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Senior Thesis

Spring 2018

Recent Progress in the Synthesis of an Antifouling Diketopiperazine Derivative Polymer

I hereby recognize and pledge to fulfill my responsibilities, as defined in the Honor Code of Allegheny College, and to maintain the integrity of both myself and the College as a whole.

Pledge: _____

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Acknowledgements

Dr. Murphree: Thank you for your countless pep-talks that almost convinced me that I was a competent chemist. I appreciate you believing in me this entire year even though I never believed in myself. You genuinely cared about me and my well-being, as well as all your students. You're a remarkable professor and human, and the knowledge you've given me these past 3 years is priceless. Again, thank you for everything.

Sistor: Thank you for all the laughs, and more importantly, the naps and snacks You always made even the hardest days manageable somehow. If I've learned anything from this year comping with you, it's that we are really bad at comping. Love you!

Ali: Thank you for spending long nights in the lab with me (especially when I had to run two columns back to back) and then helping me clean up afterwards. Not only that, but your endless support and tolerance of my obtuse personality truly allowed this process to be as painless as possible. You're the best!

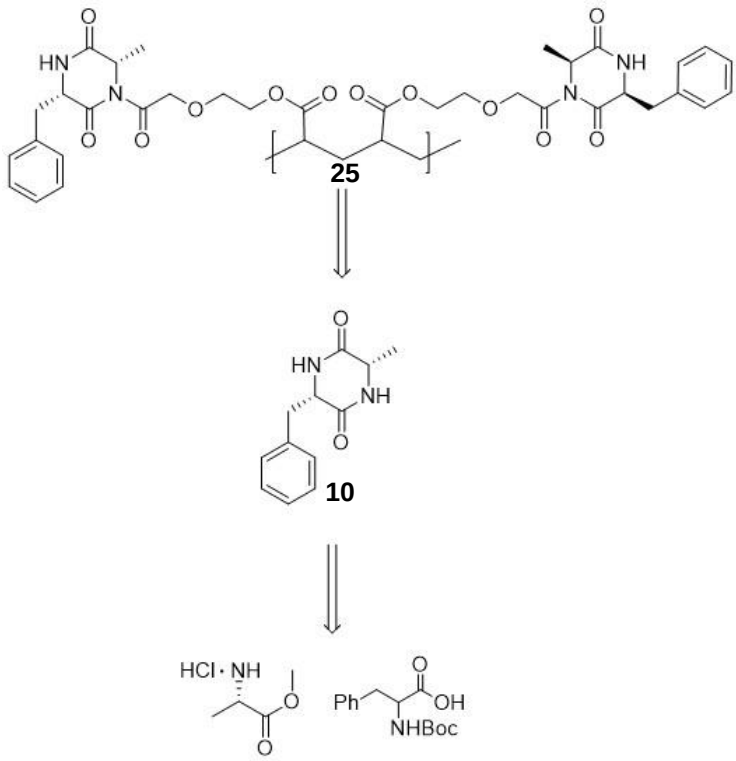
Ryan, Sadie, and Lucia: Group meeting and lab time with you guys was always a joy! Working among such smart individuals was empowering. Thanks for your positive attitudes and friendships this year!

Big Bois and Company: Thank you guys for always providing the laughs and the rational thinking I needed to survive not only this year, but throughout our college careers. Once a big boi, always a big boi. It's been a privilege having a group of friends like you ladies for 3 years. Love you all!

Mom and Dad: Last but not least, thank you both for listening to my rants when I failed and clapping for me when I succeeded. Your guidance and advice kept things in perspective and more importantly, kept me on track. You have always had the best in mind for me, and oddly enough you guys were always right. I could not be where I am today without you. Love you!

Abstract

Biofouling, or the accumulation of microbes adhered to a surface, has brought economic inefficiency in the maritime, and aquaculture fields while presenting health risks in medical fields. The first developed antifouling products, such as organotin compounds, were developed in ac polyacrylate-based paints and were shown to have several ecological and environmental issues, thus displaying the need for an environmentally friendly antifoulant. 2,5-diketopiperazines, molecules that naturally occur in secretions of marine fungi and bacteria, have been shown to exhibit antifouling activities. A retrosynthetic framework for a proposed route of synthesis to a 2,5-diketopiperazine derivative polymer is shown in Scheme 1. Recent progress of the synthesis is discussed.



Scheme 1. Retrosynthetic framework for synthesis of the 2,5-diketopiperazine derivative polymer

Introduction

Biological fouling, or biofouling, has greatly affected the medical and maritime industries both environmentally and economically. Accumulation of microorganisms on ships decrease efficiency of the ship's travel resulting in excessive fuel use, while accumulation of microorganisms on implanted devices increases the probability of infection at the implant site, ultimately leading to systemic infection. [1,5,7] This project builds upon previous literature in attempt to solve them by synthesizing an environmentally and economically feasible antifoulant with little to no negative implications.

1.1 The Mechanism of Biofouling

The biofouling process can be separated into two subsections: the biofilm created by the accumulation and adhesion of microscopic organisms and the macroscopic accumulation of macro-organisms.[1] Microorganisms include algae, fungi, cyanobacteria and macroorganisms include sponges, barnacles, mussels, and tubeworms.[2] The biofouling process begins with microscopic biofilm formation that facilitates macroscopic fouling over time.[3]

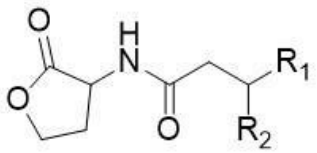
For a biofilm to form, microbes must attach irreversibly to a surface or substratum. Certain properties of the substratum can encourage irreversible attachment. Characklis *et al* noted increased microbial colonization on rougher surface as shear forces are lessened and surface area is increased. [4] The hydrophobicity of microbial cell surfaces becomes advantageous on hydrophobic surfaces, like Teflon or other plastics, because of increased hydrophobic interactions between the substratum and cell. In addition, structures on the cell surface (pili, flagella, fimbriae, or glycocalyx) can allow the microbe to overcome repulsive forces common

to all materials. [4,5] Both characteristics allow for increased irreversible attachment. Fletcher *et al*. determined that for *Pseudomnas* sp. rough, nonpolar, and hydrophobic surfaces have higher rates of irreversible attachment, and therefore biofilm formation. [6] Korber *et al*., using motile and nonmotile stains of *Pseudomonas fluorescens,* determined that the presence of cell surface structures promoted attachment to the substratum more vastly and evenly than nonmotile bacteria.[6] Irreversible attachment is also facilitated through the release of extracellular polymeric substances (EPS) which comprise of protein, polysaccharide, and proteoglycan molecules, notably fructose- and glucose- based polysaccharides. As the initial microbes continue to adhere, the EPS released forms a conditioning layer on the substratum, priming the surface for further attachment of other microbes and therefore promoting biofilm formation.[3]

Once attached, microbes divide and grow, forming multicellular aggregates known as microcolonies. The microcolonies continue to produce EPS that forms a network between them.[7] These microaggregates act as a pre-biofilm structure with the extracellular polymers as scaffolds for other microbes to adhere.[7,8] This process is relatively quick (approximately one minute), and soon the first layer of the biofilm is established. [3] The biofilm grows to form a 'mushroom' or 'tower' shape. As it thickens, bacteria arrange themselves in accordance to their metabolic mechanisms and aero tolerances.[8]

To survive as a system, bacterial colonies must communicate. Quorum sensing is the mechanism through which bacteria communicate and synchronize gene expression to monitor cell density and control behavior using molecules called autoinducers. Gram-negative bacteria use acyl-homoserine lactones (AHLs) as autoinducers, whose structure is shown in Figure 1, to

mediate communications. Gram-positive bacteria use oligopeptides as autoinducers. [8] Every species of gram-negative bacteria produces a specific AHL, restricting communication to members of that same species only. Examples of specific AHLs for a given gram-negative bacteria are shown in Figure 2, where **2** is from *Vibrio fisheri*, **3** and **4** are from *Pseudomonas aeruginosa*, and **5** is from *Vibrio harvey*.[51] The signaling system contains a synthase, *luxI*, which produces AHL variants, and a receptor, *luxR*, that binds to the AHL. *LuxR* responds to high levels of the AHL, cascading a signal across the entire biofilm that prompts changes in gene expression once the threshold level of AHL is achieved. [9,51]



1

Figure 1. Standard AHL structure where R_1 = alkyl chain from 3-11 C and R_2=H, C=O, OH.[8]

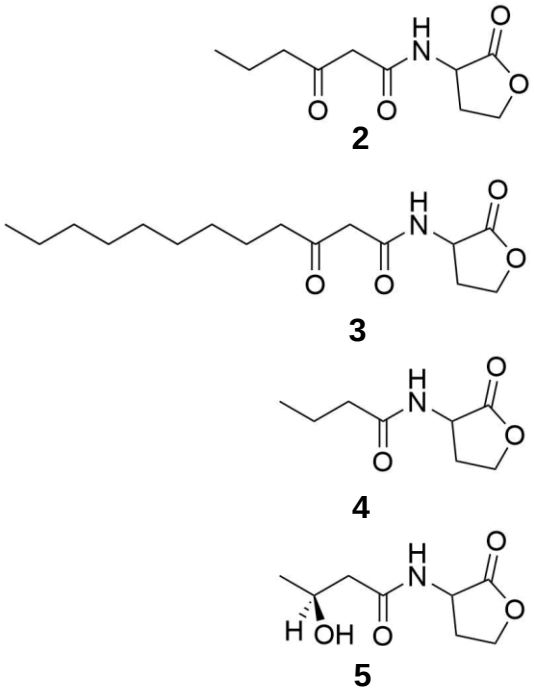


Figure 2. Examples of AHL autoinducers from various Gram-negative bacteria. [51]

Once a biofilm has been established, the extracellular matrix serves as a scaffold for macroorganisms to adhere. In about one week, spores of macroalgae and protozoa will accumulate, and in about 2-3 weeks, evolution of the larvae ensues, and the surface is covered in a vast biological communit.[3]

1.2 The Issue of Biofouling

The aforementioned process of microfouling occurs readily on medical devices, and the resulting infections have detrimental effects on the patient and the field. Elliot *et al.* found that all central valve catheters (CVCs) inserted through the skin have biofilms, and 67% of CVCs contained microorganisms despite skin and device sterilizations prior to insertion. The microbes present on the CVC, identified as *Staphylococcus aureus, Enterococcus faecalis,* and *Pseudomonas aeruginosa,* originated from the patient's skin, and depending on the site of

insertion, a biofilm on the skin surface could be present hence the ineffective sterilization.[10] Although biofilm formation on CVCs is seemingly unavoidable, the extent of the biofilm is dependent on the duration of catheterization. Short-term catheters have greater biofilm growth on the external surface, while long-term catheters' biofilm extends into the catheter inner lumen.[11] As biofilm formation progresses, growth rate levels off and cell detachment rates increase. Long-term CVCs exhibit this phenomenon, and detached cells accumulate in the bloodstream resulting in bacteremia and embolism.[10,11] The same mechanism of infection is observed during implantation of mechanical heart valves and urinary catheters as well, where almost all patients with either device become infected.[10] Because methods like skin sterilization are ineffective, approaches to pre-treating the device before implantation with antimicrobial products have been, and continue to be, explored.[11,45] For example, CVCs treated with the antibiotics minocycline, which inhibits protein synthesis in the ribosome, and rifampin, which inhibits RNA polymerase, were shown to decrease biofilm formation in patients.[11] Due to increasing antibiotic resistance, the need for alternative methods is present.

