature procedure. The aldehyde 646 was reduced by NaBH₄ to provide the alcohol 647, which was protected with a TMS group, delivering the Heck reaction precursor 648 in 84% yield. A Heck reaction of 648 assembled the dibenzofuran rings and the TMS protecting group was removed under this condition, furnishing the caging group 649. The substitution reaction of rapamycin with NDBF-OH 649 was carried out using TFA and DCM. However, no desired product was obtained after column chromatography purification (Scheme 10.16).

Scheme 10.16. Attempt of the synthesis the C16-NDBF caged rapamycin 650.
10.4 Caged iRap

Rapamycin can inhibit cell growth. In order to alleviate this effect, chemical modifications on the structural motif that contacts FRB were performed.\textsuperscript{378} Caged C-16-iRap\textsuperscript{651} added steric bulk with a planar aromatic indole ring at C16, the position that directly opposes helix 4 of FRB, for specific interaction with FRB mutants. iRap\textsuperscript{651} was synthesized in one step from rapamycin via acid-mediated carbocation formation at C-16 and subsequent quenching with 3-methylindole, according to a literature procedure.\textsuperscript{378} Caged iRap analogs\textsuperscript{652} and\textsuperscript{653} were synthesized in one step from iRap\textsuperscript{651} via chemoselective acylation with MeNPOC-NHS (571) or NDBF-NHS (564) (Scheme 10.17). Several stereoisomers (arising from the stereocenters at C16 of iRap and the $\alpha$-methyl stereocenter of the caging group) of caged iRap are possible and were isolated as a mixture, making NMR interpretation difficult. Like caged rapamycin\textsuperscript{580}, the synthetic yields of the caged iRap were low. The bioactivity of caged iRap analogs is being investigated by our collaborator Andrei V. Karginov (UNC Chapel Hill).
Scheme 10.17. Synthesis of caged iRap 652 and 653.

10.5 Conclusion and Outlook

In summary, we have developed a new method to cage rapamycin at the C40-hydroxy position. Several caged rapamycin analogs were successfully synthesized, including single-photon caged rapamycins 580 and 590, two-photon caged rapamycins 640 and 642, as well as caged iRap 652 and 653. These caged rapamycin analogs were used in biological studies by collaborators. It was found that pRap together with an iFKBP (an engineered FKBP) domain was capable of photochemically controlling kinase activity. The dRap, on the other hand, was able to photochemically regulate DNA recombination in the split Cre system and TEV protease activity, using a regular FKBP domain.

Future work will focus on the synthesis of the modified rapamycin analogs C-20-methallylrapamycin (MaRap) 654 and C-16-butylsulfonamidorapamycin (C16-BS-Rap) 655 (Figure 10.9), which can potentially reduce the inhibitory effects of rapamycin on cell
growth, hence allow heterodimerization *in vivo*.\textsuperscript{378} The synthesis of MaRap 654 and C16-BS-Rap 655 could be accomplished according to literature procedures which require HPLC purification.\textsuperscript{408, 409} The caged 654 and caged 655 can be synthesized using the same approach as for pRap.

**Figure 10.9.** The structures of MaRap 654 and C16-BS-Rap 655.

10.6 Experimental

All reactions were performed in flame-dried glassware under a nitrogen atmosphere and stirred magnetically unless indicated. Chemicals were used directly from commercial sources without further purification unless indicated. Solvents were distilled and stored with molecular sieves (3 Å for methanol and ethanol and 4 Å for all other solvents) prior to use. Toluene, xylene, dioxane were distilled from sodium/benzophenone ketyl. TEA, DIPEA, DMSO, DMF, DCE, CH\textsubscript{3}CN and pyridine were distilled from calcium hydride. Methanol and ethanol were distilled from magnesium and iodoide. CH\textsubscript{2}Cl\textsubscript{2}, THF and ether were dried by MB SPS Compact solvent purification system. All other reagent quality solvents were used...
without further purification. $^1$H and $^{13}$C NMR spectra were performed using a Varian Mercury (300 MHz and 400 MHz). Mass spectra analysis was performed by North Carolina State University facilities.

**3-Methoxy-4-(prop-2-ynyloxy)benzaldehyde (573).** Vinillin (572, 2 g, 13.14 mmol) was dissolved in DMF (14 mL). K$_2$CO$_3$ (3.63 g, 26.29 mmol) was added, followed by the addition of propargyl bromide (2.93 mL, 26.29 mmol). The reaction mixture was stirred at room temperature overnight. H$_2$O (10 mL) was added and the mixture was extracted with ether (3 x 20 mL). The combined organic layers were washed with brine (10 mL), dried over Na$_2$SO$_4$, filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc (1:1) to give 570 (2.38 g, 95%) as a light yellow solid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 9.85 (s, 1H), 7.48-7.40 (m, 3H), 7.12 (d, $J$ = 8.1 Hz, 1H), 4.84 (t, $J$ = 3.2 Hz, 2H), 3.92 (s, 3H), 2.54 (t, $J$ = 2.4 Hz, 1H).

**5-Methoxy-2-nitro-4-(prop-2-ynyloxy)benzaldehyde (574).** HNO$_3$ (20 mL) was placed in a flask and cooled to 0 °C. The aldehyde 573 (0.91 g, 4.79 mmol) was added portionwise over 10 min. The temperature was warmed to room temperature and stirred at room temperature for 4 h. The reaction mixture was poured to ice-water (10 mL) and extracted with DCM (3 x 30 mL). The combined organic layers were washed with 1 N NaOH until pH ~9, followed by H$_2$O (10 mL) and brine (10 mL), dried over Na$_2$SO$_4$, filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with DCM to give 574 (1.03 g, 91%) as a yellow solid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$
10.44 (s, 1H), 7.78 (s, 1H), 7.42 (s, 1H), 5.06 – 4.65 (m, 3H), 4.01 (s, 3H), 2.62 (t, $J = 2.4$ Hz, 1H).

1-(5-Methoxy-2-nitro-4-(prop-2-ynylox)phenyl)ethanol (575). Aldehyde 574 (500 mg, 2.13 mmol) was dissolved in dry DCM (10 mL) under argon and cooled to 0 °C. Trimethylaluminum (2.0 M in hexans, 2.34 mL 4.68 mmol) was added dropwise over 30 min. The reaction mixture was stirred at 0 °C for 1.5 h. Ice-water was added until no bubble evolution. 1 N NaOH (10 mL) was added and the mixture was stirred at room temperature for 30 min and then extracted with DCM (3 x 20 mL). The combined organic layers were washed with 1 N NaOH (10 mL), H$_2$O (10 mL) and brine (10 mL), dried over Na$_2$SO$_4$, filtered and concentrated $in vacuo$. The residue was purified by flash chromatography on silica gel, eluting with hexanes/acetone (9:1) to give 575 (452 mg, 90%) as a yellow solid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.73 (s, 1H), 7.32 (s, 1H), 7.24 (s, 2H), 5.57 (qd, $J = 6.3$, 3.6 Hz, 1H), 4.81 (d, $J = 2.4$ Hz, 2H), 3.98 (s, 3H), 2.56 (t, $J = 2.4$ Hz, 1H), 2.23 (d, $J = 3.7$ Hz, 1H), 1.55 (d, $J = 6.2$ Hz, 3H).

1-(5-Nitrobenzo[d][1,3]dioxol-6-yl)ethyl 1H-imidazole-1-carboxylate (582). Alcohol 570 (100.0 mg, 0.474 mmol) was dissolved in DCM (4 mL). CDI (307.1 mg, 1.894 mmol) and DIPEA (0.17 mL, 0.947 mmol) were added. The reaction mixture was stirred at room temperature overnight. The reaction mixture was filtered and concentrated $in vacuo$. The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc (2:1) to give 582 as a yellow solid (132.6 mg, 92%). $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.13 (s, 1H), 7.51 (d, $J = 0.8$ Hz, 1H), 7.41 (s, 1H), 7.07 (s, 1H), 6.99 (s, 1H), 6.59 (q, $J = 6.5$ Hz, 1H), 6.13 (s, 1H), 6.11 (s, 1H), 1.78 (d, $J = 6.4$, 3H).
1-(5-Nitrobenzo[d][1,3]dioxol-6-yl)ethyl 4-nitroph enyl carbonate (583). Alcohol 570 (100 mg, 0.474 mmol) was dissolved in DCM (2.3 mL). 4-Nitrophenyl chloroformate (190 mg, 0.948 mmol) and pyridine (0.19 mL, 2.37 mmol) were added. The reaction mixture was stirred at room temperature overnight. DCM (15 mL) was added to dilute the reaction mixture and the mixture was washed with 1 N NaOH (2 x 3 mL) and brine (3 mL). The organic layer was dried over Na₂SO₄, filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc (2:1) to give a yellow solid. ¹H NMR showed impurity. The yellow solid was dissolved in DCM (10 mL), washed with 1 N NaOH (2 x 2 mL) and brine (2 mL), dried over Na₂SO₄, filtered and concentrated in vacuo to give 583 (133.3 mg, 75%) as pale yellow solid. ¹H NMR (300 MHz, CDCl₃) δ 8.29 – 8.18 (m, 2H), 7.50 (s, 1H), 7.42 – 7.29 (m, 2H), 7.11 (s, 1H), 6.40 (q, J = 6.4 Hz, 1H), 6.13 (s, 2H), 1.74 (d, J = 6.4 Hz, 3H).

8-Bromo-7-[(triisopropylsilyl)oxy]quinoline-2-carbaldehyde (636). 7-[(triisopropylsilyl)oxy]quinoline-2-carbaldehyde (635, 120.0 mg, 0.364 mmol) was dissolved in AcOH (3 mL). Br₂ (28 µL, 0.546 mmol) was added dropwise. The reaction mixture was stirred at room temperature overnight. DCM (6 mL) was added to dilute the reaction and the solution was transferred to a 125 mL beaker. Saturated NaHCO₃ was added until no bubbles were generated. The mixture was extracted with DCM (3 x 6 mL). The combined organic layers were washed with brine (5 mL), dried over Na₂SO₄, filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc (12:1) to give 636 (106.1 mg, 71%) as a pale yellow solid. ¹H NMR (300 MHz, CDCl₃) δ 10.28 (d, J = 0.8 Hz, 1H), 8.43 – 8.16 (d, J = 8.3 Hz, 1H), 7.93 (d, J = 8.3
Hz, 1H), 7.72 (d, \( J = 8.9 \text{ Hz}, 1\text{H} \)), 7.32 (d, \( J = 8.9 \text{ Hz}, 1\text{H} \)), 1.48 – 1.32 (m, 3H), 1.16 (d, \( J = 7.3 \text{ Hz}, 18\text{H} \)).

\{8-Bromo-7-[(triisopropylsilyl)oxy]quinolin-2-yl\}methanol (637). Aldehyde 636 (97 mg, 0.25 mmol) was dissolved in Ethanol (1.3 mL) and cooled to 0 °C and NaBH₄ (2.8 mg, 0.074 mmol) was added. The reaction mixture was stirred at 0 °C for 3 h. After warming to room temperature, the reaction mixture was concentrated in vacuo. H₂O (2 mL) was added and extracted with ethyl acetate (3 × 2 mL). The combined organic layers were washed with H₂O (5 mL) and brine (5 mL), dried over Na₂SO₄, filtered and concentrated in vacuo to give 637 (87 mg, 89%) as a pale yellow solid. \(^1\)H NMR (300 MHz, CDCl₃) δ 8.03 (d, \( J = 8.2 \text{ Hz}, 1\text{H} \)), 7.63 (d, \( J = 8.8 \text{ Hz}, 1\text{H} \)), 7.17 (dd, \( J = 8.6, 7.2 \text{ Hz}, 2\text{H} \)), 4.91 (d, \( J = 4.1 \text{ Hz}, 2\text{H} \)), 1.39 (dt, \( J = 14.9, 7.3 \text{ Hz}, 3\text{H} \)), 1.15 (d, \( J = 7.4 \text{ Hz}, 18\text{H} \)).

7-(Diethylamino)-4-(1-hydroxybut-3-en-1-yl)chromen-2-one (596). 7-(diethylamino)-2-oxochromene-4-carbaldehyde\(^{405}\) (592, 205 mg, 0.84 mmol) was dissolved in CH₃CN (3.3 mL) and H₂O (0.8 mL). Allyltributyl tin (0.41 mL, 1.257 mmol) and ZnCl₂ (171 mg, 1.257 mmol) were added. The reaction mixture was stirred at room temperature overnight. The reaction was quenched with sat. H₂O (4 mL) and extracted with DCM (3 × 5 mL). The combined organic layers were washed with brine (4 mL), dried over Na₂SO₄, filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc (3:2) to give 596 (234.0 mg, 97%) as a green solid. \(^1\)H NMR (300 MHz, CDCl₃) δ 7.40 (d, \( J = 9.0 \text{ Hz}, 1\text{H} \)), 6.61 (d, \( J = 9.1 \text{ Hz}, 1\text{H} \)), 6.54 (d, \( J = 2.5 \text{ Hz}, 1\text{H} \)), 6.25 (s, 1H), 5.99 – 5.74 (m, 1H), 5.24 (t, \( J = 1.2 \text{ Hz}, 1\text{H} \)), 5.20 (dp, \( J = 5.7\),
1.2 Hz, 1H), 5.00 (dt, \( J = 7.5, 3.5 \) Hz, 1H), 3.40 (q, \( J = 7.1 \) Hz, 4H), 2.79 – 2.58 (m, 1H), 2.55 – 2.34 (m, 1H), 2.24 – 2.09 (m, 1H), 1.19 (t, \( J = 7.1 \), 6H).

