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A Generalized Approach to Deliver Functionalized Isoindolines in Macrocycles

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A Generalized Approach to Deliver Functionalized Isoindolines in Macrocycles

By

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B.A. in Chemistry

M.S. in Chemistry

Advisor: Simon B. Blakey, PhD

An abstract of
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Abstract

A Generalized Approach to Deliver Functionalized Isoindolines in Macrocycles

By Tiffany Taylor

Macrocyclic peptides are a growing class of compounds that have shown promise in drug development by improving pharmacological properties. There are currently four cyclization strategies to close macrocycles; however, methods of sidechain-to-sidechain macrocyclization have shown to be more difficult without sulfide bridging, lactonization, or lactamization. We aim to develop a generalized strategy for sidechain-to-sidechain macrocyclization to form an isoindoline core via an intramolecular [2+2+2] cycloaddition. Transition metal catalyzed [2+2+2] cycloaddition reactions are attractive for constructing functionalized benzene rings and heterocyclic compounds. These reactions are known to have high atom efficiency, as well as good enantio-, regio-, and diastereoselectivity. However, there is little precedent using this approach on non-natural amino acid to synthesize fused pyrrolidine motifs in a macrocyclic form. We have begun developing an effective [2+2+2] method utilizing amino acid derived alkynes and we plan to use this method to conduct macrocyclizations on linear peptides. This work will aid in the development of a generalized method to close macrocyclic peptides, as well as building a library of unnatural cyclic peptides.

A Generalized Approach to Deliver Functionalized Isoindolines in Macrocycles

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Table of Content

Introduction

Macrocyclic peptides have shown promise in drug development due to their ability to bind to protein surfaces without a defined binding pocket and improve pharmacological properties. Macrocyclization of peptides can improve pharmacological properties by forcing the peptide into a constrained conformation that helps to enhance membrane permeability, metabolic stability, and binding affinity, which increases oral bioavailability.[1] Ribosomally synthesized and post-translationally modified peptides (RiPPs) are a growing class of natural products due to their unique structures and wide range of biological activities. With the advancements in bioinformatics over the past few years, scientists have been able to discover over 100 gene clusters that encode unique RiPPs, that have shown promising biological activity.[2-3] Some RiPPs have modifications that induce rings systems causing the peptide to form a macrocycle. One subclass of these RiPPs contain C-C crosslinks between the sidechains of the amino acid residue,

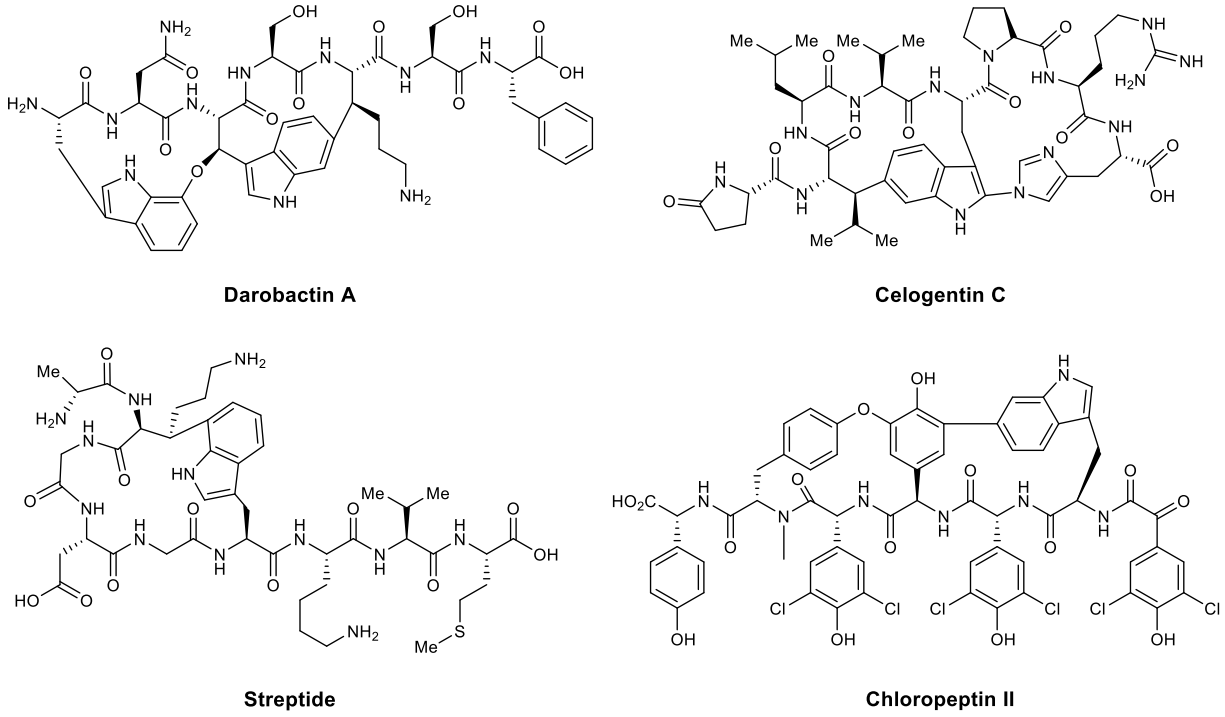


Figure 1. Examples of macrocyclic RiPP that have shown promising biological activity.

which includes pharmaceutically relevant compounds such as darobactin A, streptide, celogentin C (Figure 1).[4-8] Despite their medicinally relevant bioactivities, these compounds remain understudied in the pharmaceutical field due to there being no general design guidelines for building macrocyclic compounds because of the synthetic complexity of crosslinking sidechains. Thus, developing methods to synthesizing macrocyclic peptides will aid in the development of pharmaceuticals and allow us to explore a new chemical space of unnatural cyclic peptides. There are currently four cyclization strategies to close macrocycles: head-to-tail, tail-to-sidechain, head-to-sidechain, and sidechain-to-sidechain. However, facile sidechain-to-sidechain cyclizations are, the most used methods to achieve macrocyclization are disulfide bridging, lactonization, CuAAC (Cu(I)catalyzed azide-alkyne -1,3-dipolar cycloaddition), and ring-closing metathesis.[13-16] Recently there have been advances in generating sidechain to sidechain crosslinks in macrocycles composed of a tryptophan residue. Boger and co-workers have shown how powerful the Larock indole synthesis is when they synthesized two natural products—chloropeptin II (2011) and streptide (2019)(Figure 1). With this method, they were able to build the C-C crosslink between the sidechains of a tryptophan reside and other amino acid residues(lysine, tyrosine).[8-9] Since then, there have been two more publications (both in 2022) by Sarlah's and Baran's groups, where they were able to construct darobactin A utilizing the same methodology as Boger to close both the A and B rings of this RiPP, demonstrating the effectiveness in synthesizing this type of linkage.[10-11] The Larock indole synthesis is a powerful reaction that is used to synthesize functionalized indoles utilizing a variety of alkynes and a Pd-catalyst (Figure 2).[12] The use of Pd, high temperatures, and super stoichiometric amounts of base allows this reaction to be robust and highly selective. Larock and coworkers propose that the mechanism proceeds via a reduction of the Pd^{II} species to a Pd^{0}

species (I), then coordinates to the chlorine atom to form a new zerovalent Pd^0 complex (II) with chlorine.

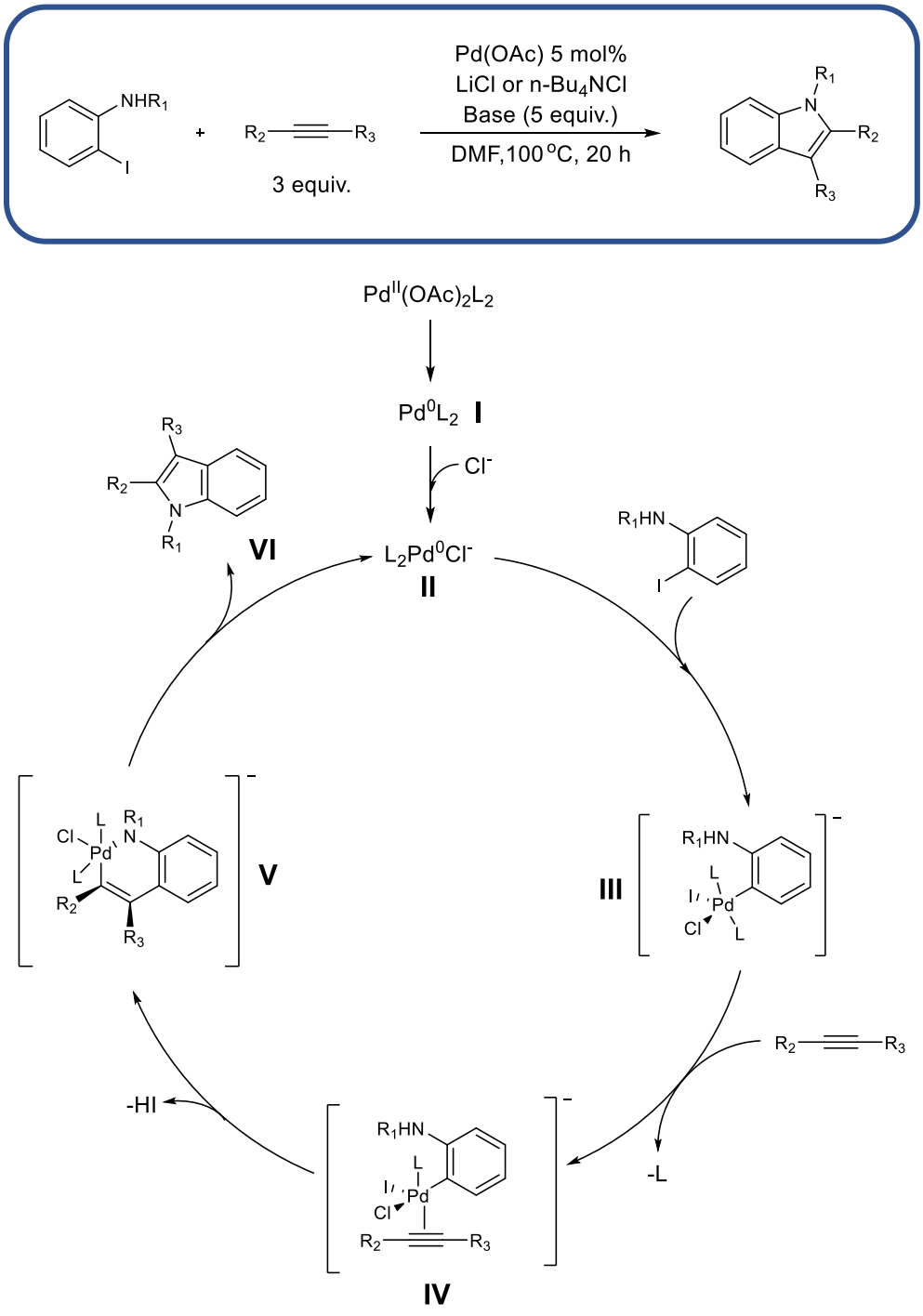


Figure 2. Larock Indole Synthesis Conditions and Proposed Mechanism.

