

SYNTHESIS OF CIPROFLOXACIN-COUPLED A_oM ESTERS AS PRE-CURSORS FOR
ANTIBACTERIAL APPLICATIONS UTILIZING CLICK CHEMISTRY

by

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ABBREVIATIONS

<i>c</i> Pe	cyclopentyl
DCM	dichloromethane
DMF	<i>N, N</i> -dimethylformamide
Equiv.	equivalent
Et	ethyl
EtOAc	ethyl acetate
FDA	Food and Drug Administration
g	gram
H ₂ O	water
h	hour(s)
LC	liquid chromatography
Hz	hertz
<i>i</i> -Pr	isopropyl
J	coupling constant
M	molar
Me	methyl
mg	milligram
MHz	megahertz
mL	milliliter
mmol	millimole

ABBREVIATIONS CONTINUED

NMR	nuclear magnetic resonance
<i>t</i> Bu	tert-butyl
TEA	triethylamine
THF	tetrahydrofuran
TLC	thin layer chromatography

ABSTRACT

Biofilm-forming bacteria pose significant challenges in the realm of drug synthesis and delivery, due to their ability to acclimate to extreme environmental conditions and resist effective antibiotics. Ciprofloxacin has traditionally been employed to combat *Pseudomonas aeruginosa*, a bacterium implicated in diseases such as cystic fibrosis and tuberculosis. However, the increasing resistance to this drug necessitates modification to enhance its efficacy upon cellular entry. One approach involves appending various molecules to the piperazine moiety of ciprofloxacin, thereby altering its antibacterial activity against *P. aeruginosa* and *Staphylococcus aureus*. Here, the inclusion of 1,2,3-triazoles and acetoxymethyl (AoM) esters are implemented to the base ciprofloxacin drug, tested against *P. aeruginosa* and *S. aureus*, and compared to the base drug for observations of improved potency. The techniques of the copper-catalyzed azide-alkyne cycloaddition (CuAAC) is primarily used to synthesize these novel 1,2,3-triazole compounds.

CHAPTER ONE

BACKGROUND

Introduction

This thesis presents research conducted in Dr. Thomas Livinghouse's laboratory, focusing on the synthesis of novel 1,2,3-triazole compounds derived from the fluoroquinolone drug ciprofloxacin. To initiate this study, pre-cursor acetoxymethyl (AoM) esters were first synthesized. Subsequently, copper-catalyzed azide-alkyne cycloaddition (CuAAC) reactions were performed using copper(II) sulfate pentahydrate as the copper source and sodium ascorbate as the reductant to generate copper(I). This chapter introduces key aspects of this research, including the foundational properties of ciprofloxacin, the significance of triazoles and ester bonds in pharmaceuticals, and the origins of the CuAAC reaction.

Biofilms and its Hazards

Biofilms are ubiquitous among bacterial organisms, contributing to nearly 60 % of all microbial infections.¹ They are defined as clusters of various bacterial cells that irreversibly adhere to surfaces and excrete a glue-like substance. Over time, with the ingestion of sufficient nutrients and moisture, these colonies continue to grow and develop in size and resistance to antibiotics. As they expand, bacterial cells secrete proteins and sugary substances called extracellular polymeric substances (EPS) that allow the cells to become connected to each other.² After their complete maturation and growth, they release more bacterial cells that then adhere to new surfaces, perpetuating the cycle (Figure 1). These cells can theoretically be of one kind, but in nature, it is more common for biofilms to have several types of bacterial cells involved. Some

of the more concerning pathogens that are capable of forming biofilms are *Pseudomonas aeruginosa* and *Staphylococcus aureus*. While these biofilms are extremely concerning, their growth on medical devices and the human body is even more alarming.

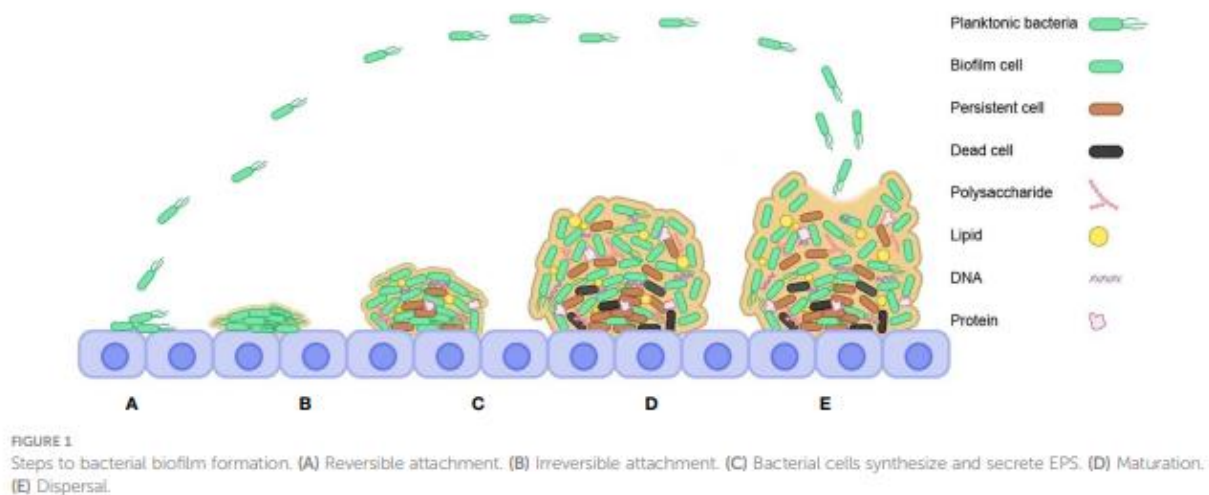


Figure 1. Life cycle of biofilms when adhering to surfaces. *Front. Cell. Infect. Microbio.* **2023**, *13*, 1 – 23.

While producing novel drugs is essential for combatting biofilm formation, it is imperative to prevent the adhesion to various surfaces, such as pacemakers, plaque within the teeth of other people, and even water lines. For instance, *P. aeruginosa* has been detected as a contaminant in dental unit water lines in concentrations of up to 12.0 %.³ Biofilms enable bacterial survival in diverse environments, including extreme temperatures, moisture levels, and pH conditions, underscoring the need for antibiotics that can permeate bacterial membranes and effectively eradicate these resilient cells.² While these cells have grown resistance towards antibiotics, there are still methods that scientists have been using to prevent the growth of these colonies.

Ciprofloxacin

Ciprofloxacin is a second-generation quinolone drug, defined by its bicyclic core present in a plethora of pharmaceuticals. It is one of, if not the most, popular fluoroquinolone drug administered to patients diagnosed with urinary tract infections (UTI's), pneumonia, and other diseases spread by *P. aeruginosa*.⁴ While its mechanism of action is not definitively known, it is known to affect the DNA gyrase and topoisomerase IV of bacterial cells, preventing DNA synthesis and replication from occurring.⁵ Its potency against Gram-positive and Gram-negative bacteria have made it a popular antibiotic to administer to patients, which can be credited to its structure (Figure 2). Unlike one of its original predecessors (nalidixic acid), ciprofloxacin contains a fluorine group, a piperazine ring, and a cyclopropyl group attached to the base quinolone structure, which has a stronger affinity for binding with DNA gyrase.

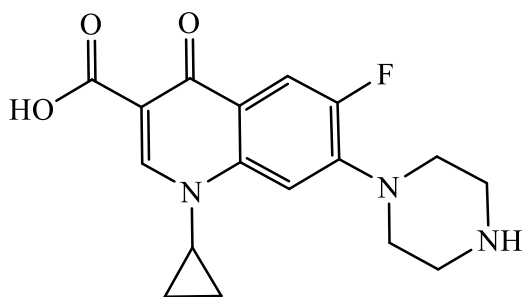


Figure 2. The base structure of ciprofloxacin.

Although this particular drug has proven to be effective against both Gram-positive and Gram-negative bacteria, the Food and Drug Administration (FDA) issued a “black box” label on the drug, warning patients and doctors about its adverse effects of tendon rupture and neuropathy. On top of its irreversible side effects, bacterial cells have been rapidly developing modes of resistance against this drug.^{4, 5} Some of these methods include a higher number of gene

mutations to prevent the ciprofloxacin molecule from binding to the DNA gyrase enzyme, and a higher concentration of efflux pumps to excrete the drug from its system without any harmful effects. The deadly combination of these two alterations within the bacterial cell have decreased the potency of ciprofloxacin, rendering it ineffective. In fact, mutations within the genes of gyrase, more specifically GyrA, have been shown to correlate with a higher number of efflux pumps to expel the free-floating ciprofloxacin molecule.⁶

Triazoles

Triazoles have gained popularity as a functional building block for basic organic chemistry and the synthesis of advanced materials. There are two main isomers of triazoles that are most prevalent in pharmaceutical drugs: 1,2,3-triazoles and 1,2,4-triazoles (Figure 3). The advantages that these building blocks possess include the ability to form $\pi - \pi$ stacking interactions and H-bonds, their stability in acidic or basic conditions, and their poor acidity and basicity in metabolic pH environments.^{7,8} This allows for the molecule to perform hydrolysis in the cell under a plethora of conditions, such as redox conditions. With these characteristics of triazoles, they have become an important scaffold for medicinal drugs.^{8,9} For the purposes of this thesis, 1,2,3-triazoles were used as building blocks for the compounds synthesized.

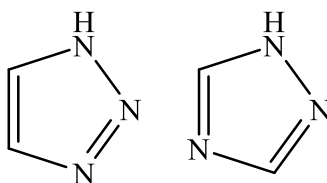


Figure 3. 1H-1,2,3-Triazole (left) and 1H-1,2,4-Triazole (right) structures.

Triazoles have become a fundamentally essential building block for a multitude of drugs geared towards treating serious bacterial diseases, such as tuberculosis and Parkinson's disease.^{9,10} Further research on these scaffolds are continuously increasing and its popularity in the pharmaceutical industry has been growing rapidly to the point where there are lead compounds being synthesized with 1,2,3-triazoles as a key component.⁹ There is a variety of techniques and methods that can be employed to synthesize these important compounds, but one of the more popular methods, which will be discussed in this thesis, is the copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction.

CuAAC Click Reaction and its Origins

During the 1960s, Rolf Huisgen introduced the concept of the formation of five-membered heterocycles via a [3 + 2] cycloaddition. Closely following the mechanism of the Diels-Alder reaction, a 1,3-dipole molecule would interact with a dipolarophile, and with an elevated level of heat, a five-membered heterocycle would be synthesized.^{11,12} Consequently, the formal charges carried by both starting materials would be neutralized (Figure 4). Some of the early limitations to this reaction were that there was a large activation energy barrier to form the products, and a 1:1 ratio of regioisomers would be produced (Figure 4).

With these limitations of the standard Huisgen [3 + 2] cycloaddition reaction, K. Barry Sharpless et al. introduced a concept of click chemistry, which is a greener chemistry technique that uses benign solvents and milder reaction conditions to achieve one regioisomer predominantly.¹³ With the introduction of a copper(I) catalyst, the 1,4-disubstituted triazole would be heavily favored over the 1,5-disubstituted triazole (Figure 5). This reaction term was originally coined by Harmuth C. Kolb, implying the concept of joining two ends of molecules

together to create a new compound, which is now a popular method for synthesizing pharmacophores.¹⁴

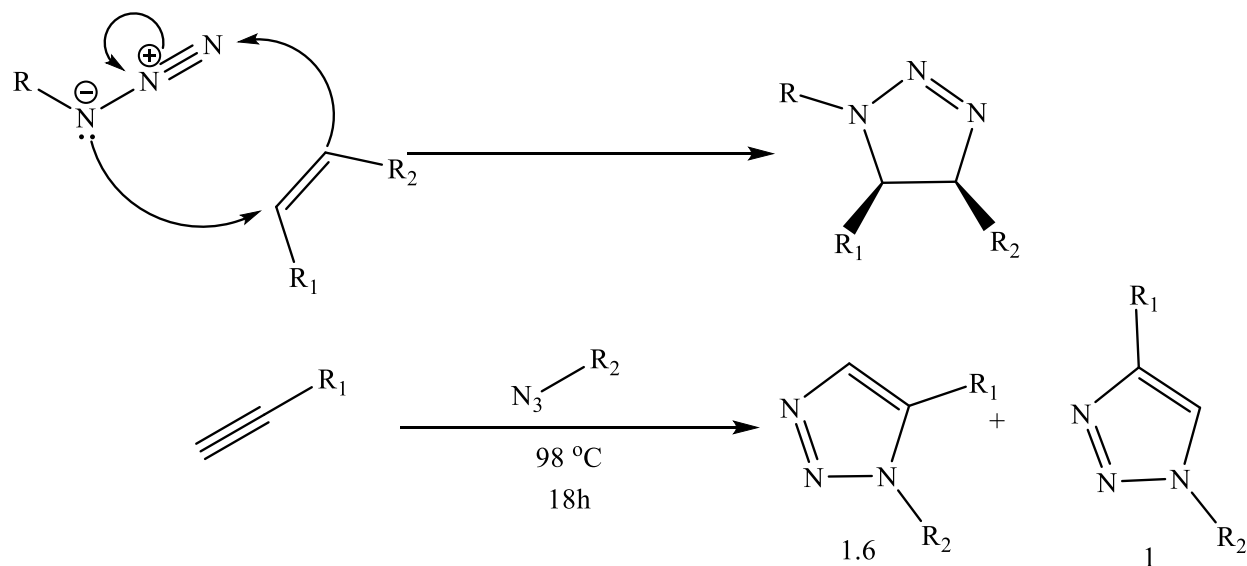


Figure 4. Huisgen 1,3-dipolar cycloaddition mechanism (top) and the regioisomeric ratio of the reaction in the original conditions (bottom).

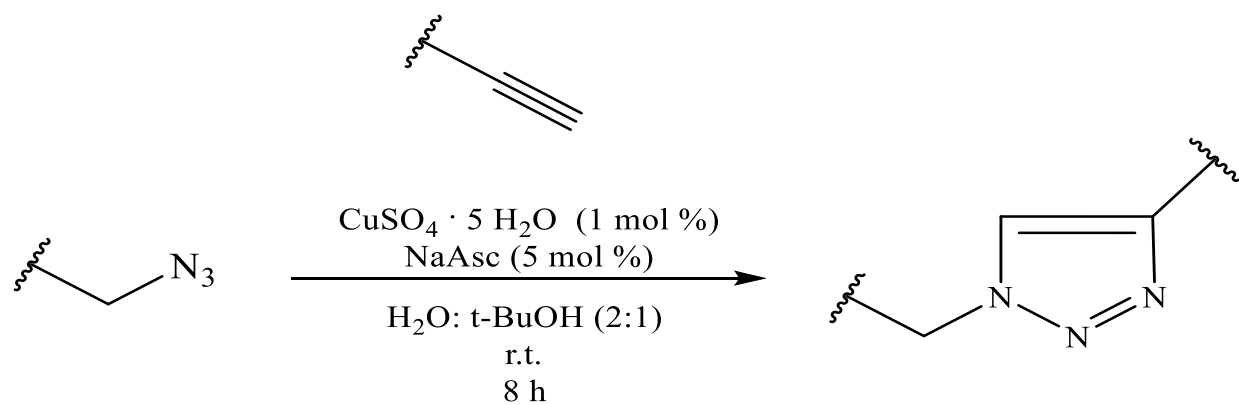


Figure 5. Copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction and its regioselectivity.

Ester Bonds in Pharmaceuticals

Although not as prevalent in pharmacophores as triazoles, ester bonds still have a beneficial role for the delivery of drugs inside the cell membrane. They are often used as a form of prodrugs, which are defined as molecules that have no biological activity by themselves. They do, however, have a masking effect for drugs that cannot permeate through the cell membrane.¹⁵ Because some molecules can have difficulty passing through the bacterial cell membrane, ester bonds can be used to mask the hydrophilicity of the molecule and allow an easier route through the membrane. These ester groups can be cleaved through esterases, allowing the free drug molecule to become active within the cell, increasing its potency both *in vivo* and *in vitro*. In fact, the FDA has approved at least thirty prodrugs over the past decade, encompassing 10 % of all medicines in the world.¹⁶

Ciprofloxacin Conjugate Syntheses

In this thesis, I am going to describe the synthesis of five novel 1,2,3-triazoles with ciprofloxacin as the base drug of interest. Along with the synthetic techniques, the minimum inhibitory concentrations (MIC) of each compound is listed and compared to the base ciprofloxacin drug, which will show the effect of the prodrug delivery method and its efficiency at killing the virulent strain of *P. aeruginosa* (PA14) and methicillin-resistant *S. aureus* (MRSA USA300 LAC) bacterial strains. Through a four-step process, these 1,2,3-triazole compounds can be synthesized in moderate yields, and has implications towards the prodrug method of ciprofloxacin.

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CHAPTER TWO

SYNTHESIS OF CIPROFLOXACIN-COUPLED A_oM ESTERS
AS PRE-CURSORS FOR ANTIBACTERIAL APPLICATIONS
UTILIZING CLICK CHEMISTRYIntroduction

Due to ciprofloxacin being an immensely popular fluoroquinolone drug, many bacterial strains have developed a strong resistance to the drug, eliminating it from their systems. In order to combat this alteration, many chemists have altered the structure of the base drug or attached molecules to improve the antibacterial properties of the drug, and even increase its scope to anti-TB, anti-cancer, and anti-tumor applications.¹⁻¹⁴ A number of these modifications involved the attachment of various molecules at the end of the piperazine molecule of ciprofloxacin, such as amino acids, thiol groups, and sugars.^{4, 5, 12} For example, Alireza Foroumadi's research group enhanced the antibacterial activity of ciprofloxacin by treating it with a thiodiazolyl functional group through a nucleophilic aromatic substitution (S_NAr) reaction, replacing the terminal hydrogen on the piperazine ring. The result of this alteration was an MIC value of 0.015 $\mu\text{g/mL}$, compared to the base value of 0.5 $\mu\text{g/mL}$.⁵ Similarly, Marvin J. Miller's research group implemented a similar methodology for attaching a functional group to the end of the piperazine ring. Their unique research was not an S_NAr reaction, but an N-capping reaction replacing a carboxylic acid with the piperazine ring. Some of the major findings were that the steric hindrance of the dimethyl group resulted in an internal lactonization when the moiety would be cleaved from the ciprofloxacin drug. Their MIC values drastically improved from 0.5 $\mu\text{g/mL}$ to

less than 0.05 $\mu\text{g/mL}$, demonstrating the positive effects of attaching moieties to the piperazine ring.⁶ One of the limitations for these findings is that the MIC values were shown against Gram-positive bacteria, but not Gram-negative. Along with the attachments of moieties to the piperazine ring, there were some alterations to the carboxylic acid of the drug,² but the tested derivatives were similar in biological activity against the bacterial strains, showing that the main source of antibacterial activity is dependent on what is attached to the piperazine ring.

In addition to the $\text{S}_{\text{N}}\text{Ar}$ reactions performed to synthesize multiple derivatives of ciprofloxacin, CuAAC reactions have also been performed to furnish multiple 1,2,3-triazoles with small molecules attached to ciprofloxacin, especially after K. Barry Sharpless won the Nobel Prize for click chemistry in 2022.¹⁵⁻²⁴ For instance, Alka Argawal's research group utilized click chemistry for multiple esters from propargylated ciprofloxacin and attached it with simple aromatic groups. The goal of these synthetic procedures was to investigate which derivatives were the most effective against both *P. aeruginosa* and *S. aureus*. Their findings ranged from MIC values of 12.5 – 25.0 $\mu\text{g/mL}$ for *P. aeruginosa* and 3.12 – 1000 $\mu\text{g/mL}$ for *S. aureus*.¹⁷ Likewise, Rahul Upadhyay's research group not only performed click chemistry with ciprofloxacin, but they further treated their compounds with lithium hydroxide to synthesize nine total scaffolds with a dicarboxylic acid structure, with ciprofloxacin as the parent drug. These two previous works do perform their analyses against both Gram-positive and Gram-negative bacteria, but their strain of *S. aureus* being tested against is not MRSA, but methicillin-susceptible *Staphylococcus aureus* (MSSA). In summary, most of, if not all, of the tested molecules were equally as effective or more active than the base ciprofloxacin molecule, further showing the importance of the 1,2,3-triazole embedded within the structure. To branch off of this

chemistry, ciprofloxacin was propargylated as closely followed by a written procedure, but different chelating attachments on the triazole component of the reaction were attached, interested in applying the effects of the chelation effect from previous work performed (Figure 6).

Danica Walsh et al. has previously reported the effect of acetoxymethyl (AoM) esters synthesis from simple phenols, and how it enhances the activity of the drug.²⁵ In her research, while there were some exceptions to the general trend, the addition of AoM esters increased the potency of the phenols being tested, which can be attributed to its characteristic of being concentrated within the cell (a characteristic that the simple phenols do not possess). Through these findings, Logan Mikesell et al. synthesized a popular intracellular fluorescent dye, Calcein Blue AM, through a base-catalyzed phenol-Mannich condensation reaction.²⁶ By using cesium carbonate as the base source, he was able to yield Calcein Blue AM in good yields, with little trouble in the workup process. In this report, the concepts of CuAAC with ciprofloxacin and the chelation effect of AoM esters are combined into a step-wise synthesis of novel 1,2,3-triazoles, using cesium carbonate as a base source for the AoM ester synthesis of azide components (Figure 6).

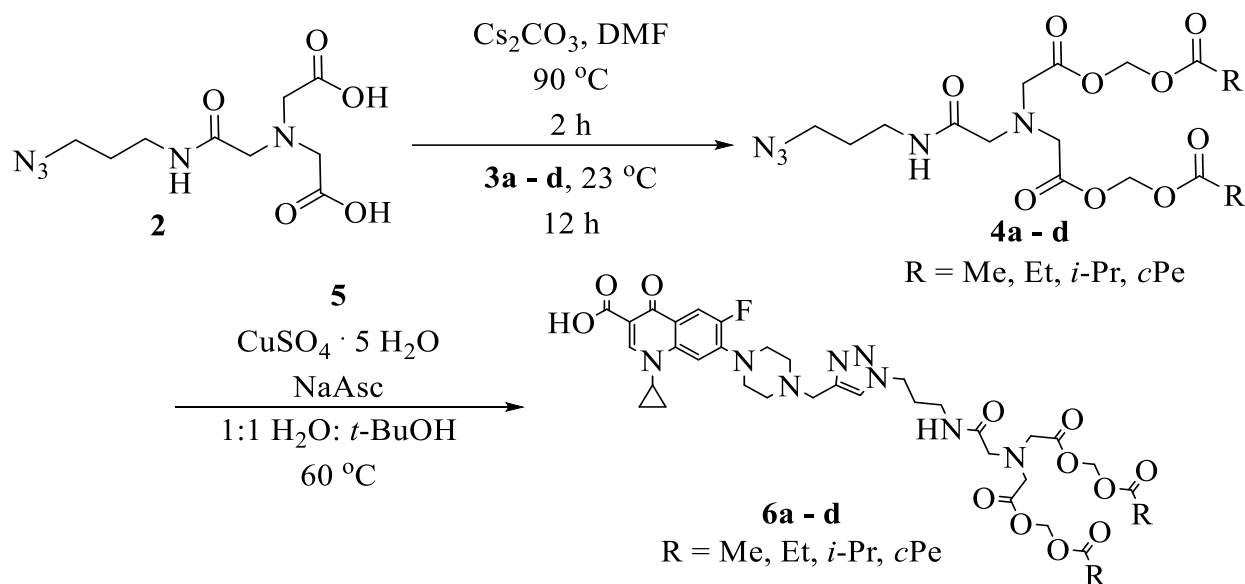


Figure 6. Synthesis of **6a – d** from **2**.