7-(Diethylamino)-4-(1,4-dihydroxybutyl)chromen-2-one (597). Alkyne 596 (24 mg, 0.084 mmol) was dissolved in THF (0.15 mL) and cooled to 0 °C. BH\(_3\)-THF (1M in THF, 125 \( \mu \)L, 0.125 mmol) was added dropwise. The reaction mixture was stirred at 0 °C for 3 h. 3 N NaOH (186 \( \mu \)L, 0.56 mmol) and H\(_2\)O\(_2\) (150 \( \mu \)L) were added. The reaction mixture was stirred at 0 °C for 2.5 h. H\(_2\)O (1 mL) was added to quench the reaction and extracted with EtOAc (3 x 2 mL). The combined organic layers were washed with brine (1 mL), dried over Na\(_2\)SO\(_4\), filtered and concentrated \textit{in vacuo}. The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc (1:2) to give 597 (16 mg, 61%) as a yellow solid. \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 7.40 (d, \( J = 9.0 \) Hz, 1H), 6.59 (s, 2H), 6.53 (s, 1H), 6.28 (s, 1H), 5.13 – 4.90 (m, 2H), 3.75 (td, \( J = 5.3, 1.8 \) Hz, 2H), 3.39 (q, \( J = 7.0 \) Hz, 4H), 2.15 – 1.97 (m, 1H), 1.80 (qd, \( J = 7.1, 4.5 \) Hz, 3H), 1.19 (t, \( J = 7.1 \) Hz, 6H).

4-[7-(Diethylamino)-2-oxochromen-4-yl]-4-hydroxybutyl methanesulfonate (598). The alcohol 597 (180 mg, 0.59 mmol) was dissolved in DCM (14 mL) and cooled to 0 °C. TEA (82 \( \mu \)L, 0.59 mmol) was added followed by slow addition of MsCl (48 \( \mu \)L, 0.59 mmol). The reaction mixture was stirred at 0 °C for 2 h. The reaction was quenched with H\(_2\)O (2 mL) and extracted with DCM (3 x 3 mL). The combined organic layers were washed with brine (3 mL), dried over Na\(_2\)SO\(_4\), filtered and concentrated \textit{in vacuo}. The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc (1:2, 1:3, 0:1) to give 598 (171 mg, 75%) as a yellow solid. \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 7.41 (d, \( J = 9.1 \) Hz, 1H), 6.63 (s, 1H), 6.56 (s, 1H), 6.27 (s, 1H), 5.05 (d, \( J = 8.1 \) Hz, 1H), 4.45 – 4.18 (m, 2H),
3.40 (q, $J = 7.1$ Hz, 4H), 2.99 (s, 3H), 2.15 (s, 1H), 1.99 (q, $J = 6.2$, 5.4 Hz, 3H), 1.81 (q, $J = 7.6$, 6.9 Hz, 1H), 1.19 (t, $J = 7.1$ Hz, 6H).

7-(Diethylamino)-4-[1-hydroxy-4-(2$\$^[4]-triaza-1,2-dien-1-yl)butyl]chromen-2-one (599). The mesylate 598 (169 mg, 0.44 mmol) was dissolved in DMF (3 mL). NaN$_3$ (71 mg, 1.10 mmol) was added. The reaction mixture was stirred at 70 °C for 1.5 h. After cooling to rt, H$_2$O (2 mL) was added to quench the reaction and extracted with ether (3 x 5 mL). The combined organic layers were washed with brine (3 mL), dried over Na$_2$SO$_4$, filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with hexanes/ EtOAc (1:1) to give 599 (121 mg, 83%) as a yellow solid. $^1$H NMR (300 MHz, CDCl$_3$) δ 7.41 (, $J = 9.1$ Hz, 1H), 6.60 (s, 1H), 6.52 (s, 1H), 6.24 (s, 1H), 5.00 (s, 1H), 4.10 (q, $J = 7.4$ Hz, 1H), 3.39 (q, $J = 6.8$, 6.3 Hz, 6H), 2.25 (s, 1H), 2.03 (d, $J = 2.1$ Hz, 1H), 1.96 (s, 0H), 1.81 (q, $J = 7.2$, 6.5 Hz, 2H), 1.33 – 1.13 (m, 6H).

4-{1-[([tert-Butyldimethylsilyl)oxy]but-3-en-1-yl}-7-(diethylamino)chromen-2-one (603). Alcohol 596 (550 mg, 1.91 mmol) was dissolved in DMF (1 mL). TBDMSCl (346 mg, 2.30 mmol) and imidazole (169 mg, 2.49 mmol) were added. The reaction mixture was stirred at room temperature overnight. The reaction was quenched with H$_2$O (4 mL) and extracted with ether (3 x 5 mL). The combined organic layers were washed with brine (5 mL), dried over Na$_2$SO$_4$, filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with hexanes/ EtOAc (4:1, 1:1) to give 596 (749 mg, 98%) as a orange solid. $^1$H NMR (300 MHz, CDCl$_3$) δ 7.46 (dd, $J = 9.0$, 1.6 Hz, 1H), 6.59 (d, $J = 9.3$ Hz, 1H), 6.52 (d, $J = 2.5$ Hz, 1H), 6.17 (d, $J = 1.6$ Hz, 1H), 5.81 (ddt, $J = 17.3$, 10.4, 7.1 Hz, 1H), 5.13 – 4.96 (m, 2H), 4.87 (dd, $J = 7.0$, 4.6 Hz, 1H), 4.10 (q, $J = 7.2$
Hz, 0H), 3.39 (q, J = 7.1 Hz, 4H), 2.61 – 2.36 (m, 2H), 1.19 (td, J = 7.1, 1.5 Hz, 6H), 0.89 (d, J = 1.7 Hz, 9H), 0.06 (s, 3H), -0.05 (s, 3H).

**Ethyl 3-hydroxyphenylcarbamate (620).** 3-aminophenol (619, 3.0 g, 27.50 mmol) was dissolved in THF (3 mL) and pyridine (2.7 mL) and cooled to 0 °C. Ethyl chloroformate (2.6 mL 27.50 mmol) was added dropwise. The reaction mixture was stirred at 0 °C for 20 h. The reaction was quenched with H$_2$O (15 mL) and extracted with DCM (3 x 10 mL). The combined organic layers were washed with brine (5 mL), dried over Na$_2$SO$_4$, filtered and concentrated *in vacuo*. Toluene (3 mL) was added to the residue and cooled to 0 °C for 1 h. The white precipitate was filtered, washed with cold toluene, dried *in vacuo* to give 620 (3.350 g, 67%) as white solid. $^1$H NMR (300 MHz, DMSO-d$_6$) $\delta$ 10.19 (s, 1H), 7.74 (d, J = 8.8 Hz, 1H), 7.58 (d, J = 2.1 Hz, 1H), 7.40 (dd, J = 8.8, 2.1 Hz, 1H), 6.50 (s, 1H), 4.96 (s, 2H), 4.16 (q, J = 7.1 Hz, 2H), 1.25 (t, J = 7.1 Hz, 3H).

**Ethyl 4-(chloromethyl)-2-oxo-2H-chromen-7-ylcarbamate (622).** The phenol 620 (250 mg, 1.38 mmol) was dissolved in methanesulfonic acid (5 mL). Ethyl 4-chloroacetoacetate (621, 0.26 mL, 1.93 mmol) was added. The reaction was stirred at room temperature for 19 h. The reaction mixture was poured to ice-H$_2$O (10 mL) and the formed precipitate was filtered, washed with ice-H$_2$O, dried *in vacuo* to give 622 (365 mg, 92%) as grey solid. $^1$H NMR (300 MHz, DMSO-d$_6$) $\delta$ 7.47 (d, J = 8.7 Hz, 1H), 6.57 (d, J = 8.7 Hz, 1H), 6.43 (d, J = 2.1 Hz, 1H), 6.23 (s, 2H), 6.17 (s, 1H), 4.86 (d, J = 0.8 Hz, 2H).

**7-Amino-4-chloromethylcoumarin (623).** The carbamate 622 (300 mg, 1.07 mmol) was suspended in a mixture of concentrated H$_2$SO$_4$ (0.9 mL) and glacial acetic acid (0.9 mL). The suspension was heated to 125 °C for 2 h. After cooling to room temperature, the brown
solution was poured into ice-H$_2$O (30 mL), neutralized with 1N NaOH till pH ~8. The formed precipitate was filtered, washed with ice-H$_2$O, dried \textit{in vacuo} to give 623 (149 mg, 67\%) as grey solid. $^1$H NMR (300 MHz, DMSO-d$_6$) $\delta$ 7.46 (d, $J = 8.7$ Hz, 1H), 6.57 (dd, $J = 8.7, 2.1$ Hz, 1H), 6.42 (d, $J = 2.1$ Hz, 1H), 6.26 – 6.13 (m, 3H), 4.86 (d, $J = 0.8$ Hz, 2H).

**{(7-Amino-2-oxo-2H-chromen-4-yl)methyl acetate (624).}** The chloride 623 (99 mg, 0.47 mmol) was dissolved in DMF (04. mL). KOAc (55.7 mg, 0.57 mmol) and TBAB (10 mg, catalytic amount) were added. The reaction mixture was stirred at 50 °C for 20 h. After cooling to room temperature, the reaction mixture was poured into H$_2$O (3 mL), cooled to 0 °C. The formed precipitate was filtered, washed with ice-H$_2$O, dried \textit{in vacuo} to give 624 (98 mg, 89\%) as grey solid. $^1$H NMR (300 MHz, DMSO-d$_6$) $\delta$ 7.35 (d, $J = 8.7$ Hz, 1H), 6.54 (dd, $J = 8.6, 2.2$ Hz, 1H), 6.42 (d, $J = 2.1$ Hz, 1H), 6.20 (s, 2H), 5.95 (d, $J = 1.3$ Hz, 1H), 5.22 (d, $J = 1.3$ Hz, 2H), 2.15 (s, 3H).

**{(7-(Ethylamino)-2-oxo-2H-chromen-4-yl)methyl acetate (625).}** The amine 624 (50 mg, 0.22 mmol) was dissolved in DMF (1.2 mL). K$_2$CO$_3$ (30 mg, 0.22 mmol) was added. The reaction mixture was stirred at room temperature for 15 min. Iodoethane (69 µL, 0.86 mmol) was added. The reaction mixture was stirred at 75 °C for 20 h. After cooling to room temperature, the reaction mixture was quenched with H$_2$O (1 mL) and extracted with EtOAc (3 x 2 mL). The combined organic layers were washed with brine (2 mL), dried over Na$_2$SO$_4$, filtered and concentrated \textit{in vacuo}. The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc (1:1) to give 625 (27 mg, 49\%) as a pale solid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.37 (d, $J = 1.5$ Hz, 1H), 6.55 – 6.37 (m, 2H), 6.12 (t, $J = 1.3$ Hz,
(7-(N-ethyl-N-(prop-2-ynyl)amino)-2-oxo-2H-chromen-4-yl)methyl acetate (626).

The amine 625 (27 mg, 0.10 mmol) was dissolved in DMF (0.6 mL). K₂CO₃ (14 mg, 0.10 mmol) was added. The reaction mixture was stirred at room temperature for 15 min. Propargyl bromide (80% in toluene, 46 µL, 0.42 mmol) was added. The reaction mixture was stirred at 75 °C for 20 h. After cooling to room temperature, the reaction mixture was quenched with H₂O (1 mL) and extracted with EtOAc (3 x 3 mL). The combined organic layers were washed with brine (2 mL), dried over Na₂SO₄, filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc (2:1) to give 626 (14 mg, 44%) as a yellow solid. ¹H NMR (300 MHz, CDCl₃) δ 7.32 (d, J = 8.8 Hz, 1H), 6.71 (d, J = 1.9 Hz, 0H), 6.66 (dd, J = 2.6 Hz, 8.8 Hz, 1H), 6.18 (d, J = 1.3 Hz, 1H), 5.21 (d, J = 1.3 Hz, 2H), 4.06 (d, J = 2.4 Hz, 2H), 3.51 (q, J = 7.1 Hz, 2H), 2.22 (t, J = 2.4 Hz, 1H), 2.17 (s, 3H), 1.24 (t, J = 7.1 Hz, 3H).

(7-(N-ethyl-N-((1-(3-hydroxypropyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-2-oxo-2H-chromen-4-yl)methyl acetate (627). The alkyne 626 (6.2 mg, 0.022 mmol), 3-azidopropan-1-ol (4.2 mg, 0.043 mmol), Na ascorbate (5.0 mg, 0.025 mmol) and CuSO₄-5H₂O (1.6 mg, 0.0065 mmol) were dissolved in H₂O (500 µL), t-BuOH (500 µL) and DCM (500 µL). The reaction mixture was heated in 40 °C for 24 h. After cooling to room temperature, the reaction mixture was concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with DCM/acetone (2:1) to give 627 (6.0 mg, 73%) as a white solid.
7-(N-ethyl-N-(prop-2-ynyl)amino)-4-(hydroxymethyl)-2H-chromen-2-one (629).

The ester 627 (165 mg, 0.55 mmol) was dissolved in MeOH (6.9 mL). K$_2$CO$_3$ (84 mg, 0.61 mmol) was added. The reaction mixture was stirred at rt overnight. The reaction mixture was concentrated in vacuo. The residue was dissolved in H$_2$O (2 mL) and extracted with EtOAc (3 x 5 mL). The combined organic layers were washed with brine (3 mL), dried over Na$_2$SO$_4$, filtered and the filtrate was concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with DCM/acetone (20:1) to give 627 (45 mg, 31%) as a yellow solid. $^1$H NMR (300 MHz, DMSO-d$_6$) $\delta$ 7.48 (d, $J$ = 8.9 Hz, 1H), 6.77 (dd, $J$ = 8.9, 2.6 Hz, 1H), 6.66 (d, $J$ = 2.5 Hz, 1H), 6.12 (d, $J$ = 1.1 Hz, 1H), 5.53 (t, $J$ = 5.6 Hz, 1H), 4.68 (d, $J$ = 5.6 Hz, 2H), 4.21 (d, $J$ = 2.4 Hz, 2H), 3.51 (q, $J$ = 6.7 Hz, 3H), 3.17 (t, $J$ = 2.4 Hz, 1H), 1.18 – 1.08 (m, 3H).

General procedure for synthesizing NHS esters. The alcohol (570, 575, 637, 564 or 629) (50 mg – 2 g scale, 1 eq) was dissolved in dry CH$_3$CN (0.2 M). To the solution were added $N,N'$-disuccinimidyl carbonate (2 eq) and TEA (3 eq). The reaction was stirred at room temperature overnight and the solvent was concentrated in vacuo. The residue was directly purified by column chromatography on SiO$_2$, eluting with hexanes/EtOAc or DCM/acetone to give the desired caging group 571, 576, 638, 641 or 630.

