

Studies of polymer-stabilized bimetallic nanoclusters and synthesis of substrate peptides for
electronic chemical biosensors

by

Zhaoyang Ren

B.S., China Agriculture University, 2019

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

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Department of Chemistry
College of Arts and Sciences

KANSAS STATE UNIVERSITY
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Abstract

Two research projects were conducted and they consist of catalytic oxidation of C-H bond utilizing bimetallic nanoclusters and synthesis of substrate peptides for electronic chemical detection of overexpressed proteases in breast cancers.

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In the second project, a series of polypeptides were designed, synthesized and attached to a ferrocene moiety in one end and a H₂N-(CH₂)₄CO linker in the opposite end. They were used to profile overexpressed proteases in breast cancers using electrochemical technique. Proteases MMP-9 and cathepsin B were investigated, since they are overexpressed in breast cancers, particularly triple-negative breast cancer (TNBC). The quantification and profiling of proteolysis rates of a group of overexpressed proteases will provide a patent of proteolytic activity. This in turn may allow the recognition of the presence and progression of a specific cancer such as TNBC.

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Approved by:

Major Professor
Dr. Duy Hua

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Dedication

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Papers Published During the Program of Study

During the study of the degree, the following academic papers have been published in which I am a co-author:

1. Fan, H., Tong, Z, Ren, Z., Mishra, K., Morita, S., Edouarzin, E., Gorla, L., Averkiev, B., Day, V. W., Hua, D. H., Synthesis and Characterization of Bimetallic Nanoclusters Stabilized by Chiral and Achiral Polyvinylpyrrolidinones. Catalytic C(sp³)-H Oxidation. *J Org Chem*, **2022**. 87(10): p. 6742-6759[1]

Partial results published in this publication will be discussed in Chapter 1 and 2.

2. Anderson, M.J., Song, Y., Fan, H., Wright, J. G., Ren, Z., Hua, D. H., Koehne, J. E., Meyyappan, M., Li, J., Simultaneous, multiplex quantification of protease activities using a gold microelectrode array. *Biosens Bioelectron*, **2020**. 165: p. 112330.[2]
3. Song, Y., Wright, J. G., Anderson, M. J., Rajendran, S., Ren, Z., Hua, D. H., Koehne, J. E., Meyyappan, M., Li, J., Quantitative Detection of Cathepsin B Activity in Neutral pH Buffers Using Gold Microelectrode Arrays: Toward Direct Multiplex Analyses of Extracellular Proteases in Human Serum. *ACS Sens*, **2021**. [3]

Partial results published in the second and third paper will be discussed in Chapter 3.

4. Cooper, A.M., Cooper, A. M., Song, H., Yu, Z., Biondi, M., Bai, J. Shi, X., Ren, Z., Weerasekara, S. M., Hua, D. H., Silver, K., Zhang, J., Zhu, K. Y., Comparison of strategies for enhancing RNA interference efficiency in *Ostrinia nubilalis*. *Pest Manag Sci*, **2021**. 77(2): p. 635-645.[4]
5. Menuet, E.M., Zhou, J., Tian, S., Brenner, R. E., Ren, Z., Hua, D. H., Kilway, K. V., Moteki, S. A., Chirality-driven self-assembly: application toward renewable/exchangeable resin-immobilized catalysts. *Org Biomol Chem*, **2022**. 20(21): p. 4314-4319.[5]

Results published in publications 4 and 5 are not discussed in this dissertation.

Chapter 1 - Synthesis and Characterization of the Chiral Substituted Polyvinylpyrrolidones and Bimetallic Nanoclusters

1.1 Background and Significance

Catalysts is a type of substance that can facilitate reaction rates by lower the reaction energy barriers to achieve the goal under mild reaction conditions. Traditional C-H activation reactions usually require a stoichiometric amount of acid, base or reagent, which leads to extensive work-up procedure and greater amounts of byproducts. We envision functionalization of an inert C(sp³)-H bond among numerous C-H bonds in a complex molecule, can be achieve by careful design of bimetallic nanocluster catalysts. Recent years, there are many publications reported this subject using transition metals catalysts[6]. The use of transition metal nanoclusters is a less explored topic.

Metals can be divided into three groups: bulky metals, nanoparticles (less than 100 nm) and nanoclusters (1-10 nm) [7]. Although the materials are the same, the size of nanoclusters is so small making the quantum phenomena starts to affect and the band structure of the metal becoming discrete energy levels[8], therefore providing unique property in catalytic[9], biological [10] and optical properties[11]. The nano-sized metal nanoclusters have large surface areas, which make them useful and popular catalysts Especially some metals have little catalytic reactivity when they are in bulk, however when the size is reduced to nanoparticle level, they show greatly enhanced reactivity, such as Au nanoparticles [12]. For the reasons mentioned above, transition metal nanoclusters act as important role in many research areas and in industry. Monometallic nanoclusters possess high reactivity due to their nano-size and various oxidation reactions have been reported, such as catalytic oxidation of alcohols and cycloalkanes. The concept of adding another metal to the mono metal catalyst makes it a bimetallic catalyst, which

may increase the catalytic property of the metal(s). In addition, interactions between the two metals may change the activity of the active metal in the catalyst. Therefore, bimetallic metal nanoparticles attracted our attention.

Bimetallic nanocluster is not simply adding two metals together to make it a bimetallic catalyst, but selective selecting two metal salts then co-reducing them to afford a mixture of alloys. Alloying is by introducing another element into a metal, which often brings out interesting changes of the properties [13]. This may improve catalytic properties of the metals by specific electronic interaction between the two metals, which changes the overall structures of the active components in the catalyst. In conclusion, the addition of another metal to the monometallic nano-catalyst may not only enhance the catalytic properties (a cooperative effect, the alloy effect and synergistic effect) but also increase their interactions with the substrate.

The two metals in bimetallic nanoclusters should be specifically picked to give enhancement of reaction rate. The standard reduction potential of the Cu is + 0.34 V, Pd + 0.915 V, Au + 1.40 V[14]. [14]. Bimetallic nanoclusters containing gold as the core metal will enhance the reactivity of the shell metal, such as copper or palladium, since gold has the highest electron negativity, 2.54, in all elements. Au metallic nanoparticles have high surface activity [15, 16]. Therefore, Cu/Au and Pd/Au two alloy systems were selected for the study to achieve our goals.

Stabilizers can be applied to prevent the aggregation of metals. In the nanostructured catalysts, polymers are widely used as the supports for stabilizing the nanoclusters [17]. Selected polymers have large surface area and can dissolve in both aqueous and organic solvents, making them promising stabilizers [18]. The functional group in branches of the polymer can help to complex the metal and can allow more active centers exposing to the substrate [19]. Polyvinylpyrrolidone (PVP) as an amphiphilic non-ionic polymer[20], possessing pyrrolidone

branch and ethylene backbone, its small branch, good stability and its excellent solubility in both water and organic solvents, serves as a useful stabilizer and it is commonly used in the synthesis of nanoclusters[21]. With PVP as the chosen stabilizer in mind, a design for achieving stereoselectivity in the catalytic oxidation reactions was made. If a stereogenic center is introduced into the pyrrolidinone ring of PVP, the modified PVP supported nanoclusters may provide a certain degree of stereo- and/or enantio-selectivity in oxidation reactions. Nanoclusters stabilized by chiral stabilizer can produced enantioselectivity and it has been reported from our laboratory previously[22],), so we tried to have chosen to study the most challenging C-H oxidation of complex molecules by the use of give attribute of chiral selective to our nanoclusters by chchirally modified PVP and bimetallic Cu/Au and Pd/Au nanoclusters along with various oxidants such as H₂O₂ and t-BuOOH..

The properties of bimetallic nanoclusters may affect by factors, such as length of the polymer, reducing agent in the reduction of metal salts to generate nano-metals. These factors will be discussed in the following sections after the synthesis of the chiral polymers.

1.2 Synthesis of the Chiral Substituted Polyvinylpyrrolidones

A group or groups can be added to PVP molecules in three different positions – indicated by arrows - to produce chiral PVPs as illustrated in Figure 1. Our goals are to synthesize chiral PVPs and use them to prepare chiral bimetallic nanoclusters for catalytic oxidation reactions, which may show enantioselectivity. Hence, we designed four chiral polymers by adding a group or groups separately at C3, C4, and C5 of PVP.

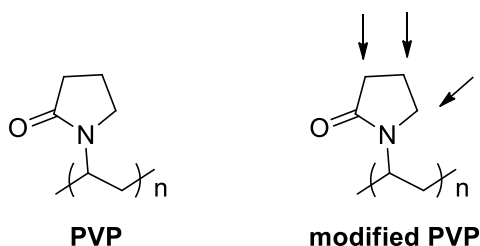


Figure 1 PVP and Its Possible Modified Position

The series of chiral substituted polyvinylpyrrolidones **1** – **4** have been synthesized in our laboratory and they were used in various oxidation reactions (Figure 2).

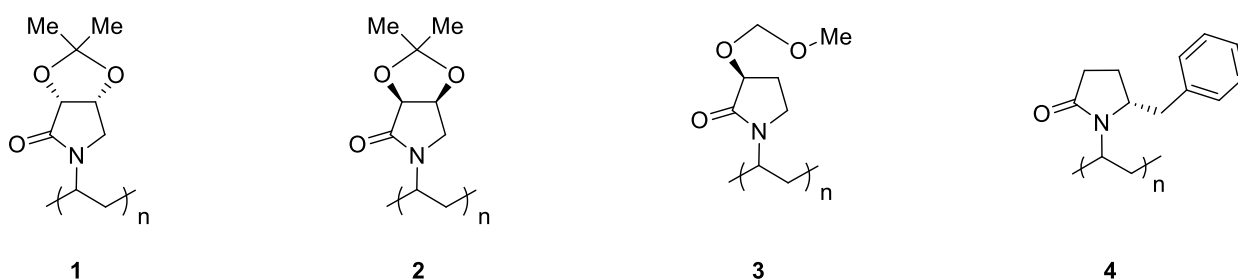


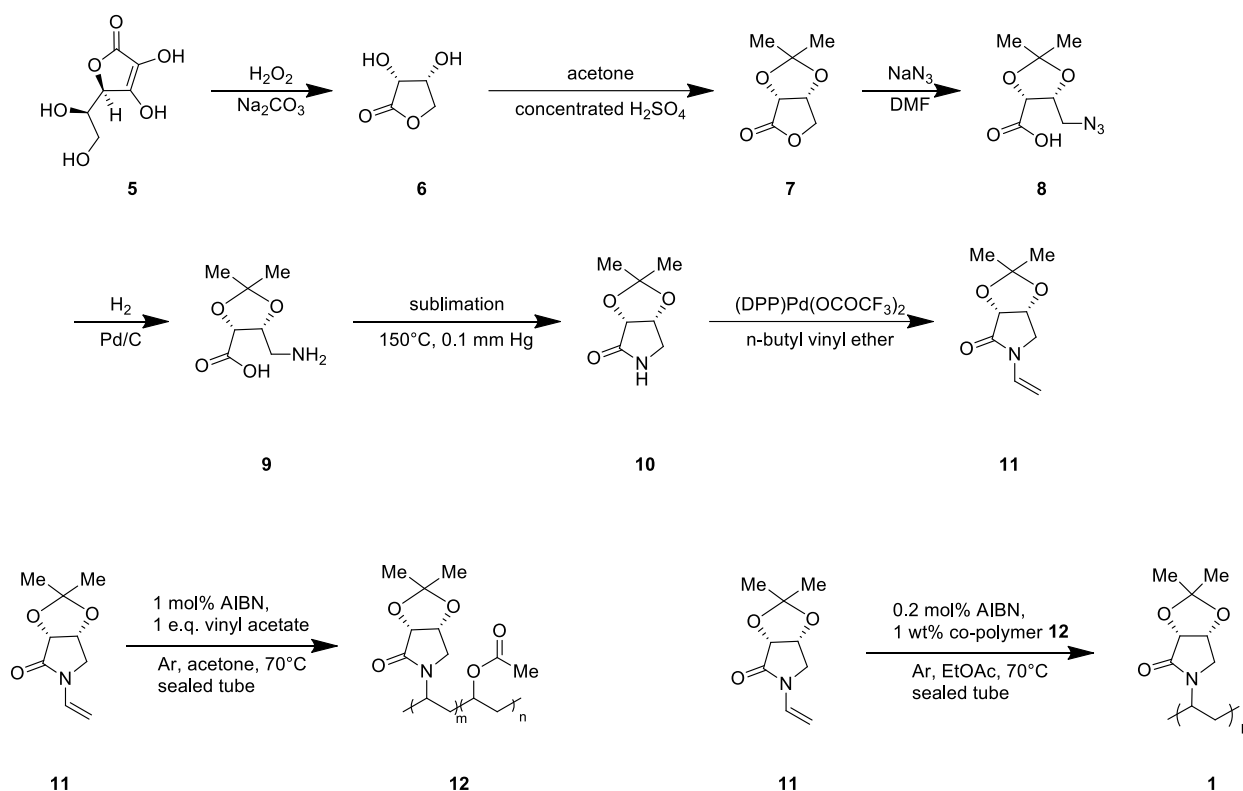
Figure 2 Designed Polymers (CSPVP 1, CSPVP 2, CSPVP 3, CSPVP 4)

Synthetic procedures leading to these four classes of chiral polymers are different, and synthesis of polymer **4** has been described previously in paper [23]. Herein, syntheses of chiral polymers **1** – **3** are described and polymer **4** was also made for comparison in the oxidation reactions.

1.2.1 Synthesis of the Chiral Substituted Polyvinylpyrrolidones **1**

The synthesis of chiral polymer **1** was carried out by several members in Hua group and I contribute to producing it in sufficient quantity for the oxidation reactions. The synthesis started from D-isoascorbic acid (**5**) (Scheme 1). It was oxidized into dihydroxy lactone **6** using hydrogen peroxide and sodium carbonate to give 93% yield of the lactone, which was protected with acetone to give acetonide product **7**. Ring opening reaction of **7** using sodium azide gave azido-acid **8** as the major product. After that, azido-acid **8** was reduced by Pd/C under hydrogen atmosphere (30 psi) in methanol to give the corresponding amino acid, which upon sublimation under high vacuum

at 130 °C to give cyclized amide **10**. Lactam **10** was used to produce vinyl lactam **11** by reacting with distilled n-butyl vinyl ether in the presence of a catalytic amount of 4,7-diphenyl-1,10-phenanthroline palladium bis(trifluoroacetate) [(DPP)Pd(OCOCF₃)₂]. N-Vinylpyrrolidone **11** serves as the precursor for the preparation of CSPVP-1 (compound **1**). We first prepared copolymer **12** by conducting a free-radical copolymerization of N-vinylpyrrolidone **11** and vinyl acetate, since this copolymer is needed as a surfactant in assisting the block polymerization process as described below.

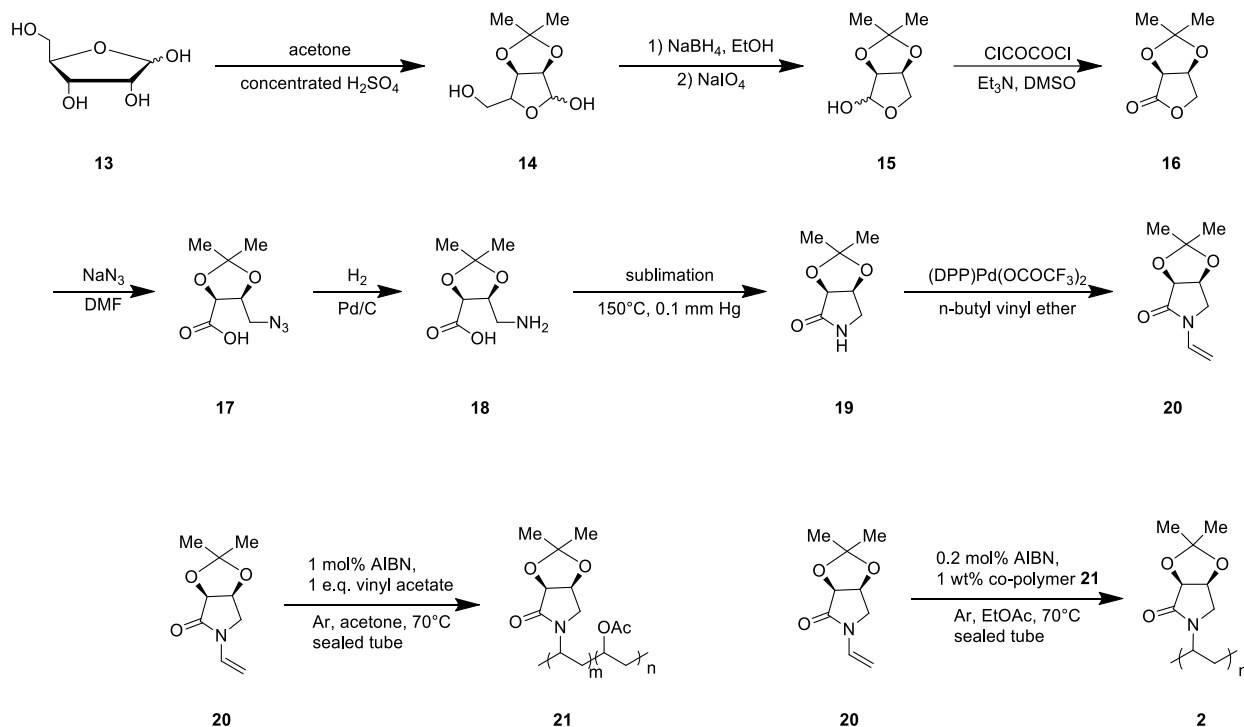


Scheme 1 Syntheses of Vinyl Lactam **11 and CSPVP-1**

1.2.2 Synthesis of the Chiral Substituted Polyvinylpyrrolidones **2**

Similar to the described method above, synthesis of chiral polymer **2** was carried out starting from D-(-)-ribose (**13**), and the synthetic sequence is shown in Scheme 2. The D-(-)-ribose was treated with acetone and catalytic amount concentrated sulfuric acid to give acetone **14**.

Compound **14** was reduced by sodium borohydride followed by oxidative cleavage with sodium periodate to give a mixture of stereoisomers **15**. With pure compound **15** at hand, a Swern oxidation was conducted to convert alcohol **15** to lactone **16**. The following steps were similar to the procedures described in polymer **1**. The lactone ring on compound **16** was opened by the treatment with sodium azide to give compound **17**, which was reduced by 30 psi H₂ and 5% Pd over carbon to give amino acid **18**. Compound **18** was heated in a sublimator to give chiral pyrrolidone **19**. Vinylation of compound **19** with *n*-butyl vinyl ether and a catalytic amount (DPP)Pd(OCOCF₃)₂ yielded polymer precursor **20**. Copolymerization with vinyl acetate and polymerization of compound **21** gave chiral polymer **2**. All polymerization reactions were conducted under nitrogen or argon system in the absence of oxygen. The presence of oxygen can terminate the polymerization chain reactions.

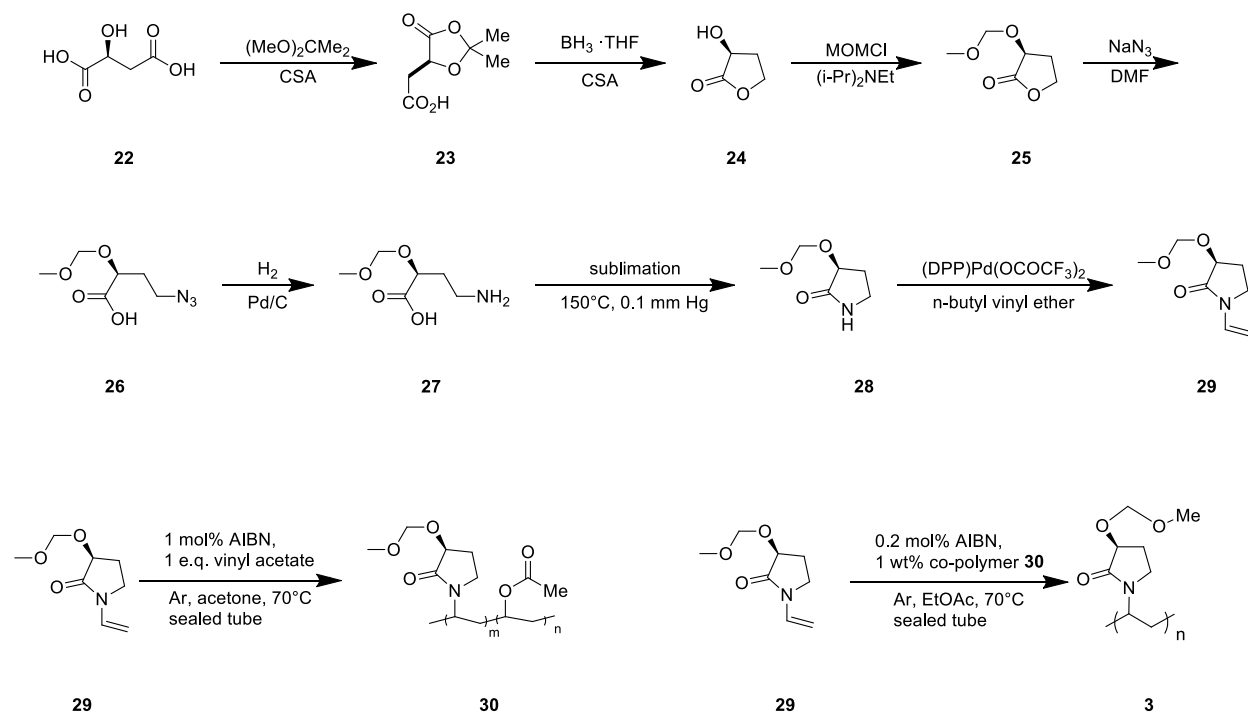


Scheme 2 Syntheses of Vinyl Lactam 20 and CSPVP-2

The design of polymer **2** is to provide a mirror-imaged molecule of polymer **1** so that the chiral selectivity of the chiral-stabilized nano-catalysts can be compared based on the stereoselectivity of the products obtained from subsequent oxidation reactions.

1.2.3 Synthesis of the Chiral Substituted Polyvinylpyrrolidones 3

The synthetic route of polymer **3** is shown in Scheme 3. The reactions of compound **22** - **24** were carried out by me by following a previously reported method[23]. L-(S)- malic acid (**22**) was treated with 2,2-dimethoxypropane, D-10- camphorsulfonic acid to give methyl protected form compound **23**. The compound **23** was later reduced by $\text{BH}_3 \cdot \text{THF}$ to give (S)-3-hydroxydihydrofuran-2(3H)-one (**24**). The rest of the steps were carried out by our graduate student Edrue Edouarzin.



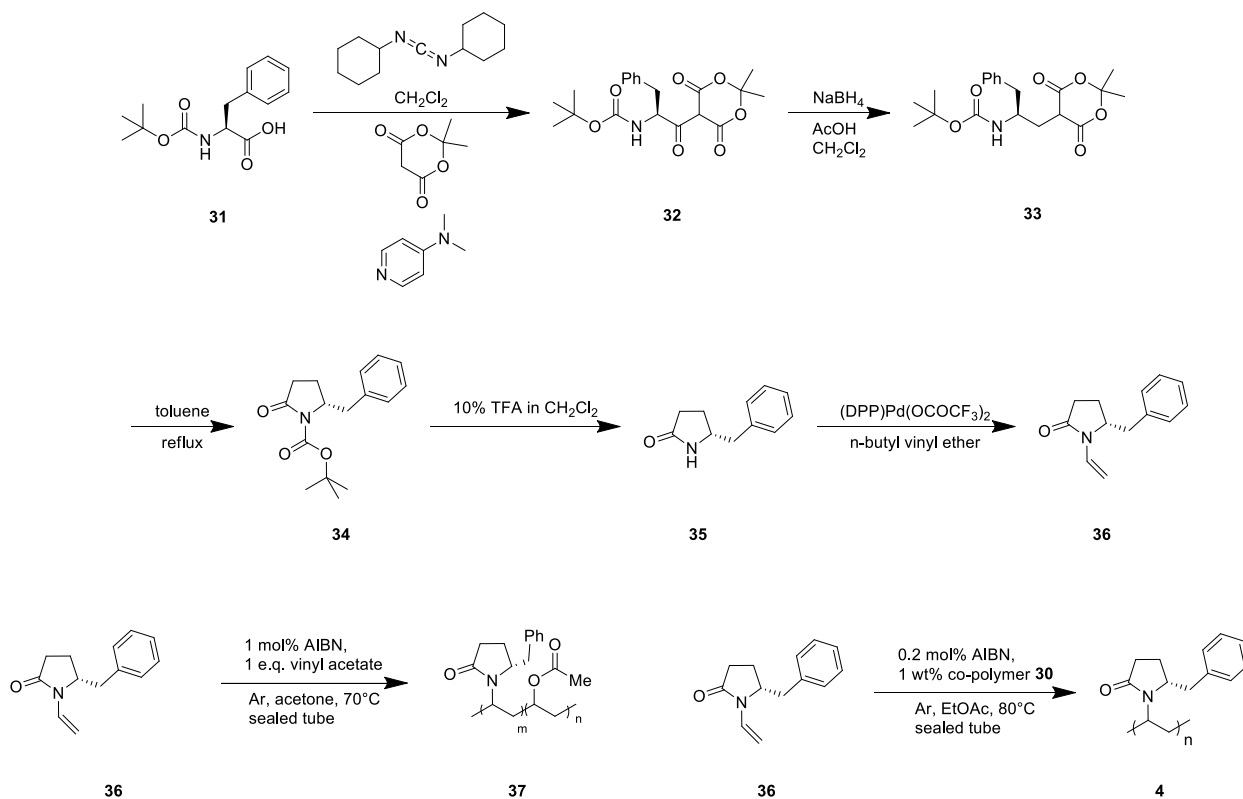
Scheme 3 Syntheses of vinyl lactam **29** and CSPVP-**3**

This polymer was designed as C3 substituted polymer to examine the induced stereochemistry and reactivity between different polymers. In terms of the solubility, polymer **3** possess the best solubility comparing to CSPVP **1**, **2** and **4**.

1.2.4 Synthesis of the Chiral Substituted Polyvinylpyrrolidones **4**

The synthetic route CSPVP **4** is shown in Scheme 4. This polymer was the original work performed by previous students in Hua's laboratory and it has been published in the JACS paper in 2016[23]. The reaction started with *N*-(tert-butoxycarbonyl)-L-phenylalanine (**31**) coupling with *N,N'*-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) to give compound **32**. The ketone function of compound **32** was reduced using sodium borohydride to give compound **33** then followed by a cyclization protocol to afford **34**. The protecting group of compound **34** was removed using TFA in dichloromethane to give compound **35**. To achieve vinylation, compound **35** was treated catalyst (DPP)Pd(OCOCF₃)₂ and *n*-butyl vinyl ether to give vinyl derivative **36**. Copolymer **37** was prepared by conducting a free-radical copolymerization of *N*-vinylpyrrolidone **65** with degassed vinyl acetate. The polymerization of compound **4** was

conducted by other members in Hua's laboratory.



Scheme 4 Syntheses of Vinyl Lactam 36 and CSPVP-4

This polymer was synthesized for the purpose of comparison of the presently designed polymers in their oxidation reactions. Comparing polymerization step of four polymers, polymer **4** required a higher temperature and longer time for its formation, possibly because of the C5-bulky benzyl functional group.

1.3 Preparation of Chiral Bimetallic Nanoclusters

One of the important factors about the nanoclusters is the cluster sizes and diverse distribution of different ratios of metal atoms in the clusters, which means the chosen method to prepare the bimetallic nanoclusters is important and reproducibility of the catalysts. In general, bimetallic nanoclusters are prepared fresh by using a chemical reduction method as described below[24]. The advantage of this method is that the nanoclusters can be obtained in a one-pot

reaction, and the process of the reaction is simple, convenient and fast[25].

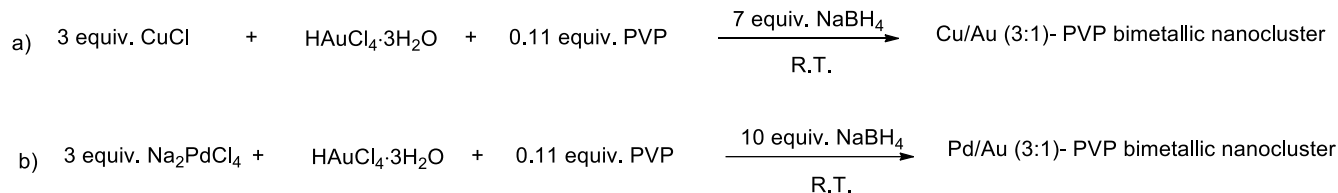


Figure 3 Preparation of Bimetallic Nanoclusters

Firstly, 0.11 equivalent amount of PVP or modified CSPVP with different molecular weights (MWs were determined using HRMS as described below) was dissolved in deionized water or distilled DMF (over CaH_2 under reduced pressure) as a stabilizer, then 3 equivalent amount of CuCl or Na_2PdCl_4 metal salt was added, then 1 equivalent amount of HAuCl_4 was added while stirring, and finally the corresponding amount of NaBH_4 was added as reducing agent to reduce both metal elements to 0 valence state, the color of the suspension will rapidly become deep dark color with the addition of reducing agent, and a homogeneous mixture of bimetallic nanoclusters was obtained then the nanoclusters were stirring for two hours at room temperature with no precipitation found.

For the Cu/Au mixture before dropping in the reducing agent NaBH_4 , CuCl presented a white powder suspended in the solution and HAuCl_4 presented a golden yellow solution, after the reducing agent was added, as the metal salt was gradually reduced to the metal, the metal mixture gradually formed nanoclusters with PVP and the mixed solution turned into a homogeneous dark reddish brown color. For the Pd/Au mixture before the drop of reducing agent NaBH_4 , Na_2PdCl_4 and HAuCl_4 showed a light total color solution, after the addition of reducing agent, as the metal salt was gradually reduced to metal, the metal mixture gradually formed nanoclusters with PVP and the mixed solution turned into a homogeneous dark brown-black color.

The ratio between PVP and metal, and between metal and metal was obtained from the previous comparative study[23], the results shows with different molar ratios of the reactants will affect the final size of the catalyst, and the bimetallic nanoclusters at 3 : 1 ratio have relatively better catalytic activity and selectivity. In the experiments that remove polymers as stabilizer, adding only two metal salts and then reducing them, black particles settled directly on the bottom of the vessel and the particles have no catalytic activity, which indicating that polymer are essential for the formation of the nanoclusters and they function as stabilizer. When the stabilizer is added to the metal salts, but no reductant (NaBH_4) is added, the color of the solution will start to change, slightly from light yellow (the color of the gold solution) to brown after stirring for a while, but the color is lighter comparing to fully functioned nanoclusters that we made. The reason for this phenomenon is that the two metals form a synergistic effect and PVP itself also possess a weak reducing property. Catalytically active bimetallic nanoparticles are formed only when stabilizers, metal salts and reducing agents are present. The absence of precipitates in the formed homogeneous dispersion mixture indicates that the presence of the stabilizer prevents the zero-valent metal from settling due to aggregation, so the polymer also acts as a dispersant in the bimetallic nanoclusters. The homogeneous solution was darkened by the concentration, but still no precipitation was observed, indicating that the bimetallic nanoclusters could be formed in both water and DMF with good solubility. The prepared nanoclusters can be stored at room temperature for several months without precipitation, and the catalytic activity was good, indicating their good stability.

1.4 Characterization of the Chiral Substituted Poly Vinylpyrrolidones and PVP Stabilized Bimetallic Nanoclusters

Chiral polymers 1 -3 were characterized by ^1H and ^{13}C NMR spectroscopy and circular dichroism (CD) spectroscopy. Their molecular weights were determined by high-resolution mass spectrometry (HRMS). The bimetallic nanoclusters were characterized by several instrumentation including circular dichroism (CD), dynamic light scattering (DLS), gel permeation chromatography (GPC-HPLC) and HRMS. Because it is hard to form uniform bimetallic nanoparticles, methods can be used to investigate the length of the polymer, the size and chirality of the nanoclusters.

1.4.1 Determination of Molecular Weights of Chiral Polymers by Gel Permeation Chromatography

Molecular weights of the polymers were measured by gel permeation chromatography (GPC) using a Shimadzu UFLC-20AD-HPLC system equipped with a ELSD-LTII (evaporative light-scattering) detector along with UV and RI detectors. Two connected size-exclusion columns, TSKgel α -400 (7.8 mm I.D. x 30 cm; 10 μm) and TSKgel α -M (7.8 mm I.D. x 30 cm; 13 μm) (from Tosoh Bioscience, Grove City, OH) were used with methanol as solvent with a flow rate of 1 mL/min. The commercial available PVPs with different length: 40,000 Dalton, 58,000 Dalton, and 360,000 Dalton molecular weights were used as a standard to give a linear equation that reveals the relativity of the retention time (X axis) and molecular weight (Y axis). Then the designed polymers (CSPVP **1**, **2**, **3**) synthesized by heat and by UV irradiation were also inject to the GPC-HPLC, then according to the retention time, the equation we got from the previous result can give us the idea of the molecular weights of polymers. The results were summarized in **Table 1**.

The results showed the polymers synthesized by heat and by UV will give different retention times which means the length of the polymer are different. The polymers we made by heat should be around 20-24k Dalton. However, CSPVP-1R and CSPVP-1S were made under same conditions and the same amount of the AIBN, the polymer length and molecule weight should be very similar. Also, the CSPVP-2 was also set up exact conditions, the weight of the polymer should vary from 20K to less than 10 K. With previous results, we know the solubility of the CSPVP-2 has good solubility towards solvents, and polymers tend to swell towards solvent that them can well dissolved in but shrink in the solvent that they have poor solubility in. So the question was asked: Is GPC-HPLC a good system to analyze the molecular weight of designed polymer? Could be the solubility and the shape of the polymer also play important roles in retention time. So another experiment was done, this time we remove the two methyl group of the CSPVP-1R then injected into GPC-HPLC. This time the retention time move to latter for three minutes. With same back bones of the polymer, by only remove the methyl groups, the retention time changes. Therefore, we decided to explore alternative methods to obtain reliable results for the polymer, leading us to turn to HRMS as a solution.

