Progress Towards the Total Synthesis of Petrosaspongiolide L and Small Molecule Control of Nucleic Acid Function

by

Austin B. Kilgore

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This thesis was presented

by

Austin B. Kilgore

It was defended on

April 2, 2020

and approved by

Dr. Seth Childers, Assistant Professor, Department of Chemistry

Dr. Paul Floreancig, Professor, Department of Chemistry

Committee Member's Name, Academic Rank, Department Affiliation

Committee Chair: Dr. Alexander Deiters, Professor, Department of Chemistry

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Austin B. Kilgore, MS

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Since their discovery in 1997, the Petrosaspongiolide family of marine natural products has gained interest due to their potency and selectivity as phospholipase A2 inhibitors. Petrosaspongiolide L is unique within this family of compounds due to its pyridine containing tetracyclic ring structure that closely resembles the biologically active Spongidines. This pyridine moiety makes it an ideal candidate to highlight a microwave-mediated [2+2+2] cyclotrimerization reaction. Two different strategies were attempted in the synthesis of a key diyne intermediate.

Conditional control of nucleic acid function has become an important method of elucidating gene function as well as a potential candidate in the treatment of multiple disorders. Endogenously, non-coding RNA utilizes the specificity of Watson-Crick base pairing to regulate gene expression. Expanding on these interactions, researchers have succeeded in manipulating these pathways by exposing cells and organisms to synthetic plasmids and oligonucleotides. Modifications to synthetic nucleic acids (backbone modifications, caging groups, etc.) have increased their stability and specificity while also allowing spatiotemporal control of their function. Surprisingly, small molecule induced bioorthogonal transformations have yet to be applied to the control of modified nucleic acids. The progress towards the design and optimization of synthetic linkers capable of cleaving a nucleic acid backbone via a simple alloc deprotection is discussed herein.

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Al(iPrO)3: aluminum isopropoxide

Alloc: allyloxy carbonyl

ASO: antisense oligonucleotide

DABCO: 1,4-diazabicyclo[2.2. 2]octane

DDQ: 2,3-dichloro-5,6-dicyano-1,4-benzoquinone

DIBALH: diisobutylaluminium hydride

DIPEA: diisopropylethylamine

DMT: dimethoxy trityl

dsiRNA: dicer substrate RNA

GSH: glutathione

HPLC: high performance liquid chromatography

LNA: locked nucleic acid

m-CPBA: meta-chloroperoxybenzoic acid

MAPK: mitogen-activated protein kinase

MeOH: Methanol

miRNA: microRNA

mRNA: messenger RNA

n-Bu4NCN: tetrabutylammonium cyanide

ncRNA: non-coding RNA

ONB: ortho-nitrobenzyl

PAGE: polyacrylamide gel electrophoresis

PCC: pyridinium chlorochromate

PLA2: phospholipase A2

PMB: p-methoxybenzyl chloride

PMO: phosphorodiamidate mopholino

PNA: peptide nucleic acid

Proc: propargyloxy carbonyl

RISC: RNA-induced silencing complex

RNAi: RNA interference

SAR: structure-activity relationship

shRNA: short hairpin RNA

siRNA: small interfering RNA

TBDMS: tert-butyldimethylsilyl

TFO: triplex forming oligonucleotide

TMS: trimethylsilyl

1.0 Progress Towards the Total Synthesis of Petrosaspongiolide L

1.1 Introduction to Petrosaspongiolides

Petrosaspongiolides are a family of 18 compounds (named Petrosaspongiolide A-R), the first group of which (A-L) was originally reported by Paloma, *et al.* after their isolation from the sea sponge *Petrosaspongia nigra* in 1997.[1] While these compounds vary in their functional group makeup, they all share a sesterterpene or norsesterterpene ring structure (**1, Figure 1A**). This fused ring structure contributes to its reported biological activity as an anti-inflammatory agent due to its ability to interact favorably with the hydrophobic binding pocket of the enzyme phospholipase A2 (PLA_2).[2-4] PLA_2 is an important enzyme in phospholipid metabolism and signal transduction, acting by catalyzing the cleavage of the sn-2 acyl bond of certain phospholipids. Although all PLA_2 enzymes catalyze this specific reaction, they are generally split into 13 groups based on size, substrate, and cellular location. Activation of these enzymes naturally occurs through phosphorylation and/or Ca^{2+} ion exchange, but their misregulation is associated with inflammatory responses common in several neural disorders as well as exposure to bee and snake venom.[5] Due to the broad range of PLA_2 enzymes and the potential presence of multiple groups within a single cell or tissue, selectivity has become perhaps the highest priority in the study of potential inhibitors. The most potent PLA_2 inhibitors have been those containing a γ-hydroxybutenolide moiety, for example Petrosaspongiolide M (**2**), which has an IC_{50} of 1.6 μM against human synovial PLA_2 (**Figure 1B**).[2] This moiety acts by covalently binding to a free lysine in the binding pocket of PLA_2 through reductive amination of the hydrolyzed butenolide ring (**Figure 1C**).[3-4] Other notable compounds containing this γ-hydroxybutenolide have been

reported as PLA_2 inhibitors, including luffolide and manoalide, the latter of which made it to phase II of clinical trials.

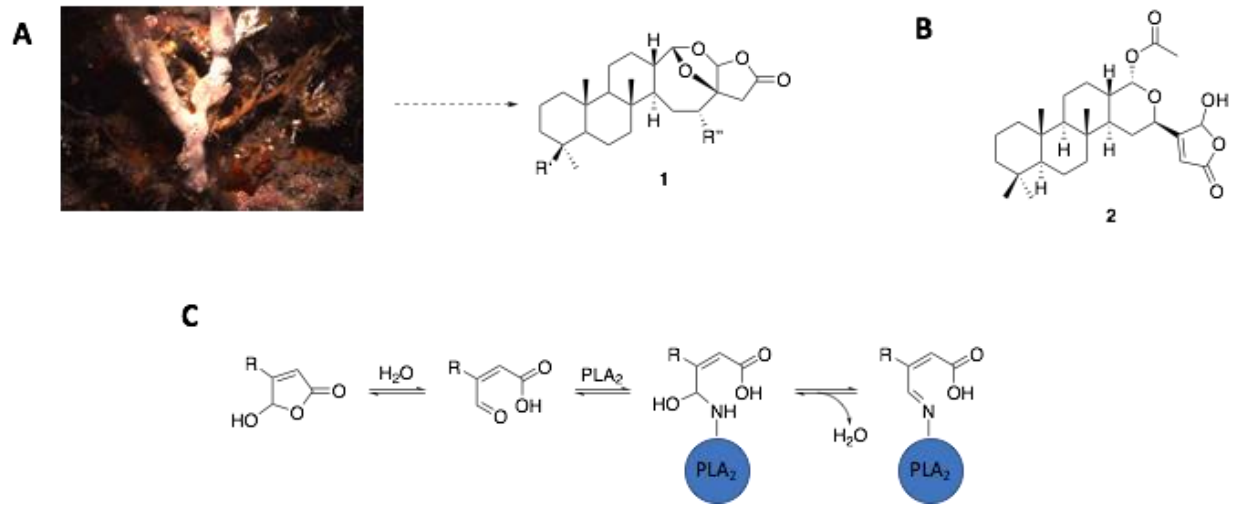


Figure 1. Introduction to Petrosaspongiolides.

(A) *Petrosaspongia nigra* sea sponge and the general structure of Petrosaspongiolide natural products. (B) Structure of Petrosaspongiolide M (2). (C) Mechanism for the covalent inhibition of PLA_2 through hydrolysis and reductive amination of the γ-hydroxybutenolide moiety.[3-4]

Petrosaspongiolide L (**3**) is unique within this family due to its tetracyclic pyridine ring structure (**Figure 2**). The biological activity of this compound has not been fully elucidated, but **3** has been reported as cytotoxic (IC_{50} = 5.7 µg/mL) towards human bronchopulmonary non-small-cell-lung-carcinoma (NSCLC-N6).[1] The structure of **3** more closely resembles the pyridinium containing Spongidines A-D (**4-7**), a class of natural products isolated from the genus *Spongia* in 2000 (**Figure 2**).[6] This initial isolation and characterization reported mild, although selective, activity of Spongidines towards human synovial PLA_2. Although these compounds are not as potent as those containing a γ-hydroxybutenolide functionality, their varied chemical makeup and PLA_2 group selectivity makes them useful in the design of novel inhibitors.

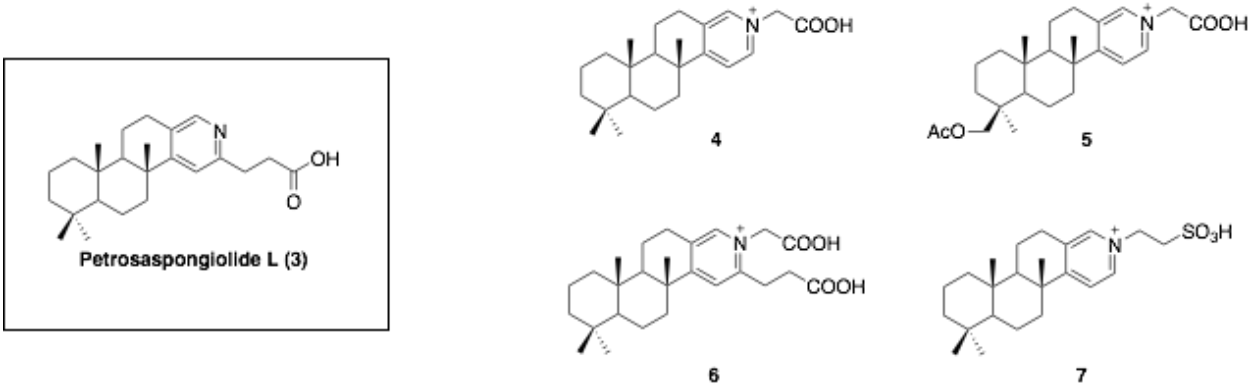
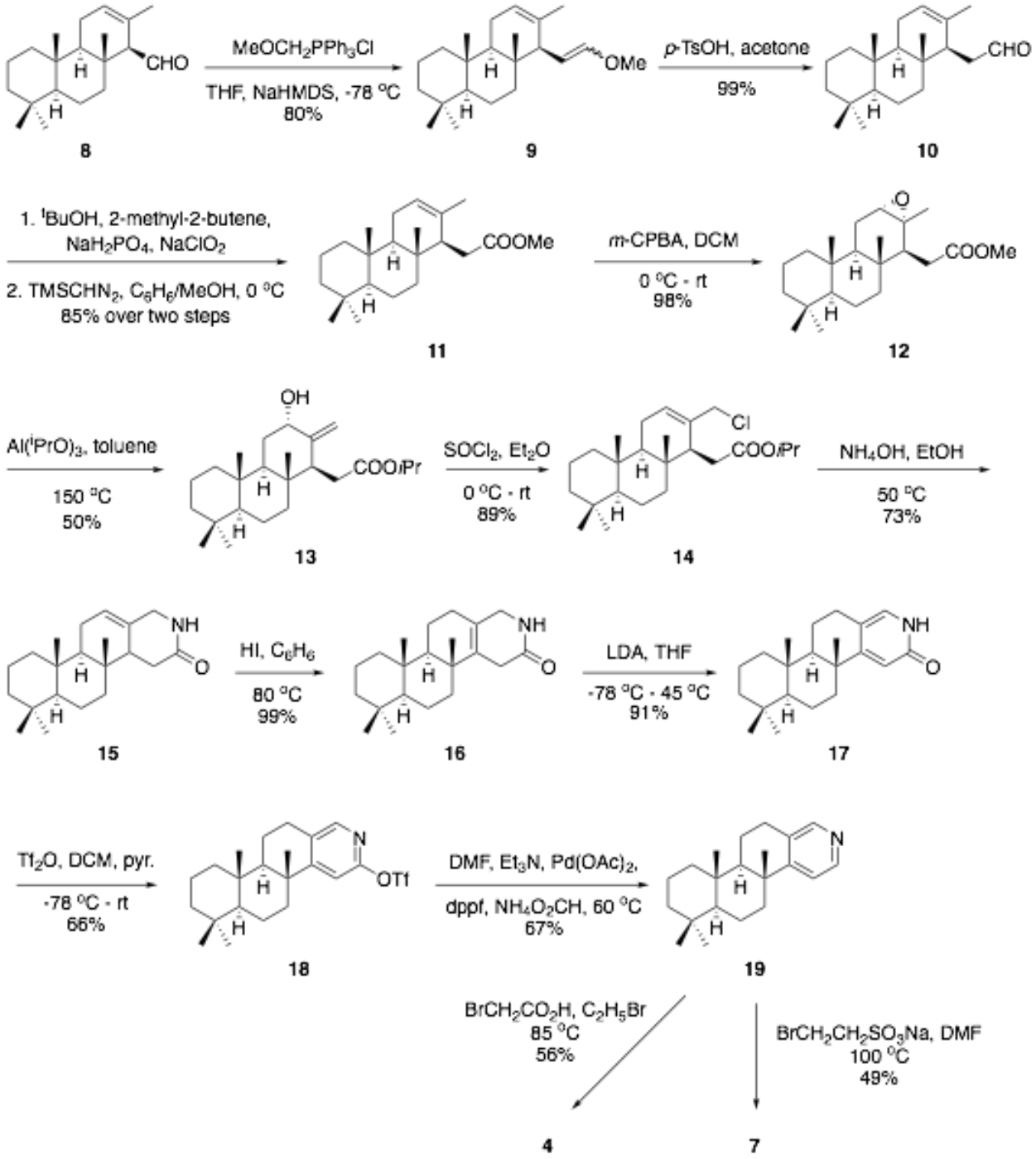


Figure 2. Spongidine and Petrosaspongiolide L Structure.

Confirmed structures of pyridine containing natural product Petrosaspongiolide L (3) and related Spongidines A-D (4-7).

There are currently two completed total syntheses of **4** and **7**, the most recent of which also included the total synthesis of Petrosaspongiolide L methyl ester (**3b**).[7-8] The first successful synthesis of **4** and **7** utilized a select aromatization of the aniline moiety followed by N-alkylation (**Scheme 1**).[7] Synthesis was initiated with **8** which was readily synthesized from the commercially available sclareol. Homologation of the aldehyde was carried out over two steps, first producing **9** through a Wittig reaction followed by quantitative hydrolysis to **10**. A two step oxidation of the aldehyde followed by direct esterification led to methyl ester **11**. *m*-CPBA was utilized to selectively epoxidize the alkene to form **12**. Epoxide formation was needed for the next allylic rearrangement in order to selectively chlorinate the exocyclic position. Ring opening was completed using $Al(_{i}PrO)_3$, which also led to an unintended transesterification forming **13**. Optimization of chlorinating conditions through varying solvents, thionyl chloride equivalence, and reaction time resulted in **14** in a high yield. The nitrogen was introduced using ammonium hydroxide which cyclized to form lactam **15**. Aromatization was initiated through quantitative isomerization to **16** using hydrogen iodide before deprotonation and oxidation in the presence of

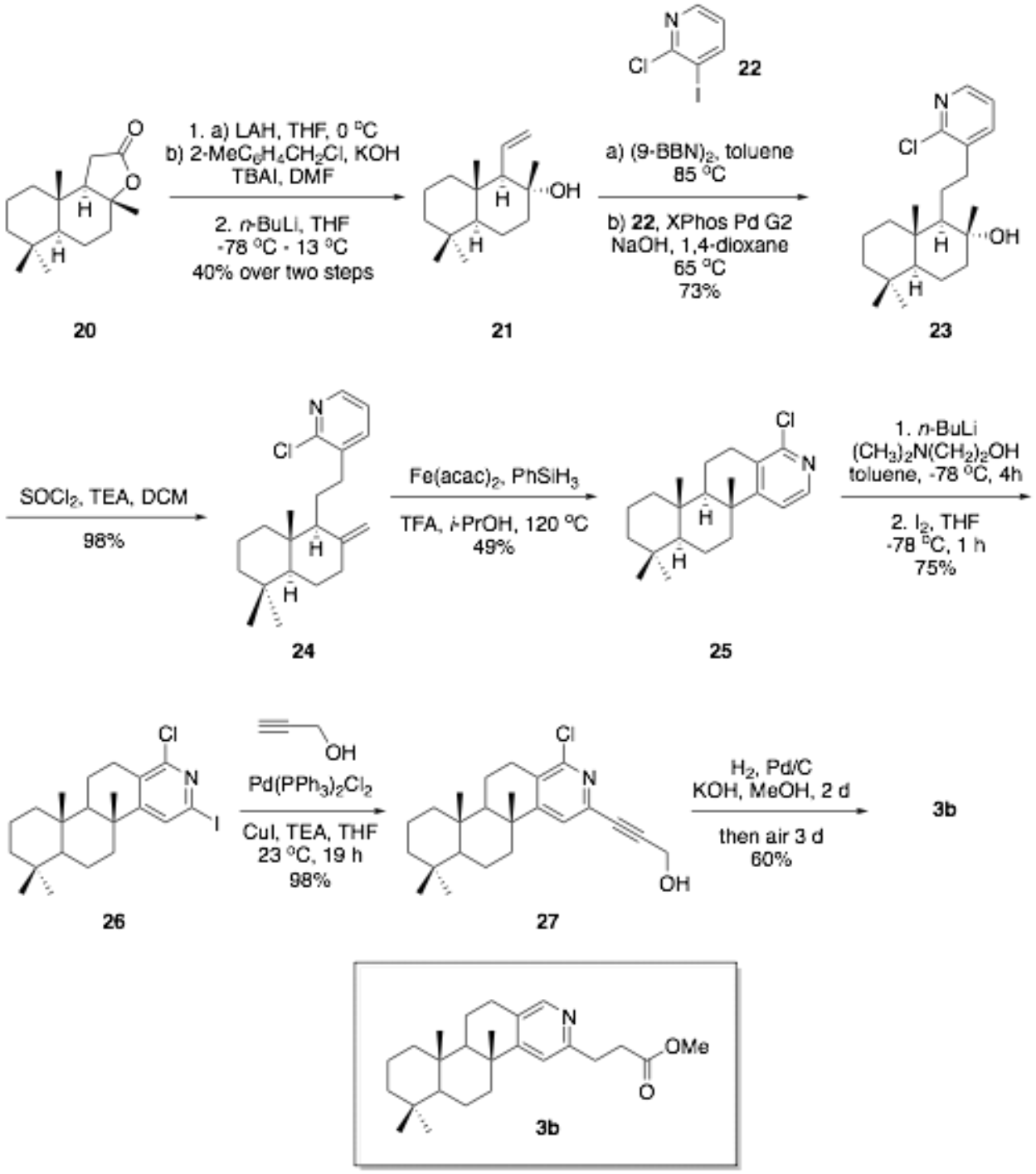
air afforded diene **17**. Triflate formation in **18** was reduced with palladium leading to the target aniline moiety in **19**. Finally, N-alkylation of **19** using bromo-derivatives of each respective side chain led to completion of both **4** and **7**.



Scheme 1. Reported Synthesis of Spongidines A and D.

The first reported total synthesis of **4** and **7** utilizing select aromatization and N-alkylation.[7]

While this synthesis allowed for variation through N-alkylation, it did not address further substitution of the pyridine ring as seen in **3**. The most recent synthesis, published by Bartels *et al.* in 2019, does address this through C-H functionalization within the pyridine ring followed by a Sonogashira coupling.[8] Compound **3b** was synthesized from commercially available sclareolide (**20**) first by its reduction and a Wittig-type fragmentation of an *in situ* benzyl ether to form **21** (**Scheme 2**). Coupling of the functionalized aniline **22** to **21** was achieved using Buchwald's precatalyst XPhos Pd G2 to form **23** in a moderate yield. Dehydration of **23** using thionyl chloride led to the exocyclic alkene found in **24** which guided cyclization through radical formation from an iron mediated hydrogen atom transfer. Attack of this tertiary radical to the electron-poor chloroaniline species closed the ring forming **25** as the only diastereomer. C-H functionalization of the aniline ring was successful through the formation of an aryllithium species that was trapped by iodine to form **26**. Propargyl alcohol was then coupled to **26** using Sonogashira coupling conditions to form compound **27**. Compound **3b** could then be completed through hydrogenation and a Heyns-type oxidation to convert the propargyl alcohol to the methyl ester. It was noted that hydrolysis of **3b** to complete **3** was confirmed by HRMS, but decomposed during isolation on silica gel.



Scheme 2. Reported Synthesis of Petrosaspongiolide L Methyl Ester.

First reported total synthesis of **3b**.[8]

While these total syntheses have been successful, efficient variability at the pyridine ring structure still remains an issue. Approaches towards the total synthesis of **3** and the development of methods to introduce new analogues helpful for SAR studies are discussed below.

1.2 Attempted approaches

The substituted pyridine ring structure provides an ideal candidate to highlight a microwave mediated [2+2+2] cyclotrimerization.[9-11] This general transformation involves the cyclization of 3 alkyne and/or nitrile moieties to form an aromatic system, most often facilitated by metal catalysts. This reaction tolerates a wide range of alkynes and nitriles which is ideal in developing varied analogues of natural products that may increase their activity towards a given target. The efficiency and fast reaction time also allows for a more high throughput method of achieving these structures.

The key precursor needed to complete this transformation for **3** is diyne **29** which is proposed to be synthesized from the commercially available sclareolide (**20, Figure 3**). Reaction of **29** with the nitrile **30** should result in the ethyl ester **31** which would only require hydrolysis to form **3**. Varying the R-group in **29** is a way of directing the regioselectivity of the cyclotrimerization reaction, but isn't anticipated to be needed in order to form the pyridine ring.[12] Knowledge of functional groups that increase activity in similar molecules could lead to a class of easily synthesized analogues depending on the nitrile or additional alkyne used.