α-Methyl-2-nitropiperonyl succinimidyl carbonate (NPOC-NHS, 571). The compound was eluted with hexanes/EtOAc (5:1) to give 571 in 89% yield as a light yellow solid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.49 (s, 1H), 7.09 (s, 1H), 6.39 (q, $J$ = 6.4 Hz, 1H), 6.14-6.12 (m, 2H), 2.79 (s, 4H), 1.73 (d, $J$ = 6.4 Hz); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 168.7,
153.0, 150.8, 148.0, 141.6, 133.2, 105.9, 105.6, 103.5, 76.5, 25.6, 22.3; MS calcd C_{14}H_{12}N_{2}NaO_{9} 375.04405, found 375.1.

2-[1-([(2,5-Dioxopyrrolidin-1-yl)oxy]carbonyl)oxyethyl]-4-methoxy-5-(prop-2-yloxy)phenyl]azinic acid (576). The compound was eluted with DCM/EtOAc (20:1, 10:1) to give 576 in 83% yield as a light yellow solid. $^{1}$H NMR (300 MHz, CDCl$_3$) δ 7.81 (s, 1H), 7.08 (s, 1H), 6.50 (q, $J$ = 6.4 Hz, 1H), 4.85 – 4.75 (m, 2H), 4.04 (s, 3H), 2.78 (s, 4H), 1.75 (d, $J$ = 6.4 Hz, 3H).

{8-Bromo-7-[(triisopropylsilyl)oxy]quinolin-2-yl}methyl 2,5-dioxopyrrolidin-1-yl carbonate (638). The compound was eluted with hexanes/EtOAc (3:1, 1:1) to give 638 in 67% yield as a light yellow solid. $^{1}$H NMR (300 MHz, CDCl$_3$) δ 8.13 (d, $J$ = 8.4 Hz, 1H), 7.64 (d, $J$ = 8.9 Hz, 1H), 7.44 (d, $J$ = 8.3 Hz, 1H), 7.21 (t, $J$ = 7.0 Hz, 3H), 5.66 (s, 2H), 2.84 (s, 4H), 1.47 – 1.28 (m, 3H), 1.15 (d, $J$ = 7.3 Hz, 18H).

4-[1-([(2,5-Dioxopyrrolidin-1-yl)oxy]carbonyl)oxyethyl]-8-oxatricyclo[7.4.0.0^{2,7}]trideca-1(13),2(7),3,5,9,11-hexaen-5-ylazinic acid (641). The compound was eluted with hexanes/EtOAc (2:1, 1:1) to give 641 in 69% yield as a light yellow solid. $^{1}$H NMR (300 MHz, CDCl$_3$) δ 8.27 (d, $J$ = 2.4 Hz, 2H), 8.14 – 8.06 (m, 1H), 7.68 – 7.55 (m, 2H), 7.45 (ddd, $J$ = 7.8, 6.7, 1.6 Hz, 1H), 6.54 (dt, $J$ = 6.6, 6.1 Hz, 1H), 2.78 (s, 4H), 1.87 (d, $J$ = 6.4 Hz, 3H).

2,5-Dioxopyrrolidin-1-yl 7-[ethyl(prop-2-yn-1-yl)amino]-2-oxo-2H-chromen-4-yl)methyl carbonate (630). The compound was eluted with DCM/acetone (20:1) to deliver 630 as a yellow solid in 58% yield. $^{1}$H NMR (300 MHz, CDCl$_3$) δ 7.32 (d, $J$ = 9.0 Hz, 1H), 6.86 – 6.67 (m, 2H), 6.28 (s, 1H), 5.42 (dd, $J$ = 8.0, 1.2 Hz, 2H), 4.08 (d, $J$ = 2.6 Hz, 2H),
3.63 – 3.48 (m, 2H), 2.85 (d, \( J = 1.8 \) Hz, 4H), 2.25 (t, \( J = 2.4 \) Hz, 1H), 1.26 (t, \( J = 7.1 \) Hz, 3H).

**But-3-yn-1-yl-4-([tert-butyldimethylsilyl]oxy)-4-[7-(diethylamino)-2-oxochromen-4-yl]butyl carbonate (607).** 3-Butyne-1-ol (20 \( \mu \)L, 0.27 mmol) was dissolved in DCM (1 mL). Coumarin-NHS 606 (50 mg, 0.089 mmol) and DMAP (22 mg, 0.18 mmol) were added. The reaction mixture was stirred at room temperature overnight. The solvent was concentrated *in vacuo*. The residue was directly purified by column chromatography on SiO\(_2\), eluting with hexanes/EtOA (2:1) to give 606 (40 mg, 88%) as a light yellow solid.

**4-([tert-Butyldimethylsilyl]oxy)-4-[7-(diethylamino)-2-oxochromen-4-yl]butyl N-(prop-2-yn-1-yl)carbamate (608).** Coumarin-NHS (606, 300 mg, 0.54 mmol) was dissolved in DMF (2.7 mL). Propargylamine (41 \( \mu \)L, 0.64 mmol) was added. The reaction mixture was stirred at room temperature for 30 min. The reaction was quenched with H\(_2\)O (3 mL) and extracted with ether (3 x 6 mL). The combined organic layers were washed with brine (5 mL), dried over Na\(_2\)SO\(_4\), filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc (1:1) to give 608 (227 mg, 85%) as a yellow solid.

**General procedure for removing a TBDMS protecting group.** The silyl ether 607, 608 and 609 (20 – 50 mg scale, 1 eq) was dissolved in THF (0.1 M) and cooled to 0 °C. TBAF (1 M in THF, 1.5 eq) was added dropwise. The reaction mixture was stirred at 0 °C for 1 h. The reaction was quenched with H\(_2\)O and extracted with ether. The combined organic layers were washed with brine, dried over Na\(_2\)SO\(_4\), filtered and concentrated *in vacuo*. 246
The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc to give 610, 611 and 612.

**4-(4-[(But-3-yn-1-yloxy)carbonyl]oxy)-1-hydroxybutyl)-7-(diethylamino)-2H-chromen-2-one (610).** The compound was eluted with Hex/EtOAc (1:3) to deliver 610 as a light yellow solid in 82% yield. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.38 (d, $J = 9.0$ Hz, 1H), 6.54 (d, $J = 9.0$ Hz, 1H), 6.43 (d, $J = 2.6$ Hz, 1H), 6.21 (d, $J = 0.8$ Hz, 1H), 5.14 – 4.84 (m, 1H), 4.30 – 4.11 (m, 4H), 3.37 (q, $J = 7.1$ Hz, 4H), 2.84 (s, 1H), 2.53 (td, $J = 6.9$, 2.7 Hz, 2H), 2.02 – 1.66 (m, 5H), 1.17 (t, $J = 7.0$ Hz, 6H).

**4-[7-(Diethylamino)-2-oxo-2H-chromen-4-yl]-4-hydroxybutyl $N$-(prop-2-yn-1-yl)carbamate (611).** The compound was eluted with Hex/EtOAc (1:3) to deliver 611 as a light yellow solid in 82% yield. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.39 (d, $J = 9.0$ Hz, 1H), 6.71 – 6.56 (m, 1H), 6.52 (d, $J = 2.6$ Hz, 1H), 6.25 (d, $J = 0.9$ Hz, 1H), 4.99 (s, 1H), 4.88 (s, 1H), 4.15 (m, $J = 5.8$ Hz, 2H), 3.95 (t, $J = 2.6$ Hz, 1H), 1.98 – 1.71 (m, 4H), 1.19 (td, $J = 7.0$, 1.0 Hz, 6H).

**4-[7-(Diethylamino)-2-oxo-2H-chromen-4-yl]-4-hydroxybutyl $N$-benzyl-$N$-(prop-2-yn-1-yl)carbamate (612).** The compound was eluted with Hex/EtOAc (1:2) to give 612 as a light yellow solid in 92% yield. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.25-7.08 (m, 6H), 6.57 – 6.30 (m, 2H), 6.19 (d, $J = 2.6$ Hz, 1H), 4.95 (br, 1H), 4.52 (s, 2H), 4.21 (s, 2H), 4.10-3.81 (m, $J = 5.8$ Hz, 2H), 3.38 (q, $J = 7.1$ Hz, 4H), 2.20 (t, $J = 2.6$ Hz, 1H), 1.98 – 1.71 (m, 4H), 1.20 (td, $J = 7.0$, 1.0 Hz, 6H).

**General procedure for synthesizing caged rapamycin.** Rapamycin (565, 20 mg, 1 eq) was dissolved in dry DCM (0.05 M). Caging-NHS (5 eq) and DMAP (2 eq) were added.
The reaction mixture was stirred at room temperature for 24 h. The reaction mixture was concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with DCM/EtOAc mixture.

**NPOC-caged rapamycin (pRap, 580).** The compound was eluted with DCM/EtOAc (10:1, 5:1, 2:1, 1:1) to give 580 as a light yellow solid in 36% yield. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.48 (s, 1H), 7.05 (s, 1H), 6.44-6.03 (m, 7H), 5.94-5.83 (m, 1H), 5.60-5.47 (m, 1H), 5.43 (m, 1H), 5.38-5.31 (m, 1H), 5.20-5.08 (m, 1H), 4.50-4.38 (m, 1H), 4.14-4.09 (m, 2H), 3.98-3.73 (m, 2H), 3.67-3.55 (m, 2H), 3.35-3.31 (m, 7H), 3.13-3.03 (m, 5H), 2.87-2.50 (m, 3H), 2.40-2.11 (m, 2H), 2.03-1.52 (m, 15H), 1.47-1.32 (m, 6H), 1.24-0.86 (m, 24H); MS calcd for [M + Na]$^+$ C$_{61}$H$_{86}$N$_2$NaO$_{19}$ 1173.5723, found 1173.7.

**1-(5-Methoxy-2-nitro-4-prop-2-ynyloxyphenyl) ethanol-caged rapamycin (NMPN-Rap, 581).** The compound was eluted with DCM/ethyl acetate (10:1, 5:1, 2:1, 1:1) to give 581 as a light yellow solid in 45% yield. $^1$H NMR (300 MHz, CDCl$_3$) δ 7.75 (s, br, 1H), 7.07 (d, 1H), 6.36-6.21 (m, 3H), 6.17-6.04 (m, 1H), 5.95-5.84 (m, 1H), 5.54-5.36 (m, 2H), 5.25 (d, $J$ = 5.4 Hz), 5.17-5.07 (m, 2H), 4.79-4.67 (m, 3H), 4.60-4.15 (m, 2H), 3.94 (s, 3H), 3.90-3.51 (m, 3H), 3.47-3.22 (m, 8H), 3.18-3.03 (m, 5H), 2.87-2.50 (m, 3H), 2.38-2.11 (m, 1H), 2.03-1.63 (m, 12H), 1.61-1.32 (m, 12H), 1.24-0.86 (m, 27H); MS calcd for [M + Na]$^+$ C$_{64}$H$_{90}$N$_2$NaO$_{19}$ 1213.60, found 1213.60.

**TIPS-BHQ caged rapamycin 639.** The compound was eluted with DCM/ethyl acetate (10:1, 5:1, 2:1, 1:1) to give 639 as a white solid in 32% yield. $^1$H NMR (300 MHz, CDCl$_3$) δ 8.07 (d, $J$ = 8.4 Hz, 1H), 7.61 (d, $J$ = 8.8 Hz, 1H), 7.40 (d, $J$ = 8.3 Hz, 1H), 7.20 (d, $J$ = 8.3 Hz, 1H), 6.48 – 5.79 (m, 6H), 5.65 – 5.02 (m, 6H), 4.79 (s, 1H), 4.68 – 4.20 (m, 1H),...
4.22 – 4.06 (m, 1H), 3.92 – 3.48 (m, 6H), 3.44 – 3.14 (m, 8H), 3.11-2.98 (m, 4H), 2.80-2.42 (m, 4H), 2.39-05 (m, 5H), 1.92-1.60 (m, 10H), 1.59 – 1.17 (m, 10H), 1.14 (d, J = 7.4 Hz, 18H), 1.10 – 0.53 (m, 22H). MS calcd for [M + Na]⁺ C₇₁H₁₀₅BrN₂NaO₁₆Si 1371.6 found 1373.7.

**BHQ-caged rapamycin 640.** Caged reapamycin 639 (8.9 mg, 0.007 mmol) was dissolved in dry MeOH (400 µL) and DCM (40 µL). KF (1.0 mg, 0.017 mmol) was added. The reaction mixture was stirred at rt for 1 h. The reaction mixture was concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with DCM/EtOAc (4:1) to give 640 (5.4 mg, 69%) as a white solid. ¹H NMR (300 MHz, CDCl₃) δ 8.09 (d, J = 8.4 Hz, 1H), 7.68 (d, J = 8.8 Hz, 1H), 7.41 (dd, J = 8.4, 4.3 Hz, 1H), 7.30 (d, J = 8.9 Hz, 1H), 6.49 – 5.74 (m, 5H), 5.61 – 5.32 (m, 3H), 5.21 (dd, J = 34.6, 5.3 Hz, 3H), 4.77 (s, 1H), 4.58 (td, J = 10.3, 4.9 Hz, 1H), 4.46 – 4.13 (m, 1H), 3.80 (d, J = 23.5 Hz, 1H), 3.73 – 3.48 (m, 2H), 3.46 – 3.14 (m, 8H), 3.11 (s, 4H), 2.84 – 2.44 (m, 3H), 2.40-2.09 (m, 3H), 2.10 (s, 1H), 2.00 – 1.65 (m, 9H), 1.62 – 1.32 (m, 12H), 1.32 – 0.63 (m, 24H). MS calcd for [M + Na]⁺ C₆₁H₈₆BrN₂NaO₁₆ 1215.5, found 1215.5.