While microscopic biofouling plagues medicine, macroscopic biofouling hinders the aquaculture industry. Cages, nets, and ropes used to collect large quantities of shellfish are particularly susceptible to biofouling due to their constant presence in a wet environment and exposure to micro and macroorganisms.[2] Aquaculture produces about $31.4 billion in revenues, and anywhere between 5-10% of the production costs can be attributed to the prevention and treatment of biofouling.[13] Treatment techniques include the physical removal of the fouled organism, biological control, where a predator species of the fouling organism is introduced to

decrease population, and the use of coatings on the ship to deter fouling. [13-14] The cost of biofouling can also be attributed to the increase in fuel use cause by the ships' increased friction and drag during travel.[13] Schultz *et al.* studied FFG-7 frigates traveling at a rate of 7.7 m s^{-1}, finding that light-macrofouled boats experienced 11% increase in resistance, while a heavily macrofouled boat experienced 80%. To achieve the same speed, 11% increase and an 86% increase in shaft power for light and heavily fouled boats respectively. [14] It is estimated that the Navy's cost allocation to prevent fouling is $56 million per year.[15] With such a large proportion of revenues being allocated to preventing the spoilage of their products and machinery and a noticeable increase in fuel consumption, the necessity for cost-effective antibiofouling preventions and treatments exists.

The reason the medical and aquaculture industries are struggling to manage biofilm formation is that biofilms are resistant to typical antimicrobial tactics, such as disinfection, physical removal, or antibiotic treatment. They exist as a complex, heterogeneous patterned layer with channels to transport nutrients and entrap particles existing in the outside environment.[4,5] The extracellular polymeric matrix resists antibiotic treating by retarding the diffusion rate via chemical reaction or physical limitations the transport rate. In addition, cell growth rates in biofilms are significantly slower than planktonic bacteria, preventing antibiotic uptake, kinetically inhibiting its mechanism of action. [5] Even countermeasures like disinfection, which kills living microbes, are ineffective as the already dead biomass that exists within the matrix provides conditions favorable for regrowth.[7] The matrix also allows the bacteria to more easily communicate with each other as they are closer, and the chance of disruption or pili

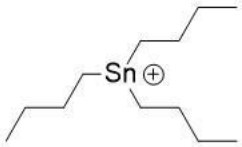
damage is reduced, thus plasmids containing resistance to antimicrobial tactics can be transferred. With no simple way to destroy the biofilms that have already formed, different approaches, such as targeting quorum sensing, have come to light.

1.3 Previous Solutions to Biofouling

Phoenicians and Carthaginians first attempted to treat biofouling using copper and lead sheathing on the bottom of ships. Other cultures during this time used materials like tar, wax, asphaltum, arsenic, and sulfur and oil mixtures to prevent biofouling from damaging their ships.[14] As technology has progressed into the mid twentieth century, compounds like organo-mercury, lead, arsenic and DDT were used, but as their use posed severe health and environmental risks, organic tin derivatives were used as a replacement.[16] The main organic tin compound used industrially was tributyltin (TBT), shown in Figure 3. Examples of its derivatives include tributyltin oxide and tributyltin fluoride, both of which have been shown to inhibit the growth of any biofilm at very low concentration.

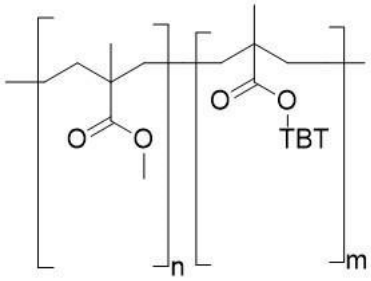
In the primary stages of TBT, its release rate from the paint was uncontrolled and exponentially decreasing, as TBT was freely associated. However, in the 1970s, copolymer paints were introduced. Tributyltin in copolymer paints was chemically bonded in a copolymer-resin system via an organotin-ester linkage, resulting in slow and controlled release of the antifouling agent over time.[16] An example includes TBT self-polishing paints. The paints are based on acrylic copolymers with TBT groups bound to the polymer chain by ester bonds, shown in Figure 3. Once submerged, the paint dissolves into sea water, facilitated by the hydrolysis of the unstable carboxyl-TBT bond due to alkaline conditions.[17] This slow and

controlled release mechanism of the antifoulant is of central relevance to this project, as the ultimate product is an acrylate-based polymer. These paints became so popular that in 1999, it is estimated that 70% of all commercial ships were protected by TBT-copolymer paints, saving $2.4 billion in fuel and preventative costs.[17]



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Figure 3. Structure of tributyltin.[14]



7

Figure 4. Structure of acrylate-TBT copolymers in antifouling paints.[17]

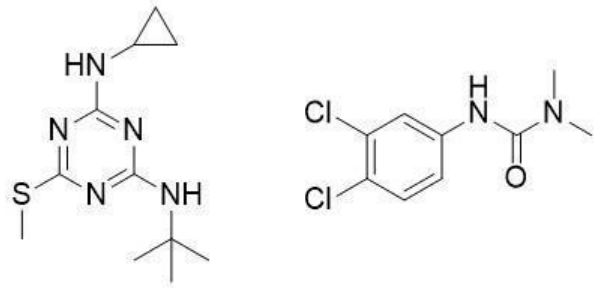
The increased use of organotin containing copolymer paints caused an accumulation of tributyltin and contaminated the water supplies. It has been shown that solutions of TBT at low concentrations, 1-2 ng L^{-1}, algae, zooplankton, and mollusks can be poisoned, deteriorating their shell. All were killed around 16 µg L^{-1}. [18] Organotin compounds cause neurotoxicity, hepatotoxicity, renal toxicity or dermal toxicity in humans.[46] In a 1984 study, six workers that had exposure to a mixture of disubstituted and trisubstituted organotin compounds were evaluated. One man died, one was hospitalized, and only three returned to work.[47] The cause of

death was primarily due to leukoencephalopathy.[48] Poisonings in France were documented in 1954, causing legislation to ban TBT use in 1982. [18,19] They were quickly followed by the United Kingdom in 1985.[20] Shortly thereafter, concentrations in Arcachon Bay in France decreased from 900ng/L in 1983 to 10ng/L in the late 1980s, and the shellfish life had noticeably recovered.[21] Eventually, the International Maritime Organization banned TBT and use its applications from vessels in 2008.[19]

To find an alternative to the recently banned tributyltin, booster biocides grew in popularity. They were combined with cuprous oxide and copper thiocyanate to make "TBT-free" coatings for boats, originally for smaller ships (less than or equal to 25m). [21,22] However, as the use of booster biocides increased, the negative environmental impacts they caused came to light. The most prominent biocide booster was the s-triazine herbicide Irgarol 1051 (**8**) (Figure 5). Concentrations of Irgarol 1051 as low as 0.4 µg/L and 10 µg/L were noted to induce acute toxicity to crustaceans and affect the growth of *Enteromorpha intestinalis* respectfully.[16] The mechanism of action for Irgarol 1051 was the inhibition of electron transport in chloroplasts during photosynthesis, preventing the cells from producing adequate amount of ATP to survive.[17]

The other most commonly used and found booster biocide was Diuron (**9**) (Figure 5). Diuron has a very broad-spectrum of antifouling properties, hence its widespread use. Diuron also inhibits photosynthesis and has found to have a similar toxicity to Irgarol 1051.[22, 23] It also has a high persistence, due to its slow rate of natural hydrolysis, and can be found in soil,

sediment, and water for up to a year. As Diuron has been classified as a teratogen, mutagen, and a carcinogen, the booster biocide is no longer approved for use in the United Kingdom.[22, 24] With the failure of two antifouling techniques, the search for an environmentally friendly and effective antifoulant was in full force.



8 **9**

Figure 5. Structures of Irgarol 1015 and Diuron. [22, 23]

To determine and compare the effectiveness and toxicity of any given antifouling product, there are certain parameters that need to be calculated. The EC50 is the minimum concentration that inhibits 50% of the organisms' settlement. The LC50 is the lethal concentration that kills 50% of the organisms' population; the lower this value, the higher acute toxicity. The therapeutic ratio (TR) is the ratio LC50/EC50, comparing the antifouling capability to its toxicity level; higher TR values indicate less toxic antifoulants while lower TR values indicate highly toxic antifoulants. If the TR is greater than 50, and the EC50 is less than 5.0 μg/mL, then the compound is considered to be a promising nontoxic, antifouling agent.[30,35]

1.4 Natural Antifouling Products: 2,5-Diketopiperazines

Within the context of environmentally friendly antifoulants, natural products of marine and terrestrial sources are a logical place to look. They already exist in nature, so the probability of any adverse effects arising is smaller than synthetic antifoulants. Studies have shown that sessile marine organisms, like the marine sponge *Acanthella cavernosa* or the sea pansy *Renilla reniformis*, are able to prevent biofouling on their surfaces by producing and secreting secondary metabolites.[25-27] The structures of these secondary metabolites have been leading molecules when synthesizing antifoulants. Compounds like terpenoids, steroids, carotenoids, phenolics, furanones, alkaloids, peptides, and lactones have all been shown to have antifouling properties.[25] For the sake of this project, the focus will be primarily based on peptide-related structures.

The large subgroup of marine *Actinomyces* are primarily popular for their antibiotic and anticancer agents, while their antifouling agent activity has been underrepresented in the literature.[28] The *Streptomyces* genus produces a wide variety of biologically active agents; over 500 species accounts for 80% of relevant secondary metabolite.[29] Another stand out microbe is the *Cladosporium* genus of fungi that have shown ability to degrade polycyclic aromatic hydrocarbons and antifungal activity.[30] Although the secondary metabolites of both organisms vary greatly in structure depending on the species, this project focuses on one specifically: the 2,5-diketopiperazine (DKP).

The 2,5-DKP, from a synthetic standpoint, is a favorable scaffold for antifouling agents as it is small, conformationally rigid, and contains chiral templates that can induce chirality into different systems. These attributes open the door for diversity with defined stereochemistry,

allowing for broad ranges of biological activities.[31] Researchers have been able to isolate several DKPs from both *Streptomyces* and *Cladosporium.* In 2012, Cho *et. al* isolated two DKPs from *Streptomyces praecox*, shown in Figure 6 (**10, 11**), and their LC50, EC50, and TR values are shown in Table 1, in addition to those of Irgarol 1051 (**8**).[28] Li, X. *et al.* isolated five distinctive DKPs from *Streptomyces fungicidicus* (Figure 6) (**10-17**); its LC50, EC50, and TR values are shown in Table 1. Qi *et al.* isolated several secondary metabolites with antifouling activity from *Cladosporium*, one of which was the 2,5-DKP shown as **17** in Figure 5, and its LC50, EC50, and TR values are shown in Table 1.

Molecules **10, 11, 14** have EC50 values below 25 μg mL^{-1} and TR values that are acceptable as good antifouling agents, indicating low toxicity and low effective concentration. A drawback to these structures, save **10** and **11**, is that they exhibit poor solubility. They lack substituents on the amide nitrogen atoms, increasing intermolecular hydrogen bonding. Molecules with aromatic groups at the 3 or 6 positions additionally engage in π–π stacking.[33] Otherwise, with moderate modifications, these scaffolds show promising futures as antifouling agents. As a result, the structure of **10** is used as a scaffold for the final product in the project.