Results and Discussion

In order to synthesize the starting material, 3-chloropropylamine was converted to 3-azidopropylamine through an $\text{S}_{\text{N}}2$ reaction, using a 3-chloropropylamine hydrochloride salt solution in water (Figure 7). NaN_3 (2.97 equiv.) was used as the nucleophilic source and was added slowly over 5 minutes. The resultant mixture was allowed to heat to a gentle reflux for 15 hours. After heating, the mixture was allowed to cool to room temperature before basifying the reactant mixture with potassium carbonate (pK_a of conjugate acid, 10.25), which was carried out in situ. The addition of the base resulted in a slushy product that had to be immediately diluted with tetrahydrofuran (THF) and more water to prevent clumping of the potassium and sodium chloride salts. Further acylation was originally performed with chloroacetic anhydride but could also be readily achieved using chloroacetyl chloride, as reported by David Davidson et al.²⁷ By cooling the reactant mixture with an ice bath to $0\text{ }^\circ\text{C}$, the acylation reaction was carried by the dropwise addition of chloroacetic anhydride/chloroacetyl chloride (1.3 equiv.). This is an

extremely exothermic reaction, so keeping it in cold temperature was vital. Because the acylation of acyl chlorides reacts more quickly than acetic anhydrides, the former needed to be added more slowly and carefully. The hydrochloride salt of 3-azidopropylamine was separated from the organic layer, and the product was furnished in good yield after all of the solvent was removed under high vacuum.

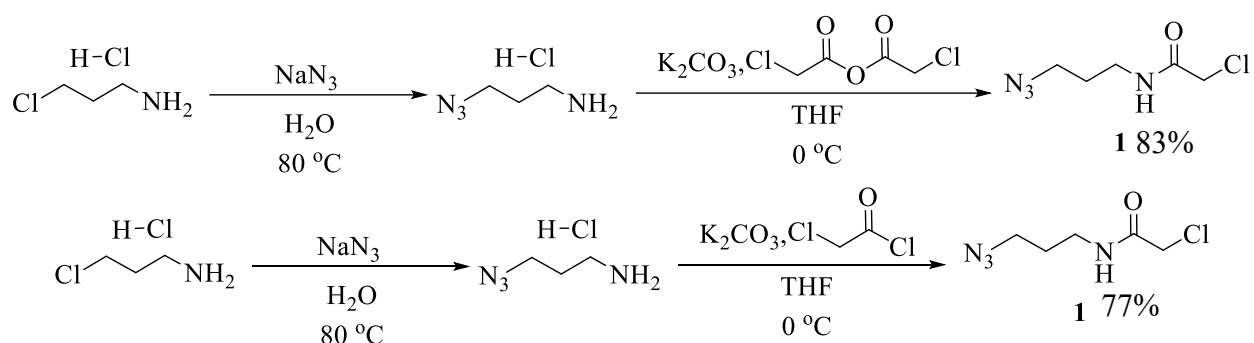
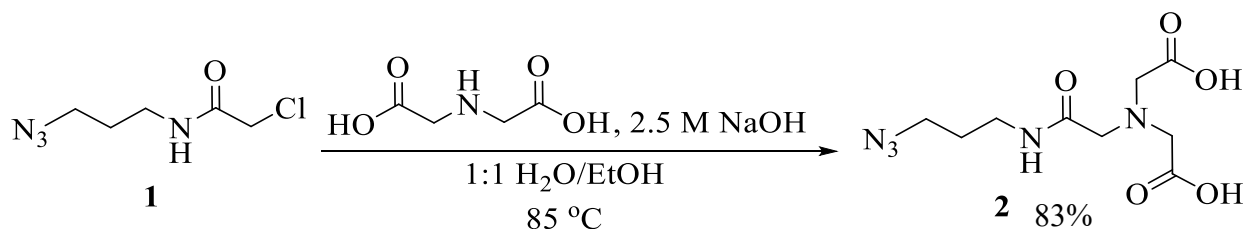
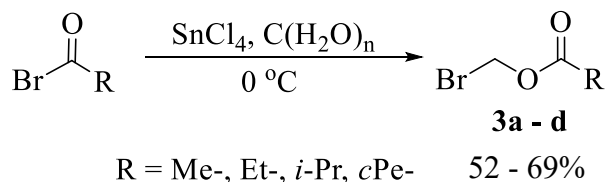


Figure 7. Synthesis of **1**.

A subsequent N-alkylation reaction was performed with iminodiacetic acid (1.0 equiv.), 2.5 M aqueous NaOH, and 50% aqueous ethanol (Figure 8). Originally, 2 equivalents of base was added to the reaction mixture to basify the reactant mixture, but after an hour of reflux, another equivalent of base was added to keep the reactant mixture in basic conditions (pH = 10). In order to avoid any cross contamination with organics that could have been formed during the reaction, diethyl ether was used to extract any organics from the reaction. After reacidifying the separated aqueous layer to a pH of 2 and removing the water in vacuo, the resulting solid was redissolved in methanol to separate out **2** from the sodium chloride salt formed, allowing for a simple filtration to be performed. The product was recovered in good yield, and after placing under high vacuum overnight, any trace amounts of methanol left was removed.

Figure 8. Synthesis of **2**.

A separate reaction was performed to synthesize the bromomethyl esters (**3a – 3d**), which was carried out by a written procedure (Figure 9).²⁶ Through the use of stannic chloride as a Lewis acid, paraformaldehyde was added very slowly in cold temperature to furnish the bromomethyl esters in high purity and moderate yield.²⁶ The bromomethyl pivalate ester was attempted to be synthesized with pivaloyl bromide as the starting substrate, but unfortunately, the reaction was unsuccessful, most likely because stannic chloride is a weaker Lewis acid, and the reaction has to be under heated conditions, as opposed to colder ones. Even when utilizing a different procedure for synthesizing the pivaloyloxymethyl ester, the resulting product was not pure enough to use for the ensuing S_N2 reaction. Due to this, a fresh bottle of pivaloyloxymethyl chloride was purchased and used.

Figure 9. Synthesis of **3a – d**.

Unfortunately, the S_N2 reaction involving carboxylic acids using cesium carbonate as the base produced esters in poor yields (~20% or less). Even after retrieving moderate to good yields of the crude material, the vast majority of that material would be lost after performing silica gel flash chromatography. This was most likely a result of the formation of the mono-substituted side product, instead of the disubstituted product. Any trace amount of water was detrimental to the progress of the reaction and had to be ensured that no water or moisture was leaking into the reaction flask, which was a difficult condition to hold. The reaction conditions of 1.5 equiv. of cesium carbonate in *N,N*-dimethylformamide was used for the majority of the syntheses of **4a** – **4e** (Figure 10). An alternate set of conditions was also performed with tetramethylammonium fluoride in acetonitrile, but there was no significant change with the purity and yield of the ester products. If anything, the yield was slightly lower, but the mono-substituted spot was not present when observing the crude product on the TLC plate. One of the advantages of using the latter method, however, was that the amount of time between the addition of base and the bromomethyl esters was drastically reduced from 2 h to 5 min.

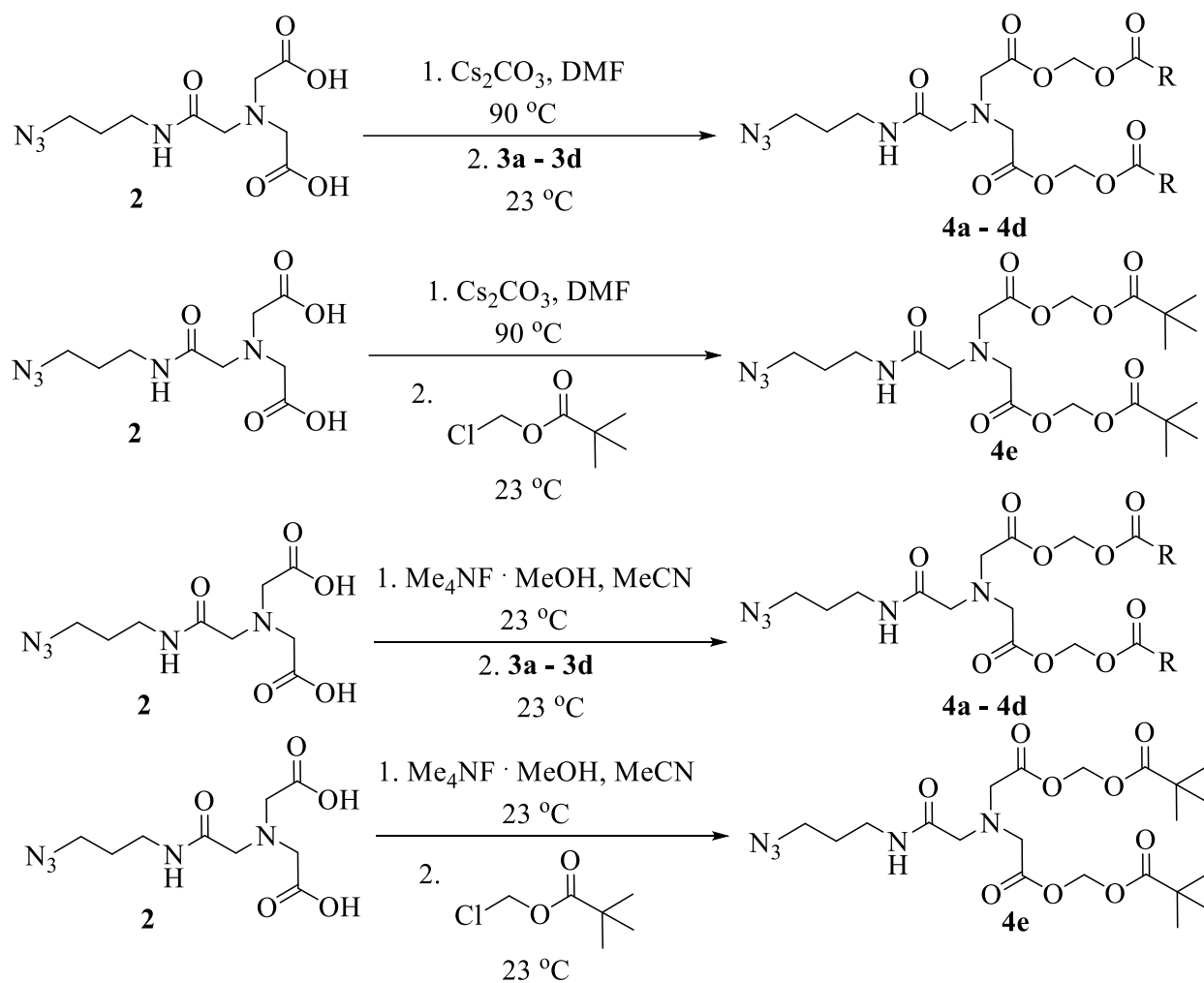


Figure 10. Synthesis of acetoxymethyl esters **4a – 4e** through two separate conditions.

Originally, a published procedure was followed that used a 1 mol % concentration of a copper source (0.1 M aqueous solution of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), and a 10 mol % concentration of the reductant (0.1 M aqueous solution of sodium ascorbate), with **5** being the source of the alkyne, as synthesized through another procedure (Figure 11).¹⁹ The solvent used for these conditions alternated from 1:1 *t*-butanol/water to 2:1 *t*-butanol/water. Under these conditions, although the product would be synthesized (as seen through TLC), there would still be starting material

present. The reaction mixture would be carried out overnight in an oil bath heated at 60 °C, but not all of the alkyne would be consumed. This could have been a result of all of the Cu(I) catalyst being used up. After increasing the concentrations of the copper(II) source and reductant to 5 mol % and 15 mol %, respectively, the reaction would be carried out to completion and a higher yield resulted from this alteration (Figure 11). On top of this change, the solvent was also heavily concentrated with *t*-butanol (10:1 *t*-butanol/water). This was confirmed by the complete consumption of the alkyne, as shown through TLC.

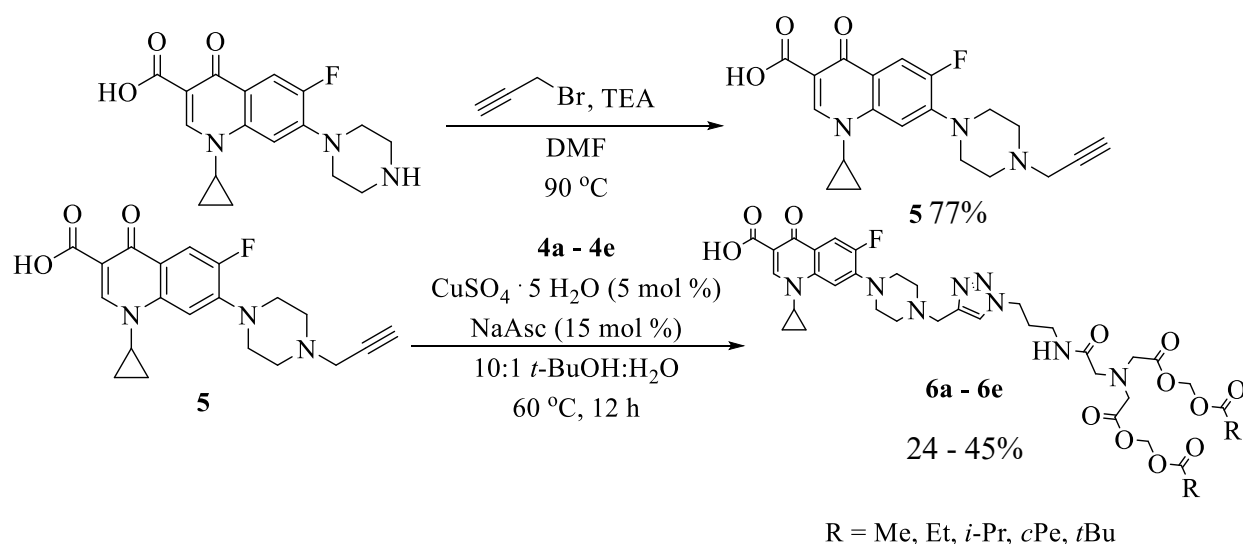


Figure 11. Synthesis of **6a – 6e** from ciprofloxacin.

The mechanism of action for this particular click reaction has been pondered for a lengthy period of time. Originally, the proposed catalytic mechanism involved only one Cu(I) molecule, but there has been some recent findings via thermochemistry that hypothesize a second Cu(I) molecule being directly involved with the ring closure (Figure 12). After coordination of the Cu(I) to the alkyne (exothermic in water), a second Cu(I) binds to the π -bond of the acetylide. The resulting intermediate coordinates with the azide, forming a six-membered

metallacycle. An ensuing ring contraction and protonolysis step forms the triazole of interest, which closes the catalytic cycle.²⁸ This can explain why the original use of 1 mol % of $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ was insufficient for using up all of the alkyne in the click reaction.

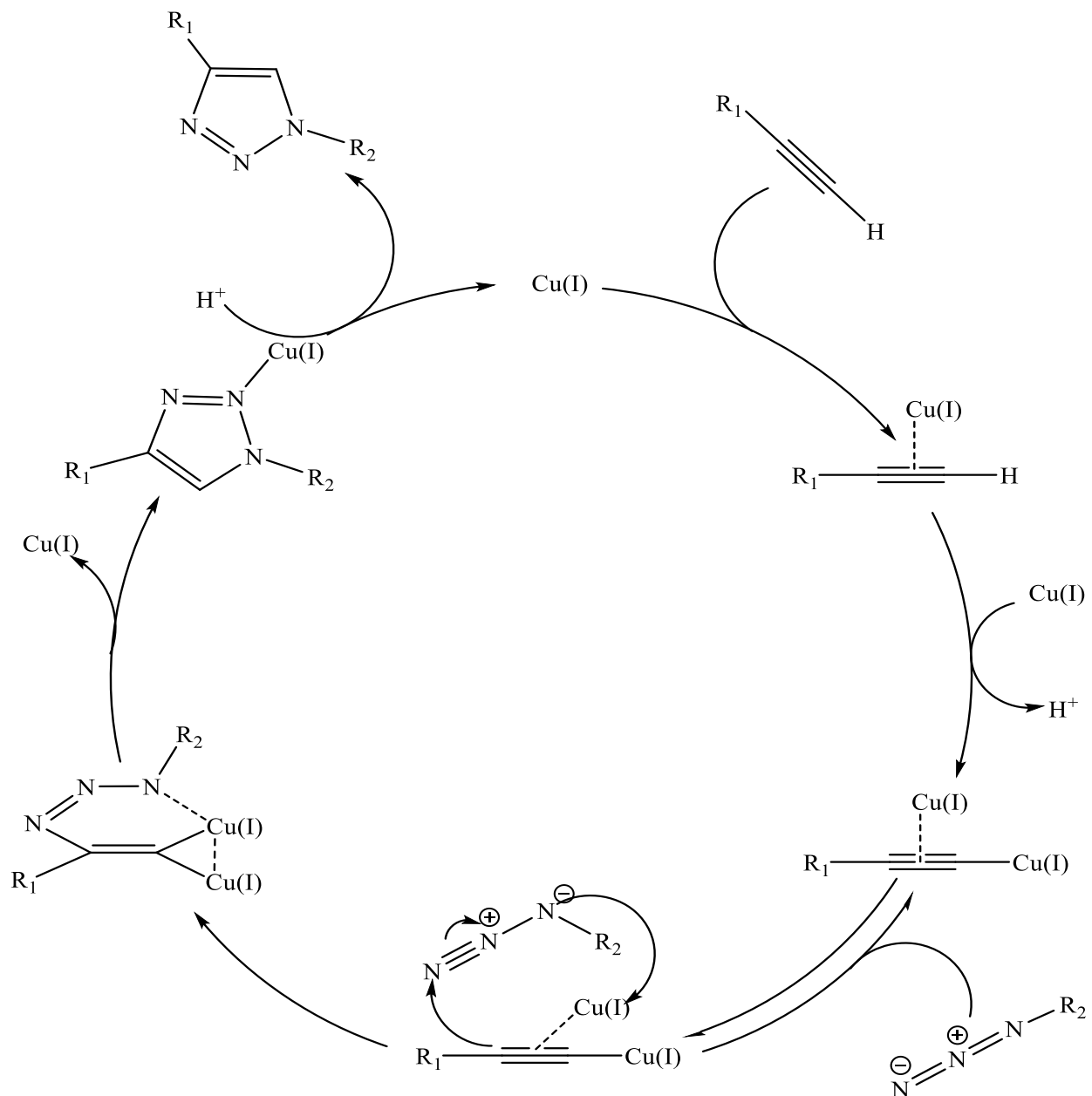


Figure 12. Catalytic cycle of the CuAAC reaction with Cu(I) as the catalyst.

Compounds **6a** – **6e** were tested against strains of *Pseudomonas aeruginosa* (PA14), as well as methicillin-resistant *Staphylococcus aureus* (MRSA USA300 LAC) by the protocol established by Heidi Koenig et al.²⁹ The results from all of the triazole compounds were compared to the original ciprofloxacin molecule, as well as the propargylated derivative of ciprofloxacin. Based on the results (Table 1), all of the triazole compounds are inferior with their biological activity against both PA14 and MRSA USA300 LAC. However, when comparing the triazole compounds to each other, the most effective compounds were **6b**, **6c**, and **6d**. The minimum inhibitory concentration (MIC) values of these compounds were 25, 26, and 28 µg/mL against the PA14 strain, respectively. Against the MRSA strain, their MIC values were 102, 211, and 112 µg/mL, respectively. The other triazole compounds synthesized were weaker in their results of MIC values, with **6e** being the least effective towards the bacterial strains.

Table 1. Biological data of triazoles **6a** – **6e** with ciprofloxacin and **5** as control groups.

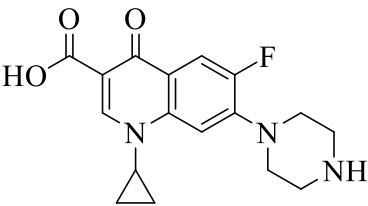
No.	Compound	PA14 MIC (µg/mL)	MRSA USA300 LAC MIC (µg/mL)
		0.04	23

Table 1 Continued.

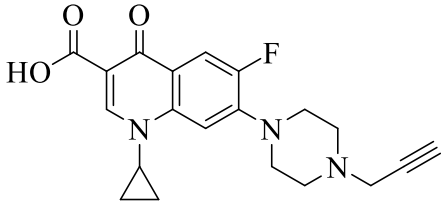
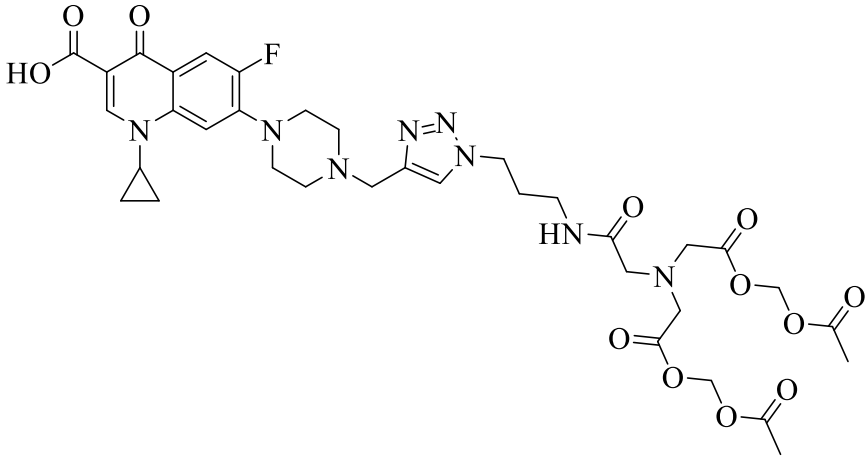
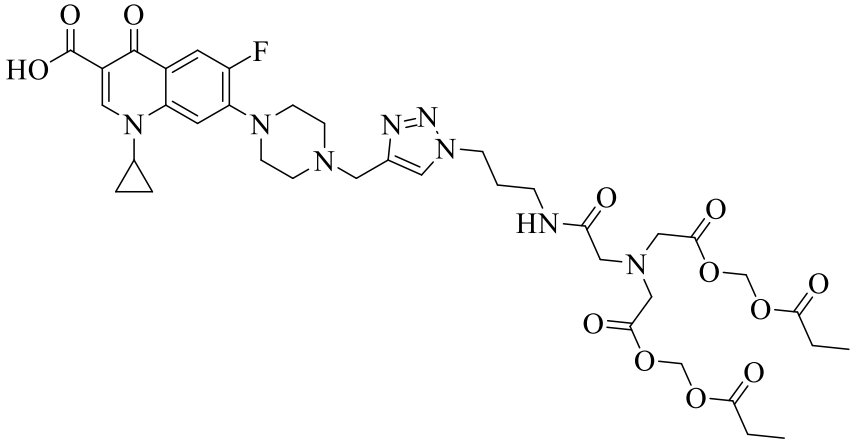
No.	Compound	PA14 MIC ($\mu\text{g/mL}$)	MRSA USA300 LAC MIC ($\mu\text{g/mL}$)
5		0.09	23
6a		49	393
6b		25	102

Table 1 Continued.