**PEGylated rapamycin 588.** NMPN-caged rapamycin (581, 7.2 mg, 0.0061 mmol), PEG-N₃ (3.5 mg, 0.00605 mmol), Cu₂SO₄·5H₂O (100 µL of 2 mg/ml aqueous solution, 0.0006 mmol), Na ascorbate (1.4 mg, 0.00696 mmol) and tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA) (1.6 mg, 0.00302 mmol) were added to t-BuOH (100 µL) and DCM (100 µL). The reaction mixture was heated in 40 °C for 24 h. After cooling to room temperature, the reaction mixture was concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with EtOAc/MeOH (1:0, 50:1, 20:1, 10:1) to give
588 (3.8 mg, 36%) as a white solid. \( ^1\text{H NMR} (300 \text{ MHz, CDCl}_3) \delta 7.87 \text{ (s, 1H)}, 7.78 \text{ (d, } J = 3.7 \text{ Hz, 1H}), 7.05 \text{ (d, } J = 3.8 \text{ Hz, 1H}), 6.47 \text{ – 6.26 (m, 3H)}, 6.11 \text{ (t, } J = 12.4 \text{ Hz, 1H}), 5.93 \text{ (d, } J = 10.5 \text{ Hz, 1H}), 5.44 \text{ (dd, } J = 37.9, 13.0 \text{ Hz, 1H}), 5.27 \text{ (s, 3H)}, 5.17-5.09 \text{ (s, 1H)}, 4.77 \text{ (s, 1H)}, 4.54 \text{ (t, } J = 4.9 \text{ Hz, 2H)}, 4.42 \text{ (s, 1H)}, 4.14 \text{ (d, } J = 6.1 \text{ Hz, 1H}), 4.02 \text{ – 3.78 (m, 7H)}, 3.61 \text{ (p, } J = 4.2 \text{ Hz, 55H}), 3.52 \text{ (dd, } J = 5.7, 3.2 \text{ Hz, 2H}), 3.44 \text{ – 3.31 (m, 6H)}, 3.32 \text{ – 3.20 (m, 2H)}, 3.20 \text{ – 2.98 (m, 3H)}, 2.93 \text{ – 2.48 (m, 1H)}, 2.30 \text{ (d, } J = 13.6 \text{ Hz, 2H}), 1.97 \text{ (d, } J = 32.2 \text{ Hz, 4H}), 1.84 \text{ – 1.50 (m, 13H)}, 1.23 \text{ (s, 5H)}, 1.18-0.62 \text{ (m, 28H)}.

\[2-(1-{[(40-Hydroxyl-rapamycin)carbonyl]oxy}ethyl)-5-{[1-(4-{4-[4-(1-[(cyclohexyloxy)carbonyl]oxy}ethyl)-2-hydroxy-5-(hydroxynitroso)phenoxymethyl]-1H-1,2,3-triazol-1-yl}butyl)-1H-1,2,3-triazol-4-yl]methoxy}-4-hydroxyphenyl]azinic acid (NMPN-caged rapamycin dimer, 590).\] The 1-(5-Methoxy-2-nitro-4-prop-2-ynyloxyphenyl) ethanol-caged rapamycin (581, 28 mg, 0.024 mmol) and 1,4-diazidobutane (1.0 mg, 0.071 mmol) were dissolved in H\(_2\)O (0.5 ml), t-BuOH (0.5 ml) and DCM (0.5 ml) in a vial. CuSO\(_4\)-5H\(_2\)O (0.5 mg, .0019 mmol), TBTA (3.8 mg, 0.0071 mmol) and sodium ascorbate (4.3 mg, 0.021 mmol) were added to the solution. The reaction mixture was heated at 40 °C for 24 h. The solvents were concentrated under reduced pressure and the resulting residue was re-dissolved in small amount of DCM and MeOH and purified by column chromatography on SiO\(_2\) (eluted with DCM/ethyl acetate 1:2, 5% MeOH in EtOAc), delivering 15 mg (85% yield) of the rapamycin dimer 590. \( ^1\text{H NMR} (300 \text{ MHz, CDCl}_3) \delta 7.74 \text{ (s, br, 2H)}, 7.63 \text{ (s, br, 2H)}, 7.06 \text{ (d, } J = 3.0 \text{ Hz, 2H}), 6.40-6.37 \text{ (m, 6H)}, 6.35-6.10 \text{ (m, 2H)}, 5.95-5.82 \text{ (m, 2H)}, 5.52 \text{ (dd, } J = 8.7 \text{ Hz and } J = 15.3 \text{ Hz, 2H}), 5.37 \text{ (d, } J = 9.9 \text{ Hz, 2H)}, 5.29-5.24 \text{ (m, 6H)}, 5.20-5.08 \text{ (m, 2H)}, 4.74 \text{ (s, 2H)}, 4.43-4.31 \text{ (m, 6H)}, 4.28-4.18 \text{ (m, 2H)}, \]
3.92 (s, 6H), 3.88-3.52 (m, 8H), 3.41-3.20 (m, 18H), 3.19-3.02 (m, 10H), 2.72-2.63 (m, 4H), 2.59-2.48 (m, 2H), 2.40-2.19 (m, 4H), 1.94 (s, br, 10H), 1.83-1.62 (m, 24H), 1.58-1.30 (m, 22H), 1.17-0.65 (m, 44H). HRMS calcd for [M + Na] C₁₃₂H₁₈₈N₁₀NaO₃₈ 2544.2984, found 2545.3106.

(4-(2-Iodophenoxy)-2-nitrophenyl)methanol (647). 4-(2-Iodophenoxy)-2-nitrobenzaldehyde³⁵³ (369 mg, 1.0 mmol) was dissolved in MeOH (5.6 mL) and cooled to 0 °C. NaN₃ (38 mg, 1.0 mmol) was added. The reaction was stirred at 0 °C for 30 min. Ice-H₂O was added to quench the reaction and the reaction mixture was extracted with ether (3 x 10 mL). The combined organic layers were washed with brine (5 mL), dried over Na₂SO₄, filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc (2:1, 1:1) to give 647 (345 mg, 93%) as a yellow solid. ¹H NMR (300 MHz, CDCl₃) δ 7.89 (dd, J = 7.8, 1.5 Hz, 1H), 7.65 (d, J = 8.5 Hz, 1H), 7.59 (d, J = 2.6 Hz, 1H), 7.37 (dddd, J = 8.1, 7.3, 1.6, 0.6 Hz, 1H), 7.20 (dd, J = 8.5, 2.7 Hz, 1H), 7.06 – 6.93 (m, 2H), 4.90 (d, J = 6.6 Hz, 2H), 2.58 – 2.44 (m, 1H).

(4-(2-Iodophenoxy)-2-nitrobenzyloxy)trimethylsilane (648). Alcohol 647 (340 mg, 0.92 mmol) was dissolved in DCM (3 mL).TMSCl (174 µL, 1.37 mmol), TEA (256 µL, 1.83 mmol) and DMAP (22 mg, 0.18 mmol) were added. The reaction mixture was stirred at rt overnight. Saturated NH₄Cl (10 mL) was added to quench the reaction and the reaction mixture was extracted with DCM (3 x 10 mL). The combined organic layers were washed with NH₄Cl (5 mL), dried over Na₂SO₄, filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc (4:1) to give 648 (340 mg, 84%) as a light yellow solid. ¹H NMR (300 MHz, CDCl₃) δ 7.88 (dd, J = 7.7, 1.6
Hz, 1H), 7.81 (dt, J = 8.7, 1.0 Hz, 1H), 7.60 (d, J = 2.6 Hz, 1H), 7.35 (ddd, J = 8.2, 7.2, 1.5 Hz, 1H), 7.01 – 6.90 (m, 2H), 5.00 (d, J = 0.8 Hz, 2H), 0.17 (s, 9H).

[4-(hydroxymethyl)-8-oxatricyclo[7.4.0.0^{2,7}]trideca-1(13),2(7),3,5,9,11-
hexaen-5-yl]azinic acid (649). The iodide 648 (200 mg, 0.45 mmol) was dissolved in DMA (4 mL). Pd(OAc)$_2$ (10.1 mg, 0.045 mmol), Cs$_2$CO$_3$ (294 mg, 0.90 mmol) were added. The reaction mixture was stirred at 90 °C for 15 h. After cooling to rt, the reaction mixture was filtered. H$_2$O (10 mL) was added to the filtrate and extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (5 mL), dried over Na$_2$SO$_4$, filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc (4:1, 2:1, 1:1) to give 649 (41 mg, 38%) as a yellow solid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.36 (d, J = 1.3 Hz, 1H), 8.27 (s, 1H), 8.03 (dt, J = 7.8, 1.1 Hz, 1H), 7.69 – 7.53 (m, 2H), 7.43 (ddt, J = 8.2, 7.0, 1.3 Hz, 1H), 5.17 – 5.02 (m, 2H), 2.68 (t, J = 6.6 Hz, 1H).
CHAPTER 11: Caged Erythromycin

11.1 Introduction

Erythromycin (656, Figure 11.1), a 14-membered macrolactone, was the first clinically useful macrolide antibiotic.\textsuperscript{410} Ever since its discovery in 1953,\textsuperscript{410,411} erythromycin and other macrolides have been widely used as effective antibiotics for the treatment of bacterial infections from gram-positive bacteria, gram-negative cocci and mycoplasmas.\textsuperscript{410}

![Figure 11.1. The structure of erythromycin.](image)

Chemical modification of erythromycin has allowed for improvement of its physicochemical properties and biological activity.\textsuperscript{412} A number of modifications have been applied to the macrolactone ring and the sugar-rings of erythromycin. For example, the 11-, 2’-, 4’’- hydroxyl moieties had been converted to ester groups.\textsuperscript{413,414} Erythromycin has been shown to have a low stability under acidic condition. To improve the acid stability of the antibiotic, the cladinose ring has been removed.\textsuperscript{412,414} Further modifications have been made at the C9 ketone including reduction to the alcohol\textsuperscript{413,415} and conversion to the oxime.\textsuperscript{415-417} Additionally, the N-methyl group has been transformed an i-Pr group, ring
expansion has also been reported, and semi-acetal has been formed inside the 14-membered ring.

The action mode of erythromycin is to disrupt the overall protein synthesis through inhibition of ribosome function. A variety of mechanism of erythromycin resistance has been identified in bacteria, namely, ribosome modification, efflux of the drug, and direct modification of erythromycin. Inactivation of erythromycin by the phosphotransferase MphA take places in certain strains of E. coli. It is revealed that the mphA gene is regulated by MphR(A) is a regulatory protein that controls expression of itself and mphA by binding upstream to a promoter (PmphR). When antibiotic is present, MphR(A) binds to the antibiotic and releases the operator, inducing the MphA expression and erythromycin resistance (Figure 11.2A). This negative feedback loop allows to activate the erythromycin resistance in the presence of antibiotic.

In collaboration with the Cropp Lab (VCU), a general erythromycin inducible system was developed to enable conditional express of EGFP in the presence of erythromycin. A photocaged erythromycin analog that cannot bind to MphR(A) can be designed for photochemical spatial and temporal control over gene expression. Crystal structure of MphR(A)/erythromycin complex (PDB 3FRQ) (Figure 11.2 C) illustrated the hydrogen bonding interactions between residue N123 and the C-9 and C-11 oxygen atoms of erythromycin, as well as residue H147 and R122 with the C-9 and C-1 carbonyl groups, respectively. The chemical modification on C9 position could possibly interrupt the hydrogen bonding between the erythromycin and MphR (A). Installing a caging group (eg. o-nitrobenzyl group) onto the erythromycin will mask its activity and the caging group can be
removed upon light irradiation, thus restoring the binding ability to MphR(A) and activating the gene expression.

Figure 11.2. (A) Erythromycin resistance gene cassette. (B) Engineered MphR(A)/promoter system developed for erythromycin-inducible gene expression. (C) Ligand binding sight of MphR(A) with bound erythromycin (yellow) indicating hydrogen bonds to the C-9 carbonyl that are disrupted by the caging group (PDB 3FRQ).424

11.2 Synthesis of Caged Erythromycin

A directly photocaged erythromycin (655) at the 2’-hydroxyl group was first explored. Several different activated caging groups, including the NHS carbonate 576, the \( p \)-nitrophenyl carbonate 583 and \( o \)-nitrobenzyl chloroformate (656), were employed to acylate 655 (Scheme 11.1). When the erythromycin was treated the NHS carbonate 576 or the \( p \)-nitrophenyl carbonate 583, no desired products were obtained. When the erythromycin was reacted with freshly prepared \( o \)-nitrobenzyl chloroformate (656) in the presence of K\(_2\)CO\(_3\), a
product that corresponded to the loss one molecule of H$_2$O (confirmed by MS analysis) was produced.

Scheme 11.1. Attempts to cage erythromycin.
Then the erythromycin was activated with diphosgene, followed by the treatment with the caging group 575. Again, this methodology did not give any desired product (Figure 11.2).

Scheme 11.2. Attemp to cage erythromycin.

Since the attempts to directly photocage erythromycin (655) at the 2'-hydroxyl group did not provide the desired caged compound, the more nucleophilic 9-oxime analog 661 of erythromycin was selected as a target (Figure 11.3) for the installation of the caging group, as 9-oxime analogs have previously been reported to retain biological activity.425, 426
In order to obtain the cage erythromycin-9-oxime, the nitrobenzyloxyamine hydrochloride (662) was first explored (Scheme 11.3). The use of Na$_2$CO$_3$ or NaOAc as a base caused the product to decompose or no reaction took place. When PPTS or pyridine were used, the reaction did not proceed to completion complete, and the caged product could not be separated from erythromycin by column chromatography.

**Figure 11.3.** The structure of erythromycin-9-oxime (661).
Scheme 11.3. Attempted syntheses of caged erythromycin-9-oxime [655] from 2-nitrobenzyloxyamine hydrochloride (662).

In order to solve the purification problem, a longer chain oxime was used to reduce the polarity of the erythromycin product. A decyl chain was used in the place of o-nitrobenzyl group. However, when the erythromycin was then treated with the decylhydroxylamine, the reaction was not complete and the purification problem was not solved.

Since the direct conversion of erythromycin (655) to caged erythromycin-9-oxime (663) using 2-nitrobenzyloxyamine hydrochloride was not successful, the synthesis of the caged erythromycin-9-oxime (661) in a two-step fashion via erythromycin-9-oxime as intermediate was carried out. The erythromycin-9-oxime (661) was obtained by reacting erythromycin with hydroxylamine in the presence of triethylamine according to a literature
The alkylation of erythromycin-9-oxime (661) with 2-nitrobenzyl bromide (664) was carried out under various conditions. However, no desired product was obtained (Scheme 11.4). This might be due to the high reactivity of 2-nitrobenzyl bromide (664), which can react with other functional groups on the erythromycin-9-oxime as well.