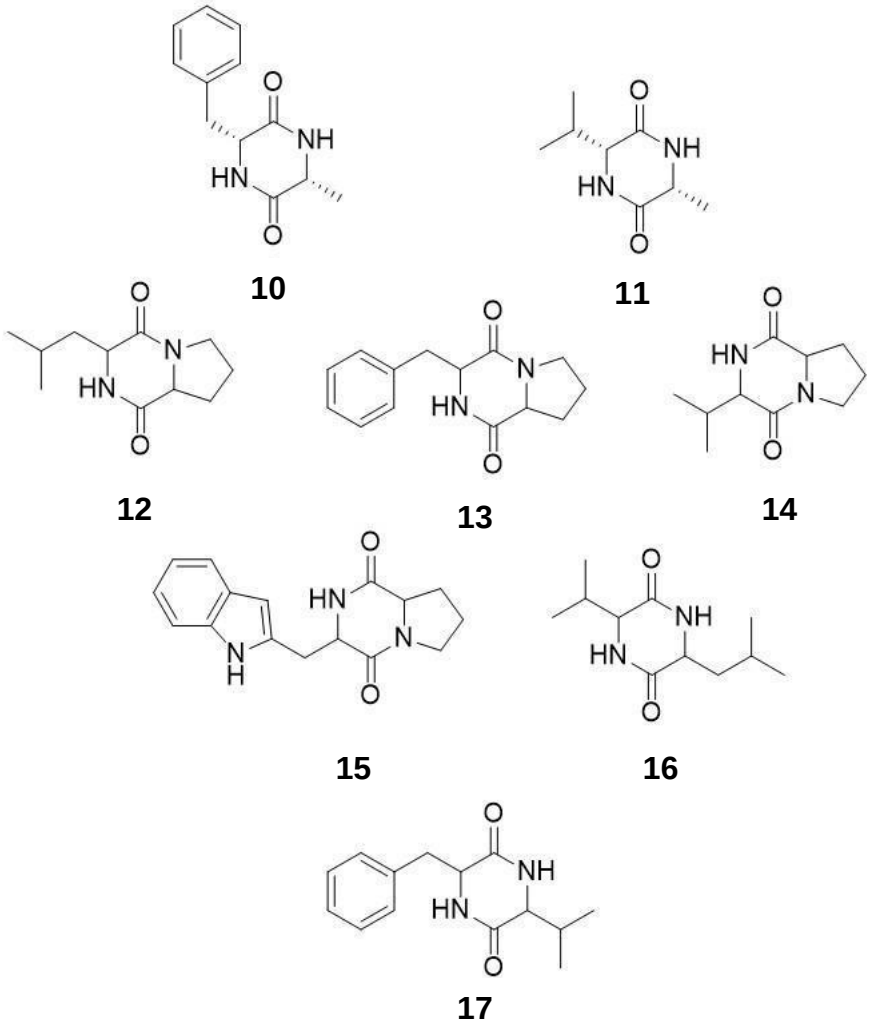


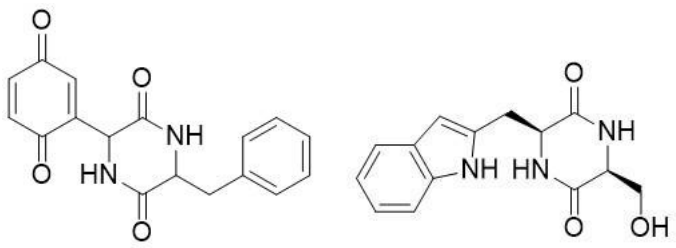
Figure 5. Structures of 2,5 diketopiperazines from Cho *et al.* (**10,11**) Li,X. *et al.* (**8-12**) and Qi *et al.* (**13**) [28,34,35]

Table 1. LC50, EC50, and TR values for **8, 10-17**.[28,34,35]

Compound (µg/mL)	LC50	EC50	TR
8	0.012	0.002	6
10	210.4	0.800	263
11	132.3	1.10	120
12	134.4	31.5	4.26
13	114.7	68.3	1.68
14	147.0	19.6	7.50
15	169.2	36.7	4.60
16	144.2	57.2	2.50
17	>200	37.82	~ 6

The mechanism of action of each DKP is as various as their structures. Studies have reported that DKPs have been able to inhibit enzymes like indoleamine 2,3-dioxygenase, an enzyme that catalyzes the conversion of L-tryptophan to N-formylnuenine in bacteria. Inhibition of nitrate production, growth of P388 cells, topoisomerase activity, and induction of apoptosis have all been identified as mechanisms of action for antifouling DKPs.[32] Fairly recently in 2016, 2,5-DKPs have shown to inhibit quorum sensing, a very crucial part of biofilm formation and integrity.[34]

Scoffone *et al.* observed the effect of ten new 2,5-DKP derivatives the quorum sensing system of *Burkholderia cenocepacia*, containing the CepI synthase, an analog of *luxI*. Four of these molecules showed anti-quorum sensing activity, acting as non-competitive inhibitors toward both CepI substrates, decreasing the efficiency of the enzyme to produce AHLs. The most active compound in this study is shown in Figure 7, (**18**). *B. cenocepacia* biofilms formed in the presences of 2,5-DKP derivatives are less structured, contain fewer cells, and are unable to occupy the entire surface as well as if there were no 2,5-DKP present.[36] Sun *et. al* focused on one 2,5-DKP derivative (**19,** Figure 7) that was isolated from *Rheinheimera aquimaris* and tested its anti-quorum sensing properties using the violacein production system which is mediated by quorum sensing. Stectrophotometrical analyses provide proof that all concentrations of **19** inhibited violacein production through competitive inhibition with several bacterial quorum sensing systems.[37]



18 **19**

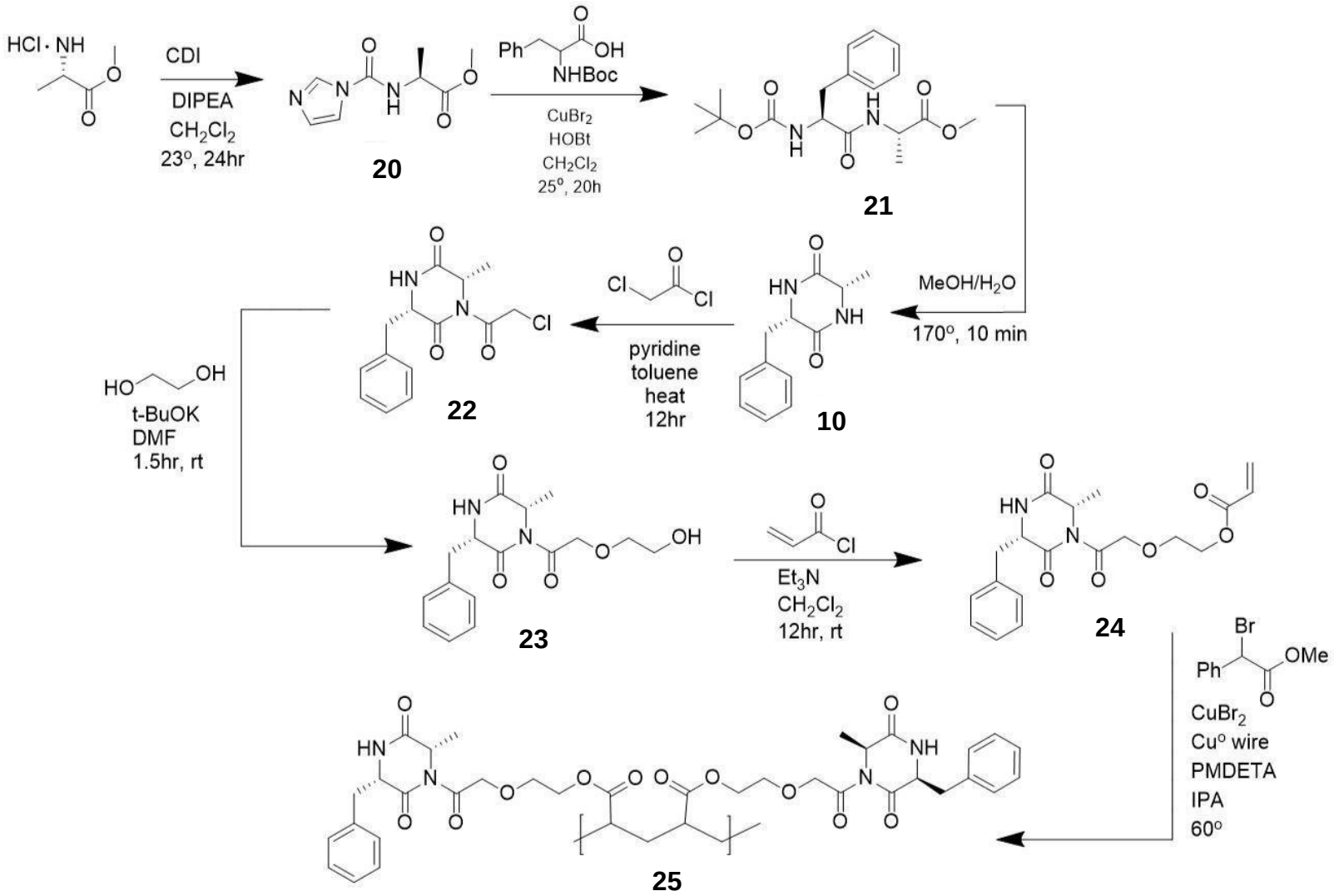
Figure 7. 2,5-DKP derivatives that successfully inhibit quorum sensing in bacteria.[36, 37]

1.5 Methodologies for Synthesis of a Diketopiperazine Polymer

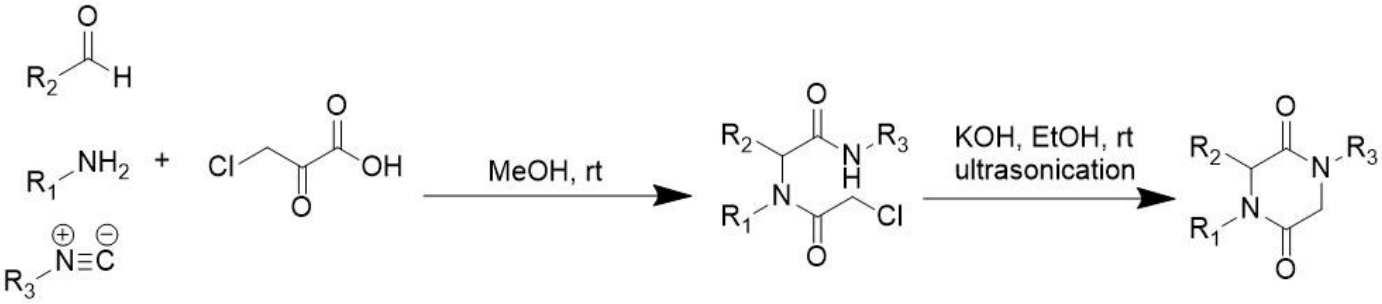
Free 2,5-DKPs engage in antifouling activity, but for these compounds to be used effectively and in larger quantities, attaching them to polymers is preferable. Polymers provide a vast variety of applications and properties necessary to apply the antifouling properties of 2,5-DKPs on the macroscopic level. The methodologies presented in Scheme 2 stem from novel and convenient synthetic strategies presented in the literature for 2,5-DKP preparation and proven techniques to modify amide and acyl chloride functionalities, resulting in a 2,5-DKP acrylate monomer (**24**). The final step is polymerization of the monomer to presumably provide an antifouling material.