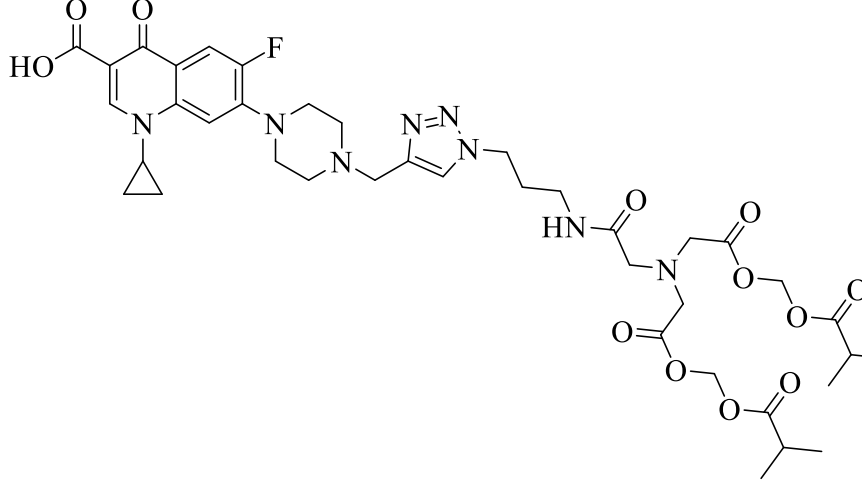
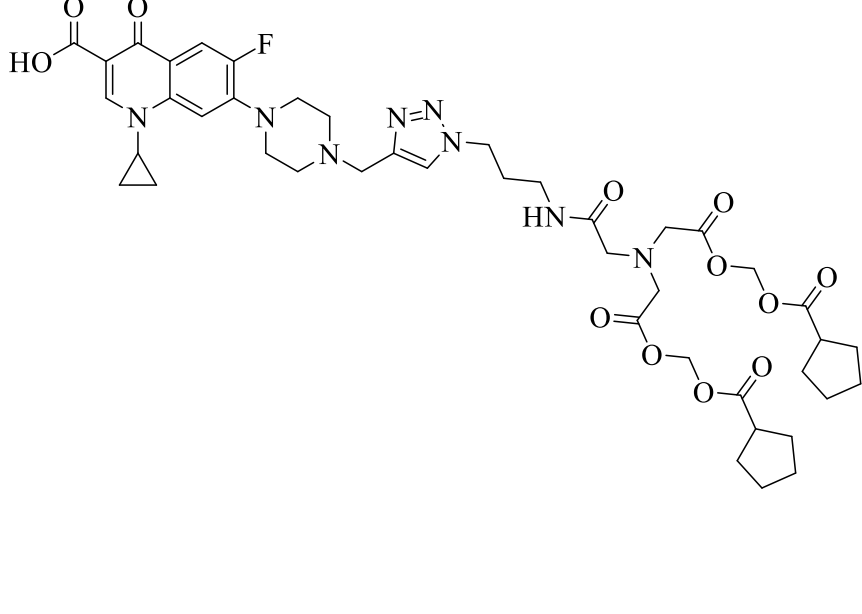
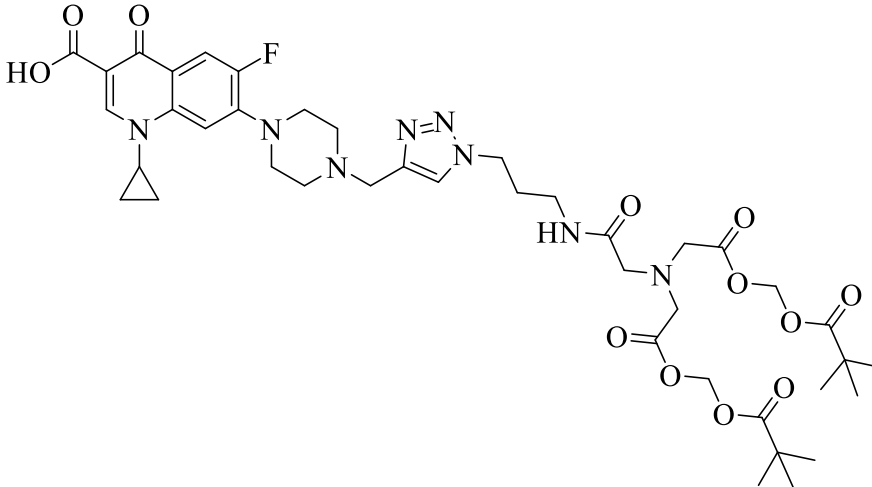
No.	Compound	PA14 MIC (μg/mL)	MRSA USA300 LAC MIC (μg/mL)
6c		26	211
6d		28	112

Table 1 Continued.

No.	Compound	PA14 MIC ($\mu\text{g/mL}$)	MRSA USA300 LAC MIC ($\mu\text{g/mL}$)
6e		54	435

Based off of the results of the MIC values, it can be concluded that the more complex the R group is, the more active the compound is against both strains of bacteria. The only exception to this trend seemed to be **6e**, where the *t*-butyl group actually reduced the effectivity against the bacterial strains.

Future Investigations

The study that was accomplished provides a wide avenue of interesting chemistry that can be explored. There are a multitude of more complex R groups that can be implemented to investigate its direct effect against PA14 and MRSA USA300 LAC. More specifically, the

continuation of chelating R groups can be applied with various hydrocarbon chains and rings known to enhance drug potency, such as an octyl chain. In addition, the synthesis of more lipophilic and hydrophilic esters can be used as comparisons against each other for the efficacy of the prodrugs being synthesized. Instead of iminodiacetic acid for the N-alkylation step, this can be replaced with a standard glyphosate molecule, in which a trisubstituted AoM ester can be synthesized and tested against the same bacterial strains. Branching off of the glyphosate, an N-capping procedure can be performed to attach a pyrrolidine and morpholine ring to the end of the carboxylic acid, where a different disubstituted AoM ester can be utilized for these pharmaceutical applications. From these results, more drugs can be synthesized for an improved outcome for combatting against bacteria that can survive in harsh conditions. More emphasis towards these click reactions with different second-generation fluoroquinolones can also prove to be an effective strategy for drug delivery and efficacy against PA14, MRSA USA300 LAC, and other harmful bacterial strains capable of creating biofilms.

Conclusion

In conclusion, a set of novel 1,2,3-triazole prodrug conjugates were synthesized and tested against PA14 and MRSA USA300 LAC bacterial strains. Through the utilization of acylation, N-alkylation, an S_N2 ester synthesis, and the well-known CuAAC technique, five unique 1,2,3-triazoles were synthesized in high purity, although yields were low to moderate. Although these triazoles were not as effective against both strains, the propionate derivative proved to be the most effective, although the isobutyrate and cyclopentyl carboxylate derivatives were very comparable to each other. These results open the door for more versatility and variety

with the triazoles synthesized and possibly, its efficacy against different strains of biofilm-forming bacteria.

Experimental Section

General Information

All compounds from commercial sources were used as received unless noted otherwise. Solvents were certified ACS grade. Compounds **4a** – **4e** and **6a** – **6e** were carried out under nitrogen using oven-dried glassware. All other reactions were carried out under ambient conditions using oven-dried glassware. Thin-layer chromatography (TLC) was performed using Silicycle glass-backed TLC plates, silica gel F-254 over glass support, 250 μ m thickness. Visualization was accomplished with UV light (254 nm) and/or a potassium permanganate stain. All ^1H , $^{13}\text{C}\{^1\text{H}\}$, and ^{19}F NMR spectra were obtained on a Bruker 400 MHz Ascend AVANCE NEO NMR Spectrometer. Chemical shifts (δ) were reported in reference to solvent peaks (residue CDCl_3 at δ 7.26 ppm for ^1H and CDCl_3 at δ 77.0 ppm for $^{13}\text{C}\{^1\text{H}\}$).

N-(3-azidopropyl)-2-chloroacetamide (**1**): A 50 mL round-bottomed flask equipped with a magnetic stirring bar and reflux condenser was charged with 3-chloropropylamine hydrochloride (1.00 g, 7.69 mmol, 1.0 equiv.), and water (4 mL), respectively. NaN_3 (1.49 g, 22.9 mmol, 3.0 equiv.) was added portionwise. The resulting mixture was heated to 80 $^\circ\text{C}$ with an oil bath for 15 h. The resulting mixture was then basified with potassium carbonate (2.31 g, 16.7 mmol, 2.2 equiv.) and diluted with THF (10 mL). Chloroacetyl chloride (800 μL , 10.0 mmol, 1.3 equiv.) was then added dropwise, and allowed to stir at 0 $^\circ\text{C}$ for 30 min. The organic layer was separated, and further extracted with the addition of ether (10 mL). The combined organic layers were dried with brine (1 x 5 mL). The organic layer was then dried with Na_2SO_4 and filtered. The

filtrate was concentrated under vacuo and further dried under high vacuum to yield **1** (1.13 g, 83%) as a yellow oil. ^1H NMR (400 MHz, CDCl_3) δ : 6.78 (s, 1H, N-H), 4.06 (s, 2H), 3.41 (dt, 4H, $J = 6.27$ Hz), 1.84 (tt, 2H, $J = 6.63$ Hz). $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ : 166.1, 49.3, 42.6, 37.6, 28.5, 25.6.

2-[Carboxymethyl-[2-(3-azidopropylamino)-2-oxoethyl]amino]diacetic acid (**2**): A 50 mL round-bottomed flask equipped with a magnetic stirring bar and reflux condenser was charged with a solution of **51** (442 mg, 2.50 mmol, 1.0 equiv.) in 50% aqueous ethanol and iminodiacetic acid (333 mg, 2.50 mmol, 1.0 equiv.), respectively. The reaction mixture was heated to reflux at 85 °C with an oil bath, and 2.5 M NaOH (4.00 mL, 4.0 equiv.) was added and left to stir overnight. The reaction mixture was then concentrated in vacuo, diluted with H_2O (5 mL), and the organics were extracted with ether (3 x 5 mL). The aqueous layer was then acidified with 6 M hydrochloric acid (1.67 mL, 4.0 equiv.), until a pH of 2 was reached. The aqueous layer was concentrated in vacuo, dissolved in methanol (MeOH), and passed through a Celite plug. The resulting yellow liquid was concentrated via rotary evaporator and further dried under a high vacuum system to yield **2** (564 mg, 83%) as an off-white solid. ^1H NMR (400 MHz, D_2O) δ : 4.05 (s, 2H), 3.87 (s, 4H), 3.32 (tt, 4H, $J = 6.48$ Hz), 1.76 (tt, 2H, $J = 6.3$ Hz) $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, D_2O) δ : 168.7, 165.5, 56.0, 55.5, 48.9, 48.7, 47.1, 37.0, 27.4.

General Procedure for Electrophilic Esters

Bromomethyl acetate (**3a**): A 25 mL round-bottomed flask equipped with a magnetic stirring bar and a yellow poly seal cap was charged with acetyl bromide (3.7 mL, 50 mmol, 1.0 equiv.) and stannic chloride (0.060 mL, 0.5 mmol, 0.01 equiv.). The mixture was cooled to 0 °C with an ice bath, and paraformaldehyde (1.3 g, 45 mmol, 0.9 equiv.) was added portionwise. The

reactant mixture was then stirred at 0 °C for 1 h. The reactant mixture was allowed to warm to room temperature, and then was purified via short-path distillation to collect **3a** (5.25 g, 69%) as a yellow oil. ^1H NMR (400 MHz, CDCl_3) δ : 5.72 (s, 2H), 2.07 (s, 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ : 168.8, 57.0, 20.8.

Bromomethyl propionate (**3b**): A 25 mL round-bottomed flask equipped with a magnetic stirring bar and yellow poly seal cap was charged with propanoyl bromide (4.5 mL, 50 mmol, 1.0 equiv.) and stannic chloride (0.060 mL, 0.5 mmol, 0.01 equiv.). The mixture was cooled to 0 °C with an ice bath, and paraformaldehyde (1.3 g, 45 mmol, 0.9 equiv.) was added portionwise. The reactant mixture was then stirred at 0 °C for 3 h. The reactant mixture was allowed to warm to room temperature and was purified via short-path distillation to collect **3b** (5.48 g, 66%) as a yellow oil. ^1H NMR (400 MHz, CDCl_3) δ : 5.83 (s, 2H), 2.42 (tt, 2H, $J = 7.49$ Hz), 1.20 (t, 3H, $J = 7.54$ Hz) $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ : 172.3, 57.1, 27.5, 8.6.

Bromomethyl isobutyrate (**3c**): A 25 mL round-bottomed flask equipped with a magnetic stirring bar and yellow poly seal cap was charged with isobutyryl bromide (5.4 mL, 50 mmol, 1.0 equiv.) and stannic chloride (0.060 mL, 0.5 mmol, 0.01 equiv.). The mixture was cooled to 0 °C with an ice bath, and paraformaldehyde (1.3 g, 45 mmol, 0.9 equiv.) was added portionwise. The reaction was left to stir at 0 °C for 3 h. The reaction mixture was allowed to warm to 23 °C and was purified via short-path distillation to collect **3c** (5.20 g, 63%) as a yellow oil. ^1H NMR (400 MHz, CDCl_3) δ : 5.84 (s, 2H), 2.63 (s, 1H, $J = 6.96$ Hz), 1.21 (d, 6H, $J = 7.01$ Hz) $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ : 174.8, 57.2, 33.9, 18.4.

Bromomethyl cyclopentanecarboxylate (**3d**): A 25 mL round-bottomed flask equipped with a magnetic stirring bar and yellow poly seal cap was charged with cyclopentanecarbonyl

bromide (3.2 mL, 5.0 mmol, 1.0 equiv.) and stannic chloride (0.0060 mL, 0.05 mmol, 0.01 equiv.). The mixture was cooled to 0 °C with an ice bath, and paraformaldehyde (0.13 g, 4.5 mmol, 0.9 equiv.) was added portionwise. The reaction was left to stir at 0 °C for 3 h, and a short-path distillation was performed to collect **3d** (2.65 g, 53%) as a black oil. ¹H NMR (400 MHz, CDCl₃) δ: 5.84 (s, 2H), 2.81 (p, 1H, *J* = 7.52 Hz), 1.95 – 1.61 (m, 8H) ¹³C{¹H} NMR (100 MHz, CDCl₃) δ: 174.5, 57.3, 43.5, 29.6, 25.8.

General Procedure for Ester Synthesis using Cs₂CO₃

A 100 mL round-bottomed flask equipped with a magnetic stirring bar and reflux condenser was charged with **2** (1.0 g, 3.66 mmol, 1.0 equiv.) and Cs₂CO₃ (1.79 g, 5.49 mmol, 1.5 equiv.) in 10 mL of *N,N*-dimethylformamide (DMF). The resulting mixture was heated at 90 °C with an oil bath overnight. The mixture was then cooled to room temperature, and then cooled in an ice bath to 0 °C, which was immediately followed by the dropwise addition of the appropriate bromomethyl ester (9.15 mmol, 2.5 equiv.) and left to stir overnight and warm to 23 °C. The reactant mixture was quenched with H₂O (50 mL), and the organics were extracted with ethyl acetate (3 x 30 mL). The combined organics were washed with water (3 x 10 mL) and brine (3 x 10 mL). The organic layer was dried with sodium sulfate and filtered. The filtrate was concentrated in vacuo. The crude product was then purified by silica gel flash chromatography to furnish the product.

Bis(acetoxymethyl) 2,2'-((2-[carboxymethyl-[2-(3-azidopropylamido)-2-oxoethyl]amino)]azanediyldiacetate (**4a**): Subjection of **2** and **3a** with the general Cs₂CO₃ procedure afforded **4a** (210 mg, 23%) as an off-yellow, viscous oil after silica gel flash chromatography (hexanes/ethyl acetate 1:1): ¹H NMR (400 MHz, CDCl₃) δ: 7.83 (s, 1H, N-H),

5.77 (s, 4H), 3.60 (s, 4H), 3.39 (s, 2H), 3.39 (tt, 4H, $J = 6.73, 4.24$ Hz), 2.13 (s, 6H), 1.83 (tt, 2H, $J = 6.7$ Hz) $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ : 170.8, 170.0, 169.5, 79.4, 59.1, 55.8, 49.1, 36.7, 28.7, 20.6.

(2,2'-((2-[Carboxymethyl-[2-(3-azidopropylamido)-2-oxoethyl]amino)]azanediyl)bis(acetyl))bis(oxy))bis(methylene) Dipropionate (**4b**): Subjection of **2** and **3b** with the general Cs_2CO_3 procedure afforded **4b** (181 mg, 21%) as an off-yellow, viscous oil after silica gel flash chromatography (hexanes/ethyl acetate 2:1): ^1H NMR (400 MHz, CDCl_3) δ : 7.77 (s, 1H, N-H), 5.72 (s, 4H), 3.53 (s, 4H), 3.31 (m, 6H), 2.34 (q, 4H, $J = 7.54$), 1.76 (tt, 2H, $J = 6.68$ Hz), 1.10 (t, 6H, $J = 7.53$ Hz) $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ : 170.9, 168.9, 167.9, 77.3, 57.0, 53.7, 47.0, 34.6, 26.6, 25.1, 6.5.

(2,2'-((2-[Carboxymethyl-[2-(3-azidopropylamido)-2-oxoethyl]amino)]azanediyl)bis(acetyl))bis(oxy))bis(methylene) Diisobutyrate (**4c**): Subjection of **2** and **3c** with the general Cs_2CO_3 procedure afforded **4c** (210 mg, 24%) as an off-yellow, viscous oil after silica gel chromatography (hexanes/ethyl acetate 3:1): ^1H NMR (400 MHz, CDCl_3) δ : 7.77 (s, 1H, N-H), 5.72 (s, 4H), 3.52 (s, 4H), 3.32 (m, 6H), 2.53 (s, 2H, $J = 7.01$ Hz), 1.76 (tt, 2H, $J = 6.74$ Hz), 1.12 (d, 12H, $J = 7.00$ Hz) $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ : 175.6, 170.4, 170.0, 79.6, 59.2, 55.7, 49.2, 36.6, 33.7, 28.8, 18.6.

(2,2'-((2-[Carboxymethyl-[2-(3-azidopropylamido)-2-oxoethyl]amino)]azanediyl)bis(acetyl))bis(oxy))bis(methylene) Dicyclopentyl carboxylate (**4d**): Subjection of **2** and **3d** with the general Cs_2CO_3 procedure afforded **4d** (70 mg, 13%) as an off-yellow, viscous oil after silica gel chromatography (hexanes/ethyl acetate 4:1): ^1H NMR (400 MHz, CDCl_3) δ : 7.85 (s, 1H, N-H), 5.78 (s, 4H), 3.59 (s, 4H), 3.38 (m, 6H), 2.78 (tt, 2H, $J = 7.49$

Hz), 1.94 – 1.58 (m, 18 H) $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ : 175.3, 170.4, 170.0, 79.5, 59.1, 55.7, 49.2, 43.4, 36.6, 29.8, 28.8, 25.8.

(2,2'-((2-[Carboxymethyl]-[2-(3-azidopropylamido)-2-oxoethyl]amino)]azanediyl)bis(acetyl))bis(oxy))bis(methylene) Dipivate (4e): Subjection of **2** and pivaloyloxymethyl chloride with the general Cs_2CO_3 procedure afforded **4e** (50 mg, 13%) as a yellow, viscous oil after silica gel flash chromatography (hexanes/ethyl acetate 4:1): ^1H NMR (400 MHz, CDCl_3) δ : 7.76 (s, 1H, N-H), 5.71 (s, 4H), 3.51 (s, 4H), 3.31 (m, 6H), 1.76 (tt, 2H, J = 6.71 Hz), 1.15 (s, 18H) $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3) δ : 174.53, 167.84, 167.43, 77.30, 56.66, 53.22, 46.70, 36.29, 34.12, 26.34, 24.34.

1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(prop-2-yn-1-yl)piperazin-1-yl)-1,4-dihydroquinolone-3-carboxylic acid (5): A 100 mL round-bottomed flask equipped with a magnetic stirring bar and a reflux condenser was charged with ciprofloxacin (994 mg, 3.0 mmol, 1.0 equiv.) and 20 mL of DMF, respectively. The reactant mixture was then treated with triethylamine (TEA) (1.26 mL, 7.5 mmol, 2.5 equiv.) and propargyl bromide (833 μL , 7.5 mmol, 2.5 equiv.). The reactant mixture was then heated at 80 $^\circ\text{C}$ with an oil bath and left to stir overnight. The reactant mixture was allowed to cool to 23 $^\circ\text{C}$, and then placed in an ice bath. Cold DI water (25 mL) was added to the reactant mixture, in which precipitate crashed out. This was filtered and washed with DI water, and then dissolved on the fritted funnel with dichloromethane/methanol (9:1) and passed through. The filtrate was concentrated in vacuo, dissolved in dichloromethane (DCM), and passed through a silica gel plug, using DCM/MeOH (9:1) as the eluent. The resulting solution was concentrated in vacuo, dried under high vacuum overnight, to furnish **5** (850 mg, 77%) as an off-white solid. ^1H NMR (400 MHz, CDCl_3) δ :

14.90 (s, 1H, COOH), 8.70 (s, 1H), 7.96 (d, 1H, $J = 13.07$ Hz), 7.30 (d, 1H, $J = 7.14$ Hz), 3.48 (dd, 1H, $J = 3.23$ Hz), 3.35 (s, 2H), 3.33 (dd, 4H, $J = 4.89$ Hz), 2.75 (dd, 4H, $J = 4.76$ Hz), 2.25 (s, 1H), 1.32 (td, 2H, $J = 7.02$ Hz), 1.14 (td, 2H, $J = 7.02, 3.88$ Hz). $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ : 177.2, 167.0, 153.7 (d, $J = 251.6$ Hz), 147.5, 145.9, 139.1, 120.0 (d, $J = 8.04$ Hz), 112.6 (d, $J = 23.67$ Hz), 108.3, 104.8 (d, $J = 3.32$ Hz), 51.4, 49.7, 46.9, 35.3, 8.2. ^{19}F NMR (376 MHz, CDCl_3) δ : -120.7.

General Synthesis of Triazole **6a** – **6e**

An oven-dried 10 mL round-bottomed flask equipped with a magnetic stirring bar and a reflux condenser was charged with **4a** – **4e** (2.0 equiv.) and **5** (1.0 equiv.) in 6 mL of 10:1 *t*-BuOH/ H_2O . 0.1 M aqueous solutions of sodium ascorbate (15 mol %) and $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ (5 mol %) were added respectively and left to stir at 60 °C overnight. The reaction was checked via thin-layer chromatography to check for complete consumption of the alkyne. The reaction was quenched with the addition of H_2O , and the organics were extracted with DCM. The combined organic layers were dried with brine (1 x 5 mL). The organic layer was further dried with Na_2SO_4 and filtered. The filtrate was concentrated in vacuo and further purified by silica gel flash chromatography to obtain **6a** – **6e**.

(((4-((1-Propylamido-(2-oxoethyl)amino)azanediyl)diacetate)-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**6a**): Subjection of **4a** and **5** with the general triazole procedure afforded **6a** (54 mg, 27%) as an off-yellow film after silica gel flash chromatography (DCM/EtOAc/MeOH 80:15:5). ^1H NMR (400 MHz, CDCl_3) δ : 15.00 (s, 1H, COOH), 8.74 (s, 1H), 7.98 (d, 1H, $J = 13.10$ Hz), 7.93 (s, 1H, N-H), 7.67 (s, 1H), 7.34 (d, 1H, $J = 7.10$ Hz), 5.77 (s, 4H), 4.43 (dd, 2H, $J = 6.79$ Hz), 3.77

(s, 2H), 3.60 (s, 4H), 3.52 (m, 1H), 3.39 (s, 2H), 3.34 (m, 4H), 3.33 (dd, 2H, $J = 6.35$ Hz), 2.76 (dd, 4H, $J = 4.52$ Hz), 2.18 (tt, 2H, $J = 6.59$ Hz), 2.11 (s, 6H), 1.37 (dt, 2H, $J = 6.75$ Hz), 1.17 (dt, 2H, $J = 6.81, 4.56$ Hz). $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ : 177.2, 170.8, 170.0, 169.5, 167.0, 153.7 (d, $J = 251.42$ Hz), 147.4, 145.8 (d, $J = 10.58$ Hz), 143.8, 139.1, 123.3, 119.9, 112.4 (d, $J = 23.48$ Hz), 108.2, 104.8, 79.4, 59.2, 55.9, 53.1, 52.4, 49.7 (d, $J = 4.87$ Hz), 47.6, 38.2, 36.1, 35.3, 31.2, 30.4, 29.7, 20.7, 8.2. ^{19}F NMR (376 MHz, CDCl_3) δ : -120.8.