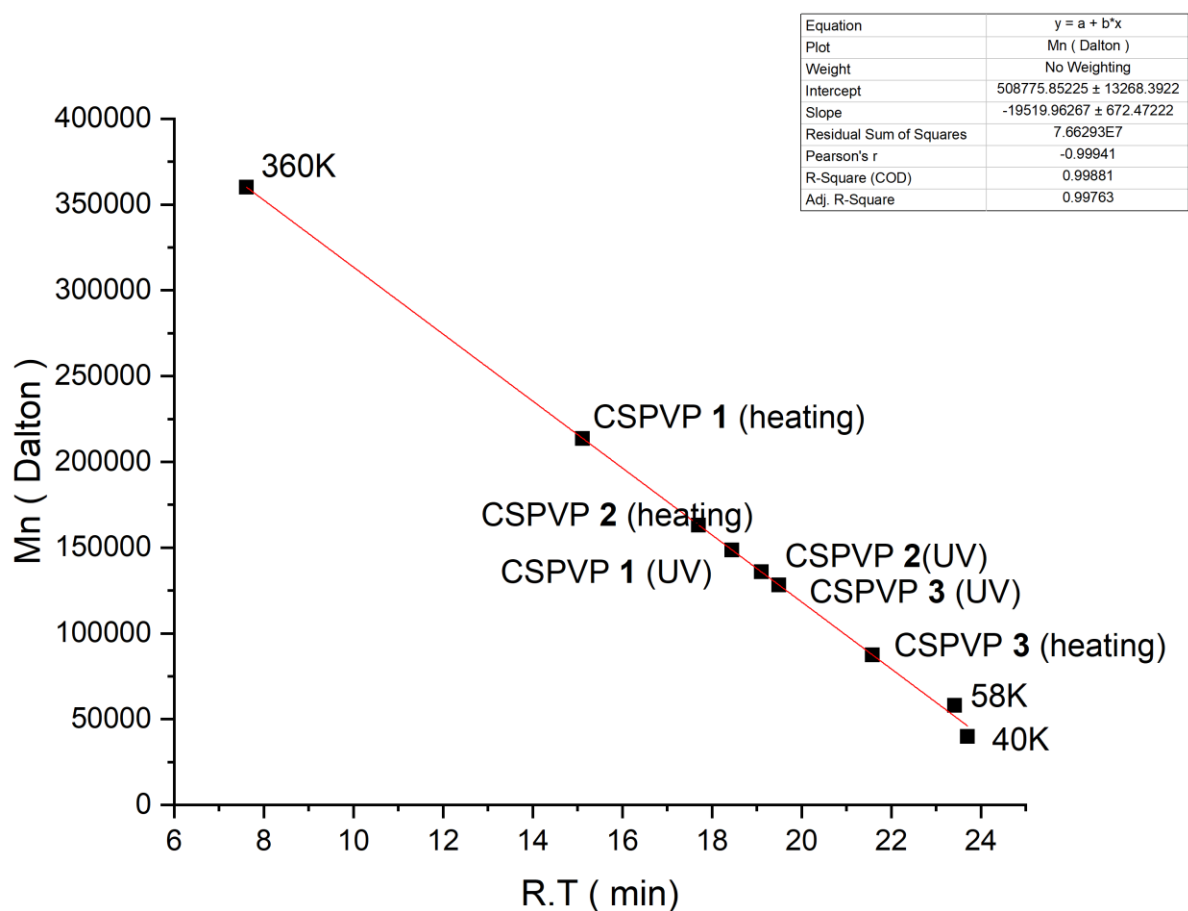


Figure 4 HPLC Chart for Molecular Weight Determination of Polymer 1, 2, 3

Table 1 Molecular Weights of PVPs and CSPVPs

Polymers	Mn (Dalton)	RT(Mn)/min	Mw (Dalton)
40K PVP	40,000	23.701	67,000
58K PVP	58,000	23.413	62,000
360K PVP	360,000	7.616	367,000
CSPVP-1, prepared by heating	213,683	15.109	243,000
CSPVP-1, prepared by UV	148,669	18.443	179,000
CSPVP-2, prepared by heating	163,177	17.699	206,000
CSPVP-2, prepared by UV	135,818	19.102	166,000
CSPVP-3, prepared by heating	87,477	21.581	99,000
CSPVP-3, prepared by UV	128,154	19.495	143,189

1.4.2 Molecular Weight Determination by High-Resolution Mass Spectrometry

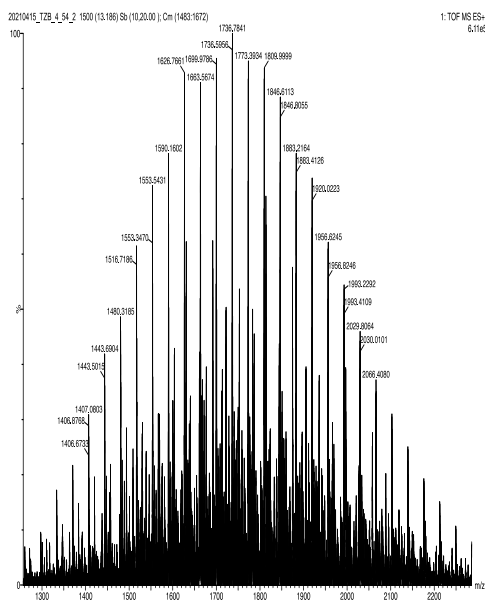
We found the solubility and structure difference may cause the incorrect measurements of polymers[26], so HRMS comes to our mind. Therefore, we turned to HRMS to determine the molecular weights. HRMS method can provide a real insight of the molecular weight of the polymer, instead of providing the circumstantial proof of average weight of the polymer, it can show the detected mass on the molecule level[27]. So we analyzed the same samples we analyzed by GPC-HPLC. All the samples were dissolved and filtered to give 1.5 mg/mL concentration in the ratio of 3 :1 in the solution of water and acetonitrile. The samples were injecting separately into solution into Xevo G2-XS QToF Quadrupole Time-of-Flight Mass Spectrometry, equipped with an ultra-performance liquid chromatography (UPLC).

A polymer sample has a distribution of polymer length, and a demonstration of data interpretation is shown in Figure 4. Since the electrospray ionization (ESI) method is utilized, multiple charge states are present.

The polymer weight was determined by GPC-HPLC only one way to provide polymer's mass. Due to structural, swelling ratio and solubility difference between the polymers[26], it may not provide a reliable data. Therefore, we turned to high-resolution mass spectrometry (HRMS). High resolution mass spectrometry method was used for absolute polymer molecular weight measurement on molecule level rather than an average of the whole polymer sample[27]. The polymer samples were prepared to a concentration of 1.5mg/mL in acetonitrile and D.I. water (3:1) then filtered before injecting 5 μ l solution into Xevo G2-XS QToF Quadrupole Time-of-Flight Mass Spectrometry equipped with an ultraperformance liquid chromatography (UPLC).

A polymer sample has a distribution of polymer length, and a demonstration of data interpretation is shown in Figure 3. The interpretation of the data is shown below. A polymer is distribution of the chain unity, there for when a polymer, therefore a typical polymer mass spectrum should have a distinct pattern of different ionized polymer mass[28]. As shown in Figure 5, it showed a typical pattern for the CSPVP 1.

Figure 5 A typical Mass Spectrum of Chiral Polymer 1.



The detected mass indicated different ionized level of the polymer gave different mass and based on calculation the ionized level and the mass of the polymer can be calculated form the pattern. Therefore the following equation can be applied[28]:

The first two mass in the pattern were used to illustrate in equation 1:

Equation 1 Illustrative Information to Calculated M (mass) and n (charge of the ion on the polymer)

$$\frac{M + n}{n} = 1443.69$$

$$\frac{M + n + 1}{n + 1} = 1407.08$$

The logic behind the equation is as follows: when one ion is added to the polymer, the mass should be $[M+1]/1$, while with two ions added to the polymer, the detected mass should be $[M+2]/2$. This calculation can be repeated until the signal cannot be detected anymore. However, there is a limitation to the maximum mass in HRMS. To overcome this, we can use the mass from the multiple ionized polymers to calculate the average mass. Assuming unknown charges on the polymer, we derived that there were n charges on the ionized polymer to give a mass of 1443.69 and $(n+1)$ charges to give a mass of 1407.08. By solving the two equations for the ionized mass, we calculated a mass of 55,409 (M) and charges of 38 (n). Applying this method to the entire pattern, we were able to determine the molecular mass of the polymer, as shown in Table 2 and 3.

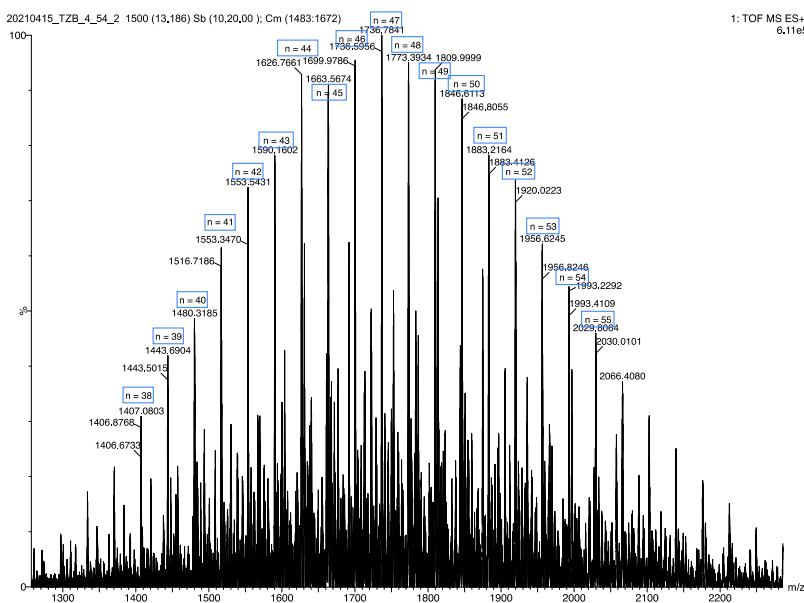
Table 2 Mass Spectrum of Chiral Polymer 1 Showing the n Values

mass peak	M value	Calculated n , number of charges	n , number of charges, after round up the calculated values
1443.6904	55409.26	38.40690	38
1407.0803			
1480.3185	58266.70	39.38753	39
1443.6904			
1516.7186	61599.57	40.64050	40
1480.3185			
1553.5431	63903.61	41.16060	41
1516.7186			
1590.1602	67379.44	42.39940	42

1553.5431			
1626.7661	70578.86	43.41267	43
1590.1602			
1663.5674	73447.02	44.17686	44
1626.7661			
1699.9786	77576.86	45.66087	45
1663.5674			
1736.7841	80125.53	46.16099	46
1699.9786			
1773.7841	83166.77	46.91308	47
1736.7841			
1809.9999	88551.58	48.95057	48
1773.7841			
1846.6113	91193.20	49.41083	49
1809.9999			
1883.2164	94900.43	50.41951	50
1846.6113			
1920.0223	98136.85	51.13898	51
1883.2164			
1956.6245	102531.73	52.42915	52

1920.0223			
1993.2292	106435.85	53.42550	53
1956.6245			
2029.8064	110501.83	54.46642	54
1993.2292			
2066.408	114484.42	55.42944	55
2029.8064			

Figure 6 A Typical Mass Spectrum of Chiral Polymer 1 with Labeled the Number of Ions



The selected sample pattern with labeled the number of ions is showed in Figure 6. Then with obtained data above, various molecular weight parameters can be achieved by using equations in Equation 2.

Equation 2 Molecular Weight Calculations

$$M_n = \frac{\sum n_i M_i}{\sum n_i}$$

$$M_w = \frac{\sum n_i M_i^2}{\sum n_i M_i}$$

$$M_z = \frac{\sum n_i M_i^3}{\sum n_i M_i^2}$$

$$M_{z+1} = \frac{\sum n_i M_i^4}{\sum n_i M_i^3}$$

$$I = \frac{M_w}{M_n}$$

M_i represents molecular weight of the ionized polymer, N_i represents the abundance of the monomer in obtained ionized polymer by dividing the mass unit of the monomer masss ($M_w = 183$), i represents polydispersity index. The results of the selected polymer are shown below in Table 4.

Table 3 Calculated M_n , M_w , M_z , M_{z+1} and I Values of Chiral Polymer 1

M_n	M_w	$I=M_w/M_n$
85,232.5236	89,051.47	1.044

Therefore, the M_n of CSPVP **1** is 85,232. Then the method was applied to all injected samples and results are shown in Table 5.

Table 5 Calculated Molecular Weights of PVPs and CSPVPs

	M_n	M_w
CSPVP 1 made by using 0.1% AIBN	130,512.28	135,323.93
CSPVP 1 made by using 0.2% AIBN	85,232.5236	89,051.47

CSPVP 1 made by using 0.4% AIBN	45,580.46	48,038.78
CSPVP 1 made by using 0.2% AIBN without co- polymer	61,269.70	70,944.24
CSPVP 2 made by using 0.2% AIBN	76,794.12	81,576.36
CSPVP 2 made by using 0.2% AIBN (repeated preparation)	74,449.88	80,021.81
CSPVP 3 made by using 0.4% AIBN	57,011.81	59,910.24
CSPVP 3 made by using 0.2% AIBN	57,297.49	59,871.49
PVP made by using 0.1 % AIBN	59,359.16	59,611.54

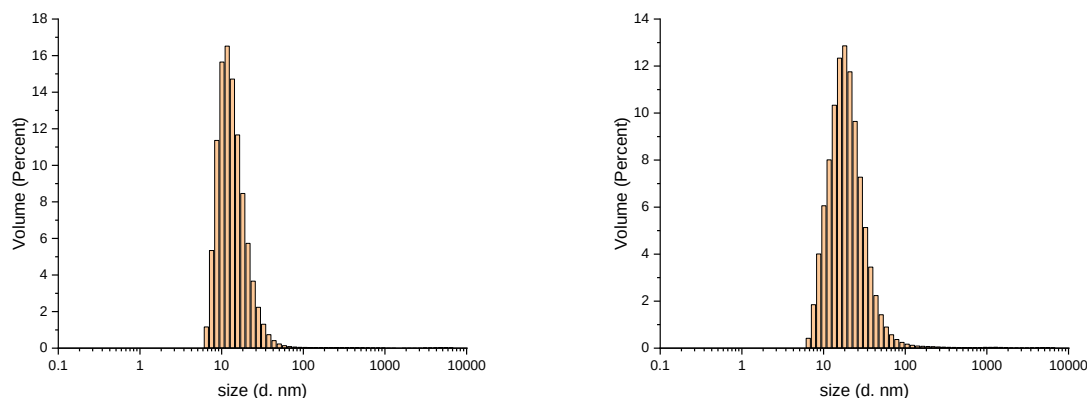
PVP made by using 0.2 % AIBN	37,341.41	42,946.46
PVP made by using 0.4 % AIBN	30,936.92	32,678.94
Copolymer 12	35,527.79	40,479.97
Copolymer 21	36,347.98	40,113.86
Copolymer 30	35,101.18	38,914.96

The mass of the polymer was determined by this method, and it was found that the trend in polymer mass matched the trend in polymer length. With the polymer mass now determined, the catalysts were evaluated based on their size, chirality, and catalytic behavior. These evaluations provide valuable insights into the catalytic effects of the catalysts.

1.4.3 Catalyst Characterization by Dynamic Light Scattering

The polymer and nanoclusters were also characterized by Dynamic Light Scattering (DLS). The polymer and nanocluster samples prepared for DLS were all filtered using a 3000 MWCO cutoff Vivaspin 20 centrifugal filter, and then washed several times with deionized water to remove inorganic salts and possible impurities. A ZetaPALS zeta potential analyzer (Brookhaven Instrument Co.) was used for the measurements, and the results are shown below. However, DLS has its limitations: when the nanoclusters aggregate, the corresponding peak will show up regardless of the quantity [29].

As it shown in Figure 7, the polymers **1** and **2** exhibit a similar distribution in aqueous solution. The majority of both polymers are in the range of 10-20 nm, but aggregation and rotation of polymer in solution resulted in the detection of larger particles, up to 100 nm in size.



In the case of CSPVP 1 supported Cu/Au and Pd/Au nanoclusters, as shown in Figure 8, the nanocluster sizes range from 60 to 100 nm, and they are about four times larger than those of the pure polymer.

Figure 7 (left) CSPVP 1, $c=0.08$ mM, (Right) CSPVP 2, $c=0.04$ mM

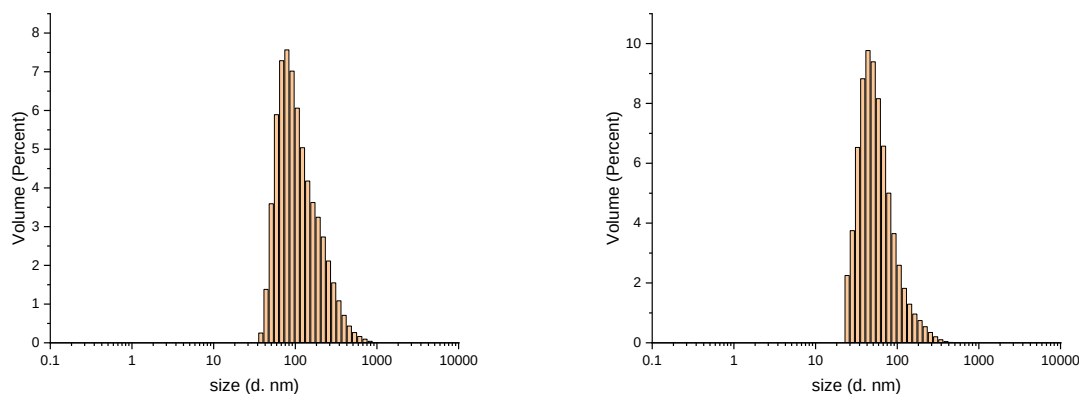


Figure 8 (left) Cu/Au CSPVP 1, $c=0.1$ mM, (right) Pd/Au CSPVP 1, $c=0.1$ mM

In the case of CSPVP 3 as shown in Figure 9, the nanocluster sizes shows around 20 nm, and PVP is in the range of 10-20 nm. In the case of CSPVP 3 supported Cu/Au and Pd/Au

nanoclusters, as shown in Figure 10, the nanocluster sizes range from 50 to 80 nm, and they are very consistent with each other and about four times larger than those of the pure CSPVP **3**.

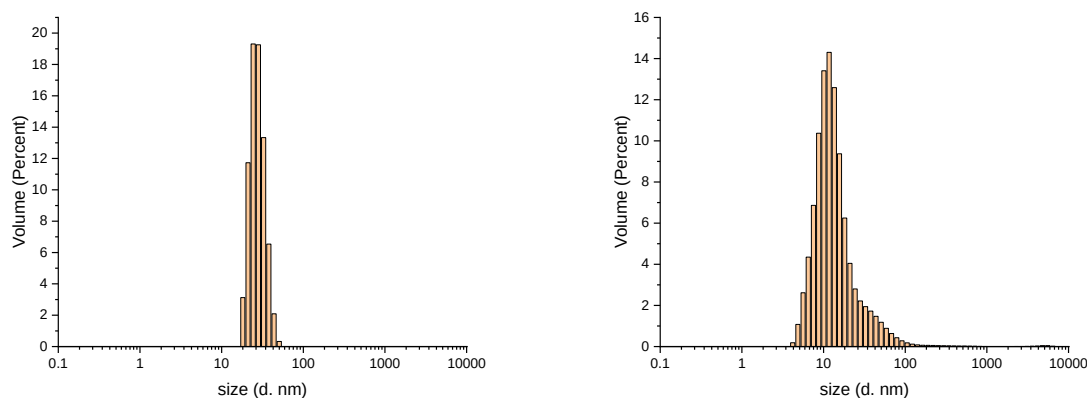


Figure 9 (left) CSPVP **3, $c=0.009$ mM, (Right) 40 K PVP, $c= 0.03$ mM**

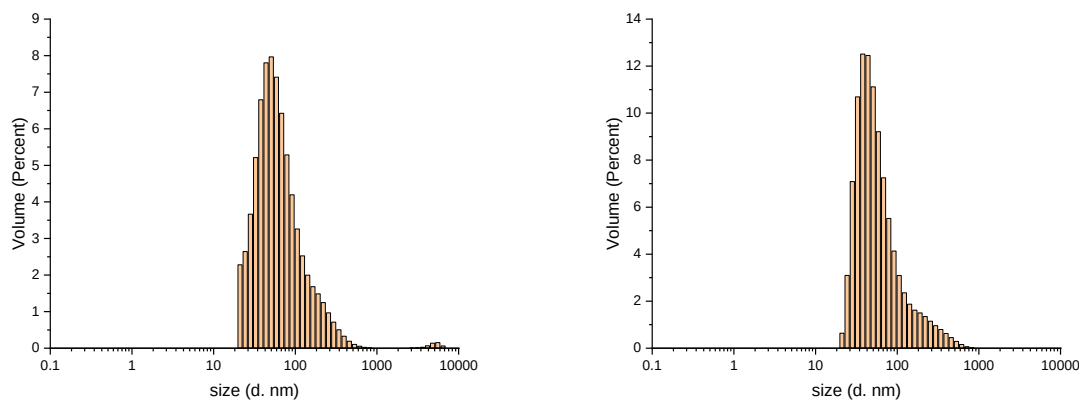


Figure 10 Cu/Au CSPVP **3, $c=0.64$ mM, (right) Pd/Au CSPVP **3**, $c=0.18$ mM**

In conclusion, the chiral polymers we synthesized are all approximately 20 nm or smaller. Once they form nanoclusters, their sizes increase and remain stable, resulting in a consistent size of the nanoclusters.

1.4.4 Catalyst Characterization by Circular Dichroism

To provide information on the chirality and sizes of the polymers and nanoclusters, as well as to offer further insight into their properties, circular dichroism (CD) method was used. Measurements were performed using a JASCO J-815 Circular Dichroism Spectropolarimeter.

Prior to measurement, all samples were prepared and filtered using 3000 MWCO cutoff Vivaspin 20 centrifugal filters and washed several times with deionized water to remove small sized impurities.

As it shown in Figure 11, the CD spectra of CSPVP 1 (black) and CSPVP 2 (red) were obtained at the same concentration. The nice mirror image of each other suggests that CSPVP 1 and 2 exhibit opposite chirality, as we expected. CSPVP 1 exhibits a strong absorption band at 223 nm and CD value of -201.13 mdeg, while CSPVP 2 exhibits a strong absorption band at 219 nm and CD value of +179.98 mdeg. The small difference in values could be caused by a slight difference in the chain length of the polymer. As for CSPVP 3, it shown a strong absorption band at 221 nm with CD value of -92.02 mdeg in Figure 12.

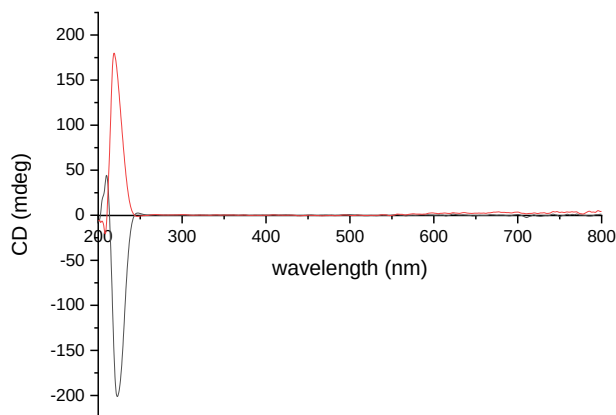


Figure 11 Circular Dichroism (CD) Spectra of 3 μ M CSPVP 1 (black), CSPVP 2 (red)

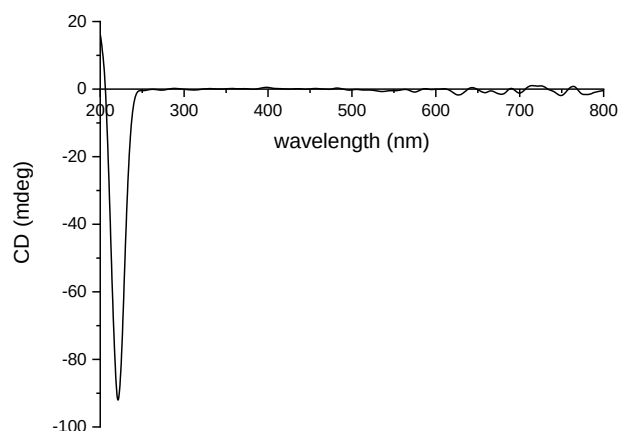


Figure 12 Circular Dichroism (CD) Spectra of 3 μ M CSPVP 3 (black)

Figure 13 shows the PVP absorption in CD. Since PVP is achiral, it shows no chirality, as expected.

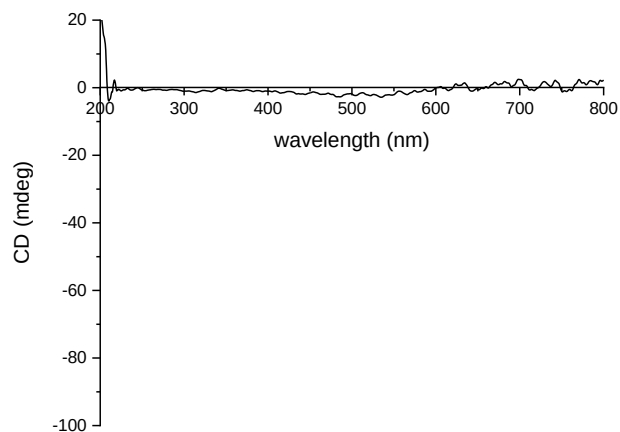


Figure 13 Circular Dichroism (CD) Spectra of 3 μ M of PVP (black)

One of our main goals was to investigate whether chirality can be introduced into the nanoclusters by chiral ligands. To test this, we conducted a control test, comparing Cu/Au and

Pd/Au nanoclusters supported by CSPVP **1** and CSPVP **2**. The results were interesting. In Cu/Au CSPVP **1** and **2** comparisons as shown in Figure 14, although the concentration increased from 3 μ M to 1.5mM, the strong bands absorption around 220 nm in polymer CSPVP **1** and **2** lowered. Instead, for Cu/Au CSPVP **1**, it shown new absorption at 215 nm, 230 nm, 238 nm and 253 nm, with corresponding CD values of - 33 mdeg, + 22 mdeg, - 31 mdeg and + 38 mdeg. For for Cu/Au CSPVP **2**, it shown new absorption at 213 nm, 228 nm, 237 nm and 252 nm, with corresponding CD values of + 33 mdeg, - 21 mdeg, + 79 mdeg and - 27 mdeg.

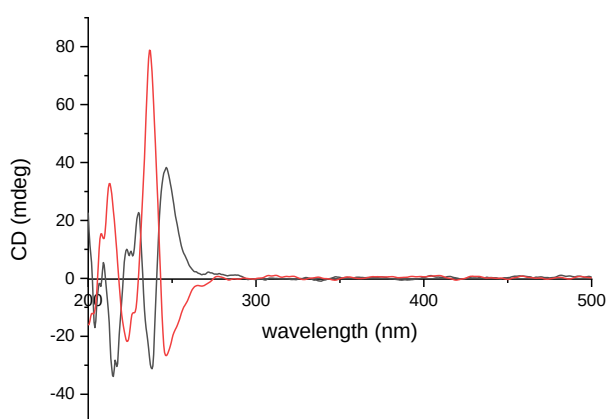


Figure 14 Circular Dichroism (CD) Spectra 1.5 mM Cu/Au CSPVP **1 (black) and 1.5 mM Cu/Au CSPVP **2** (red)**

In Pd/Au CSPVP **1** and **2** comparisons, the strong bands absorption around 220 nm in polymer CSPVP **1** and **2** decreased a lot. For Pd/Au CSPVP **1** and **2**, they also shown mirror images of each other. Pd/Au CSPVP **1** gave the strongest absorption band at 218.5 nm with CD value of -23.42 mdeg, and Pd/Au CSPVP **2** gave the strongest absorption band at 218.5 nm with CD value of + 33.71 mdeg.

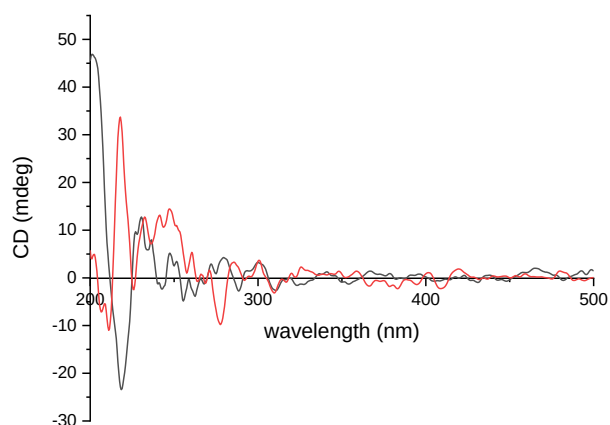


Figure 15 Circular Dichroism(CD) Spectra 1.5mM Pd/Au CSPVP 1 (black) and 1.5 mM Pd/Au CSPVP 2 (red)

By compared the spectra between polymers and their nanoclusters, a conclusion can be made when nanoclusters were made, new absorption peaks appeared in CD. Both sets of nanoclusters exhibited a mirror image comparison with similar absorption peaks but opposite chirality. This suggested that the nanoclusters were chiral, and that the chirality was introduced by the chirality of the ligands.

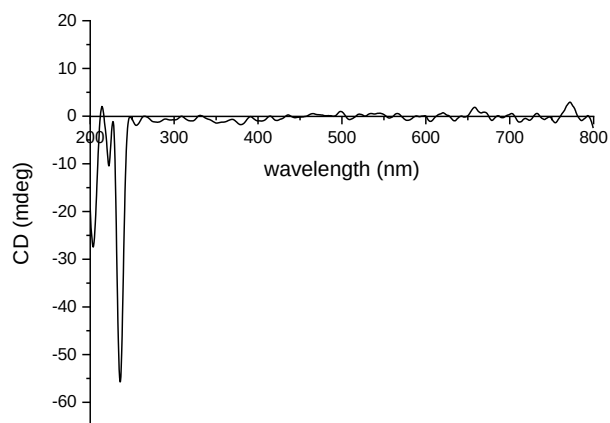


Figure 16 Circular Dichroism (CD) Spectra 1.5 mM Cu/Au CSPVP 3

As it shown in Figure 16, Cu/Au **3** gave the strongest absorption band at 236 nm with CD value of -55.72 mdeg.

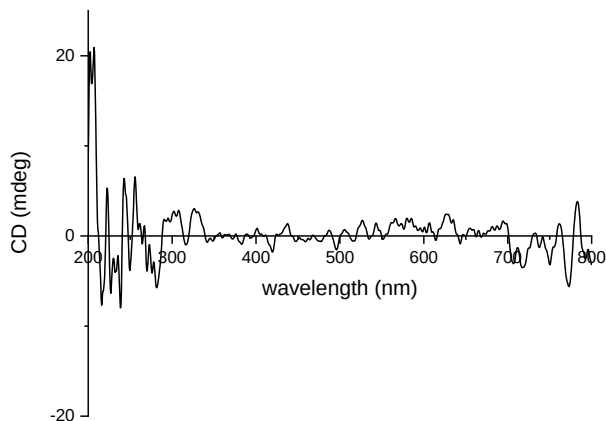


Figure 17 Circular Dichroism (CD) Spectra 1.5mM Pd/ Au CSPVP 3

As it shown in Figure 17, Pd/Au CSPVP **3** gave the strongest absorption band at 239 nm and 216 nm, with corresponding CD value of -7.7 mdeg and -7.69 mdeg.

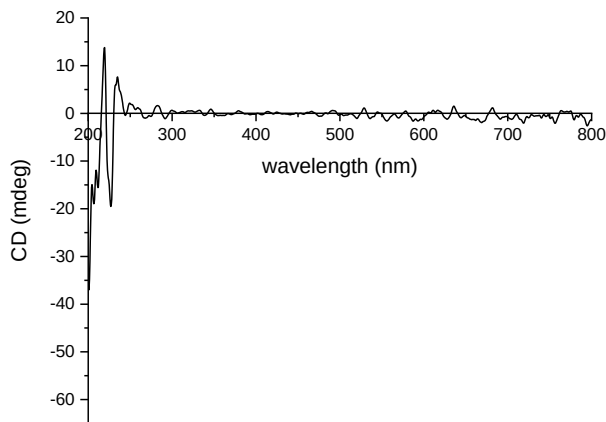


Figure 18 Circular Dichroism(CD) Spectra 1.5mM Cu: Au (3:1)/PVP

The measurements of Cu/Au PVP and Pd/Au PVP were also taken, they both shown almost no chirality in the spectra as Figure 18 and 19 presented.

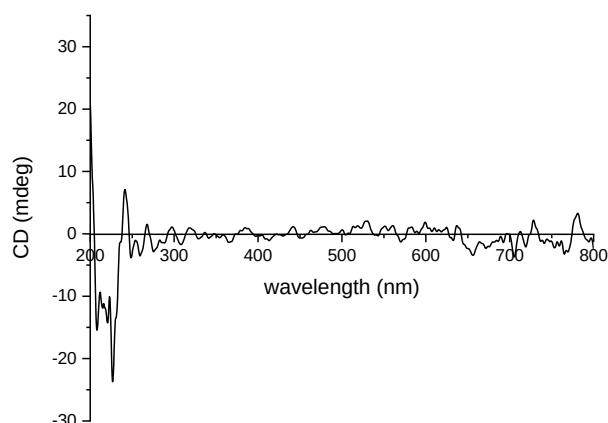


Figure 19 Circular Dichroism (CD) Spectra 1.5mM Pd/ Au PVP

The absorption of the chiral polymers and nanoclusters suggest the structures could be random coil structures. These findings provide important insights into catalytic reactions.

1.4.5 Catalyst Characterization by Fourier-Transform Infrared Spectroscopy

FTIR spectroscopy was employed to compare the structural changes of the polymers with nanoclusters, using the liquid membrane method for the measurement process[30]. A drop of the sample mixture was placed on the infrared transparent window of the Agilent Cary 630 FTIR Spectrometer to form a thin film. Then top crystal was gently press onto the sample to sandwich it between the two windows. The scan range was set to $4000\text{-}700\text{ cm}^{-1}$. Samples were prepared in 1.5 mM concentration in 2 mL deionized water, filtered by 3000 MWCO cutoff Vivaspin 20 centrifugal filters, then lyophilized to powder forms, and used in the measurement of FTIR spectra.

Figure 20 FTIR of 1.5 mM CSPVP 1 (black line), 1.5 mM Cu/Au CSPV1 (red line), 1.5 mM Pd/Au CSPVP 1 (blue line) — a) full spectrum; b) $700\text{-}1200\text{ cm}^{-1}$

a)

b)

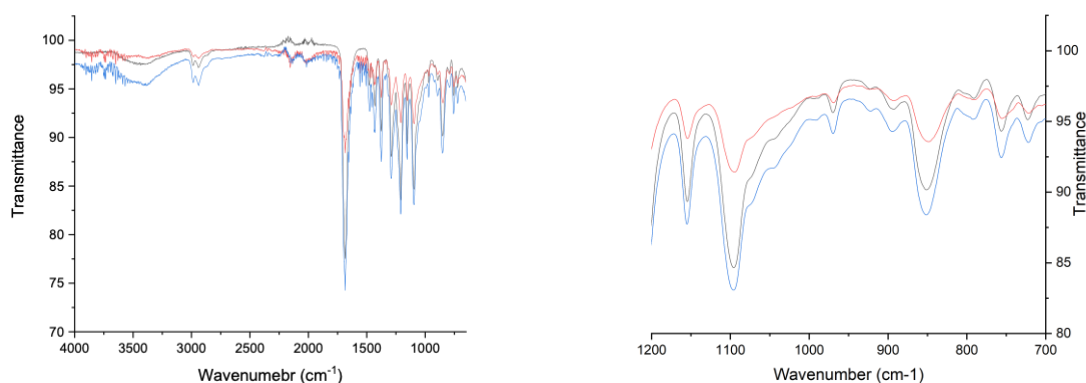


Figure 21 FTIR of 1.5 mM CSPVP 2 (black line), 1.5 mM Cu/Au CSPVP 2 (red line), 1.5 mM Pd/Au CSPVP 2 (blue line) — a) full spectrum; b) 700-1200 cm⁻¹

a)

b)

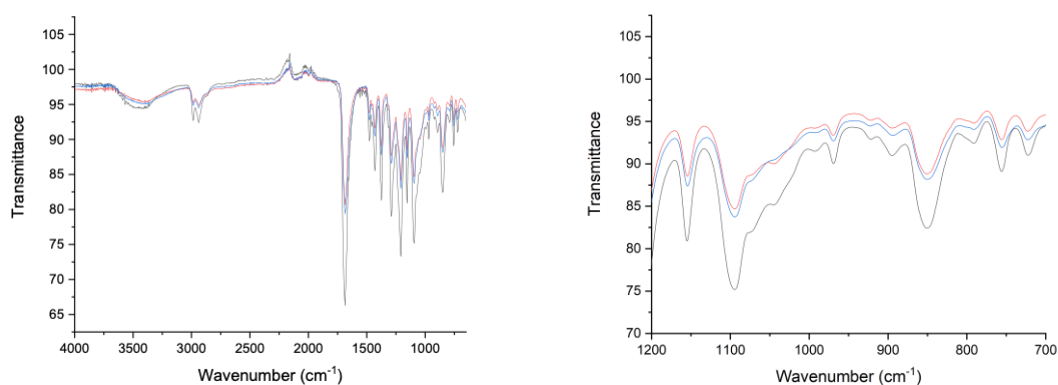


Figure 22 FTIR of 1.5 mM CSPVP-2 (black line), 1.5 mM Cu/Au CSPVP 3 (red line), 1.5 mM Pd/Au CSPVP 3 (blue line) — a) full spectrum; b) 700-1200 cm⁻¹

a)

b)

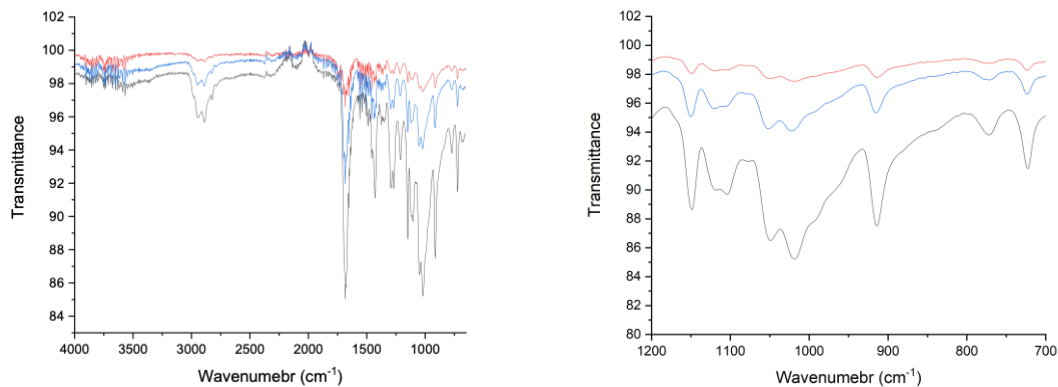


Figure 20, 21 and 22 displays the FTIR spectra of chiral PVPs and their corresponding bimetallic nanoclusters, revealing no significant changes. The signature peaks at 2988, 2938, 1685, 1289, 1018 cm^{-1} corresponding to CSPVP **1** and **2** vibrational stretching for C-H on the pyrrole ring, C=O, C-O and C-N bond. A characteristic peak was found at 1654 cm^{-1} , which could suggest corresponds to the interaction of Pd/Au nanoclusters with C=O bond. Results from FTIR studies showed the main component in the nanoclusters is chiral PVP. Chiral PVP molecules encapsulate the nanometals and provide water or DMF solubilization property of the entire bimetallic nanoclusters. The comparison shows that no absorption peaks appear or disappear in the FTIR spectra of the polymer and nanoclusters, which indicates that there is no structural change in PVP, and such a result is to be expected since PVP is a non-ionic compound.