Synthesizing the 2,5-DKP scaffold is typically done through a head-tail cyclization of a dipeptide.[32] There are several documented approaches to 2,5-DKP synthesis as noted by Borthwick (2012): via amide bond formation, *N*-alkylation, tandem cyclization, or *C*-acylation.[38] This project utilizes the first technique and will discuss it in detail later. *N*-Alkylation completed intramolecularly via Ugi-4CR (Scheme 3) presents possible epimerization

at the stereocenter if the R_1 substituent is alkyl chain and therefore cannot form the desired 2,5-DKP. Tandem cyclization can be completed via N1−C2/C3−N4 bond formation, or C2−N1−C6 bond formation under a variety of conditions. *C*- Acylation is typically completed via enolate acylation or radical mediated C-C cyclization. [38]



Scheme 2. Proposed synthesis of 2,5-diketopiperazine derivative polymer



Scheme 3. 2,5-diketopiperazine synthesis via Ugi-4CR mediated intramolecular N-Alkylation. R_1=4-$ClC_6H_4CH_2$, $PhCH_2$, c-C_6H_{11} R_2=4-ClC_6H_4, 4-MeO_6H_4, 2-naphthyl, 4-BrC_6H_4, CH_3, R_3= $PhCH_2$, c-C_6H_{11}

The first step for the synthesis of this project's final product utilizes the amide bond formation approach, as this is the most common and fiscally feasible approach. Adapted from Suppo *et.al*, two commercially available amino acids are coupled to produce a dipeptide (**17**) via activation of the amine (Scheme 10, Scheme 11). [39] These conditions are favorable for several reasons. It avoids the problems associated with carboxylic activated coupling reactions. Epimerization of the reagent can occur if sensitive amino acids (His and Cys) are used. Even when using appropriate amino acids, because the carboxyl site is activated, a C→N direction of peptide synthesis occurs. Peptides are naturally built in the N→C direction and reversing the direction of synthesis leaves the C terminal prone to formation of oxazalone intermediates, resulting diastereomeric products. Although work-arounds have been developed[51-55], these require modification of both starting amino acids. Therefore, the use of N-N'-carbonyldiimidazole (CDI) and commercially available amino acids circumvent the need for modification and presents reasonably priced reagents.[39]

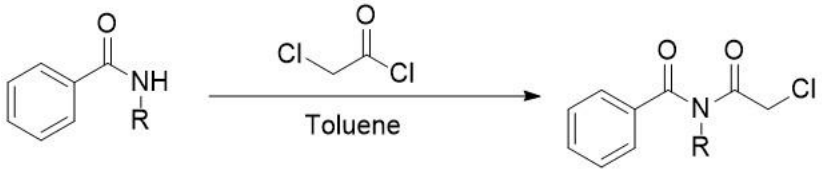
Subsequent cyclization relies on the thermal deprotection of the dipeptide to initiate intramolecular cyclization. Cyclization can be carried out under reflux conditions at high boiling solvents, such as toluene or xylene for 24 hours.[30] However, due to recent advanced in

diketopiperazine chemistry, several sets of microwave conditions for this reaction have been presented.[49,50] Tullerberg *et al* demonstrates the importance of solvent choice for this reaction, comparing the yield of the reaction ran under 12hr reflux versus 10 minute microwave conditions. Toluene provided the highest yield under reflux (38%) while the lowest under microwave conditions (8%). Water under reflux conditions provided 5% yield and 67% yield in the microwave, indicating that reflux conditions, regardless of solvent, are less successful than microwave.[49] A summary of the study's conclusions is provided in Table 2. However, at larger scales, reflux conditions prove to be more useful. This project takes advantage of the conditions developed by Nakao *et al* where a solvent mixture of 3:1 water: methanol at a 0.484mmol scale is used resulting in the 2,5-DKP (**6**).[28,40]

	Yield %	
Solvent	**Δ**	**MW**
Toluene	38	8
tert-Butanol	36	12
Acetonitrile	33	5
1,2-Dicholoethane	33	6
Toluene/2-butanol (1:4)	33	10
1,2-Dimethoxyethane	25	6
tert-Butanol/H_2O (1:1)	24	9
Benzene	17	5
CCl_4	16	5
1,4-Dioxane	10	7
Methanol	6	5
DMF	5	6
H_2O	5	67

Once the 2,5-DKP is formed, three modifications to construct the acrylate moiety are proposed. These modifications double to increase solubility and potential antifouling activity by adding substituents to the amide nitrogen.[33] The three modifications include acyl chloride and amine addition-elimination form an amide (**22**)[41], followed by the SN2 reaction between the chloride and ethylene glycol, resulting in ether and hydroxy moieties (**23**).[42] The final modification utilizes carbonyl addition to displace the chloride atom with an alpha-beta unsaturated carbonyl, providing the 2,5-DKP acrylate monomer (**24**).[43]

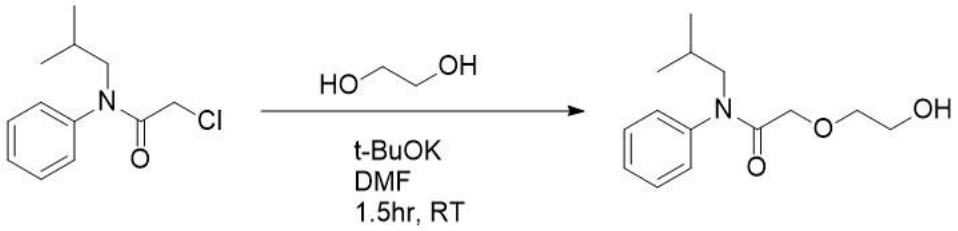
The proposed conditions for the first modification were developed by Watanabe *et al* as shown in Scheme 4. The system is applicable to this project and presents little detriments. However, there is a possibility that this reaction could occur at the amide nitrogen closest to the benzyl moiety of **10**. Due to steric hinderance in this area, it is not expected that this product is the major product, if it forms at al. Watanabe *et al* does not indicate formation of this product.



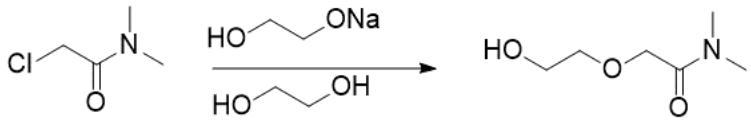
Scheme 4. Model system for amide bond formation via chloroacetyl chloride. R=$C_{22}H_{45}$.[41]

The following SN2 reaction has proposed conditions adapted from Andersen *et al*. The system is based on synthesis of GABA uptake inhibitors and exhibits a comparable reaction site for this project, shown in Scheme 5. However, because DMF is a female teratogen, other possible systems were explored. Scheme 6 shows a system developed by Trinka *et al* for

preparation of 2-chloroethoxy-acetic acid-*N*,*N*-dimethylamide.[56] The disadvantages of this protocol is that while no heat was needed for Andersen *et al*'s rendition, the reaction is heated and the reagents are added dropwise. This conflicts on the time restraints of this project. Also, the isolated yields for Andersen *et al* and Trinka *et al* are significantly different, affording 99% and 62.8% respectfully.[42,56]

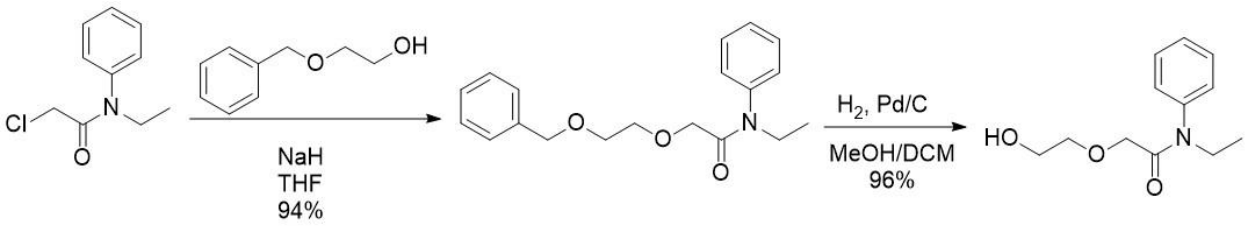


Scheme 5. Model system for the second modification by Andersen *et al*.[42]



Scheme 6. Model system for the second modification by Trinka *et al*.[56]

A third system for this reaction has potential and is developed by Yagishita *et al*, shown in Scheme 7. This system is two steps rather than one, and utilizes a protected ethylene glycol derivative, 2-benzyloxyethanol to prevent side deprotonation with sodium hydride, affording 96% isolated yield of the protected product. It is then exposed to hydrogenation conditions on Pd/C in methanol and methylene chloride, affording an overall yield across two steps of 90.2%. A possible pitfall to this system is, upon hydrogenation, the benzyl moiety of **11** has the potential to be reduced, but Yagishita *et al* does not discuss the formation of this product.[57]



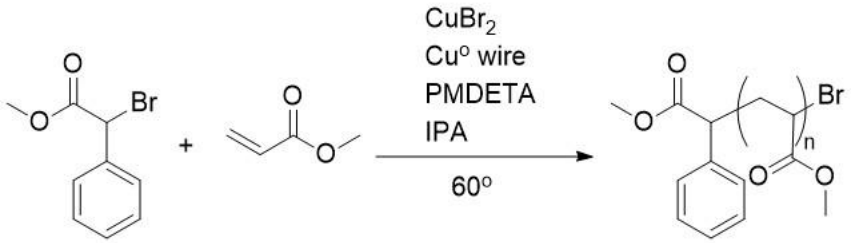
Scheme 7. Model system for the second modification by Yagishita *et al*.[57]

The final modification to achieve the 2,5-DKP acrylate monomer (**24**) is adapted from Xiao *et al*, shown in Scheme 8. The conditions proposed are versatile, as any alcohol, including phenols, can complete this reaction, affording 85-95% isolated yield depending on the reagent. The reagent acryloyl chloride is also commercially available. [43]



Scheme 8. Model system for the final carbonyl addition to create **24**, developed by Xiao *et al.* R= any alkyl or aryl group.[43]

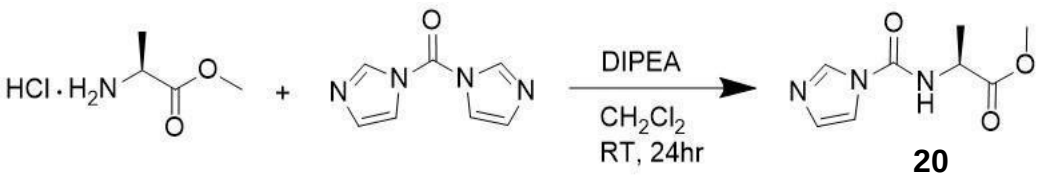
Whitfield *et al.* has developed universal conditions for polymerization of acrylates, using *N*,*N*,*N*′,*N*″,*N*″-Pentamethyldiethylenetriamine (PMDETA) as a ligand. Presumably, these conditions will provide the proposed final product, a 2,5-DKP derivative polymer, shown in Scheme 9.[44]



Scheme 9. Polymerization conditions developed by Whitfield *et al*.[44]

2. Results and Discussion

Synthesis of the Boc-protected amino-ester dipeptide (**21**) has been completed in both small and larger scales. The alpha-aminoester activation of L-alanine methyl ester hydrochloride, Scheme 10, was performed successfully at 2.86 and 7.16mmol scales (Table 3) . The product (**20**) is prone to degradation over time under air, increasing oxidation and rates of hydrolysis, noted by the failure of isolation in entry 1. The second attempt, purification was performed immediately to afford 64% **20.** In preparation of further reactions, the reaction was scaled up to 7.16 mmol (entry 3). However, with 35% yield, a significant amount of product loss is evident, presumably due to degradation of product through side reactions and/or hydrolysis from latent water contained in the starting material. Entries seven and eight were not purified, as successfully isolating product on the column was less likely as scale increased. In order to save product, the reaction was pushed forward without purification.

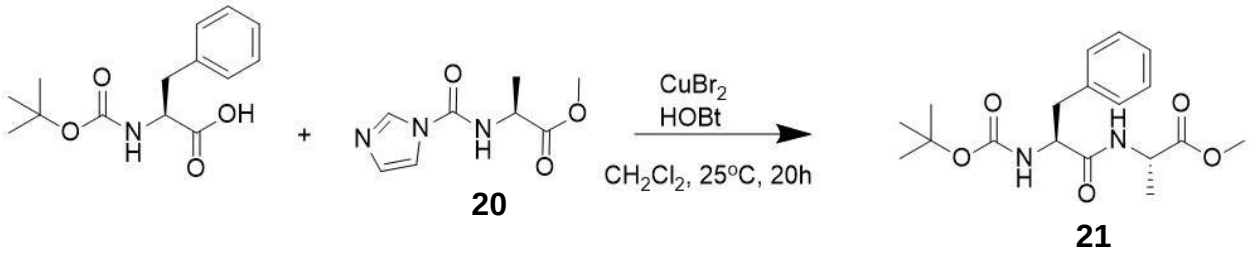


Scheme 10. Alpha-aminoester activation of L-alanine methyl ester hydrochloride.[39]

Table 3. Scale-up of alpha-aminoester activation reaction.