The new Pd^0 species then undergoes an oxidative addition with the aryl iodine to form complex (III), then coordinates with the alkyne to produce an aryl palladium intermediate (IV) which progresses through a syn-insertion into the aryl palladium bond. The halide is then displaced by the nitrogen resulting in a six-membered palladacycle (V) then undergoes a reductive elimination to afford the desired indole product (VI) and regenerates the Pd^0 catalyst (Figure 2).

We plan to utilize the main concept behind the Larock annulation and apply it towards closing macrocycles. This work can also prove useful in delivering macrocycles with an isoindoline core, which acts as an important pharmacophore in drug candidates.[28]

In previous syntheses of building macrocycles, the first step would be to build the crosslink between the sidechains of the amino acid residues to establish the stereocenters and functional handles then the rest of the peptide is constructed through peptide coupling.[4,6-11] With our research, we envision applying a late stage macrocyclization, where the linear peptide is built first then cyclization of the peptide would be conducted last. Utilizing this strategy, we would be able to synthesize linear peptide precursors using solid phase peptide synthesis (SPPS) to incorporate the desired pre-functionalized amino acids followed by our macrocyclization strategy to close the newly formed macrocycle.

<u>Methodology Background:</u>

Transition metal catalyzed [2+2+2] cycloaddition reactions are attractive for constructing functionalized benzene rings and heterocyclic compounds. These reactions are known to have high atom efficiency, as well as good enantio-, regio-, and diastereoselectivity. With a variety of transition-metals being used in catalyst complexes-Rh, Ru, Co, and Pd- rhodium catalysts are the most popular and efficient. In 1974, Muller reported on the use of a rhodium(I) mediated [2+2+2] cycloaddition to synthesize substituted benzenes in organic synthesis, which has inspired further

functionalization of benzene derivatives.[16] These reactions have been shown to work with both internal and external alkynes, symmetrical and asymmetrical dipropargyl amines, and can tolerate a variety of functional groups; in addition, they have also shown to work in both aqueous and organic solvent conditions.[17-25] In 2006, Deiters and co-workers published a general method using Wilkinson's catalyst to synthesize isoindolines on resin, which gave good yields (62%-85%); however, there was a very limited scope for this reaction (7 examples).[24] Despite the limited scope, this reaction shows that it can work on resin, which tells us that it could be compatible with peptide synthesis.

Roglans and co-workers published a general method using a cationic rhodium catalyst (Wilkinson's catalyst) and tethered dialkyne under reflux conditions to produce an isoindoline core with amino acid functionality (Figure 3).[26] They report that protection of both the C- and N-terminus on the amino acid (propargylglycine residue) is necessary due to solubility issues. With the introduction of amino acid functionality in this chemistry, it has sparked interest in using these types of reactions for applications in peptide chemistry. Notably, this group published a review in 2022 on the use of rhodium catalysts, the advantages that rhodium has over the other widely used transition metal catalysts (Co and Ru). In the review, they propose a catalytic cycle that shows the dialkyne coordinating with the cationic Rh(I)-complex (I) which gets oxidatively inserted to form a fused rhodacyclopentadiene complex (II). The rhodacyclopentadiene then coordinates with the alkyne (III) which then goes through an oxidative addition to form a rhodabicyclo [3.2.0] heptatriene (IV) (1.7 kcal/mol) which quickly converts to the more stable rhodacycloheptatriene

(V) due to the exergonic nature of the transformation (-11.8 kcal/mol). The rhodacycle then goes through a reductive elimination to produce the predicted isoindole product (VI) (Figure 3).[27]

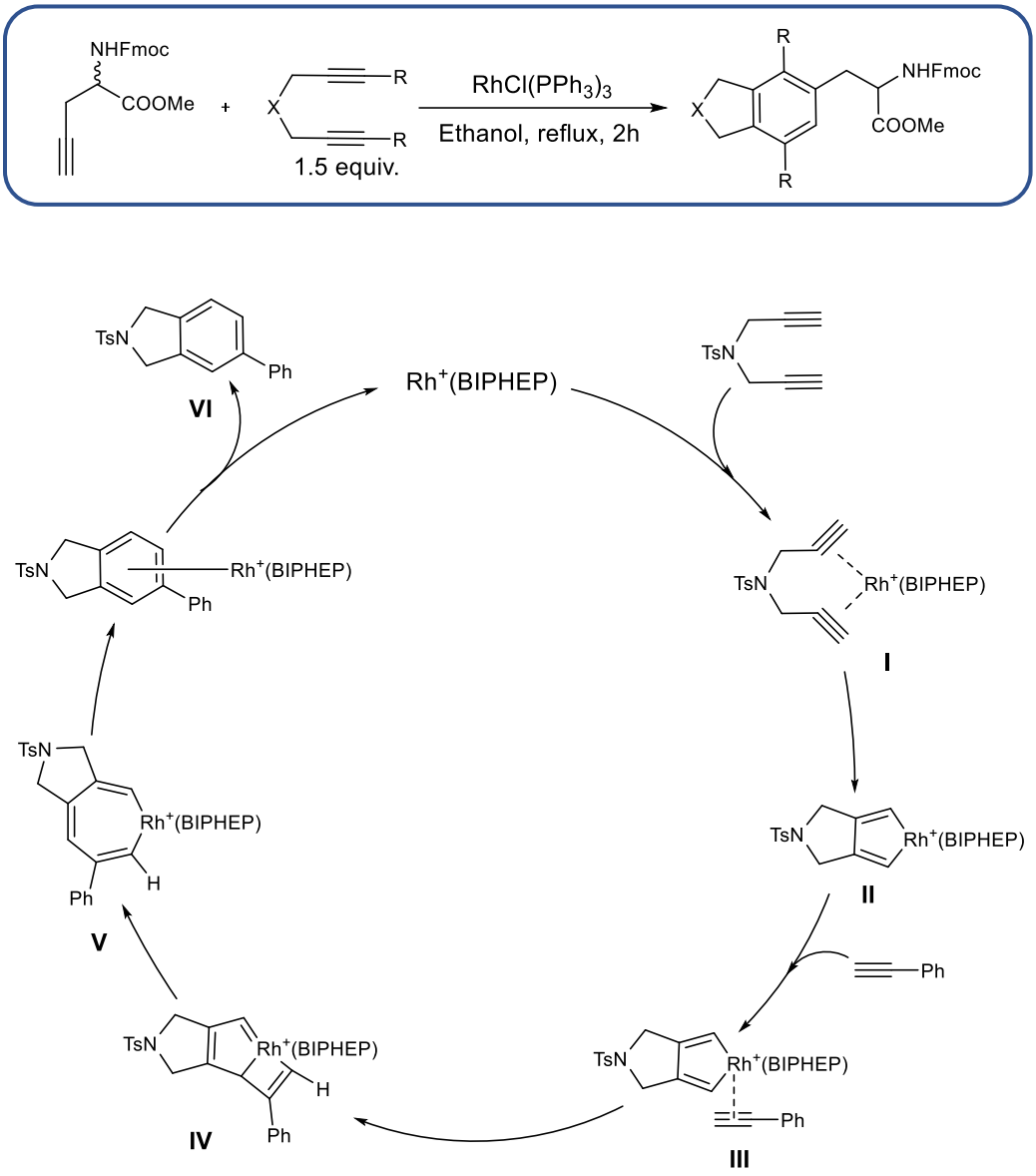


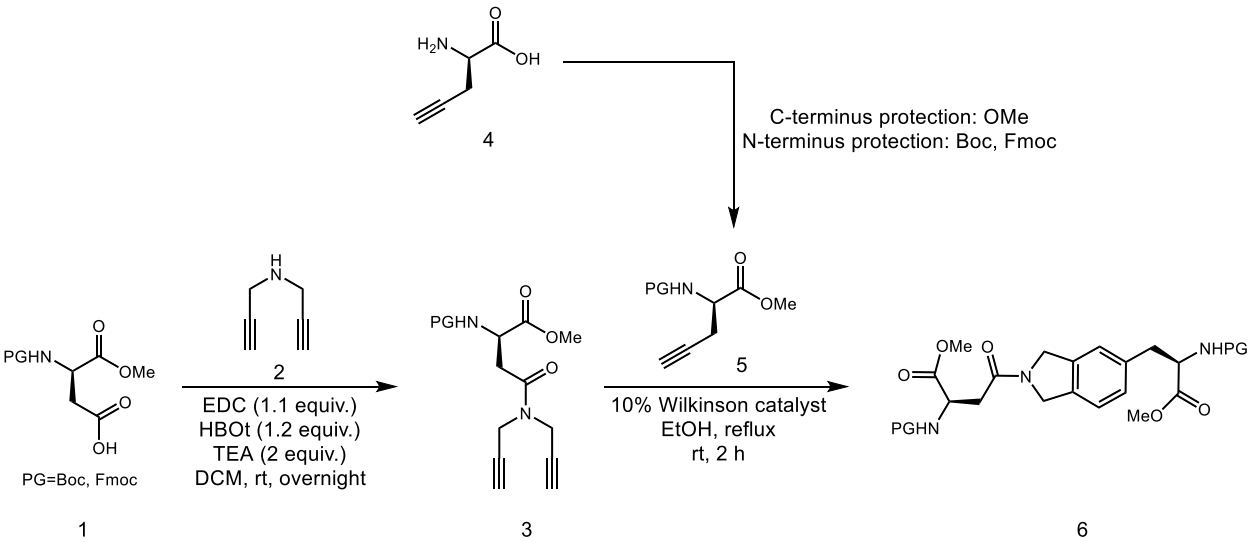
Figure 3: Roglans and co-workers [2+2+2] method (top) and proposed mechanistic pathway for a Rh catalyzed [2+2+2].

There is little precedent using this approach on non-natural amino acids to synthesize fused pyrrolidine motifs in a macrocyclic form. We believe the [2+2+2] cycloaddition is an ideal method for macrocyclization because it resembles the Larock annulation where the transition metal

complex templates the moieties for cyclization and releases ring strain to form macrocycles. This catalytic framework will hopefully offset the entropic penalty for macrocyclization. Therefore, we plan to extend Roglans group's Rh catalyzed [2+2+2] strategy to synthesize the isoindoline core between two amino acids derivatives (aspartic acid and glycine) and incorporate the core into a macrocyclic peptide.