(((4-((1-Propylamido-(2-oxoethyl)amino)azanediyl)bis(oxy))bis(methylene) dipropionate)-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**6b**): Subjection of **4b** and **5** with the general triazole procedure afforded **6b** (48 mg, 26%) as an off-yellow film after silica gel flash chromatography (DCM/EtOAc/MeOH 80:15:5). ^1H NMR (400 MHz, CDCl_3) δ : 14.99 (s, 1H, COOH), 8.72 (s, 1H), 7.96 (s, 1H, N-H), 7.95 (d, 1H, $J = 13.03$ Hz), 7.70 (s, 1H), 7.33 (d, 1H, $J = 7.12$ Hz), 5.77 (s, 4H), 4.42 (dd, 2H, $J = 6.81$ Hz), 3.79 (s, 2H), 3.59 (s, 4H), 3.53 (m, 1H), 3.39 (s, 2H), 3.36 (dd, 4H, $J = 5.13$ Hz), 3.32 (dd, 2H, $J = 6.31$ Hz), 2.78 (dd, 4H, $J = 4.86$ Hz), 2.38 (q, 4H, $J = 7.53$ Hz), 2.17 (tt, 2H, $J = 6.60$ Hz), 1.37 (dt, 2H, $J = 6.79$ Hz), 1.17 (dt, 2H, $J = 3.79$ Hz), 1.14 (dd, 6H, $J = 7.53$ Hz). $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ : 177.1 (d, $J = 2.36$ Hz), 173.0, 170.9, 170.1, 167.0, 153.7 (d, $J = 251.44$ Hz), 147.4, 145.8 (d, $J = 10.55$ Hz), 143.3, 139.1, 123.5, 119.8 (d, $J = 7.96$ Hz), 112.4 (d, $J = 23.47$ Hz), 108.1, 104.9, 79.5, 59.2, 56.0, 52.8, 52.3, 49.5 (d, $J = 4.85$ Hz), 47.6, 36.1, 35.3, 30.4, 27.2, 8.7, 8.2. ^{19}F NMR (376 MHz, CDCl_3) δ : -120.7.

(((4-((1-Propylamido-(2-oxoethyl)amino)azanediyl)bis(oxy))bis(methylene) diisobutyrate)-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**6c**): Subjection of **4c** and **5** with the general triazole

procedure afforded **6c** (35 mg, 24%) as an off-yellow film after silica gel flash chromatography (DCM/MeOH 95:5). ^1H NMR (400 MHz, CDCl_3) δ : 15.03 (s, 1H, COOH), 8.76 (s, 1H), 8.00 (d, 1H, $J = 13.11$ Hz), 7.96 (s, 1H, N-H), 7.71 (s, 1H), 7.36 (d, 1H, $J = 7.10$ Hz), 5.80 (s, 4H), 4.45 (dd, 2H, $J = 6.78$ Hz), 3.79 (s, 2H), 3.61 (s, 4H), 3.55 (m, 1H), 3.41 (s, 2H), 3.38 (dd, 4H, $J = 5.21$ Hz), 3.34 (dd, 2H, $J = 6.34$ Hz), 2.79 (dd, 4H, $J = 4.22$ Hz), 2.61 (s, 2H, $J = 6.99$ Hz), 2.20 (tt, 2H, $J = 6.55$ Hz), 1.40 (dt, 2H, $J = 6.70$ Hz), 1.20 (dt, 2H, $J = 5.20$ Hz), 1.19 (d, 12H, $J = 7.00$ Hz). $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ : 177.1, 175.6, 170.8, 170.0, 167.0, 153.7 (d, $J = 251.57$ Hz), 147.4, 145.9 (d, $J = 10.24$ Hz), 143.8, 139.1, 123.3, 119.8 (d, $J = 7.86$ Hz), 112.4 (d, $J = 23.46$ Hz), 108.1, 104.8 (d, $J = 3.39$ Hz), 79.6, 59.2, 55.9, 53.1, 52.4, 49.7 (d, $J = 4.89$ Hz), 47.6, 36.1, 35.3, 33.7, 30.4, 18.6, 8.2. ^{19}F NMR (376 MHz, CDCl_3) δ : -120.7.

(((4-((1-Propylamido-(2-oxoethyl)amino)azanediyl)bis(oxy))bis(methylene)dicyclopentyl carboxylate)-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**6d**): Subjection of **4d** and **5** with the general triazole procedure afforded **6d** (24 mg, 45%) as an off-yellow film after silica gel flash chromatography (DCM/MeOH 94:6). ^1H NMR (400 MHz, CDCl_3) δ : 14.94 (s, 1H, COOH), 8.70 (s, 1H), 7.95 (d, 1H, $J = 13.06$ Hz), 7.89 (s, 1H, N-H), 7.62 (s, 1H), 7.28 (d, 1H, $J = 7.09$ Hz), 5.72 (s, 4H), 4.37 (dd, 2H, $J = 6.76$ Hz), 3.71 (s, 2H), 3.53 (s, 4H), 3.46 (m, 1H), 3.33 (s, 2H), 3.29 (dd, 4H, $J = 5.25$ Hz), 3.26 (dd, 2H, $J = 6.34$ Hz), 2.70 (dd, 4H, $J = 7.08$ Hz), 2.11 (tt, 2H, $J = 6.51$ Hz), 1.88 – 1.51 (m, 16H), 1.31 (dt, $J = 13.60$, 6.86 Hz), 1.12 (dt, $J = 10.62$, 3.76 Hz). $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ : 177.2, 175.3, 170.8, 170.0, 167.1, 153.7 (d, $J = 252.0$ Hz), 147.4, 145.9 (d, $J = 11.33$ Hz), 143.7, 123.3, 112.6 (d, $J = 26.95$ Hz), 108.2, 104.8, 79.6, 59.2,

56.0, 53.0, 52.4, 49.7, 47.6, 43.4, 36.1, 35.2, 30.4, 29.8, 26.8, 25.8, 8.2. ^{19}F NMR (376 MHz, CDCl_3) δ : -120.8.

(((4-((1-Propylamido-(2-oxoethyl)amino)azanediyl)bis(oxy))bis(methylene) dipivate)-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**6e**): Subjection of **4e** and **5** with the general triazole procedure afforded **6e** (54 mg, 27%) as an off-yellow film after silica gel flash chromatography (DCM/MeOH 93:7). ^1H NMR (400 MHz, CDCl_3) δ : 14.92 (s, 1H, COOH), 8.71 (s, 1H), 7.96 (d, 1H, $J = 13.06$ Hz), 7.88 (s, 1H, N-H), 7.62 (s, 1H), 7.28 (d, 1H, $J = 7.09$ Hz), 5.72 (s, 4H), 4.37 (dd, 2H, $J = 6.77$ Hz), 3.99 (s, 4H), 3.60 (s, 2H), 3.32 (s, 2H), 3.30 (dd, 4H, $J = 5.31$ Hz), 3.26 (dd, 2H, $J = 6.39$ Hz), 2.70 (dd, 4H, $J = 4.08$ Hz), 1.77 (tt, 2H, $J = 6.60$ Hz), 1.31 (dt, 4H, $J = 6.79$ Hz), 1.14 (m, 20H). $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ : 177.1, 174.5, 170.7, 170.1, 169.3, 153.1 (d, $J = 148.1$ Hz), 147.4, 145.9 (d, $J = 11.31$ Hz), 144.0, 123.1 (d, $J = 47.85$ Hz), 112.4, 108.2, 104.8, 79.9, 55.9, 53.1, 52.4 (d, $J = 3.9$ Hz), 49.7 (d, $J = 5.34$ Hz), 49.3, 42.6, 38.8, 37.6, 35.2, 29.7, 28.5, 26.8, 8.2. ^{19}F NMR (376 MHz, CDCl_3) δ : -120.7.

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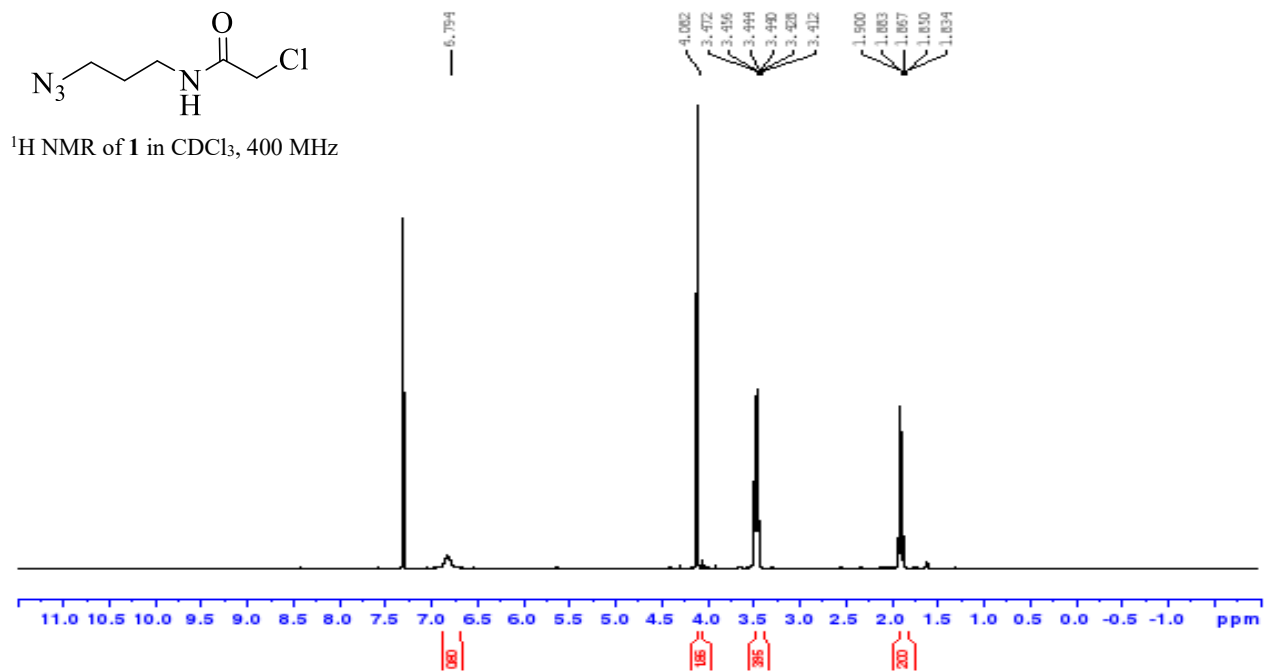
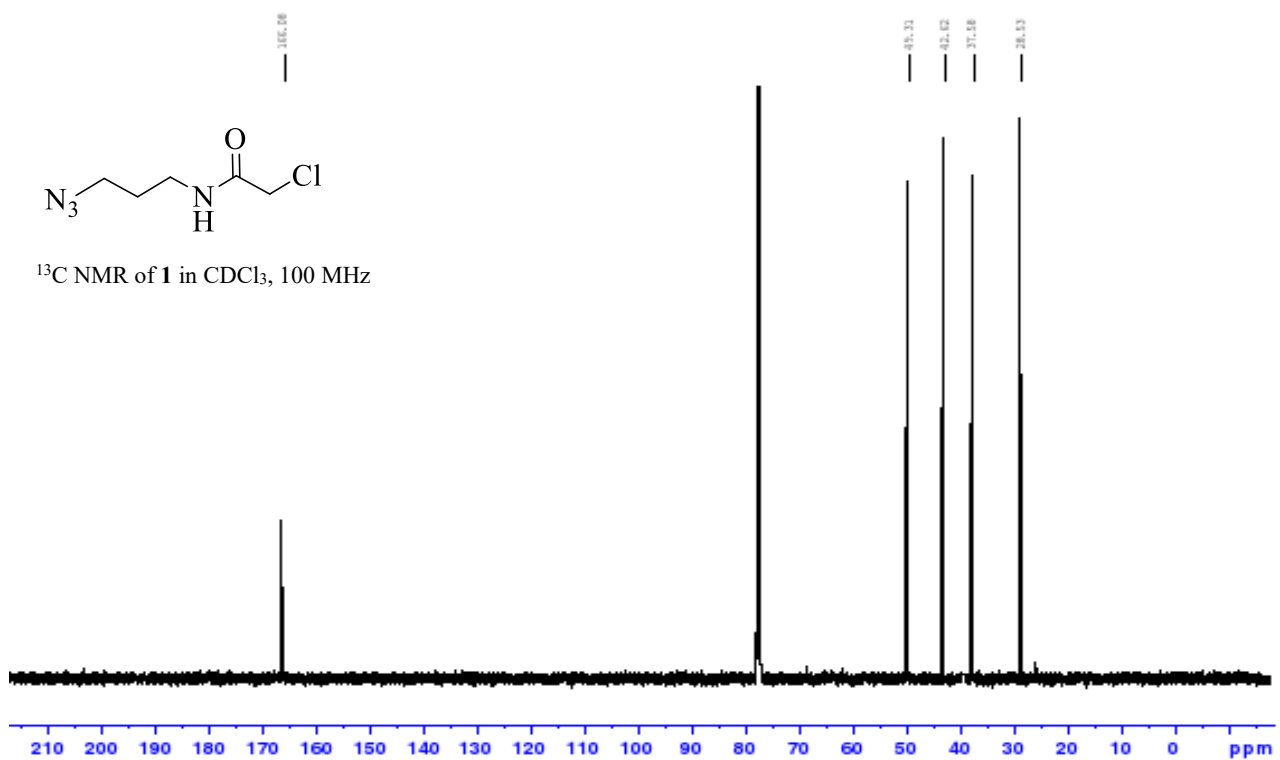
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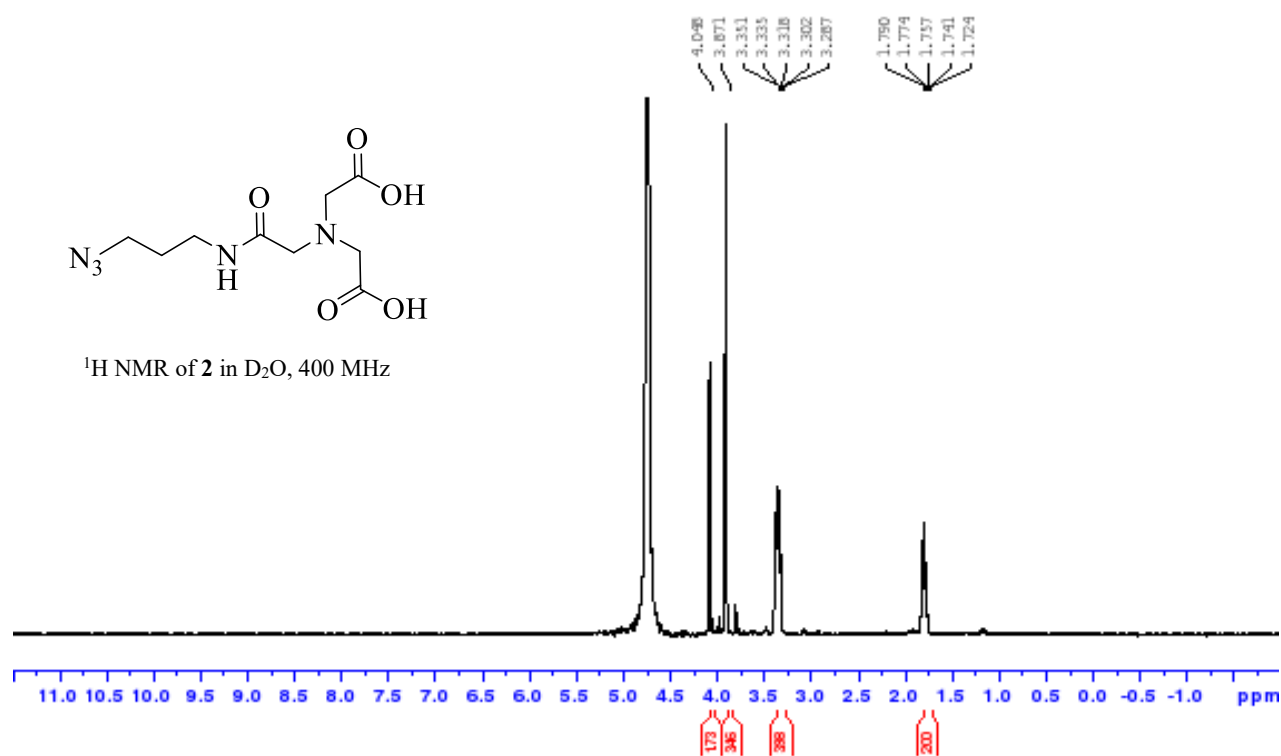
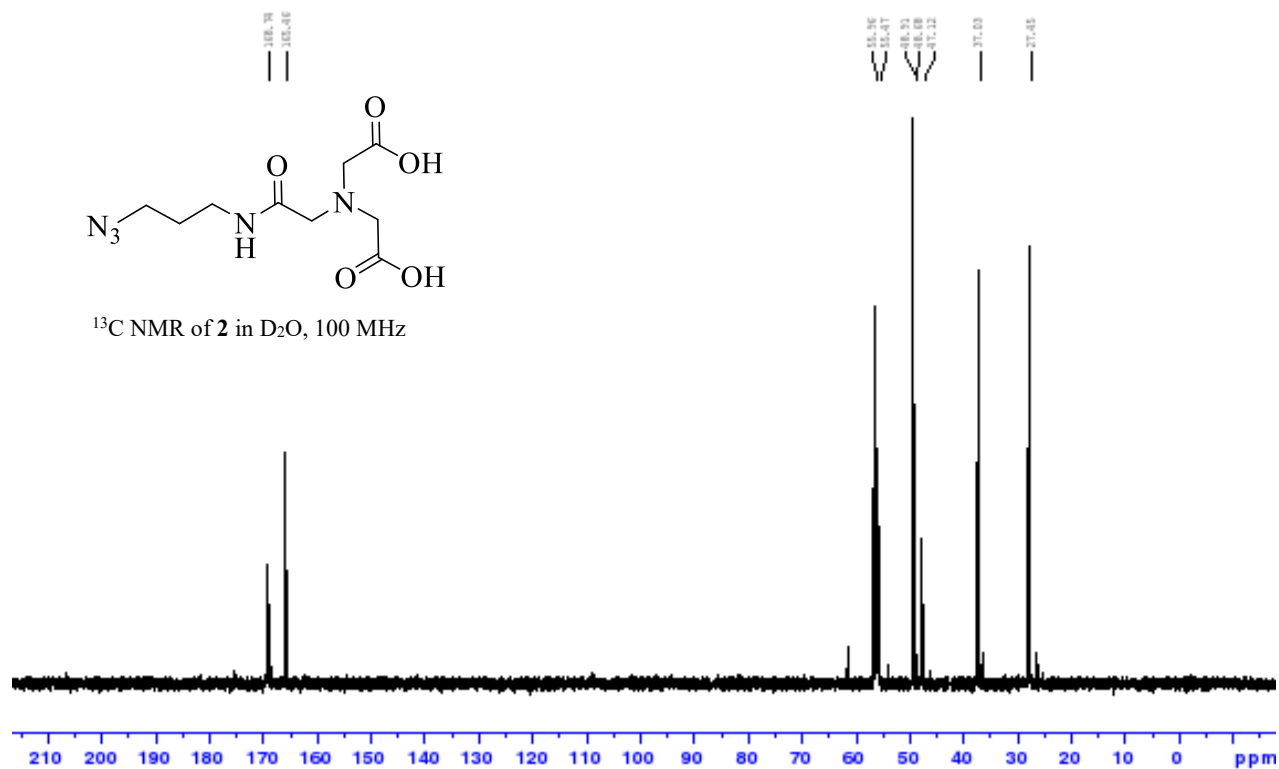
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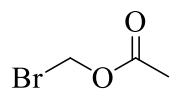
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APPENDIX

SUPPORTING INFORMATION FOR CHAPTER 2

Figure A.1. ^1H NMR of $\text{C}_5\text{H}_9\text{N}_4\text{OCl}$ (**1**).Figure A.2. ^{13}C NMR of $\text{C}_5\text{H}_9\text{N}_4\text{OCl}$ (**1**).

Figure A.3. ¹H NMR of C₉H₁₅N₅O₄ (**2**).Figure A.4. ¹³C NMR of C₉H₁₄N₅O₄ (**2**).



^1H NMR of **3a** in CDCl_3 , 400 MHz

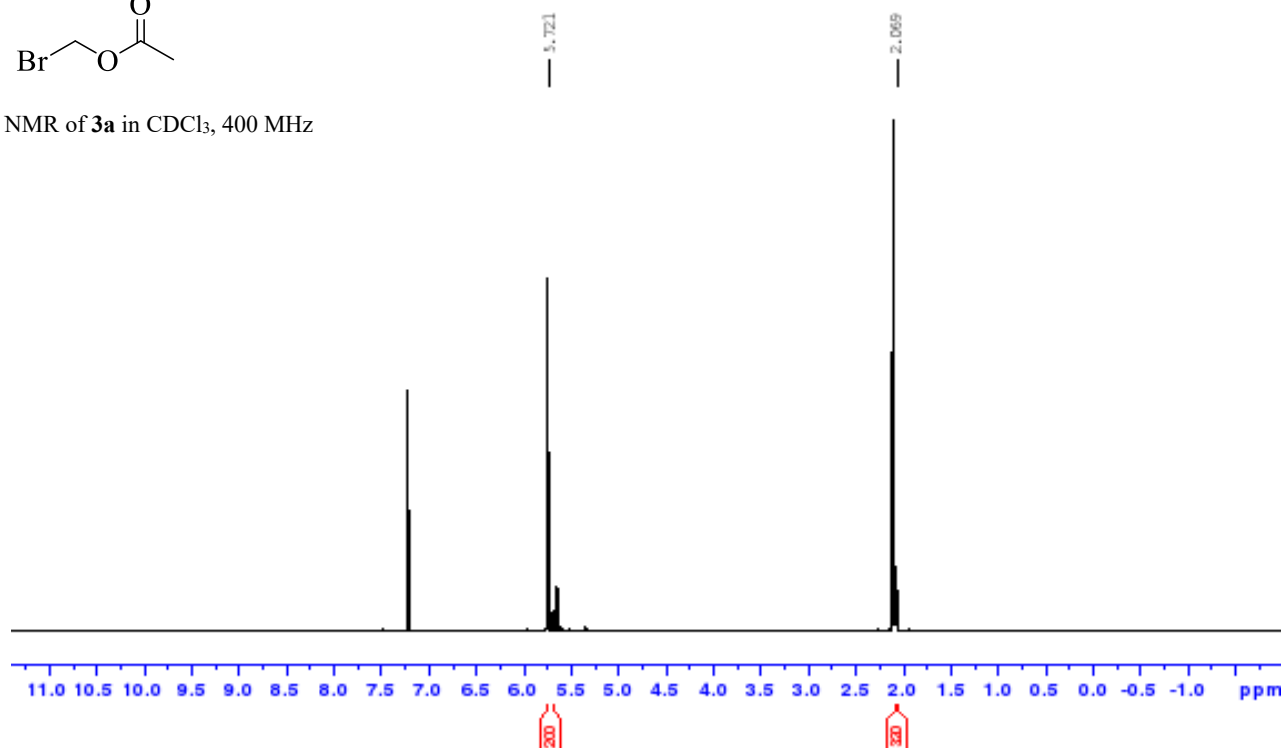
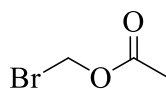


Figure A.5. ^1H NMR of $\text{C}_3\text{H}_5\text{O}_2\text{Br}$ (**3a**).