Entry	Scale (mmol)	Crude (%)	Isolated %
1	2.86	-	-
2	2.86	-	64%
3	7.16	-	55%
4	7.16	-	-
5	7.16	-	35%
6	10.3	-	18%
7	10.3	96%	-
8	10.3	99%	-

Dipeptide synthesis via coupling of **20** and Boc-L-phenylalanine (Scheme 11) has been completed successfully also in small and larger yields (Table 4).



Scheme 11. Dipeptide synthesis via coupling of **16** and Boc-L-Phenylalanine.[40]

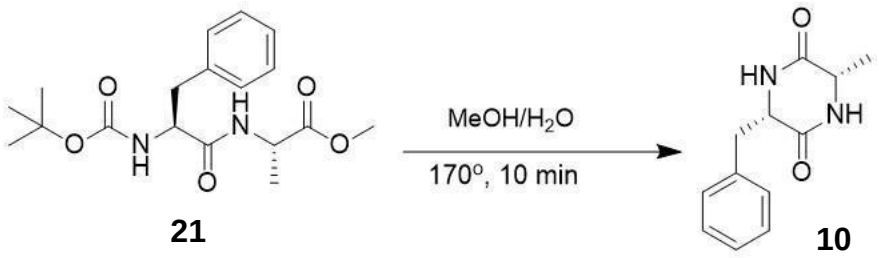
Table 4. Scale-up of dipeptide synthesis reaction.

Entry	Scale (mmol)	Crude (%)	Isolated (%)
1	1.09	-	-
2	0.77	-	-
3	1.13	-	35%
4	2.20	-	95%
5	1.17	37%	

From Table 3, **20** produced from entry 2 was used for entries 1 and 2 in Table 4. However, due to prolonged time between reactions, it was confirmed by NMR that **20** had degraded over time. Thus, **20** produced from the 7.16mmol reaction was used for entries 3 and 4 in a timely manner, resulting in relatively successful reactions for entries 3 and 4.

Entry five used all of **20** prepared from entry 6 in table 2 and was not purified before proceeding to the next reaction. Entry 3 in Table 3 shows that purification might lead to higher yields in the cyclization reactions as side reactions could be occurring.

Using **21** from entry 4, the thermal deprotection of the Boc-protection group and subsequent cyclization reaction (Scheme 12) was attempted to afford **10** in relatively high yield without any purification, even in the instance of entry 3 where impure **20** was utilized.



Scheme 12. Cyclization of Boc-L-Phe-Ala ester dipeptide forming a 2,5-DKP.

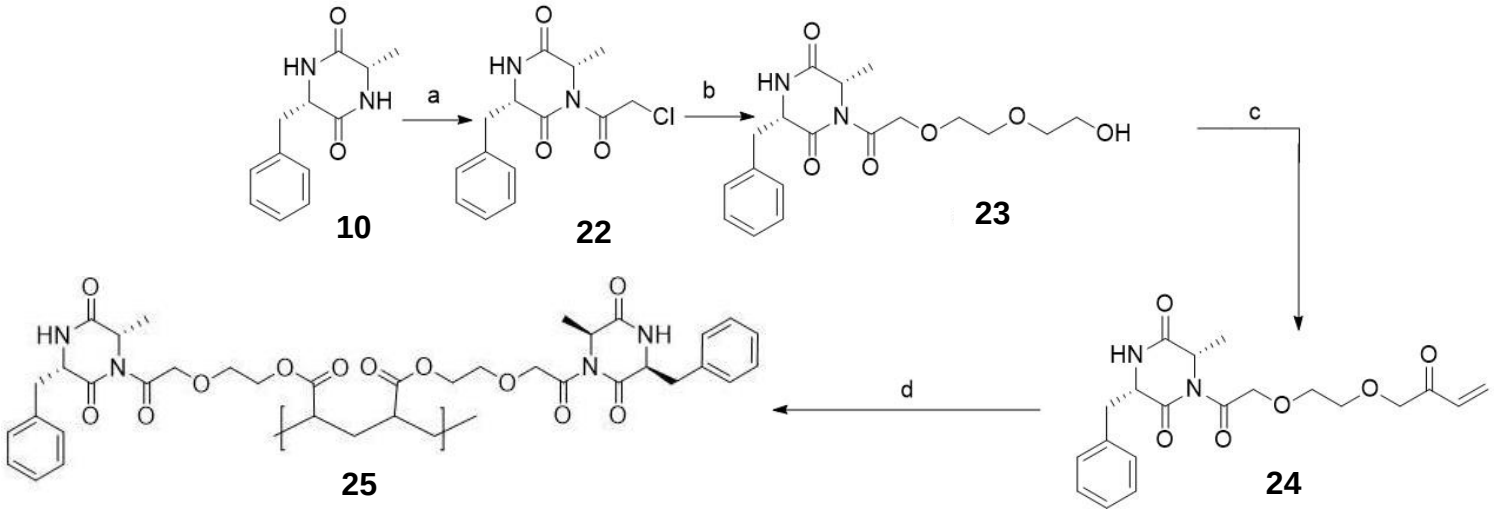
Table 5. Cyclization Reaction results

Entry	Scale (mmol)	Isolated (%)
1	0.484	51%
2	0.484	63%
3	0.484	62%

The proposed synthesis has been relatively successful thus far, presenting relatively high yields in large scales and impure reagents. These conditions prove to be effective when synthesizing **10** successfully.

3. Future Considerations

Four more reactions need to be completed in order to fully synthesize the product, beginning with diketopiperazine **10** (Scheme 13). Furthermore, the cyclization reaction should be optimized to be performed at higher scales. It is likely that microwave synthesis is not favorable in order to do this, so conditions prepared under reflux conditions should be reviewed.[49] Once the desired polymer is isolated and characterized, its antifouling activity can be evaluated using bioassays using *Streptomyces sp*. as described by Cho et al.



Scheme 13. Remaining reactions a). $ClCH_2COCl$, pyridine, toluene b) ethylene glycol, t-BuOK, DMF c) CH_2CH_2COCl, Et_3N, CH_2Cl_2 d) $PhCH_2BrCOOMe$, $CuBr_2$, Cu^o wire, PMDETA, IPA

4. Narrative Experimentals

General:

L-alanine methyl ester hydrochloride (CAS 2491-20-5), carbonyldiimidazole (CAS 530-62-1), Boc-L-phenylalanine (CAS 13734-34-4), cupric bromide (CAS 7789-45-9), HOBt hydrate (CAS 123333-53-9) were all obtained from Sigma-Aldrich Chemical Company. NMR spectra were taken on a JEOL 400 MHz instrument, and IR spectra were taking using a Cary 630 spectrometer.

Methyl (1H-imidazole-1-carbonyl-L-alaninate) (16).[39]

To a 25-mL round bottom flask, carbonyldiimidazole (CDI) (0.4604 g, 2.86 mmol) and methylene chloride (1.60 mL) were added. The flask was sealed with a septum, under nitrogen using syringe technique and placed on ice until the temperature reached approximately 0^{o}C. To a second 25-mL round bottom flask, L-alanine methyl ester hydrochloride (0.396 g, 2.86 mmol), methylene chloride (3.20 mL), and N,N- diisopropylethylamine (DIPEA) (0.500 mL, 2.86 mmol) were added. This solution was added dropwise over 40 min to the CDI solution. The resulting solution was stirred at room temperature under nitrogen for 24 hr, then concentrated via rotary evaporation. Ethyl acetate (EtOAc) (8 mL) was added and the resulting solution was washed once with deioinized water (10 mL). The aqueous layer was further extracted with EtOAc (4 mL) and the resulting organic layers were combined, dried with sodium sulfate, and concentrated via rotary evaporation to afford **16** (0.5621 g, 98%). The crude product was purified via flash chromatography (100% EtOAc) to afford **16** as a white solid (0.3677g, 64%). R_f = 0.3. mp 86-87^{o}C (lit. 90.0-92.5 ^{o}C). ^{1}H NMR (400 MHz, CDCl3): δ1.59 (d, *J* = 7.2 Hz, 3H), 3.81 (s, 3H), 4.64 (quint, 1H), 7.13 (d, *J* = 19.0 Hz, 2H), 7.46 (t, J=1.4 Hz, 1H), 8.71 (s, 1H). ^{13}C NMR (101 MHz, CDCl3) δ 17.79, 49.68, 53.04, 116.58, 129.95, 136.31, 148.78, 173.15. IR

(neat) v=3138.9, 2969.5, 2878.8, 2809.5, 1740.3, 1711.7, 1553.7, 1484.4, 1454.5, 1376.9, 1288.4, 1256.4, 1213.2, 1150.6, 1104.2, 1072.7, 754.8 cm^{-1}

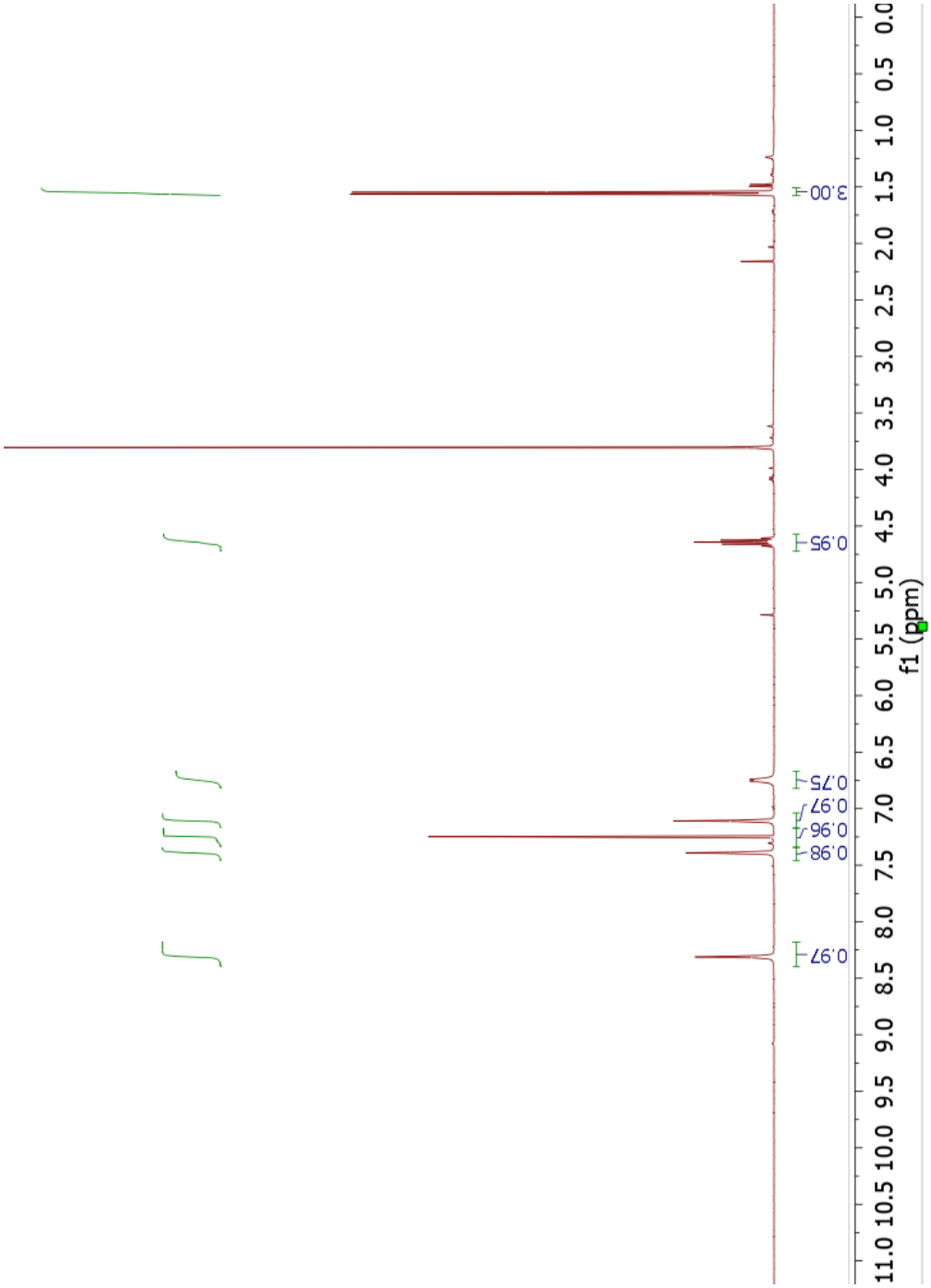
Methyl (tert-butoxycarbonyl)-L-phenylalanyl-L-alaninate (17).[39]