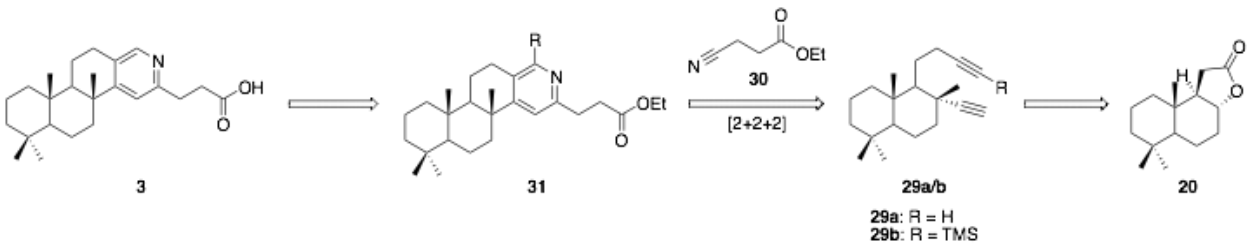
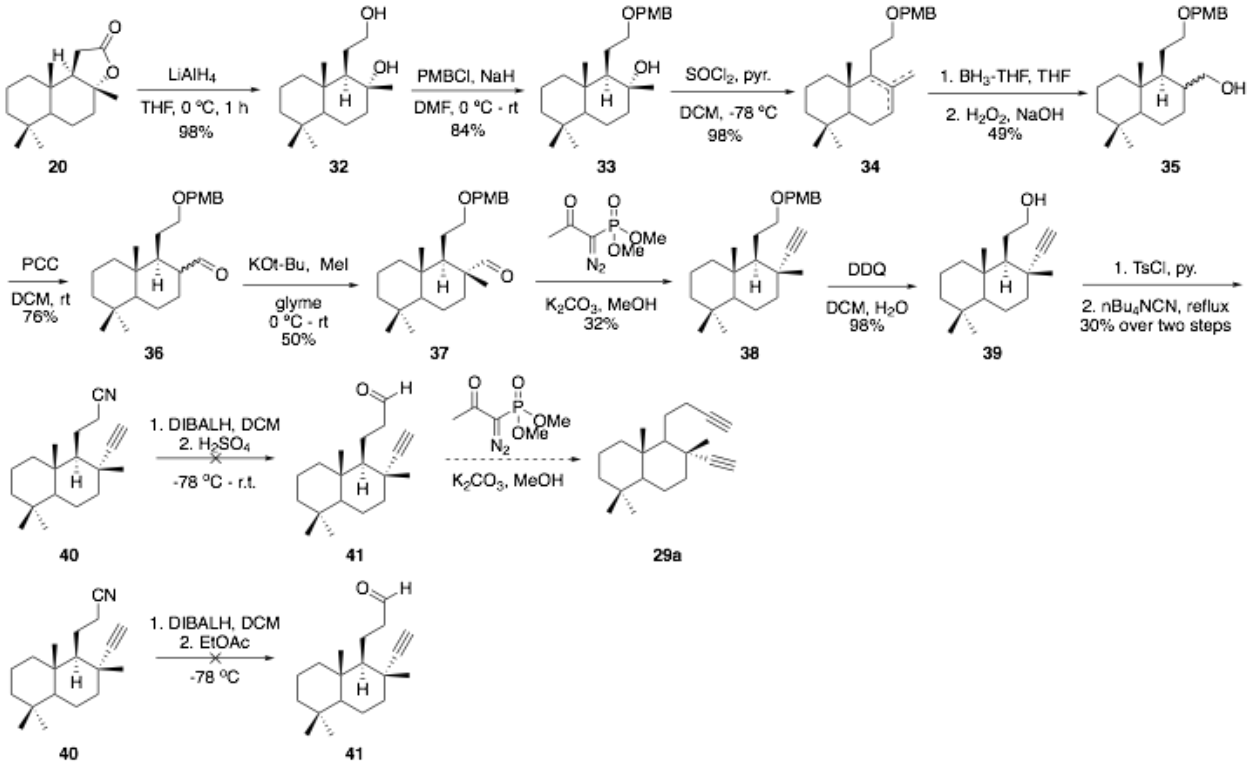


Figure 3. Retrosynthetic Analysis of Petrosapongiolide L (3).

Progress towards this class of natural products has stalled in the Deiters' lab due to issues in installing both of the alkyne groups in the cyclotrimerization precursor **29**.[13] Though unsuccessful in completing the synthesis of **3**, this previous work led us to assume that installation of the internal alkyne should be done prior to the terminal alkyne. The initial approach involved first the reduction of **20** with lithium aluminum hydride followed by the protection of the primary alcohol with *p*-methoxybenzyl (PMB) chloride to afford **33** (**Scheme 3**).[13] Dehydration of the remaining alcohol using thionyl chloride resulted in a mixture of alkene isomers **34**, the major isomer being the exocyclic alkene (~ 55% of product). BH_3•THF reacted exclusively with the major isomer and was quantitatively oxidized to **35**. This regioselectivity for this oxidation could also be seen by ^1H NMR of the remaining starting material (**34**) that contained only minor isomers. Oxidation of **35** with PCC resulted in aldehyde **36**, which was then methylated at the alpha position using sodium *tert*-butoxide and iodomethane. Installation of the first alkyne **38** was achieved through homologation of aldehyde **37** using the Ohira-Bestmann reagent and K_2CO_3 in MeOH. A Corey-Fuchs reaction was also attempted to increase the yield of this alkyne formation, but no conversion was observed.[13] The stereochemistry of the tertiary propargyl carbon has been designated as *R* based on evidence provided by 2-D NMR but a crystal structure is needed to confirm.[14] The PMB ether was deprotected using DDQ to afford alcohol **39**. This alcohol was then tosylated using tosyl chloride and reacted with *n*-Bu_4NCN to

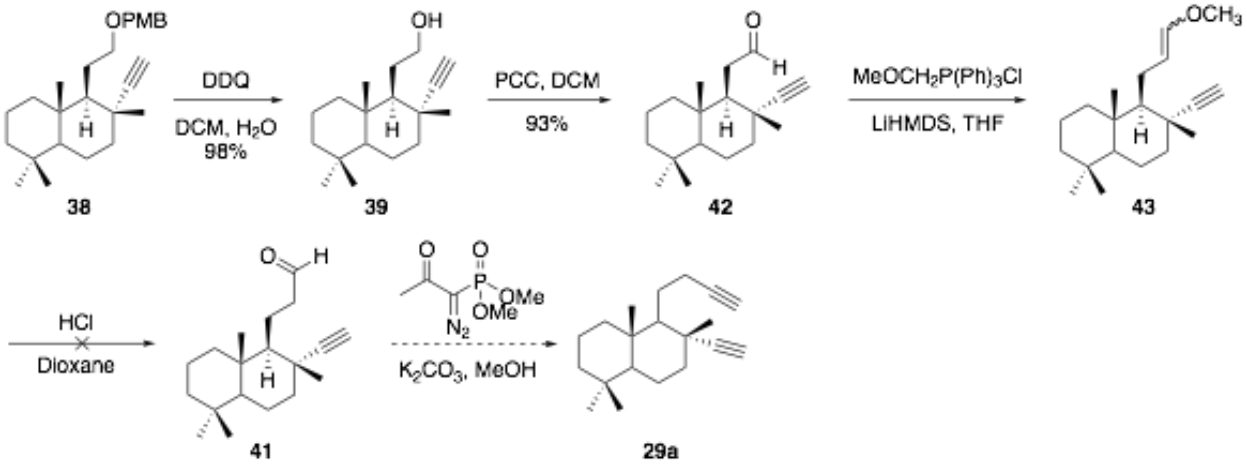
form the primary nitrile **40** in one pot.[13,15] Conversion of this nitrile to aldehyde **41** would allow for a second Seyferth-Gilbert homologation to install the final alkyne. Two different conditions were attempted for the reduction of nitrile **40**, the first of which was initiated by a single hydride transfer using DIBALH.[16] Sulfuric acid (0.5 M) was then added to convert this imine to the aldehyde seen in **41**, but no product was seen. Fear of decomposition in this acidic environment led to milder conditions using ethyl acetate and an acidic workup.[17] Again, this reaction resulted in multiple side products, but the desired product **41** was not observed. Failure to complete this synthesis led to an alternative route in installing the second alkyne.



Scheme 3. First Attempted Synthesis of Petrosaspongiolide L.

First attempted synthesis of key intermediate **29a**. See experimental section for detailed reaction conditions and characterization data.

The second approach involved a Wittig-type homologation in order to synthesize aldehyde **41**.[18] After PMB deprotection, the primary alcohol **39** was oxidized using PCC to form **42** (**Scheme 4**). This was exposed to Wittig conditions to first form the unsaturated ether **43**, which was then treated with HCl to form the target aldehyde **41** in one pot. While partial conversion to **43** was observed by ^{1}H NMR, no new aldehyde peaks were seen. Additionally compounds **42** and **43** could not be successfully isolated from the reaction mixture because of their near identical polarities and a triphenylphosphine side product. This coupled with the subsequent Seyferth-Gilbert homologation, a reaction in which the yield has already been low, led to more research into more efficient and higher yielding routes to synthesize diyne **29**.



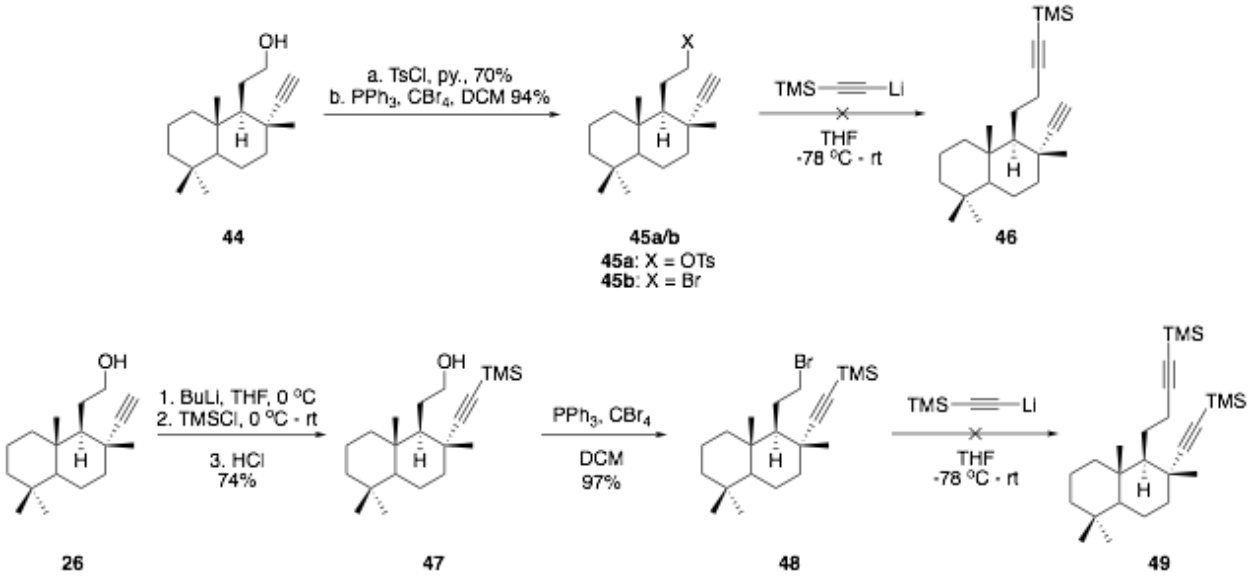
Scheme 4. Second Attempted Synthesis of Petrosaspongiolide L.

Second approach towards the synthesis of **9a** highlighting a Wittig-type transformation.

1.3 Conclusion and outlook

Two different approaches were attempted in the synthesis of an important precursor in the total synthesis of **3**. This synthesis was designed to showcase the ease and versatility of a microwave mediated [2+2+2] cyclotrimerization to form substituted pyridine ring structures in diverse natural products. The major issue in the proposed syntheses has been the addition of the terminal alkyne to a substrate already containing an internal primary alkyne. Future directions should focus on optimization of this final alkyne addition.

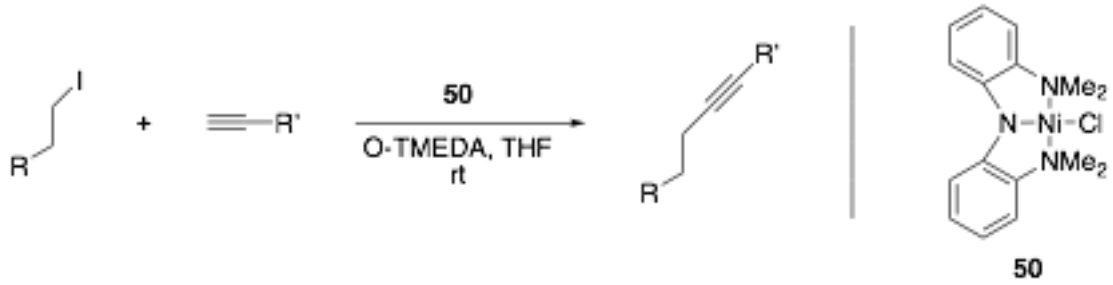
One potential approach towards the synthesis of **29** involves the direct coupling of the terminal alkyne. Past attempts, by Y. Zhou in the Deiters' lab, have included the direct addition of this alkyne to **45a/b** using trimethylsilyl (TMS) protected lithium acetylide (**Scheme 5**).[13] No conversion to **46** was observed likely due to deprotonation of the existing alkyne by the lithium reagent. To prevent this issue, the internal alkyne was first protected with TMS (**47**), but still no product was seen under the same coupling conditions.



Scheme 5. Previous Attempts of Direct Alkyne Addition.

Attempted direct couplings of the terminal alkyne using TMS protected lithium acetylide reagents by the Deiters' lab.[13]

More recently, Vechorkin, *et al*. reported that the use of a nickel pincer catalyst known as "nickamine" (**32)** could selectively couple alkynyl grignard reagents to primary alkyl iodides (**Scheme 6**).[19] This reaction offers a relatively broad scope of reaction partners, but has not yet been reported on a substrate containing another alkyne. Optimization of this reaction would need to be conducted on a similar test substrate, but could offer an efficient way of producing the desired diyne **9**.



Scheme 6. Model Study Using Nickamine.
General reaction for the coupling of primary alkynes with primary alkyl iodides using catalyst **32**.

Successful synthesis of **29** could lead to an important intermediate in the synthesis of pyridine natural products and their derivatives, especially those related to compounds **2-7**, which have proven to be inhibitors of PLA_2.

1.4 Experimental

All reactions were performed in flame-dried glassware under a nitrogen environment and stirred magnetically unless stated otherwise. All chemicals obtained directly from commercial sources were used without further purification unless indicated otherwise. ^{1}H and ^{13}C NMR spectra were obtained from a Bruker Avance III 300, 400, or 500 MHz with chemical shifts reported relative to either residual $CHCl_3$ (7.26 ppm), DMSO (2.50 ppm), or CH_3OD (3.30 ppm). Mass spectra analysis was performed by University of Pittsburgh facilities.

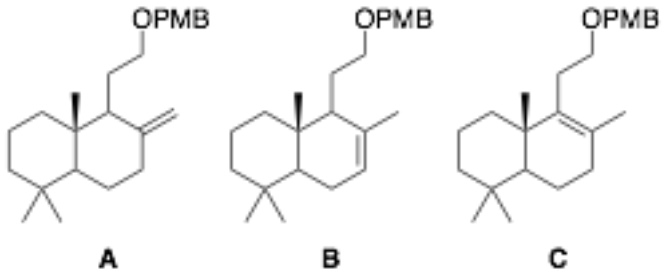
(1*R*,2*R*,8a*S*)-1-(2-Hydroxyethyl)-2,5,5,8a-tetramethyldecahydronaphthalen-2-ol (32). Lithium aluminum hydride (1.51 g, 39.7 mmol) was slowly added to a stirring solution of **28** (9.94 g, 39.7 mmol) in THF (200 mL) at 0 °C. The mixture was stirred at 0 °C for 2 hours. After the disappearance of starting material by TLC, the reaction mixture was quenched with ethyl acetate (10 mL) then concentrated *in vacuo*. Resulting solid was dissolved in diethyl ether (200 mL) then washed with 1 N HCl (100 mL), water (100 mL), and brine (100 mL). The organic layer was then dried over Na_2SO_4 (~ 3 g), filtered, then concentrated *in vacuo*. The final white solid was used without further purification (9.84 g, 98%). Analytical data matches that previously reported in the literature for this compound.[13] ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.76 - 3.82 (m, 1 H), 3.43 - 3.51 (m, 1 H), 1.87 - 1.93 (dt, J = 12.1, 3.2 Hz, 1 H), 1.62 - 1.67 (m, 5 H), 1.53 - 1.57 (m, 1 H), 1.34 - 1.49 (m, 3 H), 1.25 - 1.30 (m, 2 H), 1.20 (s, 3 H), 1.09 - 1.15 (m, 1 H), 0.92 - 0.97 (m, 2 H), 0.88 (s, 3 H), 0.79 (s, 6 H). ^{13}C NMR (500 MHz, $CDCl_3$) δ 73.2, 64.2, 59.1, 56.0, 44.4, 41.2, 39.3, 39.0, 33.4, 27.9, 24.7, 21.5, 20.5, 18.4, 15.3. HRMS (ESI^+) calcd for $C_{16}H_{30}O_2Na$ $(M + Na)^+$ 277.21380, found 277.21552.

(1*R*,2*R*,8a*S*)-1-(2-((4-Methoxybenzyl)oxy)ethyl)-2,5,5,8a-tetramethyldecahydronaphthalen-2-ol (33). The diol **32** (4.43 g, 17.42 mmol) was dissolved in

dimethylformamide (44 mL) and then cooled to 0 °C. NaH (2.09 g, 53.26 mmol) was added to the reaction mixture which was then stirred for 10 min at 0 °C. TBAI (562 g, 1.74 mmol) was added in one portion, before adding *p*-methoxybenzyl chloride (3.53 mL, 26.13 mmol) dropwise. The mixture was allowed to stir at 0 °C for 1 hour then at room temperature overnight. The reaction mixture was quenched with cold water (20 mL) , then extracted with ether (3 x 25 mL). The combined organic layer was washed with water (100 mL) and brine (100 mL), dried over Na_2SO_4 (~ 2 g), filtered, and concentrated *in vacuo*. The product was purified using flash chromatography on silica gel, eluting with hexanes/EtOAc (4:1) to give **33** as a white solid (5.44 g, 84%). Analytical data matches that previously reported in the literature for this compound.[13] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.23 - 7.26 (d, J = 8.7 Hz, 2 H), 6.85 - 6.88 (d, J = 8.7 Hz, 2 H), 4.45 (s, 2 H), 3.79 (s, 3 H), 3.58 - 3.64 (m, 1 H), 3.29 - 3.36 (m, 1 H), 1.86 - 1.92 (dt, J = 12.3, 3.1, 1 H), 1.70 - 1.80 (m, 1 H), 1.44 - 1.68 (m, 5 H), 1.34 - 1.42 (m, 3 H), 1.15 - 1.30 (m, 3 H), 1.13 (s, 3 H), 0.87 - 0.92 (m, 1 H), 0.87 (s, 3 H), 0.83 - 0.85 (m, 1 H), 0.77 - 0.78 (d, J = 1.6 Hz, 6 H). HRMS (ESI$^+$) calcd for $C_{48}H_{76}O_6$ $(2\ M + H)^+$ 749.57146, found 749.56932.

Synthesis of alkene isomers (34). Alcohol **33** (2.20 g, 5.87 mmol) and pyridine (0.950 mL, 11.74 mmol) were added to DCM (30 mL) then the reaction mixture was cooled to -78 °C. $SOCl_2$ (2.12 mL, 29.4 mmol) was added dropwise then the mixture was stirred for 1 hour. The reaction mixture was quenched with saturated $NaHCO_3$ (20 mL) and allowed to warm to room temperature. The organic layer was separated then the aqueous layer was extracted with DCM (3 x 15 mL). The combined organic layer was dried over Na_2SO_4 (~ 1 g), filtered, and concentrated *in vacuo*. The product was purified using flash chromatography on silica gel eluting with hexanes/EtOAc (9:1) to give a mixture of alkene isomers (2.04 g, 98%). Analytical data matches that previously reported in the literature for this compound.[13] Isomeric ratio (**A**:**B**:**C** = 3:1:1)

determined from diagnostic peaks in ^{1}H NMR (300 MHz, $CDCl_3$) **A**: δ 4.51 (apparent s, 1 H), **B**: δ 5.40 (bs, 1 H), **C**: δ 2.24 - 2.32 (m, 1 H).



((1*S*,8a*R*)-1-(2-((4-Methoxybenzyl)oxy)ethyl)-5,5,8a-trimethyldecahydronaphthalen-2-yl)methanol (35). Alkene isomers **34** (4.7 g, 13.27 mmol) were dissolved into tetrahydrofuran (33 mL) then cooled to 0 °C. A 1M solution of borane-tetrahydrofuran complex in tetrahydrofuran (17.3 mL, 17.3 mmol) was added dropwise, then the reaction mixture was stirred for 3 hours at 0 °C. Sodium hydroxide (30 mL, 3N solution) and hydrogen peroxide (24 mL, 30% solution) were added to the mixture which was stirred at 0 °C for 1 hour then at room temperature for an additional 1 hour. The reaction mixture was quenched with cold water (10 mL) then extracted with diethyl ether (3 x 20 mL). The combined organic layer was rinsed with sodium bisulfite (0.10M, 2 x 10 mL), saturated $NaHCO_3$ (15 mL), brine (15 mL), dried over Na_2SO_4 (~ 1 g), filtered, and concentrated *in vacuo*. The product was purified using flash chromatography on silica gel, eluting with hexanes/EtOAc (9:1 → 5:1) to give **35** as a clear oil (2.44 g, 49%). Analytical data matches that previously reported in the literature for this compound.[13] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 - 7.27 (d, J = 8.7 Hz, 2 H), 6.86 - 6.89 (d, J = 8.7 Hz, 2 H), 4.42 - 4.43 (d, J = 3.0 Hz, 2 H), 3.80 (s, 3 H), 3.68 - 3.71 (d, J = 10.0, 1 H), 3.46 - 3.51 (t, J = 6.7 Hz, 2 H), 1.94 - 1.99 (m, 1 H), 1.72 - 1.80 (m, 2 H), 1.44 - 1.64 (m, 7 H), 1.33 - 1.42 (m, 4 H), 1.24 - 1.30 (m, 1 H), 1.08 - 1.21 (qd, J = 13.7, 4.2 Hz, 1 H) 0.89 - 0.90 (d, J = 2.4 Hz, 1 H), 0.85 (s, 3 H), 0.79 (s, 3 H), 0.70 (s, 3 H).

(1*S*,8a*R*)-1-(2-((4-Methoxybenzyl)oxy)ethyl)-5,5,8a-trimethyldecahydronaphthalene-2-carbaldehyde (36). Alcohol **35** (4.50 g, 12.01 mmol) and pyridinium chlorochromate (5.18 g, 24.02 mmol) were dissolved in DCM (120 mL) and stirred at room temperature for 2 hours. The reaction mixture was filtered over silica gel and washed with excess DCM. The product was purified using flash chromatography on silica gel, eluting with hexanes/EtOAc (10:1 → 5:1) to give **36** as a clear oil (3.40 g, 76%). Analytical data matches that previously reported in the literature for this compound.[13] ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.99 (s, 1H), 7.23 - 7.26 (d, J = 8.7 Hz, 2 H), 6.87 - 6.89 (d, J = 8.7 Hz, 2 H), 4.39 - 4.49 (d, J = 7.2 Hz, 2 H), 3.80 (s, 3 H), 3.56 - 3.61 (t, J = 6.5 Hz, 2 H), 2.31 - 2.44 (m, 2 H), 1.82 - 1.96 (m, 2 H), 1.65 - 1.72 (m, 2 H), 1.52 - 1.58 (m, 2 H), 1.43 - 1.49 (m, 1 H), 1.29 - 1.40 (m, 4 H), 1.10 - 1.20 (m, 1 H), 0.89 - 0.98 (m, 3 H), 0.86 (s, 3 H), 0.78 (s, 3 H), 0.73 (s, 3 H).