^{13}C NMR of **3a** in CDCl_3 , 100 MHz

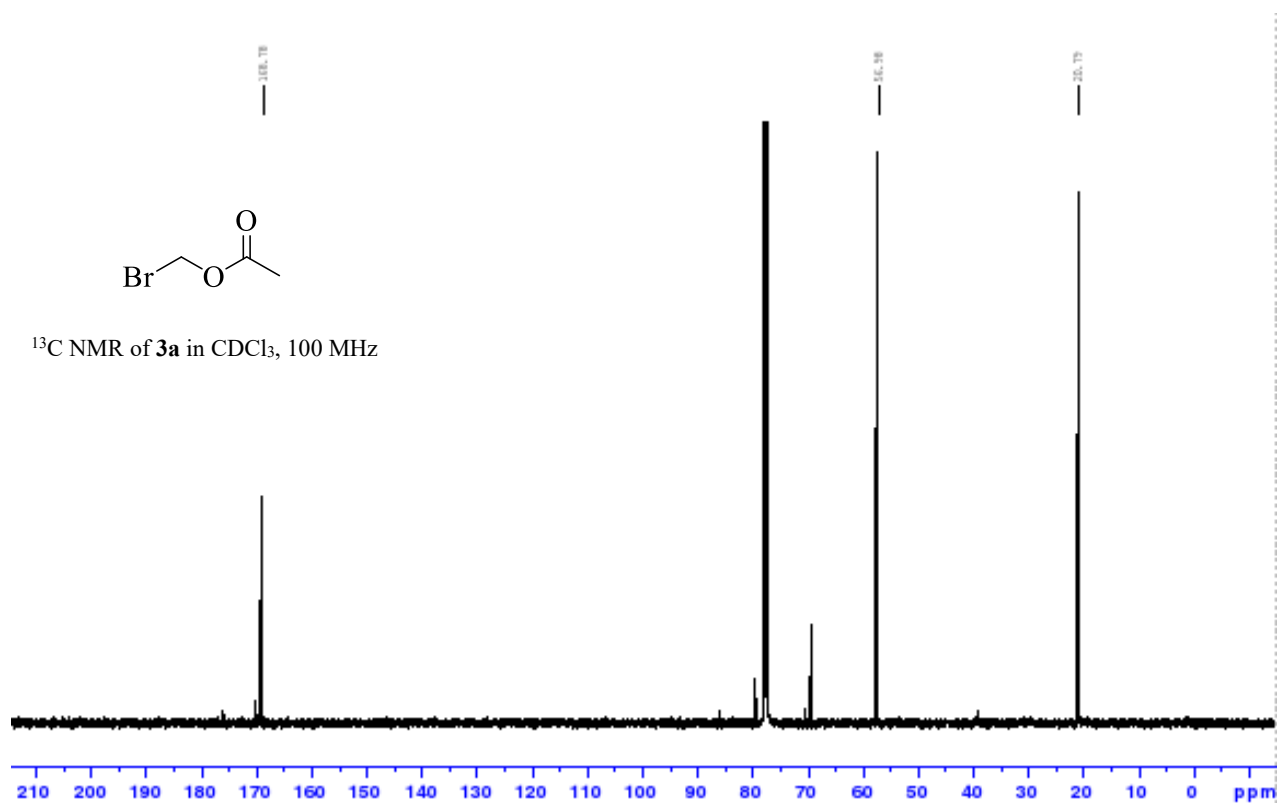
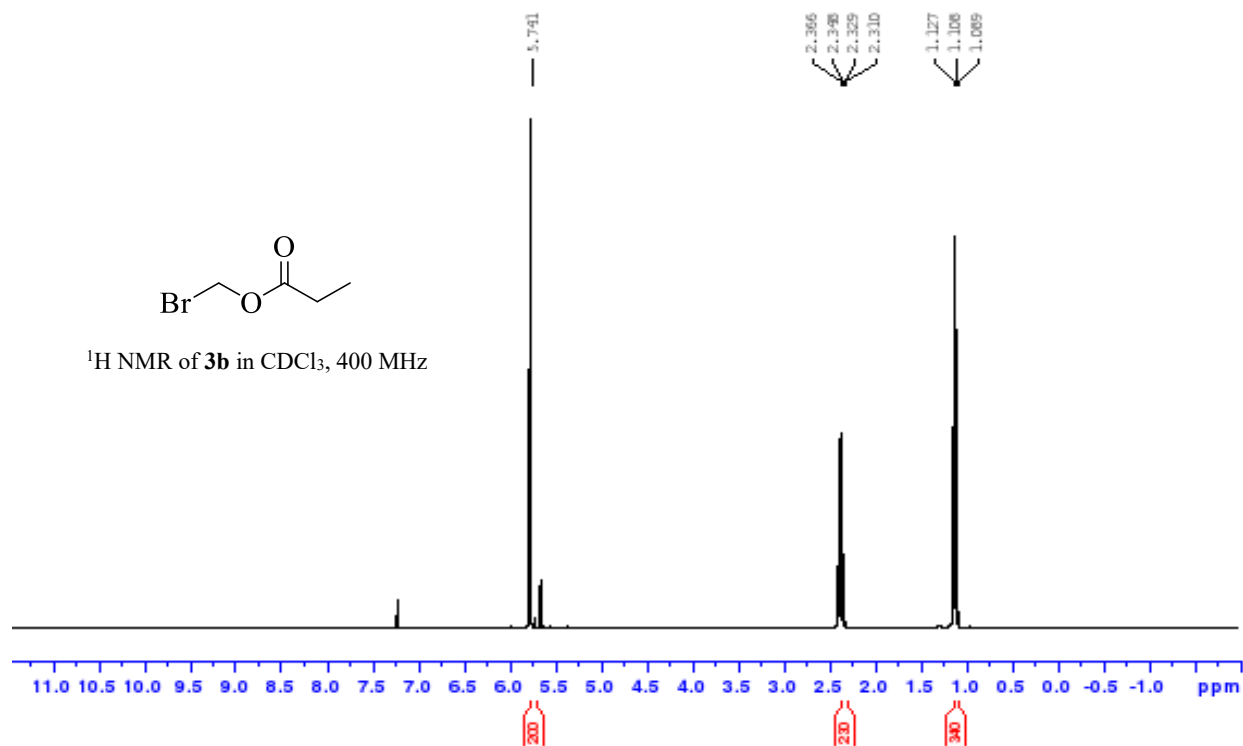
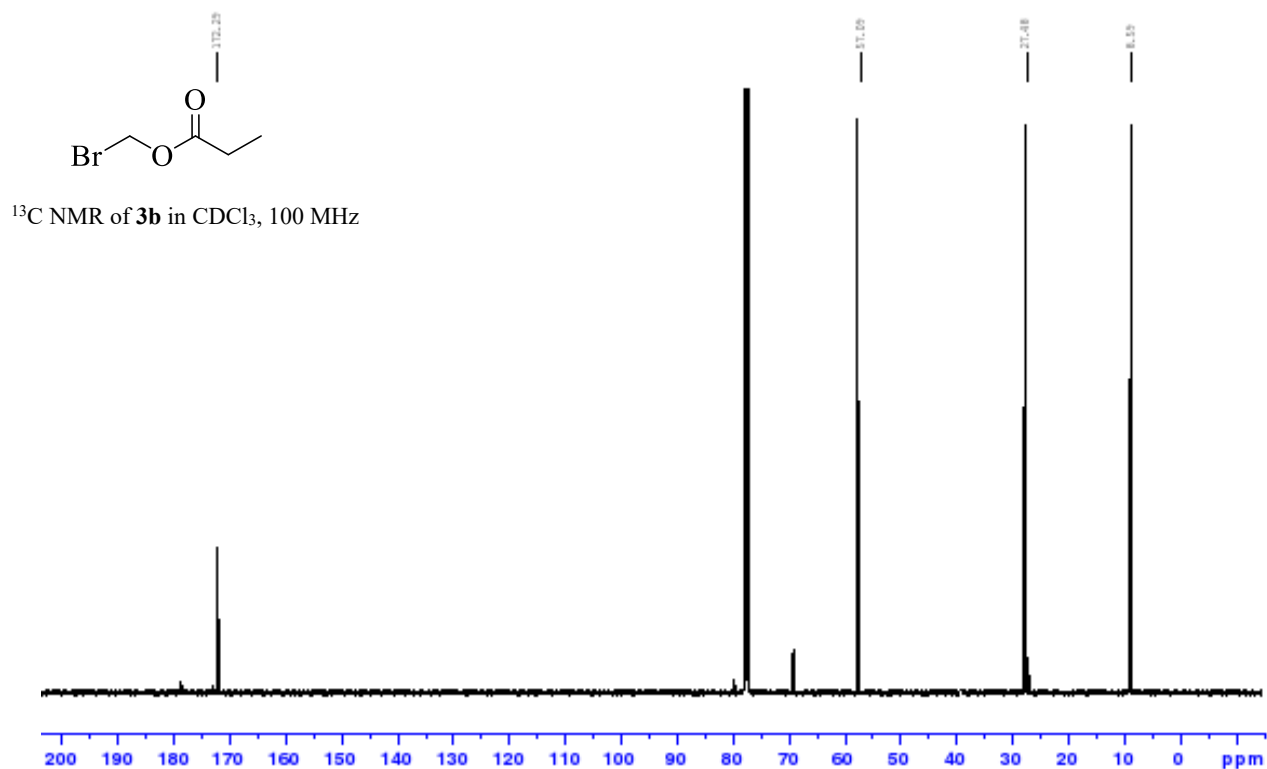
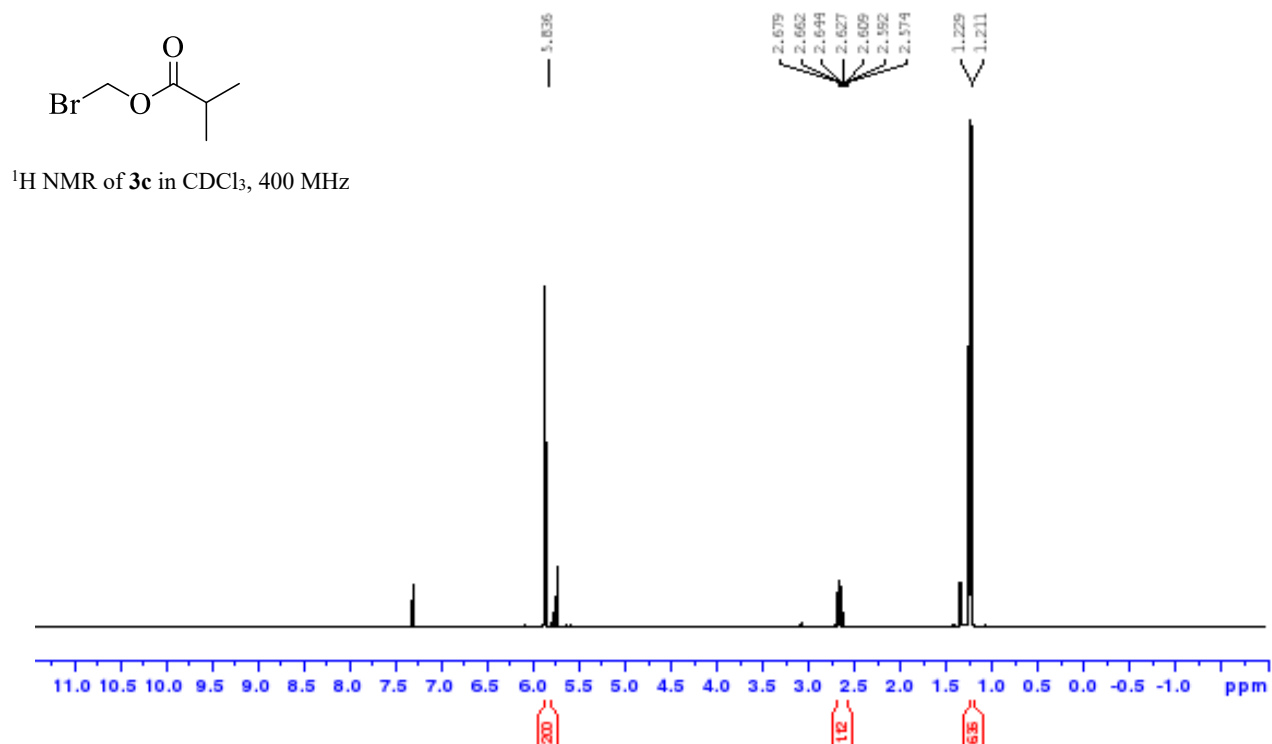
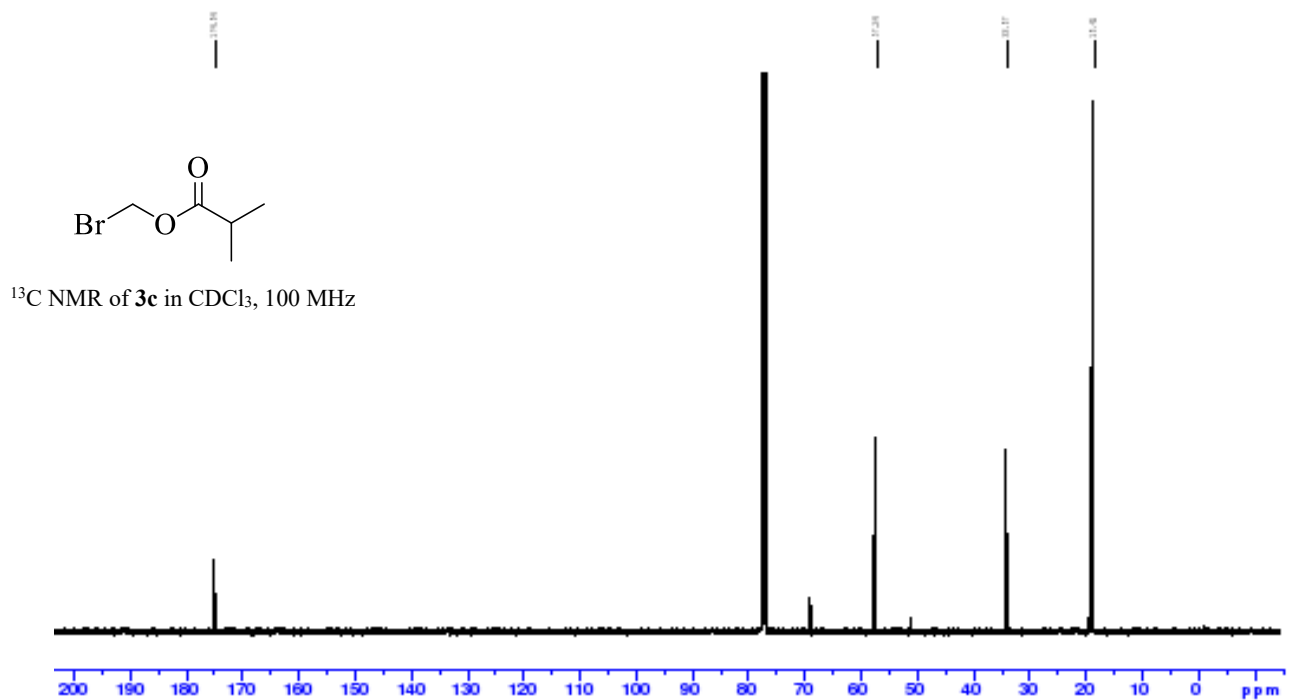
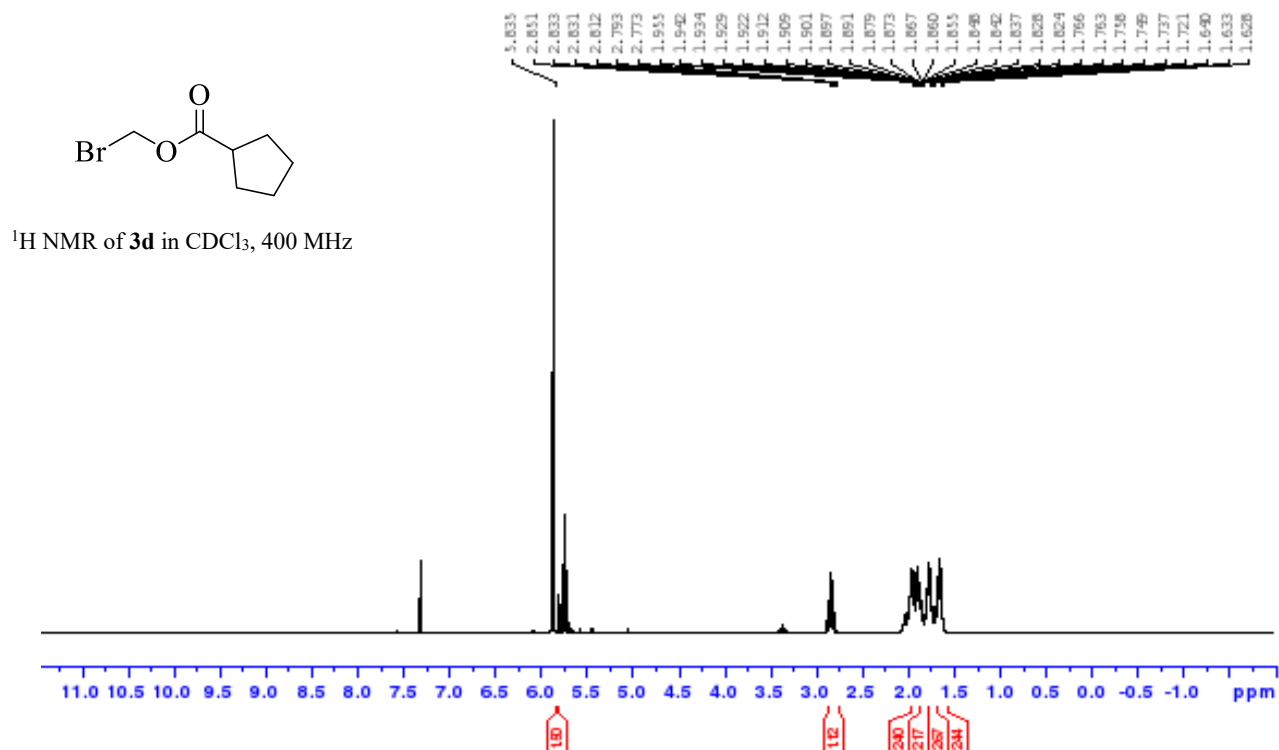
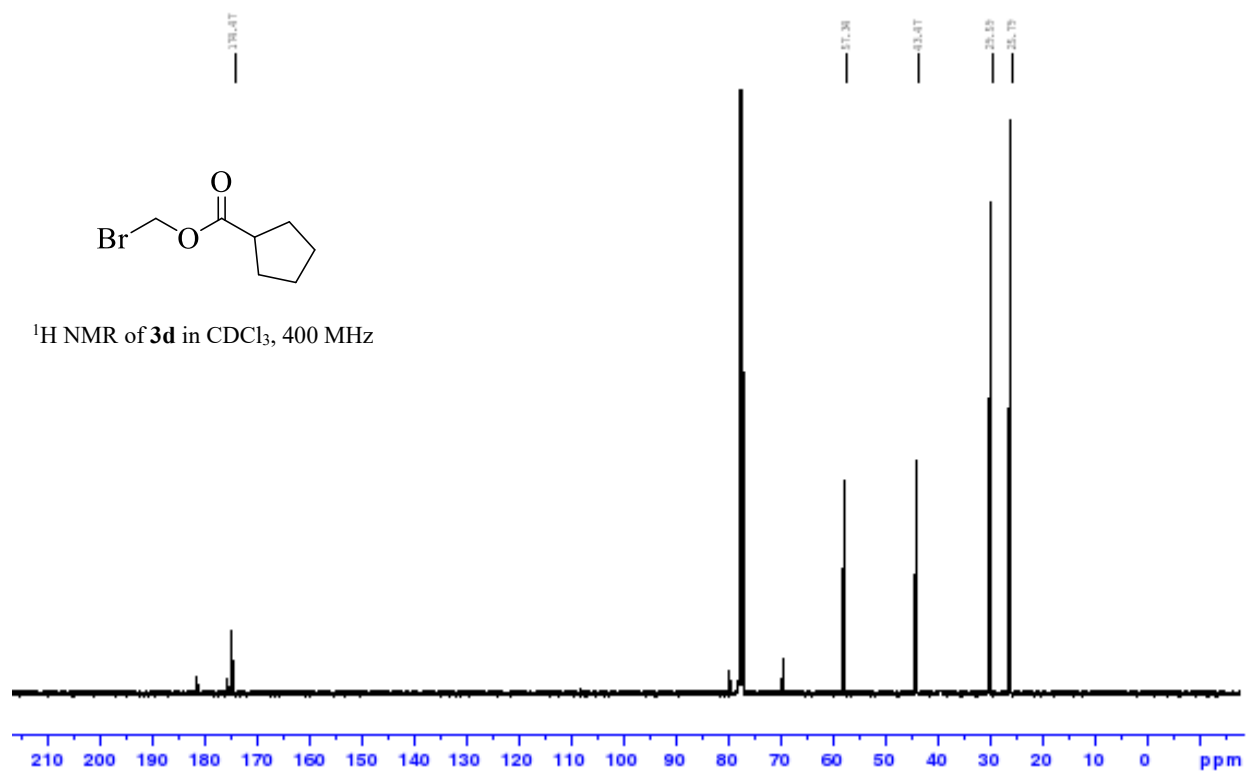
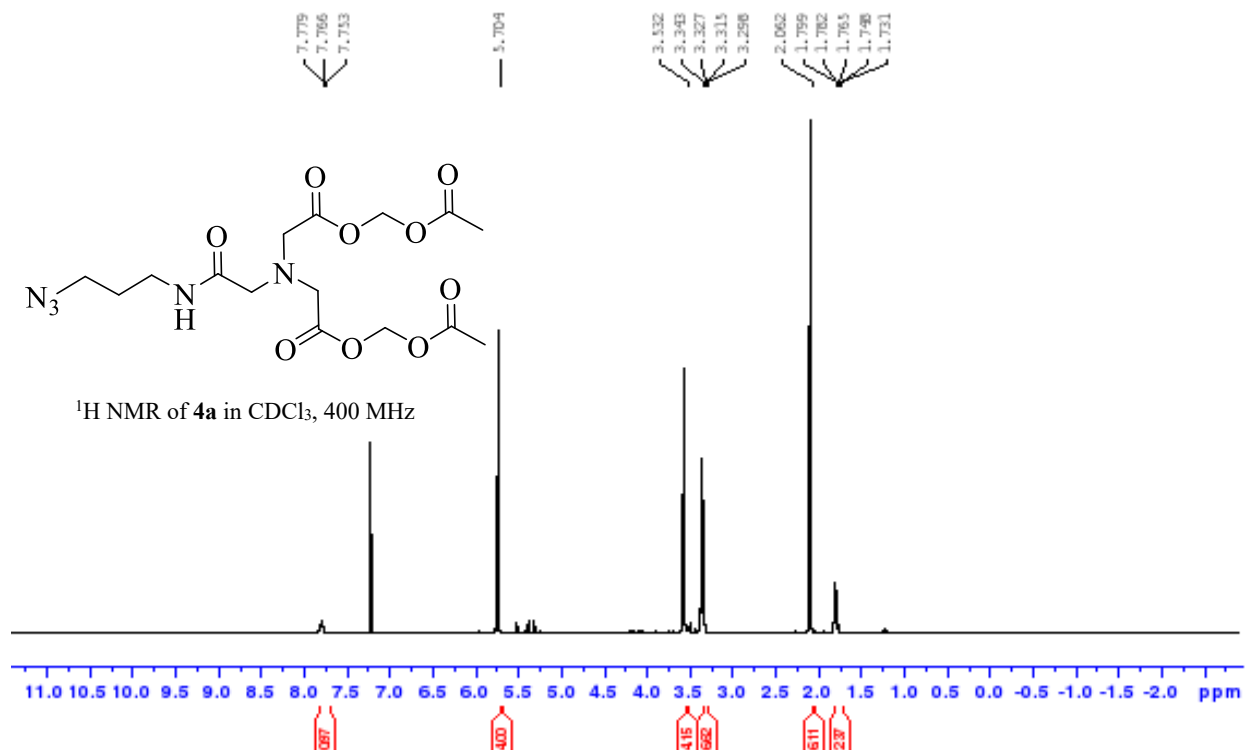
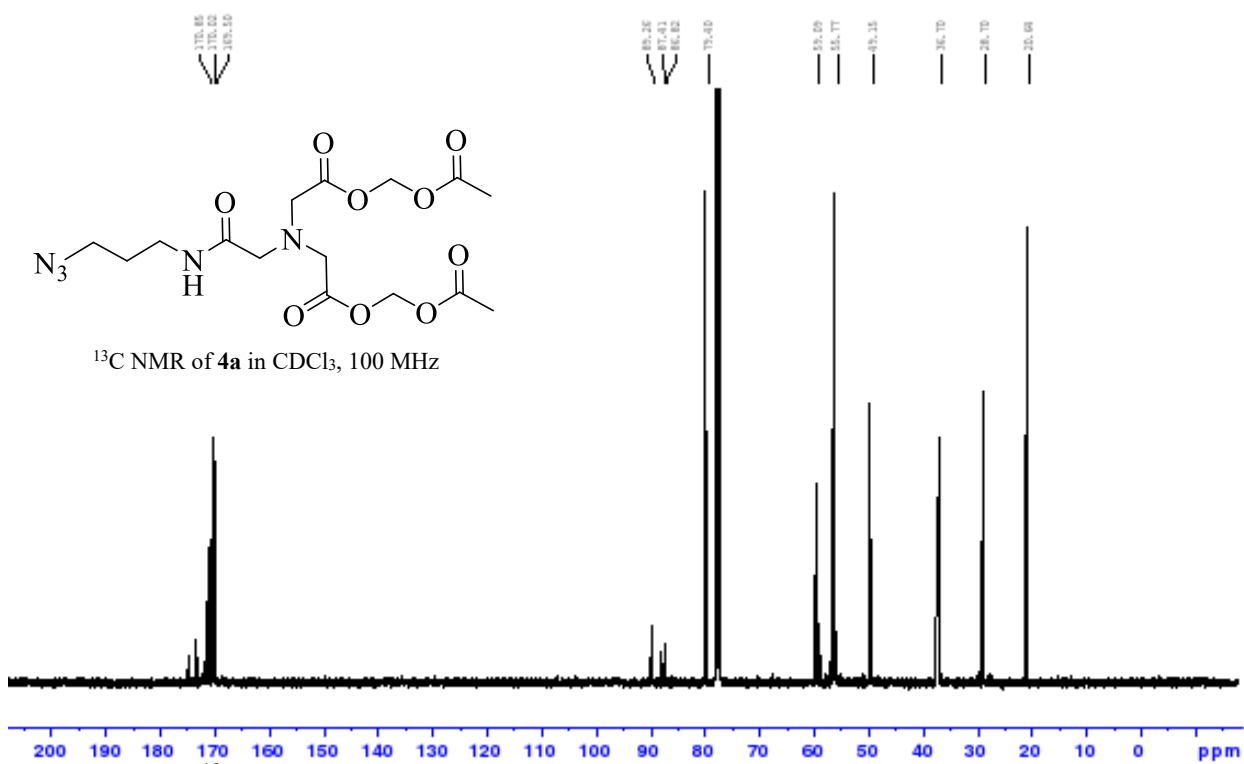