**Scheme 11.4.** Attempted syntheses of caged erythromycin-9-oxime 661 using 2-nitrobenzyl bromide (664).
Evidence in the literature supported that the erythromycin-9-oxime (661) can successfully be alkylated with alkyl chlorides. The 2-nitrobenzyl chloride (667) was synthesized from the reaction of 2-nitrobenzyl alcohol (665) with thionyl chloride. The erythromycin-9-oxime (661) was then reacted with the 2-nitrobenzyl chloride (667) in the presence of K$_2$CO$_3$ in refluxing acetone, delivering the 2-nitrobenzyl caged erythromycin-9-oxime 670 exclusively as the $E$ isomer in 91% yield. In a similar fashion, 6-nitropiperonyl alcohol (666) was converted to the 6-nitropiperonyl chloride (668), which was reacted with erythromycin-9-oxime 661, delivering the 6-nitropiperonyl caged erythromycin-9-oxime 671 in 87% yield (Scheme 11.5). The decaging of 6-nitropiperonyl caged erythromycin-9-oxime 671 was analyzed by TLC. The decaging was performed using a 360 nm UV lamp as the light source and PBS 7.4 buffer (500 µM) as the solvent. Decaging started at 2 min and was complete after 30 min of irradiation. An attempt to synthesize the caged erythromycin 672 was also made using the same fashion of making caged erythromycin 671. The known chloride 669 was reacted with erythromycin-9-oxime, however, the reaction mixture was decomposed and no desired product 672 was obtained (Scheme 11.5).
Scheme 11.5. The synthesis of caged erythromycin-9-oximes 670 and 671 and attempted synthesis of 672.

11.3 Photochemical control over gene expression using a photocaged erythromycin

The photochemical control over gene expression was performed by Laura Gardner (Deiters lab).

The light-dependent response of GFP expression to UV light was observed by employing the caged erythromycin 671 in liquid E. coli cultures. The GFP expression was under control of erythromycin resistance cassette, which shows fluorescence only in the presence of erythromycin or erythromycin-9-oxime (Figure 11.4). The caged erythromycin 671 without UV irradiation showed no fluorescence, indicating that 671 was complete inactive. Upon 5 min UV (365 nm) irradiation, the fluorescence was restored to the level of
erythromycin-9-oxime, showing almost quantitative releasing of the erythromycin-9-oxime upon irradiation.

![Fluorescence graph](image)

**Figure 11.4.** Light-activated GFP expression in the liquid mediate.

The spatial control of reporter gene expression was also obtained by treating cells containing pJZ12 and pMLEGFP with caged erythromycin (671) on agar plate followed by irradiating half of the plate at 365 nm for 5 min (Figure 11.5).
Figure 11.5. EGFP expression in a bacterial lawn, cells treated with (A) erythromycin-9-oxime (661) and (B) caged erythromycin (671). The right half of the plate was exposed to UV light for 5 min.

11.4 Conclusion and Outlook

In summary, we have successfully synthesized two caged erythromycin analogs using erythromycin-9-oxime as the intermediate. UV light irradiation of the caged erythromycin analogs releases erythromycin-9-oxime. We have demonstrated light-activatable gene expression in prokaryotic cells with an MphR(A)/promoter system that can be controlled with high spatial and temporal resolution. This technology is based on a photocaged erythromycin molecule and provides a useful tool for the precise regulation of gene expression and thus the study of gene function.

The UV light at 365 nm could be damaging to the cells, a caged erythromycin that can decage at longer wavelength will favor the light-activatable gene expression in cells. The light-triggered logic gates can be performed with lower-energy light. Future work can focus on the synthesis of the caged erythromycin analogs that can undergo decaging with lower-energy light and apply the analogs to other cell system. The DEACM caging group can be
photolysis at 405 nm with photolysis efficiency. The DEACM cage erythromycin can be synthesized using a similar method as that for 673 (Scheme 11.6).

Scheme 11.6. The proposed synthesis of DEACM caged erythromycin 673.

11.5 Experimental

All reactions were performed in flame-dried glassware under a nitrogen atmosphere and stirred magnetically unless indicated. Chemicals were used directly from commercial sources without further purification unless indicated. Solvents were distilled and stored with molecular sieves (3 Å for methanol and ethanol and 4 Å for all other solvents) prior to use. Toluene, xylene, dioxane were distilled from sodium/benzophenone ketyl. TEA, DIPEA, DMSO, DMF, DCE, CH$_3$CN and pyridine were distilled from calcium hydride. Methanol and ethanol were distilled from magnesium and iodole. CH$_2$Cl$_2$, THF and ether were dried by MB SPS Compact solvent purification system. All other reagent quality solvents were used without further purification. $^1$H and $^{13}$C NMR spectra were performed using a Varian
Mercury (300 MHz and 400 MHz). Mass spectra analysis was performed by North Carolina State University facilities.

**2-Nitrobenzyloxyamine hydrochloride (662).** N-hydroxyphthalimide (500.0 mg, 3.605 mmol) and K$_2$CO$_3$ (508.0 mg, 3.678 mmol) were dissolved in DMF (10 mL). 2-Nitrobenzyl bromide (729.0 mg, 3.371 mmol) was added. The reaction mixture was stirred at rt overnight. H$_2$O (30 mL) was added and the white precipitation was filtered, washed with H$_2$O and ether, dried *in vacuo* to give the phthalimide (785.0 mg, 86%) as a white solid. $^1$H NMR (300 MHz, DMSO-d$_6$) δ 8.11 (dt, $J = 8.1, 1.3$ Hz, 1H), 7.88 (dd, $J = 7.8, 1.5$ Hz, 1H), 7.82 (s, 1H), 7.78 (tt, $J = 7.6, 1.3$ Hz, 1H), 7.70 – 7.61 (m, 1H), 5.54 (s, 2H).

The phthalimide (804.0 mg, 2.716 mmol) was dissolved in MeOH (45 mL). Hydrazine hydride (416 µL, 8.419 mmol) was added dropwise. The reaction mixture was stirred at rt overnight. 1N HCl was added to adjust the pH to ~2. The reaction mixture was cooled in ice for 1 h. The formed precipitation was filtered and the filtrate was concentrated and the resulting residue was partitioned between DCM (30 mL) and NH$_4$HCO$_3$ (30 mL). The organic layer was dried with Na$_2$SO$_4$, filtered. 6 N HCl was added to the filtrate to adjust the pH to 2 and concentrate *in vacuo* to form white solid. This white solid was filtered, washed with cold DCM and dried *in vacuo* to give 662 (496.0 mg, 90%) as a white solid. $^1$H NMR (300 MHz, DMSO-d$_6$) δ 10.92 (br, 3H), 8.28 – 7.99 (m, 1H), 7.83 (td, $J = 7.5, 1.3$ Hz, 1H), 7.75 – 7.61 (m, 2H), 5.37 (s, 2H).

**Erythromycin-9-oxime (661).** Erythromycin (1.0 g, 1.363 mmol) was dissolved in methanol (2.5 mL). TEA (474 µL, 3.408 mmol) and hydroxylamine hydrochloride (474 mg, 6.815 mmol) were added. The reaction mixture was heated to reflux for 24 h. 2/3 Of the
solvent was removed in vacuo and the resulting residue was cooled to 0 °C for 1 h. The solid was filtered and washed with cold MeOH. The wet cake was suspended in MeOH (2.5 mL) and 20-25% NH₃ (0.65 mL) was added slowly to the solution at 10-12 °C and stirred for 30 min. H₂O (0.65 mL) was added and precipitation formed. H₂O (3 mL) was added and cooled to 0 °C. The precipitation was filtered, washed with cold H₂O and dried in vacuo to give 661 (508.1 mg, 50%) as a white solid. $^1$H NMR (300 MHz, CDCl₃) δ 5.15 – 5.00 (m, 1H), 4.90 (d, $J = 4.8$ Hz, 1H), 4.40 (d, $J = 7.2$ Hz, 1H), 4.26 (s, 1H), 4.01 (dd, $J = 14.6$, 7.8 Hz, 2H), 3.76 (s, 1H), 3.67 (s, 1H), 3.58 (d, $J = 7.5$ Hz, 1H), 3.52 – 3.38 (m, 2H), 3.30 (s, 3H), 3.22 (dd, $J = 10.1$, 7.2 Hz, 1H), 3.08 – 2.80 (m, 3H), 2.68 (d, $J = 6.9$ Hz, 1H), 2.39 (d, $J = 17.1$ Hz, 1H), 2.33 – 2.14 (m, 7H), 2.06 – 1.78 (m, 3H), 1.70 – 1.54 (m, 5 H), 1.50– 1.42 (m, 4H), 1.27 (d, $J = 6.2$ Hz, 3H), 1.22 (s, 4H), 1.20 (s, 1H), 1.17 (s, 3H), 1.15 (s, 2H), 1.11 (d, $J = 1.8$ Hz, 4H), 1.09 – 1.00 (m, 6H), 0.82 (t, $J = 7.3$ Hz, 3H).

**General procedure of converting benzyl alcohol to benzyl chloride.** Benzyl alcohol (1 eq) was dissolved in DCM (0.14 M). DMF (catalytic amount) was added followed by the slow addition of thionyl chloride (1.4 eq). The reaction mixture was stirred at refluxing for 2.5 h and cooled to rt. H₂O was added and extracted with DCM three times. The combined organic layers were washed with brine, dried over Na₂SO₄, filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, to give the corresponding benzyl chloride.

**2-Nitrobenzyl chloride (667).** Eluted with hexanes/EtOAc (4:1). Light yellow solid. 88% yield. $^1$H NMR (300 MHz, CDCl₃) δ 8.04 (dd, $J = 8.1$, 1.2 Hz, 1H), 7.75 – 7.59 (m, 2H), 7.55 – 7.44 (m, 1H), 4.96 (s, 2H).
6-Nitropiperonyl chloride (668). Eluted with hexanes/EtOAc (4:1). Yellow solid. 82% yield. $^1$H NMR (300 MHz, CDCl$_3$) δ 7.57 (d, $J = 1.6$ Hz, 1H), 7.24 (d, $J = 1.7$ Hz, 1H), 6.13 (d, $J = 1.7$ Hz, 2H), 4.92 (d, $J = 1.5$ Hz, 2H).

**General procedure of synthesizing caged erythromycin-9-oxime.** Erythromycin-9-oxime (000, 1 eq) was dissolved in acetone (0.025 M). Benzyl chloride (10 eq) and K$_2$CO$_3$ (7 eq) were added. The reaction mixture was refluxed for 16 h. After cooled to rt, the reaction mixture was concentrated in vacuo. The residue was dissolved in EtOAc and washed with saturated NaHCO$_3$ three times and brine, dried over Na$_2$SO$_4$, filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, to give the corresponding caged erythromycin-9-oxime.

2-Nitrobenzyl caged erythromycin-9-oxime (670). Eluted with MeOH/TEA/DCM (1:1:100). Yellow solid. 91% yield. $^1$H NMR (300 MHz, CDCl$_3$) δ 8.04 (dd, $J = 8.2$, 1.3 Hz, 1H), 7.66 (td, $J = 7.6$, 1.3 Hz, 1H), 7.52 (dd, $J = 7.8$, 1.4 Hz, 1H), 7.42 (td, $J = 7.8$, 7.3, 1.5 Hz, 1H), 5.43 (s, 2H), 5.04 (dd, $J = 11.1$, 2.3 Hz, 1H), 4.91 (d, $J = 4.8$ Hz, 1H), 4.40 (d, $J = 7.1$ Hz, 1H), 4.07 – 3.91 (m, 4H), 3.85 – 3.69 (m, 1H), 3.64 (s, 1H), 3.54 (dd, $J = 7.6$, 3.0 Hz, 1H), 3.51 – 3.36 (m, 2H), 3.29 (d, $J = 1.0$ Hz, 4H), 3.19 (dd, $J = 10.3$, 7.2 Hz, 1H), 3.08 – 2.94 (m, 3H), 2.94 – 2.82 (m, 1H), 2.65 (d, $J = 7.1$ Hz, 1H), 2.25 (s, 10H), 2.04 – 1.79 (m, 2H), 1.78 – 1.49 (m, 6H), 1.33 – 1.15 (m, 11H), 1.15 – 0.99 (m, 10H), 0.82 (t, $J = 7.3$ Hz, 3H). MS calcd [M+H]$^+$ C$_{44}$H$_{74}$N$_3$O$_{15}$ 884.512, found 884.5.

6-Nitropiperonyl caged erythromycin-9-oxime (671). Eluted with MeOH/TEA/DCM (1:1:100). Yellow solid. 91% yield. $^1$H NMR (300 MHz, CDCl$_3$) δ 7.59 (s, 1H), 6.97 (s, 1H), 6.11 (s, 2H), 5.38 (s, 2H), 5.05 (dd, $J = 11.1$ Hz and 1.5 Hz, 1H), 4.89
(d, J = 4.8 Hz, 1H), 4.40 (d, J = 7.5 Hz, 1H), 4.02 (s, 1H), 3.98 (d, J = 9.3 Hz, 2H), 3.86-3.71 (m, 1H), 3.66 (s, 1H), 3.56 (d, J = 10.8 Hz, 1H), 3.51-3.43 (m, 1H), 3.42 (s, 1H), 3.30 (s, 3H), 3.20 (dd, J = 7.5 Hz and 10.5 Hz, 1H), 3.03-2.97 (m, 2H), 2.91-2.82 (m, 2H), 2.67 (q, J = 6.6 Hz, 1H), 2.49-2.30 (m, 2H), 2.27 (s, 6H), 2.16 (d, J = 10.2 Hz, 1H), 2.01-1.83 (m, 2H), 1.70-1.56 (m, 4H), 1.54-1.52 (m, 1H), 1.49-1.39 (m, 4H) 1.29-1.26 (m, 4H), 1.25-1.20 (m, 5H), 1.19-1.15 (m, 5H), 1.12-1.04 (m, 12H), 0.82 (t, J = 7.2 Hz, 3H); MS calcd [M+H]+ C_{45}H_{74}N_{3}O_{17} 928.5, found 884.5.
12.1 Introduction

Nonribosomal peptides (NRPs) are short peptides that contain 2 to 50 amino acids. Unlike ribosomal peptides, which contain only the 20 proteinogenic amino acids, NRPs can be assembled from 500 different possible monomers, including non-proteinogenic amino acids, fatty acids, and α-hydroxy acids. Furthermore, while the structure of ribosomal peptides is a linear peptide chain, the structures of the NRPs can be linear, branched, cyclic or even polycyclic. The great diversity of chemical structures of NRPs gives them broad pharmacological and biological activities, such as the immunosuppressant cyclosporine A and the antibiotic penicillin.