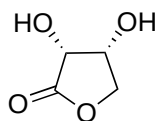
1.5 Conclusions

Bimetallic nanoclusters Cu/Au and Pd/Au were prepared in a one-pot method using chiral PVP as a stabilizer. The chiral PVP polymers were subjected to a comprehensive characterization process that included GPC-HPLC, HRMS, FTIR, and CD analyses. Similarly, the bimetallic nanoclusters were subjected to a thorough characterization process that involved HRMS, FTIR, CD and DLS, analyses to determine their structural features, sizes, and chemical compositions, including the metal contents.

1.6 Experimental Section

1.6.1 Representative Synthesis Reactions of the Precursors of CSPVP

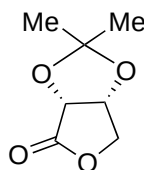
(3R,4R)-3,-3-Dihydeoxy-dihydrofuran-2(3H)-one (6) [31, 32]



6

To a RB flask, 50g of D-isoascorbic acid (**5**) (0.282 mol) was kept at 0 °C environment, a solution of 59.7 g sodium carbonate (0.568 mol) in 500 mL deionized water was added. To it, 64.4 mL 30% hydrogen peroxide (0.568 mol) was dropwise in 20 minutes, the reaction solution was stirred at 0 °C for another 10 minutes. After that, the reaction solution was heated to 42 °C for one hour. To workup the reaction, 11.4 g activated charcoal was added in small batches and kept the reaction mixture stirred at 80 °C for 30 minutes to quench the remaining hydrogen peroxide. The reaction mixture was filtered through the Celite while its hot to remove the charcoal, and 142 mL warm water was used to wash the filtration residue. The filtrate was collected and acidified with 6N HCl till pH reach to 1, then concentrated give the crude compound. The crude compound was re-dissolved in 200 mL of ethyl acetate and heated to reflux for 30 minutes and mixture were filtered through a fritted funnel to collect the desired compound while hot. The insoluble solids were then dissolved in another 200 mL ethyl acetate and repeated the above steps to extract out the desired product. The similar process was repeated three times and the filtrate was combined and placed at rest in the refrigerator at -4°C overnight. The desired product was crystallized and collected by filtration to give 28 g (83% yield) of the titled compound (**6**), **¹H NMR** (400 MHz, D₂O): δ 5.73 (dd, *J* = 7.2, 3.2 Hz, 1 H, OH), 5.32 (s, 1 H, OH), 4.37 – 4.32 (m, 1 H), 4.24 (dt, *J* = 10, 3.2 Hz, 1 H), 4.22 – 4.19 (m, 1 H), 4.03 (dd, *J* = 10, 3.2 Hz 1 H); **¹³C NMR** (400 MHz, D₂O): δ 177.1, 72.5, 70.1, 69.0. The spectral data are consistent with the previous reported spectra data.

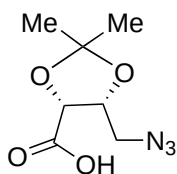
2,3-O-Isopropylidene-D-erythrone (7) [33, 34]



7

To an RB flask, 26.9 g (3*R*,4*R*)-Dihydro-3,4-dihydroxy-2(3*H*)-furanone (**6**) (0.228mol) was flame dried and 460 mL distilled acetone was added to dissolve the starting material, then 0.46 mL concentrated sulfuric acid (4.600 mmol) was added dropwise. The reaction solution was stirred at room temperature for 12 hours. To work the reaction, 76.61g sodium bicarbonate (0.912 mmol) reaction solution was added to neutralize the excess sulfuric acid and stirred for 30 minutes, followed by evaporation to remove the acetone to afford the crude. The crude was dissolved in 200 mL ethyl acetate and 100 mL deionized water then ethyl acetate was used to extract the aqueous layer for several times to obtain the desired compound. The organic layers were collected, washed with brine and dried over sodium sulfate Na₂SO₄. The mixture was filtered through a fritted funnel and the filtrate was concentrated to dryness to give crude 40.0 g. The crude was purified by column chromatography using hexane and ethyl acetate as eluent to yield 2,3-O-isopropylidene-D-erythrone (**7**) 28.1g (78% yield): ¹H NMR (400 MHz, CDCl₃): δ 4.83 (dd, *J* = 6, 4 Hz, 1 H), 4.73 (d, *J* = 6 Hz, 1 H), 4.46 (d, *J* = 11 Hz, 1 H), 4.43 (dd, *J* = 11, 4 Hz, 1 H), 1.41 (s, 3 H), 1.32 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃): δ 174.8, 113.9, 75.8, 74.8, 70.5, 26.9, 25.6. The spectral data are consistent with the previous reported spectra data.

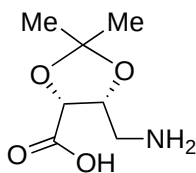
(2R,3R)-4-Azido-4-deoxy-2,3-O-isopropylidene-D-erythronic acid (8) [35]



8

To an RB flask containing a magnetic stirred bar, 4.82 g (0.031 mmol) 2,3-O-isopropylidene-D-erythronolactone (**7**) was and 6.94 g (0.1067 mmol) sodium azide NaN_3 were thoroughly dried and remaining under argon. Then, 30 mL distilled *N,N*-dimethylformamide DMF was added in to the flask, the reaction mixture was then heated under 120 °C and stirred for 12 hours. To work up the reaction, the reaction mixture was cooled to room temperature, then diluted with 500 mL of diethyl ether and 10 mL of H_2O . The mixture was shaken in a separatory funnel and the organic layer was collected and concentrated to recover the starting material, leaving 1.45 g (30% recovery) of lactone **7**. The aqueous layer was acidified by 20% aqueous HCl dropwise to pH 2, and then extracted with dichloromethane several times. The extracts were combined, washed with saturated sodium chloride, dried over sodium sulfate, filtered through a fritted funnel, and concentrated on a rotary evaporator and then under high vacuum to give 3.19 g (52% yield) of desired product **8**. **^1H NMR** (400 MHz, CDCl_3): δ 9.50 – 9.0 (bs, 1 H, OH), 4.67 (d, $J = 7.2$ Hz, 1 H), 4.60 – 4.55 (m, 1 H), 3.60 (dd, $J = 13.2, 3.2$ Hz, 1 H), 3.40 (dd, $J = 13.2, 6$ Hz, 1 H), 1.63 (s, 3 H), 1.42 (s, 3 H); **^{13}C NMR** (400 MHz, CDCl_3): δ 172.2, 112.2, 73.8, 70.8, 52.0, 27.1, 26.2. The spectral data are consistent with the previously reported spectra data.

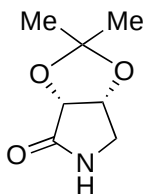
(2R,3R)-4-Amino-4-deoxy-2,3-O-isopropylidene-D-erythronic acid (9) [35]



9

To a hydrogenator bottle, 3.5 g (17. mmol) of (2R,3R)-4-azido-4-deoxy-2,3-O-isopropylidene-D-erythronic acid (**8**) and 100 mL methanol were added followed by 0.317 g of 5% Pd/C. The hydrogenator bottle was connected to a hydrogenation apparatus and maintained the system under 30 psi hydrogen gas and shaken for 12 h at 25 °C. Hydrogen gas in the bottle was released and the mixture was filtered through a pad of Celite. The solids were washed with methanol several times. The filtrate was collected and concentrated to dryness affording 3.6 g of light yellow solid. The crude solid was washed with diethyl ether three times, and the washed solid was dried under vacuum to give 2.9745 g (98% yield) of product **9** as a white solid. ¹H NMR (400 MHz, DMSO-d₆): δ 4.38 (d, *J* = 7.2 Hz, 1 H), 4.30 – 4.27 (m, 1 H), 2.76 (t, *J* = 8 Hz, 2 H), 1.39 (s, 3 H), 1.20 (s, 3 H). The spectral data are consistent with the previous reported spectra data.

(-)-(3aR,6aR)-Tetrahydro-2,2-dimethyl-4H-1,3-dioxolo[4,5-c]pyrrol-4-one (10)[35]



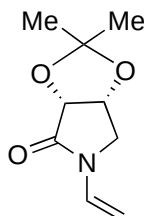
10

To a sublimator flask containing a magnetic stirred bar, 2.9745 g (14.80 mmol) of dried (2R,3R)-4-amino-4-deoxy-2,3-O-isopropylidene-D-erythronic acid (**9**) was placed at the bottom

of the sublimator and maintained under 0.1 – 0.5 mm Hg vacuum and heated at 120°C then slowly increased to 150 °C until the sublimation process was completed (no solids were observed from condensation at the cold finger or upper portion of the flask. The sublimator was cooled down to room temperature and restored to the atmospheric pressure using argon. The white solid product was carefully collected to afford 1.89 g (82% yield) of product **10**. **¹H NMR** (400 MHz, CDCl₃): δ 6.30 (bs, 1 H, NH), 4.80 (t, *J* = 5.9 Hz, 1 H), 4.60 (d, *J* = 5.9 Hz, 1 H), 3.57 (dd, *J* = 11.5, 4.8 Hz, 1 H), 3.51 (d, *J* = 11.5 Hz, 1H), 1.48 (s, 3H), 1.39 (s, 3H); **¹³C NMR** (100 MHz, CDCl₃): δ 174.4, 114.3, 75.7, 74.9, 45.9, 27.0, 25.9. This data is consistent with what has been reported previously.

(-)-(3aR,6aR)-5-Ethenyl-tetrahydro-2,2-dimethyl-4H-1,3-dioxolo[4,5-c]pyrrol-4-one (11)

[36]

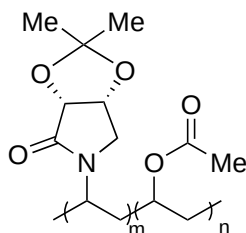


11

To a RB flask equipped with a reflux condenser and a magnetic stirred bar, 40.0 mL of freshly distilled vinyl n-butyl ether, 0.5295 g (3.369 mmol) amide **10** and 43 mg (0.067 mmol) of 4,7-diphenyl-1,10-phenanthroline palladium bis(trifluoroacetate) [(DPP)Pd(OCOCF₃)₂] were added. The reaction mixture was stirred at 75°C for 3 hours, cooled down to room temperature and then filtered through a pad of silica gel, and washed the silica gel with ethyl acetate several times. The filtrate was dried over sodium sulfate, evaporated on a rotary evaporator and then under vacuum to dryness. The residue was purified by silica gel column chromatography using a mixture of hexane: ethyl acetate, 5:1, as an eluent to afford 0.5162 g (84% yield) product **11**. **¹H**

NMR (400 MHz, CDCl₃) δ : 7.03 (dd, J = 16.0, 9.2 Hz, 1 H, CH=), 4.84 – 4.80 (m, 1 H), 4.69 (d, J = 6.4 Hz, 1 H), 4.53 (d, J = 9.2 Hz, 1 H, =CH₂), 4.49 (d, J = 16 Hz, 1 H, =CH₂), 3.65 (dd, J = 10, 1.5 Hz, 1 H), 3.64 (dd, J = 10, 4 Hz, 1 H), 1.41 (s, 3 H), 1.35 (s, 3 H); **¹³C NMR** (100 MHz, CDCl₃): δ 169.4, 129.2, 113.0, 96.7, 78.1, 72.1, 48.7, 27.2, 25.9; MS (ESI, MeOH): m/z = 184.0 ([M + H]⁺). HRMS was taken, m/z [M + H]⁺ calculated for C₉H₁₄NO₃⁺: 184.0974 and the mass was found as 184.0952.

Poly(3R,4R-3,4-dimethyl-N-vinylpyrrolidinone-co-vinyl acetate) [P(DVP-co-VAc)]
(Copolymer-1-R) (12) [37]

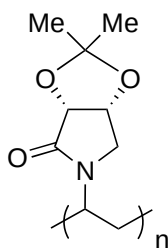


12

A solution of 4.0 mg (24.4 mmol) of AIBN in 1 mL degassed acetone was prepared inside a glove box. A sealable tube equipped with a magnetic bar was dried under high vacuum and flame, and then maintained under argon. To the sealable tube, 44 mg (0.24 mmol) of vinyl lactam **11** was added and the contain was dried under vacuum and heat, and then maintained under argon. To it, were added 0.022 mL (0.485 mmol) of distilled and degassed vinyl acetate and 100 μ L (2.18 mmol, 0.2 mol%) of AIBN from the aforementioned acetone solution in the glove box. The tube was capped with a Teflon cap containing a Teflon O-ring and transferred out of the glove box and stirred at 60 °C for 22 hours. The reaction mixture was transfer into a round-bottomed flask, diluted with 10 mL hexane, and white solid precipitated out. The supernatant solution was pipetted out and the hexane washing was repeated several times. The

solid was collected by filtration and dried under vacuum to give 46.0 mg (70% yield). $^1\text{H NMR}$ (400 MHz, CDCl_3): δ 4.89 - 4.40 (m, 3 H), 3.59 - 3.02 (m, 3 H), 2.13 (s, 3H), 2.11 - 1.21 (m, 10H); $^{13}\text{C NMR}$ (100 MHz, CDCl_3): δ 172.5 - 169.0 (m), 112.0, 77.23, 72.3, 47.0 - 42.0 (m), 37.0 - 32.0 (m), 27.3, 25.4, 21.2.

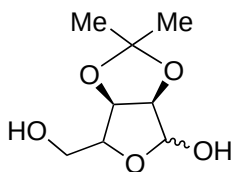
Poly[(3aR,6aR)-2,2-dimethyl-5-vinyl-dihydro-3aH-[1,3]dioxolo[4,5-c]pyrrol-4(5H)-one] (1)



1

In a glove box, a solution of 8.5 mg (52.8 mmol) AIBN in 1 mL of degassed ethyl acetate was prepared. To a sealable tube inside the glove box, a solution of 0.2 g (1.092 mmol) vinyl lactam **11** and 2.0 mg copolymer **12** (0.1 % wt.) in 0.658 mL of degassed ethyl acetate were added followed by 45 μL (2.18 mmol, 0.2 mol%) of AIBN-ethyl acetate solution that described above. The tube was capped with a Teflon cap containing a Teflon O-ring, transferred out of the glove box, and stirred at 70 $^{\circ}\text{C}$ for 22 hours. The reaction mixture was transfer into a round bottomed flask, diluted with 15 mL of hexane to precipitate out the desired polymer as a white solid. The supernatant solution was pipetted out and the hexane washing process was repeated several times. The solid was collected by filtration and dried under vacuum to give 0.1972 g (99% yield) of polymer **1**. $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 4.80 - 4.45 (bs, 2 H, CHO), 3.8 - 3.0 (m, 3 H, CHN and CH_2N), 1.80 - 1.2 (m, 8 H, one CH_2 and 2 CH_3 group); $^{13}\text{C NMR}$ (100 MHz, CDCl_3): δ 171.6 (m), 112.1 (bs, O-C-O), 78.0 (bs, C-O), 72.4 (bs, C-O), 47.0 - 43.0 (m, CHN and CH_2N), 36.5 - 31.0 (m, CH_2), 27.4 (bs), 25.4 (bs).

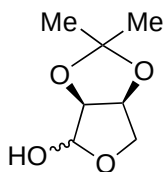
β -D-2,3-O-(1-Methylethylidene)-ribofuranose (14**)[38]**



14

To a RB flask containing a magnetic stirred bar were added 40 g (0.27 mol) of D-ribose (**13**) and 240 mL of distilled acetone followed by dropwise addition of 1.2 mL (0.0225 mol) of concentrated sulfonic acid. The reaction solution was stirred at room temperature for 3 hours, neutralized with 7.6 g (0.09 mol) of sodium bicarbonate carefully, and filtered through a pad of Celite. The solid was washed with acetone three times and the filtrates were combined and concentrated to dryness to afford 50.44 g of crude product. The crude was purified by silica gel column chromatography using a mixture of hexane and ethyl acetate in a ratio of 1:2 to afford product **14** in 49.31 g (96% yield). **¹H NMR** (400 MHz, CDCl₃): δ 5.52 (d, J = 6.0 Hz, 1H), 5.41 (d, J = 5.4 Hz, 1H), 4.81 (d, J = 5.9 Hz, 1H), 4.58 (d, J = 5.9 Hz, 1H), 4.39 (br s, 1H), 4.20 (br s, 1H), 3.71 (br s, 2H), 1.49 (s, 3H), 1.33 (s, 3H); **¹³C NMR** (100 MHz, CDCl₃): δ 112.2, 102.7, 87.6, 86.7, 81.6, 63.5, 26.3, 24.7. This data is consistent with what has been reported previously.

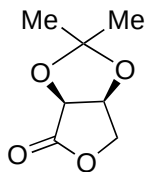
(3a*S*,4*S*,6a*S*)-2,2-Dimethyl-tetrahydrofuro[3,4-*d*][1,3]dioxol-4-ol (15**)[39]**



15

To a RB flask containing a magnetic stirred bar, were added 15.8 g (0.083 mol) of β -D-2,3-O-(1-methylethylidene)-ribofuranose (**14**) 270 mL deionized water and 100 mL ethanol. The flask was cooled over an ice-water bath and stirred vigorously for dissolving compound **14**. To this mixture, a solution of 4.72 g (0.125 mol) of sodium borohydride in 40 mL of deionized water was added dropwise while the flask was cooled at 0 °C. The mixture was then stirred at room temperature for 16 hours, acidified with 10% aqueous acetic acid until pH = 5, and added a suspension of 21.34 g (0.0998) of sodium periodate in 80 mL water dropwise. The mixture was stirred at room temperature for 4 hours, concentrated under reduced pressure to remove most of the solvent and then extracted with ethyl acetate three times. The organic extracts were combined, washed with water and brine, filtered, and concentrated under reduced pressure to afford 14.88 g of crude product **15**. The crude compound was used for next reaction without purification. α -anomer **¹H NMR** (400 MHz, CDCl₃): δ 5.41 (s, 1H), 4.85 (dd, 1 H), 4.61 (d, J = 6 Hz, 1 H), 4.12 (dd, J = 10.4, 3.6 Hz, 1 H), 4.06 (d, J = 10.4 Hz, 1 H), 2.80 – 2.70 (bs, 1 H), 1.48 (s, 3 H), 1.33 (s, 3 H) ppm; **¹³C NMR** (100 MHz, CDCl₃): δ 112.3, 101.8, 85.1, 80.0, 72.0, 26.2, 24.7 ppm; β -anomer: **¹H NMR** (400 MHz, CDCl₃): δ 5.00 – 4.90 (m, 1H), 4.76 (dd, J = 6, 3.6 Hz, 1H), 4.49 (d, J = 6 Hz, 1H), 3.95 (d, J = 10.4 Hz, 1H), 3.55 (dd, J = 10.4, 3 Hz, 1H), 1.80 – 1.60 (bs, 1H), 1.55 (s, 3H), 1.38 (s, 3H); **¹³C NMR** (100 MHz, CDCl₃): δ 113.4, 97.4, 79.6, 78.3, 67.6, 26.0, 24.9. This data is consistent with what has been reported previously.

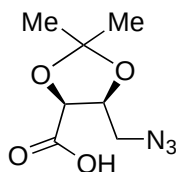
(3aS,6aS)-2,2-Dimethyl-dihydrofuro[3,4-d][1,3]dioxol-4(3aH)-one (16)[39]



16

A two-necked RB flask containing a magnetic stirred bar was dried under vacuum and hot flame and then maintained under argon. To it were added 1.0 g of dried 3 Å molecular sieves and 100 mL of freshly distilled dichloromethane, and the flask was cooled at -78 °C. To this cold solution, 5.3 mL (63 mmol) of freshly distilled oxalyl chloride and a solution of distilled 8.9 mL (0.125 mol) of dimethyl sulfoxide in 25 mL dichloromethane were added. The reaction was kept at -78 °C for 15 minutes and a solution of 4.887 g (31 mmol) of (3a*S*,4*S*,6a*S*)-2,2-dimethyl-tetrahydrofuro[3,4-*d*][1,3]dioxol-4-ol (**15**) in 25 mL of dichloromethane was added via syringe, and stirred at -78 °C for 30 minutes. Then, 27 mL (0.19 mol) distilled triethylamine was added, the temperature was kept at -78 °C for 30 minutes then warmed the flask to room temperature and stirred for additional 14 hours. The reaction mixture was diluted with 100 mL of water, acidified with 1 N HCl solution to pH ~1, and extract three times with dichloromethane. The combined organic extracts were washed with aqueous ammonium chloride, water and brine, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to dryness, yielding 4.01 g (81% yield) of product **16** as a white waxy solid. ¹H NMR (400 MHz, CDCl₃): δ 4.88 (dd, *J* = 6, 4 Hz, 1H), 4.73 (d, *J* = 6 Hz, 1H), 4.49 (d, *J* = 11 Hz, 1H), 4.42 (dd, *J* = 11, 4 Hz, 1H), 1.52 (s, 3H), 1.41 (s, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 174.1, 114.1, 75.5, 74.6, 70.2, 26.8, 25.7 ppm. ¹H NMR (400 MHz, CDCl₃): δ 4.87 (dd, *J* = 6, 4 Hz, 1H), 4.75 (d, *J* = 6 Hz, 1H), 4.49 (d, *J* = 11 Hz, 1H), 4.41 (dd, *J* = 11, 4 Hz, 1H), 1.51 (s, 3H), 1.4 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 174.1, 114.1, 75.5, 74.6, 70.2, 26.8, 25.7.

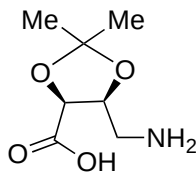
(4*S*,5*S*)-5-(Azidomethyl)-2,2-dimethyl-1,3-dioxolane-4-carboxylic acid (17**)**



17

To thoroughly dried 1.987 g (12.58 mmol) (3*S*,6*aS*)-2,2-Dimethyl-dihydrofuro[3,4-*d*][1,3]dioxol-4(3*aH*)-one (**16**) and 2.453 g (37.73 mmol) sodium azide NaN₃, an atmosphere of argon was applied. Then, 24.4 mL distilled *N,N*-dimethylformamide DMF was added in to the flask, the reaction mixture was then heated under 120 °C and stirred for 22 hours. To work up the reaction, the reaction mixture was cooled to room temperature, then diluted with 150 mL of diethyl ether and 10 mL of H₂O. The mixture was separated into two layers by separatory funnel, the organic layer was concentrated to recover the starting material of and aqueous layer was saved. The aqueous layer was acidified by 20% aqueous HCl dropwise to adjust pH to 2 then extracted with dichloromethane several times. The organic layer was combined, washed with saturated sodium chloride, dried over sodium sulfate, filtered, and concentrated to dryness to give 0.3435 g (15% recovery) lactone (**17**). while the organic layer from the acidic aqueous layer to afford 3.0 g crude desired product with DMF inside (**17**). ¹H NMR (400 MHz, CDCl₃): δ 9.52 – 9.0 (bs, 1 H, OH), 4.67 (d, *J* = 7.2 Hz, 1 H), 4.60 – 4.56 (m, 1 H), 3.60 (dd, *J* = 13.2, 3.2 Hz, 1 H), 3.40 (dd, *J* = 13.2, 6 Hz, 1 H), 1.63 (s, 3 H), 1.42 (s, 3 H); ¹³C NMR (400 MHz, CDCl₃): δ 172.2, 112.2, 73.8, 70.8, 52.0, 27.1, 26.2; MS (ESI, MeOH): *m/z* = 224.1 ([*M* + Na]⁺), 143.6, 85.6. This data is consistent with what has been reported previously[35].

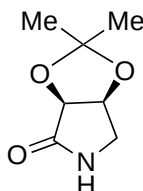
(4*S*,5*S*)-5-(Aminomethyl)-2,2-dimethyl-1,3-dioxalane-4- carboxylic acid (18**) [35]**



18

To a reduction bottle, 3.0 g (DMF include) (4S,5S)-5-(Azidomethyl)-2,2-dimethyl-1,3-dioxalane-4-carboxylic acid (**17**) was dissolved in 100 mL methanol then 0.064 g 5% Pd/C was added. The reaction mixture was connected to a hydrogenation apparatus and maintained the system under 30 psi hydrogen gas and shake for 12 h on a hydrogenator. The hydrogen was then released to normal condition and the mixture was then filtered through a pad of Celite. The residue was washed with methanol several times. The filtrate was collected and concentrate to dryness to afford 2.42 g light yellow solid. The crude solid was washed with ether for three times, and the washed solid was dried to afford 1.073 g desired product (**18**) as a white solid. **¹H NMR** (400 MHz, DMSO-d₆): δ 4.40 (d, *J* = 7.2 Hz, 1 H), 4.30 – 4.28 (m, 1 H), 2.74 (t, *J* = 8 Hz, 2 H), 1.41 (s, 3 H), 1.25 (s, 3 H). This data is consistent with what has been reported previously.

(-)-(3aS,6aS)-Tetrahydro-2,2-dihydro-3aH-[1,3]dioxolo[4,5-c]pyrrol-4(5H)-one (19**) [35]**

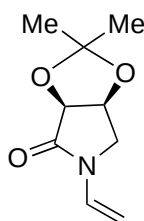


19

To a sublimator, 1.073 g (6.13 mmol) dried (4S,5S)-5-(Aminomethyl)-2,2-dimethyl-1,3-dioxalane-4-carboxylic acid (**18**) was placed at the bottom of the sublimator and heated at 120°C then slowly went to 150 °C under 0.1 – 0.5 mm Hg vacuum for three hours till the sublimation process was completed. The sublimator was cooled down to room temperature and slow restore to the atmosphere pressure. Then white crystalized were carefully collected to afford 0.7 g (73% yield) desired product (**19**). **¹H NMR** (400 MHz, CDCl₃): δ 6.31 (bs, 1 H), 4.79 (t, *J* = 5.9 Hz, 1 H), 4.58 (d, *J* = 5.9 Hz, 1 H), 3.59 (dd, *J* = 11.5, 4.8 Hz, 1 H), 3.49 (d, *J* = 11.5 Hz, 1H), 1.49 (s,

3H), 1.39 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3): δ 174.4, 114.3, 75.7, 74.9, 45.9, 27.0, 25.9; MS (ESI, MeOH): m/z = 158.4 ($[\text{M} + \text{H}]^+$). This data is consistent with what has been reported previously.

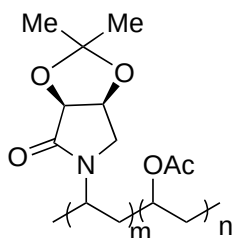
(-)-(3a*S*,6a*S*)-5-Ethenyl-tetrahydro-2,2-dimethyl-4*H*-1,3-dioxolo[4,5-*c*]pyrrol-4-one (20)
[36]



20

To a round bottom flask, 0.505 g (3.2 mmol) amide (**19**) and 0.100 g (0.153 mmol) of 4,7-diphenyl-1,10-phenanthroline palladium bis(trifluoroacetate) ($\text{DPPPd}(\text{OCOCF}_3)_2$) were dissolved in 40.0 mL distilled vinyl butyl ether. After stirred at 75°C for 13 hours, the reaction mixture was cooled down to room temperature and then filtered through a pad of silica gel, washed with ethyl acetate for servals times, dried with sodium sulfate, evaporated to dryness, purified by column chromatography using eluent hexane: ethyl acetate = 5:1 to afford 0.3222 g (74% yield) desired product (**11**). ^1H NMR(400 MHz, CDCl_3) δ : 7.05 (dd, J = 16.0, 9.2 Hz, 1 H, CH=), 4.82 – 4.79 (m, 1 H), 4.70 (d, J = 6.4 Hz, 1 H), 4.53 (d, J = 9.2 Hz, 1 H, =CH2), 4.48 (d, J = 16 Hz, 1 H, =CH2), 3.65 (dd, J = 10, 1.5 Hz, 1 H), 3.64 (dd, J = 10, 4 Hz, 1 H), 1.39 (s, 3 H), 1.35 (s, 3 H); ^{13}C NMR (100 MHz, CDCl_3): δ 169.4, 129.2, 113.0, 96.7, 78.1, 72.1, 48.7, 27.2, 25.9; MS (ESI, MeOH): m/z = 184.0 ($[\text{M} + \text{H}]^+$). HRMS was taken, $[\text{M} + \text{H}]^+$ calculated for $\text{C}_9\text{H}_{14}\text{NO}_3^+$ is 184.0974 and 184.0952 was found.

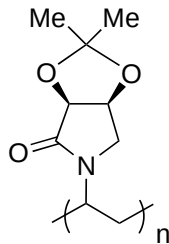
Poly(3a*S*,6a*S*-2,2-dimethyl-5-vinyl-dihydro-3a*H*-[1,3] dioxolo[4,5-*c*]pyrrol-4(5*H*)-one-co-vinyl acetate) [P(DVP-co-VAc)] (Copolymer-1-*S*) (21**) [37]**



21

A solution of 2.5 mg (15.2 μmol) AIBN in 1.25 mL degassed acetone was prepared inside the glove box. To a sealed capped tube, a solution of 90.4 mg (0.49 mmol) vinyl lactam **20** in 0.015 mL of distilled and degassed vinyl acetate followed by adding 200 μL (0.024 mmol, 0.5 mol%) of AIBN-acetone solution that prepared before in the glove box purged under positive nitrogen atmosphere. After the above steps, the tube was capped with a Teflon cap and sealed with a Teflon O-ring, then the tube was then transferred out of the glove box and stirred at 75 $^{\circ}\text{C}$ for 48 hours. The reaction mixture was transfer into a round bottom flask, followed by diluted with 10 mL hexane and white solid was precipitated out. The supernatant solution was pipetted out and repeated hexane wash several times. The solid was collected by filtration and dried under vacuum to give 17.8 mg (%) the desired compound **21**. **^1H NMR** (400 MHz, CDCl_3): δ 4.91 – 4.38 (m, 3 H), 3.64 – 3.01 (m, 3 H), 2.14 (s, 3H), 2.09 – 1.22 (m, 10H); **^{13}C NMR** (100 MHz, CDCl_3): δ 172.5 – 169.0 (m), 112.0 (s), 77.23, 72.3, 47.0 – 42.0 (m), 37.0 – 32.0 (m), 27.3, 25.4, 21.2.

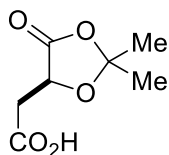
Poly[(3a*S*,6a*S*)-2,2-dimethyl-5-vinyl-dihydro-3a*H*-[1,3]dioxolo[4,5-*c*]pyrrol-4(5*H*)-one] [(+)-(1S**)] from thermal reaction (2)**



2

To a RB flask, 10.1 mg (52. mmol) AIBN was dissolved in 0.6 mL degassed ethyl acetate was prepared separately inside the glove box. To a sealed capped tube, a solution of 0.5343 g (2.91 mmol) vinyl lactam **20** and 5.4 mg previous prepared copolymer **21** (0.1 % wt.) followed by adding 57 μ l (1.77 mmol, 0.2 mol%) of AIBN-ethyl acetate solution and 0.914 mL degassed ethyl acetate that prepared before in the glove box purged under positive nitrogen atmosphere. After the above steps, the tube was capped with a Teflon cap and sealed with a Teflon O-ring, then the tube was then transferred out of the glove box and stirred at 80 °C for 26 hours. The reaction mixture was transfer into a round bottom flask, followed by diluted with 15mL hexane and white solid was precipitated out. The supernatant solution was pipetted out and repeated hexane wash several times. The solid was collected by filtration and dried under vacuum to give 0.614 g. **¹H NMR** (400 MHz, CDCl₃) δ 4.84 – 4.40 (bs, 2 H), 3.8 – 2.98 (m, 3 H), 1.80 – 1.2 (m, 8 H); **¹³C NMR** (100 MHz, CDCl₃): δ 171.6 (m), 112.1 (bs, O-C-O), 78.0 (bs, C-O), 72.4 (bs, C-O), 47.0 – 43.0 (m, CHN and CH₂N), 36.5 – 31.0 (m, CH₂), 27.4 (bs), 25.4 (bs).

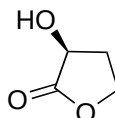
(S)-2-(2,2)-dimethyl-5-oxo-1,3-dioxolan-4-yl)acetic acid (23)[40]



23

To a round-bottom flask containing a magnetic stirred bar were added 15.0 g (0.112 mmol) of L-(S) malic acid (**22**) and 150 mL of 2,2-dimethoxypropane under argon followed by 0.5 g (2.15 mmol) of *D*-10 camphorsulfonic acid. The reaction solution was stirred at room temperature for 12 hours, neutralized with 0.20 g of sodium acetate and stirred for one hour, and filter to remove solids. The filtrate was combined, concentrated and crystallized in 150 mL of a mixture of chloroform and hexane in ratio of 1:2 to afford 16.8 g (86%) of solid compound **23**. **¹H NMR** (400 MHz, CDCl₃) δ 11.30 - 10.69 (bs, 1H), 4.71 (dd, *J* = 6.8, 4.0 Hz, 1H), 2.99 (dd, *J* = 17.2, 4 Hz, 1H), 2.86 (dd, *J* = 17.2, 6.8 Hz, 1H), 1.60 (s, 3H), 1.57 (s, 3H); **¹³C NMR** (100 MHz, CDCl₃) δ 174.8, 171.9, 111.4, 70.4, 36.0, 26.8, 25.8.