Results and Discussion

We initially hypothesized that a rhodium-catalyzed [2+2+2] cycloaddition with two amino acid sidechains (aspartic acid and glycine), would furnish an isoindoline core which could be used to close a macrocycle. To test this hypothesis, we first aimed to functionalize the protected aspartic acid (1) with the dipropargylamine (2) to deliver the amidated amino acid product (3). In addition to functionalizing the aspartic acid residue, both the N- and C-terminus of the propargyl glycine (4) residue needed to be protected to help with solubility of the compound (5). Once both starting materials were synthesized, we began the [2+2+2] cycloaddition using the method from Roglans and coworkers (Figure 4), to furnish the isoindoline core (6), which was verified to work on amino acid functionalities (Scheme 1).[26] We first chose to protect the propargyl glycine residue and the

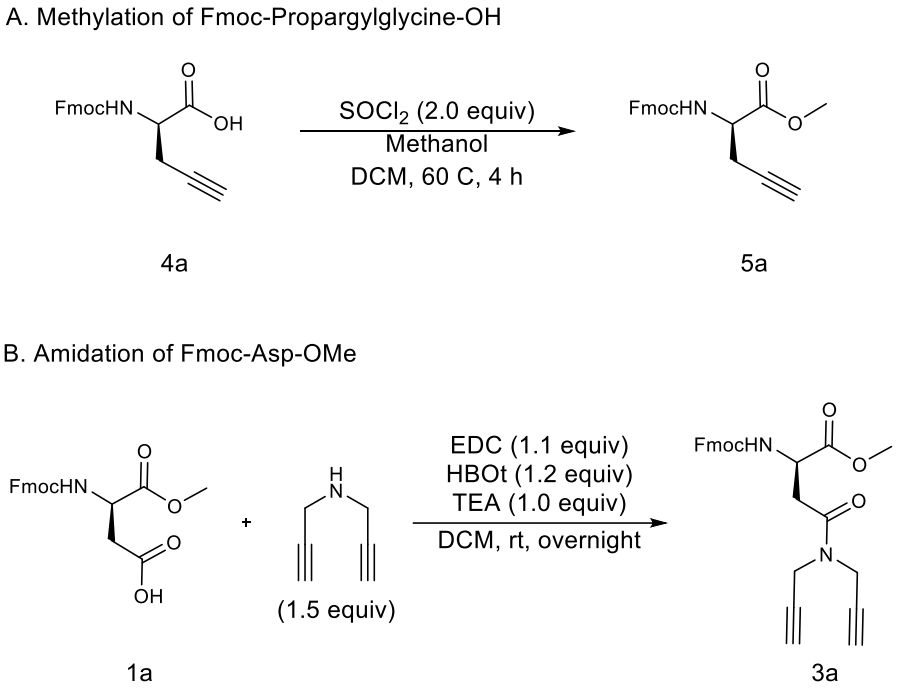


Scheme 1: Proposed Synthetic Route for the construction of the isoindoline core.

aspartic acid derivative with a Boc group on the N-terminus and a methyl ester on the C-terminus. The design of this system was pursued to indicate that if any aromatic peaks were observed it would show product formation. We observed the predicted product in an isolated yield of 6%, which indicated that the system needed to be optimized. With the same reaction conditions used in the [2+2+2] reaction, we started the optimization process focusing on changing solvent, temperature, catalyst, and protecting groups of the amino acids. Starting with the solvent screen, we expanded the previous solvent scope from Roglans group, in which they only used toluene, DCM, and ethanol. We looked at changing the solvents to incorporate more nonpolar and polar solvents. We first started with a more polar solvent, methanol, which initially showed an increase in yield, however, once confirming with mass spectroscopy it showed that other impurities were still in the sample. From this we decided to incorporate an internal standard (trichloroethylene) to help determine the yield from H^1 NMR, unfortunately our results showed that the yield had dropped slightly (4%), and the product was unable to be cleanly isolated. Next, we started to look at some non-polar solvents- acetonitrile and DMF- which gave 1% and 0% respectively, with a return of the dialkyne. From this initial screen and the previous result from the literature shows that this reaction favors a more polar solvent environment to construct the isoindoline core.

After the results of the solvent screen didn't improve much compared the initial result, we decided to start looking at incorporating biphasic systems, which has shown to be effective in previous [2+2+2] reactions. Recently, Lu and coworkers developed a method utilizing a ruthenium catalyst with water/DMA mixture to produce isoindolines for DNA-encoded library. [28] Seeing success from them, we decided to pursue this route. However, after testing their conditions on our system we did not see any of the anticipated products; in addition, we did see a return of the dialkyne starting material and two homocoupled products.

In concurrent with optimizing on the Boc-Asp-OMe and Boc-Pra-OMe system, we started to synthesize more amino acid derivatives focusing on the protecting group strategy. We decided to change the Boc protection groups to Fmoc protection groups on both amino acid derivates to see if the protecting group played a factor. Methylating the C-terminus of Fmoc-Pra-OH (4a) went smoothly with decent to good yields (41%-87%) to yield a fully protected propargylglycine amino acid (5a) (Scheme 2a). We were also able to amidate the Fmoc-Asp-OMe (1a) to make the amino acid dialkyne in 11% (3a) (Scheme 2b).Once all peaks were confirmed by H^1 and C^{13} NMR, we now had all the starting materials needed to run a [2+2+2] with a global Fmoc protection strategy. Having all precursors made for the [2+2+2] reaction, we were ready to test the conditions on our substrates. However, our biggest obstacle has been the ability to increase yields. With this in mind, we decided to recreate the literature precedented reaction with a tosyl protected dialkyne and Fmoc-Pra-OMe to see what could be causing the yields to be low. Previous literature precedents had shown that this reaction works well with sulfide groups, carbenes, or bulky-protecting groups attached to the nitrogen on the dialkyne, but not an amide group. Running this key precedent could tell us what could be causing the reaction to have low yields such as condition of the catalyst, the substrates being used, and amount of water and air the reaction can be exposed to. From using the exact conditions from the paper with the same substrates (tosyl-protected dipropargyl amine and Fmoc-propargylglycine-OMe), we will be able to determine what is causing the reaction yield to be low. We started by testing the reactivity of the catalyst that we had previously; this resulted in a <10% yield. From this result, we thought that the rhodium complex could have changed oxidation states over time from a Rh(I) complex to a Rh(III) complex (most stable oxidation state of rhodium).



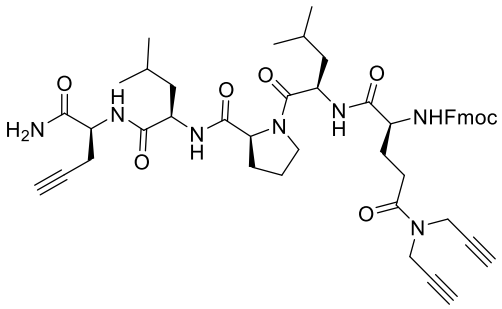
Scheme 2: Functionalizing starting materials needed for [2+2+2] reactions.

Rerunning the experiment using a new bottle of catalyst showed to increase the yield slightly to 32%; however once both a new bottle of catalyst and distilled ethanol was used the yield increased significantly to 78%. Once I was able to reproduce the literature precedent with comparable yields, I moved these conditions to my new system of Fmoc-Pra-OMe and Boc-Asp dialkyne-OMe. Changing the propargyl glycine derivative from Boc protected to Fmoc protected, was made to ensure that one variable was being changed between the literature precedent reactants and our strategy's reactants. We revised the solvent screen conditions utilizing protic and aprotic solvents (Table 1), which resulted in DCM being the best candidate for this system.

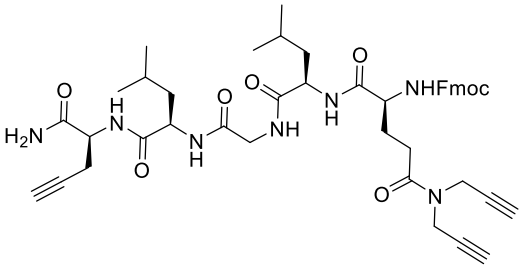
Next steps will involve building a linear peptide. First, I started out using solution phase peptide synthesis, which was not very robust, due to only being able to produce about 30% of the 2mer peptide. This strategy would not be as efficient or effective for synthesizing these linear peptides. I decided to then utilize a new strategy using SSPS (manual and automatic) and incorporating the noncanonical amino acids at the end of the linear peptide. As a proof-of-principle reaction, we will synthesize two linear five-residue peptide (yielding a 21-membered macrocycle) and incorporate either a proline (to add rigidity) or a glycine (to give more flexibility), which are known to help during the cyclization of macrocycles.[29] Once the five-residue linear peptides are built and confirmed by mass spectroscopy, we plan to use our strategy to close the macrocycle with the isoindoline core (Figure 4).

Temp.	Solvent	Results
78 ºC	Ethanol	63%
78 ºC	HFIP	31%
78 ºC	DCE	56%
78 ºC	Methanol	42%
78 ºC	THF	60%
78 ºC	DCM	81%

Table 1: Solvent Screen of [2+2+2] cycloaddition of Fmoc-Pra-OMe and Boc-Asp(dialkyne)-OMe



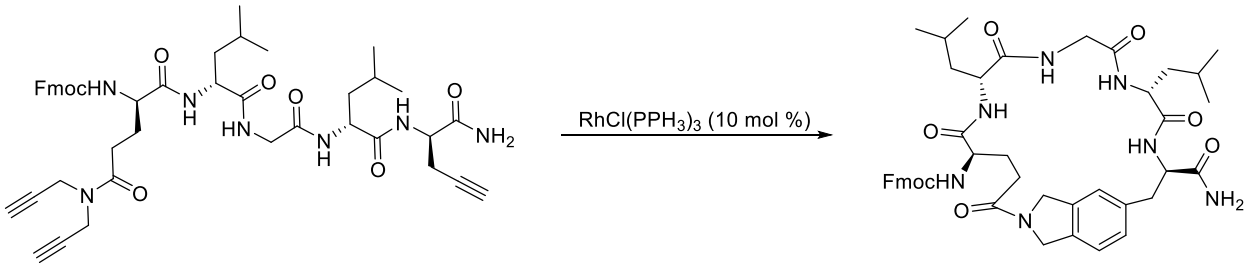
Peptide 1:
Pra-Leu-Pro-Leu-Glu*



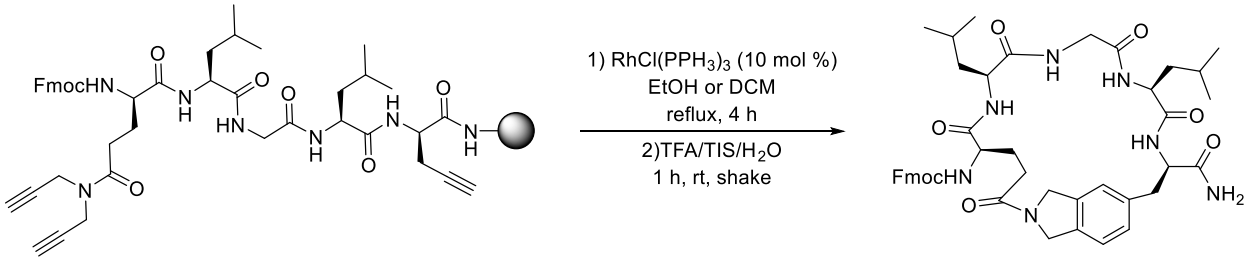
Peptide 2:
Pra-Leu-Gly-Leu-Glu*

Figure 4: Linear Peptides

I first attempted the cyclization of peptide 2 with the glycine amino acid in solution phase (Scheme 4) with ethanol or DCM for 2.5 hours at reflux. After analyzing the compounds via mass spectroscopy and NMR, there was no indication of cyclized product or linear product. After unsuccessful attempts using solution phase to cyclize, I decided to move towards using resin bound methods. Once we synthesized linear peptide on resin, we do not cleave the peptide from the resin. Instead, we run our reaction with the resin still on the peptide and will cleave it from the resin once the reaction is complete. After using our optimized method on the resin bound peptide, there was no evidence of the predicted product, only return of the triphenylphosphine oxide ions from the catalyst.