To a 10-mL round bottom flask, **9** (0.2193 g, 1.13 mmol) and CH_2Cl_2 (1.15 mL) were added. The resulting solution was stirred gently, then charged with Boc-L-phenylalanine (0.434 g, 1.69mmol, 1.5 eq), hydroxybenzotriazole hydrate (HOBt) (0.015g, 0.113mmol, 0.1 eq), and cupric bromide (0.025 g, 0.113 mmol, 0.1eq). The vessel was closed with a septum equipped with a needle and allowed to run for 5 days. The reaction was diluted with CH_2Cl_2 (7 mL) and quenched with 0.5 N HCl (4 mL). The resulting solution was transferred to a separatory funnel. The aqueous layer was separated and further extracted with CH_2Cl_2 (7 mL) The organic layers were combined and washed with a solution of saturated sodium bicarbonate (10mL). The aqueous layer was further extracted with CH_2Cl_2 (5 mL). The organic layers were combined, dried over sodium sulfate, and concentrated via rotary evaporation to afford **17** (0.2601g) The crude product was purified via flash chromatography (7:3 Hexane:EtOAc) to afford **10** (0.137g, 37%) as a white solid. mp 98-100^{o}C (lit. 108-110.5 ^{o}C), ^{1}H NMR (400 MHz, $CHCl_3$) δ 1.33 (d, *J*=7.2 Hz, 3H), 1.39 (s, 9H), 3.05 (d, *J* = 6.7 Hz, 2H), 3.69 (s, 3H), 4.10 (q, *J* = 7.1 Hz, 1H), 5.08 (m, 1H), 6.52, (m, 1H), 7.32 – 7.15 (m, 5H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 18.33, 28.32, 38.47, 48.16, 52.51, 55.63, 80.24, 125.16, 127.52, 129.06, 136.65, 155.51, 171.06, 172.91. IR (neat): 3323.4, 2984.1, 2946.6, 1751.7, 1692.8, 1655.5, 1522.4, 1445.9, 1385.8, 1366.2, 1250.5, 1159.4, 1049.5, 988.9, 859.6, 664.4 $cm^{-1.}$

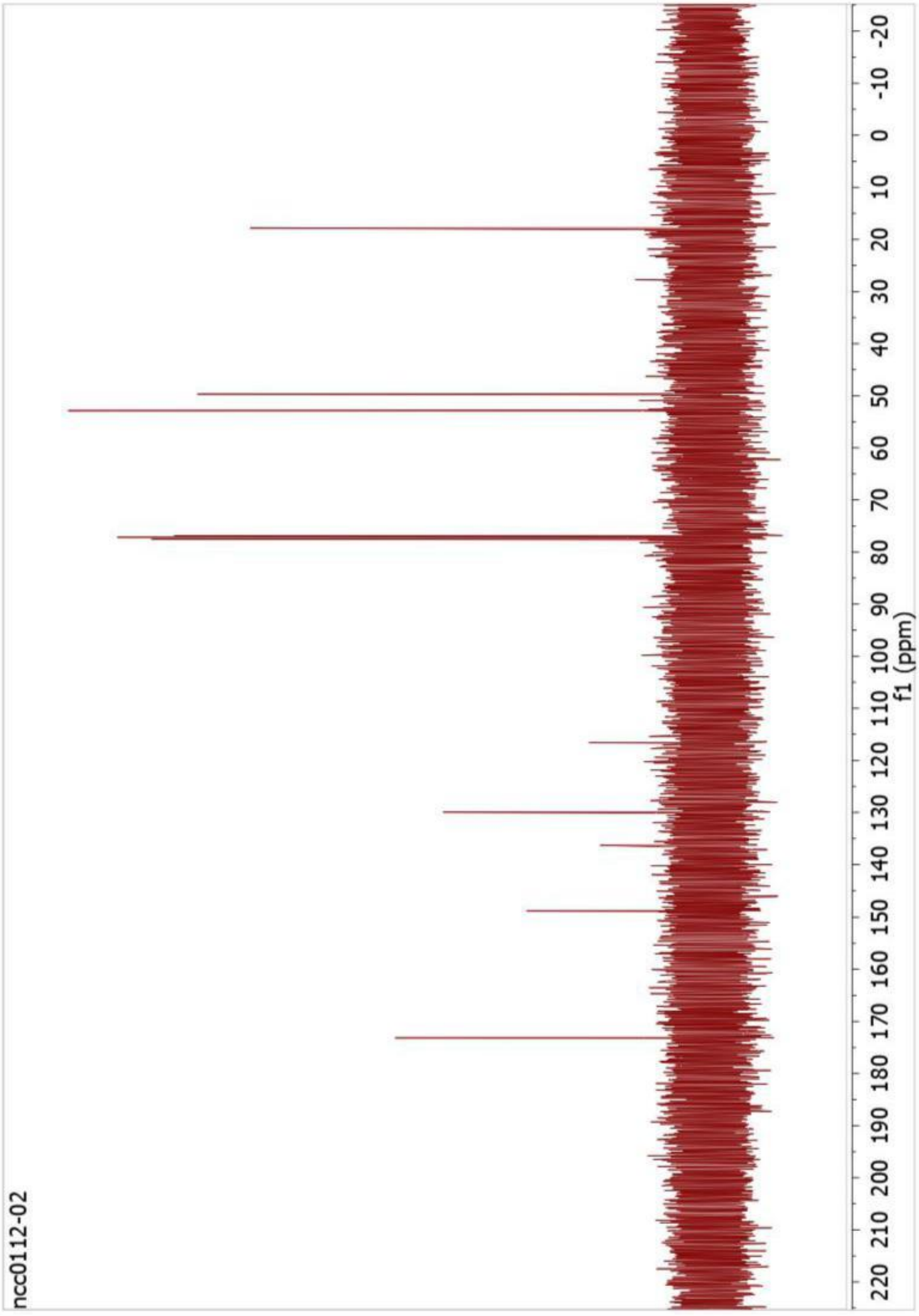
(3S,6S)-6-benzyl-3-methyl-2,5-diketopiperazine (6)[40]

To a 2-5mL microwave test tube, **17** (0.1704g, 0.485mmol), DI water (3mL), and methanol (1mL) were added. The reaction mixture was irradiated in a Biotage microwave synthesizer for 10 min at 170°C. The reaction mixture was transferred to a round bottom flask. Addional methanol (3mL) was used to assist transfer. The mixture was concentrated via rotary evaporation then washed with MeOH (3mL) to afford **6** (95.5mg, 50.5%) as a white solid. mp >240°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ2.52– 2.47 (m, 3H), 2.62 (dd, *J* = 13.4, 8.2 Hz, 1H), 2.85 (dd, *J* = 13.4, 4.9 Hz, 1H, 2.95 (dd, *J* = 13.4, 4.8 Hz, 1H), 3.13 (dd, *J* = 13.4, 3.7 Hz, 1H),), 4.19 – 4.15 (m, 1H), 7.18 – 7.12 (m, 1H), 7.24 – 7.19 (m, 1H), 7.28 (dd, *J* = 8.2, 6.7 Hz, 1H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 20.8, 37.8, 43.6, 51.2, 55.5, 127.9, 130.4, 133.1. IR (neat): 3035, 2949, 2911, 1681, 1590, 1133 cm^{-1}

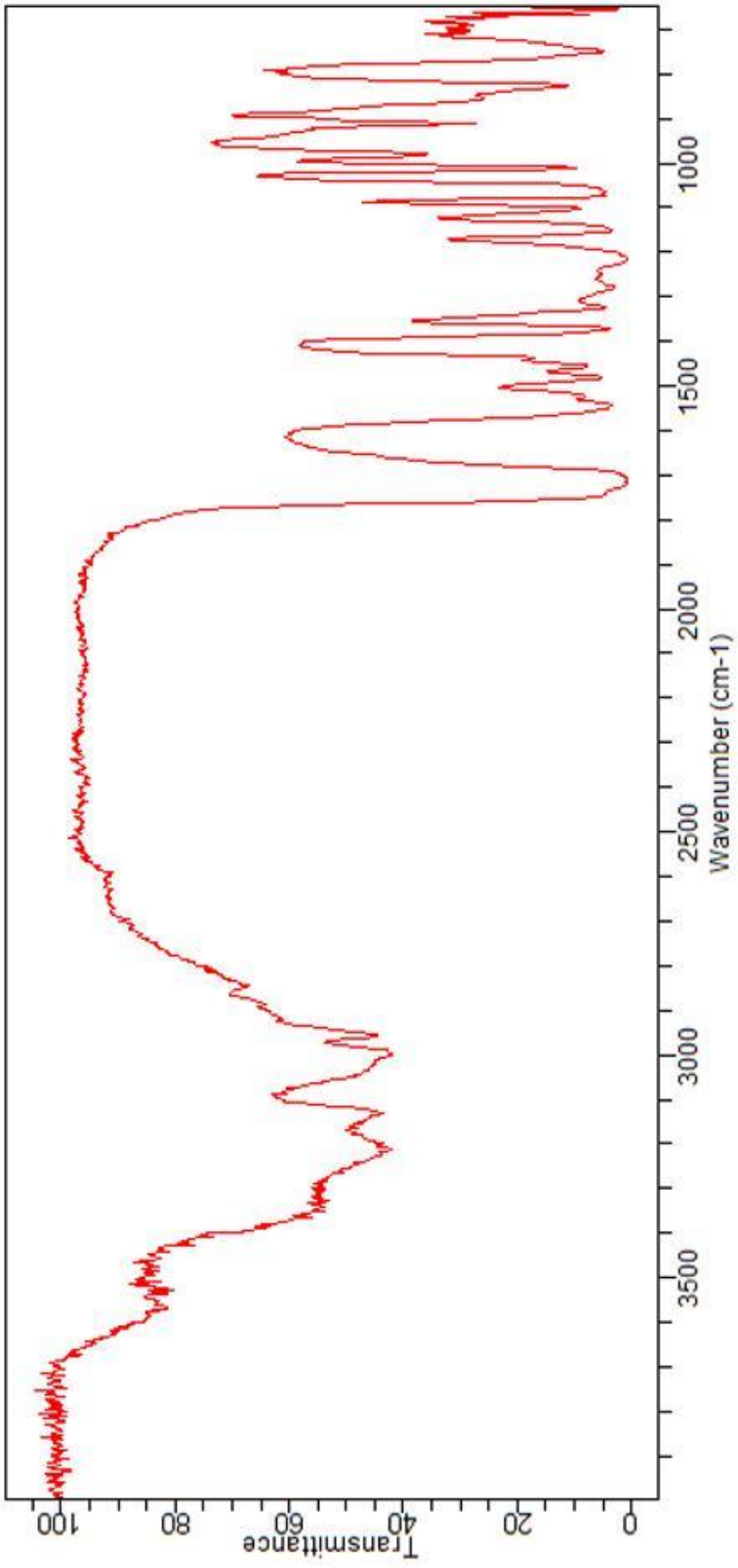
Methyl (1H-imidazole-1-carbonyl-L-alaninate) (20) ^{1}H NMR in CDCl3. JEOL400MH