(S)-3-Hydroxydihydrofuran-2(3H)-one (**24**)[40]

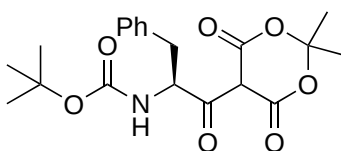


24

A two-necked RB flask containing a magnetic stirred bar was dried under vacuum and hot flame and then maintained under argon. To it were added 2.83 g (recovered) of (S)-2-(2,2-dimethyl-5-oxo-1,3-dioxolan-4-yl)acetic acid and 21 mL of freshly distilled THF under argon. The flask was cooled over an ice-water bath and 17.89 mL (0.102 mol) of BH₃•THF (1 M solution in THF) was added dropwise over 30 min. The solution was then stirred at room

temperature for 16 h. Additional 11.93 mL of $\text{BH}_3 \cdot \text{THF}$ (1 M solution in THF) was again added dropwise and stirred for 2 hours. The mixture was diluted with 40 mL of methanol and the contain was concentrated on a rotary evaporator to remove trimethyl borate. The addition of methanol and evaporation process was repeated three times, and the weight of the product remained unchanged, affording 2.50 g of crude product. This crude material, (S)-5-(2-hydroxyethyl)-2,2-dimethyl-1,3-dioxolan-4-one, was dissolved in 33 mL of chloroform and was added 0.134 g (0.577 mmol) of 10-camphorsulfonic acid. The resulting solution was stirred at room temperature for 16 hours, basified with 0.05 g (0.595 mmol) of sodium bicarbonate followed by concentration to dryness under reduced pressure and purification on silica gel column chromatography using a mixture of hexane and ethyl acetate in a ratio of 2:1. The column purification afforded 0.91 g (56% yield) of product 24. $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 4.53 (dd, $J = 10, 8.4$ Hz, 1H), 4.45 (dt, $J = 10, 7$ Hz, 1H), 4.24 (ddd, $J = 8.4, 6.8, 1.6$ Hz, 1H), 3.78–3.58 (bs, 1H), 2.61 (dddd, $J = 10, 8, 6, 2$ Hz, 1H), 2.30 (dddd, $J = 10, 8.8, 8.4, 4$ Hz, 1H); $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ 178.2, 67.4, 65.3, 30.9.

tert-butyl (S)-(1-(2,2-dimethyl-4,6-dioxo-1,3-dioxan-5-yl)-1-oxo-3-phenylpropan-2-yl)carbamate (32) [41]

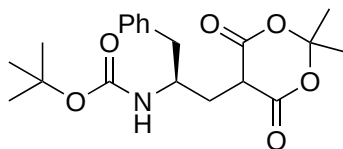


32

To a RB flask containing a magnetic stirred bar were added 4.0 g (15.6 mmol) of *N*-Boc-*O*-tert-butyl-L-phenylalanine (**31**), 3.37 g (23.4 mmol) of Meldrum's acid (2,2-dimethyl-1,3-dioxane-4,6-dione), 2.86 g (23.4 mmol) of 4-(dimethylamino)pyridine (DMAP) and 100 mL of dichloromethane under argon. The solution was cooled to 0 °C, added a solution of 3.508 g

(17.00 mmol) of *N,N'*-dicyclohexylcarbodiimide (DCC) in 20 mL of dichloromethane dropwise, and stirred at room temperature for 16 hours under argon. The reaction mixture was filtered to remove the precipitated *N,N'*-dicyclohexylurea byproduct, and the filtrate was collected, washed with 50 mL of 5% HCl, water, dried over anhydrous Na₂SO₄, filtered and concentrated to dryness, yielding 6.95 g of crude compound **32**. This compound was used in the following step without purification. ¹H NMR (400 MHz, CDCl₃) δ 7.3–7.15 (m, 5 H), 4.5–4.4 (m, 1 H), 4.3–4.2 (m, 1 H), 3.9–3.8 (m, 1 H), 2.8–2.7 (m, 2 H), 1.7 (s, 3 H), 1.65 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃) δ 207.5, 170.8, 153.1, 135.8, 129.6 (2 C), 128.9 (2 C), 127.4, 105.7, 80.3, 62.7, 60.3, 33.8, 28.4, 26.8.

***tert*-Butyl (*R*)-(1-(2,2-dimethyl-4,6-dioxo-1,3-dioxan-5-yl)-3-phenylpropan-2-yl)carbamate[41]**

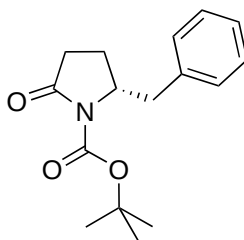


33

A flame-dried R.B. flask containing a magnetic stirred bar and 3.9 g (0.01 mol) of compound **32** was dried and maintained under argon. It was cooled to 0 °C and added dichloromethane. To it, 3.46 mL acetic acid (0.01 mol) and 0.98 g of NaBH₄ (0.026 mol) were added slowly at 0°C and the solution was kept 0 °C for three hours before it was restored to room temperature to react for another 12 hours. To workup the reaction, 60 mL of water was added in the reaction solution and then transferred to a separatory funnel, and extracted with dichloromethane several times. The dichloromethane layers were combined, washed with water, and brine. The

organic layer was dried over anhydrous magnesium sulfate, filtered through a fritted funnel then filtrate was concentrated to give 5.2 g of desired compound as a white solid. This compound was used in the subsequent step without further purification. ¹H NMR (400 MHz, CDCl₃) δ 7.33–7.17 (m, 5 H), 4.46–4.41 (m, 1 H), 4.25–4.18 (m, 1 H), 3.94–3.89 (m, 1 H), 2.88–2.83 (m, 2 H), 2.34–2.12 (m, 2 H), 1.82 (s, 3 H), 1.79 (s, 3 H), 1.24 (s, 9 H); ¹³C NMR (100 MHz, CDCl₃) δ 174.7, 150.2, 137.3, 129.6 (2 C), 129.0 (2 C), 127.1, 83.2, 59.2, 39.7, 31.3, 28.3 (3 C), 21.8.

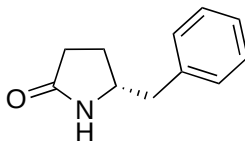
***tert*-Butyl (*R*)-2-benzyl-5-oxopyrrolidine-1-carboxylate (**34**) [41]**



34

To a RB flask containing a magnetic stirred bar, 5.2 g (13.8 mmol) of *tert*-butyl (*R*)-(1-(2,2-dimethyl-4,6-dioxo-1,3-dioxan-5-yl)-3-phenylpropan-2-yl)carbamate **33** was dried thoroughly and then dissolved in 310 mL of toluene. The solution was heated to reflux under argon for 6 hours. To workup the reaction, the temperature of the reaction solution was settled to room temperature. After that, the solution was concentrated and purified by column chromatographed using hexane: ethyl acetate = 4:1 as eluent to give 2.61g of desired product as a yellow oil. (overall yields after three steps: 61%). ¹H NMR (400 MHz, CDCl₃) δ 7.34–7.29 (m, 2 H), 7.28–7.24 (m, 1 H), 7.20–7.17 (m, 2 H), 4.39–4.34 (m, 1 H, CHN), 3.13 (dd, J = 13.5, 4 Hz, 1 H), 2.73 (dd, J = 13.5, 9.6 Hz, 1 H), 2.35–2.27 (m, 2 H), 2.20–1.90 (m, 1 H), 1.85–1.76 (m, 1 H), 1.57 (s, 9 H); ¹³C NMR (100 MHz, CDCl₃) δ 174.8, 150.1, 137.3, 129.6 (2 C), 129.0 (2 C), 127.1, 83.3, 59.3, 39.7, 31.3, 28.4 (3 C), 21.8. MS (ESI, MeOH): *m/z* = 298.1.

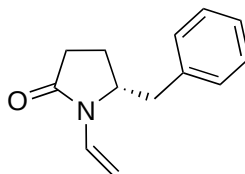
(R)-5-Benzyl-1-vinylpyrrolidin-2-one



35

To a RB flask containing a magnetic stirred bar, 2.61 g of tert-butyl (*R*)-2-benzyl-5-oxopyrrolidine-1-carboxylate **34**, (9.5 mmol) was dissolved in 13.05 mL of 10% trifluoroacetic acid in dichloromethane, and the reaction solution stirred at room temperature for 2 hours. To workup the solution, the reaction solution was transferred into a separatory funnel, then 50 mL dichloromethane was added and washed the organic layer with an 10% aqueous NaHCO₃ solution to remove the excess of TFA. The organic layer was latter washed with water and brine, then dried over magnesium sulfate, concentrated to give 1.67 g of crude compound **35** as a brown oil. This material was used in the subsequent step without further purification. ¹H NMR (400 MHz, CDCl₃) δ 7.35–7.14 (m, 5 H), 5.95–5.89 (bs, 1 H), 3.95–3.85 (m, 1 H), 2.82 (dd, J = 13.4, 5.6 Hz, 1 H), 2.71 (dd, J = 13.4, 7.6 Hz, 1 H), 2.33–2.18 (m, 3 H), 1.89–1.80 (m, 1 H); ¹³C NMR(400 MHz, CDCl₃) δ 178.1, 137.8, 129.3 (2 C), 129.2 (2 C), 127.1, 55.9, 43.2, 30.3, 27.2;

(R)-5-Benzyl-1-vinylpyrrolidin-2-one (36) [36]

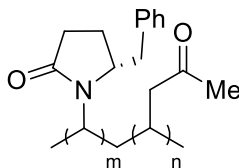


36

To a RB flask containing a magnetic stirred bar, 1.15 g of dried tert-butyl (*R*)-2-benzyl-5-oxopyrrolidine-1-carboxylate **35** (6.54 mmol) was dissolved in 20 mL of distilled *n*-butyl vinyl

ether, then 0.0414 g catalyst (DPP)Pd(TFA)₂ was added to the reaction solution and the mixture was stirred at 75°C for 16 hours under argon. The reaction mixture was restored to room temperature before filtered it through a pad of silica gel using a fritted funnel, the filtrate was collected and concentrated, and purified by silica gel column chromatography using hexane and ethyl acetate = 1:1 as an eluent to afford 0.93 g (71 % yield) of pure compound **36** as a yellow oil. ¹H NMR (400 MHz, CDCl₃) δ 7.36–7.24 (m, 3 H), 7.18–7.14 (m, 2 H), 7.08 (dd, J = 16.4, 9.6 Hz, 1 H, =CH), 4.63 (d, J = 16.4 Hz, 1 H, =CH₂), 4.62 (d, J = 9.6 Hz, 1 H, =CH₂), 4.23 - 4.16 (m, 1 H, CHN), 3.01 (dd, J = 14, 3 Hz, 1 H), 2.83 (dd, J = 14, 8 Hz, 1 H), 2.24–2.17 (m, 1 H), 2.10–1.91 (m, 3 H); ¹³C NMR (100 MHz, CDCl₃) δ 173.7, 137.1, 129.7, 128.9 (2 C), 128.4, 127.1 (2 C), 95.3, 57.0, 36.6, 30.1, 22.8;

Poly (5-substituted benzyl N-vinyl pyrrolidone-co-vinyl acetate) (37)



37

To a sealed tube containing magnetic stirred bars, 89.4 mg (0.44 mmol) of vinyl product **36** was flame dried then kept under argon. To a round-bottom flask, 3.5 mg (0.021 mmol) of azobisisobutyronitrile (AIBN) was also flame dried then kept under argon. The above mentioned glass containers were transferred into a gloved box to conduct the experiments at oxygen-free and water-free environment. For the RB flask, 0.5 mL degassed and distilled acetone was added to give AIBN acetone solution. To the sealed tube, the prepared solution of 0.075 mL AIBN acetone solution (0.0044 mmol), 81.9 µL of distilled and degassed vinyl acetate (0.89 mmol) and 0.104 mL of degassed and distilled acetone were added in. The sealed tube was later transferred

out of the glove box, and keep under reflux for 30 hours. To workup the reaction, the reaction was cooled to room temperature and added dropwise in to cold solution hexane to precipitate the desired compound copolymer **37**, The white sold was collected using centrifugation, 5 mg of copolymer was obtained, the remaining starting materiel was recovered. $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 7.21–6.78 (m, 3 H), 6.74–6.31 (m, 2 H), 3.50–3.01 (m, 3 H), 2.49–1.01 (m, 13 H). This material was used in the subsequent polymerization without purification.

1.6.2 Representative Preparation Steps for Bimetallic Nanocluster Stabilized by PVP or CSPVP

Materials

Chemicals were purchased from commercial suppliers such as Fisher Scientific Inc., including PVP, sodium tetrachloropalladate(II) (Na_2PdCl_4), polyvinylpyrrolidone (MW = 40,000) (PVP), tetrachloroauric (III) acid trihydrate (99%) (HAuCl_4), copper(I) chloride (CuCl), and sodium borohydride (NaBH_4).

Solvents such as ethyl alcohol, toluene, acetone, and acetonitrile were of analytical grade and were used without further purification. *N,N*-Dimethyl formamide (DMF) was distilled over CaH_2 under reduced pressure at 5 mmHg and 70 °C (bath temperature).

Catalyst Preparation

The general procedure for Cu/Au PVP and Pd/Au bimetallic nanoclusters catalysts were prepared as followed. $\text{Na}_2\text{Pd}_4\text{Cl}_2$, HAuCl_4 and polymer were added to water and mixed well followed by the addition of NaBH_4 to reduce the metal chlorides. The solution was stirred at room temperature for 30 minutes and used. Bimetallic nanoclusters Cu/Au-PVP or Pd/Au-PVP in either deionized water or DMF were prepared separately.

(1) A representative example for the preparation of Cu/Au(3:1)-PVP bimetallic nanoclusters in H₂O: To prepare a 25 mM Cu/Au(3:1)-PVP bimetallic nanocluster in H₂O, 0.110 g (2.75 μ mol) of PVP was dissolved in 2.875 mL H₂O in a round-bottom flask (RB) with a magnetic stirred bar, 7.4 mg (75 μ mol) of CuCl (stored in a glove box under nitrogen atmosphere) and 0.625 mL (25 μ mol) aqueous solution of 40 mM HAuCl₄·3H₂O were added separately. After stirring for 5 min, a solution of 4.8 mg (0.125 mmol) of NaBH₄ in 0.5 mL H₂O was added and it was stirred at 25 °C for 2 hours to give a 25 mM-solution of Cu/Au-PVP in H₂O.

(2) A representative example for the preparation of a 25 mM Cu/Au(3:1)-PVP bimetallic nanocluster in DMF. PVP 0.110 g (2.75 μ mol) was dissolved in 3.25 mL DMF in a RB flask with a magnetic stirred bar. To it, 7.4 mg (75 μ mol) CuCl and 0.25 mL (25 μ mol) of 100 mM HAuCl₄·3H₂O in DMF were added in sequence. After stirring for 5 min, a solution of 4.8 mg (0.125 mmol) of NaBH₄ in 0.5 mL DMF was added and the solution was stirred at 25 °C for 2 hours to give a 25 mM-solution of Cu/Au-PVP in DMF.

(3) A representative example for the preparation of Pd/Au(3:1)-PVP bimetallic nanocluster in H₂O: To prepare a 25 mM Pd/Au(3:1)-PVP bimetallic nanocluster in H₂O, 0.138g of PVP (3.45 μ mol) was dissolved in 1.8 mL deionized water in a RB flask with a magnetic stirred bar, 2.4 mL of 40 mM aqueous solution of Na₂PdCl₄ (96 μ mol) and 0.78 mL 40 mM of aqueous solution of HAuCl₄·3H₂O (32 μ mol) were added separately. After stirring the solution for 5 min, to it, 6 mg (0.15 mmol) of NaBH₄ was added and the solution was stirred at 25 °C for 2 hours to give a 25 mM solution of Pd/Au-PVP in H₂O.

(4) A representative example for the preparation of Pd/Au(3:1)-PVP bimetallic nanocluster in DMF: To prepare a 25 mM Pd/Au(3:1)-PVP bimetallic nanocluster in DMF,

0.138g of PVP (3.45 μmol) was dissolved in 1.8 mL deionized water in a RB flask with a magnetic stirred bar, 2.4 mL of 40 mM DMF solution of Na_2PdCl_4 (96 μmol) and 0.78 mL 40 mM of DMF solution of $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ (32 μmol) were added separately. After stirring the solution for 5 min, to it, 6 mg (0.15 mmol) of NaBH_4 was added and the solution was stirred at 25 $^\circ\text{C}$ for 2 hours to give a 25 mM solution of Pd/Au-PVP in DMF.

The nanocluster appearances were taken as shown in Figure 1. The prepared Cu/Au(3:1)-PVP bimetallic nanoclusters present a deep red color and the prepared Pd/Au PVP give deep brown color. There is no precipitation was observed for the Cu/Au PVP and Pd/Au PVP solution.

When the Cu/Au-PVP bimetallic nanocluster solution was used in the C-H oxidation reactions, the color of the reaction changes into transparent light yellow but no precipitate shows up. The color change could be caused by the oxidation state of the metals. Occasionally, the aggregated oligomers precipitated out and they were dispersed by vigorous stirring.

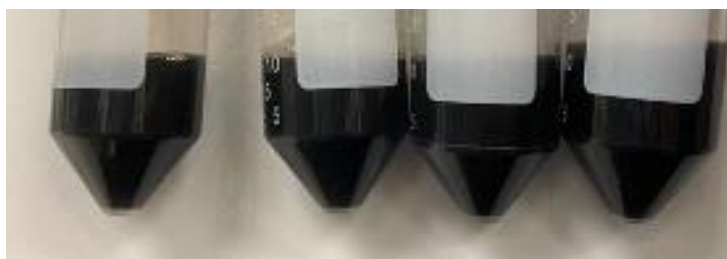


Figure 23 Solutions and colors of Cu/Au-PVP, Cu/Au(-)-1R, Cu/Au(+)-1S, Cu/Au(-)-2 catalysts

Chapter 2 - Catalytic C-H Oxidation Reactions

2.1 Background and Significance

In catalytic C-H oxidation, heterogeneous and homogeneous catalysts are available. Catalyst can be divided into two major categories: heterogeneous and homogeneous. In a homogeneous reaction, all substances are all in one phase. However, in a heterogeneous reaction, reaction phases are more than one, which means catalysts and reactants are not miscible with each other. Advantages of the homogeneous catalyst are many compared to heterogeneous catalyst, beneficial points are the homogeneous catalysts are not easy to recover and recycle, the supports or ligands of the catalysts can be modified and changed, high utilization of the catalysts, high selectivity and milder conditions[42]. The main advantages of the heterogeneous catalysts are easy to recovery of the catalyst, recycling, economic and green, and therefore largely used in industries[43]. The majority of the homogeneous catalysts do not require solid support [44]. In order to have the same advantages that homogenous and heterogeneous catalytic system, our system was adopted a similar system of biphasic catalysis system[45]. For a biphasic catalysis system, catalysts were dissolved in aqueous phase. In our system, the nanoclusters are suspended in the solvents. It is not really soluble in the solvents, but because of the small sizes, they are simialr to buphasic catalysis sytem therefore they can react rapidly. To allow the reaction happens, the reaction should stir vigorously, allowing the reaction reacts in the interface of two phases[46]. This liquid-nanocluster system was keeping the catalysts to retain the characteristic of the heterogeneous catalysis that is reactants can be absorbed on the active site of the catalyst, then catalysts link to the reactants making them more reactive, then the reaction happens, followed by the detachment of the products. At the same time, the system also keeps the advantages of the homogeneous catalysis. In our study, our nanocluster catalysts were mainly

prepared in aqueous solution and the reactant was dissolved in acetonitrile. Acetonitrile and water are miscible, thereby allowing the substrates to dissolve in the system, providing a rapid reaction[47, 48].

2.2 Oxidation of Ambroxide

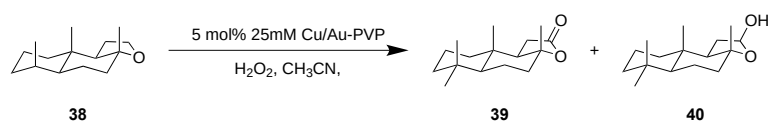
(-)-Ambroxide is a natural terpenoid and its possible oxidized product, (+)-sclareolide, is an antifungal compound. Ambroxide was chosen as the model reaction to investigate the differences between polymers and bimetallic nanoclusters. The results of this study enabled us to gain insights into the optimal reaction conditions for catalytic oxidation reactions and to enhance the yields of desired products.

As shown in Entry 1 of Table 4, when the ambroxide was dissolved in a mixture of water and acetonitrile, heated to 70 °C for three days, and no catalyst or oxidant was added, no product was produced, and 12% of the starting material was decomposed. Entries 2-4 detail the synthesis of monometallic nanoclusters (Au PVP nanocluster, Cu PVP nanocluster, and Pd PVP nanocluster) and their use in catalytic oxidation reactions, using 2 equivalents of 30% H₂O₂ as the oxidant, and heating at 70 °C for three days. The results of the study showed that monometallic nanoparticles had a negligible effect on the oxidation reactions. For Cu-PVP and Au-PVP nanoclusters, no products were obtained, while for Pd-PVP nanocluster, only 1% of the desired product, sclareolide, and 2% scleral were produced. Overall, the study indicated that monometallic nanoclusters have limited catalytic activity in these reactions. Under the same condition, Cu/Au-PVP nanocluster gave better conversion (20%) than Pd/Au-PVP (14%) for this chemical as shown in entry 5-6. Then, three chiral polymer-supported Cu/Au nanoclusters, CSPVP **1**, CSPVP **2**, and CSPVP **3**, were used to prepare the corresponding nanoclusters and set up an oxidation reaction under the same conditions. The Cu/Au-CSPVP **1** nanocluster-supported

nanoclusters gave the highest yield in bimetallic nanocluster oxidation, higher not only than CSPVP 2 and 3 but also than PVP. Meanwhile, CSPVP 2 and 3 reacted with low yields of 1% and 2%, respectively, as shown in entries 7-9. These results indicate that our nanoclusters exhibit chiral selectivity. Specifically, for ambroxide, CSPVP 1 matches its chirality, while CSPVP 2 and 3 do not. This also proves once again that our nanoclusters are chiral. With entry 7 results in mind, we increased the amount of the oxidant from 2 equivalent to 30 equivalent, and the nanocluster was more concentrated from 5 mM to 25mM, and the yields increased to 64% as shown in entry 10. The previous synthesized CSPVP-iPr was used for comparison of CSPVP 1 the entry 11, with same condition, it gave 54% yield, relatively lower than Cu/Au CSPVP 1.

With screen condition shown in table 4, the best condition for this chemical would be entry 10: 5 mol% 25 mM Cu/Au CSPVP 1 nanocluster as catalyst, 30 equivalent 30% H₂O₂ as oxidant and heated at 70 °C for three days to give 64% of the (+)-sclareolide and 23% of the recovery.

Table 4 Oxidation Reaction Results of Ambroxide



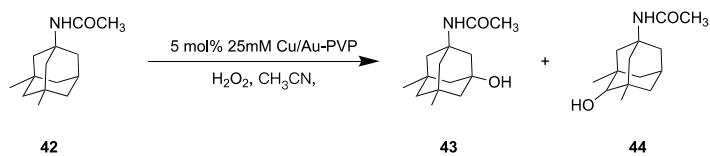
entry	polymer	catalyst composition	mol % catalyst	oxiditdant	reaction time	temperature	yields 39 40		recovered
1	none	none	none	none	72 hours	70 °C	0%	0%	88%
2	PVP	Au-PVP	5 mol%	2 eq. H ₂ O ₂	72 hours	70 °C	0%	0%	87%
3	PVP	Cu-PVP	5 mol%	2 eq. H ₂ O ₂	72 hours	70 °C	0%	0%	87%
4	PVP	Pd-PVP	5 mol%	2 eq. H ₂ O ₂	72 hours	70 °C	1%	2%	83%
5	PVP	Pd/Au-PVP	5 mol%	2 eq. H ₂ O ₂	72 hours	70 °C	14%	1%	72%
6	PVP	Cu/Au-PVP	5 mol%	2 eq. H ₂ O ₂	72 hours	70 °C	20%	0%	67%
7	CSPVP 1	Cu/Au-CSPVP 1	5 mol%	2 eq. H ₂ O ₂	72 hours	70 °C	37%	0%	51%
8	CSPVP 2	C/Au-CSPVP 2	5 mol%	2 eq. H ₂ O ₂	72 hours	70 °C	1%	0%	84%
9	CSPVP 3	Cu/Au-CSPVP 3	5 mol%	2 eq. H ₂ O ₂	72 hours	70 °C	2%	0%	83%
10	CSPVP 1	25mM Cu/Au-CSPVP 1	5 mol%	30 eq. H ₂ O ₂	72 hours	70 °C	64%	0%	23%
11	CSPVP-iPr	Cu/Au-CSPVP-iPr	5 mol%	30 eq. H ₂ O ₂	72 hours	70 °C	54%	0%	15%

2.3 Oxidation of *N*-Substituted Memantine

Memantine is an antagonist for *N*-Methyl-D-Aspartate (NMDAR) receptor and it was often used to treat neurotoxicity related diseases such as Alzheimer's diseases but it also has side effect such as headache. Therefore, we prepared *N*-Substituted Memantine analogues that may help to reduce the side effect.

In Table 5, entries 1 and 2, Cu/Au-PVP nanoclusters and Pd/Au nanoclusters were used for comparison. The oxidation occurred at the tertiary and secondary C-H positions of *N*-Substituted Memantine. Under the same conditions, Cu/Au-PVP showed better conversion to compound **43** (22%) and compound **44** (6%) than the Pd/Au-PVP catalyzed reaction, with a yield of 8.3% for compound **43** and 1% for compound **44**. Then the CSPVP **1** was used to compare with PVP under same condition but lower temperature in entry 1. As entry 4 results

shown, Cu/Au CSPVP **1** nanocluster slight increased the reaction with lowered temperature and higher recovered starting material. To improve the yield, entry 3 was designed, with more equivalent nanoclusters and 20 equivalent of oxidant, the yields improved tremendously while maintain at 50°C.

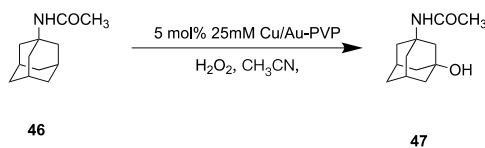


entry	polymer	catalyst composition	mol % catalyst	oxiditdant	reaction time	temperature	yields 43 44		recovered
1	PVP	Cu/Au-PVP	1.3 mol%	3 equiv H ₂ O ₂	30 hours	80 °C	22%	6%	50%
2	PVP	Pd/Au-PVP	1.3 mol%	3 equiv H ₂ O ₂	3 days	80 °C	8.3%	1%	83%
3	PVP	Cu/Au-PVP	10.0 mol%	20 equiv H ₂ O ₂	3 days	50 °C	38%	17%	12%
4	CSPVP-1R	1.92 mM Cu/Au-CSPVP-1R	1.3 mol %	3 equiv H ₂ O ₂	30 hours	50 °C	23%	4%	60%

Table 5 Oxidation Results of the *N*-Substituted Memantine

2.4 Oxidation of *N*-Substituted Adamantine

Adamantine is also the antagonist for NMDAR receptor, so we also investigated the oxidation conditions for *N*-Substituted Adamantine.



entry	polymer	catalyst composition	mol % catalyst	oxiditdant	reaction time	temperature	yields 47	recovered
1	PVP	Cu/Au-PVP	5 mol%	20 equiv H ₂ O ₂	3 days	80 °C	35%	48%
2	PVP	Cu/Au-PVP	5 mol%	20 equiv H ₂ O ₂	3 days	90 °C	51%	19%

Table 6 Oxidation Results of the *N*-Substituted Admantine

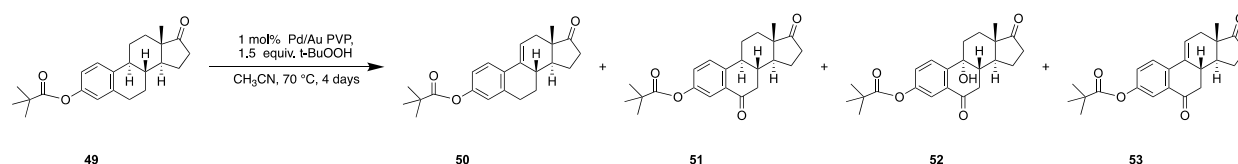
In shown in table 6 entry 1 and 2, 5 mol% Cu/Au-PVP nanocluster and 20 equivalent H₂O₂ was used for the reaction, and the reaction was stirred at 80 °C for three days, the oxidation

happen in the tertiary C-H, and the yield is 35%. To increase the yields of the reaction, the reaction temperature was increased to 90 °C, and the yield increased to 51%.

2.5 Oxidation of O-Piv-Estrone

Estrone is a type of sex hormone, and its complex ring system is worth investigating. To avoid oxidation happen at the OH group on the estrone, we protected the OH group with Pivaloyl protecting group. The reaction conditions were listed in table 7. In entry 1 and 2, CSPVP **1** and PVP were set up using the same condition, and four oxidation products were found. CSPVP **1** supported nanocluster gave better conversion of the starting material than PVP supported nanoclusters. In entry 1 and 3, Pd/Au-CSPVP **1** and **2** were set up using the same condition, and Pd/Au-CSPVP **2** gave more conversion of the compound **52** (17%) than Pd/Au-CSPVP **1** (9%). Then Cu/Au PVP reaction was also set up under similar condition, the reaction only gave two products **51** and **52**. To improve the yields of the entry 4, we increased the amount of the catalyst from 1mol% to 3mol% and added 4 equivalents of oxidant. With these adjusted conditions, the reaction managed to give 45% yield of compound **51** and 24% compound **52** within 18 hours and 50°C temperature.

Table 7 Oxidation Results of the O-Piv-Estrone



entry	polymer	catalyst composition	mol % catalyst	oxidizant	reaction time	temperature	yields				recovered
							50	51	52	53	
1	CSPVP 1	25 mM Pd/Au CSPVP 1	1 mol%	1.5 equiv t-BuOOH	4 days	70 °C	20%	20%	9%	2%	33%
2	PVP	25 mM Pd/Au PVP	1 mol%	1.5 equiv t-BuOOH	4 days	70 °C	8%	16%	9%	0%	58%
3	CSPVP 2	25 mM Pd/Au CSPVP 2	1 mol%	1.5 equiv t-BuOOH	4 days	70 °C	2%	23%	17%	0%	46%
4	PVP	25 mM Cu/Au PVP	1 mol%	1.5 equiv t-BuOOH	4 days	70 °C	0%	20%	18%	0%	54%
5	CSPVP 1	25 mM Cu/Au CSPVP 1	3 mol%	4.0 equiv t-BuOOH	18 hours	50 °C	0%	45%	24%	0%	5%

2.6 Proposed Reaction Mechanism

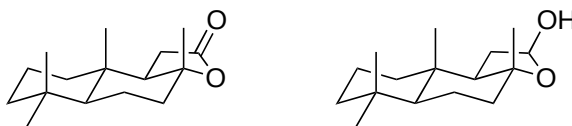
Although the mechanism for C-H oxidation catalyzed by bimetallic nanoclusters is unknown, Dr. Duy Hua proposed a reasonable mechanism based on the various oxidation products. The metal Cu or Pd and Au react with designed CSPVP to give M^0/Au^0 -CSPVP nanoclusters. When the reaction system is introduced to peroxide as the oxidant, the peroxide reacts with M^0/Au^0 -CSPVP to give a peroxide complex, and the oxidation state of Cu or Pd changes from M^0 to M^2 . Then, the nanocluster complex $M^2=O$ takes away a $C(sp_3)$ -H on the substrate, forming the nanocluster complex M^1-OH and a $C(sp_3)$ radical. The $C(sp_3)$ radical takes away the OH group on the M^1-OH complex, giving the oxidized product $C(sp_3)-OH$, and leaving M^1-OH to return to the M^0/Au^0 -CSPVP state.

Figure 24 Proposed Mechanism for Oxidation Reaction

taken using deuterated chloroform with tetramethylsilane (TMS) inside as an internal standard. The chemical shift for signal on ^1H NMR are given in unites of δ relative to TMS ($\delta = 0$), and for ^{13}C , the signals are relative to CDCl_3 ($\delta = 0$). Liquid chromatography–mass spectrometry data were from Waters Inc. The high-resolution mass spectra were obtained from aa Xevo G2-XS QToF of Quadeupole Time-of -Flight Mass Spectrometry equipped with an ultraperformance liquid chromatography from Waters Int. FTIR spectra were taken form Agilent Cary 630 FTIR. Optical rotation were taken from Perkin-Elmer 241 polarimeter.

The Cu/Au, Pd/ Au CSPVP bimetallic nanoclusters were prepared in one-pot method using chiral PVP as stabilizer. The chiral PVP polymers were characterized by GPC-HPLC, HRMS, FTIR and CD. The bimetallic nanoclusters' structure, size and chemical composition were also characterized by HRMS, FTIR, CD and DLS

Catalytic Oxidation of Ambroxide (38). Formation of Sclareolide (39) and Sclaral (40) (ZR-)



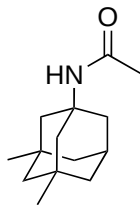
39

40

To a sealed tube, 100 mg (0.423 mmol) ambroxide **38**, 0.6 mL of acetonitrile and 0.85 mL of a 25-mM aqueous solution of Cu/Au(3:1)- PVP (5 mol% equiv., 0.0053 mmol Au, 0.0159 mmol Cu, 0.058 μmol PVP) were added. To it, 0.95 mL (8.46 mmol; 2 equivalents) of 30% hydrogen peroxide was added and the mixrture was stirred at 80°C for 3 days. To workup the reaction, the mixture was cooled to room temperature, transferred into a separatory funnel, and added 20 mL of deionized water. Then the aqueous layer was extracted three times with

dichloromethane, 100 mL each. The organic layers were combined and washed with water and brine, dried over anhydrous magnesium sulfate, filtered through a fritted funnel, and concentrated on a rotary evaporator and then under high vacuum to give 73.6 mg of crude product. It was purified through a silica gel column and used a mixture of hexane and ethyl acetate in a ratio of 6:1 as an eluent to recover 18.6 mg (18.9% recovery) of ambroxide (**15**) and obtain 48.1 mg (45% yield) of product **39** and 0.8 mg (0.7% yield) of **40**. Sclareolide **39** ^1H NMR (400 MHz, CDCl_3): δ 2.42 (dd, $J = 16.4, 14.8$ Hz, 1H), 2.25 (dd, $J = 16.4, 6.8$ Hz, 1 H), 2.12 – 2.08 (m, 1 H), 1.98 (dd, $J = 14.8, 6.4$ Hz, 1 H), 1.93 – 1.85 (m, 1 H), 1.75 – 1.62 (m, 2 H), 1.51 – 1.36 (m, 4 H), 1.35 (s, 3 H), 1.20 (td, $J = 13.6, 4.4$ Hz, 1 H), 1.11 – 1.03 (m, 2 H), 0.93 (s, 3 H), 0.90 (s, 3 H), 0.85 (s, 3 H); ^{13}C NMR (100 MHz, CDCl_3) δ 177.3, 86.6, 59.3, 56.8, 42.4, 39.7, 38.9, 36.3, 33.4, 33.3, 28.9, 21.8, 21.1, 20.8, 18.3, 15.3 ppm. Scleral **40** ^1H NMR (400 MHz, CDCl_3), as a mixture of two diastereomers: δ 9.17 (s, 1H, OH of major), 0.06 (s, 1H, OH of minor), 5.62-5.56 (m, 1H, CHO), 2.05-1.90 (m, 2H), 1.83-1.53 (m, 4H), 1.50-1.37 (m, 3H), 1.35 -1.28 (m, 1H), 1.27 (s, 3H), 1.24-1.12 (m, 2H), 1.10-0.92 (m, 2H), 0.83 (s, 3H), 0.83, 3H) ppm. ^{13}C NMR (100 MHz, CDCl_3) δ 108.4 (OCO of major), 106.2 (OCO of minor), 84.3 (CO of minor), 82.4 (CO of major), 59.9 (major), 57.6 (minor), 57.1 (minor), 56.9 (major), 42.4 (major), 39.9 (minor), 39.8 (minor), 39.7 (major), 39.6 (major), 36.2 (major), 36.0 (minor), 33.5 (major), 33.4 (minor), 33.1 (2C), 28.1 (minor), 27.4 (major), 23.4 (major), 23.0 (minor), 21.0 (2C), 20.8 (minor), 20.4 (major), 18.3 (major), 18.2 (minor), 15.1 (2C) ppm.

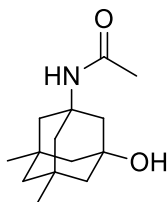
Synthesis of *N*-Acetyl-memantine (**42**) (ZR-2-11)



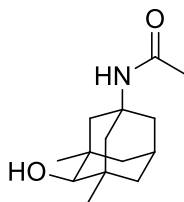
42

To a dried RB flask equipped with a magnetic stirred bar under argon were added 0.50 g (2.80 mmol) of memantine (**41**) and 10 mL of dichloromethane. The flask was cooled over an ice-water bath and to it, was added 0.96 mL (7 mmol) of triethylamine followed by 0.24 mL (3.5 mmol) of acetyl chloride via syringes in dropwise fashion. The mixture was stirred at 0 °C for 10 minutes and then at room temperature for 5 hours. The solution was transferred into a separator funnel and diluted with 100 mL of water. It was extracted three times with dichloromethane, 50 mL each. The combined extract was washed with water and brine, dried over anhydrous magnesium sulfate, and concentrated to give 0.603 g (97% yield) of compound **42**: **¹H NMR** (400 MHz, CDCl₃) δ 5.25 – 5.15 (bs, 1H, NH), 2.15 – 2.11 (m, 1H), 1.90 (s, 3H, CH₃CO), 1.82 – 1.81 (m, 2H), 1.64 – 1.63 (m, 4H), 1.36 (d, J = 16 Hz, 2H), 1.30 (d, J = 10.4 Hz, 2H), 1.16 (d, J = 10.4 Hz, 2H), 0.84 (s, 6H) ppm; **¹³C NMR** (100 MHz, CDCl₃) δ 169.3, 53.5, 50.6, 47.7 (2C), 42.8 (2C), 42.4, 40.2, 32.3, 30.1 (2C), 24.7 ppm.