(1*R*,2*R*,8a*S*)-1-(2-((4-Methoxybenzyl)oxy)ethyl)-2,5,5,8a-tetramethyldecahydronaphthalene-2-carbaldehyde (37). Sodium tert-butoxide (451 mg, 4.70 mmol) was dissolved into dimethoxyethane (34 mL) and then cooled to 0 °C. A solution of **36** (250 mg, mmol) in iodomethane (2.09 mL, 33.55 mmol) was slowly added and the reaction mixture was stirred at room temperature for 2 hours. Another 3 eq of sodium tert-butoxide (193 mg, 2.01 mmol) was added in 3 portions over 30 min. Diethyl ether (20 mL) was added to dilute the mixture before being quenched with cold water (20 mL). The aqueous layer was extracted with ether (3 x 15 mL), then the combined organic layer was washed with brine (20 mL), dried over Na_2SO_4 (~ 2 g), filtered, and concentrated *in vacuo*. The product was purified using flash chromatography on silica gel, eluting with hexanes/EtOAc (10:1) to give **37** as a clear oil (130 mg, 50%). Analytical data matches that previously reported in the literature for this compound.[13] ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.79 (s, 1 H), 7.25 - 7.28 (d, J = 8.7 Hz, 2 H), 6.87 - 6.90 (d, J =

8.7 Hz, 2 H), 4.45 (s, 2 H), 3.81 (s, 3 H), 3.38 - 3.48 (m, 2 H), 2.20 - 2.26 (dt, J = 13.3, 3.2 Hz, 1 H), 1.98 - 2.08 (m, 1 H), 1.69 - 1.81 (m, 1 H), 1.61 - 1.66 (m, 1 H), 1.54 - 1.57 (m, 1 H), 1.50 - 1.52 (m, 1 H), 1.26 - 1.48 (m, 5 H), 1.06 - 1.18 (m, 2 H), 0.95 - 1.03 (m, 4 H), 0.82 - 0.90 (m, 6 H), 0.75 (s, 3 H), 0.71 (s, 3 H).

(4a*S*,5*R*,6*R*)-6-Ethynyl-5-(2-((4-methoxybenzyl)oxy)ethyl)-1,1,4a,6-tetramethyldecahydronaphthalene (38). Aldehyde **37** (200 mg, 0.52 mmol), potassium carbonate, (216 mg, 1.56 mmol) and dimethyl (1-diazo-2-oxopropyl)phosphonate (248 mg, 1.30 mmol) were added to MeOH (3.7 mL) and the mixture was stirred at room temperature for 20 hours. Additional dimethyl (1-diazo-2-oxopropyl)phosphonate (248 mg, 1.30 mmol) was added and the reaction mixture was stirred for 24 hours. Diethyl ether (10 mL) was added to dilute the mixture before washing with water (10 mL), saturated $NaHCO_3$ (10 mL), and water (10 mL). The combined organic layer was dried over Na_2SO_4 (~ 1 g), filtered, and concentrated *in vacuo*. The product was purified using flash chromatography on silica gel, eluting with hexanes/EtOAc (9:1) to give **38** as a clear oil (63 mg, 32%). Analytical data matches that previously reported in the literature for this compound.[13] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 - 7.28 (d, J = 8.7 Hz, 2 H), 6.86 - 6.89 (d, J = 8.7 Hz, 2 H), 4.44 (s, 2 H), 3.81 (s, 3 H), 3.37 - 3.45 (m, 2 H), 2.05 (s, 1 H), 1.92 - 1.98 (dt, J = 13.0, 3.2 Hz, 1 H), 1.75 - 1.81 (m, 2 H), 1.52 - 1.71 (m, 5 H), 1.21 - 1.44 (m, 3 H), 1.16 (s, 3 H), 1.05 (s, 3 H), 0.83 (s, 3 H), 0.85 (s, 3 H), 0.74 - 0.79 (m, 2 H), 0.59 - 0.61 (t, J = 3.7 Hz, 1 H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 159.1, 130.8, 129.1, 113.7, 91.2, 72.4, 72.2, 70.1, 56.2, 55.5, 55.3, 42.7, 42.0, 39.3, 39.3, 36.5, 33.5, 33.3, 31.5, 27.8, 21.8, 19.7, 18.3, 14.9.

2-((1*R*,2*R*,8a*S*)-2-Ethynyl-2,5,5,8a-tetramethyldecahydronaphthalen-1-yl)ethan-1-ol (39). PMB ether **38** (56 mg, 0.15 mmol) was dissolved in DCM/H_2O (1.7 mL, 10:1) then cooled to 0 °C. 2,3-Dichloro-5,6-dicyano-*p*-benzoquinone (50 mg, 0.22 mmol) was added and the

mixture was stirred for 1.5 hours at 0 °C. The reaction mixture was quenched with saturated $NaHCO_3$ (3 mL) and then extracted with DCM (3 x 5 mL). The combined organic layer was washed with brine (10 mL), dried over Na_2SO_4 (~ 500 mg), filtered, and concentrated *in vacuo*. The product was purified using flash chromatography on silica gel, eluting with hexanes/EtOAc (8:1) to give **39** as a clear oil (45 mg, 98%). ^1H NMR (300 MHz, $CDCl_3$) δ 3.52 - 3.68 (m, 2 H), 2.06 (s, 1 H), 1.93 - 2.00 (dt, J = 13.0, 3.2 Hz, 1 H), 1.62 - 1.77 (m, 4 H), 1.52 - 1.59 (m, 2 H), 1.39 - 1.44 (m, 1 H), 1.24 - 1.37 (m, 3 H), 1.19 (s, 3 H), 1.08 - 1.17 (m, 1 H), 1.06 (s, 3 H), 0.86 (s, 3 H), 0.83 (s, 3 H), 0.74 - 0.82 (m, 2 H), 0.63 - 0.65 (t, J = 3.7 Hz, 1 H). ^{13}C NMR was not determined for this compound. HRMS (ESI^+) calcd for $C_{18}H_{31}O$ $(M + H)^+$ 263.2375, found 263.2362.

3-((1*R*,2*R*,8a*S*)-2-Ethynyl-2,5,5,8a-tetramethyldecahydronaphthalen-1-yl)propanenitrile (40). Alcohol **39** (20 mg, 0.08 mmol) and tosyl chloride (29 mg, 0.15 mmol) were added to pyridine (0.70 mL) then stirred at room temperature for 18 hours. The reaction mixture was quenched with cold water (1 mL) and extracted with ether (3 x 1 mL). The combined organic layer was washed with 1 N HCl (2 mL), saturated $NaHCO_3$ (2 mL), brine (2 mL), dried over Na_2SO_4 (~ 100 mg), filtered, and concentrated *in vacuo*. Resultant crude oil was used without further purification (18 mg).

Crude tosylate (18 mg) and tetrabutylammonium cyanide (32 mg, 0.12 mmol) were dissolved into dimethylformamide (0.5 mL) and the mixture was heated to 60 °C and stirred for 4 hours. Cold water (1 mL) was added to quench the reaction mixture before extracting with diethyl ether (3 x 1 mL). The combined organic layer was washed with 1 N HCl (1 mL), saturated $NaHCO_3$ (1 mL), water (1 mL), dried over Na_2SO_4 (~ 100 mg), filtered, and concentrated *in vacuo*. The product was purified using flash chromatography on silica gel,

eluting with hexanes/EtOAc (10:1) to give **40** as a clear oil (7 mg, 30% over two steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.35 - 2.41 (m, 2 H), 2.09 (s, 1 H), 1.94 - 2.00 (dt, J = 13.0, 3.2 Hz, 1 H), 1.81 - 1.88 (m, 1 H), 1.55 - 1.68 (m, 4 H), 1.30 - 1.46 (m, 4 H), 1.23 (s, 1 H), 1.08 - 1.19 (m, 2 H), 1.05 (s, 3 H), 0.87 (s, 3 H), 0.83 (s, 3 H), 0.79 - 0.81 (m, 1 H), 0.73 - 0.75 (t, J = 3.7, 1 H). Quantity of product was not sufficient to obtain a ^{13}C NMR. HRMS was not determined for this compound.

2-((1*R*,2*R*,8a*S*)-2-Ethynyl-2,5,5,8a-tetramethyldecahydronaphthalen-1-yl)acetaldehyde (42). An identical procedure was followed as for compound **36** (43 mg, 0.16 mmol). The product was purified using flash chromatography on silica gel eluting with hexanes/EtOAc (9:1)to give **42** as a clear oil. (40 mg, 93%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.81 - 9.82 (apparent t, 1 H), 2.51 - 2.75 (m, 2 H), 2.10 (s, 1 H), 1.97 - 2.04 (dt, J = 13.0, 3.2 Hz, 1 H), 1.67 - 1.70 (m, 1 H), 1.57 - 1.64 (m, 4 H), 1.45 - 1.53 (m, 1 H), 1.35 - 1.42 (m, 4 H), 1.14 - 1.17 (m, 3 H), 1.04 (s, 3 H), 0.90 - 0.99 (m, 2 H), 0.88 (s, 3 H), 0.83 (s, 3 H), 0.62 - 0.80 (m, 1 H). HRMS (ESI^{+}) calcd for $C_{18}H_{29}O$ $(M + H)^{+}$ 261.2218, found 261.2203.

Towards the synthesis of 3-((1*R*,2*R*,8a*S*)-2-Ethynyl-2,5,5,8a-tetramethyldecahydronaphthalen-1-yl)propanal (41).

(Methoxymethyl)triphenylphosphonium chloride (16 mg, 0.046 mmol) was dissolved in tetrahydrofuran (200 µL) and cooled to - 78 °C while stirring. A 1 M solution of lithium bis(trimethylsilyl)amide (46 µL) in tetrahydrofuran was added to the cooled mixture and stirred at -78 °C for 30 min. A solution of **42** (10 mg, 0.038 mmol) in tetrahydrofuran (200 µL) was added to the stirring solution dropwise. The mixture was stirred at - 78 °C for 1 hour, then allowed to warm to room temperature before being cooled back to 0 °C. The reaction mixture was diluted with ether (1 mL), washed with water (1 mL), brine (1 mL), dried over Na_2SO_4 (~ 25

mg), filtered, and concentrated *in vacuo*. The crude product was used without further purification.

A solution of 2 N HCl (500 μL) was added to a solution of crude **43** (10 mg) in dioxane (1 mL) at room temperature and was stirred for 20 minutes. Diethyl ether (1 mL) was added to the reaction mixture and then washed with water (1 mL), brine (1 mL), dried over Na_2SO_4, filtered, then concentrated *in vacuo*. Product could not be purified or characterized fully.

2.0 Metal Catalyzed Cleavage of Nucleic Acids

2.1 Introduction to conditional control of nucleic acid function

Manipulation and control of nucleic acid activity has been a major approach in discovering gene function and as a potential method of therapy in multiple diseases and disorders.[20-34] Study of these cellular processes requires an understanding of their basic parts. At the base of these processes is the storage of information within a cell's nucleus. All cellular information is stored in the form of double stranded DNA which is made up of unique sequences of the nucleotides adenine (A), thymine (T), guanine (G), and cytosine (C). Hybridization of these double stranded macromolecules is due to specific hydrogen bonding interactions following Watson-Crick base pairing (A:T, C:G). This sequence specificity is vital not only to their stability, but to their interaction with proteins and other nucleic acids controlling their function. Information stored in DNA is ultimately transcribed into messenger RNA (mRNA) which is then translated into proteins that elicit a certain function. An important difference to note is the replacement of T in RNA with uridine (U), which shares the same specificity of binding with A. Each step in this "central dogma" can be manipulated in order to turn a gene completely on or off, or vary its level of expression.

The direct use of nucleic acid agents in perturbing gene function has become a widely used tool utilizing the selectivity of their inherent base pairing. Endogenously, non-coding RNA (ncRNA) are highly involved in post-transcriptional gene regulation, most notably through the

RNA interference (RNAi) pathway (**Figure 4**).[21-26] RNAi refers to translational repression of mRNA transcripts by either microRNA (miRNA) or the synthetically related small interfering RNA (siRNA). miRNA are transcribed in the nucleus and transported to the cytoplasm as short hairpins or stem-loops. Further processing by Dicer results in a linear double stranded complex denoted as the guide and passenger strand. The guide strand is loaded onto an important protein complex known as the RNA-induced silencing complex (RISC), which is activated through removal of the passenger strand. This guide strand selectively binds complete or partial mRNA complements and suppresses its function by sterically hindering translational machinery or degradation of mRNA through deadenylation, decapping, or cleavage. As this process was elucidated, it became clear that exogenous RNAi agents could be introduced as an efficient way of selectively silencing gene function. These RNAi agents can be introduced in multiple forms including: (i) transfected plasmids containing target sequences, (ii) premature Dicer substrate RNAs (dsiRNA), (iii) or directly as mature small interfering RNA (siRNA) or miRNA mimics.

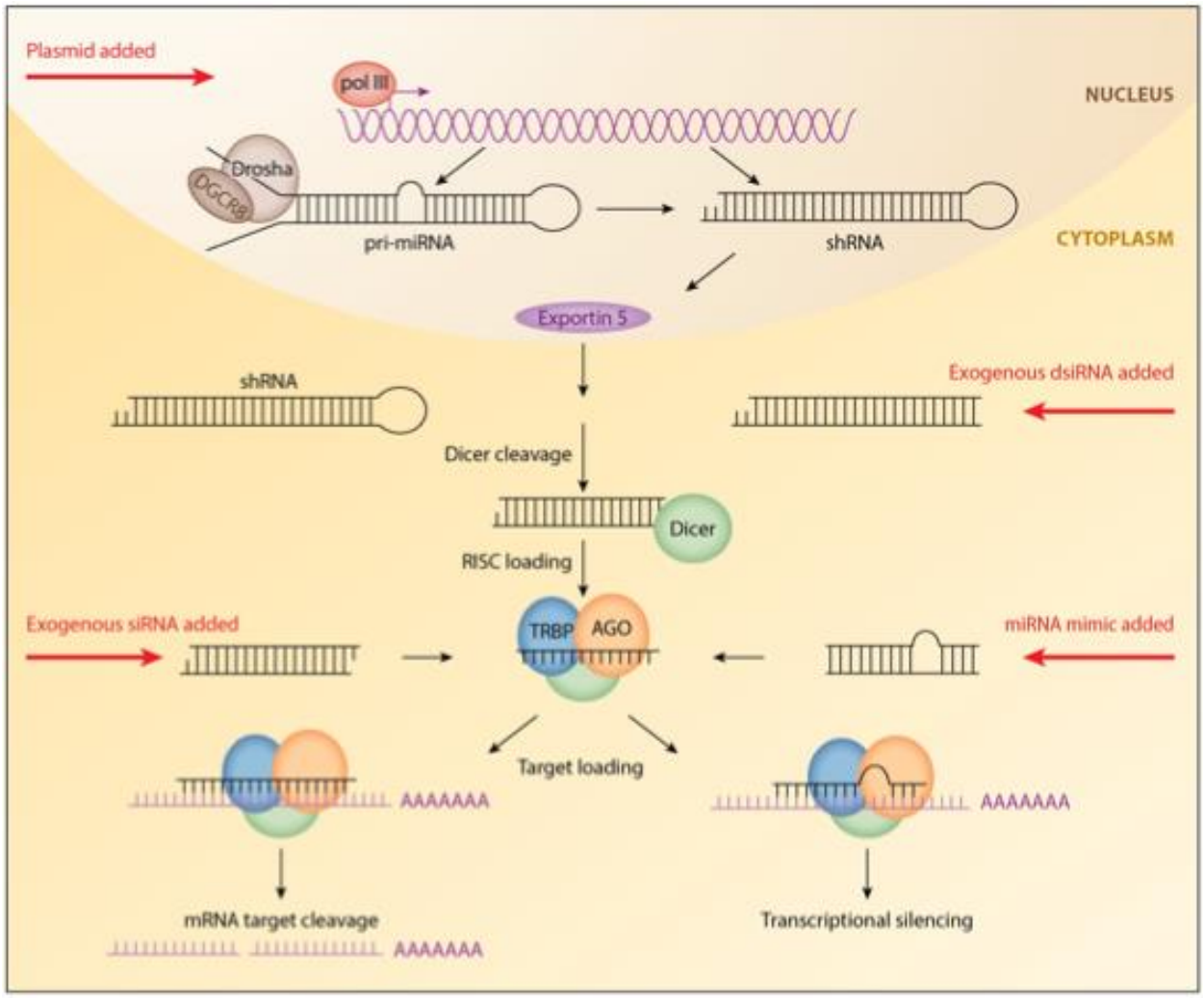


Figure 4. Mechanism of RNA Interference.

Mechanism of RNAi by cytosolic miRNA and siRNA. See the above paragraph for a more detailed discussion of important components.[26]

In addition to RNAi, several antisense agents have been developed that silence or hinder their target's function without the need of RISC.[26-30] The simplest of these are single stranded DNA molecules generally denoted as antisense oligonucleotides (ASOs), which can selectively bind mRNA forming an important RNA-DNA duplex (**Figure 5A**).[27] This duplex can block mRNA function through multiple mechanisms including steric blocking of translation or through

mRNA degradation by the recruitment RNAse H. Modified ASOs have already been utilized as therapeutics in the FDA approved fomivirsen and mipomersen.[26] Other examples of antisense agents include triplex forming oligonucleotides (TFOs) and transcription factor decoys. TFOs are short strands of RNA that bind in either the major or minor groove of a preformed RNA duplex (**Figure 5B**).[31] These triplexes are formed through both Watson-Crick and the less common Hoogsteen base pairing, often requiring further stabilization from protein complexes or metal ions (ie. Mg^{2+}). Although TFOs have not been widely applicable to many processes, their interactions have been found to successfully interfere with the function of multiple ribonucleoproteins (ie. telomerase, ribosomes, etc.). Lastly, transcription factor decoys are a unique form of antisense agents made up of specific promoter sequences that manipulate gene function at the transcriptional level (**Figure 5C**).[32-34] These sequences recruit protein transcription factors which prevents them from interacting with the endogenous genome. Decoys have become promising methods in studying multiple pathways including those associated with cell proliferation, differentiation, and inflammation.

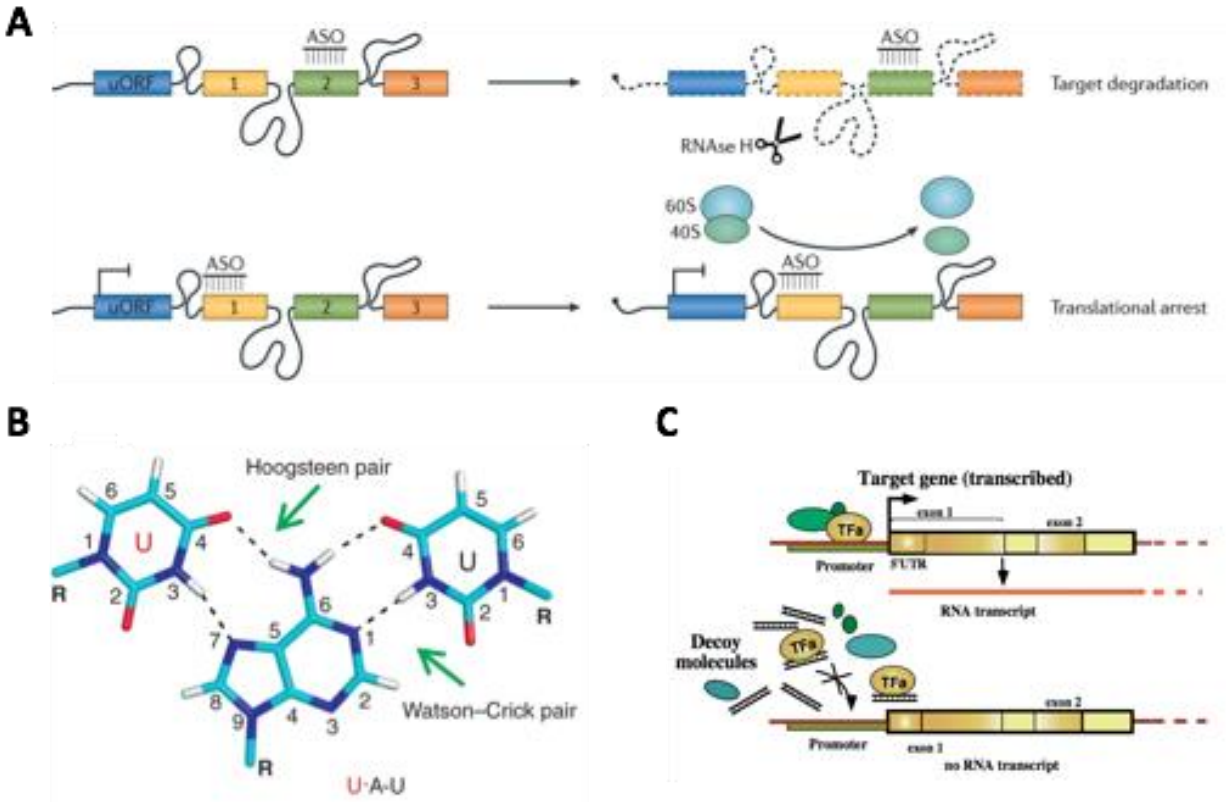


Figure 5. Additional Nucleic Acid Agents in Gene Study.