Nonribosomal peptides (NRPs) are synthesized by nonribosomal peptide synthetases (NPRSs), which are a family of large multidomain enzymes. These enzymes can repetitively catalyze chemical reactions of NRP monomers to generate a large number of secondary peptidic metabolites. NPRSs contain sets of functional domains referred to as “modules”, and each module is responsible to add one specific amino acid into the peptidic product. Usually, the sequences of the peptidic products are reflected by the order of the modules of the NPRSs; thus, variations of bioactive molecules can be assembled by modulating the constituents and specificities of the NPRSs. Generally, in order to elongate peptide intermediates, a module is built up by at least three different domains: the adenylation (A) domain, the thiolation (T) domain (also known as peptidyl carrier protein (PCP) domain) and the condensation (C) domain. The A domain (~550 residues) recognizes a specified amino acid monomer, activates it by adenylation and then transfers the
activated monomer onto the 4’-phosphopantetheiny1 (PPE) arm of the T (or PCP) domain (~80 residues). The T domain of the current and previous modules loads two covalently-bound monomers into the active site of the C domain (~450 residues). The C-domain locates in between the A and T domains and catalyzes the formation of peptidic bond between the two covalently bonded monomers, elongating the growing peptidic chain and leading to the synthesis of NRPs (Figure 12.1). After condensation, the peptides are attached to the PPE arm of the T domain again and will be carried to the C domain of the next module to continue the elongation. A thioesterase (TE) domain is often found in the final module to release the peptidic products. Some other optional domains, such as epimerization domain (E-domain), methyl-transferase domain (M-domain) for N-methylation and the cyclization domain (Cy-domain) also exist in the module to further modify the substrates.
Since NRPSs are modular, it is possible to construct novel NRPs by adding, removing or substituting domains or modules of NRPSs. In order to fully exploit these macromolecular machines, a better understanding of the reactions at the C domain is critical. The C domains show significant substrate selectivity for the nucleophilic acceptor substrate from the current module and enantioselectivity for the electrophilic donor substrate from the
It was found that the histidine motif in the C domains plays an essential role in the enzyme function. However, the mechanism of peptide bond formation catalyzed by the C domain remains unclear; and the binding interactions as well as specificity determinations are still not well understood.

The Schmeing lab at McGill University uses X-ray crystallography to solve the structures of the C domain. To obtain a structure of the C-domain in complex with substrate mimics will help with the evaluation of the reaction mechanism of the protein. New Coenzyme A (CoA) analogs are designed as non-reactive donor substrate (674), donor substrate (675) and acceptor substrate (676) (Figure 12.2). These substrates have a phosphopantetheine (Ppant) moiety and also additional functional groups. The Ppant moiety can act as a T-domain alternative and the additional functional group will reveal more information of the reaction mechanism. The C-domain will be soaked and co-crystallized with the substrates. With information from the X-ray structures of the complexes, the active site residues involved in the reaction will be identified.

![Figure 12.2. The structures of NRPS substrate mimics.](image-url)
12.2 Synthesis of the non-Reactive Donor Substrate 674

We first started the synthesis of the non-reactive donor substrate 674. Upon analysis, we anticipated that the non-reactive donor substrate 674 could be assembled from four parts, phosphate 677, pantothenic acid (678), ethylenediamine (679) and 3-propyloxiran-2-yl)methanol (680).

![Scheme 12.1. Analysis of the non-reactive donor substrate 674.](image)

Initially, we planned to first link 679 and 680. (2S,3S)-3-Propyloxirane-2-carboxylic acid (680) was synthesized from (E)-hex-2-en-1-ol (681) via a Sharpless epoxidation, followed by oxidation with RuCl₃ and NaIO₄, according to a literature procedure. Tert-Butyl 2-aminoethylcarbamate (683) was coupled to the acid 680 under EDCI/HOAt/TEA coupling condition, delivering the amide 684. However, the removal of the Boc group was problematic, since the epoxide turned out to be sensitive to acid. The Boc group was replaced by an Fmoc group, whose deprotection is usually accomplished under basic conditions. (9H-fluoren-9-yl)methyl 2-aminoethylcarbamate (685) was synthesized in two
steps from tert-butyl 2-aminoethylcarbamate (683) through protecting 683 with Fmoc and removal of Boc. The acid 680 was reacted with the Fmoc protected amine 685 in the presence of EDCI, HOBt and DIPEA to generate the amide 686. Again, the removal of Fmoc group was problematic (Scheme 12.2). This was probably also due to the sensitivity of the epoxide moiety to nucleophiles.  

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\text{Scheme 12.2. Attempted synthesis of 687.}
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In order to minimize the difficulties arising from the sensitivity of the epoxide moiety, the coupling of 678 and 679 was carried out first, followed by the assembly of the epoxide moiety (680) at a later stage. The commercially available pantothentic acid hemi calcium salt was neutralized with HCl and then protected to form the acetal 690. However,
further reaction of 690 with the amine 683 did not give the desired product 691 (Scheme 12.3).

Scheme 12.3. Attempt of the synthesis of 691.

A different protecting group, di-\(t\)-butyldisilyl (DTBS) group, a common di-functional protecting group for diols,\(^{438, 441}\) was applied to the pantothenic acid. The pantothenic acid hemi calcium salt (688) was stirred with di-\(t\)-butylsilylditriflate in DMF to produce the protected acid 692, which was coupled to the amine 683, delivering the amide 693. But the deprotection of the Boc group in 693 did not produce the desired product 694 (Scheme 12.4). This was probably due to the low stability of the DTBS under acidic condition as the DTBS was removed too under these conditions.
The acid 692 was reacted with the Fmoc-protected amine 685 to give the amide 695. Several conditions were examined to remove the Fmoc group (Scheme 12.5). Both diethyl amine/THF\(^{442}\) and NH\(_3\)/MeOH can successfully remove the Fmoc group, leaving other moieties untouched. Due to the much shorter time of diethylamine deprotection condition compared to NH\(_3\)/MeOH, we chose diethylamine as the deprotection reagent and the amine 696 was obtained. The amine 696 was coupled with the acid 680, forming the amide 697. After the DTBS group was removed by TBAF, the resulting diol 698 was treated with freshly prepared dibenzylchlorophosphonate 700 (prepared from the reaction of dibenzylphosphate (699) with NCS),\(^{443}\) delivering the phosphate 701. The removal of the benzyl protecting groups from the phosphate 701 was conducted with H\(_2\), Pd/C, and TEA in MeOH,\(^{444}\) producing the non-reactive donor substrate 674 (Scheme 12.5). This non-reactive donor substrate 674 was sent to our collaborator Dr. Thomas Martin Schmeing (Mcgill University).
Scheme 12.5. The synthesis of the non-reactive donor substrate 674.

12.3 Synthesis of the Donor Substrate 675

The structure of the donor substrate 675 is very similar to that of the non-reactive donor substrate 674. Therefore, it was predicted that 675 could be assembled in a similar way as the non-reactive donor substrate 674. Retrosynthetic analysis of the donor substrate 675 showed that 675 can be made from four different parts: phosphate 677, pantothenic acid (678), 2-aminoethanethiol (702) and 3-propyloxiran-2-yl(methanol (680) (Scheme 12.6).
Following a similar route as that for the non-reactive donor substrate, we started the synthesis with 2-aminoethanethiol (702). The amine 702 was protected with a trityl group according to a literature procedure to give 703, which underwent a smooth coupling reaction with the acid 692, delivering the amide 704. The trityl group was removed by TFA/TES, generating the thiol 705. The thiol 705 was reacted with the acid 680, producing 706 in good yield. Surprisingly, removal of the silyl protecting group with TBAF in THF led to decomposition of the compound 706. Instead, HF-pyridine was used and the silyl was successfully removed to give the diol 707, which was reacted with freshly prepared chlorophosphonate (700), resulting in the phosphate substrate 708. However, removal of the benzyl group using the same condition as that of non-reactive donor substrate 708 was not successful (Scheme 12.7). Several other conditions were explored, such as TFA/DCM, H₂/Pd/C, H₂/Pd(OH)₂, but no desired product 675 was obtained. It was assumed that the thiol ester or the epoxide moiety is not stable to the hydrogenation conditions. Thus, a different protecting group for the phosphate that doesn’t require hydrogenation for removal was
needed. PMB group as another common used protecting group on phosphate can undergo deprotection under oxidative condition.\textsuperscript{446} \{bis[(4-Methoxyphenyl)methoxy]phosphanyl]bis(propan-2-yl)amide (711)\} was prepared following a literature procedure.\textsuperscript{446} This phosphoramidite 711 was reacted with the diol 707, producing the phosphate 712. The phosphate 712 was treated with DDQ, furnishing the donor substrate 675 together with the DDQ-byproduct (Scheme 12.7). However, the purification of 675 from the DDQ-byproduct by C18 reversed column chromatography was successful. The mixture of donor substrate 675 and DDQ-byproduct was sent to Dr. Thomas Martin Schmeing (Mcgill University) for further HPLC purification.
Scheme 12.7. The synthesis of the donor substrate 675.

12.4 Synthesis of the Acceptor Substrate 676

Similar to the donor substrate 675, we envisioned that the acceptor substrate 676 can be assembled by four parts: (A) phosphate 677, (B) pantothenic acid (678), (C) ethylenediamine (702) and (D) (S)-serine (713) (Scheme 12.8).
The synthesis of the acceptor substrate 676 commenced with the protected thiol 705. Cbz-Ser(Bzl)-OH was selected to achieve global deprotection of both CBz and Benzyl groups with H₂/Pd/C. The thiol 705 and acid 714 were coupled to give the amide 716 in 80% yield. After removing the silyl protecting group of 716, the resulting diol 718 was reacted with freshly prepared chlorophosphonate (700) to give the phosphate 720 in 76% yield. Several conditions for removing the protecting groups were examined (Scheme 12.9), but no desired product 676 was obtained. In order to solve this problem, the Cbz-Ser(Bzl)-OH (713) was replaced with Boc-Ser(Bzl)-OH (715). The phosphate 721 was obtained using a similar way as that for 720, however, the removal of the protecting groups was still problematic (Scheme 12.9).
To solve the deprotection problem, PMB protected serine 723 was used instead. The PMB protected serine 723 was synthesized in one step from commercially available Boc-Ser-OH (722) and then reacted with the thiol 705, producing 724 in 85% yield. The silyl group was removed with HF-pyridine to give 725, which underwent a phosphorylation reaction with 711, generating the phosphate 726. The phosphate 726 was stirred with TFA/TES/DCM and the Boc group was removed as well as the PMB groups on the phosphate. The resulting residue was further treated with DDQ, delivering the desired acceptor substrate 676 together with the DDQ-byproduct (Scheme 12.10). This mixture was sent to Dr. Thomas Martin Schmeing (Mcgill University) for further HPLC purification.
Scheme 12.10. The synthesis of the acceptor substrate 676.

12.5 Synthesis of Minimal Compounds (SNAC Analogs).

Since the synthesis and purification of the donor substrate and acceptor substrate are problematic, structurally similar but simpler compounds were designed (Figure 12.3). The structurally simpler SNAC molecules 727-729 have similar structure on the donor side of the substrates 674, 675 and 676, which could possibly play the role of the donor site.
Figure 12.3. The structures of minimal donor 727, minimal non-reactive donor 728, and minimal acceptor 729.

The minimal donor 727 was synthesized in one step from the coupling reaction of the acid 680 and the thiol 730. In a similar fashion, the minimal non-reactive donor 728 was generated from the acid 680 and amine 731. The minimal acceptor 729 is a known compound, and the synthesis commenced with Boc-Ser(t-Bu)-OH (732) according to literature report. The 732 was reacted with the thiol 730 to give thiol ester 733, which underwent a global deprotection with 4 N HCl in dioxane, delivering the desired minimal acceptor 729 in 96% yield (Scheme 12.11). All the minimal compounds were sent to Dr. Thomas Martin Schmeing (Mcgill University).

Scheme 12.11. The synthesis of the minimal compounds 727, 728 and 729.
12.6 Synthesis of PPANT Analog

Carrier proteins (CPs) are used to deliver substrates in the natural product biosynthetic pathways. The posttranslational modification (PTM) is required to attach a Ppant group from coenzyme A onto CPs’ conserved serine site, converting CPs from its inactivate state to activate state (Figure 12.4).

**Figure 12.4** Posttranslational modification of CPs. Ppant group is transferred to conserved Ser on CPs from Coenzyme A through the catalysis of phosphopantetheinyl transferases (PPTase). The crystal structure shown here is AcpP (PDB:1T8K).

Modification of the Ppant group will presumably allow different substrates to be accepted by KS and AT domains, expanding their substrate specificities, and ultimately enriching the library of polyketide analogs. The substrate 735 (Figure 12.5) was designed, which has a similar structure as the pantetheine chain with an additional alkyne group. The alkyne group will react with the azide modified CP via [3+2] cycloaddition reaction. Accordingly, an unnatural amino acid with azide group will be incorporated into acyl carrier
proteins (ACPs), yielding the mutant ACPs. The click chemistry can attach 735 onto mutant ACPs, transferring the CPs from inactivate state to activate state (Figure 12.5). This chemical modification bypasses the substrate specificity of PPTases and allows a more diverse panel of CPs to be prepared. Since starting material for the Ppant analog 735 is expensive, an alternative analogue 736 was designed to test the click reaction.