Catalytic Oxidation of 42. Formation of N-3-hydroxy-5,7-dimethyl-adamantan-1-yl acetamide (43) and N-4- hydroxy-3,5-dimethyl-adamantan-1-yl acetamide (44) (ZR-3-21)



43



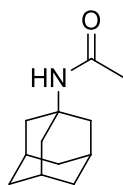
44

To a sealed tube equipped with a small magnetic stirred bar, were added 300 mg (1.36 mmol) of *N*-acetyl-memantine (**42**) and 3 mL of acetonitrile followed by 6.8 mL of a 25 mM aqueous solution of Cu/Au(3:1)- PVP (5 mol% equiv. , 51 μ mol of Cu, 17 μ mol of Au, and 1.8 μ mol of PV). To the reaction solution was added 0.184 mL (2.0 equiv., 2.71 mmol) of 30% hydrogen peroxide and the tube was heated over an oil bath at 80°C for 54 hours. To workup the reaction, the mixture was cooled to room temperature then transferred into a separator funnel, diluted with 20 mL of deionized water, and extracted three times with ethyl acetate, 100 mL each. The ethyl acetate extracts were combined, washed with water and brine, dried over anhydrous magnesium sulfate, filtered through a fritted funnel, and concentrate under reduced pressure to give 73.6 mg of crude product. It was purified by subjection into silica gel column chromatography and using a gradient mixture of dichloromethane and methanol as eluents to recover 150 mg (50% recovery) of starting material **42**, 71 mg (22% yield) of compound **43**, and 19 mg (6% yield) of compound **44**.

Compound **43**: $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 5.20 – 5.15 (bs, 1H, NH), 2.03 (s, 1H, OH), 1.91 (s, 3H, CH_3CO), 1.87 – 1.85 (s, 2H), 1.66 (d, J = 16 Hz, 2H), 1.59 (d, J = 16 Hz, 2H), 1.42 (d, J = 10.4 Hz, 2H), 1.33 (d, J = 10.4 Hz, 2H), 1.14 (dt, J = 12.4, 2 Hz, 1H), 1.09 (dt, J = 12.4, 2 Hz, 1H), 0.93 (s, 6H); $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ 169.4, 70.3 (C-O), 55.1 (C-N), 50.4 (2C), 49.4, 47.7, 46.5 (2C), 34.2 (2C), 29.1 (2C), 24.6.

Compound **44**: $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 5.16 - 5.13 (bs, 1H, NH), 3.36 (s, 1H, CHO), 2.10 – 2.02 (m, 2H), 1.91 (s, 3H, CH_3), 1.75 – 1.65 (m, 4H), 1.50 – 1.35 (m, 3H), 1.22 – 1.18 (m, 2H), 0.90 (s, 3H), 0.86 (s, 3H); $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ 169.4, 76.9, 52.8, 49.9, 47.2, 42.2, 41.0, 36.5, 36.4, 33.9, 32.2, 29.2, 25.6, 24.7.

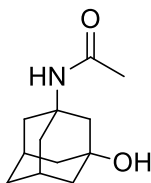
Synthesis of *N*-Acetyl-amantadine (**46**) (ZR-3-135)



46

To a RB flask, were added 3.0 g (19.84 mmol) of adamantane-1-amine (**45**) and 30 mL of tetrahydrofuran. The flask was cooled over an ice-water bath and added 3.21 mL (39.67 mmol) of pyridine dropwise. The solution was stirred for 15 minutes, warmed to room temperature, and stirred for 2 hours. To workup the reaction, 20 mL H₂O was added into the solution and the mixture was concentrated to remove the THF on a rotary evaporator. The reaction mixture was transferred into a separatory funnel and extracted three times with dichloromethane. The organic layers were combined and washed successively with 1N HCl solution, aqueous NaHCO₃ solution, water, and brine. The organic layer was dried over anhydrous MgSO₄, filtered, and concentrated to give 2.2 g (58 % yield) of product **46**: ¹H NMR (400 MHz, CDCl₃) δ 5.20 – 5.10 (bs, 1H, NH), 2.03 (s, 3H), 1.98 (s, 6H), 1.91 (s, 3H), 1.66 (s, 6H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 169.3, 51.9, 41.6 (3C), 36.3 (3C), 29.4 (3C), 24.7 ppm.

Catalytic Oxidation of 46. Formation of *N*-3-Hydroxyadamantan-1-yl acetamide (47)

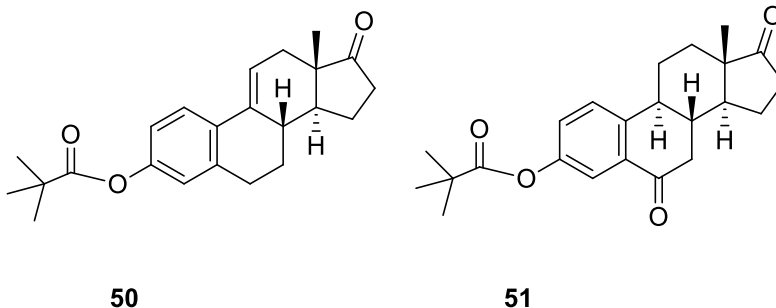


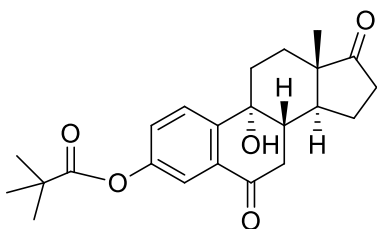
47

To a sealed tube equipped with a magnetic stirred bar, were added 50 mg (0.26 mmol) of *N*-acetyl-amantadine (**46**) and 0.55 mL of acetonitrile followed by 5.5 mL of a 10-mM aqueous

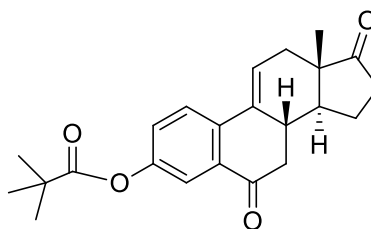
solution of Cu/Au(3:1)-PVP (5 mol% equiv. , 9.7 μmol of Cu, 3.23 μmol of Au, and 0.3 μmol of PVP). To the stirred solution, 0.05 mL (1.5 equiv., 0.4 mmol) of 30% hydrogen peroxide was added and the reaction mixture was kept at 80°C for 54 hours. To workup the reaction, the mixture was cooled to room temperature then transferred into a separatory funnel and added 10 mL of deionized water. The solution was extracted three times with 50 mL each of dichloromethane and the combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, filtered through a fritted funnel, and concentrate to give 66.3 mg of crude product. It was purified by subjecting to a silica gel column and using a mixture of dichloromethane and methanol (9:1) as an eluent to recover 14 mg (28 % recovery) of starting material **46** and 17.3 mg (31% yield) of compound **47**: $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 5.25 – 5.17 (bs, 1H), 2.29 – 2.22 (m, 2H), 1.85 (s, 3H), 2.08 – 1.50 (m, 13H); $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ 169.8, 69.6, 54.8, 49.5, 44.5, 40.8, 35.3, 31.0, 25.0.

Catalytic oxidation of O-Pivaloyl Estrone (49). Formation of (8S,13S,14S)-13-methyl-17-oxo-7,8,12,13,14,15,16,17-octahydro-6H-cyclopenta[a]phenanthren-3-yl pivalate (50), (8R,9S,13S,14S)-13-methyl-6,17-dioxo-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-3-yl pivalate (51), (8S,9R,13S,14S)-9-hydroxy-13-methyl-6,17-dioxo-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-3-yl pivalate (52) and (8S,13S,14S)-13-methyl-6,17-dioxo-7,8,12,13,14,15,16,17-octahydro-6H-cyclopenta[a]phenanthren-3-yl pivalate (53)





52



53

To a sealed tube equipped a magnetic stirred bar, were added 100 mg(0.282 mmol) of *O*-piv-estrone (**49**) and 6 mL of acetonitrile followed by 1.76 mL of a 1.6-mM aqueous solution Pd/Au (3:1)- PVP (1 mol%; 2.82 μ mol of Pd, 0.9 μ mol of Au, 0.1 μ mol of PVP). Subsequently, to it, 0.054 mL (1.5 equiv., 0.42 mmol) of 70% tert-butyl hydroperoxide was added and the mixture was stirred at 60°C over an oil bath for 96 hours. To workup the reaction, the mixture was cooled to room temperature, transferred into a separatory funnel, and diluted with 10 mL of saturated sodium metabisulfite aqueous solution to reduce the excess of oxidants. Then, 50 mL dichloromethane was added to the aqueous layer and the mixture was separated on a separatory funnel. The aqueous layer was extracted twice with dichloromethane. The dichloromethane layers were combined, washed with water and brine, dried over anhydrous magnesium sulfate, filtered and concentrate under reduced pressure to give the crude product. It was then purified on a silica gel column chromatography using a gradient mixture and hexane and diethyl ether to give 76.2 mg (76% recovery) of starting materials **49**, 7.4 mg of compound **52** (6% yield). 4.7 mg (5% yield) of compound **50** (5% yield), and 4.3 mg (4% yield) of compound **51**. Compounds **50** and **51** are inseparable by silica gel column chromatography, hence yields were based on ^1H NMR analysis and the weight of the mixture. Weight of compound **53** is less than 1 mg (< 1% yield). Compound **50**: ^1H NMR (400 MHz, CDCl_3) δ 7.59 (d, J = 8.8 Hz, 1H), 6.85 (dd, J = 8.8, 2.8 Hz, 1H), 6.79 (d, J = 2.8 Hz, 1H), 6.24 (dt, J = 5.6, 2.8 Hz, 1H, =CH), 2.94 – 2.83 (m, 2H), 2.55 – 2.45 (m, 1H), 2.44 – 2.35 (m, 1H), 2.32 – 1.93 (m, 4H), 1.70 – 1.40 (m, 4H), 1.34 (s, 9H),

0.94 (s, 3H) ppm; ^{13}C NMR (150 MHz, CDCl_3) δ 221.5, 177.2, 149.9, 137.3, 135.2, 131.7, 125.1, 121.7, 119.3, 118.9, 47.8, 46.2, 39.1, 38.02, 36.2, 34.0, 29.6, 27.6, 27.6 (3C), 22.5, 14.5 ppm. Compound **51**: ^1H NMR (400 MHz, CDCl_3) δ 7.73 (d, J = 2.8 Hz, 1H, C4-H), 7.45 (d, J = 8.4 Hz, 1H, C2-H), 7.25 (dd, J = 8.4, 2.8 Hz, 1H, C1-H), 2.88 (dd, J = 16.4, 3.2 Hz, 1H), 2.65 – 2.46 (m, 3H), 2.34 (dd, J = 16.4, 13.2 Hz, 1H), 2.23 – 2.02 (m, 4H), 1.74 – 1.53 (m, 4H), 1.36 (s, 9H), 0.93 (s, 3H) ppm; ^{13}C NMR (150 MHz, CDCl_3) δ 219.6 (C17), 196.5 (C6), 177.0 (CO₂), 149.9, 143.6, 133.5, 127.2, 126.6, 120.0, 47.6, 43.1, 39.2, 39.1, 35.7, 31.2, 27.1 (3C), 25.1, 21.4, 13.6 ppm; Compound **52**: ^1H NMR (400 MHz, CDCl_3) δ 7.74 (d, J = 2.8 Hz, 1H, C4-H), 7.61 (d, J = 8.4 Hz, 1H, C1-H), 7.29 (dd, J = 8.4, 2.8, 1H, C2-H), 2.90 (dd, J = 17.5, 12.8 Hz, 1H), 2.62 (dd, J = 17.5, 4 Hz, 1H), 2.55 – 2.45 (m, 2H), 2.37 (td, J = 12, 4.4 Hz, 1H), 2.25 – 2.14 (m, 2H), 2.16 – 1.95 (m, 2H), 1.88 – 1.84 (m, 1H), 1.65 – 1.56 (m, 2H), 1.36 (s, 9H, t-Bu), 0.93 (s, 3H, Me) ppm; ^{13}C NMR (150 MHz, CDCl_3) δ 219.4 (C17), 196.7 (C6), 176.9 (C=O ester), 151.3 (C3), 144.2, 133.1, 127.4, 125.5, 120.8, 69.4 (C9), 47.5, 43.5, 40.9, 39.1, 36.7, 35.8, 32.0, 27.4, 27.1 (3C's), 21.2, 12.8 ppm; Compound **53**: ^1H NMR (400 MHz, CDCl_3) δ 7.69 (d, J = 2.4 Hz, 1H, C4-H), 7.67 (d, J = 8.8 Hz, 1H, C2-H), 7.25 (dd, J = 8.8, 2.4, 1H, C1-H), 6.50 (dt, J = 5.2, 2.4 Hz, 1H, C11-H), 2.99 (dd, J = 15.8, 4.6 Hz, 1H), 2.86 – 2.77 (m, 1H), 2.57 (dd, J = 18.5, 10.4 Hz, 1H), 2.43 – 2.34 (m, 2H), 2.26 (q, J = 9.6 Hz, 1H), 2.24 – 2.15 (m, 1H), 1.86 – 1.80 (m, 1H), 1.73 – 1.67 (m, 1H), 1.43 – 1.37 (m, 1H), 1.36 (s, 9H, t-Bu), 0.95 (s, 3H, Me) ppm; ^{13}C NMR (150 MHz, CDCl_3) δ 220.0 (C17), 196.2 (C6), 176.9 (C=O ester), 150.7 (C3), 137.9, 133.4, 131.7, 127.6, 126.1, 124.5, 119.4, 48.4, 45.9, 42.8, 39.1, 37.8, 36.1, 34.0, 27.1 (3C's), 22.2, 14.2 ppm.

Chapter 3 - Design, Synthesis, and Electrochemical

Evaluation of Substrate Peptides for Profiling of

Cancer Overexpressed Proteases

3.1 Background and Significances

This project is in collaboration with Dr. Jun Li's group members. In Hua's laboratory, group members including me design and synthesize short substrate peptides, such as hexapeptides, containing six amino acid residues. The peptides including a linker in one end and an electronic reporter – ferrocene group – in the other end of the substrate peptides are given to Dr. Li's graduate students and they performed the immobilization onto electronic chips followed by electronic measurement. Results were provided to Hua's group for optimization of the substrate peptides to maximize selectivity with the specific proteases.

Protease is a type of enzyme that can break down the amide bond of the polypeptide chain into small fragments of peptides or amino acids. This process calls proteolysis. Proteolysis can be executed by binding to substrates' specific amino acid residues through hydrophobic and electrostatic interaction. Proteases bind to compatible conformation of specific substrate peptides through hydrogen bonding. When the living organism is healthy, proteases are involved in many proteins that regulate the physiological activities of the body, and when the organism is abnormal, proteases are overly active, such as metathesis in cancers. Thereby, measurement of abnormal overexpression of specific proteases can be used as a measure of certain diseases, such as cancer. Proteases can appear to be overly expressed in the early stages of cancers[49]. A typical example is matrix metalloproteinase 9 (MMP 9). By comparing normal cells with tumor cells, the expression of MMP-9 in diseased or cancerous cells is 9 times higher than normal [50]. Then, the

question is how to measure overexpressed proteases of a disease selectively in the presences of many other proteases from human fluids for instance. In addition, measurement of activity of the overexpressed proteases in disease cells will provide a more accurate diagnostic tool for the disease.

Our goals are to quantify the overexpressed level and activity of target proteases to determine whether a disease, such as breast cancer, can be diagnosed. Many challenges of detection of activities of selective proteases are followed: (i) With the large number of proteases in the human body and their complex structures and functions, how to accurately identify a specific target protease is the primary problem; (ii) Even though proteases are overexpressed, they are likely to be undetectable due to the low concentrations of proteases; (iii) The target proteases to be detected are not in an isolated system, but in an intricate environment, which means that the influence of the environment needs to be considered. To achieve this goal, a number of biosensors were reported including electrochemical biosensors, enzyme biosensors, immunosensors, etc.[51, 52] .

The use of a substrate peptide, a biological small peptide, as a component of protease recognition has several advantages, such as good recognition, reasonable stability, environmental friendliness, low economic cost, and easy preparation. Moreover, short substrate peptides consist of several amino acids, and different amino acid sequences may alter their reactivity or proteolysis towards a protease. This is due to differences in the side chain of the amino acid residues, charges, hydrophobic force and folding conformation. In our study, the short substrate peptides, such as hexapeptides, are assembled on the electrode surface as a tool for specific recognition, and at the end of the substrate peptide, a reporter such as a ferrocene group, is attached. The cleavage of the substrate peptide by specific protease will lead to a decrease of the

electrochemical signal. This cleavage of short substrate peptide by the protease is a proteolytic process through the hydrolysis of a specific amide bond of a given amino acid residue of the substrate peptide. The general idea is firstly to select the proteases that are overexpressed in the targeted cancers, such as breast cancer in our studies; secondly design substrate peptide sequences that can specifically recognize the target proteases; thirdly synthesis the peptide including a linker in one end of the peptide and an electronic tag, such as ferrocene, at the other end. Immobilization of the substrate peptides to the electronic chips for the study of proteolysis with a specific protease through the decrease of electronic signals. Next, a sample fluid obtained from the living organism containing the protease will be used to determine the proteolysis activity and quantification of the protease. Finally, selectivity of the substrate peptides with other proteases is needed to generate a profile of the specific protease. More than one protease including cathepsin B, MMP-9 and Adam 17 are chosen for the studies.

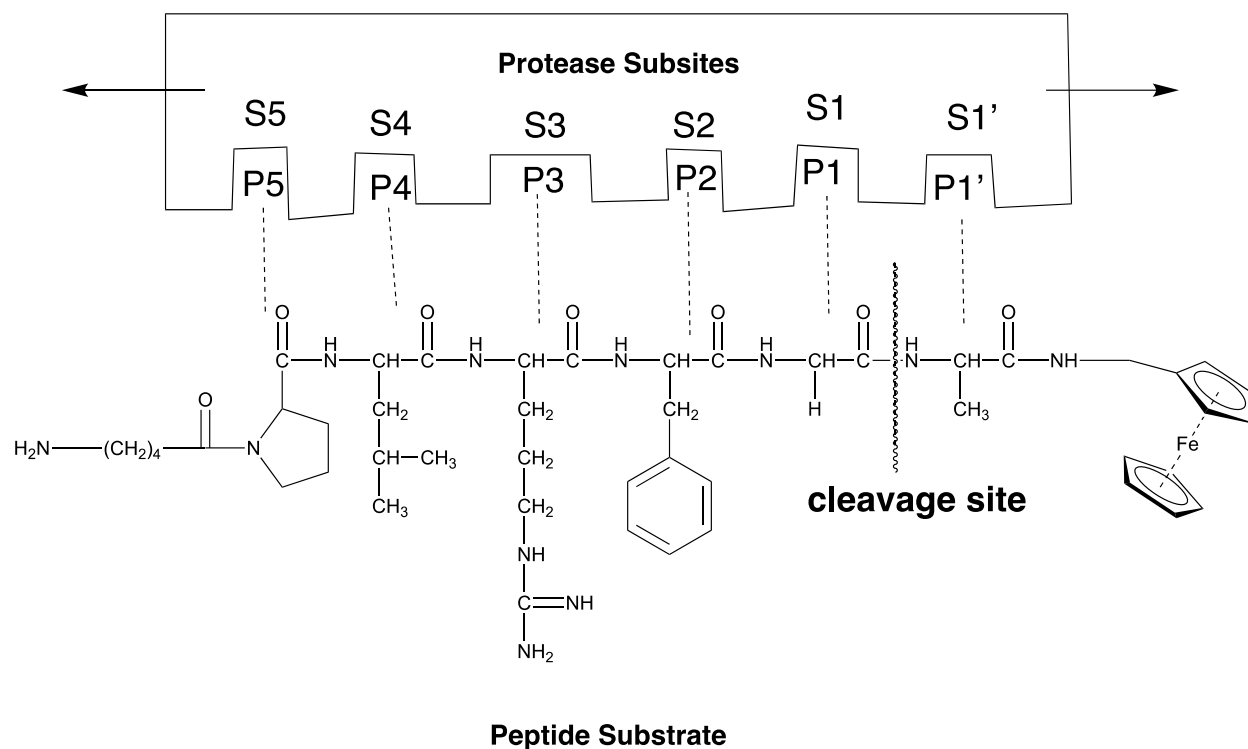
The focus of the research are three proteases: cathepsin B, Adam 17 and MMP-9, These proteases possess unique proteolysis selectivity, and their overexpression levels represent different stages of breast cancer. Cathepsin B is a type of cysteine proteases, and its overexpression can occur. Its abnormal expression allows the invasion of cancerous cells to adjacent cells, resulting in spreading of cancer and cancer metastasis. Cathepsin B is associated with a variety of diseases, including arthritis, breast cancers, colon cancer and other diseases[53].

ADAM metalloproteinase domain 17 (Adam 17), is a protease that is located on the membrane and is important for various normal and abnormal biological processes. Its abnormal regulation associated with many diseases, such as cancer.

MMP-9 is being studied for the diagnosis of early stage of ovarian carcinoma and Alzheimer's disease [54] MMP-9 is associated with the formation of the amyloid- β which caused Alzheimer's disease [55], but this finding was recently questioned [56].

With target enzymes were selected, a series of peptide substrates were designed to selective cleaved off by the target proteases as shown in figure 25. The substrate peptides and target enzyme were dissolved in buffer and incubate them together with the designed chip for several hours, then measure the AC voltammetry. This step was carried out by students in Dr. Jun Li's group. The changes in voltage can be used to determine the proteolysis rate of the process and therefore can be used to profile the activity of the enzyme.

Figure 25 Schematic Diagram of the Substrate Peptides Being Cleaved

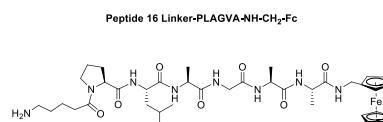
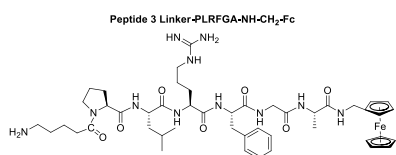


In this approach, the cleavage rate between different substrate peptides and the target enzyme is measured, and a library can be built with those informations. Then, by placing different substrate peptides on the chips and measuring the reaction rate, we can compare it with

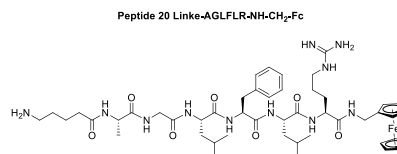
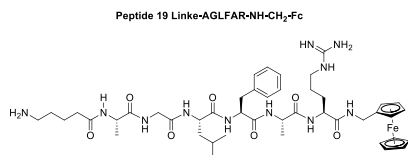
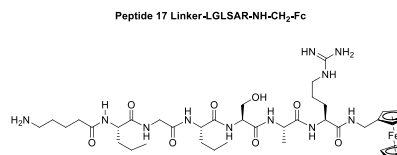
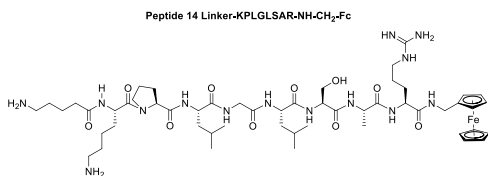
the substrate library to determine which enzyme was overexpressed. This technique, known as profiling, can be widely used in proteomics research to identify and quantify the activity of different enzymes in a biological sample. It is a valuable tool for measuring of many physiological and pathological processes, such as cancer.

3.2 Synthesis of Substrate Peptides

PeptidesSequence designed for Protease Cathepsin B



PeptidesSequence designed for Protease Adam 17



PeptidesSequence designed for Protease MMP-9

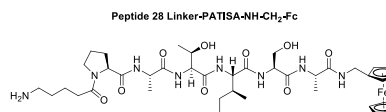
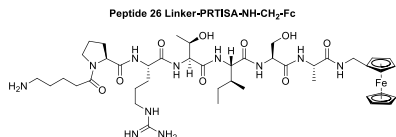


Table 8 Peptide Sequences for Selected Protease

Peptides 16, 17, 18, 19, 20, and 26 were all synthesized using the same solid-phase peptide synthesis method and a CEM Discover microwave synthesizer, following is a example of the peptide synthesis route of peptide 17.

Peptides 16, 17, 18, 19, 20, and 26 were all synthesized using the same solid-phase peptide synthesis method and a CEM Discover microwave synthesizer, following is a example of the peptide synthesis route of peptide 26.

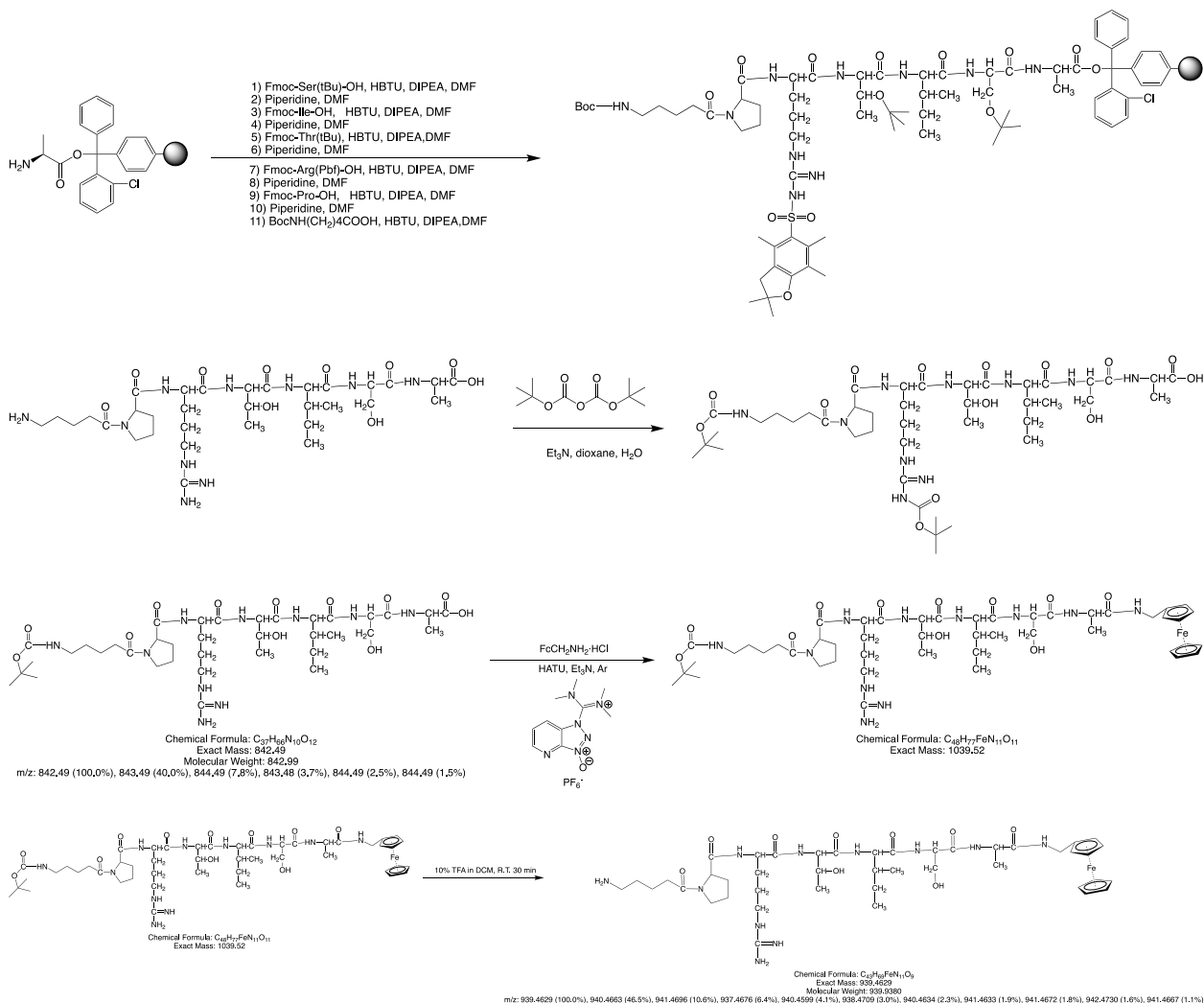
In the peptide synthesis process, the resin-protected amino acid is coupled with an amine-protected amino acid, and this step is repeated until there is no primary free amine detected in a ninhydrin test. The N-Fmoc of the protected amino acid from resin is then deprotected using a 20% piperidine in DMF solution. This procedure is repeated until the desired peptide sequence is obtained. The peptide is cleaved from the resin using a solution of TFA and (i-Pr)₃SiH in water. The resulting crude peptide is then precipitated with hexane and diethyl ether, dried, and characterized using LC-MS.

To carry out a coupling reaction between ferrocene and the peptide, all primary amines in the peptide sequence must be protected. To achieve this, the peptide is dissolved in a mixture of p-dioxane and water (1:1) and treated with triethyl amine as a base to react with di-tert-butyl decarbonate (Boc₂O). The *N*-Boc-protected peptide crude product is used without further purification and coupled with 1-(aminomethyl)ferrocene·HCl salt and 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU) in DMF. The product is then filtered and purified using preparative HPLC to obtain pure N-Boc-HN(CH₂)₄CO-peptide 26-CH₂-Fc product.

The *N*-Boc-HN(CH₂)₄CO- peptide 26-CH₂-Fc product is dissolved in dichloromethane and reacted with TFA to remove the Boc protecting group. Finally, the product is filtered and purified using preparative HPLC again to obtain H₂N(CH₂)₄CO- peptide 26-CH₂-Fc.

The substrate peptides can be functionalized onto the electrode, and AC voltammetric measurement can be used. By detecting the change in signal due to electron transfer on the ferrocene, the status of the polypeptides can be determined. After the protease cleaves the redox probe, the ferrocene end will detach. Using LC-MS and HPLC methods, the cleavage site can be determined.

Figure 26 A representative Synthesis of Linker-peptide-26-Fc



3.3 Evaluation of the Cleave Site of the Peptides

The peptides mentioned above were evaluated using HPLC. The HPLC graph of the substrate peptide in buffer was compared with the HPLC graph of the substrate peptide that had been incubated with the enzyme in buffer.

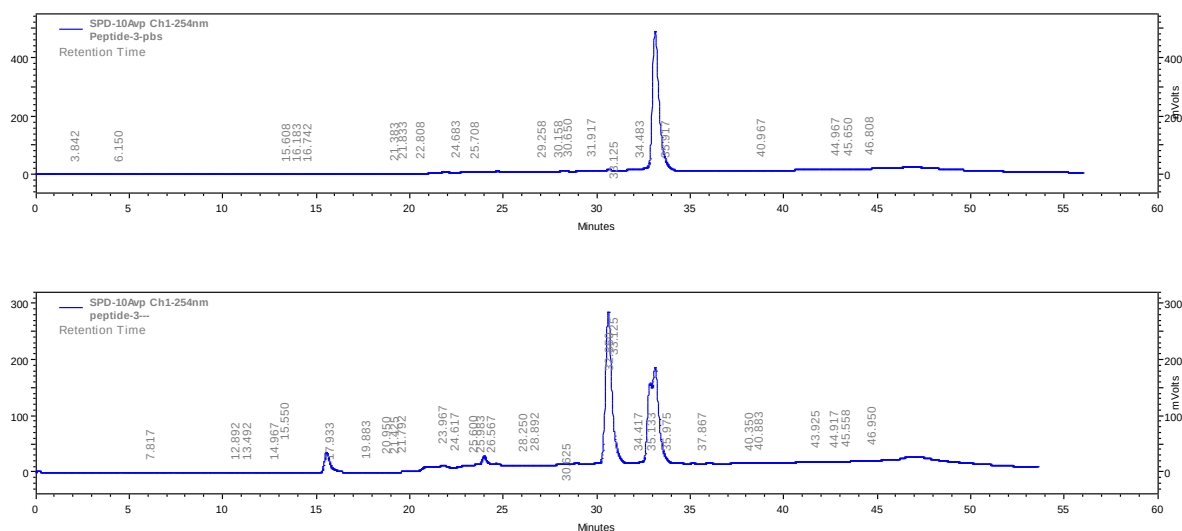


Figure 27 HPLC results of the For Linker-peptide 3-Fc in 254 nm (a) The Linker-peptide 3-Fc incubated in 0.5X PBS buffer (b) The Linker-peptide 3-Fc incubated with Cathepsin B after 2.5 h incubation in 0.5X PBS buffer

By comparing the two HPLC graphs, we observed that in the HPLC results of the sample containing linker-peptide 3 and buffer, only one peak appeared with a retention time of 33.13 minutes, which corresponds to the peak of peptide 3. When peptide 3 was incubated with the target protease Cathepsin B, the resulting sample showed two major peaks at 30.6 and 33.13 minutes, indicating the presence of a cleavage product in addition to the starting material peptide 3. LC-MS analysis confirmed that the taller peak corresponds to the cleavage product Ala-Fc, while the lower peak corresponds to linker-peptide 3-Fc. This provides evidence that peptide 3 was indeed cleaved by Cathepsin B.

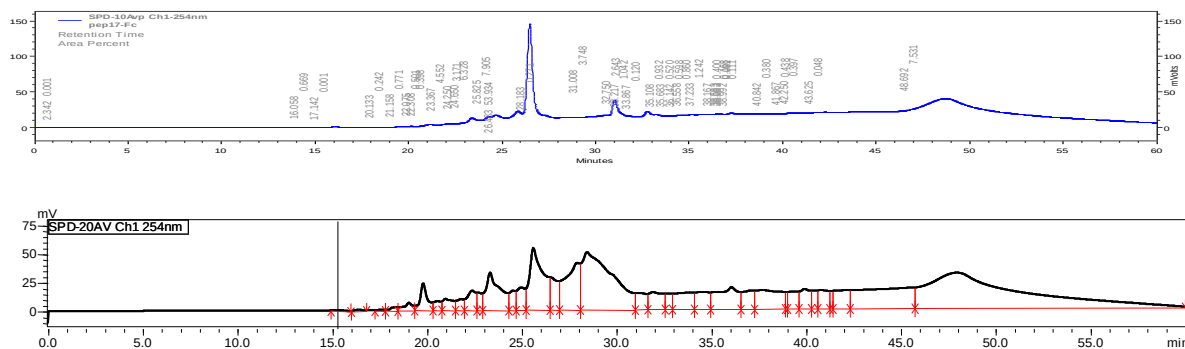


Figure 28 HPLC results of the For Linker-peptide 17-Fc in 254 nm (a) The Linker-peptide 17-Fc incubated in 1X PBS buffer (b) The 6.35 mM Linker-peptide 3-Fc incubated with 0.3 μ M Adam 17 after 2.5 h incubation in 0.5X PBS buffer 6.35 Mm peptide 17, incubated in 37 degrees for 2 hours

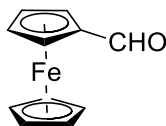
The initial evaluation of the peptide 17 is incubated to peptide 17 with Adam 17 to eluate the affinity between them, and based on the HPLC graph, the peptide 17 can be cleaved off and results of the LC-MS has confirmed the cleavage site is between Leu and Ser and fraction is Ser-Ala-Arg- NH-CH₂-Fc.

3.4 Conclusions

Multiple peptides designed and synthesized can recognized by target proteases, by reacting by each other, the biochemical signal can be detected by electrochemical methods, which allowed to quantify the target protease in the complex systems. An electrochemical method for the detection of protease B activity in complex samples was provided. The ELISA device equipped with functionalized peptide complexes provide a less costly way to detect the protease B activity than using sensors specifically bound to enzymes, DNA, etc., and the device has distinct signal changes, amplified detection signal, stable detection, good photoelectric conversion ability and shorter detection times make it a good device that has high sensitivity, low detection limit, good stability, reproducibility and low chance of false positive detection, laying a good foundation for becoming a commercial assay one day.