(**A**) Representative mechanisms of gene silencing through ASO binding and either degradation from recruitment of RNAse H or sterically hindered translational arrest.[29] (**B**) TFO binding motif highlighting the Watson-Crick and Hoogsteen faces of a U-A-U triplex.[31] (**C**) Mechanism of transcriptional silencing using decoys to inhibit production of a target mRNA.[33]

Initial utilization of these agents, especially as therapeutics, was hindered by issues surrounding both their structure and invoked cellular responses. Synthetic nucleic acid molecules, as foreign agents, can be recognized by the cell and induce an innate immune response that can degrade the antisense agent and/or cause apoptosis.[27,35] Additionally, off-target silencing is often seen because complete complementarity to target sequences (ie. mRNA) is not always required for silencing. Fortunately, recent improvements in their design has led to a

resurgence of this technology as both an experimental tool and a promising therapeutic. Chemical modifications to the nucleic acid backbone have dramatically increased stability against nucleolytic attack, decreased recognition by common endo- and exonucleases, and strengthened hybridization affinity for its target (**Figure 6**).[27] Common modifications include phosphorothioate linkages, phosphorodiamidate morpholino (PMO), peptide nucleic acids (PNAs), locked nucleic acids (LNAs), and alkylation of the 2'-OH of the ribose sugar (2'-OMe, 2'-MOE). The choice of modification depends heavily on the application, but currently there are examples of each one of these in clinical trials.[26]

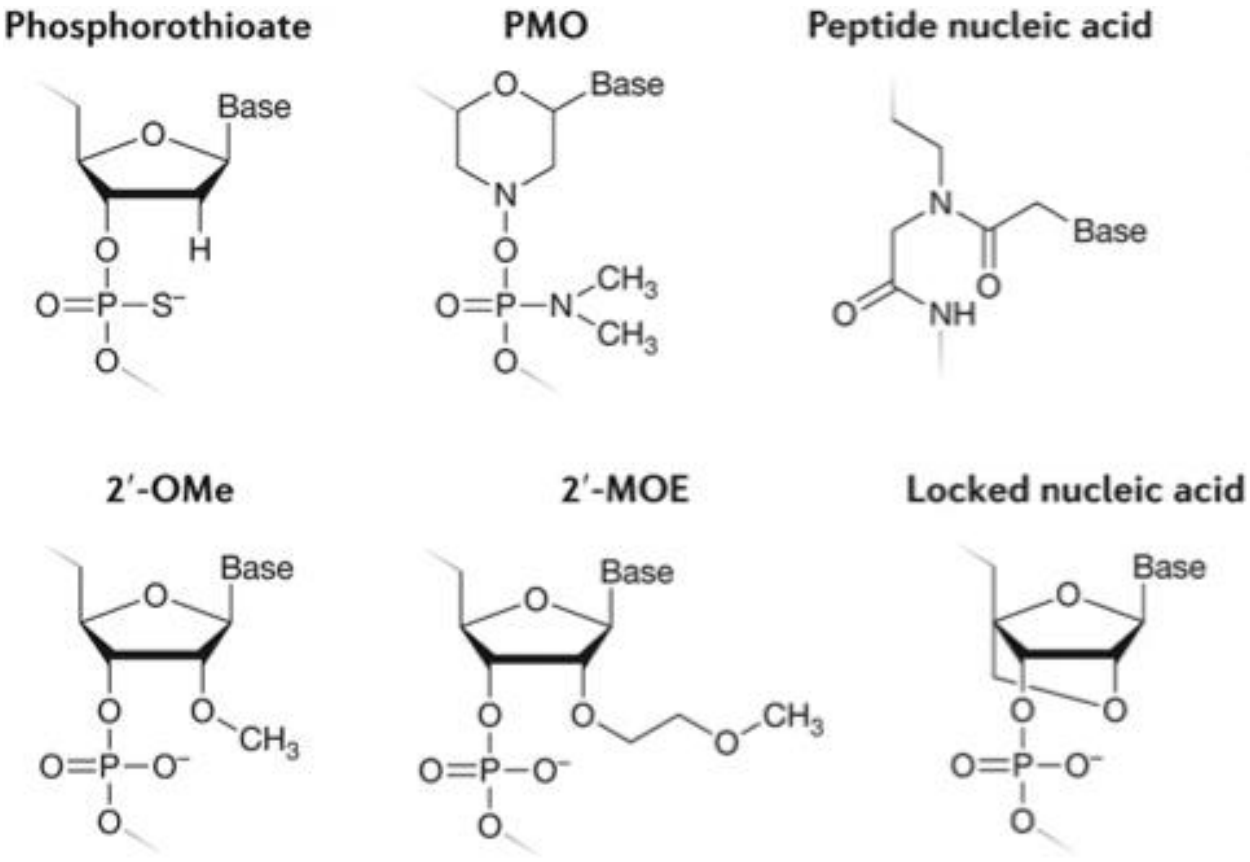


Figure 6. Common Nucleic Acid Backbone Modifications.

Common backbone modifications used to increase stability and binding affinity of synthetic nucleic acid agents.[27]

Additional modifications can be made to antisense agents to give further control of their function through inducible changes at these sites. An example of this type of modification heavily researched in the Deiters' lab is the incorporation of photolabile groups into nucleic acid structures.[36] These most often contain o-nitrobenzyl ethers (ONB, **Figure 7**), which can be removed through a light activated Norrish reaction. Incorporation of these linkers into the backbone of a synthetic antisense agent has been utilized as both an OFF and ON switch. An OFF switch is illustrated in **Figure 7A**, where cleavage prevents hybridization to its target strand therefore turning off its function. Circularizing the strand, as seen in **Figure 7B**, prevents hybridization and renders this agent inactive until cleavage at the linker. This cleavage converts the nucleic acid to its active linear form and allows a conditional ON switch of function. Another useful strategy is seen in **Figure 7C**, where certain bases are protected with ONB analogues that disrupt Watson-Crick bonding to its target strand. Upon irradiation, these protecting groups are removed and function is effectively turned on.

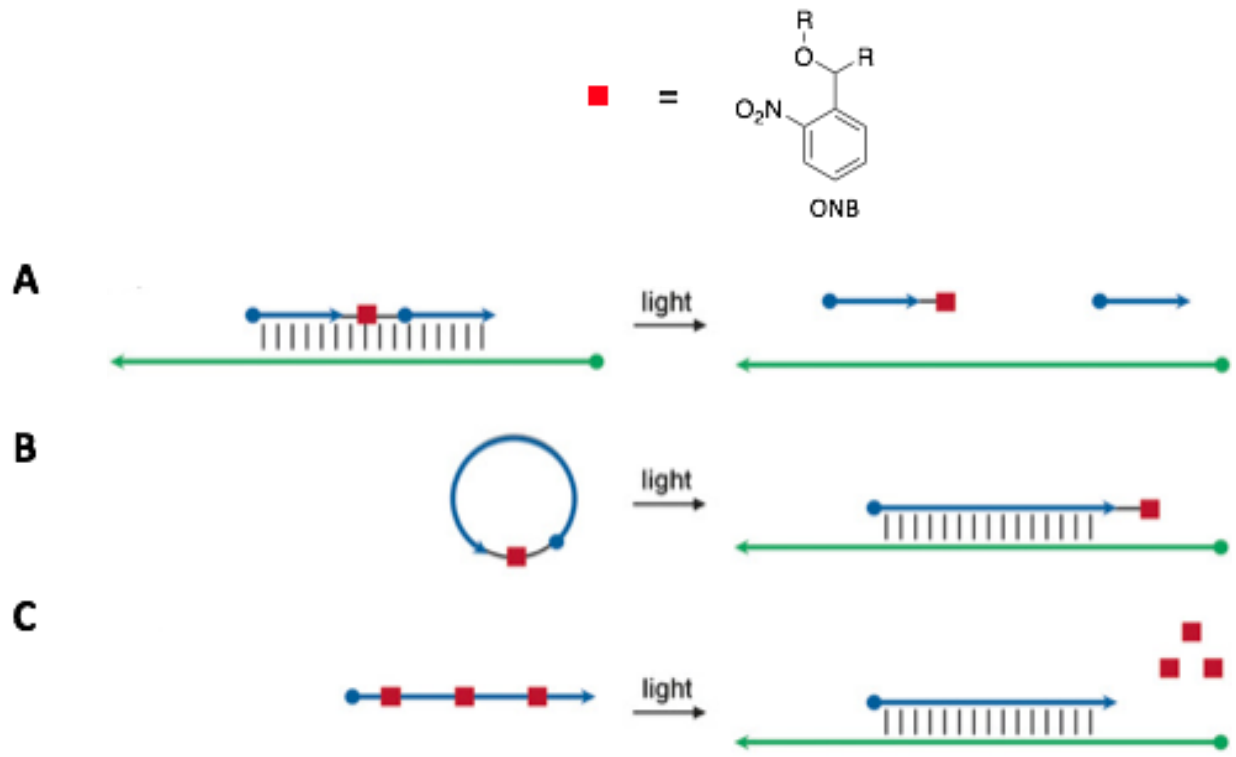


Figure 7. Light Controlled Nucleic Acid Function.

Representative examples of using light controlled cleavage of ONB groups to probe antisense activity. (A) Light induced cleavage within the backbone preventing hybridridization with its target. (B) Circular nucleic acid that is linearized through a similar cleavage event. (C) ONB protected nucleobases that are released through light activation allowing hybridization to its target[36]

While light activated cleavage and deprotection has proven to be a useful way of controlling nucleic acid function, there are some limitations to its use. This transformation requires an external light source which may not be capable of reaching certain tissues or cells within a model organism. Certain wavelengths of UV light can also cause unintended damage to the tissue limiting the maximum time of exposure and increasing off-target toxicity.[37] Recently, chemically induced transformations have been utilized as a way to replace photolabile groups.[37-

[39] These new protecting groups have been incorporated into proteins inducing activity through release of essential amino acids. Interestingly, these small molecule controlled transformations have not yet been applied to the manipulation of modified nucleic acids. Substitution of photolabile groups with chemically inducible linkers or protecting groups in antisense agents could be a promising method in discovering gene function.

2.2 Introduction to metal-catalyzed allyl- and propargyloxycarbonyl deprotection in biological systems

While metal catalysis has had a profound impact on reaction kinetics and the total possible transformations in organic synthesis, introducing metals to aqueous and cellular environments poses many difficult obstacles.[37-41] Many metal complexes are not stable to oxygen and water, as well as the complex mixture of cellular components that include strong nucleophiles (e.g. thiols).[37] These catalysts must also be highly reactive and specific for their target substrate. Low cellular concentrations of potential targets and even lower concentrations of the metal catalyst increases this need for both stability and reactivity. There is also a level of toxicity that comes with the use of certain metals and ligands in biological systems.[37,38] Overcoming all of these obstacles has been relatively slow, but over the last few decades many labs have presented promising examples of these transformations.

In searching for simple, bioorthogonal chemical transformations, chemical biologists have discovered a metal catalyzed deallylation and depropargylation stable to cellular conditions.[(18-20)] These conversions take advantage of traditional Tsuji-Trost chemistry in

deprotecting allyl and propargyl ethers, esters, and carbamates. This mechanism (shown below with ruthenium as an example) involves first an addition of the allyl group of the allyloxy carbonyl (alloc) to the metal catalyst (**Figure 8**).[37] Oxidative addition of this new ligand facilitates the release of a free amine and CO_2. Regeneration of the active catalyst requires a nucleophilic attack on the allyl fragment (ie water, thiols) which dissociates allowing further catalyst activity.

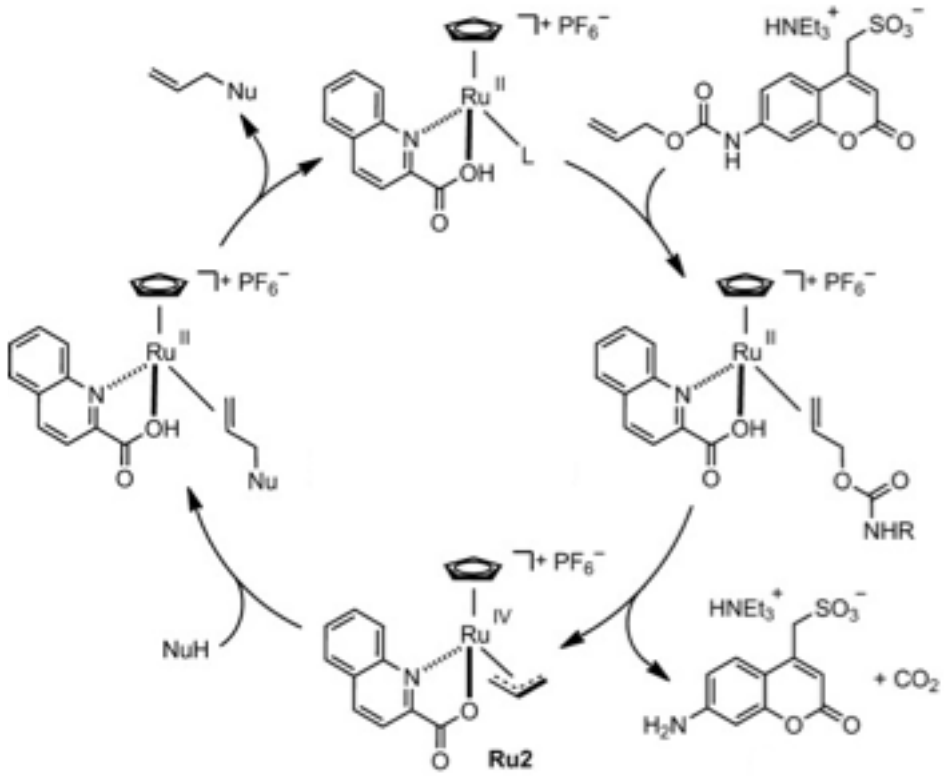


Figure 8. Tsuji-Trost Mechanism.

Representative example of Tsuji-Trost mechanism in the metal catalyzed deprotection of alloc protected coumarin.[37]

Traditional utilization of this transformation relies on palladium(0) catalysts composed of a diverse range of ligands. In applying this to biological systems, the Chen group has had success in the deprotection of propargyloxy carbonyl (proc) protected amines (**Figure 9**).[38] Simple and commercially available palladium catalysts (**Figure 9A**) were taken up by cells and could efficiently activate protein function without significant cytotoxicity (**Figure 9B**). Specifically, the protein OspF, which acts through the irreversible dephosphorylation of mitogen-activated protein kinases (MAPKs), was activated through proc deprotection of an essential active site lysine. Slight palladium catalyzed alloc deprotection was also observed in model studies, but never increased above ~25% yield under the conditions tested.

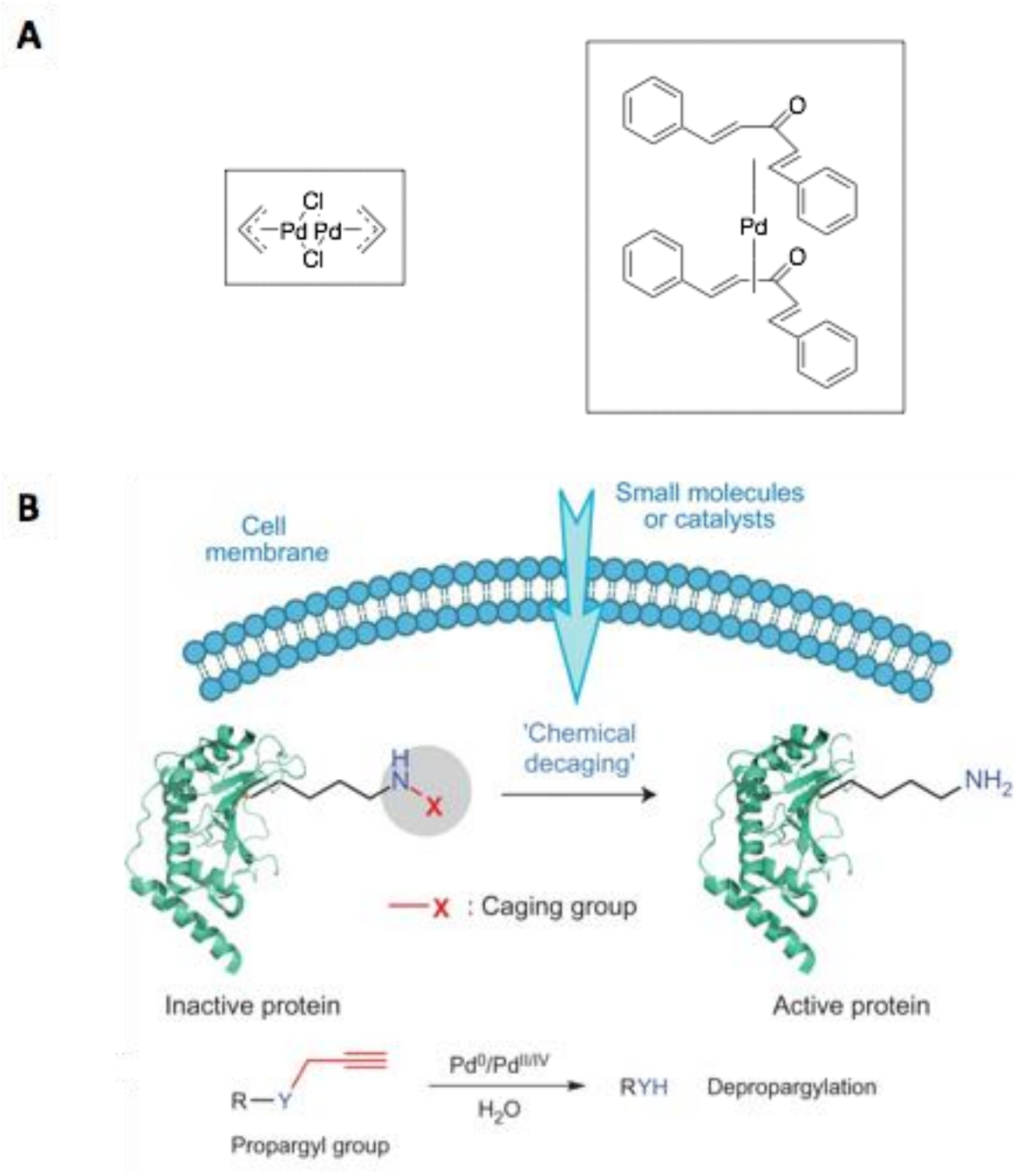


Figure 9. Palladium Catalyzed Proc Deprotection.

Work done by the Chen group using palladium catalysts to deprotect essential lysines to activate protein function.[38]

Convergent work done by the Megger's lab has focused on the use of ruthenium catalysts in the deprotection of alloc protected amines.[37,39] Their work not only led to the increased stability and reactivity of multiple generations of catalysts, but they have also been utilized in very complex biological mixtures. Most recently, this work has led to the development of **Ru9** (as denoted below) that was found to mildly deprotect alloc protected doxorubicin in blood serum (**Figure 10**).[39]

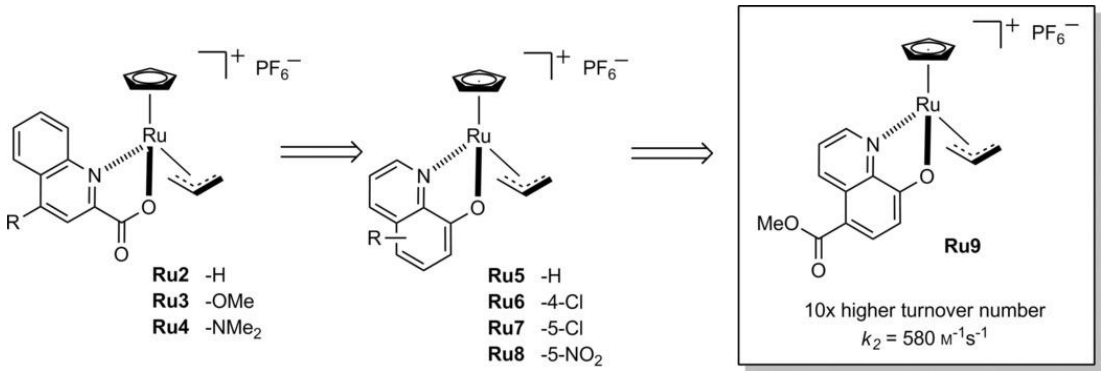


Figure 10. Water Stable Ruthenium Catalysts.

Recent generations of ruthenium catalysts synthesized and utilized in the Megger's lab.[39]

We speculated that this transformation could also be applied to the conditional control of nucleic acids. It was hypothesized that a cleavable linker could be incorporated into a short nucleic acid oligomer containing an alloc protected aryl amine para to a benzylic phosphate (**Figure 11**). The presence of a ruthenium or palladium catalyst could deprotect this amine and allow cleavage through a subsequent 1,6-elimination. This cleavage event would silence the function of its specific oligomer and could be applied to a broad range of nucleic acid agents. The progress towards developing this method is discussed in further detail below.

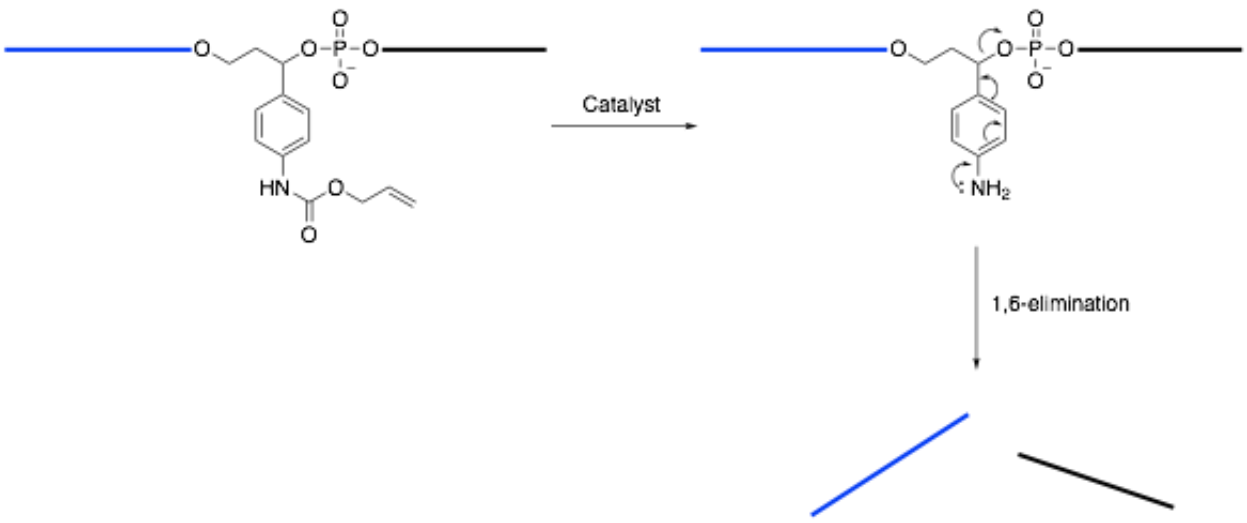


Figure 11. Mechanism of Metal Catalyzed DNA Cleavage.

General design for a metal catalyzed cleavage of a DNA linker. Alloc deprotection of the aryl amine results in an inherent 1,6-elimination cleaving the phosphate backbone.

2.3 Catalyst screening using model systems

A set of test catalysts were screened for their ability to efficiently and completely deprotect the alloc protecting group under biologically relevant conditions. Catalysts were selected based on literature precedent and their ease of synthesis. Two ruthenium catalysts (**51** and **52**) and one palladium catalyst (**53**) were selected as potential candidates.[37-39] Catalysts **51** and **52** were readily synthesized according to literature procedures, while **53** was commercially available and used without further modification (**Figure 12**).[39]

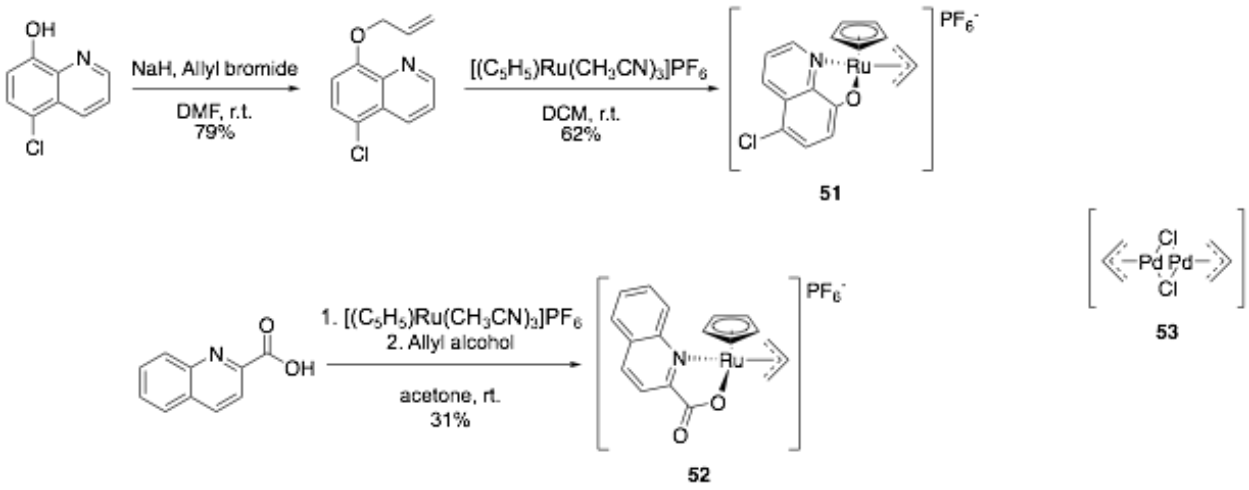


Figure 12. Catalysts Screened for Alloc and Proc Deprotection.