Scheme 4a: Macrocyclization of Linear Peptide 2 in solution



Scheme 4b: Macrocyclization of Linear Peptide 2 on resin

Conclusion and Future Work

In conclusion, after synthesizing the essential amino acid precursors and optimizing the intermolecular reaction of a [2+2+2] cycloaddition, we were able to evaluate our new method on the intramolecular system of linear peptides. Despite no reactivity being observed, this project shows promise and offers the ability to open new chemical space. A generalized approach to close macrocycles will start to become more relevant in the pharmaceutical field relying on new ways to introduce key pharmacophores into medicine. Future work in this area could lead to synthesis of different fused heterocycles that can add value to macrocyclic peptides. This would also allow scientists to start building libraries of unnatural amino acid macrocycles.

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Experimental Section

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General Information

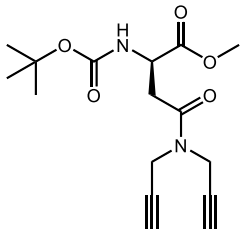
All reactions were conducted under a nitrogen atmosphere using anhydrous solvents, oven- or flame-dried glassware, and standard Schlenk technique. Anhydrous dichloromethane (DCM) and tetrahydrofuran (THF) were obtained by passage through activated alumina using a Glass Contours solvent purification system. Anhydrous methanol (MeOH), N, N-dimethylformamide (DMF), acetonitrile (MeCN), and triethylamine (TEA) was purchased and used with no further purification. Absolute ethanol (EtOH) was distilled and sparged with N_2, and N,N-Diisopropylethylamine (Hünigs base, DIPEA) was distilled from ninhydrin then KOH, then sparged with N_2, and both were stored over activated 4Åmolecular sieves. Solvents used in workup, extraction, and purification were used as received from commercial suppliers without further purification. All other reagents were purchased from Ambeed, Combi-Blocks, Oakwood Chemicals, Strem Chemicals, or Sigma-Aldrich and used as received, unless otherwise stated.

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Varian Inova 600 spectrometer, a Bruker 600 spectrometer, or a Varian Inova 400 spectrometer. Chemical shifts δ values were reported in parts per million (ppm) and coupling constants (*J* -values) in Hz. Multiplicity was indicated using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, qn = quintet, m = multiplet, b = broad, dd = doublet of doublets, ddd = doublet of doublet of doublets, ddt = doublet of doublet of triplets, qd = quartet of doublets, td = triplet of doublets.

Liquid chromatography mass spectroscopy (LCMS) and high-resolution mass spectra (HRMS) were obtained using a Thermo Electron Corporation Finigan LTQFTMS (Mass Spectrometry Facility, Emory University). Analytical High Pressure Liquid Chromatography (HPLC) was performed on an Agilent 1260 series utilizing a Pursuit C18 column (4.6 x 250 mm, 5 μM.) Analytical thin layer chromatography (TLC) was carried out on glass backed Silicycle SiliaPure® 0.25 mm silica gel 60 plates and visualized with UV light, p-anisaldehyde, bromocresol green, or potassium permanganate ($KMnO_4$). Flash column chromatography was performed using Silicycle SiliaFlash® F60 silica gel (40-60 μM). Flash column chromatography was performed using Silicycle SiliaFlash F60 silica gel (40-60 μM) on a Biotage Isolera One System.

Experimental Section:

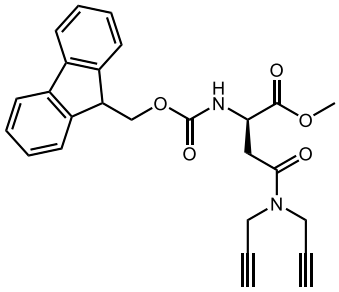
General Procedure for amino acid sidechain amidation:



Methyl N2-(tert-butoxycarbonyl)-N4,N4-di(prop-2-yn-1-yl)-L-asparaginate (S1)

Synthesized according to a reported procedure with slight modifications[1]: To a flame dried 50 mL three-necked round bottom was added Boc-L-Asp-OMe (265 mg, 1.07 mmol, 1 equiv) and hydroxybenzotriazole (HOBt) (174 mg, 1.29 mmol, 1.2 equiv). This was then purged with N_2. DMF (0.5 M, 1.5 mL) was added and allowed solution to stir and cool to 0^{o}C for 10 mins. After reaching 0 ^{o}C, 1-ethyl-3-(3-dimethylaminopropyl)carboiimide (EDCI) (226 mg, 1.18 mmol, 1.1 equiv), dipropargylamine (100µL, 1.07 mmol, 1 equiv), and TEA (299 µL, 2.15 mmol, 2.0 equiv) was added. This mixture was allowed to stir for 16 hours and warm up to room temperature. Upon completion, the reaction was quenched with ethyl acetate and washed with water (3x 15mL), sodium bicarbonate (2x 10 mL), and brine. Organic layers were combined and dried over anhydrous Na_2SO_4. The crude product was purified by flash chromatography on silica gel with a gradient eluent of ethyl acetate and hexanes (elutes between 30% to 40% ethyl acetate). Isolated in 51% yield (176 mg) as a yellow-brown viscous liquid.

^{1}H NMR (600 MHz, $CDCl_3$) δ 5.72 (d, J= 9.2 Hz, 1H), 4.59 (dt, J= 8.5, 3.9 Hz, 1H), 4.35-4.23 (qd, 2H), 4.16 (s, J= 2.5 Hz, 2H), 3.73 (s, 3H), 3.22 (dd, J=16.8, 4.0 Hz, 1H), 2.89 (dd, J=16.9, 4.0 Hz, 1H), 2.32 (t, J=2.5 Hz, 1H), 2.24 (t, J=2.5 Hz, 1H), 1.43 (s, 9H).



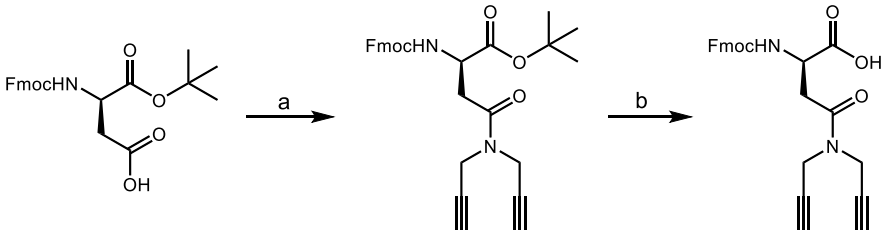
Methyl N2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N4,N4-di(prop-2-yn-1-yl)-L-asparaginate (S2)

Synthesized according to a reported procedure with slight modifications[1]: To a flame dried 50 mL three-necked round bottom was added Fmoc-L-Asp-OMe (1.98 g, 5.37 mmol, 1 equiv) and HOBt (871 mg, 6.44 mmol, 1.2 equiv). This was then purged with N_2. DMF (0.5 M, 7 mL) was added and allowed solution to stir and cool to 0^{o}C for 10 mins. After reaching 0 ^{o}C, EDCI (1.13 g, 5.91 mmol, 1.1 equiv), dipropargylamine (600 µL, 5.37 mmol, 1 equiv), and TEA (756 µL, 5.42 mmol, 1.01 equiv) was added. This mixture was allowed to stir for 18 hours and warm up to

room temperature. Upon completion, the reaction was quenched with ethyl acetate and washed with water (3x 25 mL), sodium bicarbonate (2x 15 mL), and brine. Organic layers were combined and dried over anhydrous Na_2SO_4. The crude product was purified by flash chromatography on silica gel with a gradient eluent of ethyl acetate and hexanes (elutes between 25% to 35% ethyl acetate). Isolated in 11% yield (260 mg) as an orange-yellow liquid.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.76 (d, *J* = 2.9 Hz, 2H), 7.61 (dd, 2H), 7.40 (t, *J* = 7.9, 1.8 Hz, 2H), 7.32 (td, *J*= 7.5, 1.2 Hz, 2H), 6.06 (d, *J* = 9.1 Hz, 1H), 4.70 (dt, *J* = 8.4, 3.8 Hz, 1H), 4.47-4.42 (m, 1 H), 4.39-4.28 (m, 2H), 4.28-4.22 (m, 2H), 4.19 (d, *J* = 2.5 Hz, 2H), 3.77 (s, 3H), 3.29 (dd, *J* = 16.9, 4.0 Hz, 1H), 2.94 (dd, *J* = 16.9, 4.0 Hz, 1H), 2.31 (t, *J* = 2.6 Hz, 1H), 2.26 (t, *J* = 2.5 Hz, 1H).

Synthesis of Fmoc-protected aspartic acid dialkyne



S3 **S4**

Scheme S1: Synthesis of Fmoc-protected aspartic acid dialkyne. Reagents and conditions: a) Fmoc-L-Asp-OtBu, HOBt hydrate, DCM, EDCI, dipropargylamine, DIPEA, 22 h; b) TFA, DCM, 7 h

Tert-butyl N2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N4,N4-di(prop-2-yn-1-yl)-L-asparaginate (S3)

Synthesized according to a reported procedure with slight modifications[1]: To an oven dried 100 mL two-necked round bottom was added Fmoc-L-Asp-OtBu (1.5g, 3.6 mmol, 1 equiv) and HOBt hydrate (840 mg, 4.4 mmol, 1.2 equiv). This was then purged with N_2. DCM (0.3 M, 10 mL) was added and allowed solution to stir and cool to 0^{o}C for 10 mins. After reaching 0 ^{o}C, EDCI (840 mg, 4.4 mmol, 1.2 equiv), dipropargylamine (400 µL, 3.6 mmol, 1 equiv), and DIPEA (690 µL, 4 mmol, 1.1 equiv) was added. This mixture was allowed to stir for 22 hours and warm up to room temperature. Upon completion, the reaction was quenched with ethyl acetate and washed with water (3x), sodium bicarbonate (2x), and brine. Organic layers were combined and dried over anhydrous Na_2SO_4. The crude product was purified by flash chromatography on silica gel with a gradient eluent of ethyl acetate and hexanes (elutes at 20% ethyl acetate). Isolated in 74% yield (1.3 g) as a white solid (crystals).