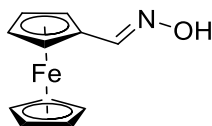
3.5 Synthetic Experimental Section

Synthesis of ferrocenecarboxaldehyde



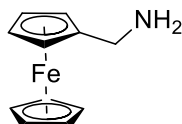
The starting material 7.0 g ferrocene (0.038 mol) was flame dried under vacuum then later changed into argon atmosphere. To it, a solution of 37.56 mL triethyl orthoformate (0.226 mol) in dried 113 mL dichloromethane were added to the flask. The flask was cooled down to 0 °C then was slowly added 44.1 g of ground aluminum chloride (0.331 mol) powder at 0 °C. The reaction mixture was stirred vigorously for 2 hours under argon at room temperature. The reaction mixture was poured into 0 °C Na₂S₂O₄ aqueous solution and stirred for another 2 hours. Emulsified layer was formed. To remove the emulsifier layer, the reaction mixture was filtered to give a better separation of aqueous layer and dichloromethane layer. A separate funnel was then used to separate aqueous layer and dichloromethane layer. The organic layer was combined, washed with sodium bicarbonate aqueous solution, water, brine then dried over Magnesium sulfate anhydrous solid. The dichloromethane layer was filtered, concentrated and purified by column chromatography to give desired product 5.8 g (73% yield). ¹H NMR (400 MHz, CDCl₃): δ 9.96 (s, 1H), 4.48 (d, 2H), 4.61 (d, 2H), 4.28 (s, 5H); ¹³C NMR (100 MHz, CDCl₃): δ

Synthesis of 1-(aldehyde oxime)ferrocene



To a mixture of 1.5 g of ferrocene carboxaldehyde (7.01 mmol) and 0.97 g of hydroxylamine hydrochloride (14.02 mmol) were added to 100 mL of ethanol, then the reaction mixture was heated to reflux. To it, 3.8 g sodium acetate (28.04 mmol) was added in portions to the hot reaction solution and refluxed for 4 hours. The reaction solution was diluted with 100 mL sodium bicarbonate aqueous solution; then aqueous layer was extracted with 100 mL ether for 5 times. Then organic layers were combined and washed with brine but gave a poor separation. So ethyl acetate was used to extract the organic layer from the brine layer. The organic layer was then dried over magnesium sulfate, filtered and concentrated to give the black crude 1.44 g. The crude was used for next reaction without future purification. ^1H NMR (400 MHz, CDCl_3): δ 8.00 (s, OH), 7.22 (s, 1 H), 4.47 (d, 2H), 4.29 (s, 2H), 4.16 (s, 5H); ^{13}C NMR (100 MHz, CDCl_3): δ

Synthesis of 1-(aminomethyl)ferrocene

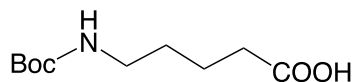


To a RB flask, 0.72 g of oxime (3.14 mmol) was dissolved in 30 mL distilled THF, 0.72 g of lithium aluminum hydride (18.9 mmol) was slowly added into the reaction solution and stirred 16 hours at room temperature. To workup the reaction, the reaction solution was added with 50 mL toluene and 2 mL ethyl acetate, followed by adding 16 drops of 15% NaOH to precipitate inorganic salt. The salt was washed with 50 mL toluene: methanol = 80: 20 mixture solution. The filtrate was concentrated to give yellow solid and gained solid was dissolved in dichloromethane and filtered again. The excess of dichloromethane was used to wash the residue. Then the filtrate was concentrated to give crude product and the crude product was

purified by column chromatography using dichloromethane and methanol 9 :1 as eluent to give 0.8068 g (62%) the desired product as yellow oil.

^1H NMR (400 MHz, CDCl_3): δ 4.20-4.00 (m, 9 H), 3.56 (s, 2 H), 1.87 (br, 1H); ^{13}C NMR (100 MHz, CDCl_3): δ 68.32 (5 C), 67.66 (4 C), 67.66

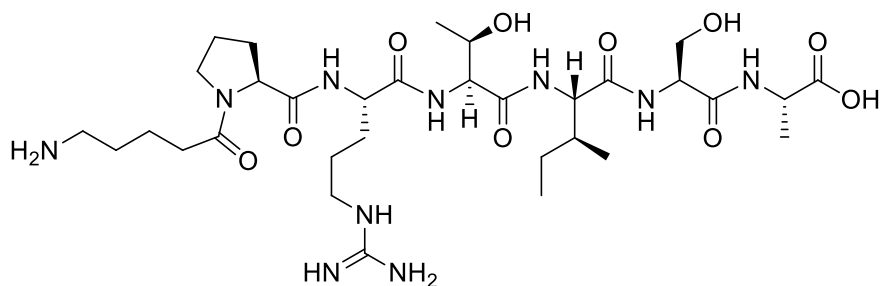
5-((tert-butoxycarbonyl)amino)pentanoic acid



To a RB flask, 1.0 g 5-aminovateic acid (8.54mmol) was dissolved in 25 mL dioxane : H_2O = 1 :1. To it, 1.8 mL (12.81mmol) trimethylamine was added then the reaction mixture was stirred at room temperature for 5 minutes. After that, 2.24 g di-tert-butyl dicarbonate (10.24 mmol) was added to the reaction mixture and stirred at room temperature for 16 hours. The reaction mixture was transferred in a separatory funnel then extracted with 50 mL ethyl acetate to remove the excess of di-tert-butyl dicarbonate. The aqueous layer was then acidified 1 N hydrogen chloride till pH 4-5 and extracted with 50 mL ethyl acetate for three times. The organic extract was wash with water, brine, dried over magnesium sulfate, filtered and concentrate to give the desired product 1.49 g desire product (81 % yield).

A Representative Synthesis of peptide 26-Fc ($\text{NH}_2-(\text{CH}_2)_4\text{-CO-Pro-Arg-Thr-Ile-Ser-Ala-NH-CH}_2\text{-Fc}$)

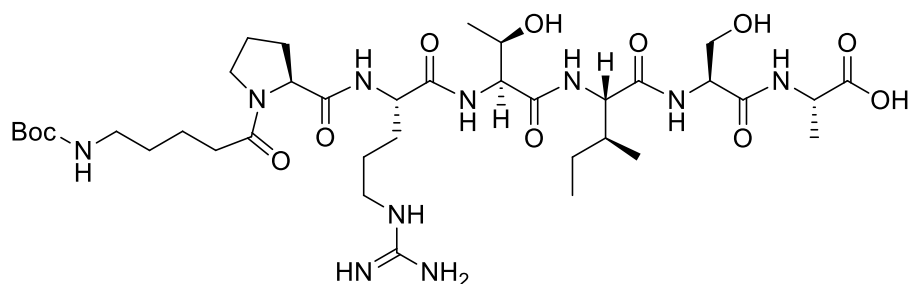
Synthesis of $\text{NH}_2-(\text{CH}_2)_4\text{-CO-Pro-Arg-Thr-Ile-Ser-Ala}$



To 1.0 g of H-Ala-2-chlorotrityl Resin (0.45mmol), 12 mL dichloromethane was added at room temperature and stirred for 30 minutes. The dichloromethane was removed used vacuum suction then the resin was washed with dimethylformamide for 5 times to remove remaining dichloromethane inside the resin. To it, 0.461g of (2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate HBTU (1.21 mmol) and 0.518 g of Fmoc-Ser(tBu)-OH (1.35 mmol) dissolved in 4.2% (*N,N*-Diisopropylethylamine DIPEA in 12ml DMF was added. The mixture was subjected to microwave irradiation (20W, 70 °C) with stirring for 8 minutes. Then liquid phase was removed used vacuum suction and washed with DMF three times. This process was repeated for three times. The reaction was checked by ninhydrin test and the resins didn't change color which means the absence of amine. Then a 12ml solution of 20% piperidine in DMF was added to the N-Fmoc protected amino acid resin and subjected to microwave irradiation (20W, 3 min, 70 °C). This process was repeated. The reaction mixture was filtered, and the resin was washed with DMF three times. The reaction was checked by ninhydrin test and the resins turn blue which means the exists of free amine. The following reaction sequence was carried out: (i) coupling with N-Fmoc-Ile-OH; (ii) deprotection of Fmoc group with piperidine in DMF; (iii) coupling with N-Fmoc-Thr(tBu)-OH; (iv) deprotection with piperidine/DMF; (v) coupling with N-Fmoc-Arg(Pbf)-OH(vi) deprotection with piperidine/DMF; (vii) coupling with N-Fmoc-Pro-OH; (viii) deprotection with piperidine/DMF; (x) coupling with N-Boc-NH(CH₂)₄CO₂H (later referred as "linker") ; The linker attached hex peptide linked with 2-

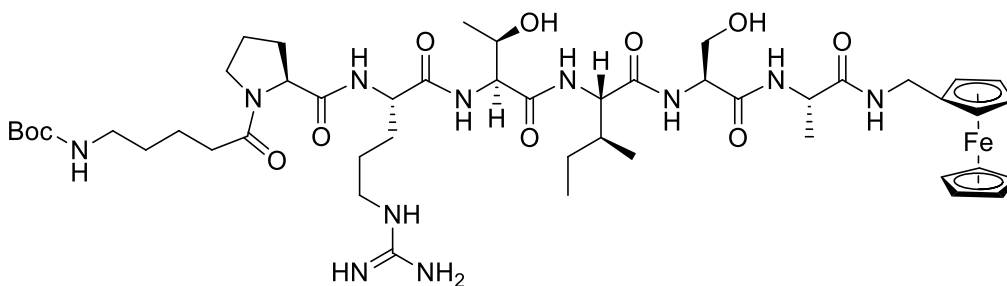
chlorotrityl resin were washed with DCM for three times, then treated with 12 mL of a cleavage solution consisted of 95% distilled trifluoroacetic acid TFA, 2.5% triisopropylsilane TIPS, and 2.5% water. The mixture was stirred at room temperature for 4 hours and filtered to get filtrate. The remaining resins were washed with TFA then treated with a cleavage solution consisted of 95% TFA, 2.5% TIPS, and 2.5% water for 4 hours and filtered to get filtrate. The filtrate was using hexane: ether= 1:1 to precipitated to the desired product as white solid to give 0.270g (80.8% yield) .

Synthesis of Boc-NH-(CH₂)₄-CO-Pro-Arg-Thr-Ile-Ser-Ala



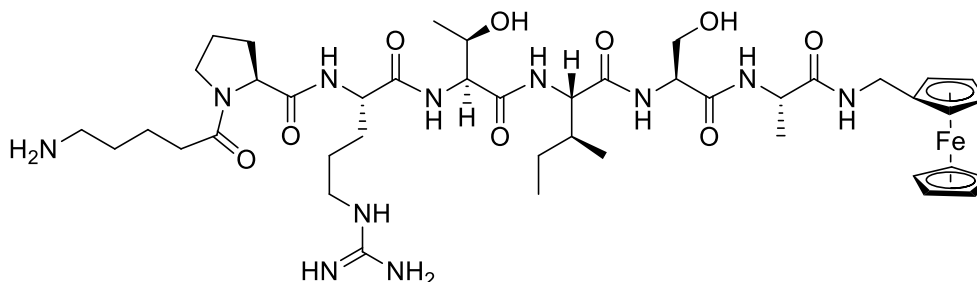
To a RB flask, 0.260 g of H₂N(CH₂)₄CO-Pro-Arg-Thr-Ile-Ser-Ala-OH (0.35 mmol) was dissolved in 20 mL of p-dioxane: water =1:1 were added 0.6 mL of distilled triethylamine (3.5 mmol) and 0.38 g of di-tert-butyl dicarbonate (1.75 mmol). The solution was stirred at room temperature for 3 hours. The reaction solution was concentrated and used for next step without purification.

Synthesis of Boc-NH-(CH₂)₄-CO-Pro-Arg-Thr-Ile-Ser-Ala-NH-CH₂-Fc



To a RB flask, 42 mg *N*-Boc-HN(CH₂)₄CO- Pro-Arg-Thr-Ile-Ser-Ala-OH (0.050 mmol) was flame dried to remove any moisture in the compound, then dried. 24.7 mg of 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate HATU (0.065 mmol) was added. To it, 0.3 mL of DMF was added and a solution of 11.8 mg of 1-(aminomethyl)ferrocene (0.055 mmol) in 1 mL of DMF. The solution was stirred at room temperature for 13 hours. The reaction was concentrated and dried to give the crude. The crude was purified by column chromatography to give 33.2 mg.

Synthesis of NH₂-(CH₂)₄-CO-Pro-Arg-Thr-Ile-Ser-Ala-NH-CH₂-Fc



A solution of 8 mg of Boc-NH(CH₂)₄-CO-pep26-CH₂-Fc (0.007 mmol) in 0.3 mL 10% TFA in dichloromethane was stirred at room temperature for 30 minutes. The crude was added to 1 mL deionized water then gently concentrated on a rotary evaporator, then toluene was added to remove TFA, this step was repeated for three times. Then the crude was lyophilized and purified to give the desired product 5.8 mg (88% yield).

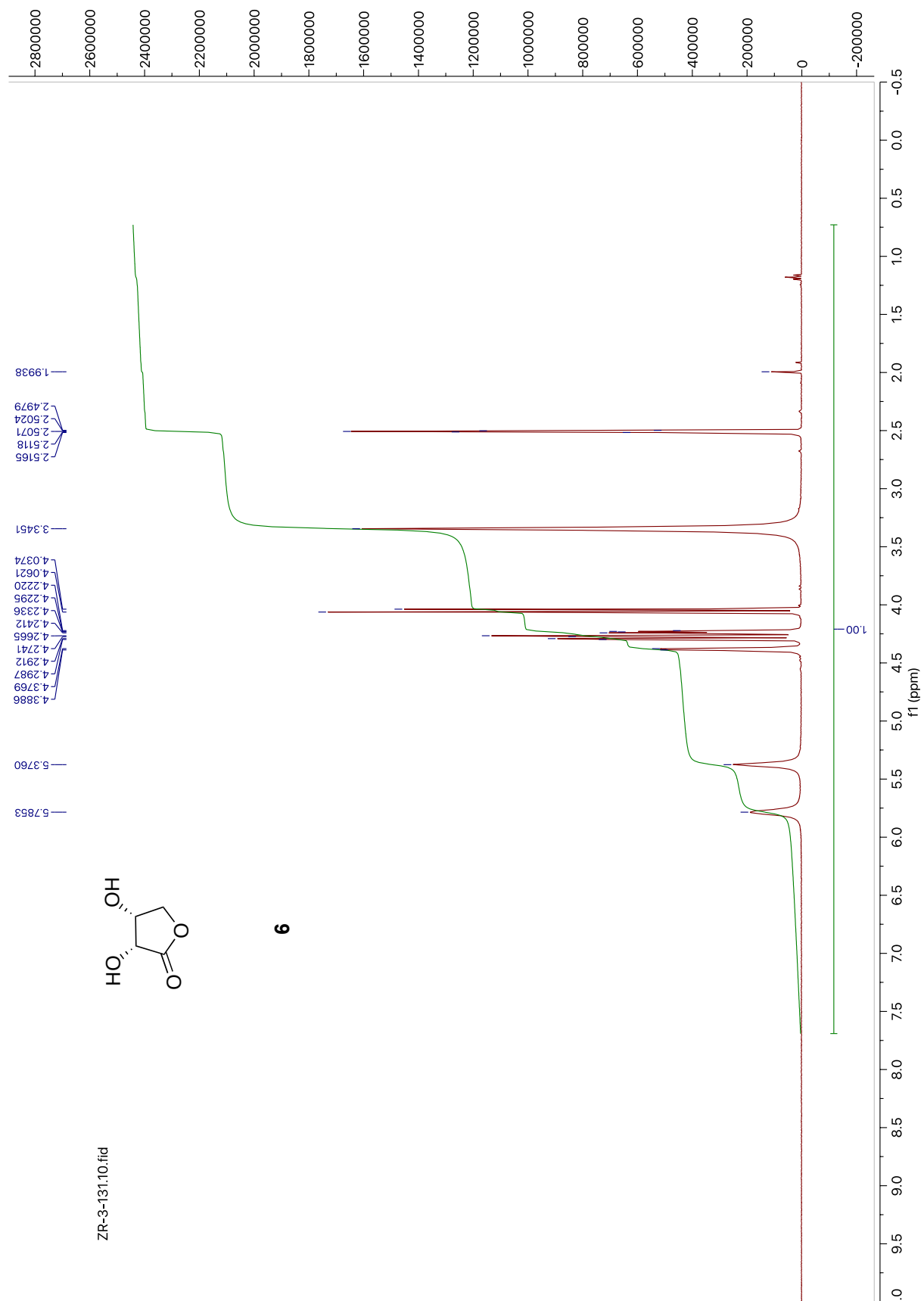
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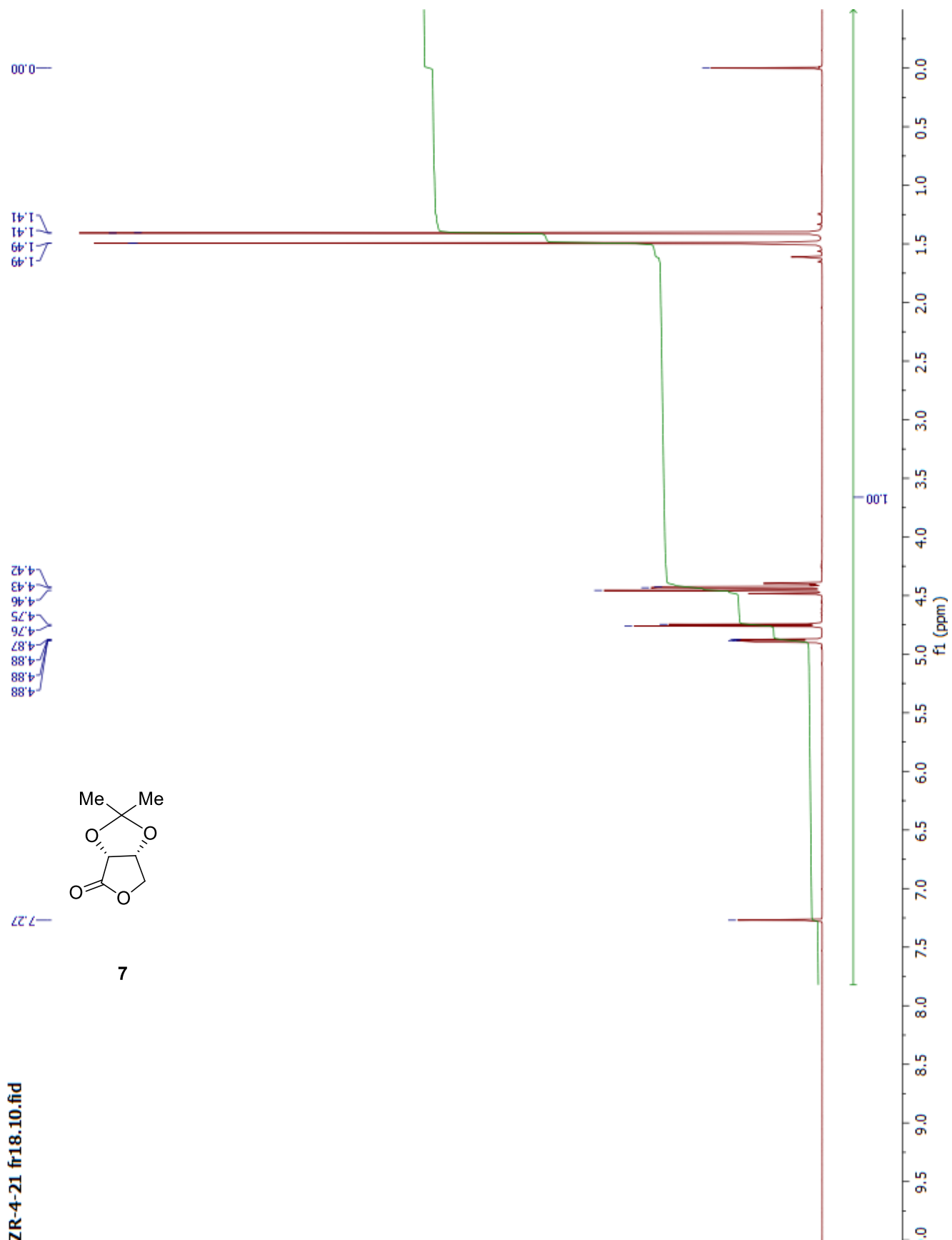
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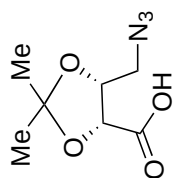
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All NMR were taken in CDCl₃, unless labeled otherwise.

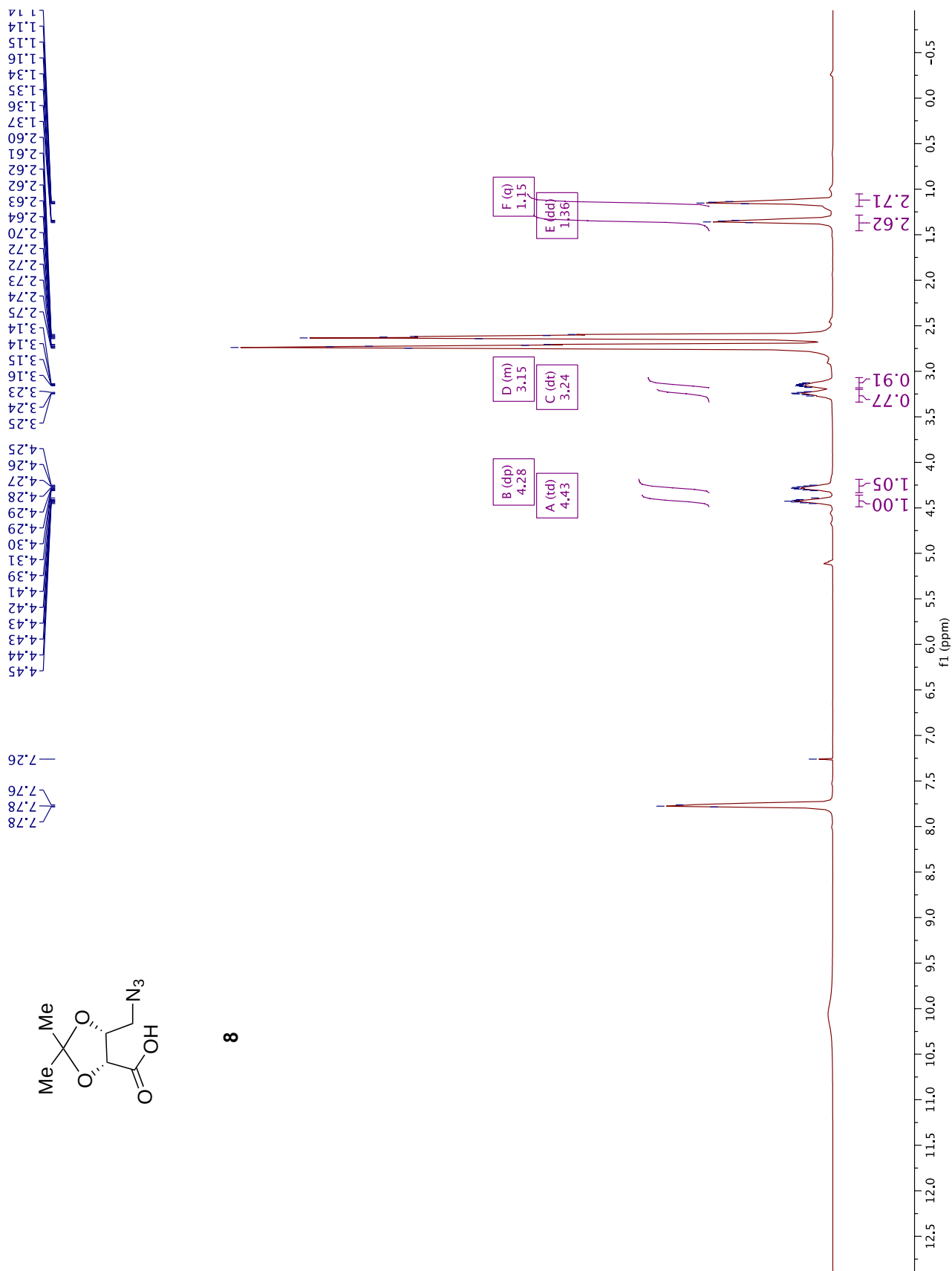


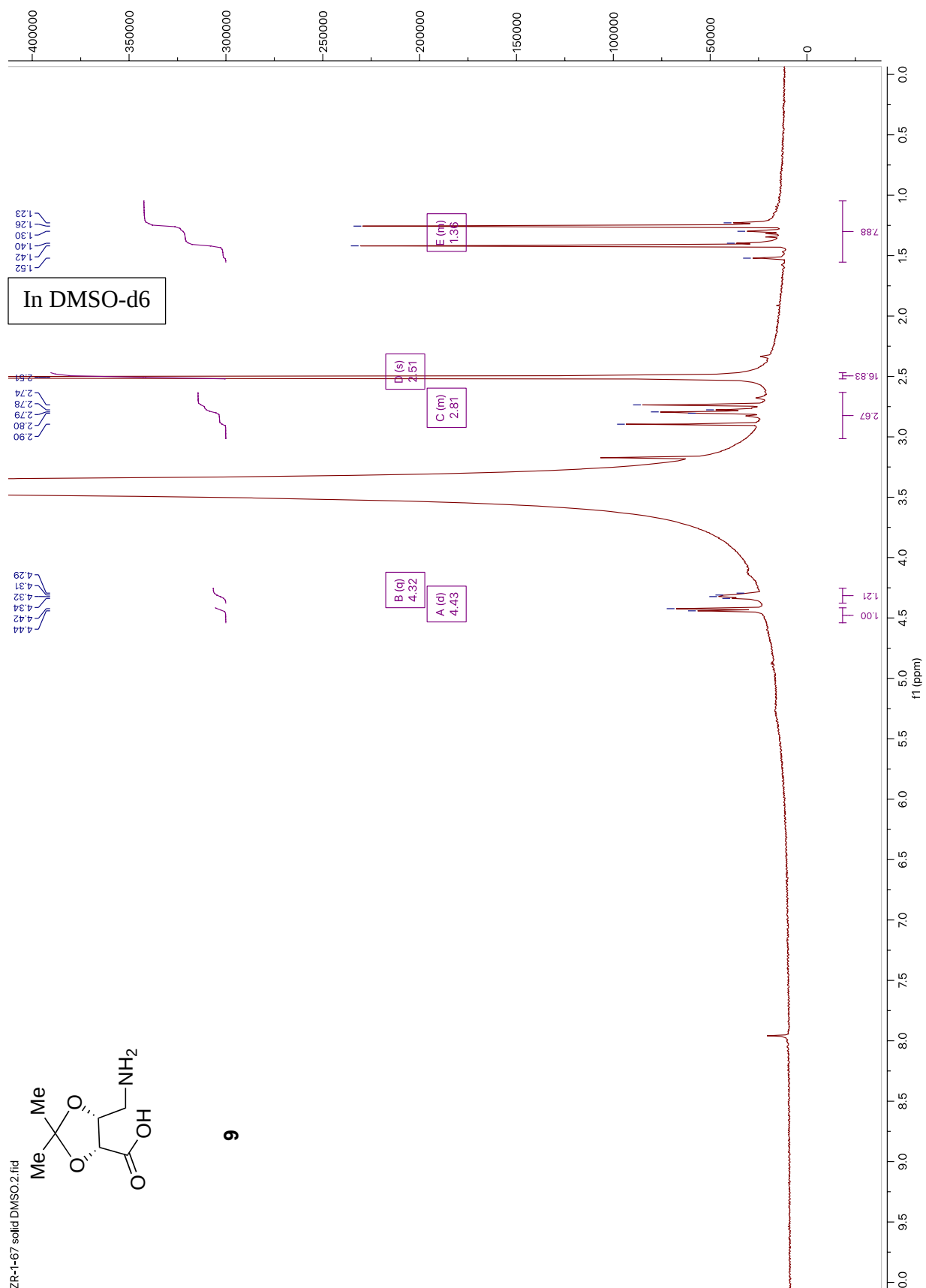
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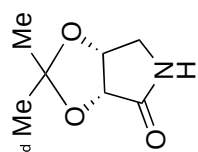




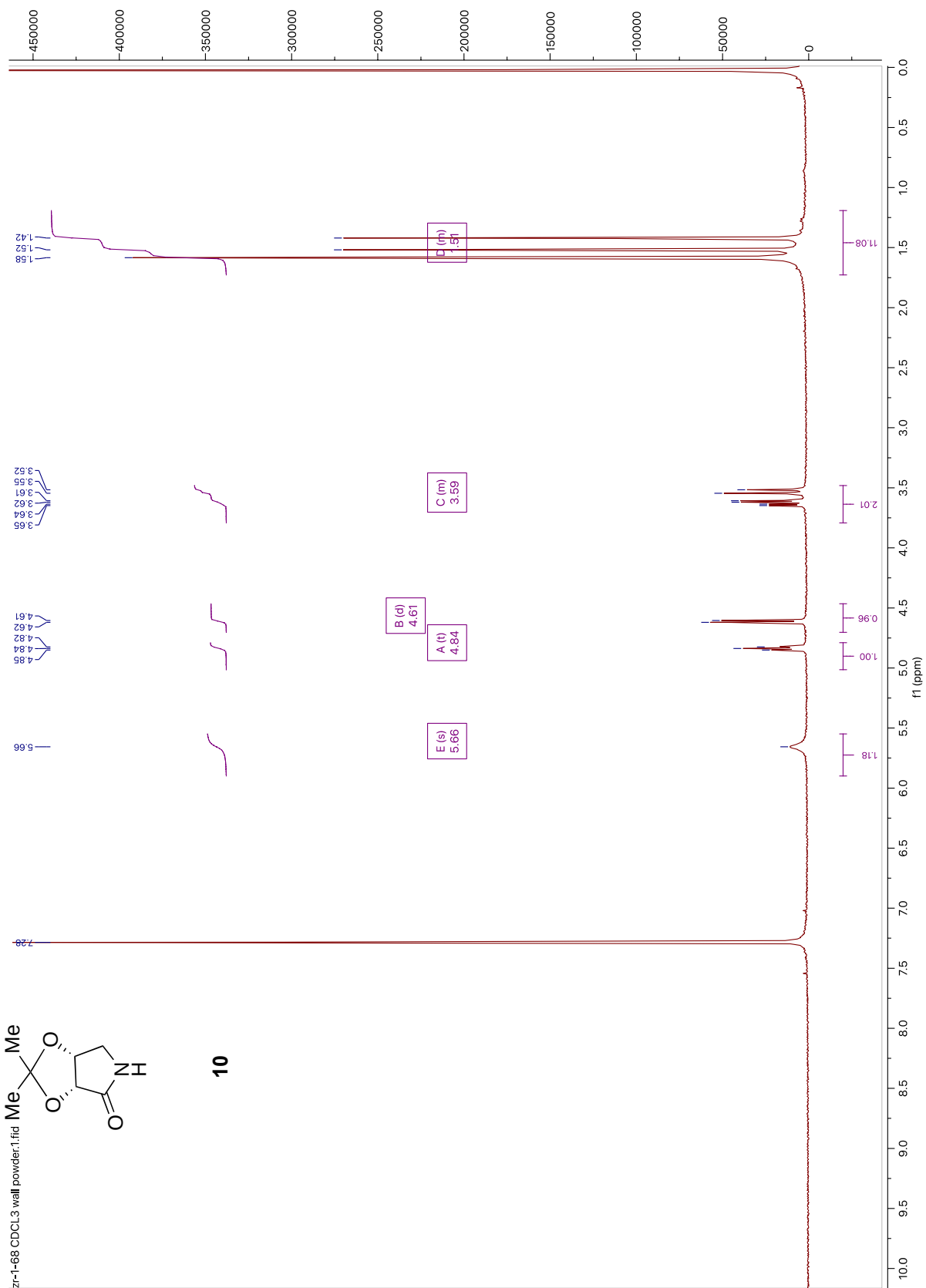
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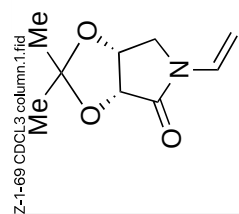




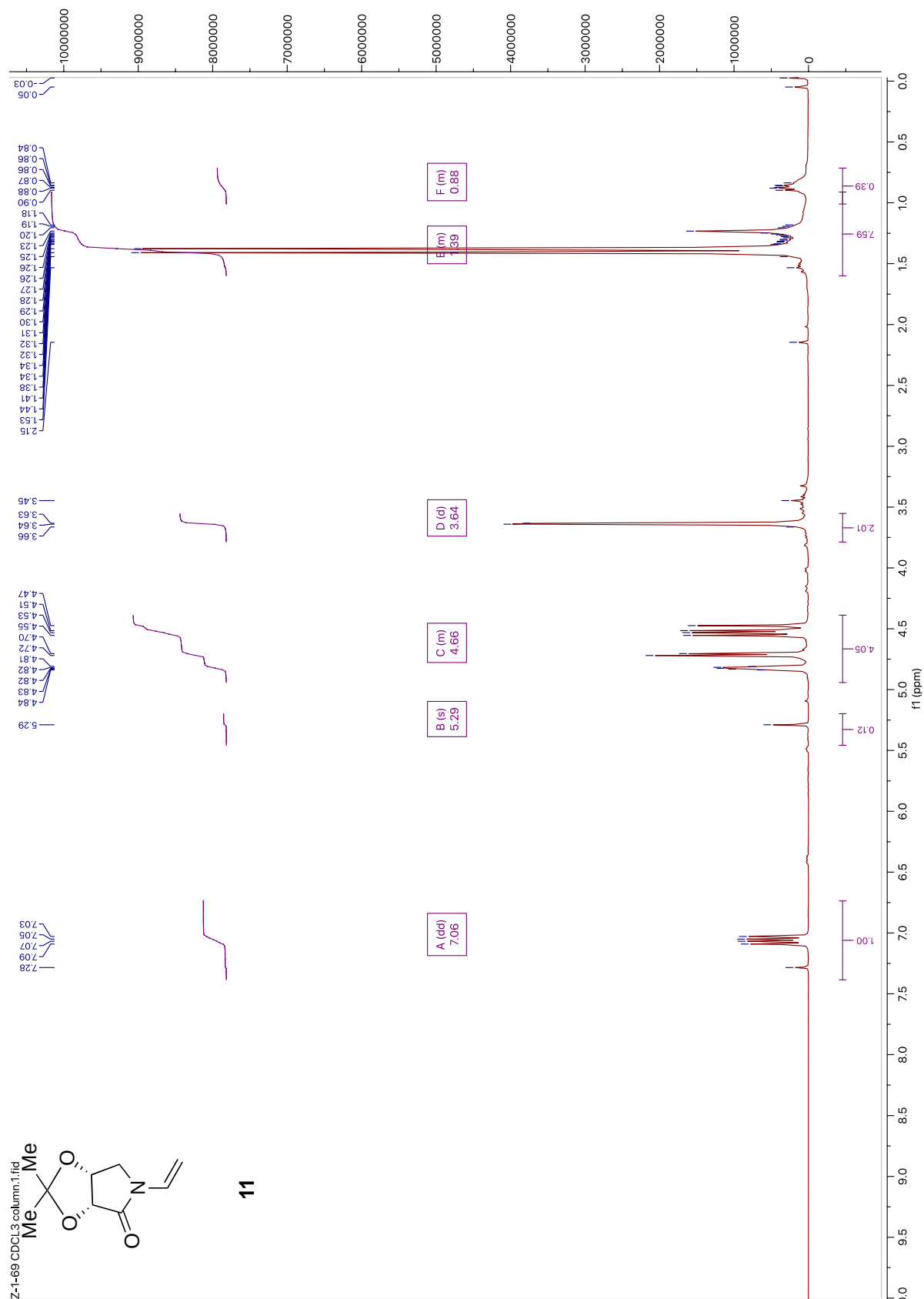


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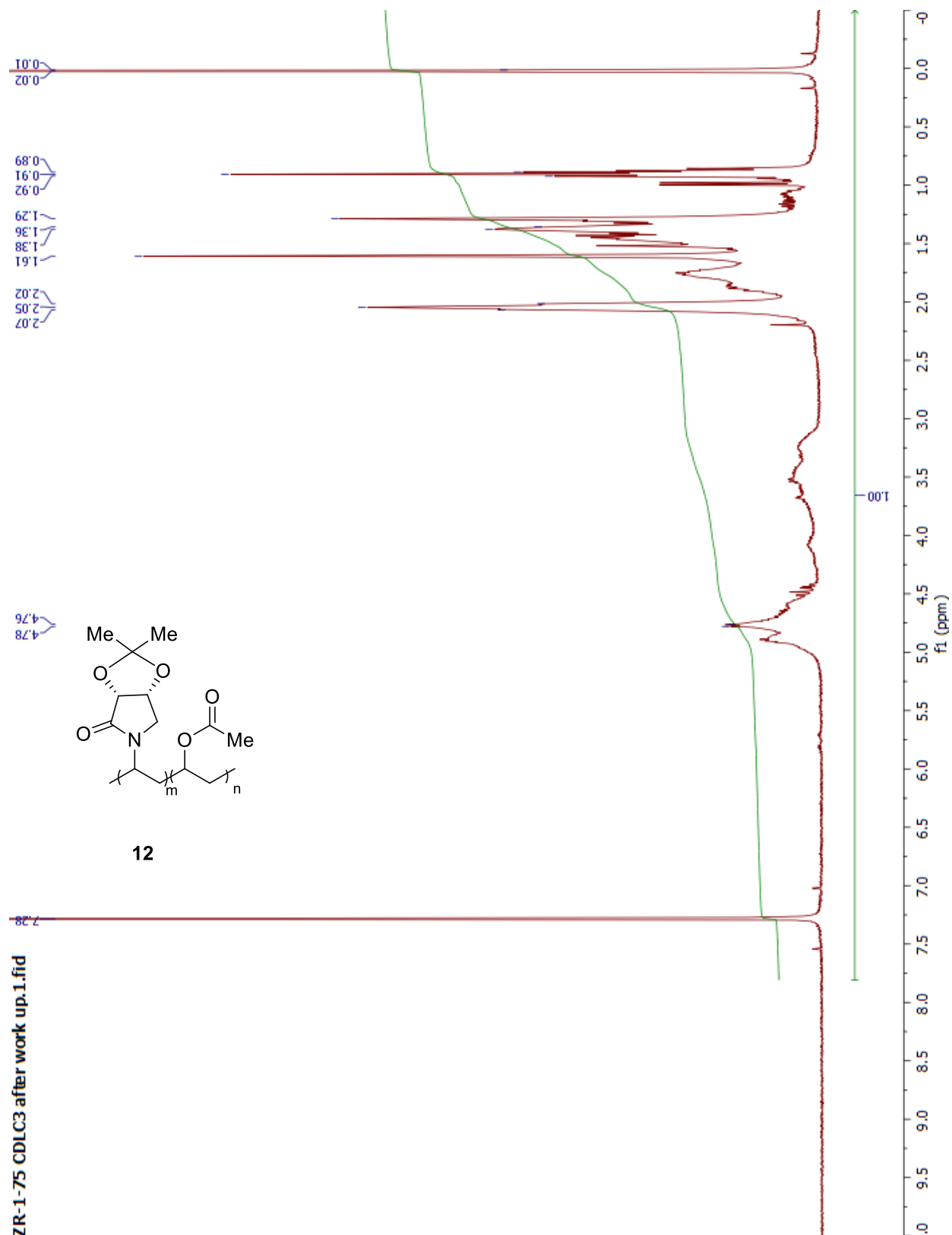