Selected catalysts for screening of alloc and proc deprotection under biologically relevant conditions. **51** and **52** were synthesized according to literature protocols and **53** was commercially available and used as received.[37-39]

First, the ability of **51 - 53** to deprotect aryl amines in biologically relevant media was tested using both alloc and proc protected aniline. The reactions were monitored using reverse phase high performance liquid chromatography (HPLC), measuring absorbance (254 nm) of compounds eluting from a gradient of 5-95% acetonitrile in water over 20 minutes. The reactions were run in a 200:1 PBS (pH = 7.4)/DMSO mixture containing 500 μM of each substrate and 5 mM glutathione (GSH). The GSH concentration was selected based on common cellular conditions (1-10 mM) and functioned as an endogenously found nucleophile capable of regenerating active catalysts (See mechanism in **Figure 8**).[37,39] Initial experiments using alloc aniline found that this reaction was temperature dependent, as there was no observable deprotection by any of the catalysts after 24 hours when run at room temperature (**Table 1**). Increasing the temperature of the reaction to 37 °C resulted in higher rates of deprotection for both **51** and **52**. The most complete deprotection after 24 hours was seen by reaction with **51**,

which resulted > 95% deprotection at a 10% catalyst loading (**Figure 13**). Comparable deprotection was also observed using **52**, but as expected was outperformed by **51** at varying concentrations. This is especially prevalent at a 5% catalyst loading where **51** performed 20% better than **52**. This trend matches literature reports for both catalysts, as the chloro-substituent acts as an ideal electron withdrawing group for ruthenium activation.[39] This success was not seen with **53** as there was no observable deprotection after 24 hours under the same conditions.

Table 1. Catalyst Screening of Alloc Deprotection.

Catalyst	Catalyst loading (mol %)	Temperature	% Deprotection
51	10	rt	0
	5	37 °C	80
	10	37 °C	> 95
52	10	rt	0
	5	37 °C	60
	10	37 °C	85
53	10	37 °C	0
Conditions: 500 μM alloc aniline, 5 mM GSH, 200:1 PBS Buffer (pH = 7.4):DMSO. Monitored via HPLC and compared to known standards. No side products were observed under these conditions. HPLC chromatograms in SI.			

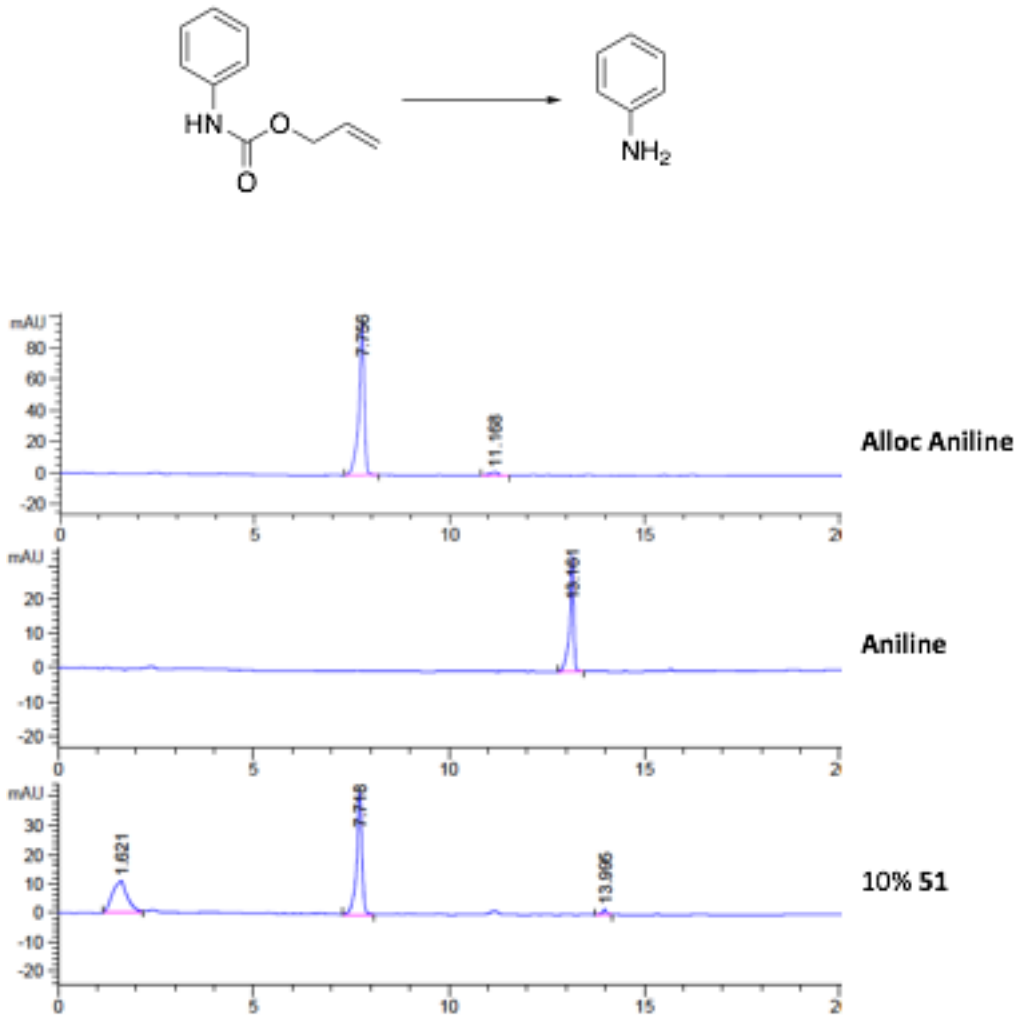


Figure 13. Alloc Deprotection by Catalyst 51.

Representative HPLC chromatograms for the deprotection of **54** to form **55** in the presence of 10% **51**. Conditions for the elution of each compound are discussed above.

Based on the literature precedent for comparable deprotection of proc protected amines, especially by palladium catalysts, a similar experiment was conducted with proc protected aniline, again monitored by HPLC.[38] Surprisingly, no catalyst tested resulted in the emergence of an aniline peak.

As a result of these findings, alloc protection was chosen for the design of a DNA cleavable linker, which is discussed in detail below. As the best performing catalyst, **51** was chosen to be used in further experiments.

2.4 Design, synthesis, and elucidation of linker 54

An alloc protected linker was designed based on the previously reported ONB group (**Figure 14**).[36] Substitution of this photolabile nitro group with an alloc protected amine provides another method to induce cleavage. This alloc linker design also takes advantage of the phosphoramidite method of nucleic acid synthesis.[42] The phosphoramidite method provides an easy and mild way of incorporating modified "bases" into DNA, in this case **54,** which can be synthesized from reaction with **56** and the commercially available **55**. Compound **56** could be constructed from 4-nitrobenzaldehyde (**57**) as discussed below. The automated nucleic acid synthesis that follows incorporates and oxidizes the attached phosphoramidite to the phosphate backbone endogenously found in nucleic acids.[42]

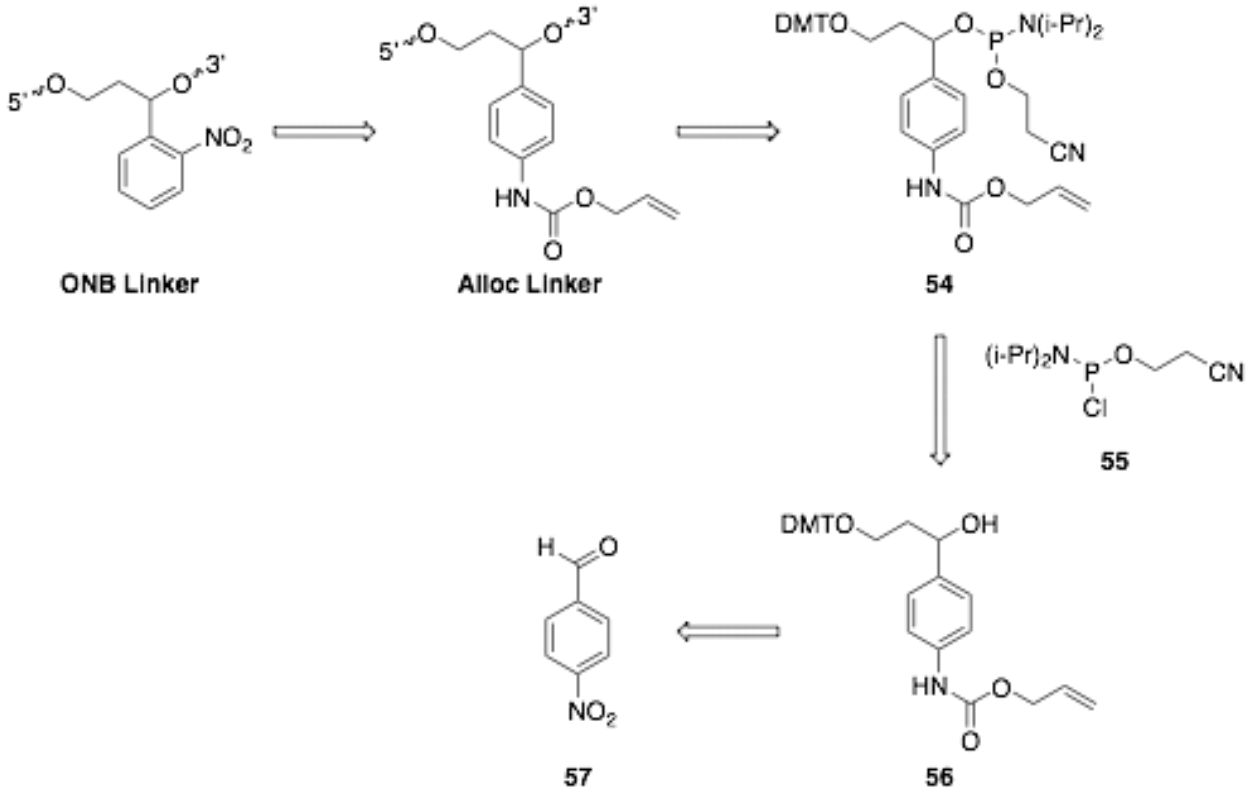
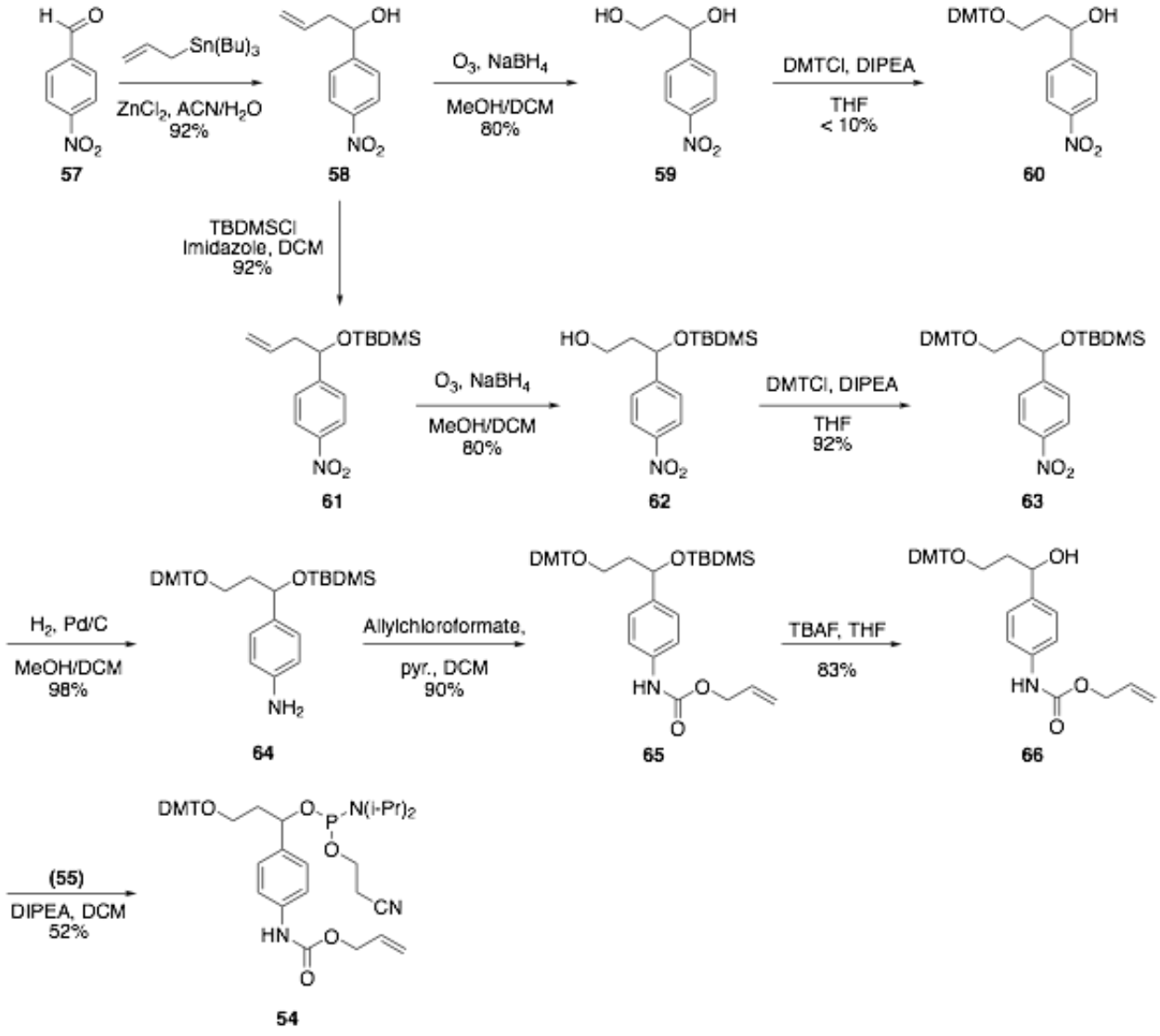


Figure 14. Retrosynthetic Analysis of Linker 54.

Design of alloc linker derived from the previously reported ONB linker. The phosphoramidite method is utilized for incorporation of **54**, which can be synthesized from **57** as discussed below.[36,42]

Synthesis of **54** began from the relatively simple and commercially available **57**. Allylation of **57** with allyltributylstannane led to alkene **58** (**Scheme 7**).[43] Initially, ozonolysis followed by a reductive workup led to diol **59**. Reaction of **59** with dimethoxytrityl chloride (DMTCl), an essential protecting group commonly used in DNA synthesis, was very low yielding in producing **60**. Although the primary alcohol should react preferentially with the DMTCl due to its increased nucleophilicity and availability, it was determined that both alcohols were reacting forming a mixture of mono- and di-protected products.[44,45] To combat this issue,

the benzylic alcohol of **58** was protected with *tert*-butyldimethylsilyl (TBDMS) chloride to form **61**.[46] Ozonolysis of **61** resulted in the primary alcohol **62**, which was then protected with DMTCl to form **63** in a 92% yield.[47] This was followed by the reduction of the nitro group with H_2 gas and 10% Pd/C forming the primary amine present in **64**. This amine was then protected with allylchloroformate to form **65**.[48] The final steps involved deprotection of the silyl ether (**66**) followed by synthesis of phosphoramidite **54** after reaction with **55** and DIPEA.[44]



Scheme 7. Synthesis of Linker **54**.

Synthesis of potential DNA cleavable linker **54**. See experimental section for detailed reaction parameters.

This modified linker (**54**) was incorporated at the 20th position of a random 30-mer sequence for *in vitro* cleavage studies. The modified oligomer was purified via polyacrylamide gel electrophoresis (PAGE) and ethanol precipitation before a mass was confirmed by HRMS (See experimental).

Cleavage of the modified oligomer was tested with increasing concentrations of **51** and visualized by PAGE. If there was cleavage at this position, there should be an observable shift of the modified oligomer. No cleavage was observed after 24 hours, even at stoichiometric concentrations of **51** (**Figure 15**).

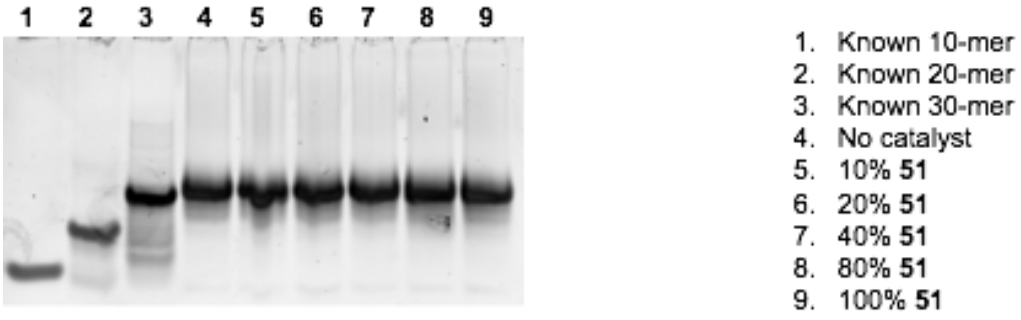


Figure 15. Gel for Model Oligonucleotide Containing 54.

In vitro cleavage experiment of base modified 30-mer using increasing concentrations of catalyst **51** (% relative to oligomer concentration). Reaction conditions: 10 μM oligomer, 5 mM GSH, in 200:1 PBS/DMSO. Run at 37 °C for 24 hours. Analyzed via 20% acrylamide gel run at 120 V for 1 hour. Bands visualized using SYBR gold stain.

There are multiple possibilities for why this cleavage did not occur, but it would be difficult to determine that from the data presented. It is possible that the modified oligomer forms

a structure (ie. hairpin, stem-loop) that sterically hinders the alloc protected amine, which would not allow the catalyst to reach its target. The second possibility for the lack of cleavage is that the phosphate of the oligomer backbone is not acidic enough to induce cleavage through a 1,6-elimination mechanism. There is a certain level of inherent stability of this phosphate backbone needed to prevent cleavage or hydrolysis in cellular conditions, which supports this conclusion, but still cannot be observed via PAGE.[50] To assess these concerns, additional model systems were tested, ultimately leading to the design of a new linker.

2.5 Design, synthesis, and elucidation of linker 87

A model system was designed to test the hypothesis that the negatively charged phosphate was stable to cleavage via a 1,6-elimination mechanism. Alkylation of the phosphate would remove the excess charge on the phosphate decreasing its pka from 7 to ~2.[49] This increase in acidity should result in a better leaving group and aid in backbone cleavage. Alkylated phosphates have previously been utilized in modified nucleic acids without a major decrease in hydrolytic stability.[50] The first model substrate mimicked compound **54** containing an alloc protected amine para to a secondary benzyl phosphate (**Figure 16**). Two analogues were designed that varied the charge on the phosphate by alkylating the R' position. These analogues could be tested via HPLC in the presence of catalyst **51** for both alloc deprotection (**69**) and elimination of the benzyl phosphate (**70**), following the reaction seen in **Figure 16**.

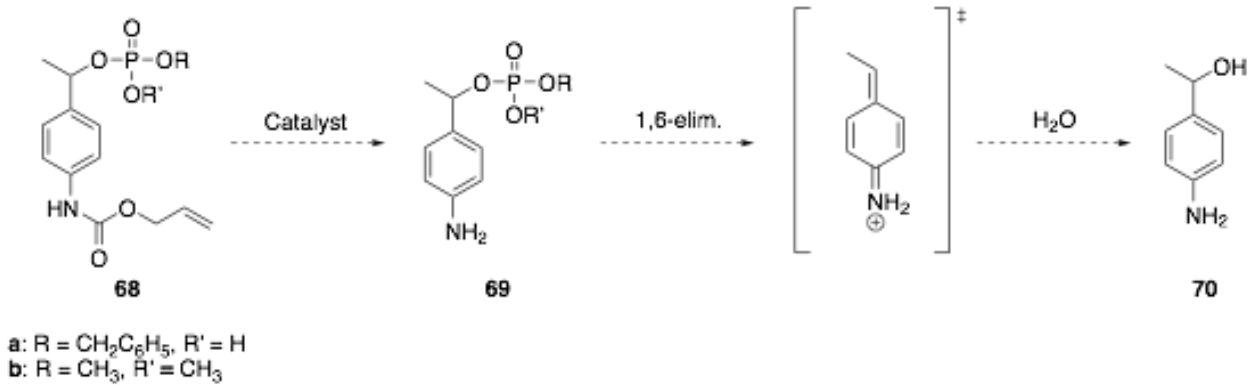
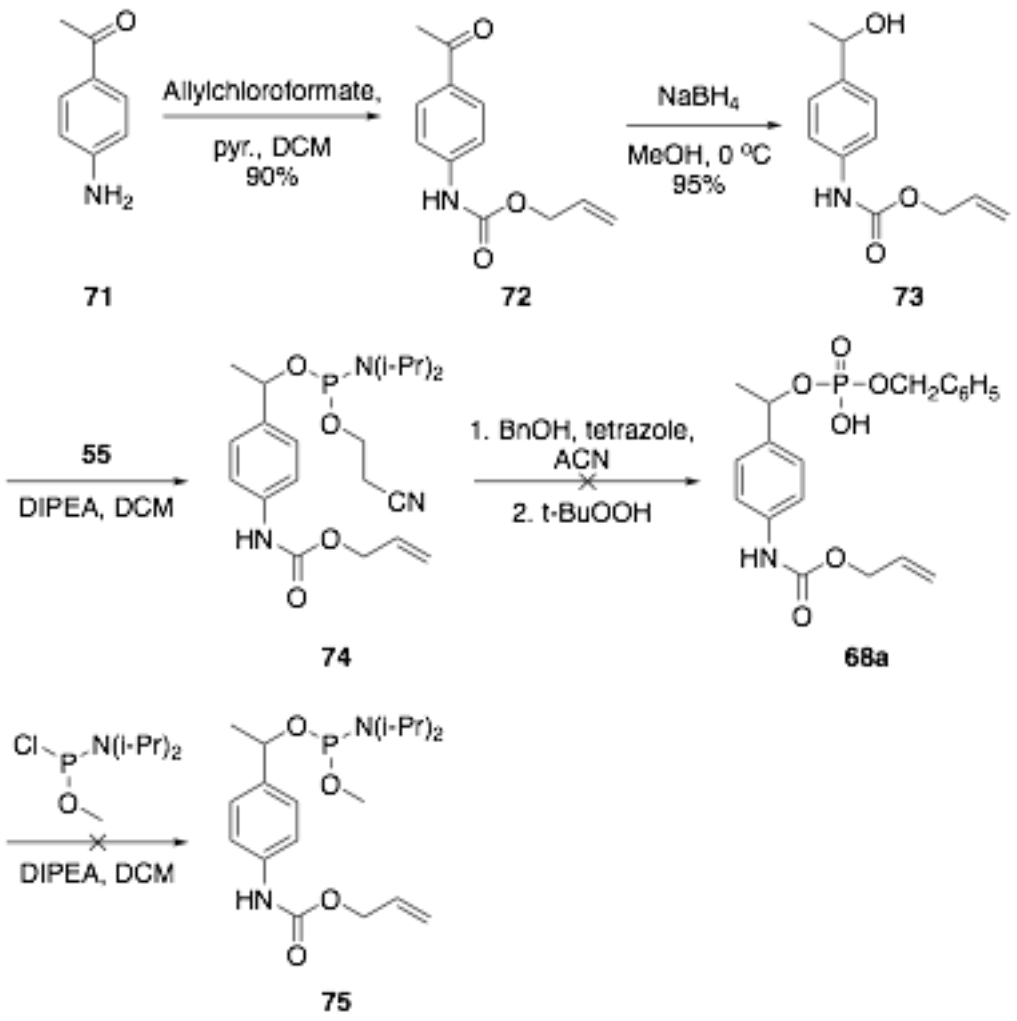


Figure 16. Mechanism for Test Substrates in HPLC Study.