**Figure 12.5.** The Ppant analogs onto CPs via click reaction.

The synthesis of 736 commenced with but-3-yn-1-ol (737). The alcohol 737 was oxidized to but-3-ynoic acid (738) by Jones’ reagent. Interestingly, low yield was obtained while the reaction was conducted with magnetic stirring, but when the stirring was replaced
by shaking, desired but-3-ynoic acid (738) was obtained in 68% yield. The acid 739 was coupled to 2-(tritylthio)ethanamine (703), delivering the amide 740. Removal of the Fmoc protecting group was performed with diethylamine in THF to give the free amino 741, which was subsequently reacted with the but-3-ynoic acid (738). However, the only product after the coupling reaction was N-(2-(2-(tritylthio)ethylcarbamoyl)ethyl)buta-2,3-dienamide (743) instead of the desired 742 (Scheme 12.12). This was possibly caused by the coupling reagents leading to isomerization of the propargyl moiety to the allene moiety.


In order to solve the isomerization problem, an additional carbon was inserted to form 746. The compound 746 was synthesized in two steps from amine 741. Amine 741 was reacted with the commercially available pent-4-ynoic acid (744), producing the amide 745. After the trityl group was removed by stirring with TFA/TES, the thiol 746 was obtained in
54% yield (Scheme 12.13). The click reaction of thiol 746 with protein azide is being tested to by collaborator Zhixia Ye (Williams lab, NCSU). To date, the click reaction of 746 with protein-azide has not been successful. More [3+2] cycloaddition conditions will be carried out by collaborator.


12.7 Summary and Outlook

In summary, the non-activate donor substrate 674 and minimal substrates 727-729 have been successfully synthesized. The donor substrate 675 and acceptor substrate 676 and have been synthesized and the purification is being conducted with HPLC in Schmeing’s lab (Mcgill University). The soaking and X-ray experiment will be performed in Schmeing’s lab.

12.8 Experimental

All reactions were performed in flame-dried glassware under a nitrogen atmosphere and stirred magnetically unless indicated. Chemicals were used directly from commercially sources without further purification unless indicated. Solvents were distilled and stored with
molecular sieves (3 Å for methanol and ethanol and 4 Å for all other solvents) prior to use. Toluene, xylene, dioxane were distilled from sodium/benzophenoneketyl. TEA, DIPEA, DMSO, DMF, DCE, CH$_3$CN and pyridine were distilled from calcium hydride. Methanol and ethanol were distilled from Magnesium and iodine CH$_2$Cl$_2$, THF and ether were dried by MB SPS Compact solvent purification system. All other reagent quality solvents were used without further purification. $^1$H and $^{13}$C NMR spectra were performed using a Varian Mercury (300 MHz and 400 MHz). Mass spectra analysis was performed by North Carolina State University facilities.

**General procedure I: Peptide coupling reaction using EDCI/HOBt.** Acid (20 mg-1 g scale, 1 eq), amine (2 eq), EDCI (2.5eq) and HOBr (3eq) were dissolved in THF (0.014 M) (or DMF (0.4 M)). DIPEA (6 eq) was added dropwise. The reaction mixture was stirred at rt overnight and was concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc mixture to give the corresponding amides.

**General procedure II: Coupling of acid and thiol using EDCI/HOBt.** Acid (20 mg-1 g scale, 1 eq), thiol (1.2 eq), EDCI (1.2 eq), HOBr (1.5 eq) and DMAP (0.2 eq) were dissolved in DCM (0.07 M). DIPEA (6 eq) was added dropwise. The reaction mixture was stirred at rt overnight and was concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc mixture to give the corresponding amides.

**General procedure III: The synthesis of amides from Fmoc carbamates.** Fmoc carbamate (105 mg, 0.168 mmol) was dissolved in DCM (9.5 mL/mmol). Et$_2$NH (5 mL/mmol) was added dropwise. The reaction mixture was stirred at rt for 20 h and was concentrated in
The resulting free amine was used directly without further purification. To the obtained free amine (2 eq) were added the acid (1 eq), EDCI (2.5 eq) and HOBt (3 eq) and THF (70 mmol/ml) (or DMF (2.5 mmol/ml)). DIPEA (6 eq) was added dropwise, the reaction mixture was stirred at rt overnight, and was subsequently concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc mixture to give the corresponding amides.

**General procedure IV: The removal of the di-tert-butylsilyl group using TBAF.** The silyl ether (20-500 mg scale, 1 eq) was dissolved in THF (33 ml/mmol) and the reaction mixture was cooled to 0 °C. TBAF (1 M in THF, 4 eq) was added dropwise. The reaction mixture was stirred at 0 °C for 2 h and was concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with DCM/acetone mixture to give the corresponding diols.

**General procedure V: The removal of the di-tert-butylsilyl group using HF-pyridine.** The silyl ether (20-500 mg scale, 1 eq) was dissolved in DCM (0.12 M) in a dry plastic tube and the reaction mixture was cooled to 0 °C. HF-pyridine (~6.4 M in DCM, 13.7 µL, 4 eq) was added dropwise. The reaction mixture was stirred at 0 °C for 2 h, saturated NaHCO₃ (3 ml/mmol) was added, and the reaction mixture was stirred at 0 °C for 5 min. After warming to rt, the mixture was purified by flash chromatography on silica gel, eluting with MeOH/EtOA give the corresponding diols.

**General procedure VI: Diol phosphorylation with dibenzylchlorophosphonate 700.** The diol (50 mg, 1 eq) was pre-dried by dissolving it in dry pyridine (2 mL) followed by removal of the solvent it in vacuo three times. It was then dissolved in dry pyridine (0.22 M)
and the solution was cooled to −40 °C. The freshly prepared solution of dibenzylchlorophosphonate (700) in toluene was added dropwise. The reaction mixture was stirred at −40 °C for 2 h and stored at −20 °C freezer overnight. The reaction mixture was warmed to rt and a small amount of H₂O was added. The mixture was concentrated in vacuo. The residue was dissolved in EtOAc, washed with 1 N H₂SO₄ (2 x 2 mL), saturated NaHCO₃ (2 x 2 mL), and saturated Na₂SO₄, dried over Na₂SO₄, filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with chloroform/acetone mixture to give the corresponding phosphates.

**General procedure VII: Diol phosphorylation with 711.** The diol (50 mg, 1 eq), {bis[(4-methoxyphenyl)methoxy]phosphanyl}bis(propan-2-yl)amine (711, 1.05 eq), and tetrazole (1 eq) were dissolved in CH₃CN (0.12 M). The reaction was stirred at rt for 1 h and cooled to −40 °C. t-BuOOH (1.1 eq) was added dropwise. The reaction mixture was stirred at 0 °C for 2 h, H₂O was added to quench the reaction and it was extracted with EtOAc (3 x 2 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with chloroform/acetone mixture to give the corresponding phosphates.

**3-((R)-2,2-di-tert-Butyl-5,5-dimethyl-1,3,2-dioxasilinane-4-carboxamido)propanoic acid (692).** Pantothenic acid hemi calcium salt (688, 2.0 g, 8.40 mmol) was dissolved in DMF (mL) and cooled to 0 °C. Di-tert-butylsilanedi(triflate) (4.5 mL, 12.6 mmol) was added dropwise. The reaction mixture was stirred at 0 °C for 2 h. TEA was added to adjust the pH till ~3. H₂O (10 mL) was added and extracted with ethe (3 x 30 mL). The combined organic layers were washed with brine (10 mL), dried over Na₂SO₄, filtered and concentrated in
vacuo. The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc (1:1, 0:1) to give 692 (1.44 g, 49% yield) as a white solid. $^1$H NMR (300 MHz, CDCl$_3$) δ 7.36 (t, $J = 6.4$ Hz, 1H), 4.41 (s, 1H), 4.02 (d, $J = 11.6$ Hz, 1H), 3.72 – 3.54 (m, 1H), 3.54 – 3.41 (m, 2H), 2.59 (t, $J = 5.9$ Hz, 2H), 1.06 (s, 18H), 1.02 (d, $J = 1.0$ Hz, 6H).

3-[(4$R$)-2,2-Di-tert-butyl-5,5-dimethyl-1,3,2-dioxasilinan-4-yl]formamido]-N-(2-[(2$R$,3$S$)-3-propyloxiran-2-yl]formamido)ethyl)propanamide (697). The compound was synthesized using general procedure III in 81% yield as a light yellow solid. $^1$H NMR (300 MHz, CDCl$_3$) δ 7.31 (t, $J = 6.4$ Hz, 1H), 6.72 – 6.59 (m, 1H), 6.55 (d, $J = 5.3$ Hz, 1H), 4.39 (s, 1H), 4.00 (d, $J = 11.6$ Hz, 1H), 3.62 (dq, $J = 12.7$, 6.1 Hz, 1H), 3.55 – 3.39 (m, 2H), 3.16 (d, $J = 2.1$ Hz, 1H), 2.92 (ddd, $J = 5.7$, 4.7, 2.1 Hz, 1H), 2.40 (t, $J = 6.1$ Hz, 2H), 1.71 – 1.33 (m, 4H), 1.06 (d, $J = 3.1$ Hz, 18H), 1.00 (d, $J = 3.7$ Hz, 6H), 0.94 (t, $J = 7.1$ Hz, 3H).

(2$R$)-2,4-Dihydroxy-3,3-dimethyl-N-{2-[(2-[(2$R$,3$S$)-3-propyloxiran-2-yl]formamido]ethyl}carbamoyl[ethyl]butanamide (698). The compound was eluted with DCM/acetone (1:1) to give 698 in 87% yield as a light yellow solid. $^1$H NMR (300 MHz, CDCl$_3$) δ 7.51 (t, $J = 6.1$ Hz, 1H), 7.37 (d, $J = 5.9$ Hz, 1H), 7.10 (d, $J = 5.5$ Hz, 1H), 4.98 (s, 1H), 4.51 – 4.02 (m, 1H), 3.96 (s, 1H), 3.63 – 3.19 (m, 6H), 3.16 (d, $J = 2.0$ Hz, 1H), 3.03 – 2.92 (m, 1H), 2.41 (d, $J = 6.2$ Hz, 2H), 1.70 – 1.34 (m, 3H), 0.93 (d, $J = 6.8$ Hz, 6H), 0.86 (s, 3H); $^{13}$C NMR (101 MHz, CD$_3$OD) δ 176.15, 174.29, 171.89, 77.38, 70.42, 59.98, 56.01, 40.47, 40.01, 39.88, 36.72, 36.52, 34.87, 21.48, 21.03, 20.24, 14.32.

Dibenzylchlorophosphonate 700. The compound was synthesized according to a literature procedure. 443 Dibenzyl phosphate (85 µL, 0.386 mmol) was dissolved in dry toluene (0.5
mL). NCS (51 mg, 0.38 mmol) was added and the reaction mixture was stirred at rt for 2.5 h. The reaction mixture was filtered under an N₂ atmosphere and the filtrate was used directly in the next step.

(2R)-4-[[bis(Benzyloxy)phosphoryl]oxy]-2-hydroxy-3,3-dimethyl-N-{2-[(2R,3S)-3-propyloxiran-2-yl]formamido}ethyl)carbamoyl]ethyl)butanamide (701). The compound was synthesized using general procedure VI and purified by column chromatography on SiO₂, eluted with DCM/acetone (2:1, 1:1) to give 701 in 68% yield as a white solid. ¹H NMR (400 MHz, CDCl₃) δ 7.33 (dd, J = 4.7, 2.5 Hz, 10H), 6.83 (t, J = 5.5 Hz, 1H), 6.71 (t, J = 5.7 Hz, 1H), 5.30 – 4.81 (m, 4H), 3.97 (dd, J = 10.0, 6.9 Hz, 1H), 3.90 (d, J = 2.4 Hz, 1H), 3.69 – 3.54 (m, 2H), 3.54 – 3.18 (m, 5H), 3.15 (d, J = 2.1 Hz, 1H), 2.96 (ddd, J = 6.0, 4.8, 2.1 Hz, 1H), 2.52 – 2.28 (m, 2H), 1.68 – 1.32 (m, 4H), 1.02 (d, J = 3.1 Hz, 3H), 0.93 (t, J = 7.2 Hz, 3H), 0.82 (s, 3H). ³¹P NMR (162 MHz, CDCl₃) δ 0.21.

Non-reactive donor substrate triethylamine salt 674. The phosphate 701 (4.8 mg, 0.007 mmol) and TEA (2.1 µL, 0.015 mmol) were dissolved in MeOH (0.19 mL). Pd/C (10%, 0.6 mg) was added. The reaction mixture was purged with H₂ and stirred at rt under H₂ for 2 h. Additional Pd/C (10%, 0.6 mg) was added and the reaction was stirred at rt for additional 8.5 h. The mixture was filtered through a pad of celite, washed with MeOH. The filtrate was concentrated in vacuo to give 674 (4.2 mg, 99%) as a white solid. ¹H NMR (300 MHz, CD₃OD) δ 4.00 (s, 1H), 3.84 (dd, J = 9.9, 6.1 Hz, 1H), 3.50 (ddd, J = 13.8, 8.7, 5.3 Hz, 3H), 3.28 (d, J = 1.3 Hz, 2H), 3.24 – 3.11 (m, 6H), 3.10 – 2.99 (m, 1H), 2.41 (t, J = 6.8 Hz, 2H), 1.70 – 1.42 (m, 4H), 1.38 – 1.24 (m, 11H), 1.03 (s, 3H), 1.01 (s, 1H), 0.88 (s, 3H). ³¹P NMR
(121 MHz, CD$_3$OD) $\delta$ 0.81. MS calcd M$^+$ C$_{17}$H$_{31}$N$_3$O$_9$P 452.1803, found 452.1797. MS calcd. [M + Na] C$_{17}$H$_{32}$N$_3$NaO$_9$P 476.1774, found 476.1724.

3-([(4R)-2,2-di-tert-Butyl-5,5-dimethyl-1,3,2-dioxasilinan-4-yl]formamido)-N-{2-[(triphenylmethyl)sulfanyl]ethyl}propanamide (704). The compound was synthesized using general procedure I, eluted with hexanes/EtOAc (2:1, 1:1) to give 704 (96% yield) as a white solid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.42 – 7.35 (m, 6H), 7.31 – 7.25 (m, 5H), 7.23 – 7.16 (m, 4H), 5.53 (s, 1H), 4.37 (s, 1H), 4.06 – 3.94 (m, 1H), 3.70 – 3.33 (m, 4H), 3.02 (p, $J$ = 6.2 Hz, 2H), 2.38 (t, $J$ = 6.4 Hz, 2H), 2.34 – 2.23 (m, 2H), 1.08 – 1.03 (m, 18H), 1.00 (s, 3H), 0.98 (s, 3H).