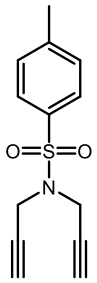
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.61 (dd, *J*= 7.5, 4.9 Hz, 2H), 7.39 (t, *J* = 7.4 Hz, 2H), 7.31 (t, *J* = 1.2 Hz, 2H), 6.03 (d, *J* = 8.9 Hz, 1H), 4.57 (dt, *J* = 8.4, 3.9 Hz, 1H), 4.42 (dd, *J* = 10.5, 7.2 Hz, 1H), 4.36 (dd, *J* = 17.6, 2.5 Hz, 1H), 4.33– 4.25 (m, 2H), 4.23 (d, *J* = 7.4 Hz, 1H), 4.19 (d, *J* = 2.5 Hz, 2H), 3.24 (dd, *J* = 16.8, 4.1 Hz, 1H), 2.89 (dd, *J* = 16.8, 3.9 Hz, 1H), 2.30 (t, *J* = 2.4 Hz, 1H), 2.24 (t, *J* = 2.5 Hz, 1H), 1.46 (s, 9H).

^{13}C (400 MHz, $CDCl_3$) δ 170.03, 169.92, 156.40, 144.10, 143.93, 127.78, 127.18, 125.36, 120.06, 82.31, 78.12, 73.46, 72.71, 67.28, 47.25, 36.35, 35.98, 27.99

Tert-butyl N2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N4,N4-di(prop-2-yn-1-yl)-L-asparaginate (S4)

Synthesized according to a reported procedure with slight modifications[2]: To an oven dried 25 mL round bottom was added S3 (500 mg, 1.03 mmol, 1 equiv). This was then purged with N_2. Dry DCM (0.3 M, 3 mL) was added followed by trifluoroacetic acid (TFA) (783 µl, 10.3 mmol, 10 equiv) and allowed solution to stir for 7 hours at room temperature. Reaction was tracked via TLC until completion. The solvent was removed, and the crude product was concentrated. The crude product was purified by flash chromatography on silica gel with a gradient eluent of ethyl acetate and hexanes (elutes at 40% ethyl acetate). Isolated in 72% yield (318 mg) an orange-yellow liquid.

^1H NMR (400 MHz, $CDCl_3$) δ 10.75 (s, 1H), 7.77 (dq, *J* = 8.3, 0.8Hz, 2H), 7.61 (dd, *J*=7.5, 4.0 Hz, 2H), 7.41 (t, 2H), 7.32 (td, *J* = 7.4, 1.2 Hz, 2H), 6.41 (d, *J* = 7.2 Hz, 1H), 4.88 (m, 1H), 4.48 (dd, *J* = 10.5, 7.1 Hz, 1H), 4.36 (dd, *J* = 17.6, 2.5 Hz, 1H), 4.33– 4.25 (m, 2H), 4.23 (d, *J* =7.4 Hz, 1H), 3.35 (dd, *J* = 16.8, 4.1 Hz, 1H), 3.04 (dd, *J* = 16.8, 3.9 Hz, 1H), 2.36 (t, *J* = 2.5 Hz, 1H), 2.30 (t, *J* = 2.5 Hz, 1H)

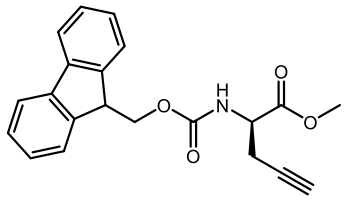


4-methyl-N,N-di(prop-2-yn-1-yl)benzenesulfonamide (S5)

Synthesized according to a reported procedure[3]: To a flame dried 50 mL round bottom was added p-toluenesulfonyl chloride (819 mg, 4.3 mmol, 1 equiv) and 4-(dimethylamino)pyridine (DMAP) (10 mol%, 52.5 mg). This was then purged with N_2. Dry DCM (0.2 M, 20 mL) was added followed by bis(prp-2-yn-1-yl)amine (400 µL, 4.30 mmol, 1 equiv) and TEA (898 µL, 6.44 mmol, 1.5 equiv). The mixture was allowed to stir at room temperature for 19 hours. Upon completion, the reaction was quenched with water and washed with DCM (3x) and brine. Organic layers were combined and dried over anhydrous Na_2SO_4. The crude product was purified by flash chromatography on silica gel with a gradient eluent of ethyl acetate and hexanes (elutes at 20% ethyl acetate). Isolated in 79% yield (841 mg) as a light yellow-brown solid.

^1H NMR (600 MHz, $CDCl_3$) δ 7.68 (dd, *J* = 8.3, 1.5 Hz, 2H), 7.27 (ddt, *J* = 7.9, 1.5, 0.7 Hz, 2H), 4.13 (dd, *J* = 2.5, 1.4 Hz, 4H), 2.39 (s, 3H), 2.15-2.13 (m, 2H).

General Procedure for protection of propargyl glycine:

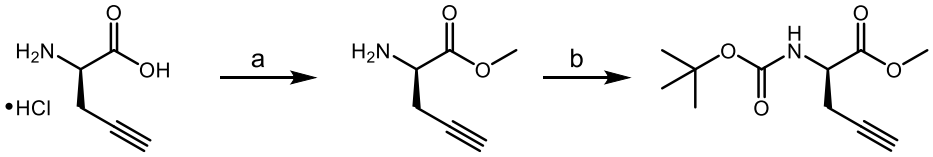


Methyl (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)pent-4-ynoate (S6)

Synthesized according to a reported procedure[4]. To a flame dried 50 mL three-necked round bottom was added (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)pent-4-ynoic acid (500 mg, 1.49 mmol, 1 equiv). This was purged with N_2. Dry methanol (0.2 M, 5mL) was added and then cooled the reaction to 0 ^oC. After reaching 0 ^oC, thionyl chloride ($SOCl_2$) (219 µL, 2.98 mmol, 2 equiv) was added dropwise. The reaction was then heated to 60 ^oC and allowed the mixture to stir for 4 hours under N_2. DCM was added (2 mL) to quench the reaction help with solubility before concentrating. The crude product was purified by flash chromatography on silica gel with a gradient eluent of ethyl acetate and hexanes (elutes at 20% ethyl acetate). Isolated in 87% yield (452 mg) as a white solid.

^1H NMR (600 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.5 Hz, 2H), 7.62 (dd, *J* = 7.5, 3.3 Hz, 2H), 7.41(t, *J* = 7.5 Hz, 2H), 7.33 (tt, *J* = 7.5, 1.5 Hz, 2H), 5.70 (d, *J* = 8.3 Hz, 1H), 4.57 (dt, *J* = 8.9, 4.8 Hz, 1H), 4.41(d, *J* = 7.2 Hz, 2H), 4.26 (t, *J* = 7.3 Hz, 1H), 3.81 (s, 3H), 2.81 (dd, *J* = 4.8, 2.6 Hz, 2H), 2.09 (s, 1H).

Synthesis of Boc-protected propargylglycine methyl ester



S7 **S8**

Scheme S2: Synthesis of Boc-protected propargylglycine methyl ester. Reagents and conditions: a) L-Propargylglycine·HCl, $SOCl_2$, MeOH, 18.5 h; b) Di-tert-butyl dicarbonate, MeCN, TEA, 14.5 h

Methyl (S)-2-aminopent-4-ynoate (S7)

Synthesized according to a reported procedure with slight modifications[5]: To a flame dried 50 mL three-necked round bottom was added L-propargylglycine·HCl (325 mg, 2.17 mmol, 1 equiv). This was purged with N_2. Dry methanol (0.4 M, 5 mL) was added, and solution was cooled to 0 ^oC. After reaching 0 ^oC, thionyl chloride (500 µL, 20.7 mmol, 3.1 equiv) was added dropwise. Allowed reaction to stir for 18.5 hours at room temperature under N_2. Upon completion via TLC (stained with bromocresol green), reaction was stopped by evaporating off

the solvent and the crude product was concentrated in *vacuo*. The resulting crude compound S6 (brown oil) was then used directly in the next step.

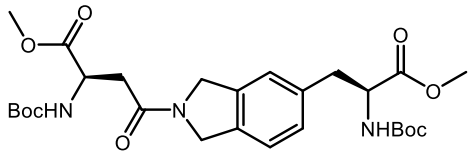
^{1}H NMR (600 MHz, $CDCl_3$) δ 8.74 (s, 2H), 4.50 (s, 1H), 3.89 (s, 3H), 3.10 (d, 2H), 2.31 (s, 1H)

Methyl (S)-2-((tert-butoxycarbonyl)amino)pent-4-ynoate (S8)

Synthesized according to a reported procedure with slight modifications[5]: In a flame dried 100 mL round bottom flask was added di-tert-butyl-dicarbonate (1.04 g, 12.23 mmol, 1.2 equiv) and purged under N_2. Dry MeCN (0.3M, 10 mL) was added to crude compound S6 (506 mg, 10.22 mmol, 1 equiv) under N_2 and then transferred to the 100 mL round bottom flask followed by the addition of TEA (664 μL, 12.23 mmol, 1.2 equiv). The reaction was allowed to stir at room temperature for 14.5 hours. Reaction was stopped by adding 1M $KHSO_4$ then washed with DCM (3x) and sodium bicarbonate. The combined organic layers were then dried over anhydrous $NaSO_4$. The crude product was purified by flash chromatography on silica gel with a gradient eluent of ethyl acetate and hexanes (elutes between 10 and 15% ethyl acetate). Isolated in 15% yield (140 mg) as a clear, viscous liquid.

^{1}H NMR (600 MHz, $CDCl_3$) δ 5.39 (d, *J* = 8.6 Hz, 1H), 4.31 (dt, *J* = 8.5, 5.1 Hz, 1H), 3.61 (s, 3H), 2.62-2.53 (m, 2H), 1.95 (t, *J* = 2.7 Hz, 1H), 1.29 (s, 9H)

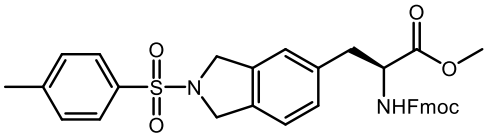
General Procedure for intermolecular [2+2+2] cycloaddition:



Methyl (S)-2-((tert-butoxycarbonyl)amino)-4-(5-((R)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)isoindolin-2-yl)-4-oxobutanoate (S9)

Synthesized according to a reported procedure with slight modifications[6]: In a flame dried 25 mL three necked round bottom was added ($Rh(I)(PPh_3)_3Cl$) (Wilkinson's catalyst) (20.4 mg, 22 μmol, 10 mol%) and propargyl glycine derivative (S7) (50 mg, 0.22 mmol, 1.0 equiv). This was then purged with N_2. Methanol (4 mL) was added then heated to the required temperature. The dialkyne derivative (S1) (106 mg, 0.33 mmol, 1.5 equiv) was added dropwise as solution in ethanol (1 mL) over 30 mins. Once added, the reaction was allowed to reflux and stir for 2 hours. The solvent was removed, and the crude compound concentrated. The crude product was purified by flash chromatography on silica gel with a gradient eluent of ethyl acetate and hexanes (elutes between 40%-60% ethyl acetate). Isolated in 16% yield (19 mg) as a light-yellow viscous liquid. m/z= 549.27