Designed substrates **68a** and **68b** to test the deprotection and elimination of varied phosphates in biologically relevant media.

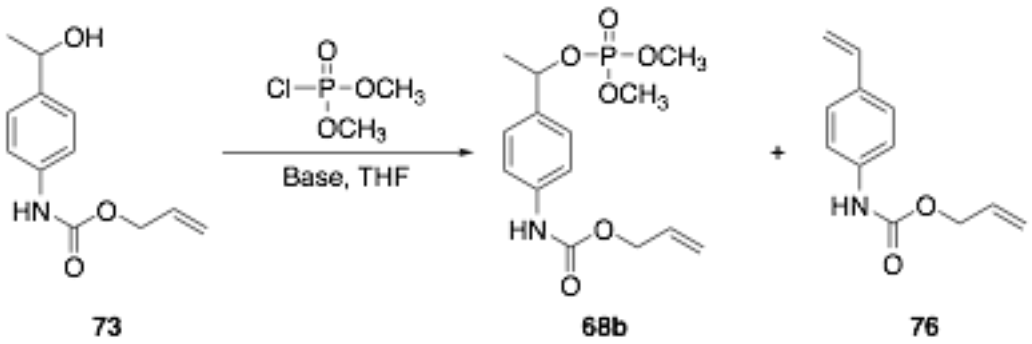
The synthesis of **68a** was initiated by the alloc protection of commercially available 4-aminoacetophenone (**71**) using the previously discussed procedure to form **72** (**Scheme 8**).[48] Reaction of **72** with sodium borohydride at 0 °C led to the selective reduction of the ketone resulting in **73**. The benzylic alcohol was then coupled with **55** to form phosphoramidite **74**.[44] Compound **74** was seen by both ^{1}H and ^{31}P NMR but could not be separated from unknown side products. This crude mixture was pushed forward in attempts towards **68a** including a one pot reaction consisting of a substitution of the alkylated amine with benzyl alcohol followed by an oxidation by *tert*-butyl hydroperoxide. No product (**68a**) was seen under these conditions. An alternative route using *N,N*-diisopropylmethylphosphonamidic chloride instead of **55** was attempted but resulted in a mixture of oxidized products not including the desired **75**.[44] These oxidized products could not be isolated and therefore were not characterized.



Scheme 8. Attempted Synthesis of **68a**.

At the same time, an attempt towards the synthesis of **68b** was conducted (**Scheme 9**). This synthesis began with intermediate **73**, which was synthesized as discussed previously. Compound **73** was first coupled with dimethoxy chlorophosphate in the presence of 1,4-diazabicyclo[2.2.2]octane (DABCO).[51] As this reaction progressed, two new spots were observed by TLC, one more polar (as expected) than the starting material and one less polar. Interestingly, as the reaction time increased, there was a near complete disappearance of the expected product spot and an increase in UV activity of the less polar side product. This side product was characterized and found to be styrene **76**. It was postulated that the trialkyl

phosphate was acidic enough to induce a DABCO mediated elimination reaction. This raised concerns about the stability of a trialkyl phosphate in aqueous media because the pka of DABCO (8.8) is relatively close to physiological pH (7.0 - 7.4).[49] To test this hypothesis, a study was conducted monitoring the formation of **68b** and **76** in the presence of three different bases: pyridine (pka = 5.2), DABCO (pka = 8.8), and DIPEA (pka = 10.8).[49] This reaction was monitored by low resolution liquid chromatography mass spectrometry (LCMS). Both reactions with pyridine and DABCO resulted in a mixture of **68b** and **76**, while in the presence of DIPEA only **76** was detected. These results supported the hypothesis that relatively weak bases led to at least partial elimination of this neutral phosphate and could lead to unwanted cleavage of a base modified oligomer subjected to aqueous conditions.

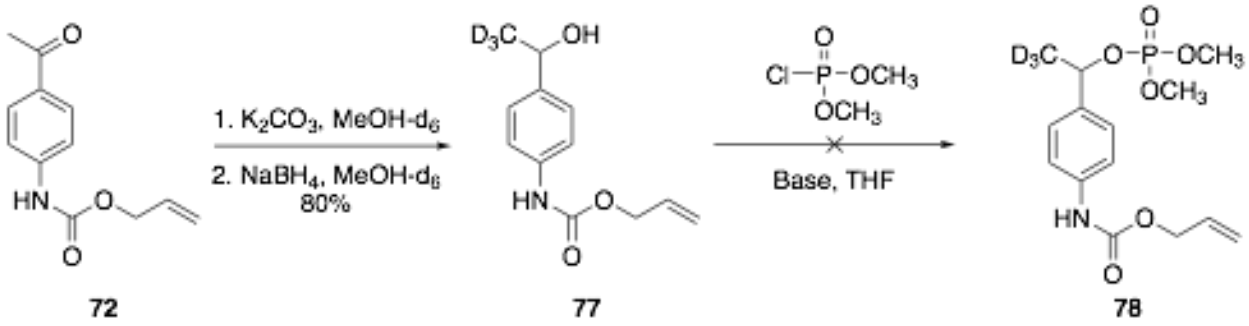


Scheme 9. Attempted Synthesis of **68b**.

Attempts towards the synthesis of **68b**. Three bases of different strengths were used (pyridine, DABCO, and DIPEA) and all resulted in **76** as the major product.

As an initial attempt to prevent this elimination process from occurring, a deuterated analogue was synthesized (**Scheme 10**). Though hydrogen and deuterium are identical electronically, the increased mass of deuterium increases the strength of its covalent bonds.[52]

Replacing the protons alpha to the phosphate with deuterium decreases the acidity at that position and could prevent unwanted styrene formation. Alloc protected **72** was reacted with potassium carbonate in deuterated methanol then reduced with sodium borohydride to form benzyl alcohol **77**. Conversion to phosphate **78** again resulted in a styrene major product in the presence of both pyridine and DABCO.[51] These results led to the conclusion that the adjacent carbon to the benzyl phosphate would need to be modified further to avoid unwanted elimination in biologically relevant media.



Scheme 10. Attempted Synthesis of **78**.

A primary analogue (**81**) was synthesized to exclude the risk of styrene formation and to test whether 1,6-elimination could occur (**Figure 17**). Analogue **81** was synthesized in two steps from 4-aminobenzyl alcohol (**79**) starting with alloc protection to form **80** (**Figure 17A**).[48] Phosphate addition in the presence of DABCO was successful in producing **81** in a 49% yield.[51]

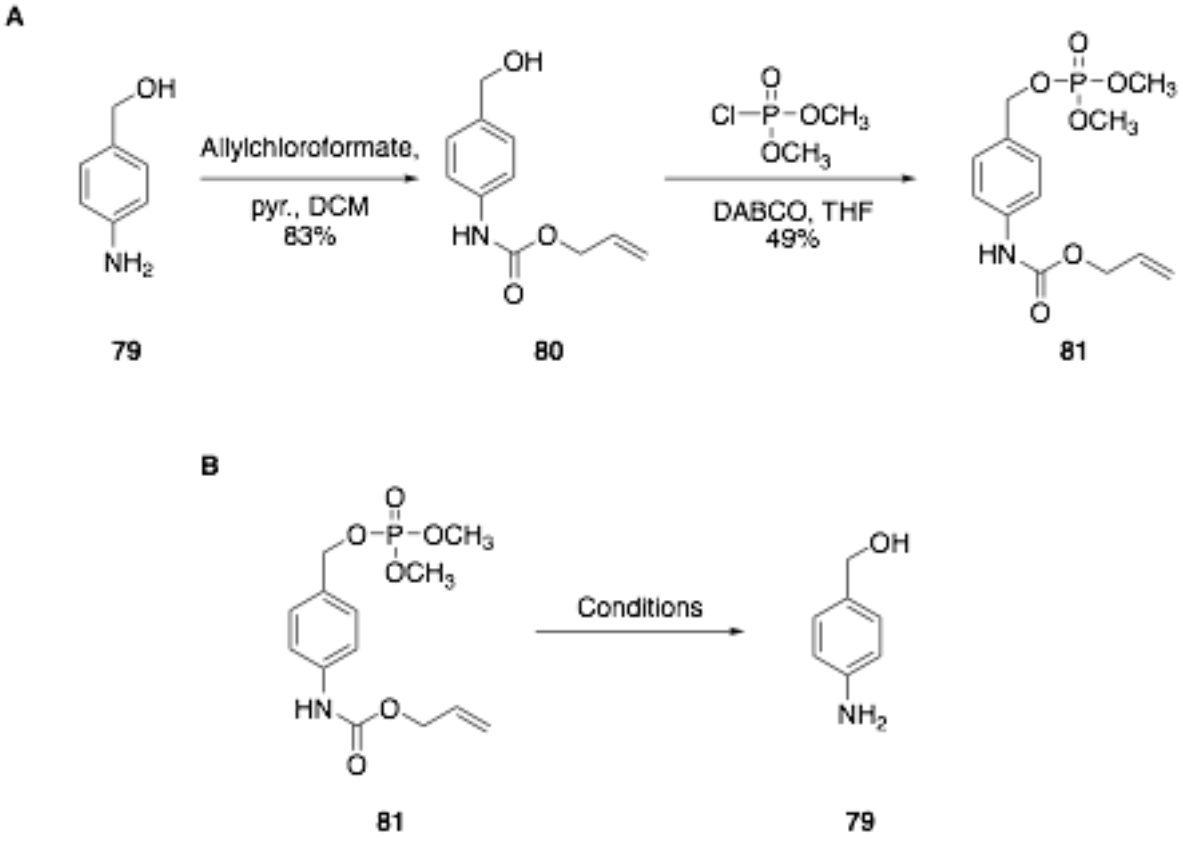


Figure 17. Synthesis of Primary Model Substrate 81.

(**A**) Successful synthesis of primary analogue **81**. (**B**) HPLC experiment conducted to test reaction of **81** to **79**. Conditions: 500 µM alloc aniline, 5 mM GSH, 200:1 PBS Buffer (pH = 7.4):DMSO with **51** (catalyst loading designated below)

After successful synthesis of test substrate **81**, an HPLC study was conducted to test conversion of **81** to **79** in the presence of **51** (**Figure 17B**). Initially, the reaction progress was monitored at specific time points over 24 hours at a 10% loading of **51** (**Figure 18A)**. Within 30 minutes the reaction stalled at between 30-40% deprotection of starting material (**Figure 18B**). Though the overall yield was low, deprotection did lead to the appearance of the desired 1,6-elimination product **79** at a 27% yield after 24 hours.

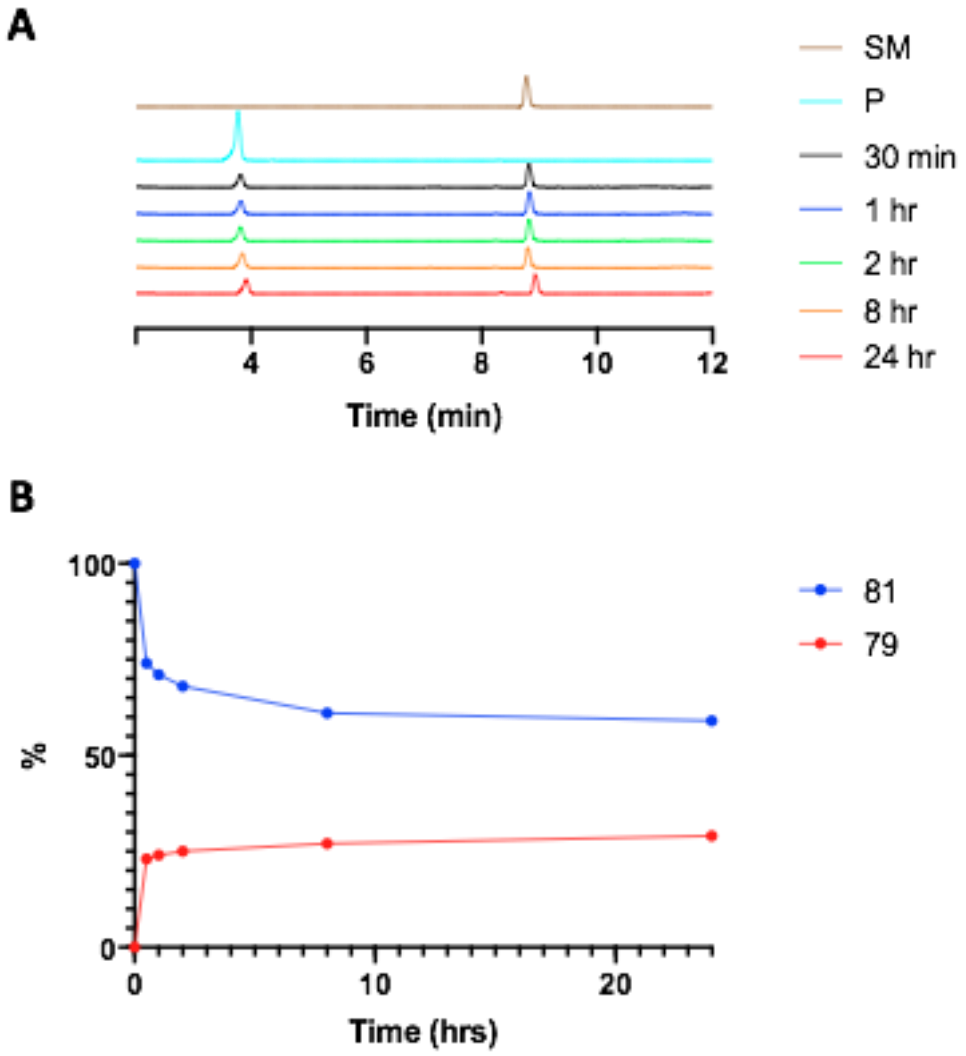


Figure 18. Time Based Deprotection Study of 81.

Conversion of **81** to **79** monitored via HPLC. (**A**) HPLC chromatogram of reaction progress at specific time points. (**B**) Graphical representation of the yield of reaction calculated from absorption values for each substrate at 254 nm. Conditions: 500 µM alloc aniline, 5 mM GSH, 10% **51**, 200:1 PBS Buffer (pH = 7.4):DMSO. The reaction was run at 37 °C and checked at time points: 0.5, 1, 2, 8, and 24 hours.

A similar experiment was conducted with increasing concentrations of **51** and analyzed via HPLC after 12 hours. There was a direct relationship between the concentration of catalyst

added and the overall yield of the reaction (**Figure 19**). A 60% catalyst loading was required for complete disappearance of **81**. Though production of **79** was observed, there were no major differences in its absorption at different catalyst loadings even with complete disappearance of **81**. Side products were observed, which could include deprotected **81** unable to cleave the benzylic phosphate bond or products formed after elimination from the attack of unexpected nucleophiles (ie. GSH).

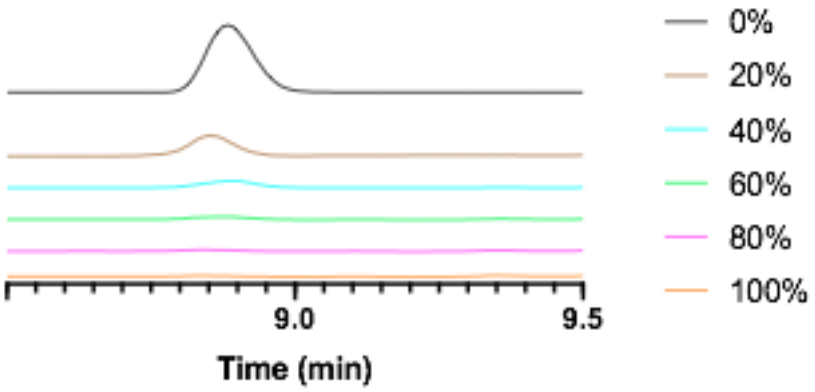
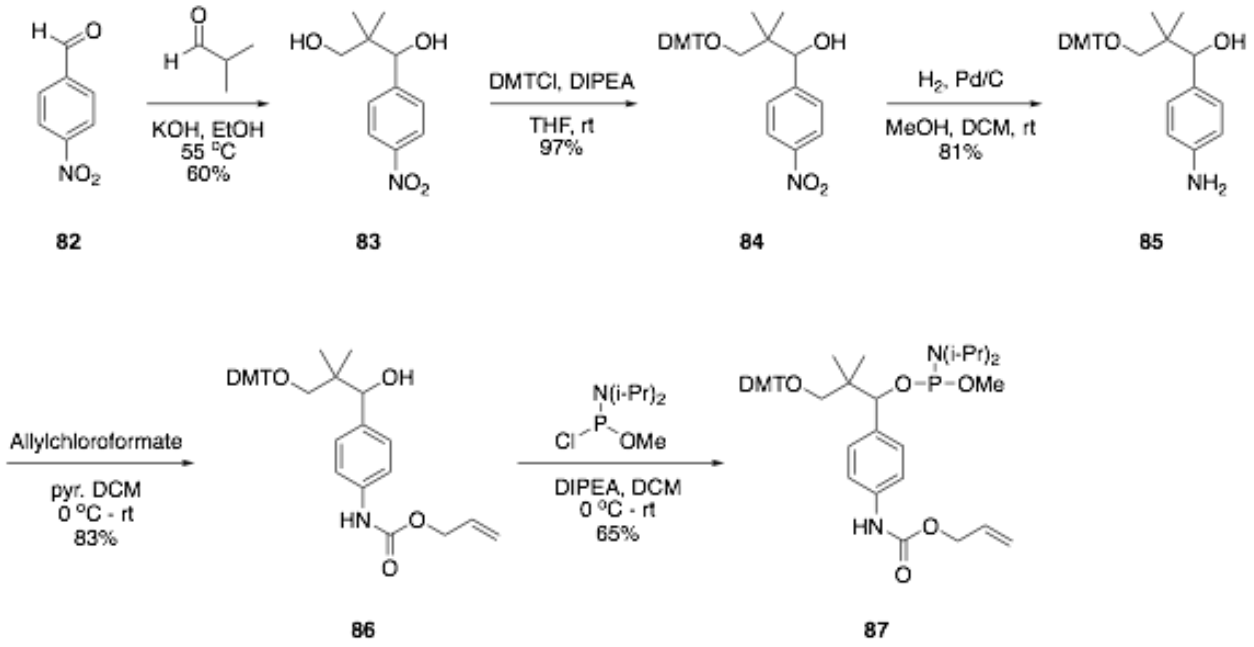


Figure 19. Concentration Based Deprotection Study of 81.

HPLC trace of **81** with increasing concentrations of **51** compared to a no catalyst control (0%). Conditions: 500 µM alloc aniline, 5 mM GSH, 200:1 PBS Buffer (pH = 7.4):DMSO. The reaction was run at 37 °C for 12 hours.

From the mild success of test substrate **81**, a new linker was designed that would test if any cleavage could be observed in a synthesized oligomer (**87**, **Scheme 11**). This linker includes the neutral phosphate as above, but replaces the adjacent methylene with a geminal dimethyl functionality, preventing styrene formation. Synthesis of this new linker began with commercially available 4-nitrobenzaldehyde (**82**), which was converted to **83** through an aldol-Cannizzaro coupled reaction (**Scheme 11**).[53] The primary alcohol of **83** was selectively protected

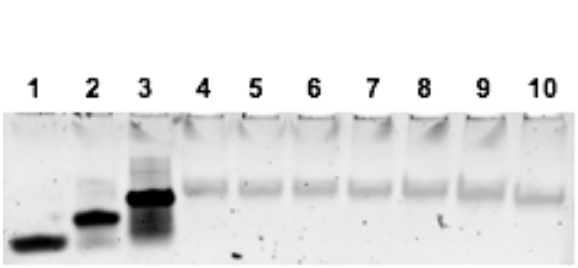
with DMTCl to produce **84** in a high yield.[44] This selectivity was not seen in the synthesis of linker **67**, and is most likely due to the increased steric bulk surrounding the secondary alcohol by the geminal dimethyl moiety. Reduction of the nitro group, under the same conditions as previously presented, led to aryl amine **85**. The linker **87** was completed through the alloc protection of this aryl amine (**86**) and addition of the methoxy phosphoramidite in the presence of DIPEA.[48,51]



Scheme 11. Synthesis of Linker **87**.

Linker **87** was successfully incorporated into the same 30-mer construct, though coupling yields for the modified linker were very low. This is not surprising as the geminal dimethyl moiety increases bulk and is most likely hindering coupling of **87** to the subsequent nucleobase. As an attempt to increase coupling efficiency, a test tetramer was used to test different reaction conditions. Unfortunately, doubling the concentration of the stock solution of **87** as well as the number of times it is pulsed over the reaction column did not increase the coupling efficiency

significantly compared to standard conditions. Despite the decreased yield during oligomer synthesis, the 30-mer construct was purified as previously discussed and confirmed by HRMS (See experimental). A cleavage study was conducted in the presence of increasing concentrations of **51** and visualized via PAGE after 12 hours. Disappointingly, no shift was observed even at stoichiometric catalyst concentrations (**Figure 20**).



1. Known 10-mer
2. Known 20-mer
3. Known 30-mer
4. No catalyst
5. 10% **51**
6. 20% **51**
7. 40% **51**
8. 60% **51**
9. 80% **51**
10. 100% **51**

Figure 20. Gel for Model Oligonucleotide Containing 87.