(R)-N-((2-(2-Mercaptoethylcarbamoyl)ethyl)-2,2-di-tert-butyl-5,5-dimethyl-1,3,2-dioxasilinan-4-carboxamide (705). Compound 704 (100.0 mg, 0.151 mmol) and triethylsilane (26 $\mu$L, 0.166 mmol) were dissolved in DCM (2.5 mL). TFA (75 $\mu$L, 0.986 mmol) was added over 10 min and the reaction mixture was stirred at rt overnight. Saturated NaHCO$_3$ was added until the mixture pH ~7 and extracted with DCM (3 x 2 mL). The combined organic layers were dried over Na$_2$SO$_4$, filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc(1:1, 1:2, 0:1) to give 705 (39 g, 63% yield) as a white solid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.34 (br, 1H), 6.24 (br, 1H), 4.41 (s, 1H), 4.02 (d, $J$ = 11.8 Hz, 1H), 3.72 – 3.32 (m, 5H), 2.64 (dt, $J$ = 8.4, 6.5 Hz, 2H), 2.53 – 2.43 (m, 2H), 1.33 (t, $J$ = 8.5 Hz, 1H), 1.08 (s, 9H), 1.06 (s, 9H), 1.02 (d, $J$ = 2.1 Hz, 6H).

3-([(4R)-2,2-Di-tert-butyl-5,5-dimethyl-1,3,2-dioxasilinan-4-yl]formamido)-N-{2-[(2R,3S)-3-propyloxirane-2-carbonylsulfanyl]ethyl}propanamide (706). The compound
was synthesized using general procedure II, eluted with hexanes/EtOAc (1:2) to give 706 in 81% yield as a white solid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.32 (t, $J = 7.1$ Hz, 1H), 6.60 (br, 1H), 6.45 (br, 1H), 4.41 (s, 1H), 4.02 (d, $J = 11.9$ Hz, 1H), 3.62 (dt, $J = 12.7$, 6.6 Hz, 1H), 3.56 – 3.43 (m, 1H), 3.44 – 3.26 (m, 4H), 3.19 (d, $J = 2.2$ Hz, 1H), 2.94 (td, $J = 6.1$, 5.3, 2.0 Hz, 1H), 2.42 (t, $J = 6.1$ Hz, 2H), 1.63 – 1.41 (m, 4H), 1.07 (s, 9H), 1.06 (s, 9H), 1.01 (s, 3H), 1.00 (s, 3H), 0.95 (t, $J = 7.2$ Hz, 3H).

(2$R$)-2,4-Dihydroxy-3,3-dimethyl-N-[2-((2$R$)-3-propyloxirane-2-carbonylsulfanyl)ethyl]carbamoyl)ethyl]butanamide (707). The compound was synthesized using general procedure V, eluted with MeOH/EtOAc (1:19) to give 707 in 92% yield as a white solid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.39 (t, $J = 6.1$ Hz, 1H), 6.38 (t, $J = 5.9$ Hz, 1H), 3.99 (s, 1H), 3.55 (q, $J = 6.1$ Hz, 2H), 3.47 (s, 2H), 3.45 – 3.29 (m, 2H), 3.17 – 2.87 (m, 5H), 2.49 – 2.35 (m, 2H), 1.75 – 1.38 (m, 4H), 0.99 (s, 3H), 0.95 (t, $J = 7.2$ Hz, 3H), 0.90 (s, 3H).

(2$R$)-4-[(bis(Benzyloxy)phosphoryl)oxy]-2-hydroxy-3,3-dimethyl-N-[2-((2$R$)-3-propyloxirane-2-carbonylsulfanyl)ethyl]carbamoyl)ethyl]butanamide (708). The compound was synthesized using general procedure VI, eluted with CHCl$_3$/acetone (2:1, 1:1) to give 708 in 69% yield as a white solid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.40 – 7.25 (m, 11H), 6.53 (t, $J = 5.9$ Hz, 1H), 5.15 – 4.88 (m, 4H), 3.98 (dd, $J = 10.1$, 7.3 Hz, 1H), 3.87 (s, 1H), 3.67 – 3.44 (m, 3H), 3.42 – 3.28 (m, 3H), 3.09 (dd, $J = 6.3$, 4.8, 1.9 Hz, 1H), 2.97 (t, $J = 6.9$ Hz, 2H), 2.38 (td, $J = 5.9$, 2.6 Hz, 2H), 1.70 – 1.34 (m, 4H), 1.02 (s, 3H), 0.93 (t, $J = 7.2$ Hz, 3H), 0.79 (s, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 198.43, 172.67, 171.54, 135.70, 135.65, 135.64, 135.59, 128.90, 128.84, 128.19, 128.14, 73.80, 73.65, 73.59, 69.93, 69.89,
{Bis[(4-Methoxyphenyl)methoxy]phosphanyl}bis(propan-2-yl)amine (711). 4-Methoxybenzyl alcohol (0.61 mL, 4.95 mmol) and DIPEA (0.95 mL) were dissolved in THF (2.2 mL) and the solution was cooled to 0°C. N-(dichlorophosphino)-N-isopropylpropan-2-amine (500 mg, 2.47 mmol) was added. The reaction was stirred at 0 °C for 10 min and rt for 3 h. The reaction was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc (10:1) to give 711 (413 mg, 41% yield) as colorless liquid. $^1$H NMR and $^{31}$P NMR match the literature report.

(2R)-4-({bis[(4-Methoxyphenyl)methoxy]phosphoryl}oxy)-2-hydroxy-3,3-dimethyl-N-[2-((2R,3S)-3-propyloxirane-2-carbonylsulfanyl)ethyl]carbamoyl]ethyl]butanamide (712). The compound was synthesized using general procedure VII, eluted with CHCl$_3$/acetone (2:1, 1:1) to give 712 in 22% yield as a white solid. $^1$H NMR (300 MHz, CDCl$_3$) δ 7.34 – 7.17 (m, 6H), 6.94 – 6.78 (m, 4H), 6.48 (t, $J = 5.8$ Hz, 1H), 5.00 – 4.88 (m, 4H), 3.95 (dd, $J = 10.2$, 7.6 Hz, 1H), 3.85 (s, 1H), 3.78 (s, 6H), 3.51 (dt, $J = 7.5$, 5.2 Hz, 3H), 3.43 – 3.30 (m, 3H), 3.10 (ddd, $J = 6.5$, 4.9, 1.9 Hz, 1H), 2.97 (t, $J = 6.5$ Hz, 2H), 2.38 (td, $J = 5.9$, 3.1 Hz, 2H), 1.70 – 1.35 (m, 4H), 1.03 (s, 3H), 0.94 (t, $J = 7.2$ Hz, 3H), 0.78 (s, 3H).

$^{31}$P NMR (121 MHz, CDCl$_3$) δ 0.54.

(2S)-2-{{(tert-Butoxy)carbonyl}amino}-3-[(4-methoxyphenyl)methoxy]propanoic acid (723). Boc-Ser-OH (722, 400 mg, 1.95 mmol) was dissolved in DMF (10 mL) and the reaction mixture was cooled to 0 °C. NaH (60% in mineral oil, 194.9 mg, 4.87 mmol) was
added and the reaction was stirred at r0 °C for 1 h. The solution of PMBCl (278 µL, 2.048 mmol) in DMF (1 mL) was added and the reaction was stirred at rt overnight. H2O (5 mL) was added and extract with ether (2 x 10 mL). The aqueous layer was acidified with 10% citric acid until pH ~3 and extracted with ether (3 x 20 mL). The combined organic layers were washed with brine (5 mL), dried over Na2SO4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with DCM/acetone (1:1) to give 723 (218 g, 34% yield) as a white solid. 1H NMR matched the literature report.451

**tert-Butyl-N-[(2S)-1-[(2-(3-[(4R)-2,2-di-tert-butyl-5,5-dimethyl-1,3,2-dioxasilinan-4-yl]formamido)propanamido)ethyl]sulfanyl]-3-[(4-methoxyphenyl)methoxy]-1-oxopropan-2-yl]carbamate (724).** The compound was synthesized using general procedure II, eluted with hexanes/EtOAc (1:2) to give 724 in 85% yield as a white solid. 1H NMR (300 MHz, CDCl3) δ 7.37 (t, J = 6.5 Hz, 1H), 7.18 (d, J = 8.5Hz, 2H), 6.86 (d, J = 8.8Hz, 2H), 5.92 (s, 1H), 5.45 (d, J = 8.9 Hz, 1H), 4.40 (d, J = 3.3 Hz, 4H), 4.06 – 3.97 (m, 1H), 3.91 (d, J = 9.7 Hz, 1H), 3.79 (s, 3H), 3.67 – 3.26 (m, 6H), 3.00 (t, J = 6.6 Hz, 2H), 2.33 (d, J = 6.1 Hz, 2H), 1.45 (d, J = 1.4 Hz, 9H), 1.07 (s, 9H), 1.06 (s, 9H), 1.03 – 1.00 (m, 6H).

**tert-Butyl-N-[(2S)-1-[(2-3-[(2R)-4-(bis[(4-methoxyphenyl)methoxy]phosphoryl)oxy]-2-hydroxy-3,3-dimethylbutanamido]propanamido)ethyl]sulfanyl]-3-[(4-methoxyphenyl)methoxy]-1-oxopropan-2-yl]carbamate (726).** The compound was synthesized using general procedure VII, eluted with MeOH/EtOAc (1:19, 2:23) to give 726 in 25% yield as a white solid. 1H NMR (300 MHz, CDCl3) δ 7.33 – 7.20 (m, 4H), 7.20 – 7.12 (m, 2H), 6.89 – 6.78 (m, 6H), 6.29 (q, J = 5.5 Hz, 1H), 5.55 (dd, J = 8.7, 5.6 Hz, 1H), 5.01 – 4.85 (m, 4H), 4.45 – 4.29 (m, 3H), 4.13 – 3.83 (m, 4H), 3.77 (d, J = 3.1 Hz, 9H), 3.66 – 3.26
(m, 7H), 3.00 (t, J = 6.4 Hz, 2H), 2.31 (t, J = 6.1 Hz, 2H), 1.44 (d, J = 4.9 Hz, 9H), 1.03 (s, 3H), 0.79 (s, 3H).

(2R,3S)-S-2-Acetamidoethyl 3-propyloxirane-2-carbothioate (minimal donor, 727). The compound was synthesized using general procedure II, eluted with hexanes/EtOAc (1:2, 0:1) to give 727 in 81% yield as a white solid.

$^1$H NMR (400 MHz, CDCl$_3$) δ 5.99 (br, 1H), 3.50 – 3.29 (m, 3H), 3.09 (t, J = 4.7Hz, 1H), 3.02 – 2.95 (m, 2H), 1.93 (s, 2H), 1.68 – 1.37 (m, 4H), 0.93 (t, J = 7.4Hz, 3H).

$^{13}$C NMR (101 MHz, CDCl$_3$) δ 198.55, 170.50, 60.45, 60.03, 39.41, 33.59, 27.76, 23.34, 19.16, 13.91.

(2R,3S)-N-(2-Acetamidoethyl)-3-propyloxirane-2-carboxamide (minimal non-reactive donor, 728). The compound was synthesized using general procedure I, eluted with DCM/acetone (1:1). 81%. White solid.

$^1$H NMR (300 MHz, CDCl$_3$) δ 6.56 (br, 1H), 6.03 (br, 1H), 3.47 – 3.27 (m, 4H), 3.19 (d, J = 2.1 Hz, 1H), 2.94 (ddd, J = 6.0, 4.8, 2.1 Hz, 1H), 1.96 (s, 3H), 1.69 – 1.35 (m, 4H), 0.95 (t, J = 7.2 Hz, 3H).

N-(2-(2-(Tritylthio)ethylcarbamoyl)ethyl)pent-4-ynamide (745). The compound was synthesized using general procedure I, eluted with hexanes/EtOAc (1:1) to give 745 in 99% yield as a white solid.

$^1$H NMR (300 MHz, CDCl$_3$) δ 7.44 – 7.35 (m, 6H), 7.33 – 7.16 (m, 9H), 6.43 (t, J = 6.0 Hz, 1H), 5.67 (t, J = 5.8 Hz, 1H), 3.48 (q, J = 6.0 Hz, 2H), 3.07 (q, J = 6.2 Hz, 2H), 2.53 – 2.37 (m, 4H), 2.31 (q, J = 6.1, 5.2 Hz, 4H), 1.95 (t, J = 2.6 Hz, 1H).

N-(2-(2-Mercaptoethylcarbamoyl)ethyl)pent-4-ynamide (746). The compound 745 (23.0 mg, 0.049 mmol) and TES (8.6 µL, 0.054 mmol) were dissolved in DCM (0.75 mL). TFA (24 µL, 0.314 mmol) was added and the reaction mixture was stirred at rt for 1 h. Saturated NaHCO$_3$ solution was added till pH ~7. The mixture was extracted with DCM (3 x 2 mL).
The combined organic layers were washed with brine (2 mL), dried over Na$_2$SO$_4$, filtered, and filtrate was concentrated \textit{in vacuo}. The residue was purified flash chromatography on silica gel, eluting with EtOAc/MeOH (20:1) to give \textbf{746} (6.0 mg, 54\% yield) as a white solid.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 6.50 (s, 1H), 6.29 (s, 1H), 3.54 (q, $J = 5.9$ Hz, 3H), 3.42 (q, $J = 6.3$ Hz, 2H), 2.66 (dt, $J = 8.4$, 6.4 Hz, 2H), 2.55 – 2.42 (m, 4H), 2.42 – 2.34 (m, 2H), 1.97 (t, $J = 2.7$ Hz, 1H).

**General procedure for removing PMB.** Phosphate or PMB ether (1 eq) was dissolved in DCM/H$_2$O (110:1, 0.15 M). DDQ (9 eq) was added. The reaction was stirred at rt overnight. DCM was added to dilute the reaction mixture and extracted with H$_2$O 3 times. The combined aqueous layers were concentrated and dried under high vacuum to give a mixture of the corresponding substrate and DDQ-byproduct.
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