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.21 (d, *J* = 7.9 Hz, 1H), 7.18 (d, *J* = 8.0 Hz, 1H), 7.06 (s, 1H), 5.86 (d, *J* = 9.1 Hz, 1H), 4.98 (d, *J* = 8.3 Hz, 1H), 4.79-4.71 (m, 4H), 4.64-4.59 (m, 1H), 4.57 (s, 1H), 3.76 (s, 3H), 3.72 (d, *J* = 3.5 Hz, 3H), 3.15 (t, *J* = 14.7 Hz, 2H), 3.04 (dd, *J* = 13.7, 6.3 Hz, 1H), 2.84 (dd, *J* = 16.6, 4.1 Hz, 1H), 1.44 (s, 3H), 1.42 (d, J= 2.7 Hz, 3H)

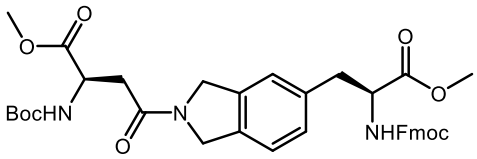


Methyl (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(2-tosylisoindolin-5-yl)propanoate (S10)

Synthesized according to a reported procedure[6]: In a flame dried 50 mL three necked round bottom was added Wilkinson's catalyst (26.5 mg, 28.6 μmol, 10 mol%) and propargyl glycine derivative (S5) (100 mg, 286 μmol, 1.0 equiv.). This was then purged with N_2. Ethanol (8 mL) was added then heated to the required temperature. The dialkyne derivative (S4) (106 mg, 429 μmol, 1.5 equiv.) was added dropwise as solution in ethanol (2 mL) over 30 mins. Once added, the reaction was allowed to reflux and stir for 2 hours. The solvent was removed, and the crude compound concentrated. The crude product was purified by flash chromatography on silica gel with a gradient eluent of ethyl acetate and hexanes (elutes at 40% ethyl acetate). Isolated in 81% yield (138 mg) as an orange-brown liquid. m/z= 596.20

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.76-7.72 (m, 2H), 7.55 (t, *J* = 6.6 Hz, 2H), 7.46-7.35 (m, 2H), 7.37-7.26 (m, 4H), 7.06 (d, *J* = 7.8 Hz, 1H), 6.93 (d, *J* = 7.4 Hz, 1H), 6.85 (s, 1H), 5.19 (d, *J* = 8.2 Hz, 1H), 4.63-4.60 (m, 1H), 4.56 (s, 2H), 4.54 (s, 2H), 4.46 (dd, *J*=10.7, 7.0 Hz, 1H), 4.36 (dd, *J*=10.7, 6.7 Hz, 1H), 4.22-4.16 (m, 1H), 3.71 (s, 3H), 3.09 (dd, *J* = 13.9, 5.6 Hz, 1H), 3.02 (dd, *J* = 13.9, 5.9 Hz, 1H), 2.38 (s, 3H)

^{13}C (400 MHz, $CDCl_3$) δ 171.69, 155.47, 143.79, 143.72, 143.65, 141.36, 141.33, 136.63, 135.63, 135.10, 133.7, 129.86, 128.85, 127.81, 127.60, 127.08, 125.01, 124.95, 123.45, 122.78, 120.05, 120.07, 66.81, 54.76, 53.58, 53.46, 52.46, 47.19, 38.04, 21.52



methyl (R)-4-(5-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methoxy-3-oxopropyl)isoindolin-2-yl)-2-((tert-butoxycarbonyl)amino)-4-oxobutanoate (S11)

Synthesized according to a reported procedure[6]: In a flame dried 50 mL three necked round bottom was added Wilkinson's catalyst (12 mg, 28.6 μmol, 10 mol%) and propargyl glycine derivative (S5) (45 mg, 286 μmol, 1.0 equiv.). This was then purged with N_2. Ethanol (8 mL) was added then heated to the required temperature. The dialkyne derivative (S4) (62 mg, 429 μmol, 1.5 equiv.) was added dropwise as solution in ethanol (2 mL) over 30 mins. Once added, the reaction was allowed to reflux and stir for 2 hours. The solvent was removed, and the crude compound concentrated. The crude product was purified by flash chromatography on silica gel with a gradient eluent of ethyl acetate and hexanes (elutes at 40% ethyl acetate). Isolated in 61% yield (53 mg) as a yellow-orange liquid. m/z= 671.28

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.6 Hz, 2H), 7.59-7.50 (m, 2H), 7.40 (t, 2H), 7.28 (t, 2H), 7.19 (d, *J* = 7.8 Hz, 1H), 7.15 (d, *J* = 8.2 Hz, 1H), 7.01 (s, 1H), 5.87 (dd, *J* = 9.3, 4.4 Hz, 1H), 5.26 (t, *J* = 7.3 Hz, 1H), 4.77-4.68 (m, 3H), 4.67 (d, *J* = 5.1 Hz, 2H), 4.62 (dd, *J* = 8.6, 4.2 Hz, 1H), 4.45 (ddd, *J* = 10.0, 7.0, 2.6 Hz, 1H), 4.41-4.32 (m, 1H), 4.18 (t, *J* = 6.8 Hz, 1H), 3.76 (s, 3H), 3.74 (d, *J* = 6.2 Hz, 3H), 3.11 (m, 2H), 2.81 (ddd, *J* = 16.6, 8.5, 4.2 Hz, 2H), 1.45 (s, 9H)

Solvent Optimization Table for Compound S11

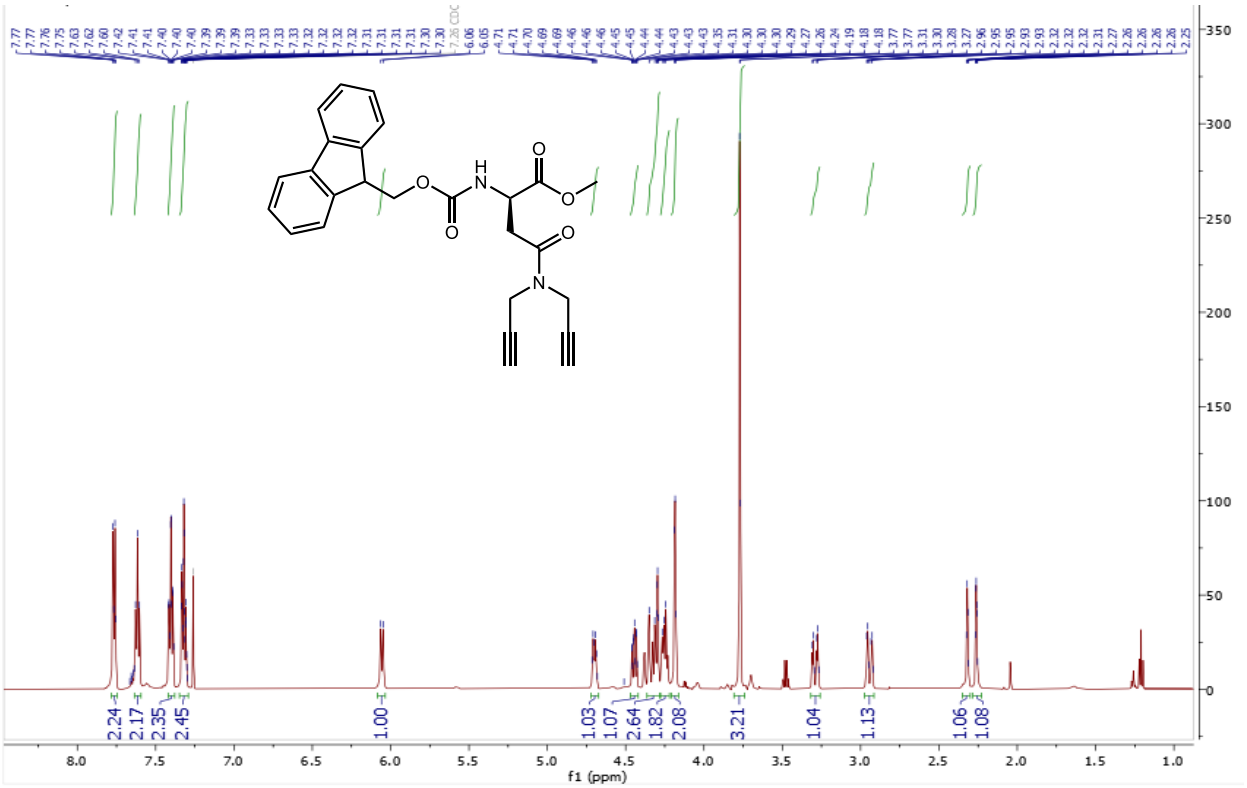
Catalyst	Temperature	Solvent	Results
[RhCl(PPh$_3$)$_3$]	**78 ºC**	**Ethanol**	**63%**
[RhCl(PPh$_3$)$_3$]	**78 ºC**	**HFIP**	**31%**
[RhCl(PPh$_3$)$_3$]	**78 ºC**	**DCE**	**56%**
[RhCl(PPh$_3$)$_3$]	**78 ºC**	**Methanol**	**42%**
[RhCl(PPh$_3$)$_3$]	**78 ºC**	**THF**	**60%**
[RhCl(PPh$_3$)$_3$]	**78 ºC**	**DCM**	**81%**

Catalyst Optimization Table for Compound S11

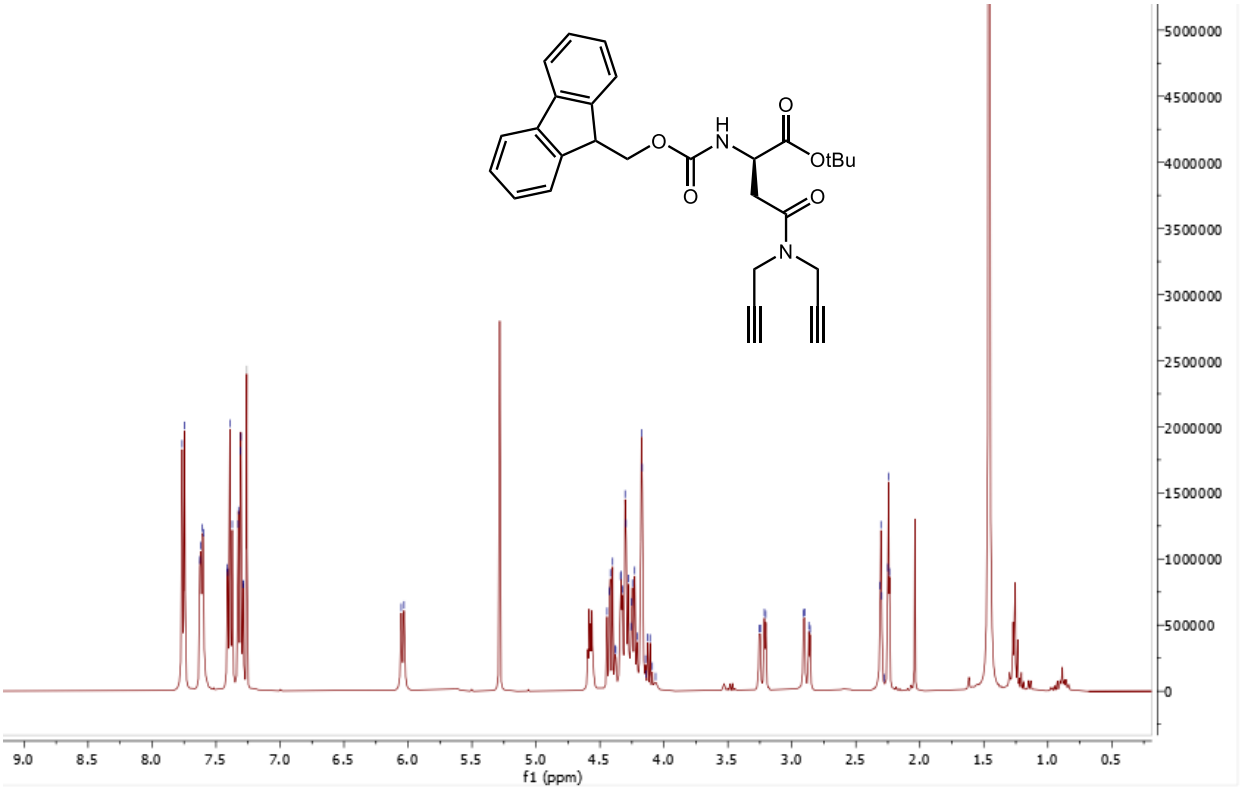
Catalyst	Temperature	Solvent	Results
Cp*RuCl(COD)	**60 ºC**	**DMA/H$_2$O**	**43%**
Cp*RuCl(COD)	**23 ºC**	**DCE**	**35%**