In vitro cleavage experiment of base modified 30-mer using increasing concentrations of catalyst **51** (% relative to oligomer concentration). Reaction conditions: 10 μM oligomer, 5 mM GSH, in 200:1 PBS/DMSO. Run at 37 °C for 24 hours. Analyzed via 20% acrylamide gel which was run at 120 V for 1 hour. Bands visualized using SYBR gold stain.

2.6 Conclusions and outlook

Multiple model substrates were tested in the design of two DNA linkers to induce cleavage through a simple alloc deprotection. Metal catalyzed alloc deprotection was found to be an efficient bioorthogonal transformation stable to biologically relevant conditions.[37-39] Issues in this design were rooted in the inability to induce efficient cleavage of a benzylic phosphate

through 1,6-elimination originating from an aryl amine. Both linkers **54** and **87** were successfully synthesized and incorporated into DNA, but were not capable of cleaving in the presence of catalyst **51**.

There could be many reasons for why this cleavage was not observed when tested at the oligomer level. The unknown conformation of the 30-mer tested may have hindered the ability of the catalyst to reach the alloc protected linker, thus preventing backbone cleavage. Further experiments could decrease the length of the modified oligomer to decrease likelihood of steric interference. This would also allow for analysis via HPLC or mass spectrometry making it easier to see the subtle decrease in mass following alloc deprotection or 1,6-elimination. Another possible solution would be to incorporate this linker into a nucleic acid strand with a more defined structure. This could be achieved through either: (i) linearizing the construct through hybridization to its complement, or (ii) circularizing by annealing the 5'- and 3'-ends (ie. circular PMOs). These constructs could be tested through PAGE analysis using the methods discussed previously.

A modeling software was used to generate a potential 2-D structure of the 30-mer construct synthesized in both gel studies (**Figure 21**).[59] The position of the modified base is denoted (blue arrow), and was varied to ensure it was not participating in a base pair interaction (G in the software). Based on this diagram, the modified linker is at the base of a loop structure, but it would be hard to determine whether this position is sterically hindered enough to prevent access by the catalyst. Further studies could vary the sequence of this 30-mer to test whether sequence does play a major role in linker availability.

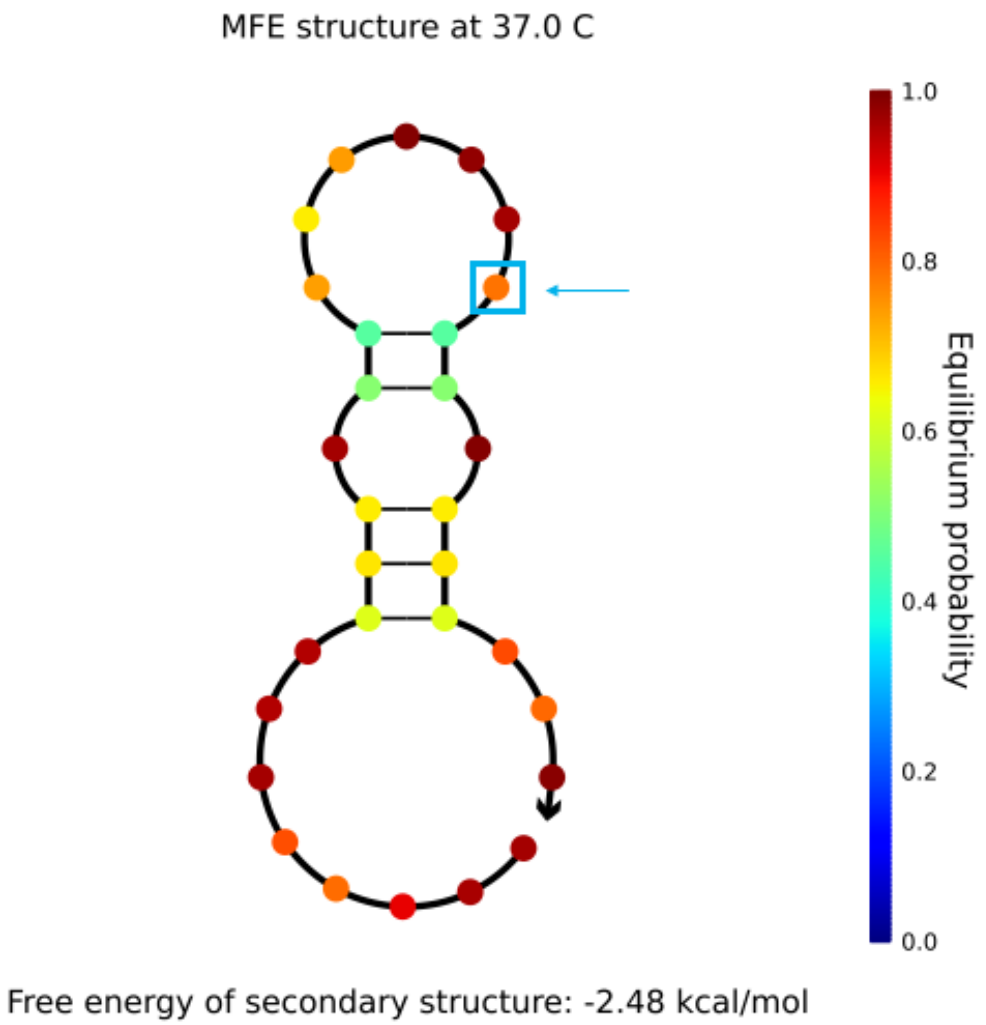


Figure 21. 2-D Model of Random 30-mer Construct. [59]

Another important variable that has not been fully elucidated for this transformation is how decreasing the concentration may alter the kinetics of the catalysts used. Recently, the Koide group developed a general method for determining how concentration and temperature affect the kinetics of an allyl ether deprotection of the Pittsburgh Green fluorophore.[54] This method could be easily applied to our system as activation of a fluorophore has already been accomplished via alloc deprotection. Alternatively, NMR studies of simple alloc protected

amines could be studied under different reaction conditions, but may not be as applicable to biological systems if conducted in organic or deuterated solvents.

2.7 Experimental

All reactions were performed in flame-dried glassware under a nitrogen environment and stirred magnetically unless stated otherwise. All chemicals obtained directly from commercial sources were used without further purification unless indicated otherwise. ^{1}H NMR spectra were obtained from a Bruker Avance III 300, 400, or 500 MHz with chemical shifts reported relative to either residual $CHCl_3$ (7.26 ppm) or CH_3OD (3.30 ppm). Mass spectra analysis was performed by University of Pittsburgh facilities.

General procedures.

Dimethoxytrityl (DMT) protection of primary alcohols. A mixture of the primary alcohol (1 eq), dimethoxytrityl chloride (1.5 eq), and DIPEA (3 eq) was combined in THF (0.08 M) and allowed to stir at room temperature overnight. The reaction mixture was quenched with water, then extracted with diethyl ether (3 x). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The product was purified using flash chromatography on silica gel eluting with a mixture of hexanes, ethyl acetate and 1% triethylamine.[44]

Alloc protection of aryl amines. The amine (1 eq) was dissolved in DCM (0.1 M) then cooled to 0 °C. Pyridine (2 eq) was added and the reaction mixture was stirred for 10 minutes. Allylchloroformate (1.2 eq) was added dropwise and the mixture was stirred at room temperature for 1 hour. The reaction was quenched with water then extracted with DCM (3 x). The combined organic layer was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The product was purified using flash chromatography on silica gel eluting with a mixture of hexanes and ethyl acetate.[48]

Reduction of aryl nitro groups. The aryl nitro (1 eq) was dissolved in a mixture of MeOH/DCM (1:1, 0.04 M) and stirred while 10% Pd/C (10% wt) was added. The reaction mixture was vigorously stirred at room temperature while H_2 gas bubbled through for 3 hours from a balloon. After the disappearance of starting material by TLC, the mixture was filtered through celite and washed with excess DCM. The filtrate was concentrated *in vacuo* to give the product that was used without further purification.

Towards synthesis of 67.

1-(4-Nitrophenyl)but-3-en-1-ol (58). Allyltributyltin (2.15 mL, 6.95 mmol) and zinc chloride (947 mg, 6.95 mmol) were added to a solution of **57** (1.00 g, 6.62 mmol) in acetonitrile/water (10:1, 33 mL). The reaction mixture was stirred overnight at room temperature. After disappearance of starting material by TLC, the mixture was concentrated *in vacuo* and redissolved in diethyl ether (20 mL). The ether mixture was then washed with water (15 mL), brine (15 mL), dried over Na_2SO_4 (~ 500 mg), filtered, and concentrated *in vacuo*. The product was purified using flash chromatography on silica gel eluting with hexanes/EtOAc (4:1) to give **58** as a white solid (1.18 g, 92%).[43] Analytical data matches that previously reported in

the literature for this compound.[55] ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 - 8.18 (d, J = 8.7 Hz, 2 H), 7.50 - 7.52 (d, J = 8.7 Hz, 2 H), 5.72 - 5.82 (m, 1 H), 5.13 - 5.18 (m, 2 H), 4.83 - 4.86 (q, J = 4.2 Hz, 1 H), 2.51 - 2.57 (m, 1 H), 2.40 - 2.48 (m, 2 H).

3-((*tert*-Butyldimethylsilyl)oxy)-3-(4-nitrophenyl)propan-1-ol (61). Compound **58** (622 mg, 3.22 mmol), *tert*-butyldimethylsilyl chloride (728 mg, 4.83 mmol), and imidazole (658 mg, 9.66 mmol) were dissolved in DCM (30 mL) and stirred at room temperature overnight. The reaction mixture was washed with saturated $NaHCO_3$ (20 mL), water (20 mL), dried over Na_2SO_4 (~300 mg), filtered, and concentrated *in vacuo*. The product was purified using flash chromatography on silica gel eluting with hexanes/EtOAc (9:1) to give **61** as a white solid (910 mg, 92%).[46] Analytical data matches that previously reported in the literature for this compound.[56] ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 - 8.20 (dt, J = 8.8, 2.0 Hz, 2 H), 7.45 - 7.48 (dt, J = 8.8, 2.0 Hz, 2 H), 5.66 - 5.80 (m, 1 H), 4.95 - 5.05 (m, 2 H), 4.78 - 4.82 (t, J = 6.0 Hz, 1 H), 2.34 - 2.50 (m, 2 H), 0.89 (s, 9 H), 0.06 (s, 3 H), -0.10 (s, 3 H).

3-((*tert*-Butyldimethylsilyl)oxy)-3-(4-nitrophenyl)propan-1-ol (62). Compound **61** (30 mg, 0.10 mmol) was dissolved in a mixture of MeOH/DCM (1:1, 5 mL) and cooled to -78 °C. Ozone was bubbled through the reaction mixture from a Welsbach T-series ozone generator via a pasteur pipette until saturated (~10 min, turned light blue) then allowed to stir under the same conditions for an additional 10 minutes. The ozone source was removed and $NaBH_4$ (20 mg, 0.50 mmol) was added slowly to the mixture over 15 minutes. The reaction mixture was allowed to warm back up to room temperature and was then concentrated *in vacuo*. The product was purified using flash chromatography on silica gel eluting with hexanes/EtOAc (9:1) to give **62** as a clear oil (25 mg, 80%).[47] ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18 - 8.21 (dt, J = 8.8, 2.0 Hz, 2 H), 7.49 - 7.51 (dt, J = 8.8, 2.0 Hz, 2H), 5.02 - 5.06 (m, 1 H), 3.65 - 3.80 (m, 2 H), 1.90 - 1.96 (m,

2H), 0.91 (s, 9H), 0.08 (s, 3H), -0.12 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$) δ 152.4, 126.5, 123.7, 72.7, 59.5, 42.3, 25.7, 18.1, -4.7, -5.1. HRMS (ESI+) calcd for $C_{15}H_{26}NO_4Si$ $(M+H)^+$ 312.1565, found 312.1565.

(3-(Bis(4-methoxyphenyl)(phenyl)methoxy)-1-(4-nitrophenyl)propoxy)(*tert*-butyl)dimethylsilane (63). Synthesized according to the general procedure for DMT protection of primary alcohols (220 mg, 0.72 mmol). The product was purified using flash chromatography on silica gel eluting with hexanes/EtOAc(20:1) and 1% TEA to give **63** as a clear oil (405 mg, 92%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07 - 8.09 (d, J = 8.8 Hz, 2 H), 7.35 - 7.40 (m, 4 H), 7.18 - 7.29 (m, 7 H), 6.79 - 6.82 (d, J = 8.8 Hz, 4 H), 4.93 - 4.97 (t, J = 6.2 Hz, 1 H), 3.79 (s, 6 H), 3.15 - 3.20 (m, 1 H), 2.96 - 3.03 (m, 1 H), 2.00 - 2.11 (m, 1 H), 1.77 - 1.87 (m, 1 H) 0.80 (s, 9 H), -0.06 (s, 3 H), -0.16 (s, 3 H). ^{13}C NMR data not included for this compound. HRMS (ESI+) calcd for $C_{43}H_{44}NO_6Si$ $(M + H)^+$ 614.2932, found 614.2955.

4-(3-(Bis(4-methoxyphenyl)(phenyl)methoxy)-1-((*tert*-butyldimethylsilyl)oxy)propyl)aniline (64). Synthesized according to the general procedure for reduction of an aryl nitro group (1.51 g, 2.46 mmol). The filtrate was concentrated *in vacuo* to give a clear oil (1.41 g, 98%) that was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 - 7.44 (d, J = 7.1 Hz, 2 H), 7.28 - 7.32 (d, J = 8.8 Hz, 4 H), 7.15 - 7.24 (m, 3 H), 7.00 - 7.03 (d, J = 8.3 Hz, 2 H), 6.79 - 6.82 (d, J = 8.8 Hz, 4 H), 6.57 - 6.60 (d, J = 8.3, 2 H), 4.69 - 4.73 (dd, J = 8.0, 4.8 Hz, 1 H), 3.79 (s, 6 H), 3.06 - 3.14 (m, 2 H), 1.80 - 2.05 (m , 2 H), 0.75 (s, 9 H), -0.14 (s, 3 H), -0.22 (s, 3 H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 158.3, 145.1, 136.7, 135.9, 130.0, 128.2, 127.7, 127.0, 126.5, 114.8, 113.0, 85.9, 72.1, 60.9, 55.2, 25.8, 18.1, -4.6, -5.1. HRMS (ESI+) calcd for $C_{36}H_{46}NO_4Si$ $(M + H)^+$ 584.31906, found 584.31778.

Allyl(4-(3-(bis(4-methoxyphenyl)(phenyl)methoxy)-1-((*tert*-butyldimethylsilyl)oxy)propyl)phenyl)carbamate (65). Synthesized according to general protocol for alloc protection of aryl amines (425 mg, 0.73 mmol). The product was purified using flash chromatography on silica gel eluting with hexanes/EtOAc (9:1) and 1% TEA to give **65** as a clear oil (410 mg, 84% yield) ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 - 7.43 (m, 2 H), 7.27 - 7.32 (m, 6 H), 7.15 - 7.24 (m, 5 H), 6.79 - 6.82 (d, J = 8.8 Hz, 4 H), 6.58 (bs, 1 H), 5.91 - 6.04 (m, 1 H), 5.33 - 5.40 (dq, J = 17.2 , 1.5 Hz, 2 H), 5.24 - 5.28 (dq, J = 17.2, 1.3 Hz, 2 H), 4.76 - 4.80 (dd, J = 8.0, 4.8 Hz, 1 H), 4.65 - 4.68 (dt, J = 5.7, 1.2 Hz, 2 H), 3.79 (s, 6 H), 3.02 - 3.18 (tt, J = 6.5, 9.1 Hz, 2 H), 1.78 - 2.07 (m, 2 H), 0.76 (s, 9 H), -0.12 (s, 3 H), -0.21 (s, 3 H). ^{13}C NMR and HRMS data not determined for this compound.

Allyl (4-(3-(bis(4-methoxyphenyl)(phenyl)methoxy)-1-hydroxypropyl)phenyl)carbamate (66). Compound **65** (300 mg, 0.45 mmol) and *tetra-n*-butylammonium fluoride (235 mg, 0.90 mmol) were dissolved in THF (5 mL) and stirred at room temperature overnight. The reaction was quenched with saturated $NaHCO_3$ (5 mL) then extracted with diethyl ether (3 x 5 mL). The combined organic layer was washed with water (10 mL) and brine (10 mL), dried over Na_2SO_4 (~100 mg), filtered, and concentrated *in vacuo*. The product was purified using flash chromatography on silica gel eluting with hexanes/EtOAc (9:1 - 3:1) and 1% TEA to give **66** as a clear oil (207 mg, 83%).[44] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 - 7.44 (m, 10 H), 7.16 - 7.23 (m, 3 H), 6.81 - 6.86 (dt, J = 8.9, 3.1 Hz, 4 H) 6.59 (bs, 1 H), 5.91 - 6.04 (m, 1 H), 5.24 - 5.40 (qt, J = 17.2, 1.5 Hz, 2 H), 4.86 - 4.97 (m, 1 H), 4.66 - 4.68 (dd, J = 5.7, 1.4 Hz, 2 H), 3.79 - 3.89 (m, 8 H), 1.93 - 2.01 (m, 2 H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 158.5, 144.7, 135.9, 132.4, 130.0, 128.1, 127.9, 126.8, 126.5, 118.3, 113.2, 86.8, 73.2, 62.0, 60.4, 55.2, 21.1, 14.2. HRMS (ESI-) calcd for $C_{34}H_{34}NO_6$ (M - H)- 552.23806, found 552.24647.

Allyl(4-(3-(bis(4-methoxyphenyl)(phenyl)methoxy)-1-(((2-cyanoethoxy)(diisopropylamino)phosphaneyl)oxy)propyl)phenyl)carbamate (67). Alcohol **66** (50 mg, 0.09 mmol) was dissolved in DCM (0.5 mL) then cooled to 0 °C. Diisopropylethylamine (47 µL, 0.27 mmol) was added and the reaction was stirred for 10 minutes. Compound **55** (21 µL, 0.09 mmol) was added dropwise then the reaction mixture stirred at room temperature overnight. The reaction was quenched with MeOH (1 mL) then concentrated *in vacuo*. The product was purified using flash chromatography on silica gel eluting with hexanes/EtOAc (4:1) and 1% TEA to give **67** as a clear oil (35 mg, 52%).[44] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 - 7.45 (m, 2 H), 7.28 - 7.34 (m, 6 H), 7.17 - 7.24 (m, 3 H), 7.01 - 7.05 (dd, J = 8.0, 6.5 Hz, 2 H), 6.79 - 6.83 (t, J = 8.9 Hz, 4 H), 5.75 - 5.87 (m, 1 H), 5.07 - 5.12 (m, 2 H), 4.92 - 5.04 (m, 1 H), 4.53 - 4.57 (m, 2 H), 3.82 - 3.98 (m, 2 H), 3.78 - 3.79 (d, J = 3.67, 6 H), 3.58 - 3.60 (m, 1 H), 3.40 - 3.41 (m, 1 H), 2.37 - 2.57 (m, 2 H), 1.03 - 1.18 (m, 12 H). ^{13}C NMR (500 MHz, $CDCl_3$) δ 158.4, 145.2, 135.5, 132.6, 130.0, 129.8, 128.3, 128.2, 127.7, 126.7, 126.6, 117.1, 117.1, 113.0, 65.9, 61.3, 60.4, 55.2, 46.2, 43.2, 43.1, 43.0, 24.6, 24.5, 23.5, 21.0, 20.2, 14.2, 11.4. ^{31}P NMR (400 MHz, $CDCl_3$) δ 131.1 - 131.7, 147.1 - 147.9. HRMS (ESI^+) calcd for $C_{43}H_{53}N_3O_7P$ $(M + H)^+$ 754.36156, found 754.36292.

Synthesis of catalyst 51.

8-(Allyloxy)-5-chloroquinoline. 5-Chloroquinolin-8-ol (20 mg, 0.11 mmol), sodium hydride (6 mg, 0.22 mmol), and allyl bromide (20 µL, 0.22 mmol) were added to DMF (400 µL) and stirred at room temperature for 1 hour. The reaction mixture was quenched with water (500 µL) and extracted with diethyl ether (3 x 1 mL). The combined organic layer was dried over Na_2SO_4 (~20 mg), filtered, and concentrated *in vacuo*. The product was purified using flash

chromatography on silica gel eluting with hexanes/EtOAc (1:1) to give 8-(allyloxy)-5-chloroquinoline as an off white solid (19 mg, 79%). Analytical data matches that previously reported in the literature for this compound.[39] ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.97 - 8.99 (dd, J = 4.2, 1.6 Hz, 1 H), 8.49 - 8.53 (dd, J = 8.6, 1.7 Hz, 1 H), 7.47 - 7.55 (m, 2 H), 6.95 - 6.98 (d, J = 8.4 Hz, 1 H), 6.11 - 6.24 (m, 1 H), 5.42 - 5.49 (dd, J = 17.3, 1.4 Hz, 1 H), 5.31 - 5.35 (dd, J = 10.5, 1.3 Hz, 1 H), 4.84 - 4.85 (d, J = 5.4 Hz, 2 H), ^{13}C NMR (400 MHz, $CDCl_3$) δ 153.5, 149.8, 140.9, 133.0, 132.8, 127.1, 126.3, 122.3, 122.3, 118.6, 109.2, 70.0. HRMS (ESI^+) calcd for $C_{12}H_{11}NOCl$ $(M + H)^+$ 220.05237, found 220.05538.

Catalyst 51. Tris(acetonitrile)cyclopentadienylruthenium(II) hexafluorophosphate (38 mg, 0.09 mmol) was dissolved in DCM (1 mL). 8-(allyloxy)-5-chloroquinoline (19 mg, 0.09 mmol) was added and the reaction mixture was stirred for 30 minutes. The solvent was removed *in vacuo* then the remaining solid was filtered and washed with ethyl acetate (3 x 5 mL) to give **51** as an orange solid (21 mg, 62%). Analytical data matches that previously reported in the literature for this compound.[39] ^{1}H NMR (400 MHz, acetone-d_6) δ 8.99 - 9.00 (d, J = 4.2 Hz, 1 H), 8.52 - 8.55 (d, J = 9.4 Hz, 1 H), 7.61 - 7.66 (dd, J = 5.2, 8.4 Hz, 1 H), 7.41 - 7.46 (t, J = 8.0 Hz, 1 H), 7.07 - 7.09 (d, J = 7.7 Hz, 1 H), 6.97 - 7.00 (d, J = 7.7 Hz, 1 H), 6.34 (s, 5 H), 4.66 - 4.74 (m, 2 H), 4.38 - 4.47 (m, 2 H), 4.22 - 4.24 (m, 1 H). HRMS (ESI^+) calcd for $RuC_{17}H_{15}NOCl$ $(M - PF_5)^+$ 385.98802, found 385.98954. (ref)

Catalyst test substrates.