11



ZR-1-75 CDLC3 after work up.1.fid



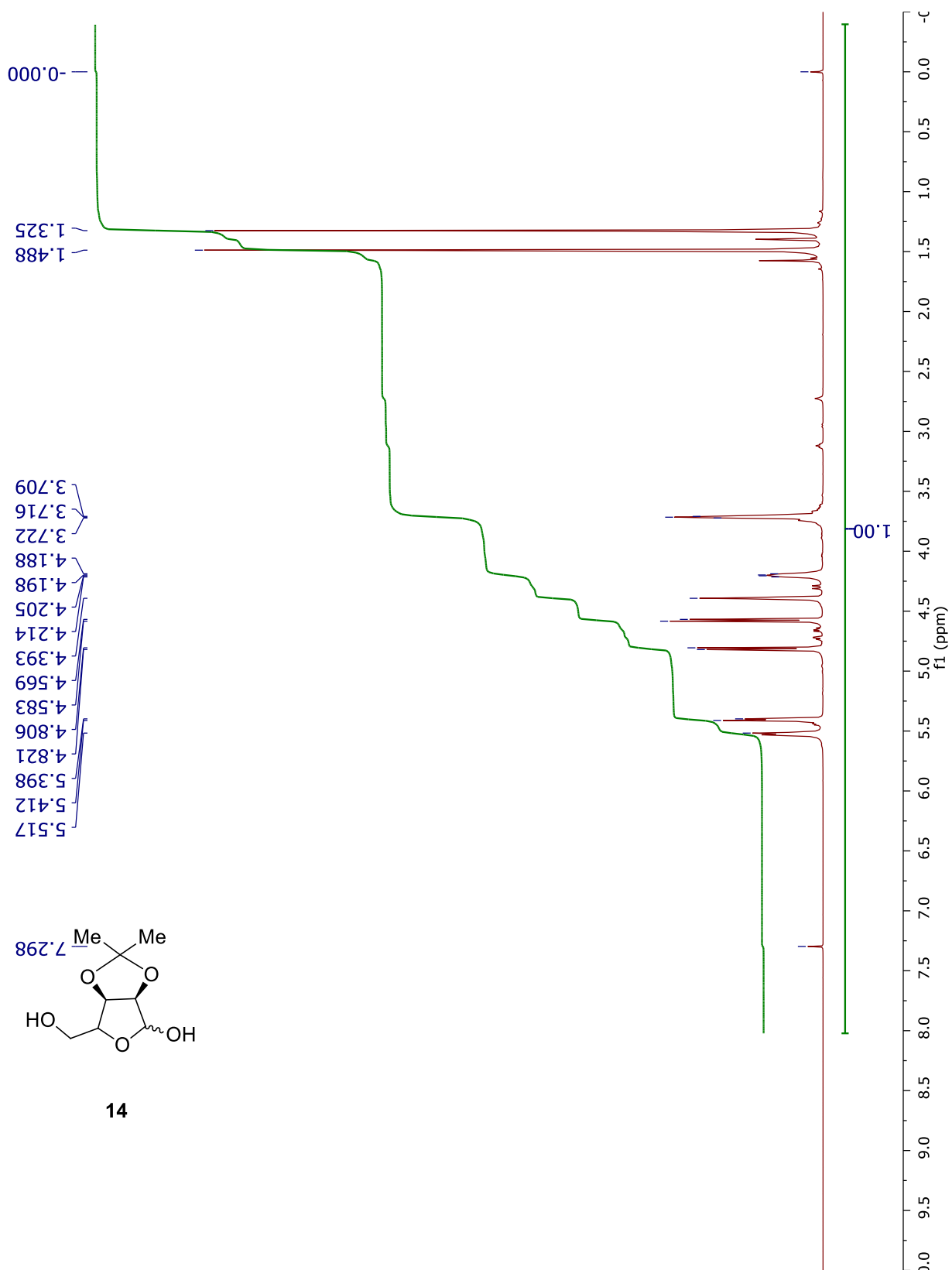
Chemical structure of compound 1 is shown below the spectrum:

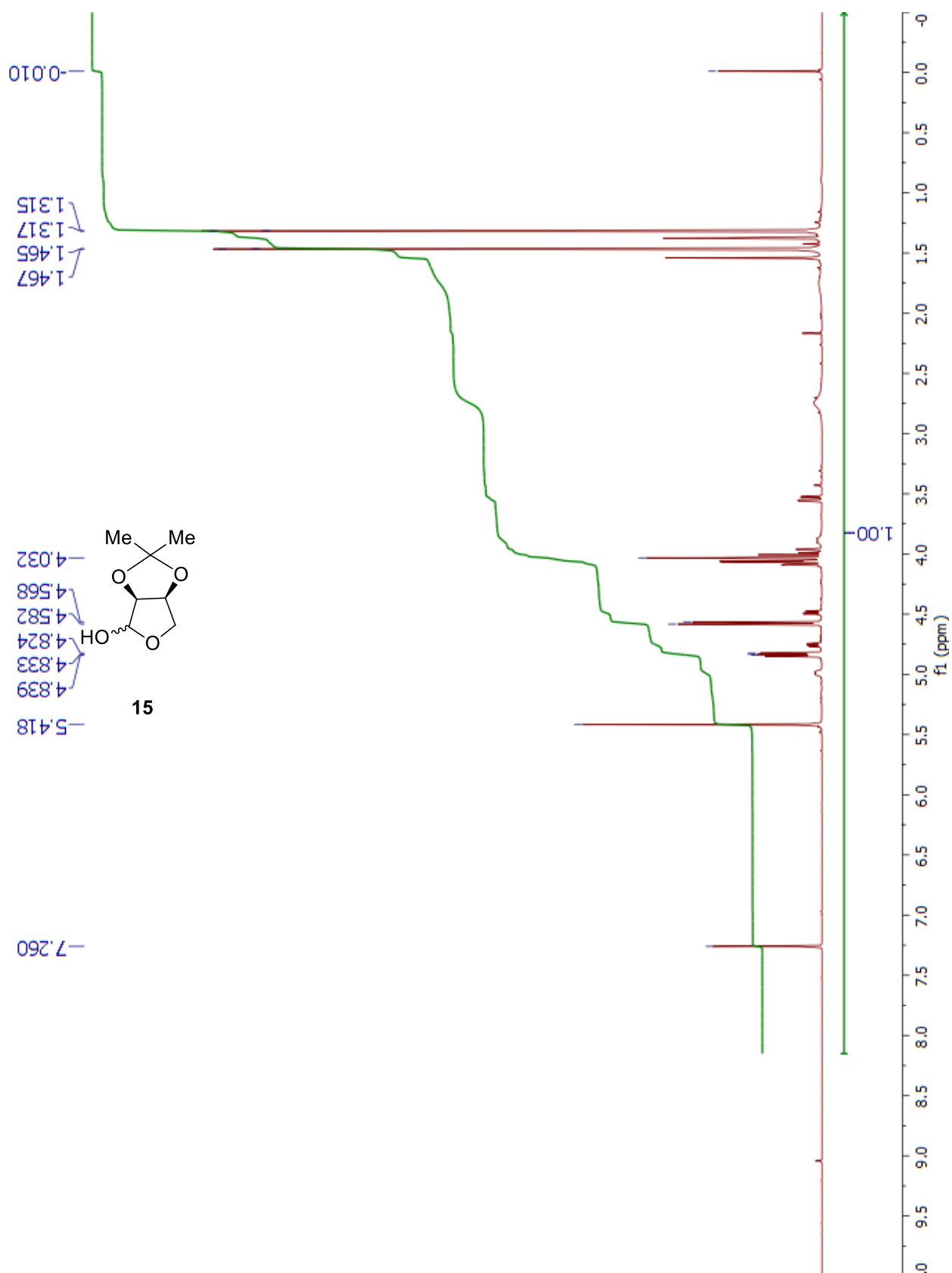
CN1CCCC1(C)C2CCCC2

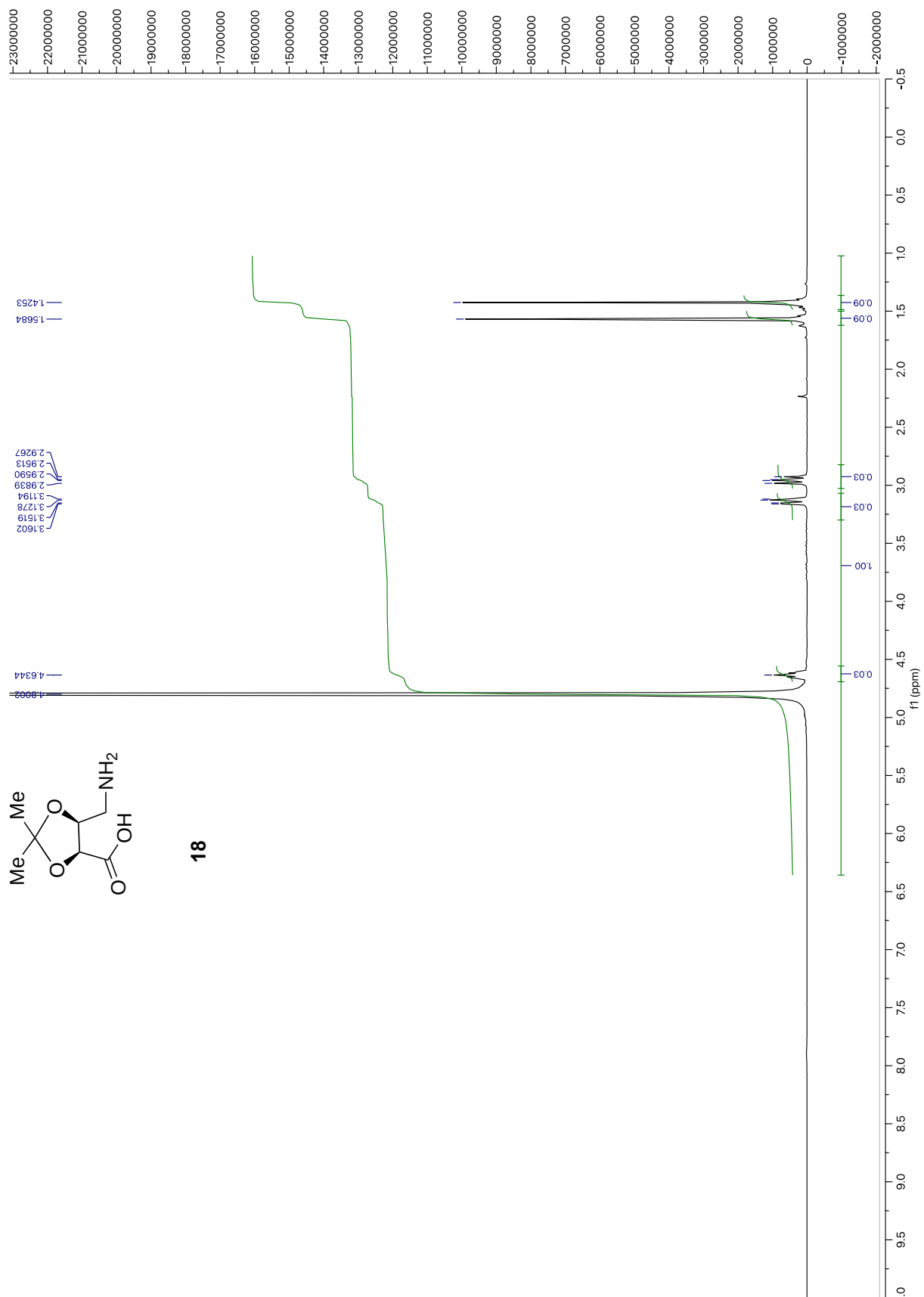
The spectrum displays the following chemical shifts (ppm):

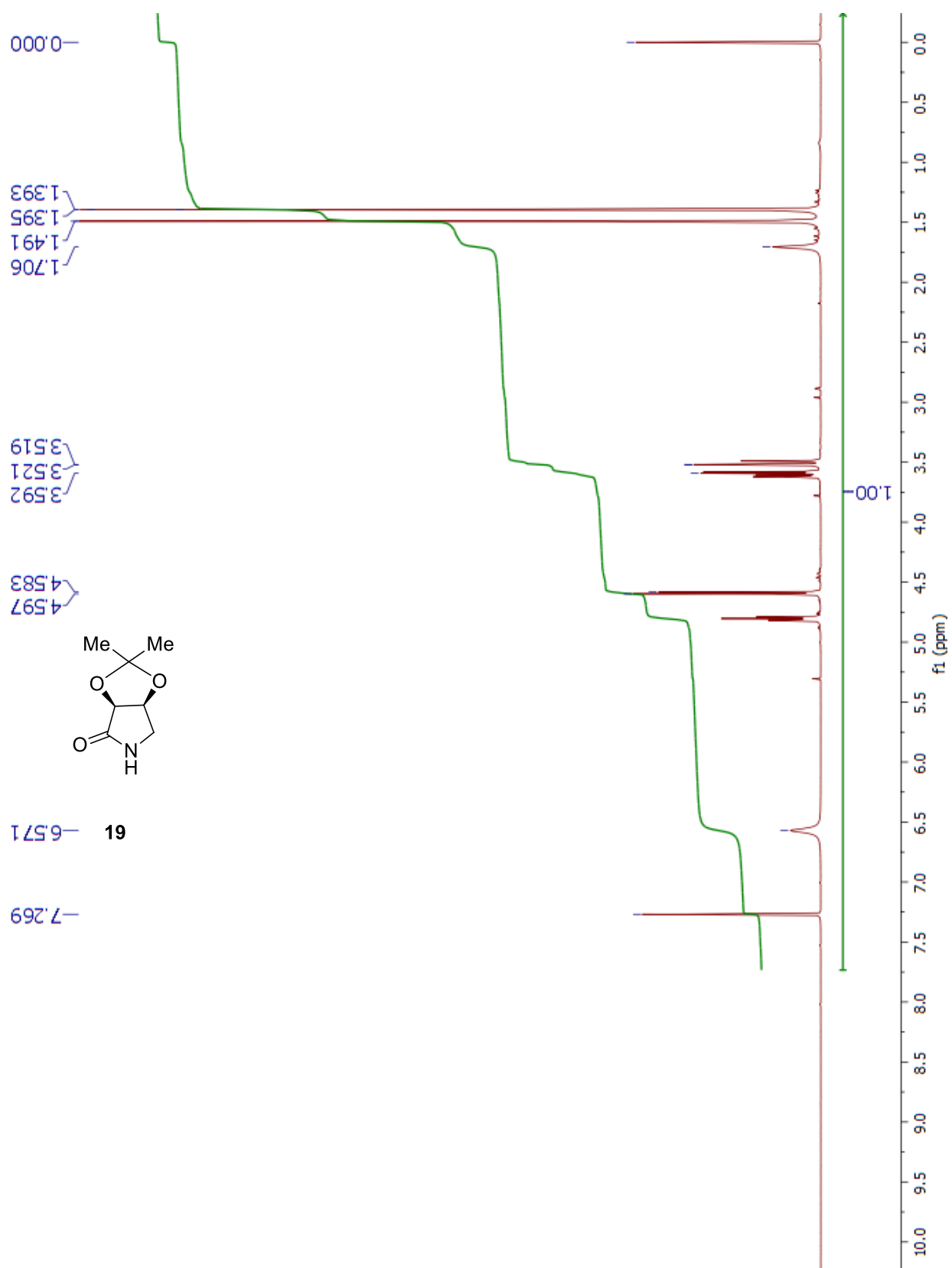
- 7.28, 7.29, 7.35
- 5.97
- 5.01
- 4.77, 4.59, 4.57, 4.55
- 3.79, 3.74, 3.67, 3.66, 3.60, 3.56, 3.54, 3.50, 3.45, 3.23, 3.13
- 2.13, 2.06, 1.89, 1.77, 1.52, 1.51, 1.44, 1.37, 1.26, 0.99, 0.97, 0.93, 0.91, 0.87, 0.85
- 0.08

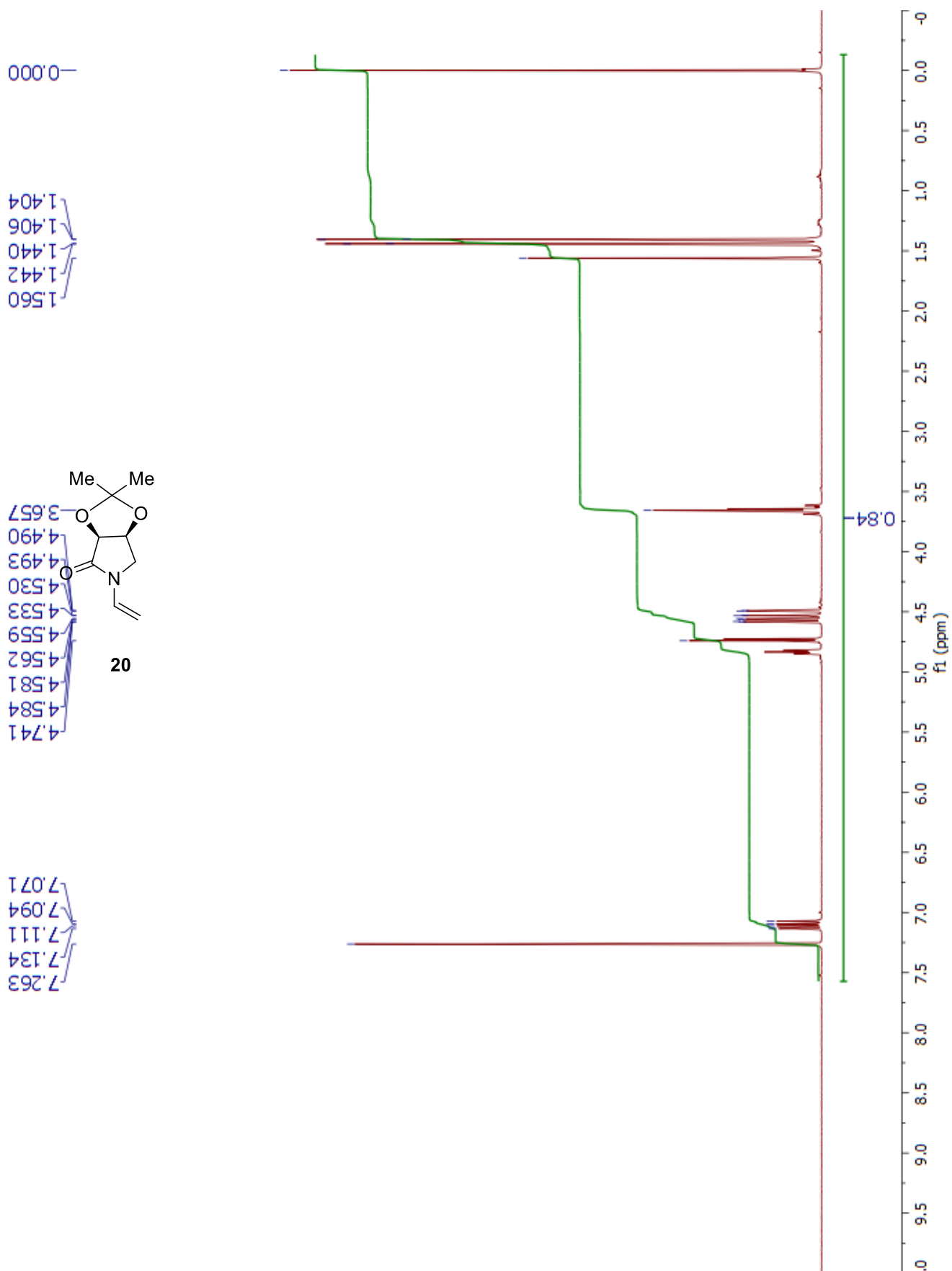
The integration curve shows a broad peak at ~7.2 ppm with an integral of 2.90, and a multiplet at ~4.5 ppm with an integral of 1.00.