Spectra Data

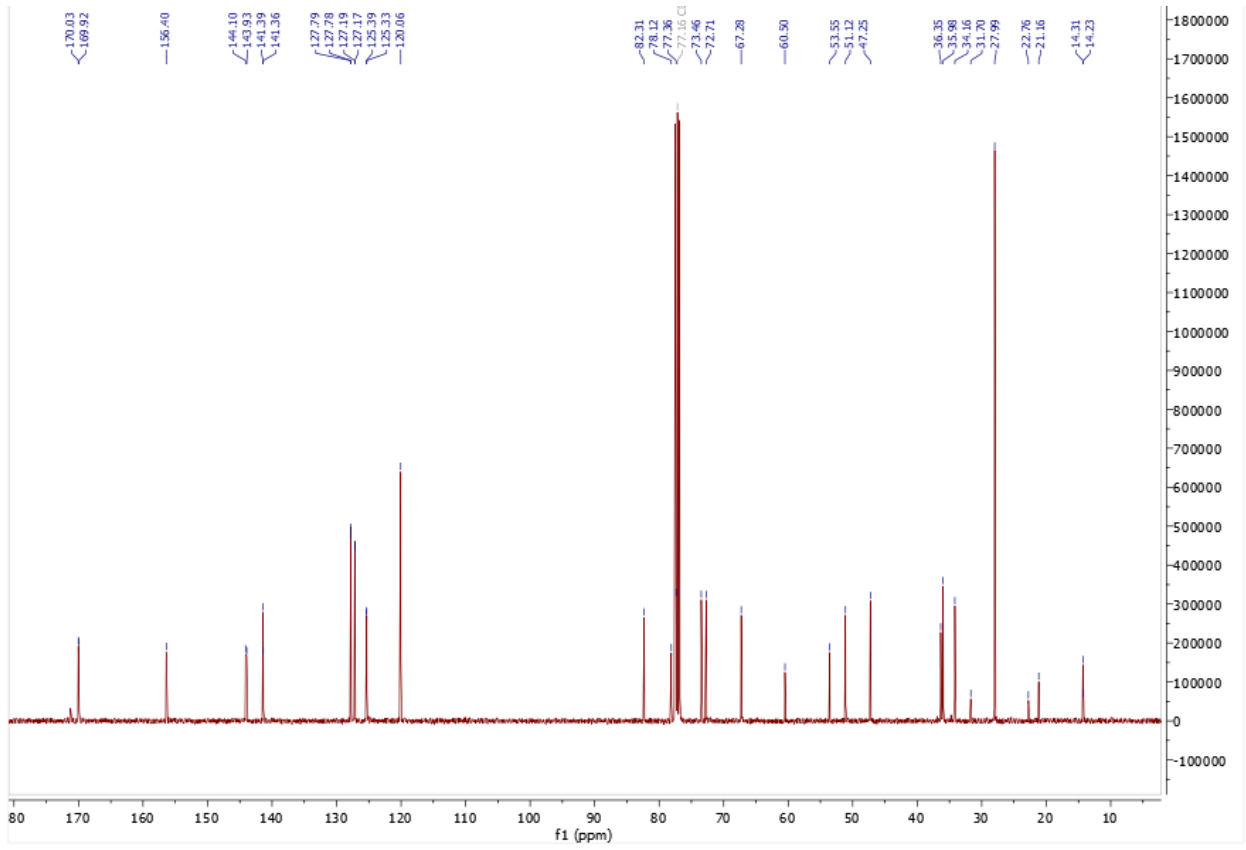
^{1}H NMR of Compound S2



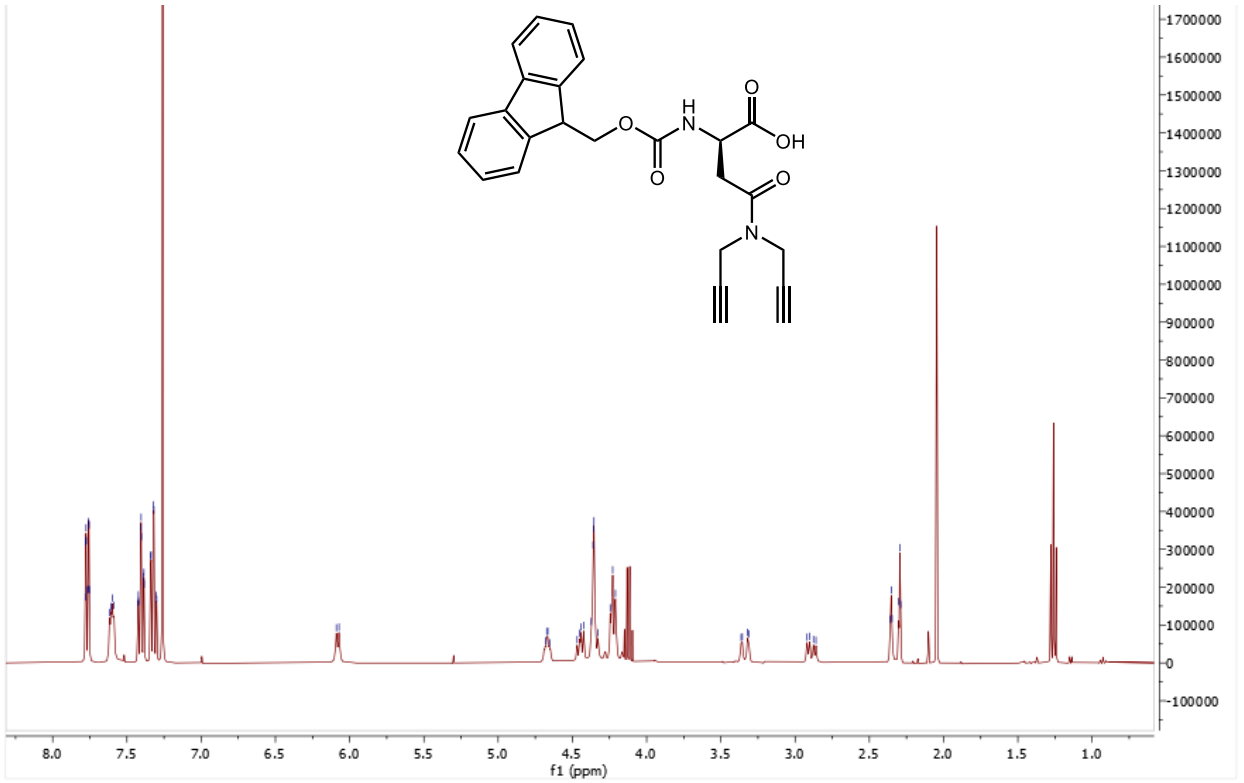
^{1}H NMR of Compound S3



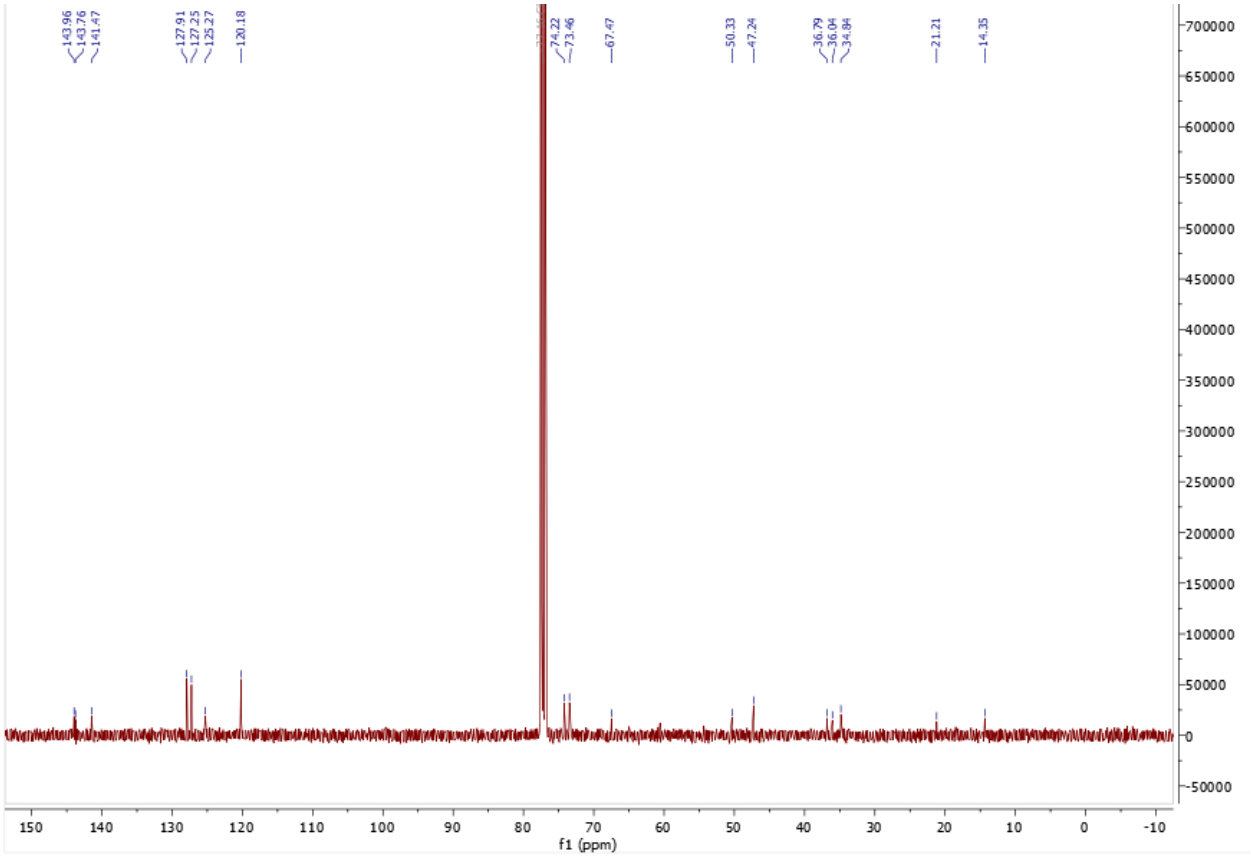
^{13}C NMR of Compound S3



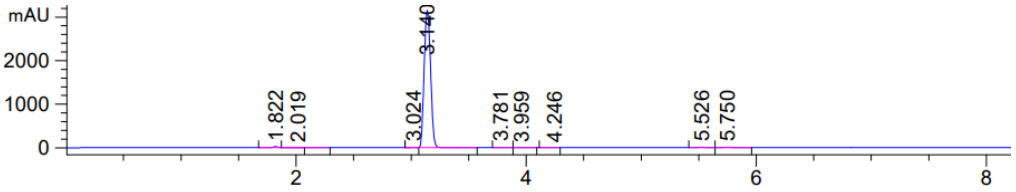
^{1}H NMR of Compound S4



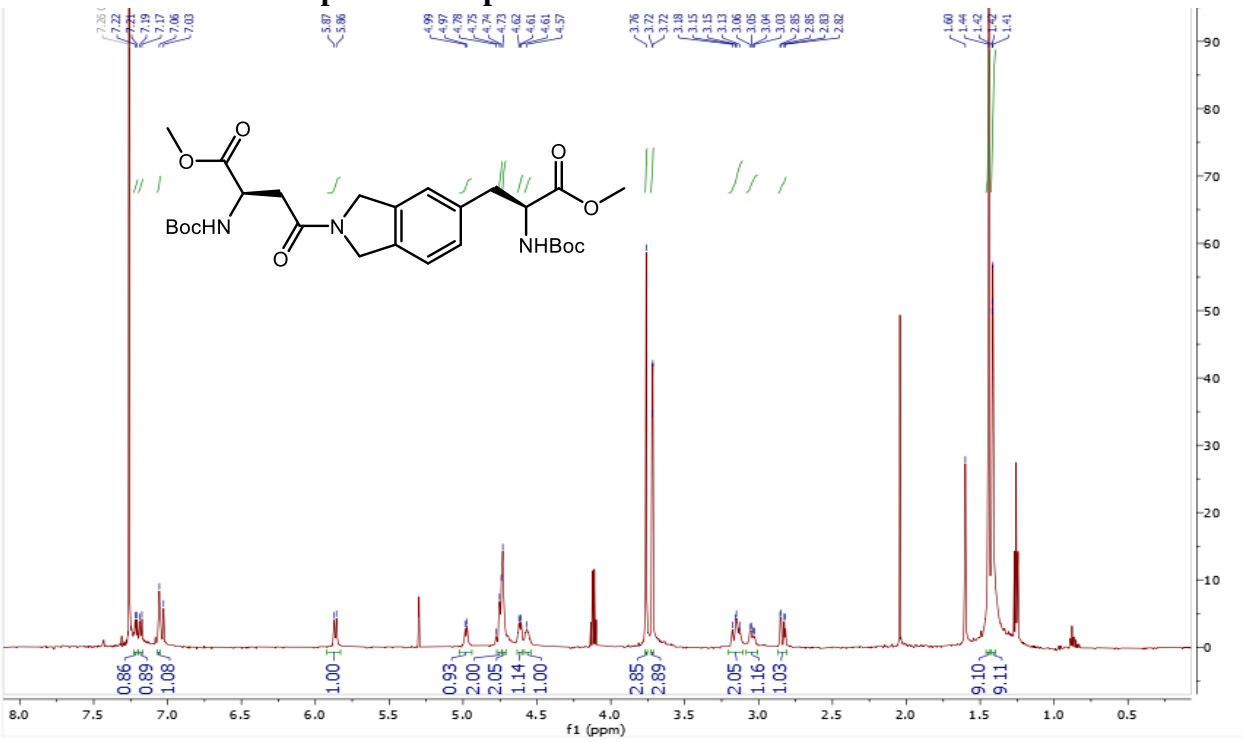