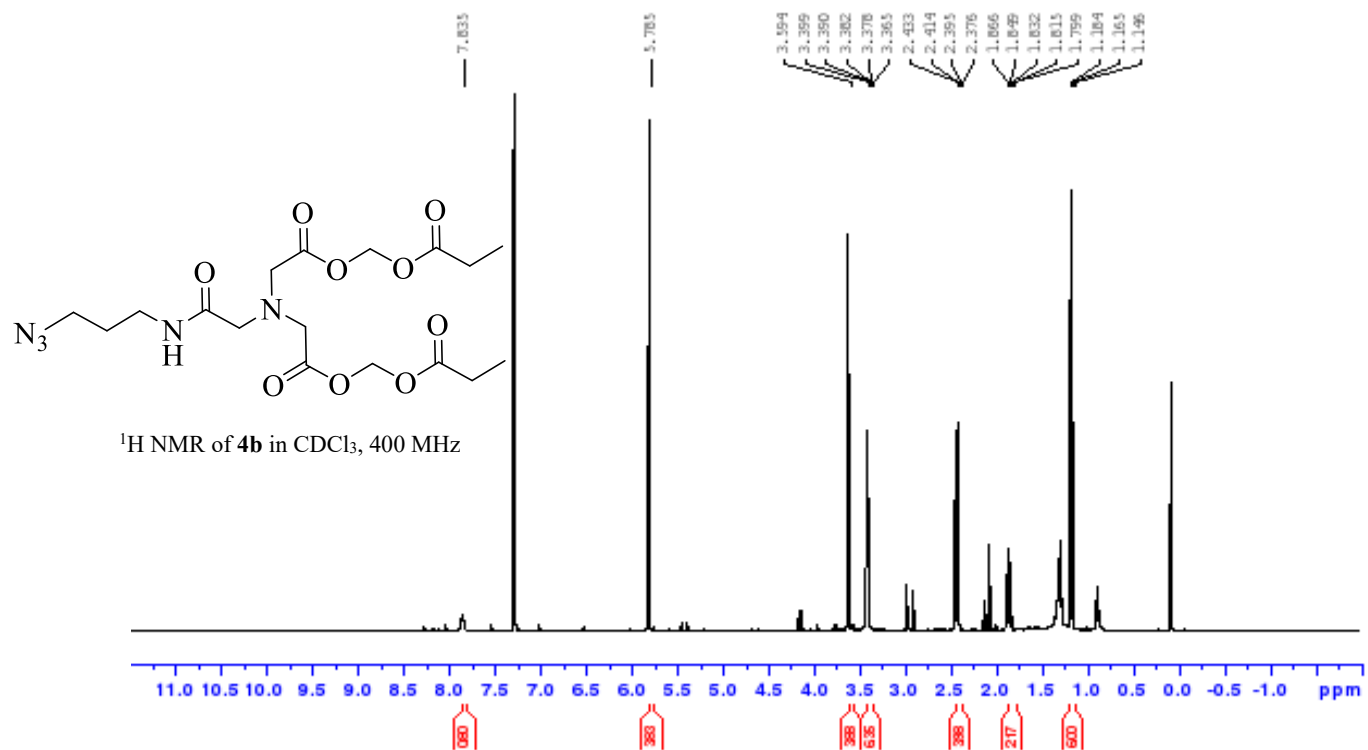
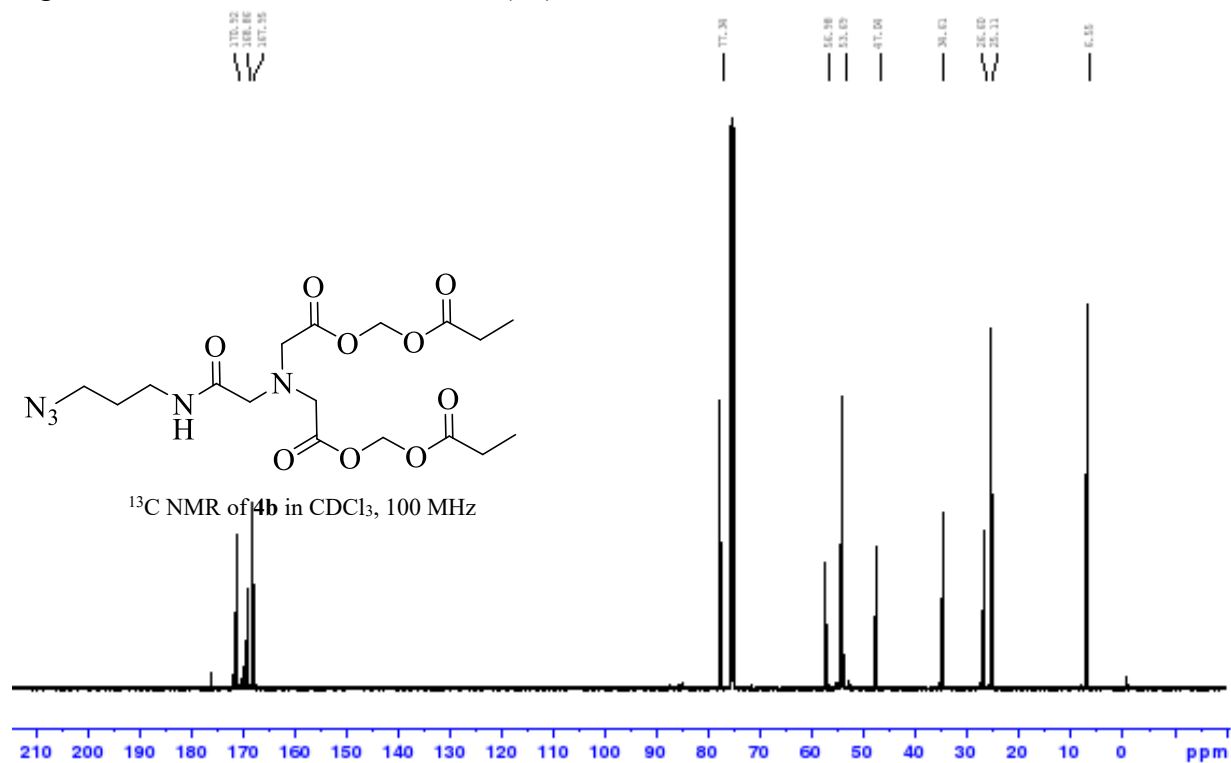
Figure A.6. ^{13}C of $\text{C}_3\text{H}_5\text{O}_2\text{Br}$ (**3a**).

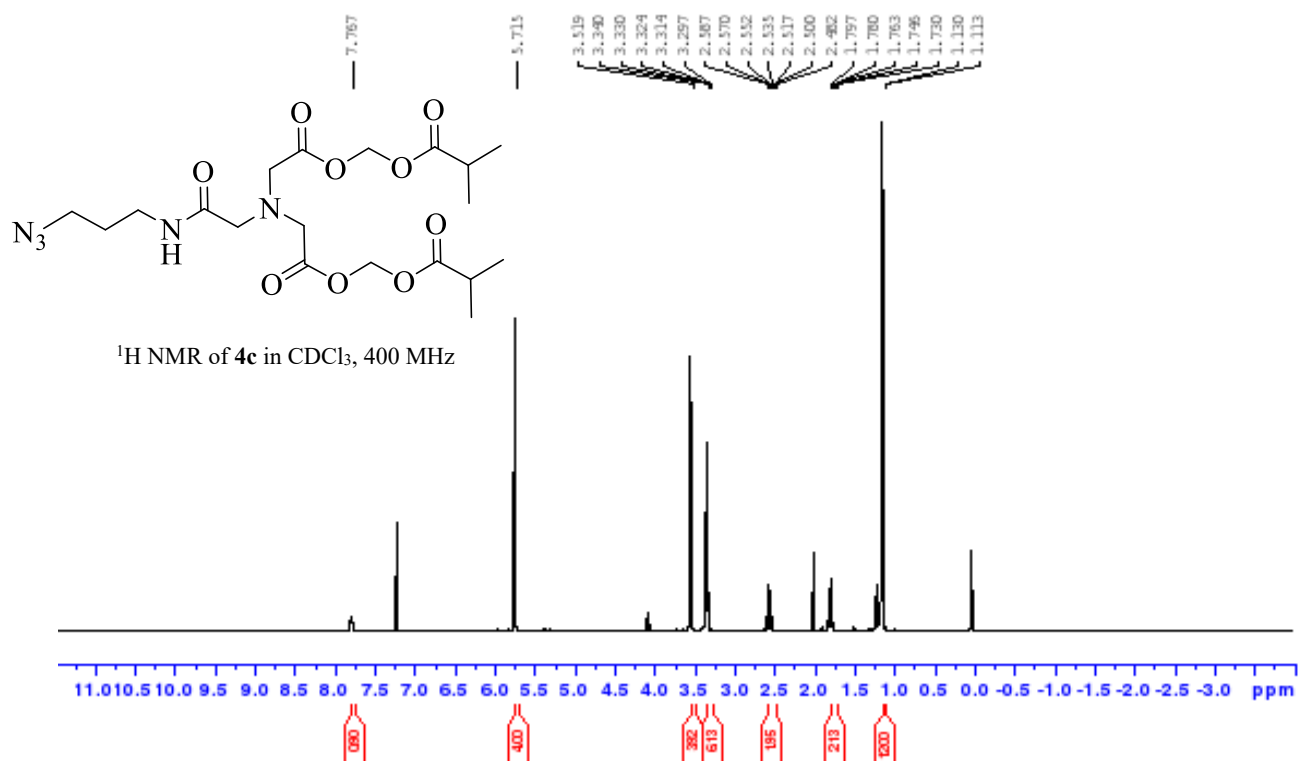
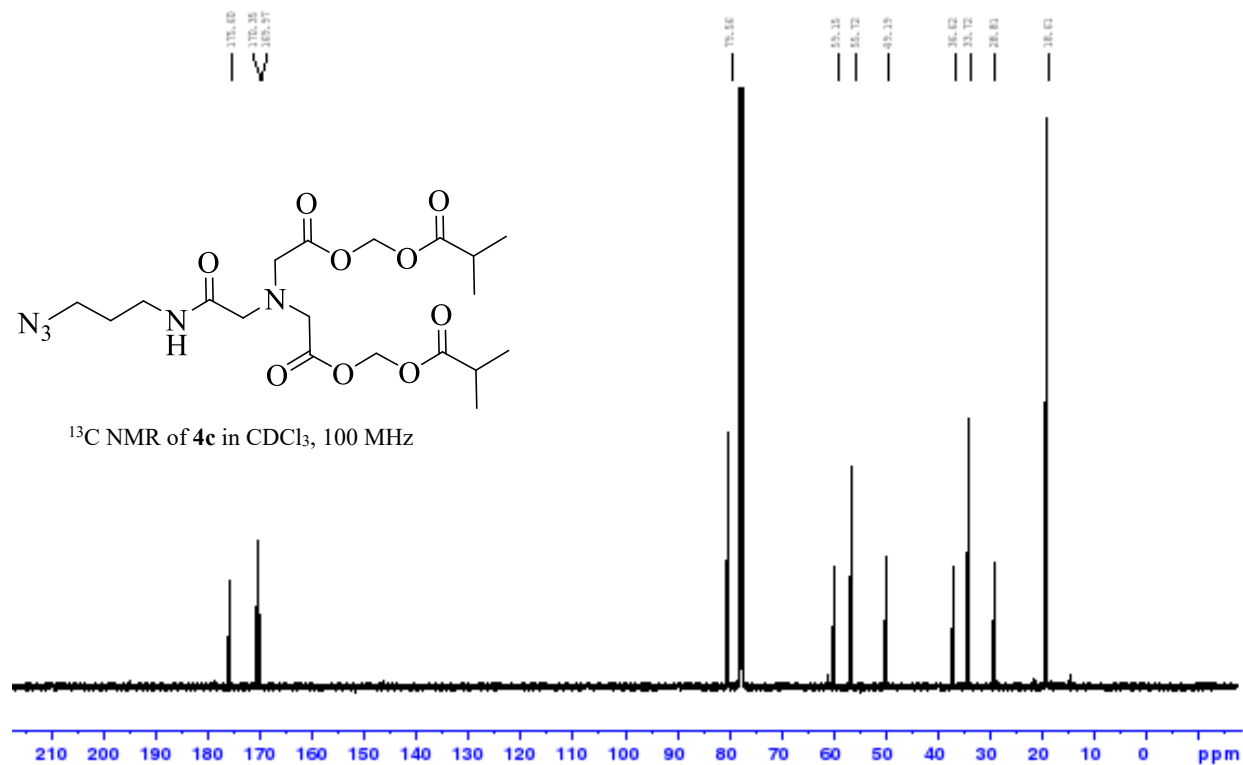
Figure A.7. ^1H NMR of $\text{C}_4\text{H}_7\text{O}_2\text{Br}$ (**3b**).Figure A.8. ^{13}C NMR of $\text{C}_4\text{H}_7\text{O}_2\text{Br}$ (**3b**).

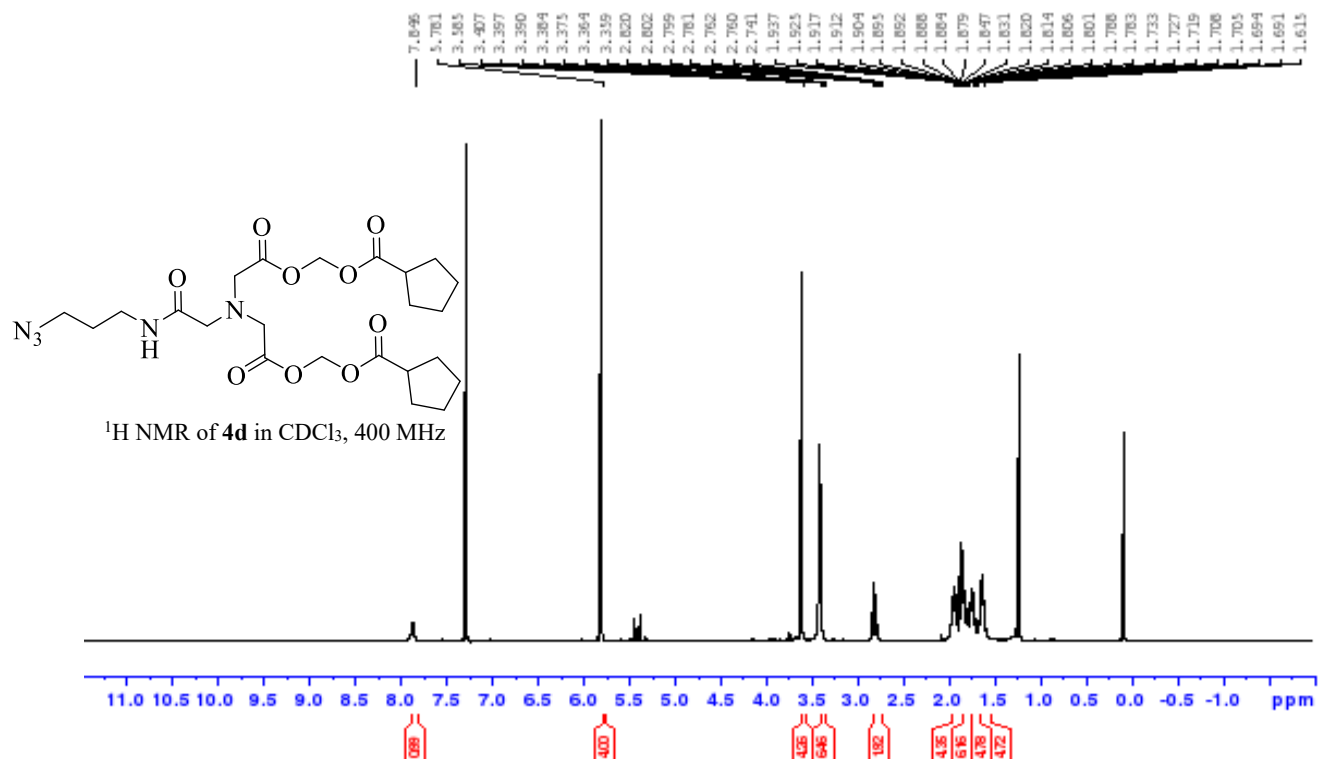
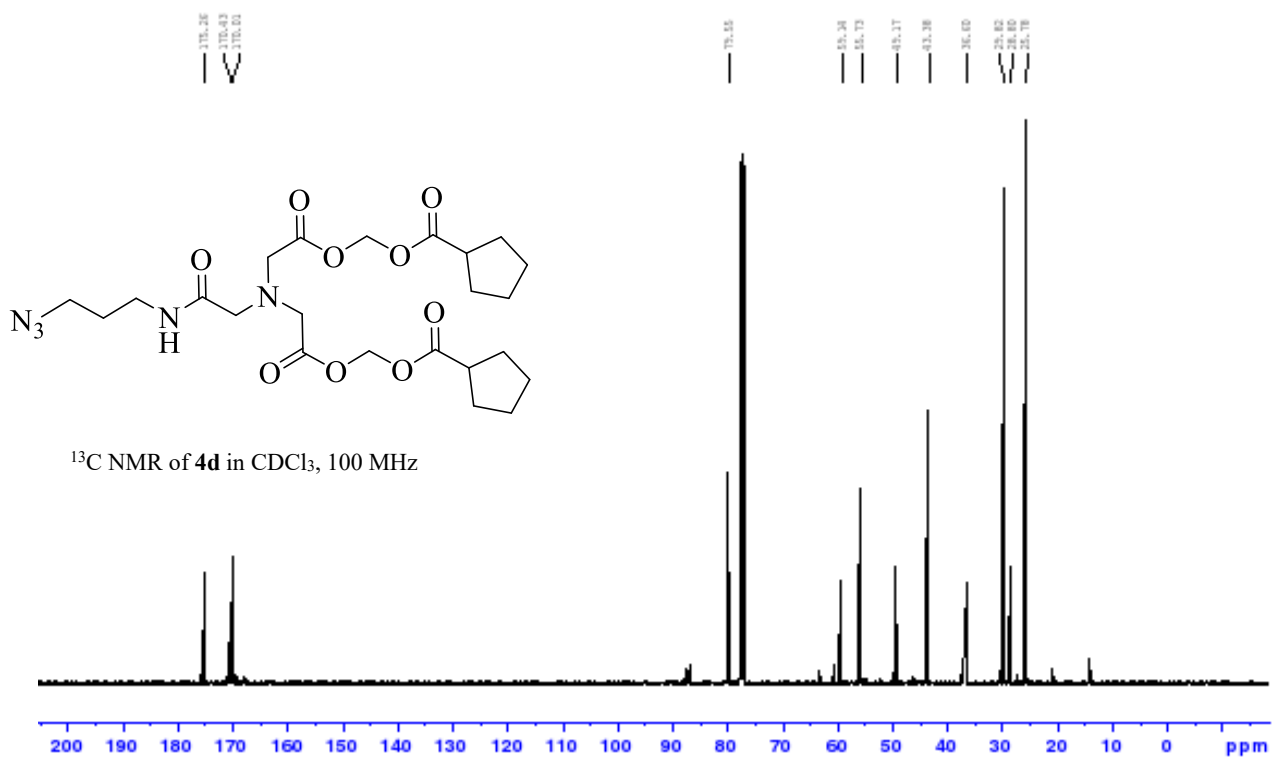
Figure A.9. ^1H NMR of $\text{C}_5\text{H}_{10}\text{O}_2\text{Br}$ (**3c**).Figure A.10. ^{13}C NMR of $\text{C}_5\text{H}_{10}\text{O}_2\text{Br}$ (**3c**).