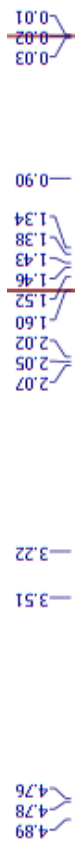


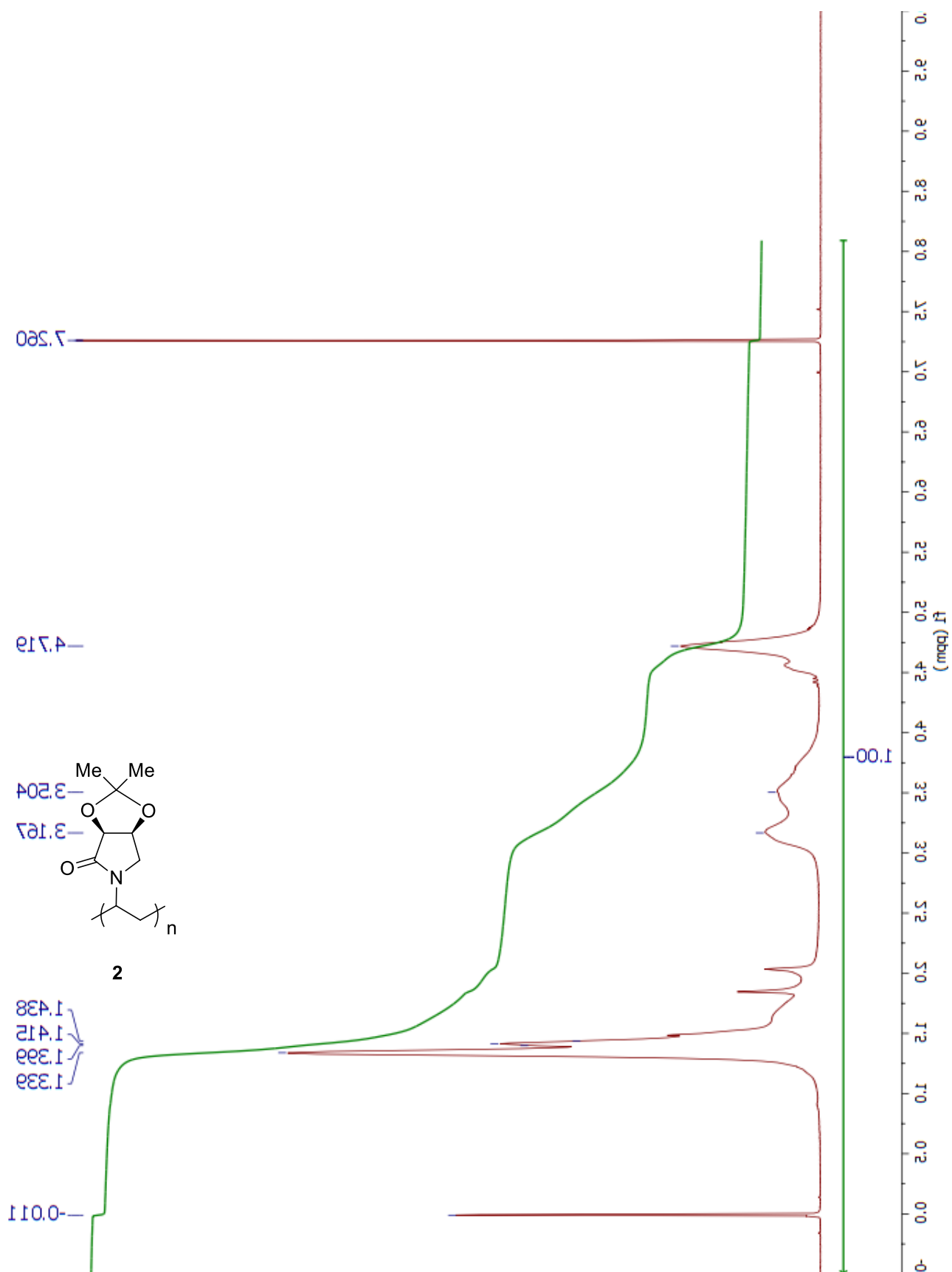


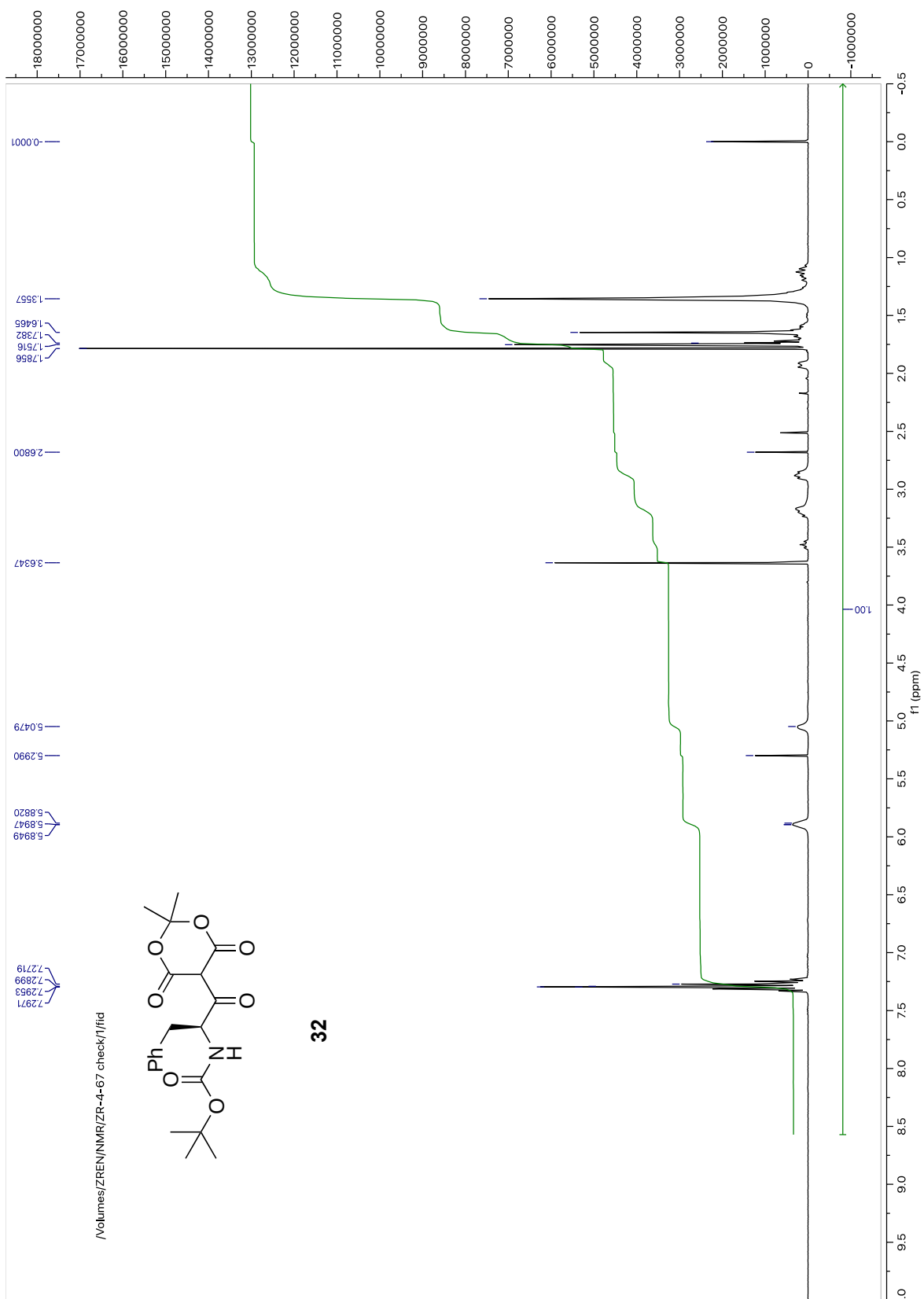


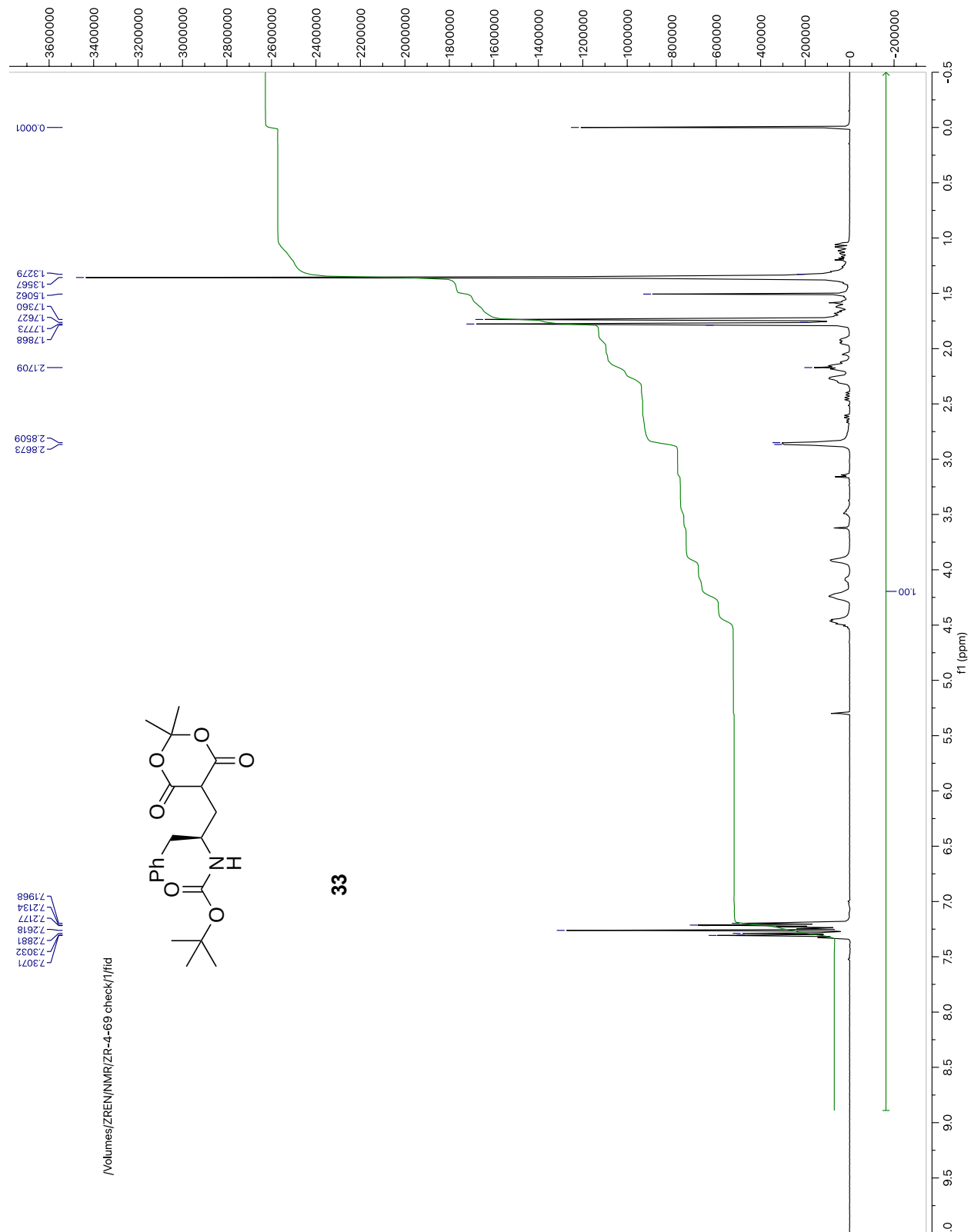


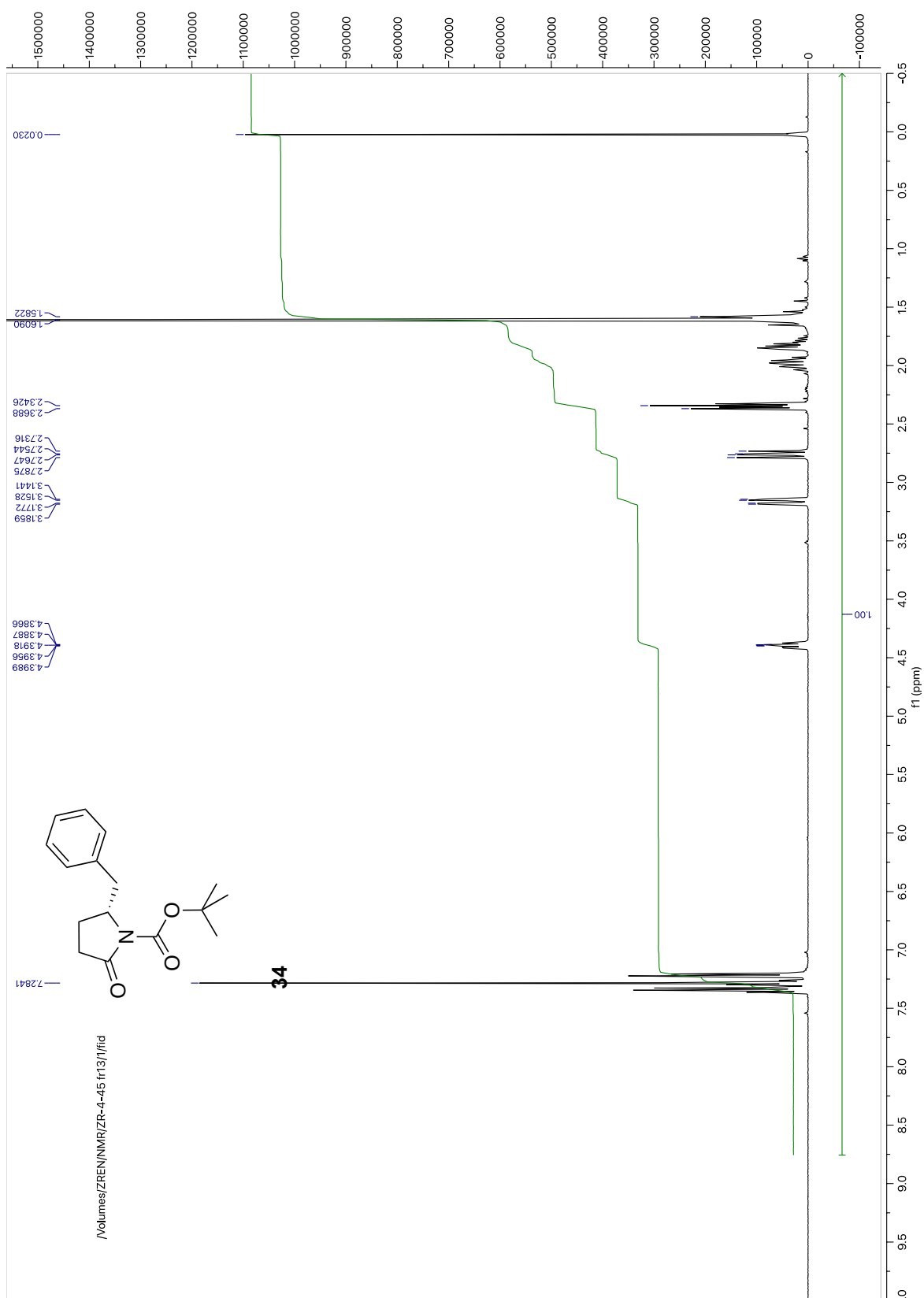


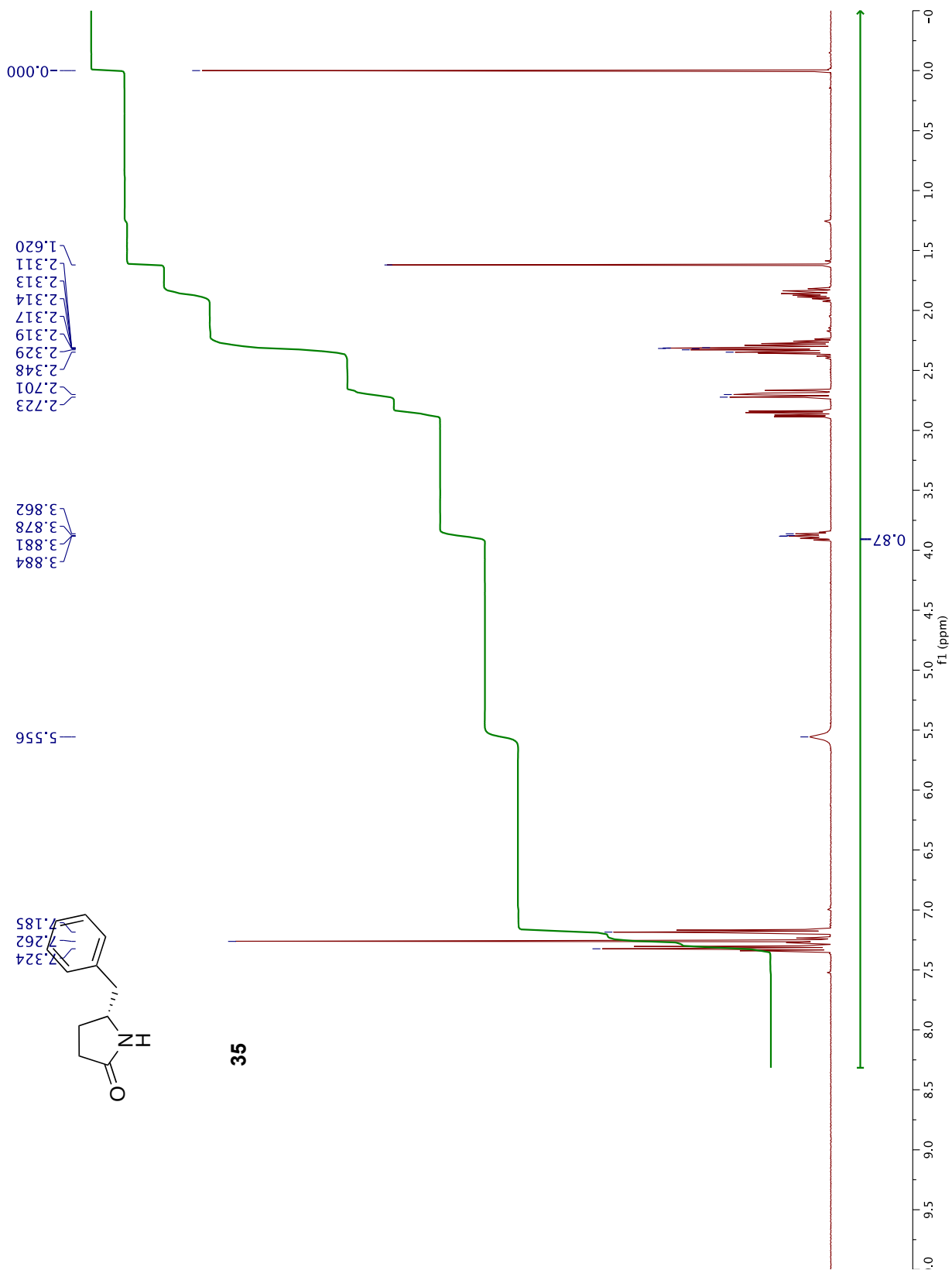


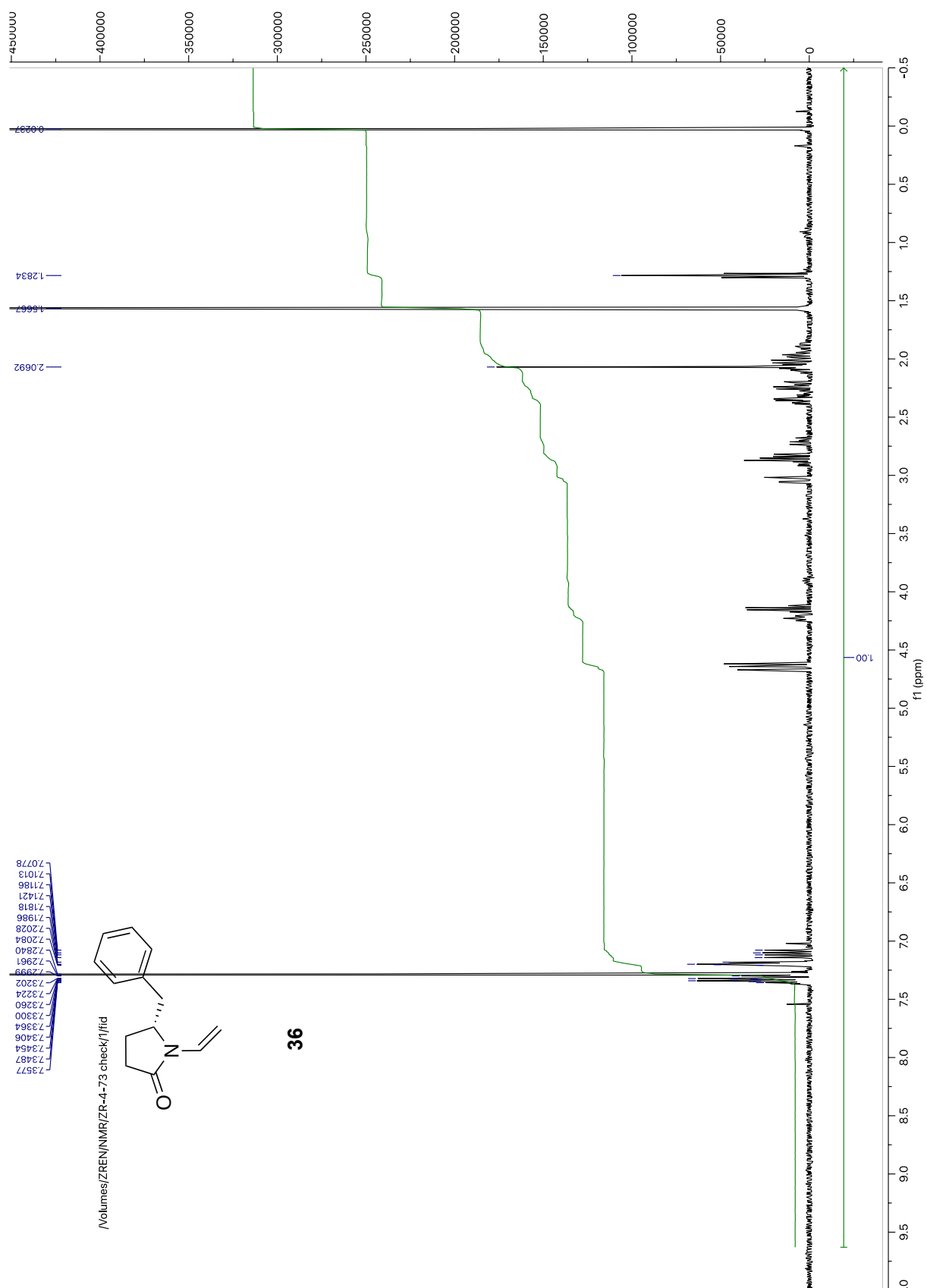




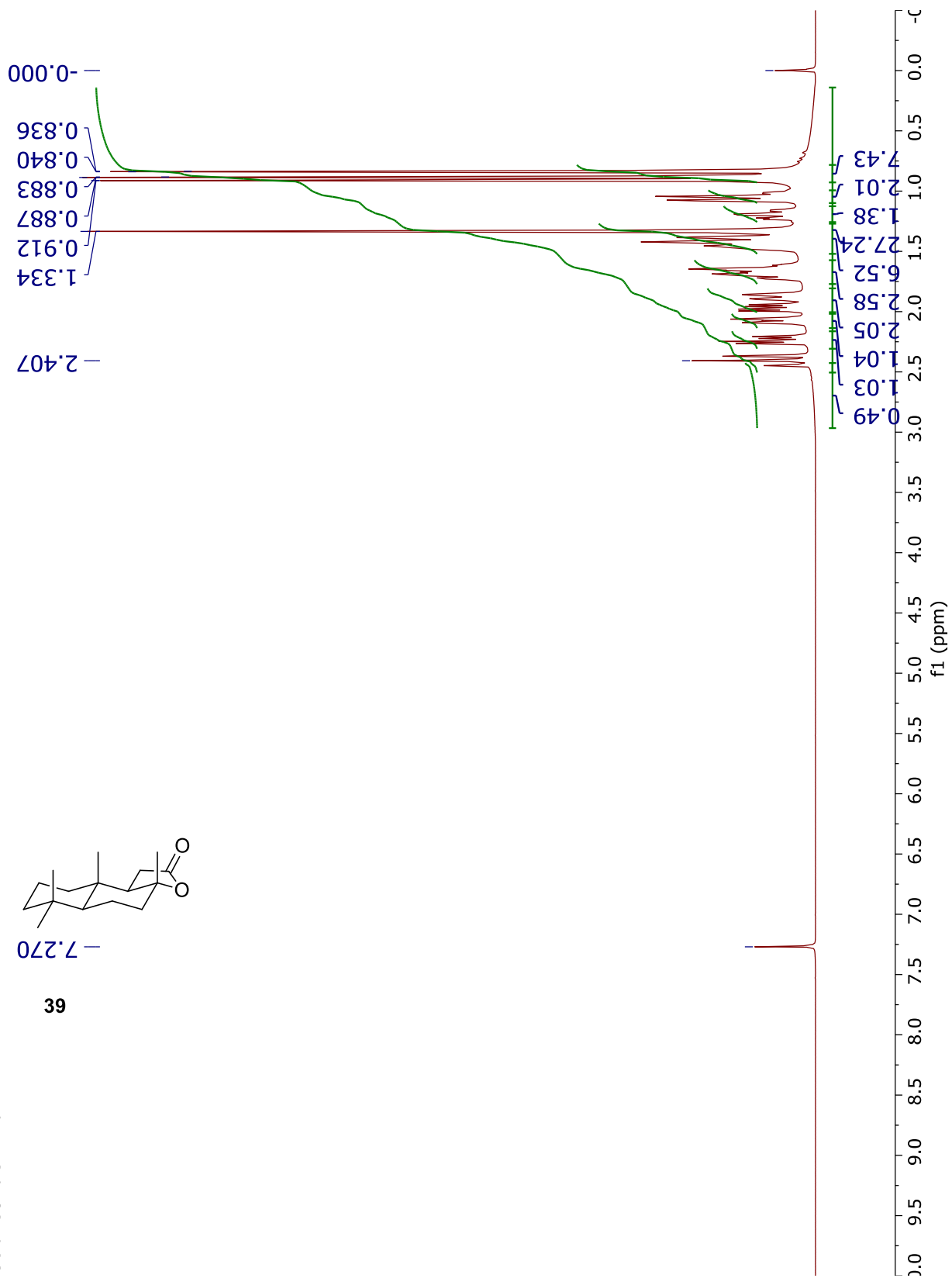


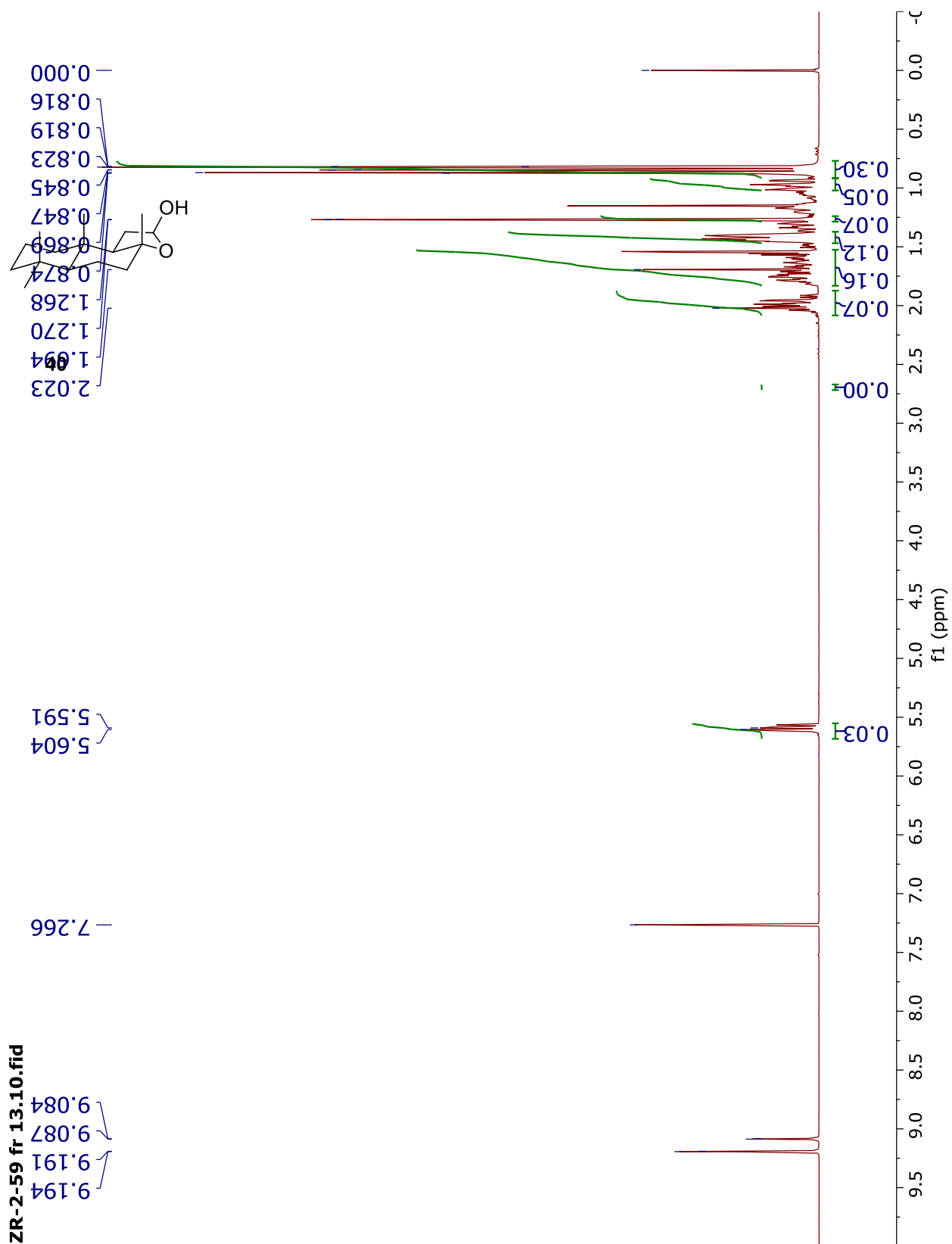


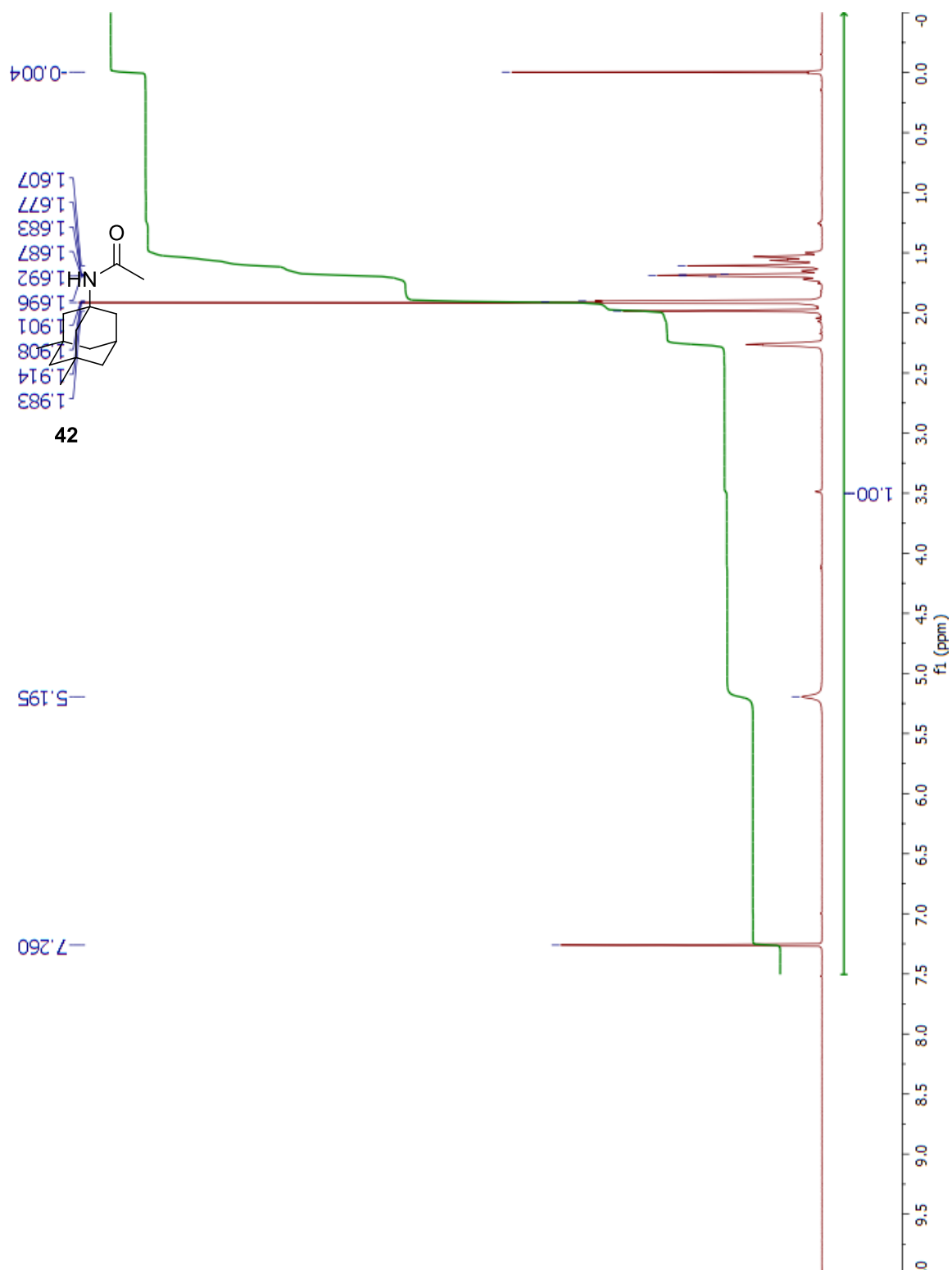


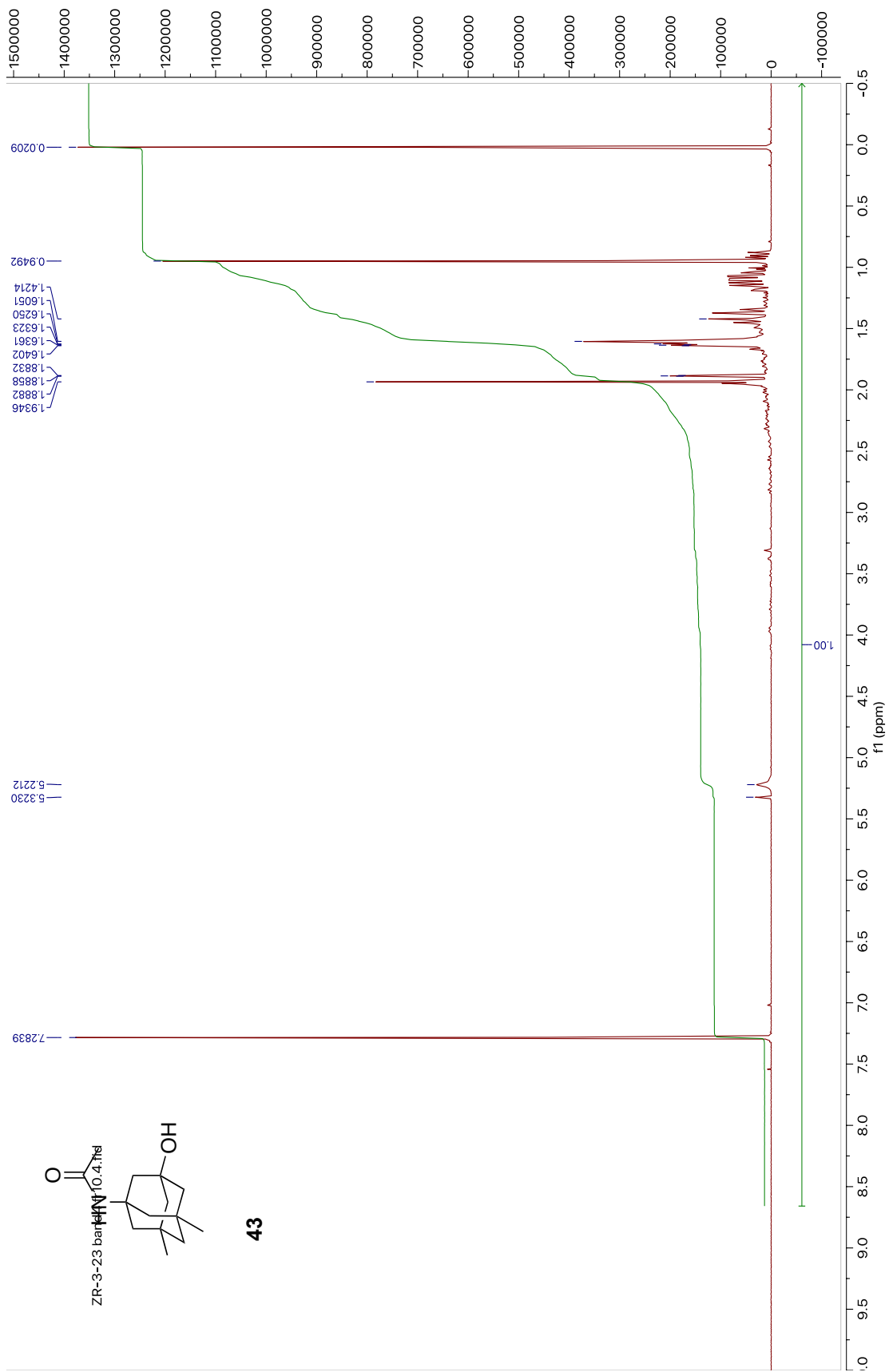


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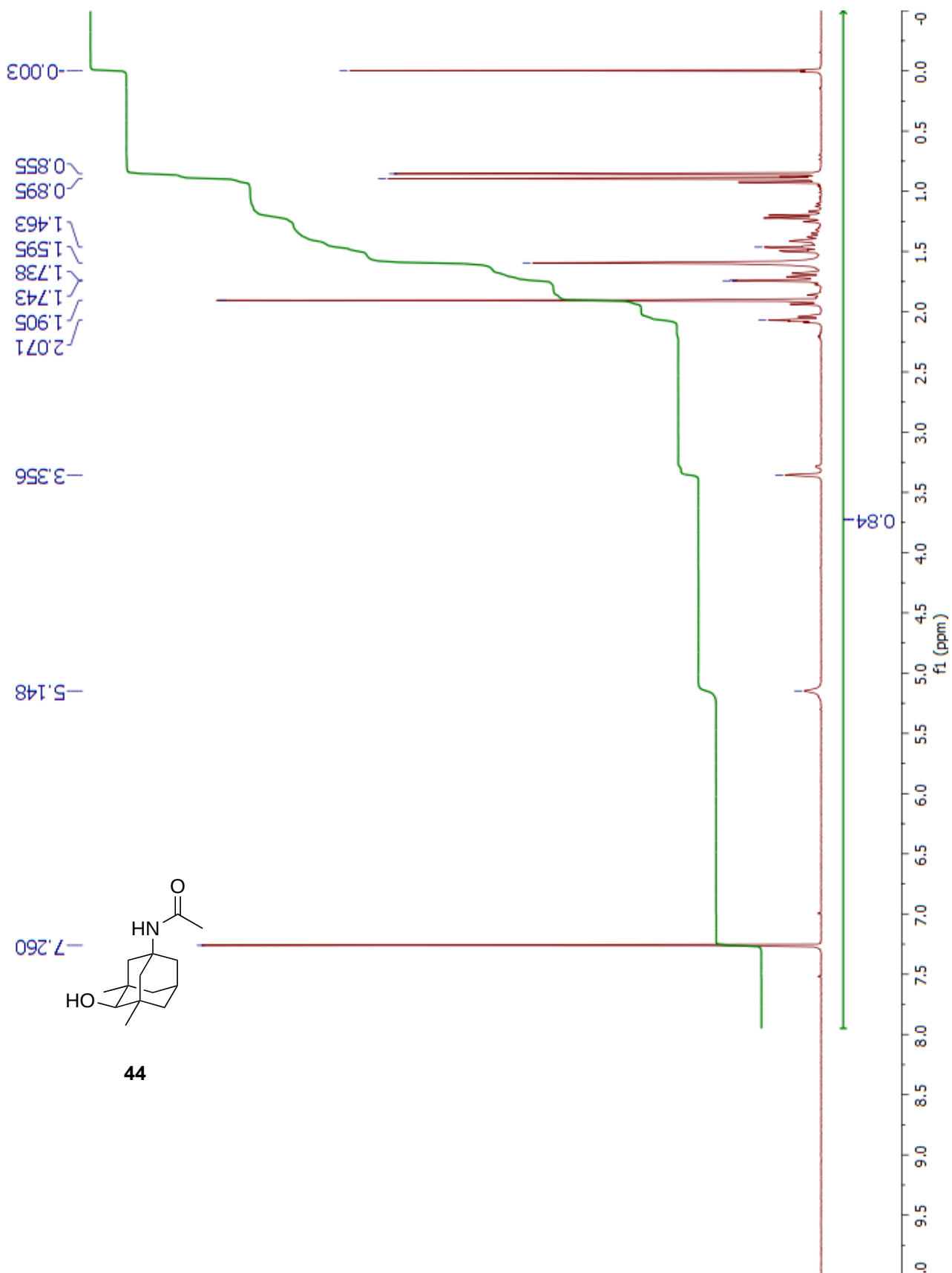




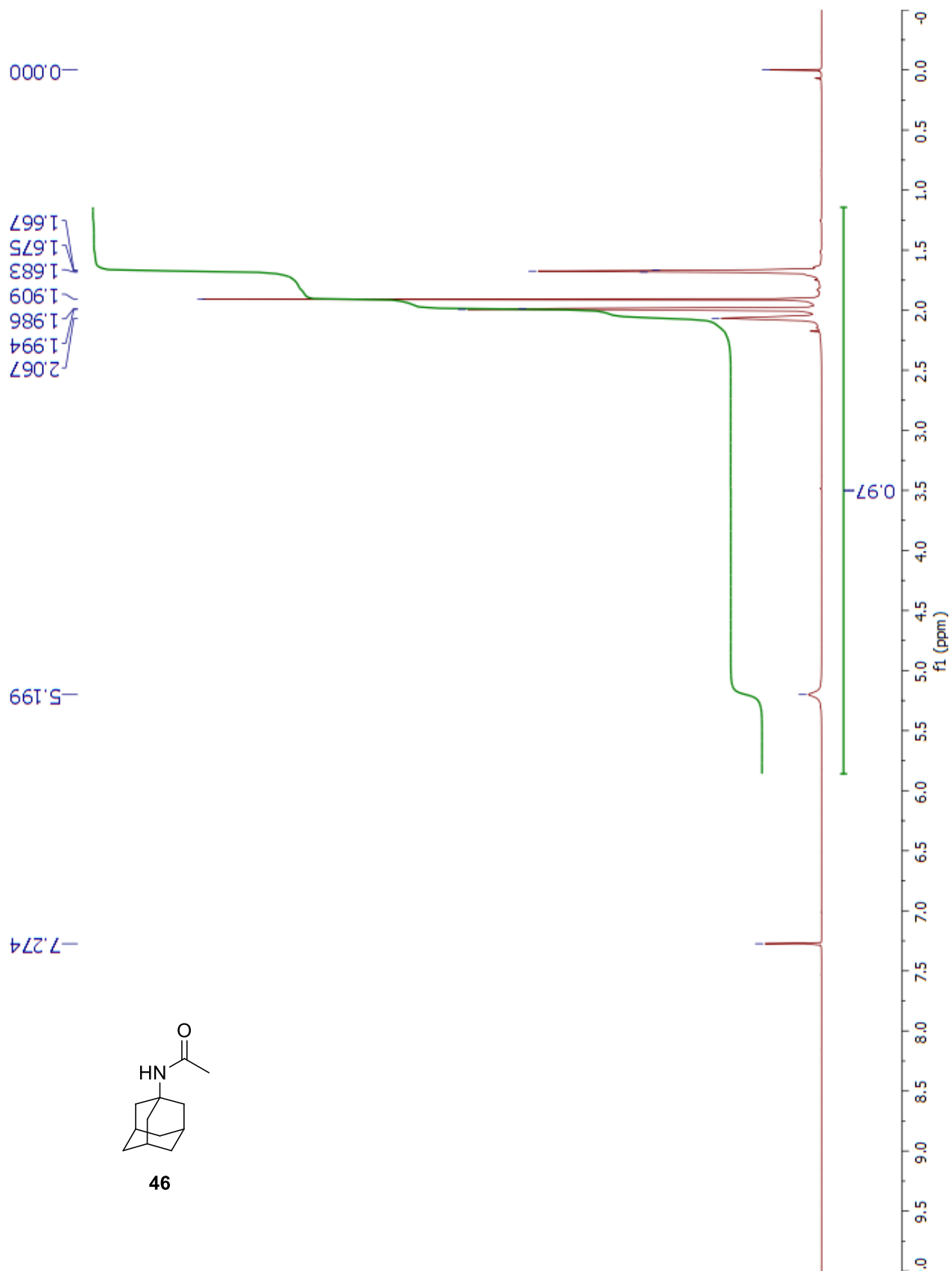
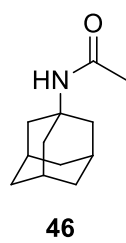


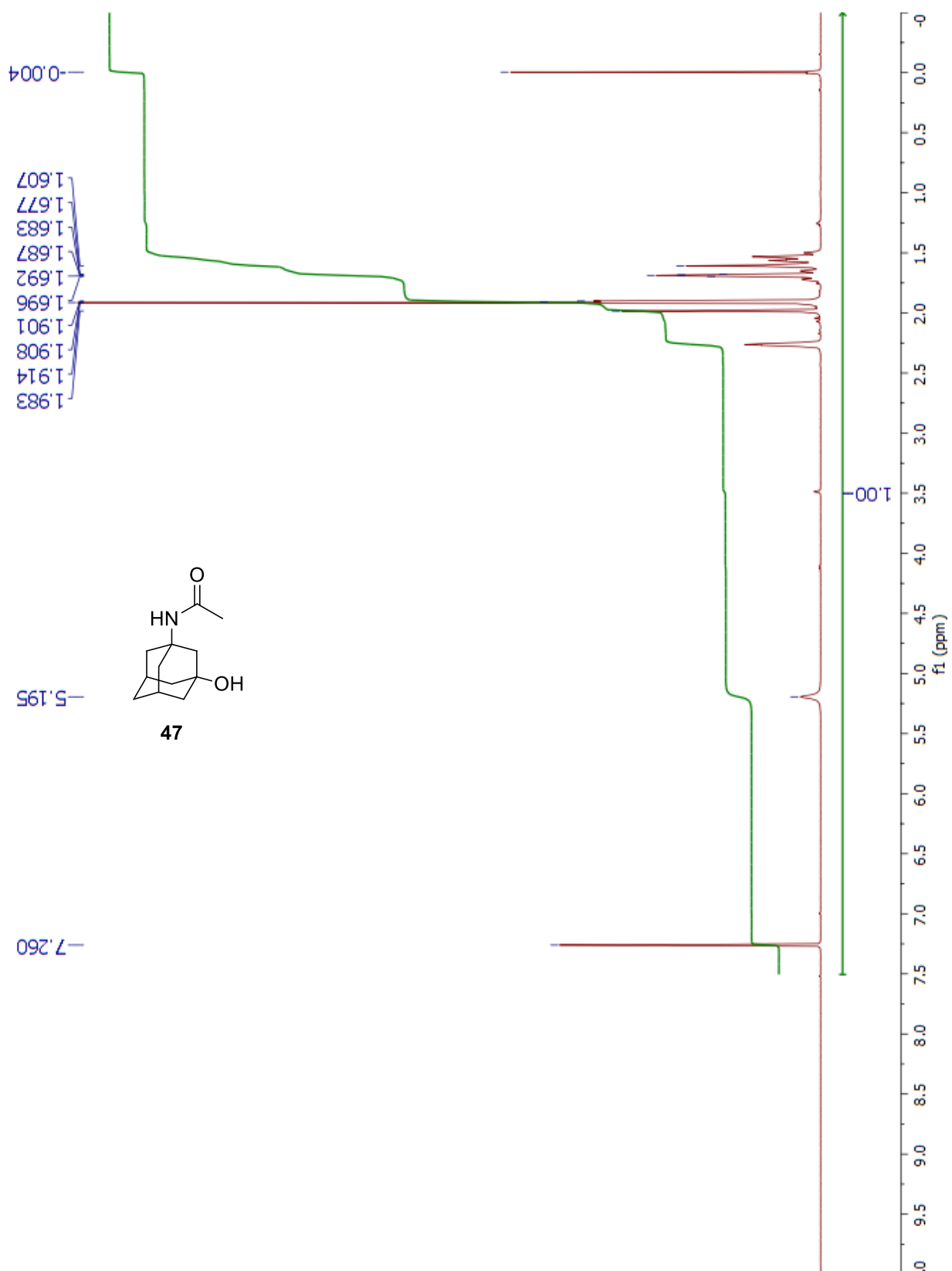


ZR-3-23 b4fr9 peak 2.10.fid



ZR-1-146.10.fid





zr-2-117 fr16-17.10.fid