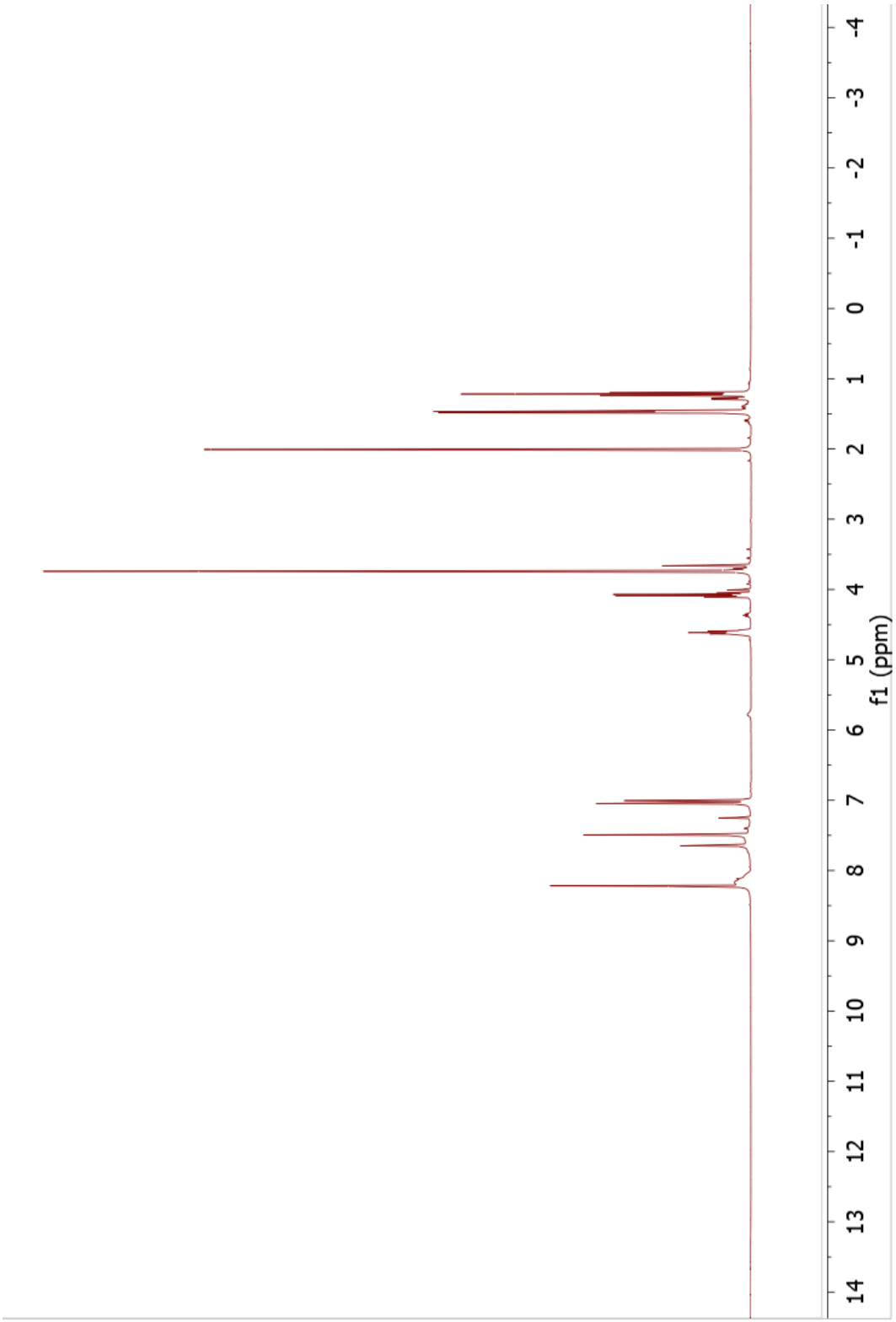
Methyl (1H-imidazole-1-carbonyl-L-alaninate) (20) ^{13}C NMR in CDCl3. JEOL101MHz



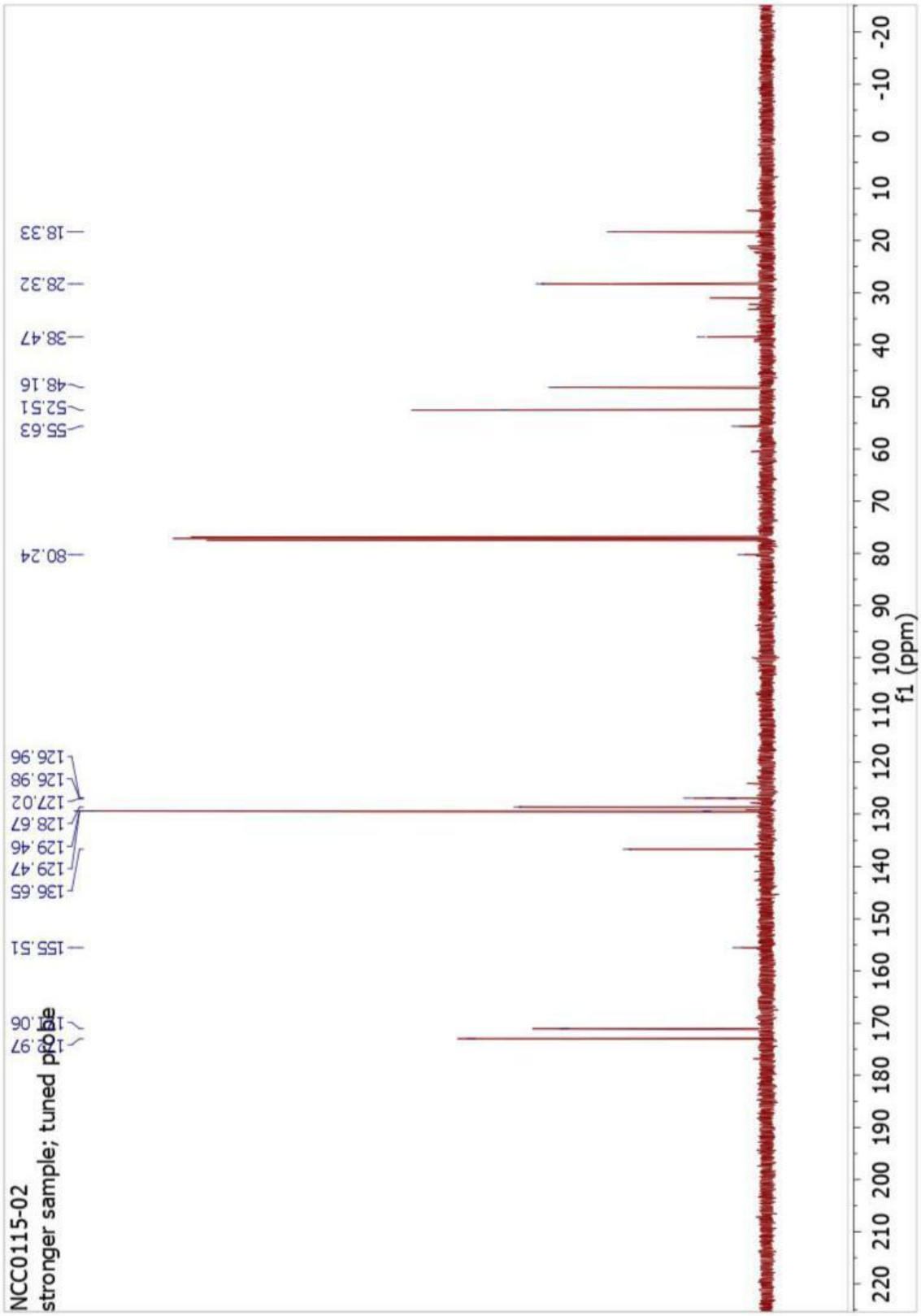
Methyl (1H-imidazole-1-carbonyl-L-alaninate) (9) [13] IR (neat)



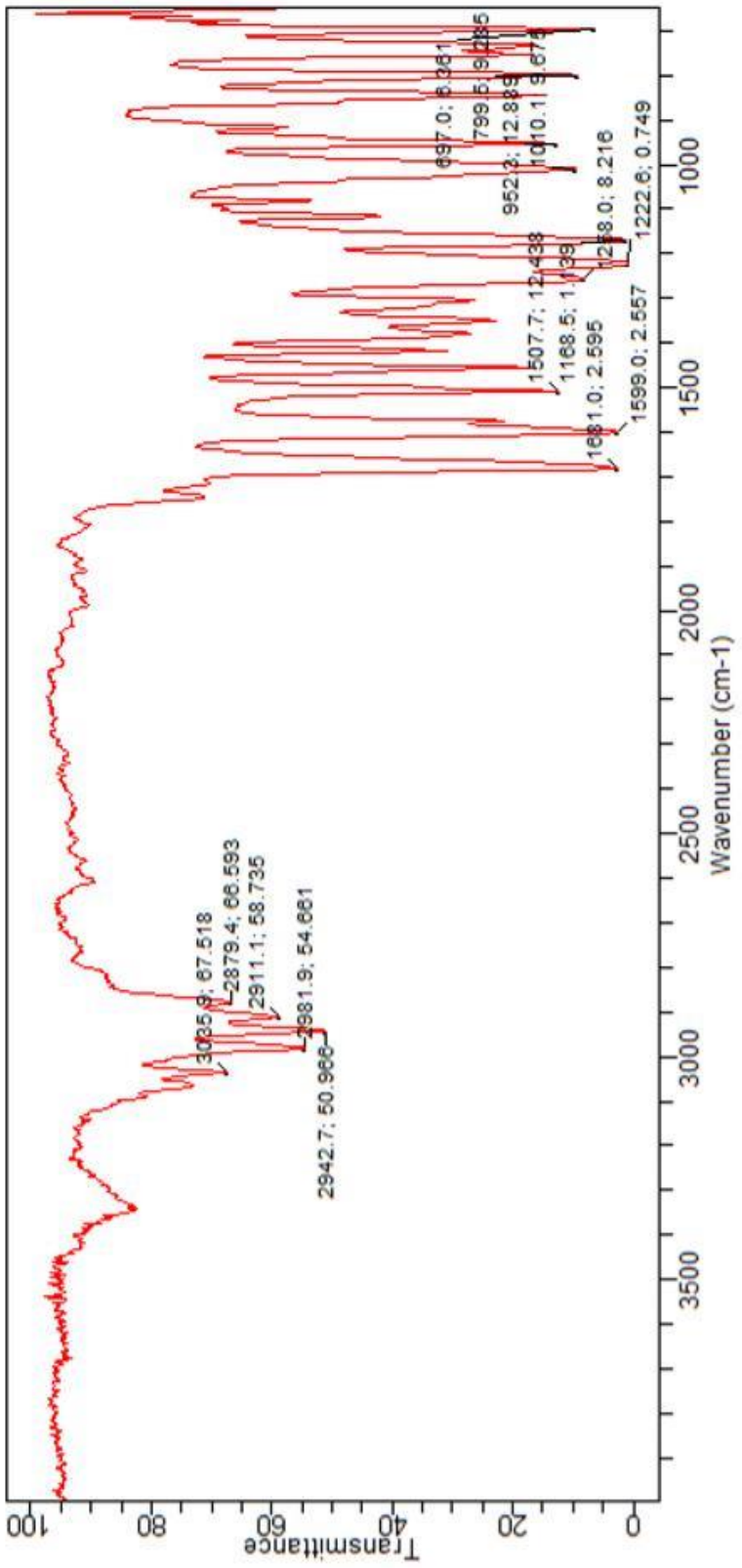
Methyl (tert-butoxycarbonyl)-L-phenylalanyl-L-alaninate (10) ^{1}H NMR in CDCl3. JEOL400MHz