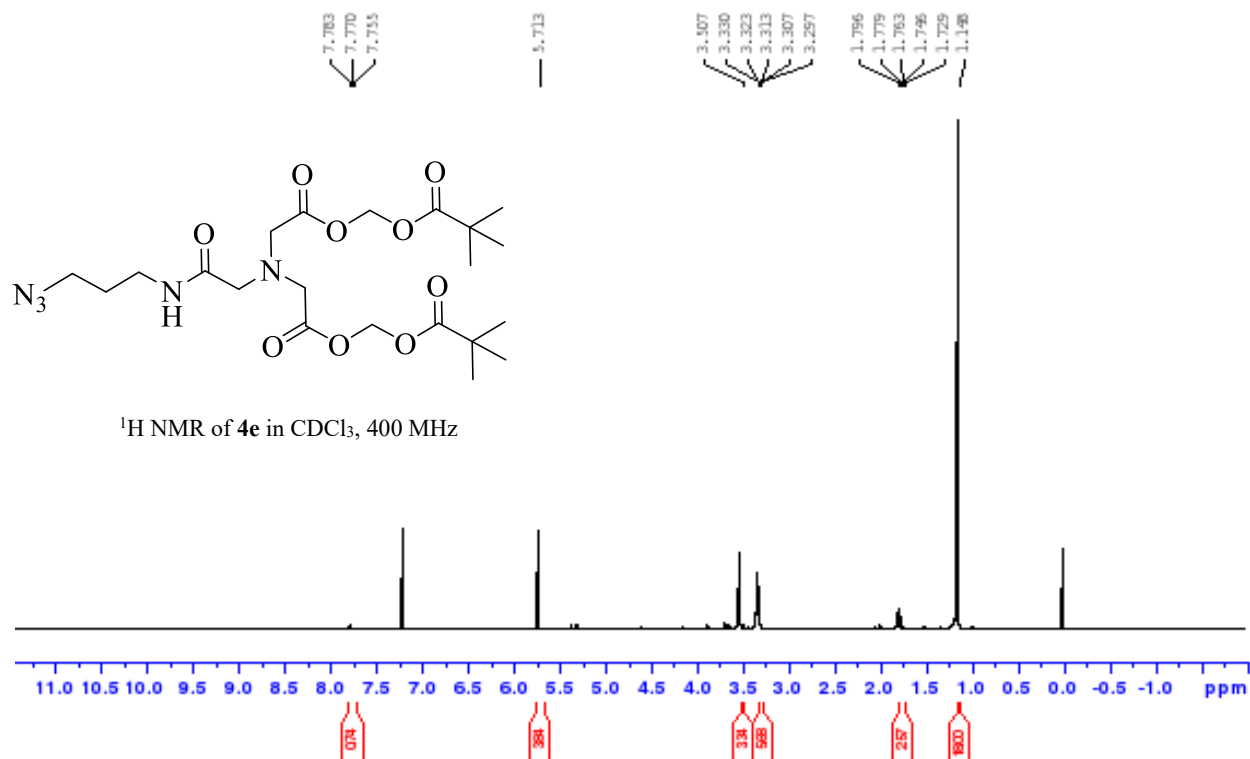
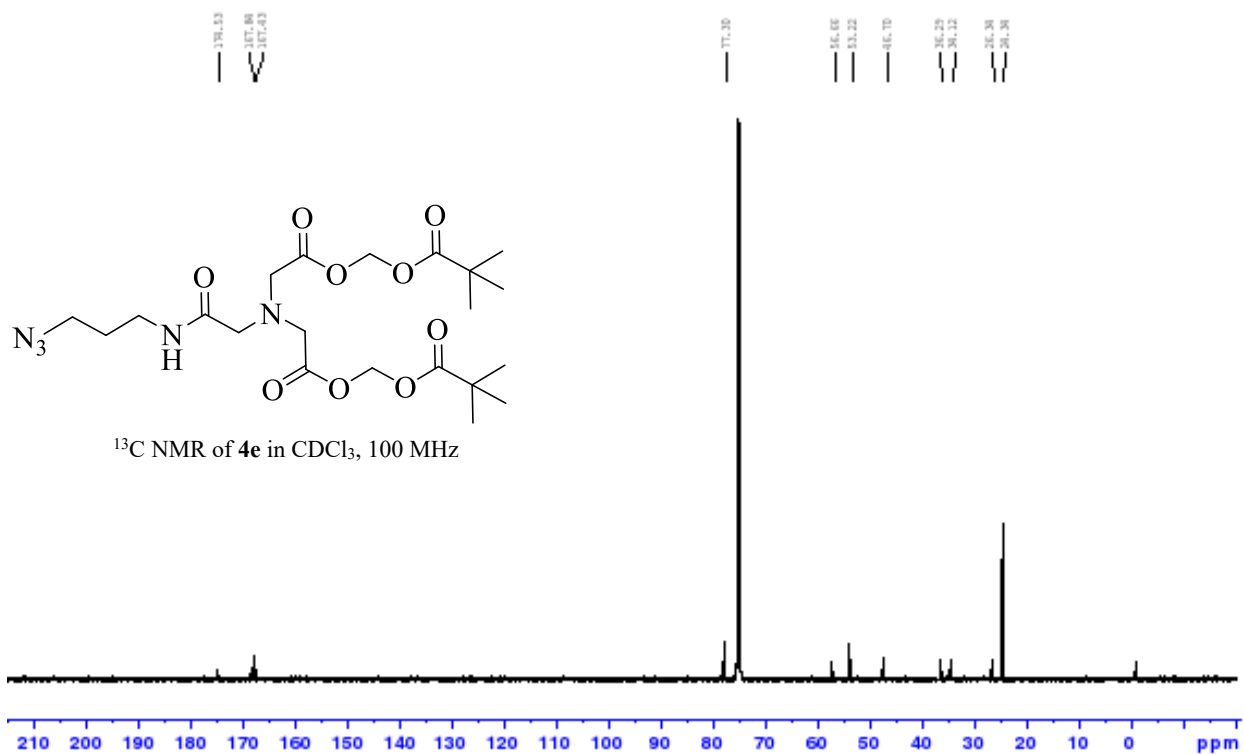
Figure A.11. ^1H NMR of $\text{C}_7\text{H}_{11}\text{O}_2\text{Br}$ (**3d**).Figure A.12. ^{13}C NMR of $\text{C}_7\text{H}_{11}\text{O}_2\text{Br}$ (**3d**).

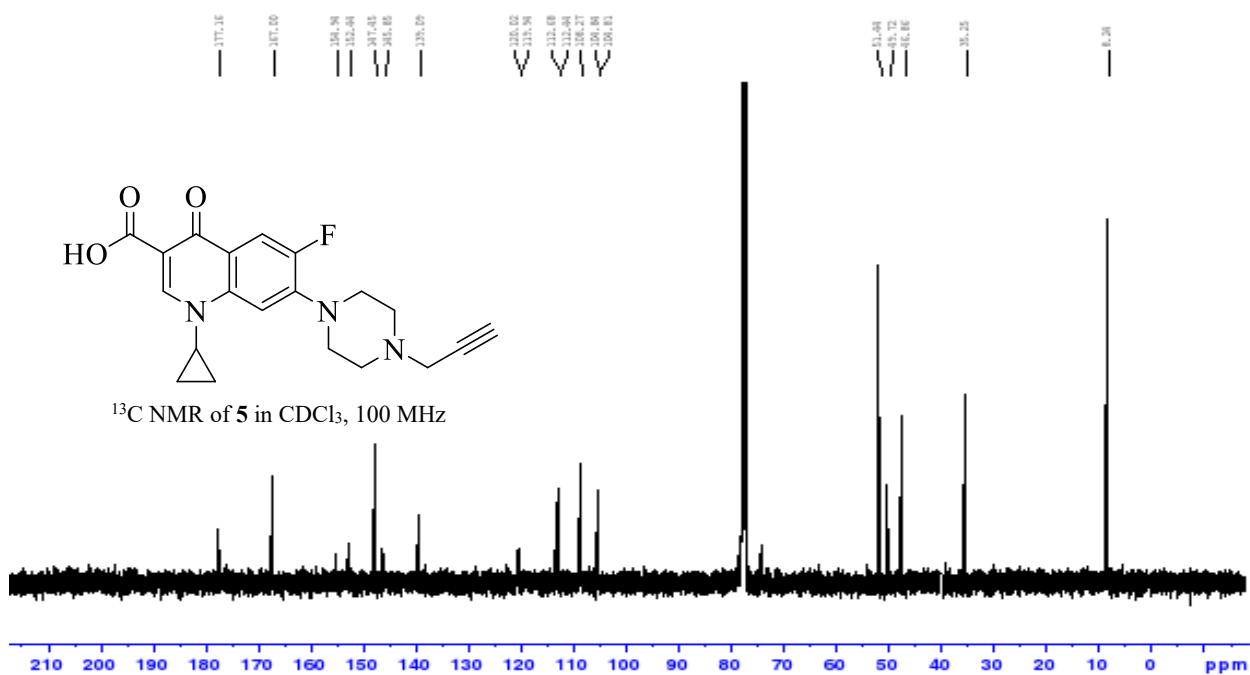
Figure A.13. ^1H NMR of $\text{C}_{15}\text{H}_{23}\text{N}_5\text{O}_9$ (**4a**).Figure A.14. ^{13}C NMR of $\text{C}_{15}\text{H}_{23}\text{N}_5\text{O}_9$ (**4a**).

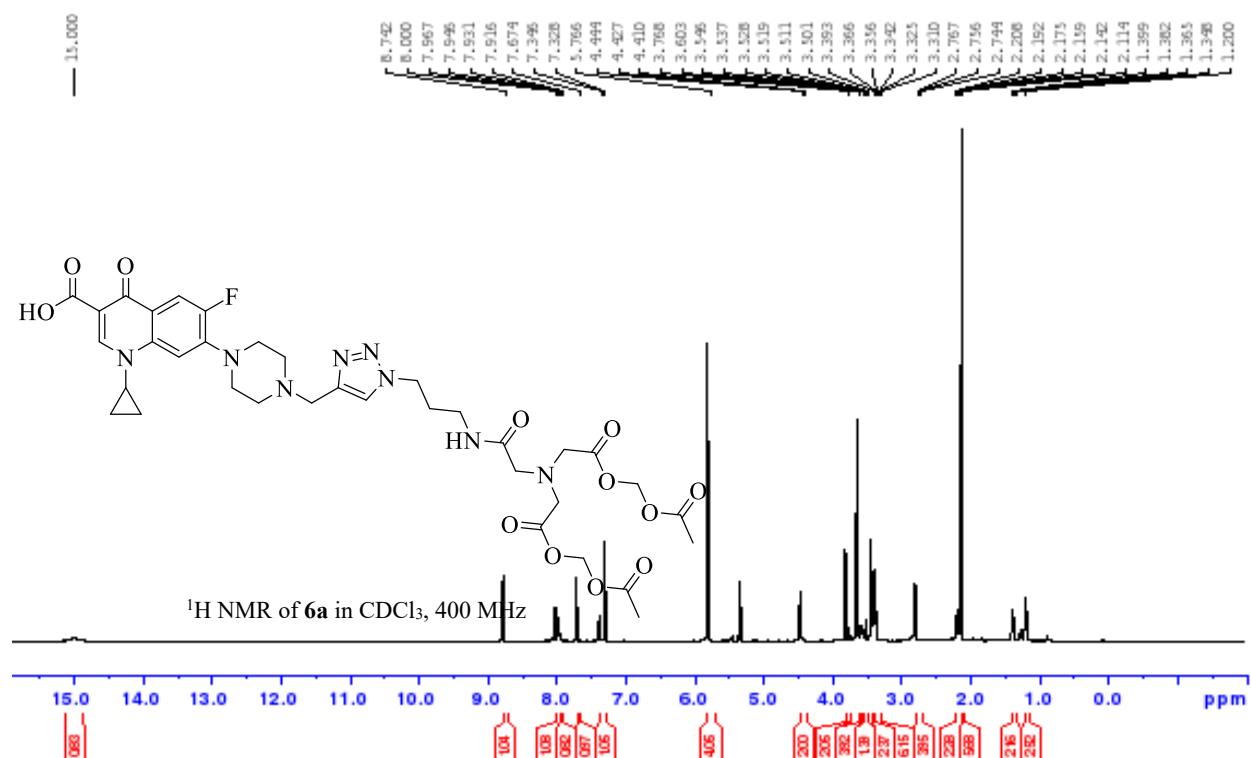
Figure A.15. ¹H NMR of C₁₇H₂₇N₅O₉ (**4b**).Figure A.16. ¹³C NMR of C₁₇H₂₇N₅O₉ (**4b**).

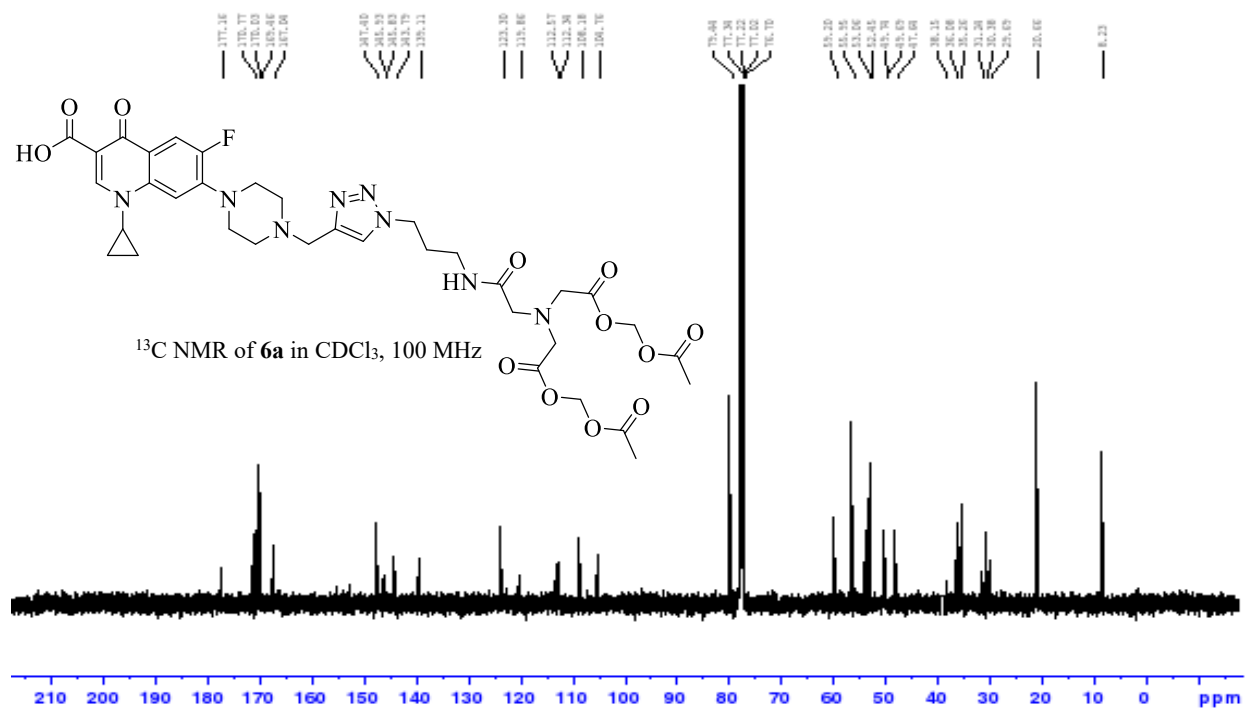
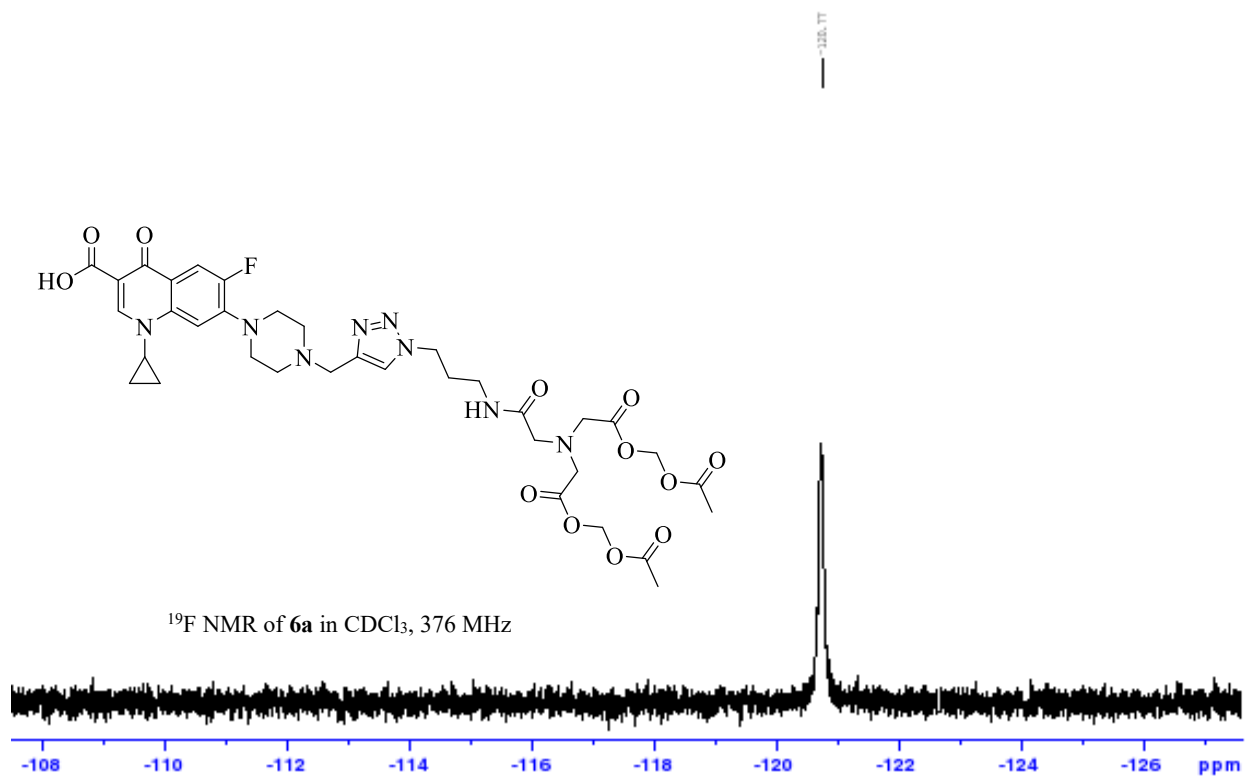
Figure A.17. ^1H NMR of $\text{C}_{19}\text{H}_{31}\text{N}_5\text{O}_9$ (**4c**).Figure A.18. ^{13}C NMR of $\text{C}_{19}\text{H}_{31}\text{N}_5\text{O}_9$ (**4c**).

Figure A.19. ¹H NMR of C₂₅H₃₃N₅O₉ (**4d**).Figure A.20. ¹³C NMR of C₂₅H₃₃N₅O₉ (**4d**).

Figure A.21. ^1H NMR of $\text{C}_{21}\text{H}_{33}\text{N}_5\text{O}_9$ (**4e**).Figure A.22. ^{13}C NMR of $\text{C}_{21}\text{H}_{33}\text{N}_5\text{O}_9$ (**4e**).





Figure A.27. ¹³C NMR of C₃₅H₄₃N₈O₁₁F (**6a**).Figure A.28. ¹⁹F NMR of C₃₅H₄₃N₈O₁₁F (**6a**).

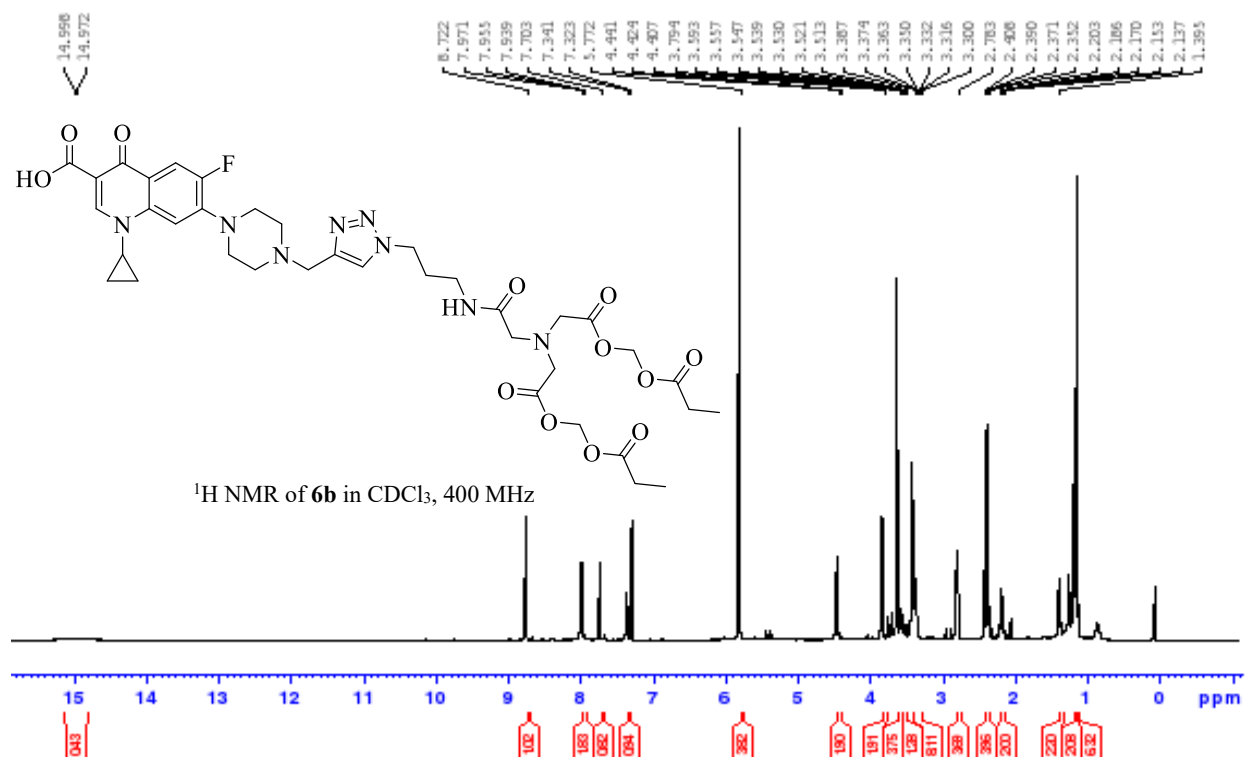


Figure A.29. ^1H NMR of $\text{C}_{37}\text{H}_{47}\text{N}_8\text{O}_{11}\text{F}$ (**6b**).