Allyl phenylcarbamate. Synthesized according to the general procedure for alloc protection of aryl amine (50 mg, 0.54 mmol). The product was purified using flash chromatography on silica gel eluting with hexanes/EtOAc (9:1 - 8:1) to give the product as a

yellow solid (94 mg, 98% yield). Analytical data matches that previously reported in the literature for this compound.[57] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 - 7.39 (m, 2 H), 7.29 - 7.33 (m, 2 H), 7.05 - 7.09 (t, J = 7.3 Hz, 1 H), 6.63 (bs, 1 H), 5.92 - 6.02 (ddt, J = 17.2, 10.5, 5.7 Hz, 1 H), 5.34 - 5.39 (dq, J = 17.2, 1.5 Hz, 1 H), 5.25 - 5.28 (dq, J = 10.4, 1.3 Hz, 1 H), 4.66 - 4.68 (m, 2 H).

Prop-2-yn-1-yl phenylcarbamate. Aniline (49 µL, 0.54 mmol) and $NaHCO_3$ (135 mg, 1.61 mmol) were added to DCM (1.35 mL) and cooled to 0 °C. Propargyl chloroformate (58 µL, 0.59 mmol) was added to the cooled mixture dropwise over 15 minutes. The reaction mixture was allowed to stir for 2 hours under the same conditions. The reaction was quenched with water (1 mL) then extracted with DCM (3 x 1 mL). The combined organic layer was dried over Na_2SO_4 (~20 mg), filtered, and concentrated *in vacuo*. The product was purified using flash chromatography on silica gel eluting with hexanes/EtOAc (9:1 - 8:1) to give prop-2-yn-1-yl phenylcarbamate as a yellow solid (48 mg, 51%). Analytical data matches that previously reported in the literature for this compound.[58] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 - 7.40 (m, 2 H), 7.29 - 7.34 (m, 2 H), 7.06 - 7.11 (m, 1 H), 6.68 (bs, 1 H), 4.78 - 4.79 (d, J = 2.5 Hz, 2 H), 2.51 - 2.52 (t, J = 2.5 Hz, 1 H).

Cleavage and elimination test substrates.

Allyl (4-acetylphenyl)carbamate (72). Synthesized according to the general procedure for alloc protection of aryl amine (200 mg, 1.48 mmol) The product was purified using flash chromatography on silica gel eluting with hexanes/EtOAc (3:1) to give **72** as a yellow oil (293 mg, 90%). Analytical data matches that previously reported in the literature for this compound.[57] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 - 7.95 (d, J = 8.8 Hz, 2 H), 7.47 - 7.50 (d, J = 8.8 Hz, 2 H),

6.84 (bs, 1 H), 5.91 - 6.04 (ddt, J = 17.2, 10.5, 5.8 Hz, 1 H), 5.34 - 5.42 (dq, J = 17.2, 1.5 Hz, 1 H), 5.27 - 5.36 (dq, J = 10.4, 1.2 Hz, 1 H), 4.68 - 4.71 (m, 2 H), 2.57 (s, 3 H).

Allyl (4-(1-hydroxyethyl)phenyl)carbamate (73). Compound **72** (100 mg, 0.46 mmol) was dissolved in methanol (2.3 mL) and cooled to 0 °C. Sodium borohydride (35 mg, 0.91 mmol) was added in one portion then the reaction mixture was stirred for 2 hours. The reaction was quenched with water (2 mL) then extracted with diethyl ether (3 x 2 mL). The combined organic layer was dried over Na_2SO_4 (~20 mg), filtered, and concentrated *in vacuo*. The product was purified using flash chromatography on silica gel eluting with hexanes/EtOAc (5:1) to give **73** as a white solid (97 mg, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 - 7.38 (m, 4 H), 6.64 (bs, 1 H), 5.92 - 6.01 (m, 1 H), 5.34 - 5.39 (dd, dd, J = 17.2, 1.5 Hz, 1 H), 5.25 - 5.28 (dd, J = 10.4, 1.2 Hz, 1 H), 4.86 - 4.90 (q, J = 6.4 Hz, 1 H), 4.66 - 4.67 (d, J = 5.7 Hz, 2 H), 1.47 - 1.49 (d, J = 6.4 Hz, 3 H). ^{13}C NMR and HRMS data was not determined because this was only studied as a model system.

Allyl (4-(acetyl-d3)phenyl)carbamate (77). Compound **72** (300 mg, 1.4 mmol) and potassium carbonate (20 mg, 0.14 mmol) were added to methanol-d_4 (6 mL) and stirred at room temperature. Complete deuteration was confirmed after 30 minutes from the disappearance of the alpha-keto protons via ^{1}H NMR. The reaction mixture was then cooled to 0 °C. Sodium borohydride (106 mg, 2.8 mmol) was added to the mixture which was allowed to stir for 2 hours. The mixture was quenched with water (3 mL) before the methanol-d_6 was removed *in vacuo*. The remaining aqueous solution was extracted with diethyl ether (3 x 1 mL). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo* resulting in a white solid (245 mg, 80%), which was used without further purification. ^{1}H NMR (300 MHz, MeOH-d_4) δ 7.38 - 7.40 (m, 2 H), 7.26 - 7.29 (m, 2 H), 5.93 - 6.06 (m, 1 H), 5.32 - 5.40 (dq, J = 17.2,

1.6 Hz, 1 H), 5.20 - 5.25 (dq, J = 10.5, 1.4 Hz, 1 H), 4.76 (bs, 1 H), 4.61 - 4.64 (dt, J = 17.2, 1.6 Hz, 2 H). ^{13}C NMR and HRMS data was not determined because this was only studied as a model system.

Allyl (4-(hydroxymethyl)phenyl)carbamate (80). Synthesized according to the general procedure for alloc protection of an aryl amine (90 mg, 0.81 mmol). The product was purified using flash chromatography on silica gel eluting with hexanes/EtOAc (5:1 - 0:1) to give **80** as a yellow oil (139 mg, 83%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 - 7.40 (m, 4 H), 6.63 (bs, 1 H), 5.91 - 6.04 (m, 1 H), 5.33 - 5.40 (dd, J = 17.2, 1.5 Hz, 1 H), 5.25 - 5.29 (dd, J = 10.4, 1.3 Hz, 1 H), 4.65 - 4.69 (m, 4 H). ^{13}C NMR and HRMS data was not determined because this was only studied as a model system.

Allyl (4-(((dimethoxyphosphoryl)oxy)methyl)phenyl)carbamate (81). Alcohol **80** (50 mg, 0.24 mmol) and DABCO (40 mg, 0.36 mmol) were dissolved in THF (2.4 mL) and stirred at room temperature. Dimethyl chlorophosphate (39 µL, 0.36 mmol) was added dropwise and then the mixture was stirred for 12 hours. The reaction mixture was concentrated *in vacuo*.
The product was directly purified using flash chromatography on silica gel eluting with hexanes/EtOAc (5:1 - 0:1) to give **81** as a yellow oil (38 mg, 49%).[51] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 - 7.44 (d, J = 8.3 Hz, 2 H), 7.30 - 7.32 (d, J = 8.5 Hz, 2 H), 7.25 (bs, 1 H), 5.89 - 5.99 (m, 1 H), 5.32 - 5.36 (dd, J = 17.2, 1.5 Hz, 1 H), 5.22 - 5.25 (dd, J = 10.4, 1.2 Hz, 1 H), 5.01 - 4.99 (d, J = 8.7 Hz, 2 H), 4.63 - 4.65 (d, J = 5.7 Hz, 2 H), 3.71 (s, 3 H), 3.68 (s, 3 H). ^{31}P NMR (400 MHz, $CDCl_3$) δ 1.26. ^{13}C NMR and HRMS data was not determined because this was only studied as a model system.

Towards synthesis of 87.

2,2-Dimethyl-1-(4-nitrophenyl)propane-1,3-diol (83). Potassium hydroxide (40 mg, 0.66 mmol) in ethanol (0.5 mL) was added slowly to a stirring solution of **82** (100 mg, 0.66 mmol) and isobutyraldehyde (120 µL, 1.2 mmol). The mixture was allowed to cool (~10 min), then heated to 60 °C and stirred for 5 hours. The reaction mixture was then concentrated *in vacuo* before being resuspended in water (1 mL) and extracted with diethyl ether (3 x 1 mL). The combined organic layer was washed with water (3 mL), dried over Na_2SO_4 (~100 mg), filtered, and concentrated *in vacuo*. The product was purified using flash chromatography on silica gel eluting with hexanes/EtOAc (2:3) to give **83** as an off-white solid (98 mg, 66%).[53] ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 - 8.22 (m, 2 H), 7.51 - 7.54 (m, 2 H), 4.79 - 4.81 (d, J = 3.2, 1 H), 3.55 - 3.61 (m, 3 H), 2.19 - 2.23 (t, J = 5.0 Hz, 1 H), 0.88 (s, 6 H). ^{13}C NMR (500 MHz, $CDCl_3$) δ 148.9, 147.3, 128.5, 122.9, 80.9, 77.3, 72.2, 39.3, 22.5, 18.7. HRMS was not determined for this compound.

3-(Bis(4-methoxyphenyl)(phenyl)methoxy)-2,2-dimethyl-1-(4-nitrophenyl)propan-1-ol (84). Synthesized according to the general procedure for the DMT protection of a primary alcohol (440 mg, 1.95 mmol). The product was purified using flash chromatography on silica gel eluting with hexanes/EtOAc (4:1 - 2:3) and 1% TEA to give **84** as a colorless oil (1.06 g, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 - 8.04 (d, J = 8.8 Hz, 2 H), 7.44 - 7.47 (m, 2 H), 7.33 - 7.36 (m, 5 H), 7.27 - 7.30 (m, 4 H), 6.85 - 6.89 (m, 4 H), 4.68 - 4.69 (d, J = 3.8 Hz, 1 H), 3.98 - 3.99 (d, J = 3.8 Hz, 1 H), 3.82 (s, 6 H), 3.08 - 3.11 (d, J = 9.2 Hz, 1 H), 3.01 - 3.04 (d, J = 9.2 Hz, 1 H), 0.92 - 0.93 (d, J = 4.5 Hz, 6 H). ^{13}C NMR (500 MHz, $CDCl_3$) δ 158.7, 148.9, 147.1, 144.3, 135.2, 130.1, 129.1, 128.5, 128.1, 128.0, 127.9, 127.8, 127.1, 122.6, 113.3, 113.2, 86.9, 80.5, 71.0, 60.4, 55.3, 39.5, 23.1, 20.2. HRMS (ESI$^+$) calcd for $C_{32}H_{33}NO_6Na$ $(M + Na)^+$ 550.22001, found 550.21864.

1-(4-Aminophenyl)-3-(bis(4-methoxyphenyl)(phenyl)methoxy)-2,2-dimethylpropan-1-ol (85). Synthesized according to the general procedure for an aryl nitro reduction (1.06 g, 2.01 mmol). The filtered product was concentrated *in vacuo* to give **85** as a colorless oil (805 mg, 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 - 7.48 (d, J = 7.2 Hz, 2 H), 7.22 - 7.37 (m, 7 H), 6.93 - 6.96 (d, J = 8.4 Hz, 2 H), 6.84 - 6.87 (m, 4 H), 6.51 - 6.54 (d, J = 8.4 Hz, 2 H), 4.49 - 4.50 (d, J = 3.4 Hz, 1 H), 3.80 (s, 6 H), 3.56 - 3.60 (m, 3 H), 3.09 - 3.12 (d, J = 8.9 Hz, 1 H), 2.98 - 3.01 (d, J = 8.9 Hz, 1 H), 0.89 (s, 3 H), 0.85 (s, 3 H). ^{13}C NMR (500 MHz, $CDCl_3$) δ 158.5, 145.2, 144.6, 135.8, 131.6, 130.2, 128.6, 128.2, 127.9, 126.8, 114.2, 113.2, 86.6, 80.9, 71.4, 55.2, 39.6, 23.4, 19.8. HRMS (ESI$^+$) calcd for $C_{32}H_{36}NO_4$ $(M + H)^+$ 498.26389, found 498.26314.

Allyl(4-(3-(bis(4-methoxyphenyl)(phenyl)methoxy)-1-hydroxy-2,2-dimethylpropyl)phenyl)carbamate (86). Synthesized according to the general procedure for alloc protection of an aryl amine (800 mg, 1.61 mmol). The product was purified using flash chromatography on silica gel eluting with hexanes/EtOAc (5:1) and 1% TEA to give **86** as a colorless oil (774 mg, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 - 7.47 (d, J = 7.2 Hz, 2 H), 7.28 - 7.37 (m, 8 H), 7.16 - 7.23 (m, 1 H), 7.08 - 7.11 (d, J = 8.6 Hz, 2 H), 6.82 - 6.87 (m, 4 H), 6.56 (bs, 1 H), 5.90 - 6.03 (m, 1H), 5.33 - 5.39 (dd, J = 17.2, 1.5 Hz, 2 H), 5.24 - 5.28 (dd, J = 10.4, 1.3 Hz, 1 H), 4.65 - 4.67 (d, J = 5.6 Hz, 2 H), 4.55 - 4.56 (d, J = 3.3 Hz, 1 H), 3.81 (s, 6 H), 3.74 - 3.75 (d, J = 3.5 Hz, 1 H), 3.09 - 3.12 (d, J = 8.9 Hz, 1 H), 2.99 - 3.02 (d, J = 8.9 Hz, 1 H), 0.90 (s, 3 H), 0.86 (s, 3 H). ^{13}C NMR (500 MHz, $CDCl_3$) δ 158.6, 144.5, 136.6, 135.6, 132.5, 130.1, 128.3, 128.1, 128.0, 126.9, 118.2, 113.2, 86.7, 80.7, 71.4, 65.8, 55.2, 39.5, 23.3, 19.8. HRMS (ESI$^+$) calcd for $C_{36}H_{39}NO_6$ $(M + Na)^+$ 604.26696, found 604.26595.

Allyl(4-(3-(bis(4-methoxyphenyl)(phenyl)methoxy)-1-((dimethoxyphosphoryl)oxy)-2,2-dimethylpropyl)phenyl)carbamate (87). Compound **86** (50 mg, 0.09 mmol) was dissolved

in THF (860 µL) and cooled to 0 °C. DIPEA (45 µL, 0.26 mmol) was added and the mixture was stirred for 10 minutes under the same conditions. *N,N*-diisopropylmethylphosphonamidic chloride (38 µL, 0.17 mmol) was added and the reaction mixture was stirred overnight. The reaction was quenched with methanol (1 mL) then concentrated *in vacuo*. The product was purified using flash chromatography on silica gel eluting with hexanes/EtOAc (5:1) and 1% TEA to give **87** as a colorless oil (56 mg, 65%).[44] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 - 7.42 (m, 2 H), 7.28 - 7.35 (m, 5 H), 7.25 - 7.27 (m, 1 H), 7.19 - 7.21 (m, 1 H), 7.08 - 7.11 (m, 2 H), 7.01 - 7.03 (m, 1 H), 6.79 - 6.82 (m, 4 H), 6.53 (bs, 1 H), 5.92 - 6.00 (m, 1 H), 5.33 - 5.37 (dd, J = 17.2, 1.5 Hz, 1 H), 5.24 - 5.26 (d, J = 10.4 Hz, 1 H), 4.74 - 4.81 (m, 1 H), 4.64 - 4.65 (d, J = 5.6 Hz, 2 H), 3.80 - 3.83 (d, J = 13.9 Hz, 2 H), 3.79 (s, 6 H), 3.54 - 3.61 (m, 1 H), 3.37 - 3.43 (m, 1 H), 1.08 - 1.30 (m, 12 H), 0.88 - 0.89 (d, J = 6.7 Hz, 6 H), 0.71 (s, 3 H). ^{13}C NMR (500 MHz, $CDCl_3$) δ 158.3, 145.3, 145.2, 136.5, 136.4, 136.3, 136.2, 132.5, 130.5, 130.4, 130.3, 129.0, 128.7, 128.6, 128.5, 127.6, 126.5, 118.2, 117.2, 112.9, 112.8, 85.6, 80.5, 78.8, 69.1, 69.0, 65.7, 55.2, 53.4, 50.7, 50.5, 50.4, 47.4, 43.1, 43.0, 42.8, 42.7, 40.8, 24.7, 24.2, 22.9, 22.5, 21.7, 21.2, 21.0, 20.2. ^{31}P NMR (400 MHz, $CDCl_3$) δ 149.8, 146.9. HRMS (ESI$^+$) calcd for $C_{43}H_{58}N_2O_8P$ $(M + H_3O)^+$ 761.39253, found 761.39107.

Additional procedures.

HPLC analysis studies. HPLC analysis was performed on either a Shimadzu or Agilent 1200 series model HPLC. Reaction aliquots were run on an Agilent InfinityLab Poroshell 120 EC-C18 column (4.6 x 150 mm, 4 µm particles with Phenomenex SecurityGuard C18 kit) at 40 °C eluting with a 5 - 95% gradient of acetonitrile in water. The chromatograms were generated

using absorbance values of eluting compounds at 254 nm and analyzed either via Prism GraphPad (Shimadzu HPLC) or ChemStation (Agilent 1200 series).

DNA synthesis and purification. DNA synthesis of modified oligonucleotides was performed using standard β-cyanoethyl phosphoramidite chemistry, with appropriately protected canonical bases and phosphate linkages on an Applied Biosystems model 394 automated DNA/RNA synthesizer at a 0.2 mM scale with solid-phase supports. All commercial reagents were obtained from Glen Research. Linkers **54** and **87** were dissolved in acetonitrile to a final concentration of 0.07 M. Standard synthesis cycles provided by Applied Biosystems were used with 10 min coupling times for **54** and **87**. Oligonucleotides were eluted from the solid-phase supports with 1 mL of ammonium hydroxide methylamine (AMA, 1:1) and deprotected at room temperature overnight. The full-length modified oligonucleotides were purified by PAGE gel band excision and elution into PBS buffer, pH 7.4. Concentration of modified oligonucleotide was determined by absorbance at 260 nm via a nanodrop spectrophotometer. Mass was confirmed via electrospray ionization mass spectrometry (ESI-MS) performed by University of Pittsburgh facilities.

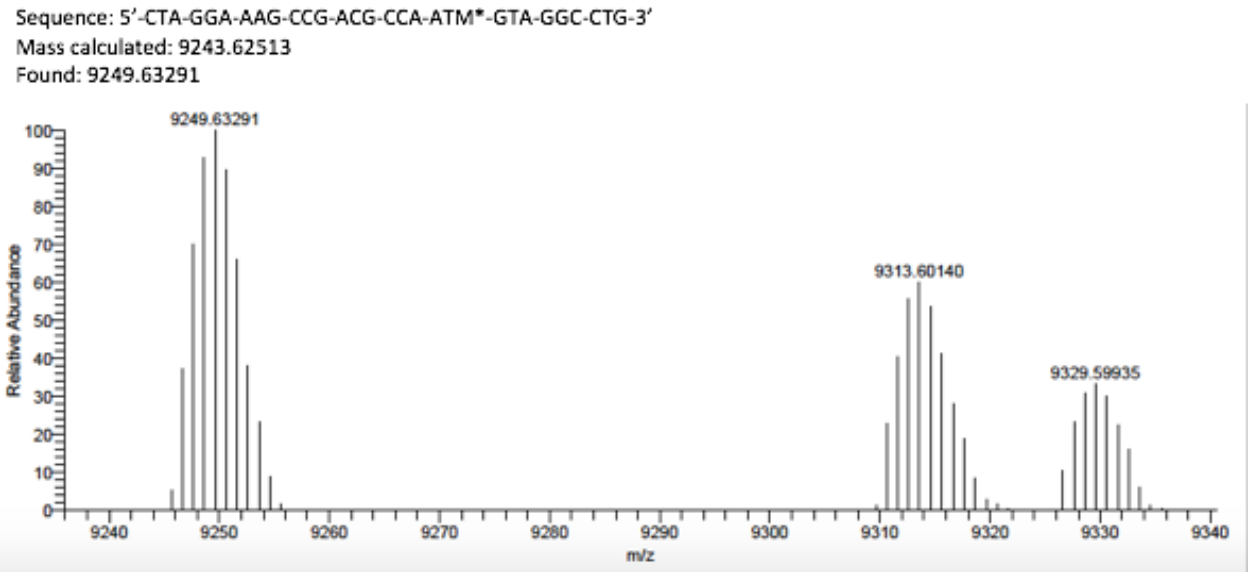


Figure 22. HRMS of 30-mer Containing Linker 54.

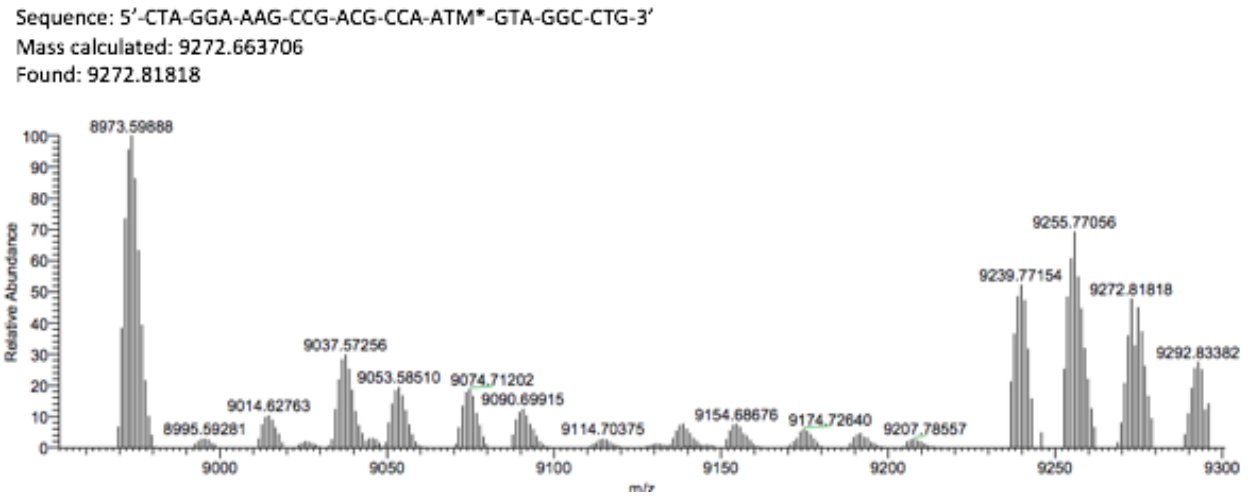


Figure 23. HRMS of 30-mer Containing Linker 87.

***In vitro* DNA studies.** DNA cleavage studies were conducted in 0.2 mL PCR tubes containing 10 μM of the designated oligonucleotide and 5 mM GSH in a PBS (pH = 7.4)/DMSO mixture (200:1). Reaction vessel was securely placed in an incubator and shaken at 37 °C for the

allotted time. Reaction mixtures were diluted with undyed glycerol loading buffer and analyzed via a 20% acrylamide gel run at 120 V for 1 hour. Resultant gel was stained with Invitrogen SYBR gold according to manual protocol and imaged using ChemiDoc software.

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