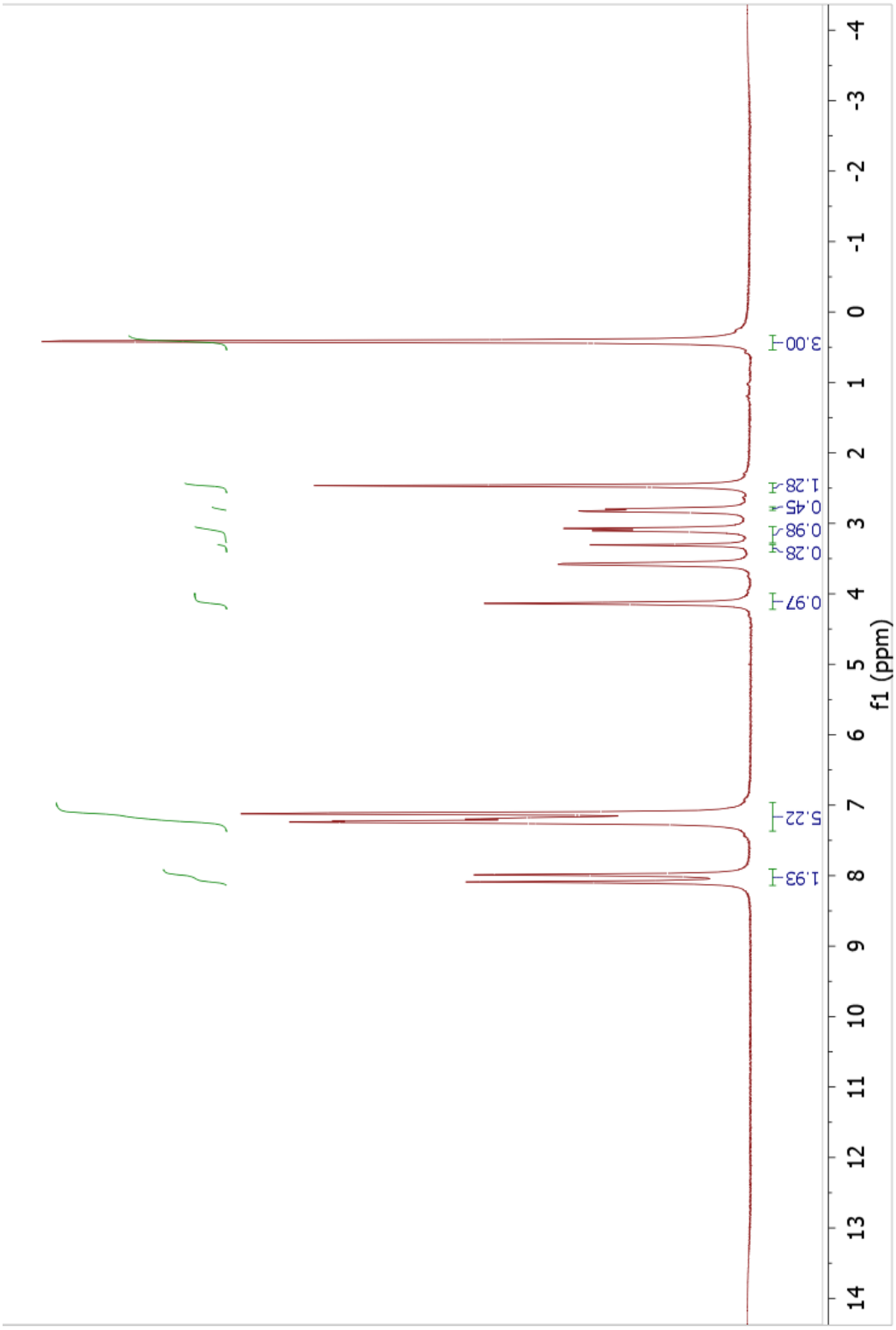
Methyl (tert-butoxycarbonyl)-L-phenylalanyl-L-alaninate (10) ^{13}C NMR in CDCl3. JEOL101MHz



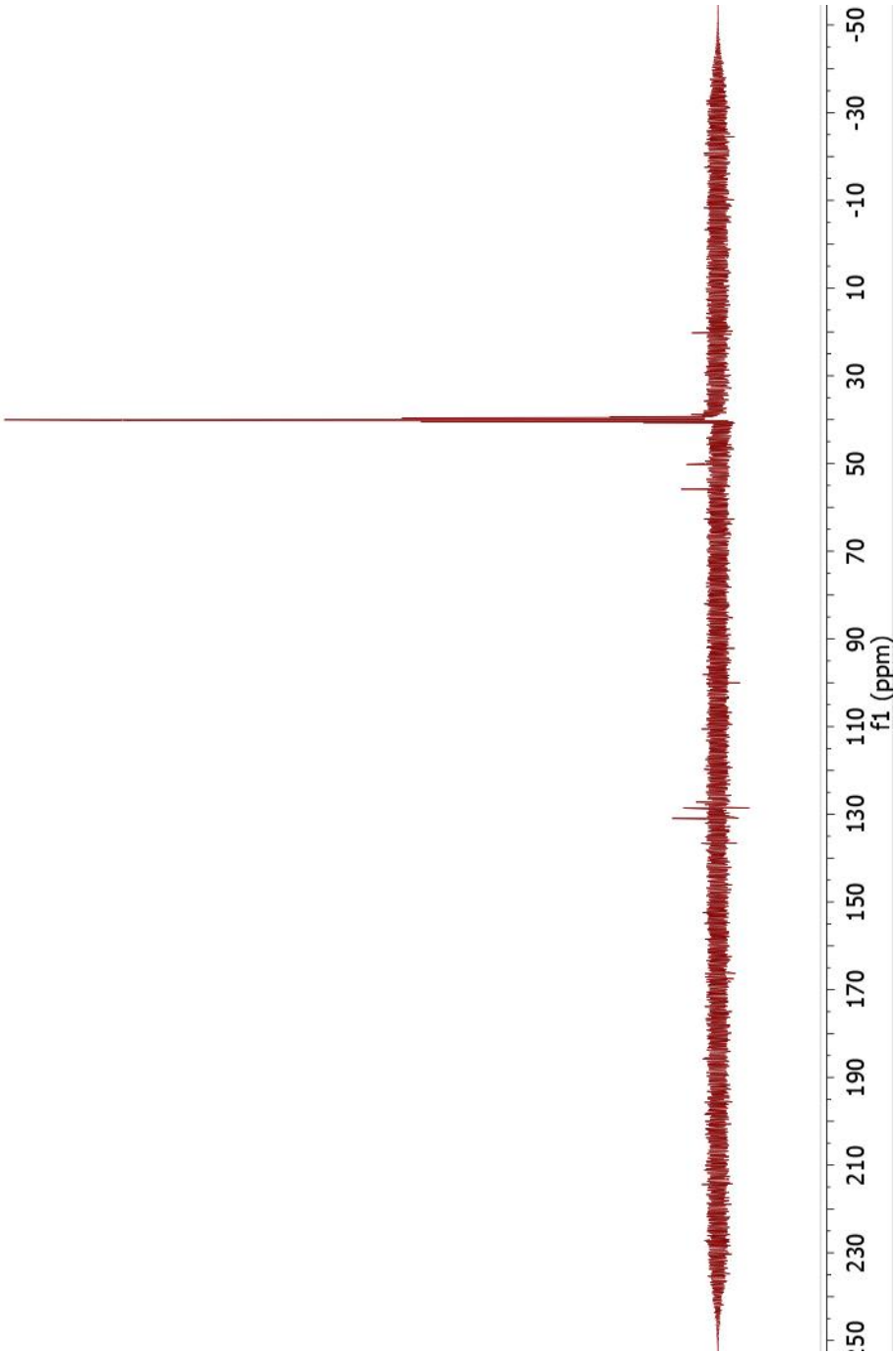
Methyl (tert-butoxycarbonyl)-L-phenylalanyl-L-alaninate (10)[39] IR (neat)



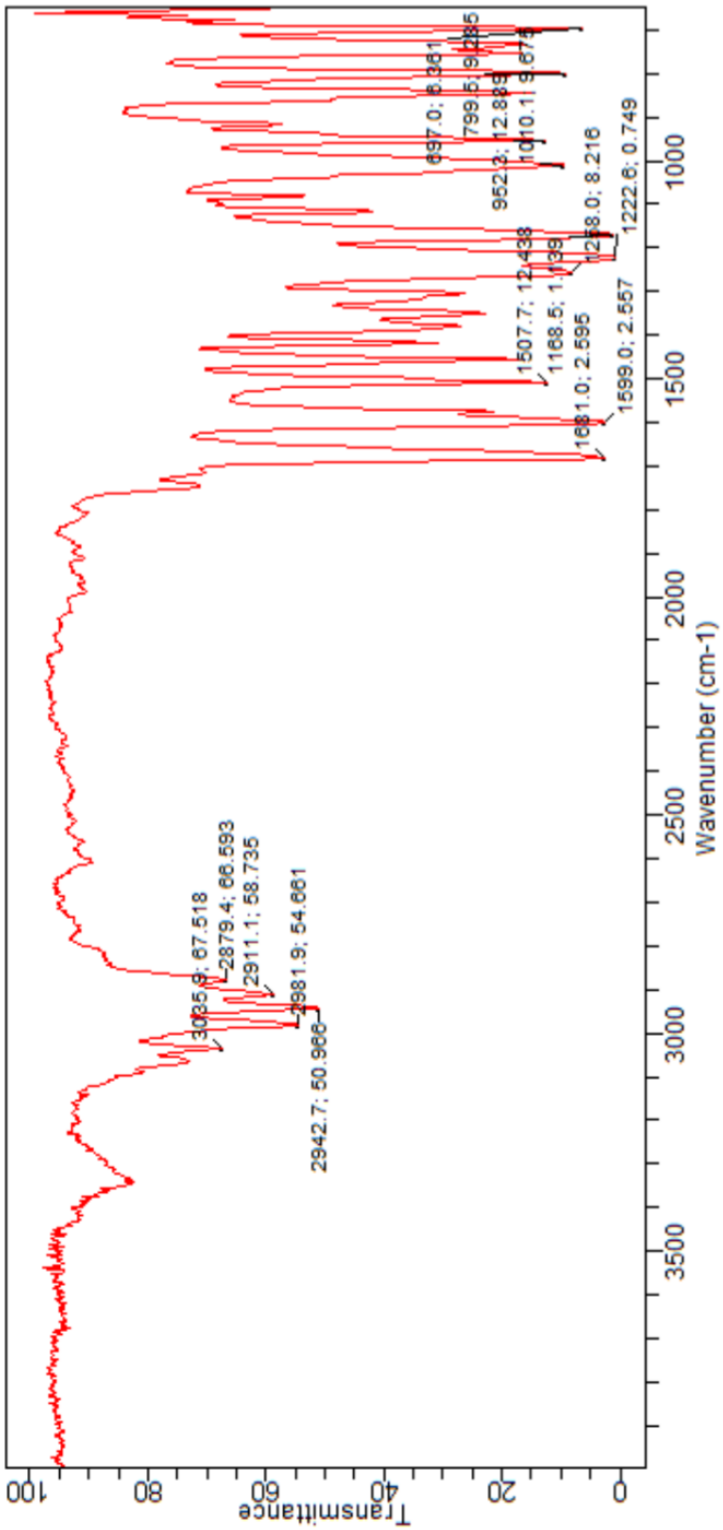
(3S,6S)-6-benzyl-3-methyl-2,5-diketopiperazine (6)^{401}H NMR in DMSO-d$_6$. JEOL400MH



(3S,6S)-6-benzyl-3-methyl-2,5-diketopiperazine (6)[40] ^{13}C NMR in DMSO-d_6. JEOL101MH



(3S,6S)-6-benzyl-3-methyl-2,5-diketopiperazine (6)[40] IR (neat)



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