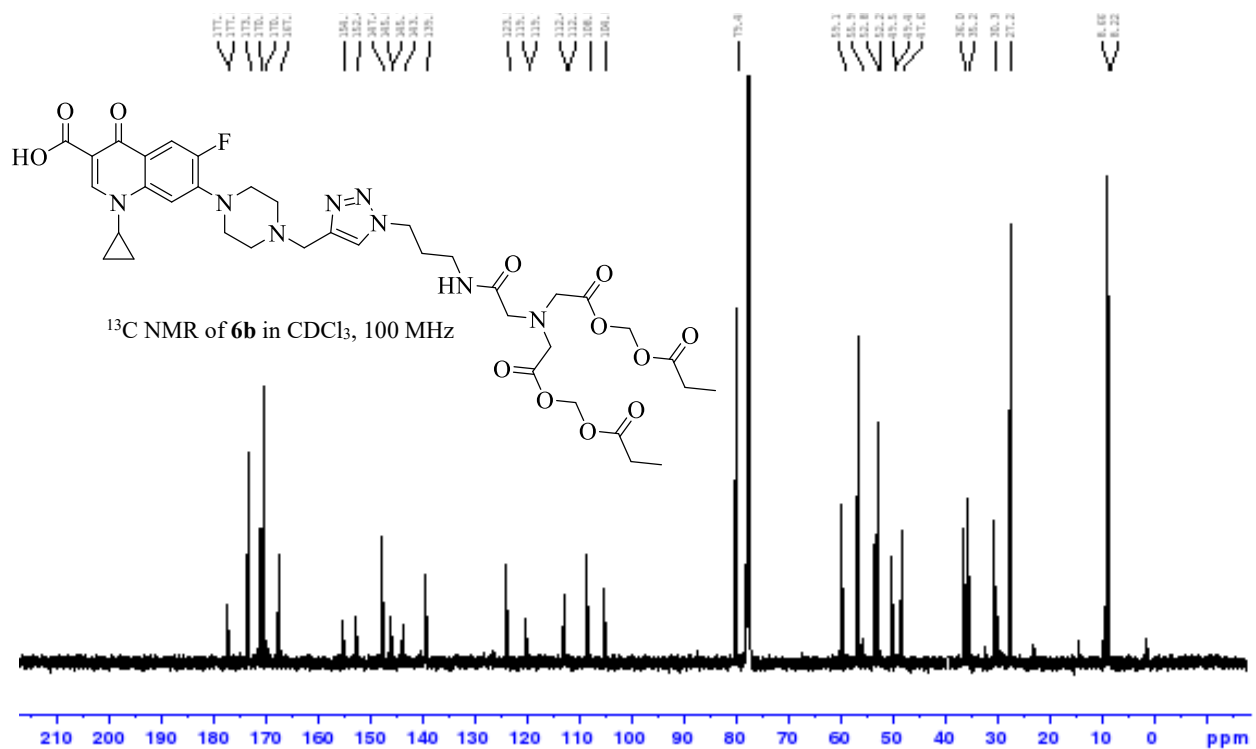
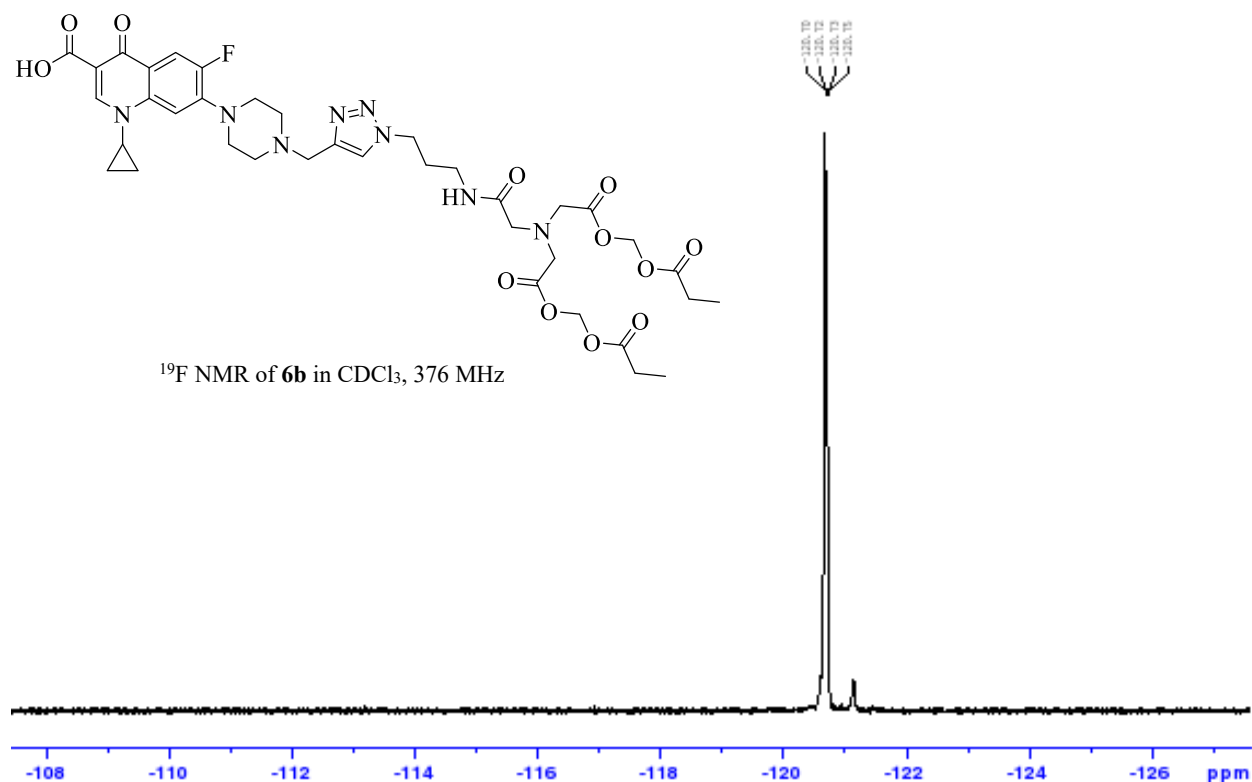
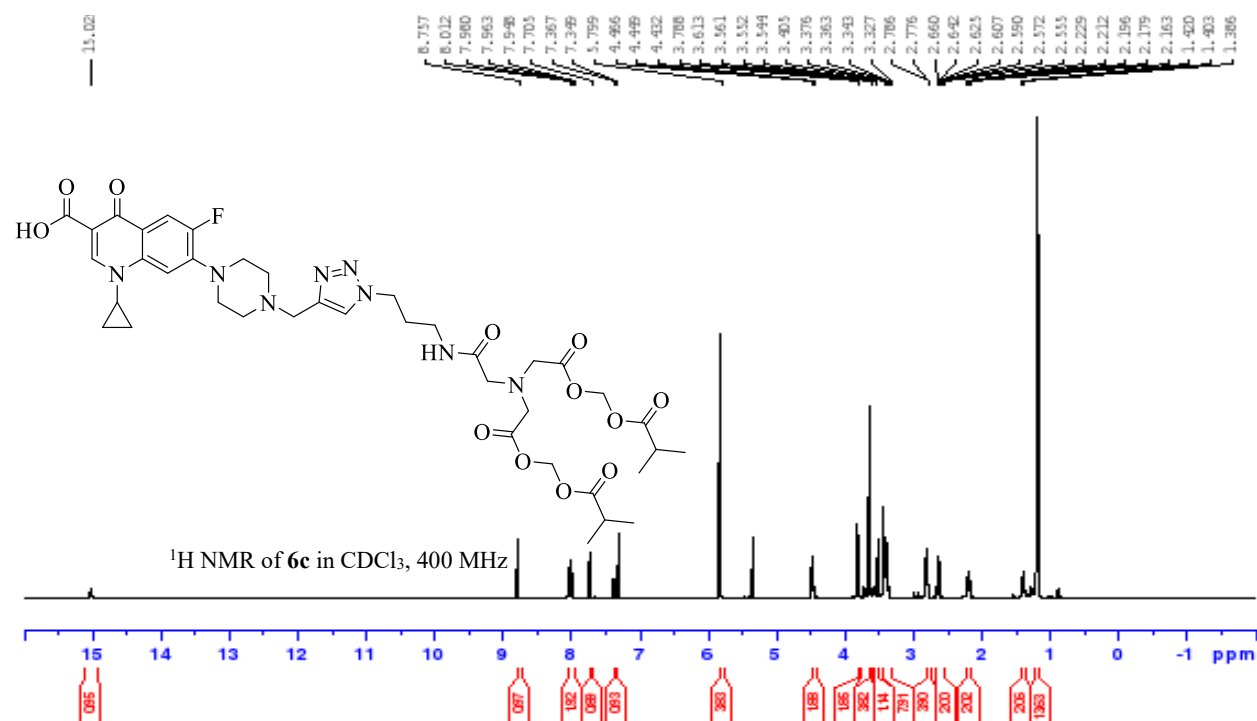
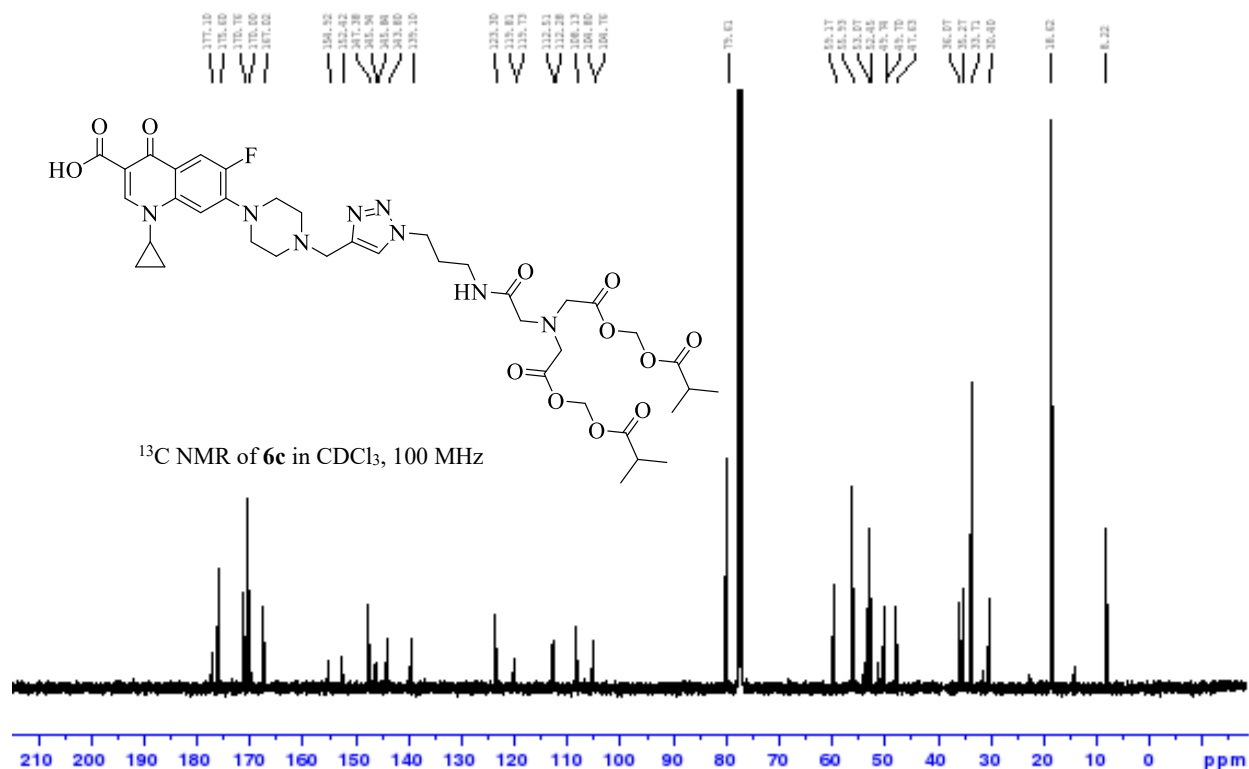
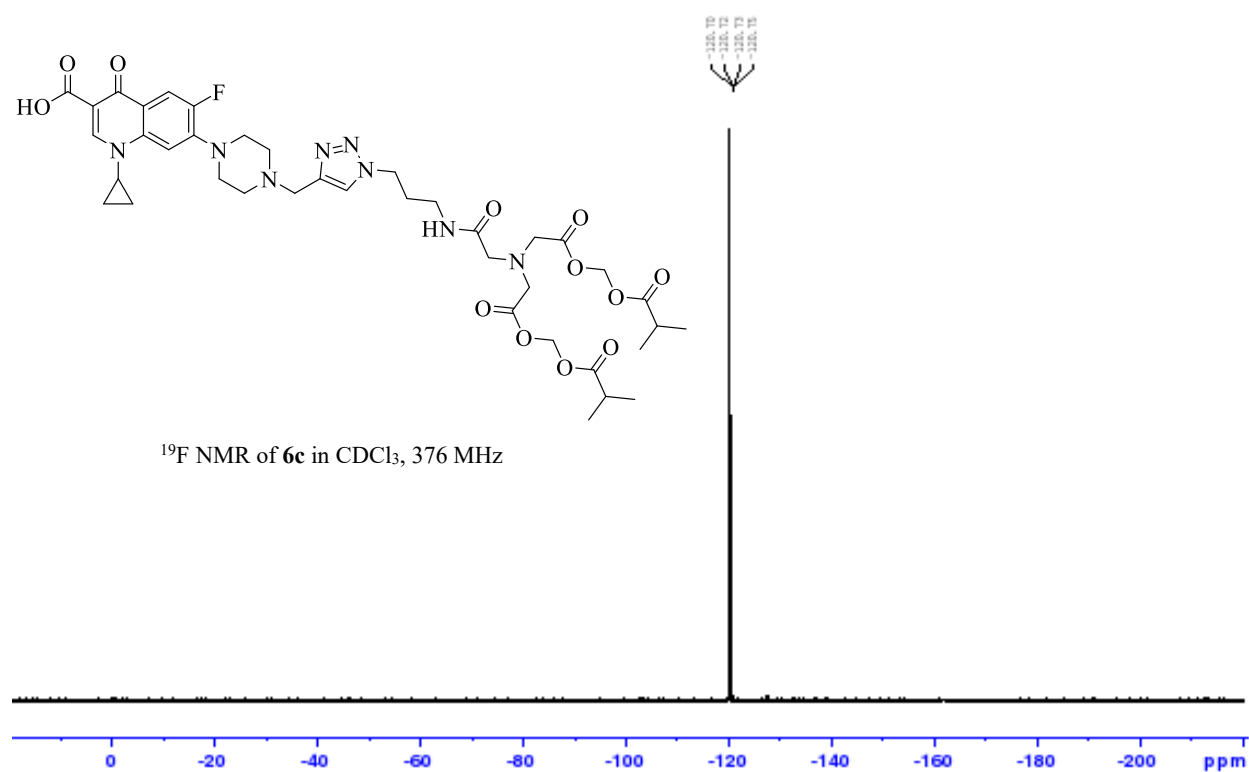
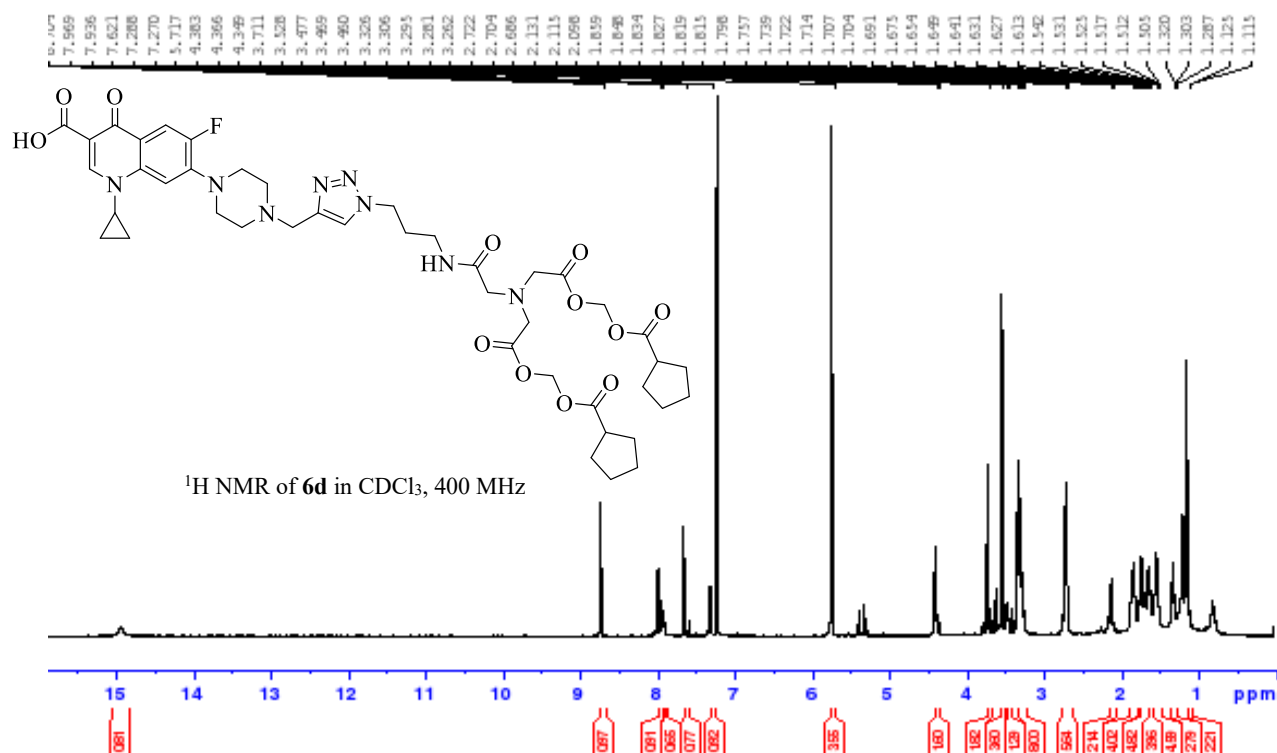
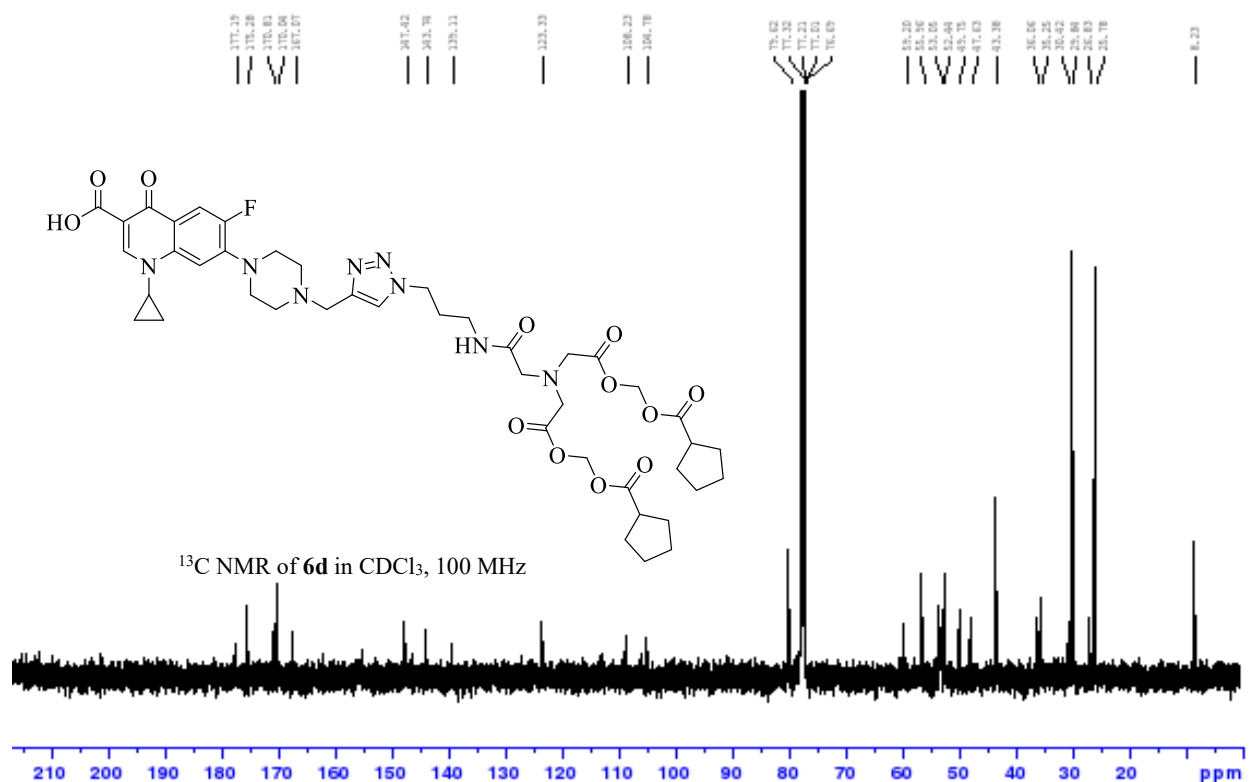


Figure A.30. ^{13}C NMR of $\text{C}_{37}\text{H}_{47}\text{N}_8\text{O}_{11}\text{F}$ (**6b**).

Figure A.31. ¹⁹F NMR of C₃₇H₄₇N₈O₁₁F (**6b**).Figure A.32. ¹H NMR of C₃₉H₅₁N₈O₁₁F (**6c**).

Figure A.33. ¹³C NMR of C₃₉H₅₁N₈O₁₁F (**6c**).Figure A.34. ¹⁹F NMR of C₃₉H₅₁N₈O₁₁F (**6c**).

Figure A.35. ¹H NMR of C₄₃H₅₅N₈O₁₁F (**6d**).Figure A.36. ¹³C NMR of C₄₃H₅₅N₈O₁₁F (**6d**).

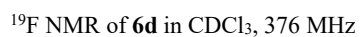


Figure A.37. ^{19}F NMR of $\text{C}_{43}\text{H}_{55}\text{N}_8\text{O}_{11}\text{F}$ (**6d**).

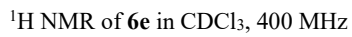
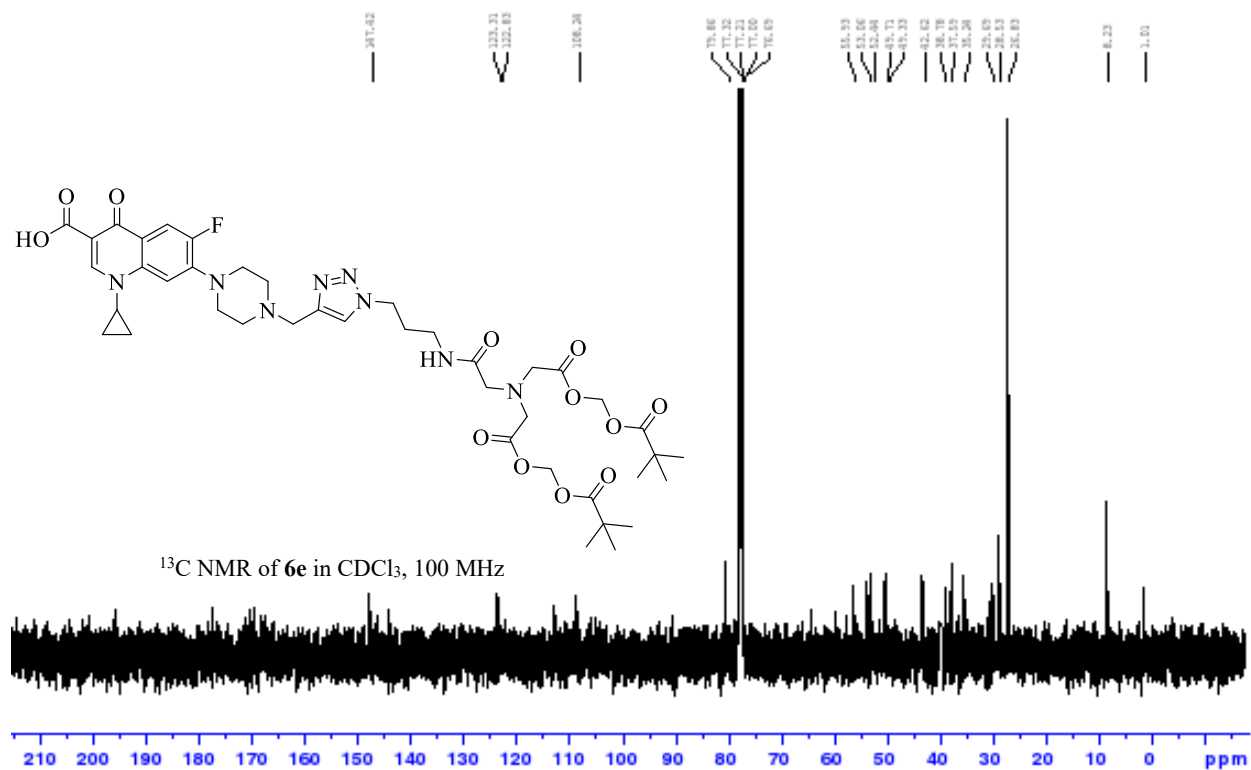
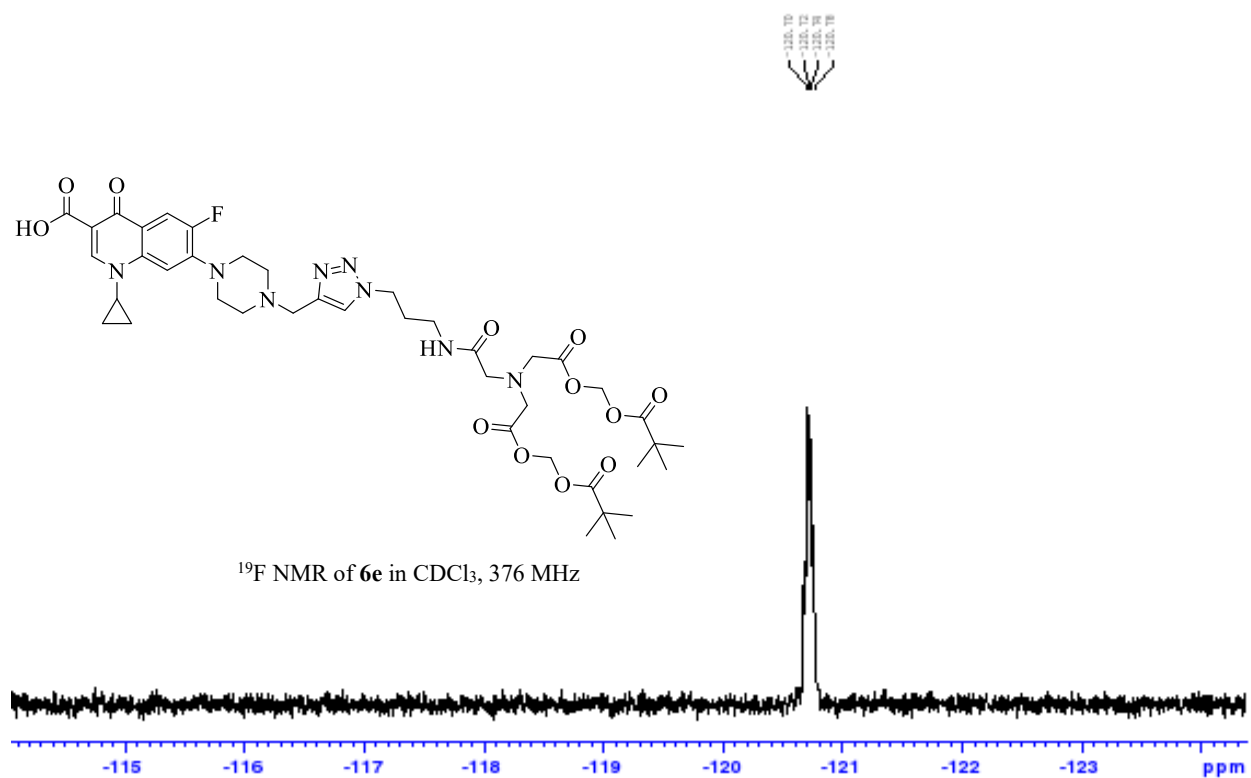


Figure A.38. ^1H NMR of $\text{C}_{41}\text{H}_{55}\text{N}_8\text{O}_{11}\text{F}$ (**6e**).

Figure A.39. ¹³C NMR of C₄₁H₅₅N₈O₁₁F (**6e**).Figure A.40. ¹⁹F NMR of C₄₁H₅₅N₈O₁₁F (**6e**).