^{13}C NMR of Compound S4

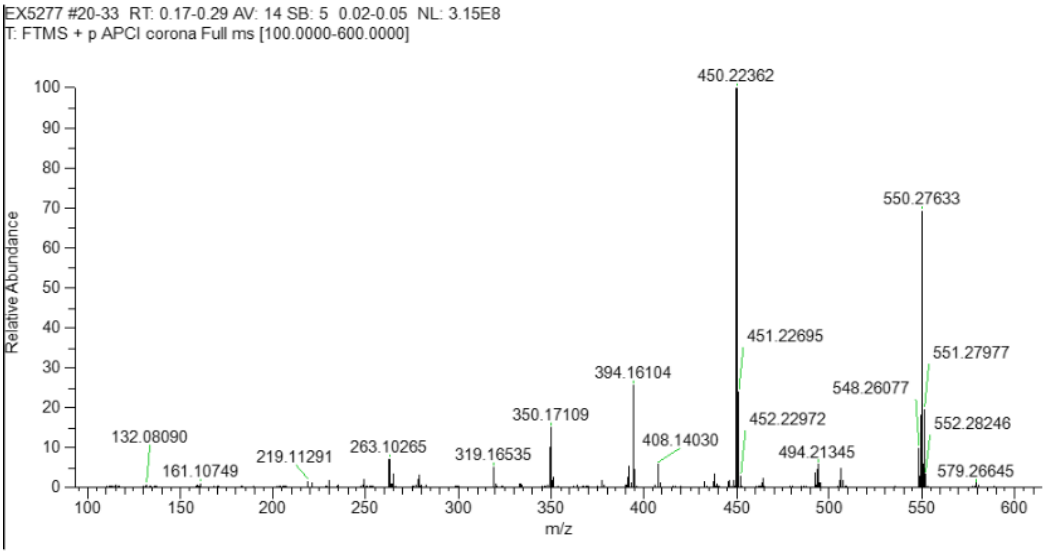


HPLC Trace of Compound S4



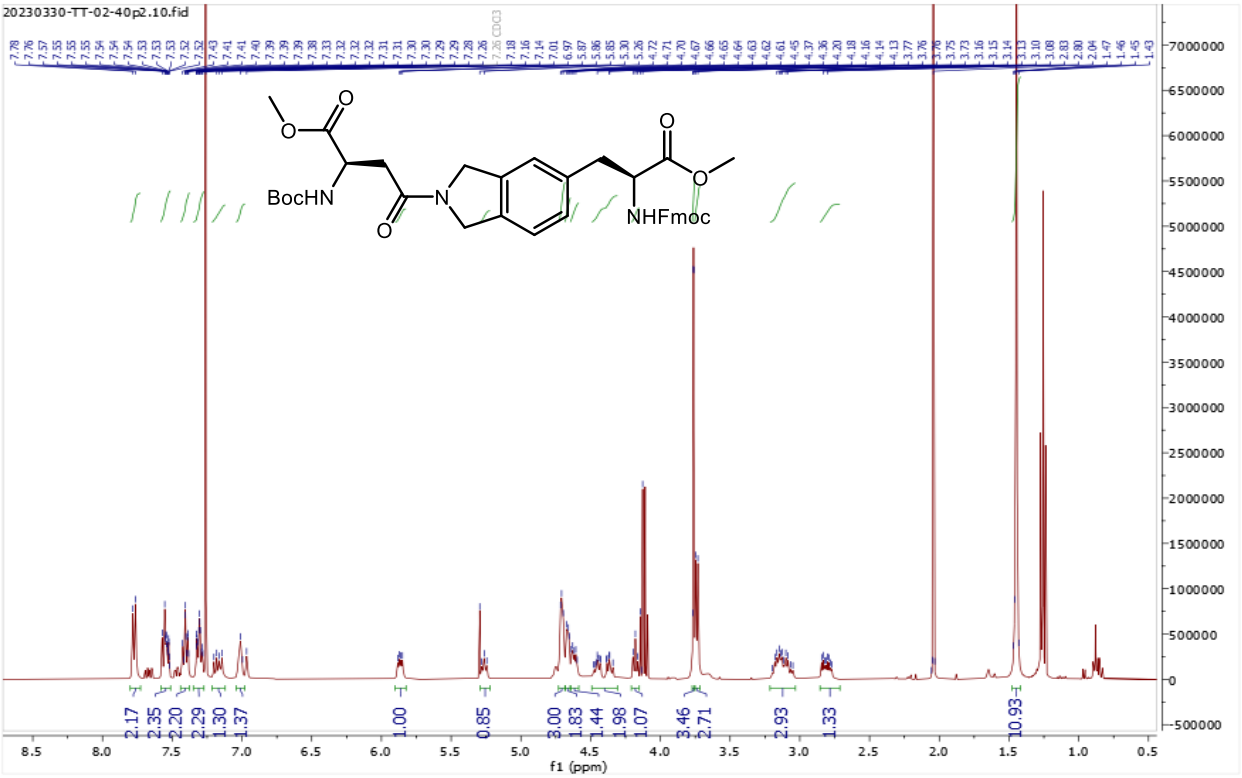
^{1}H NMR and Mass Spec. of Compound S9

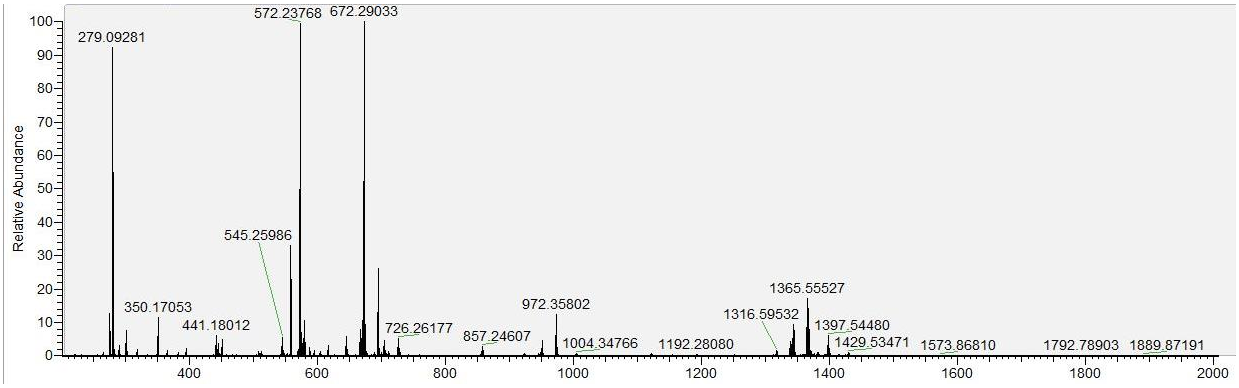




m/z= 549.27

^{1}H NMR and Mass Spec. of Compound S11





m/z=671.28

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