1999

Attempted Synthesis of an Insulin Mimic: An Ethylenediaminetetraacetic Acid (EDTA) Structural Analog in Complex with Vanadium

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Introduction

Diabetes mellitus is the most common endocrine disease and the third leading cause of death in the United States. Approximately 6 million cases have been diagnosed, and it is estimated that there are another 4 million more borderline but undiagnosed cases (1). Diabetes is characterized by an abnormally high level of glucose in the blood. Under normal body conditions, insulin is secreted by the islets of Langerhans in the pancreas when blood glucose levels are high, allowing for the reuptake of glucose into the cells from the blood. However, diabetic patients suffer from insulin-related deficiencies in two forms known as type I and type II diabetes. Type I diabetes is a result of elevated blood glucose levels due to inadequate secretion of insulin. Fewer than 10% of diabetics are type I. Type II diabetes is a result of insensitivity to insulin. Type II diabetic patients produce normal or elevated levels of insulin, but these patients have a shortage of insulin receptors. Insulin, therefore cannot bind to the insulin receptors, and blood glucose levels remain high. Approximately 90% of diabetes case are type II (1).

In the last few years, chemists have been experimenting with insulin-mimetic agents that can by-pass the insulin receptors, while still having the effect of lowering blood glucose levels. The insulin mimics that have been studied are effective for type I and type II diabetes because they produce the same effects as insulin, and they do not interact with the insulin receptors. The insulin-mimetic agents completely by-pass the insulin receptors on the cell surface instead of binding to the receptors. Therefore, the insufficiency of insulin secretion (type I) and a shortage of insulin receptors (type II) can be overcome by these insulin-mimetic agents.

The insulin-mimetic agents that have been studied are complexes of vanadium (a metal cation) with ethylenediaminetetraacetic acid (EDTA), and structural analogs of EDTA. Insulin binds specifically to insulin receptors, which are a specific tyrosine kinase. This tyrosine kinase (insulin receptor) is activated when insulin binds to it, and activation of the tyrosine kinase induces transcription of glucokinase. Glucokinase is the enzyme that phosphorylates glucose (by adding a phosphate group) to glucose-6-phosphate in the blood when glucose levels are high. Glucose-6-phosphate is then removed from the blood, and it is eventually stored as glycogen in the liver (1). The vanadium-EDTA complexes are believed to have an insulin-mimetic effect by bypassing the insulin receptors, and binding to non-specific tyrosine kinases. These non-specific tyrosine kinases then induce transcription of glucokinase just as the specific tyrosine kinase (insulin receptors) induces transcription of glucokinase. Therefore, the vanadium-EDTA complexes are able to produce the same effect as insulin by following a separate pathway for induction of glucokinase, which is the enzyme required to phosphorylate glucose (2).

EDTA and its structural analogs are organic compounds that are commonly used to bind metal cations, such as vanadium. EDTA has the structure shown in Figure 1 (3).

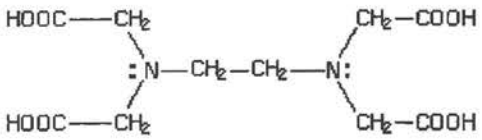


Figure 1: Structure of Ethylenediaminetetraacetic Acid

As the pH of a solution containing EDTA (or structural analogs) is raised (hydrogen ion concentration is lowered), the carboxyl groups (-COOH) are deprotonated to form

carboxylate anions (-COO^-) groups, which are negatively charged. The negative charge on the carboxylate anions, as well as the lone pairs of electrons on the nitrogen atoms of EDTA allows for EDTA to interact with metal cations, such as vanadium, which are electron deficient. A common example is the ethylenediaminetetraacetatocalcium complex (Figure 2) (4).

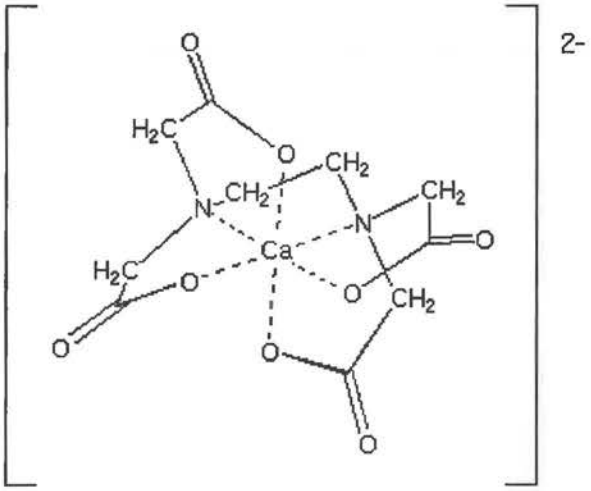


Figure 2: Structure of Ethylenediaminetetraacetatocalcium Complex

In this structure, Ca^{2+} interacts with the electrons on the oxygen atoms of the –COO^- groups and with the lone pairs of electrons from the nitrogen atoms. EDTA is a hexadentate ligand because Ca^{2+} and EDTA interact at six different binding sites. Vanadium interacts with EDTA and its structural analogs in a very similar manner as that shown in Figure 2.

In this research project, an original structural analog of EDTA was proposed for synthesis. Following the rules for naming a compound set forth by the International Union of Pure and Applied Chemistry (IUPAC), the compound was named N-(salicylidene)-N',N'-ethylenediaminediacetic acid. The purpose of the project was to

design a synthesis of this compound (Figure 3) in complex with vanadium. N-(salicylidene)-N',N'-ethylenediaminediacetic acid in complex with vanadium has possibilities as an insulin mimic.

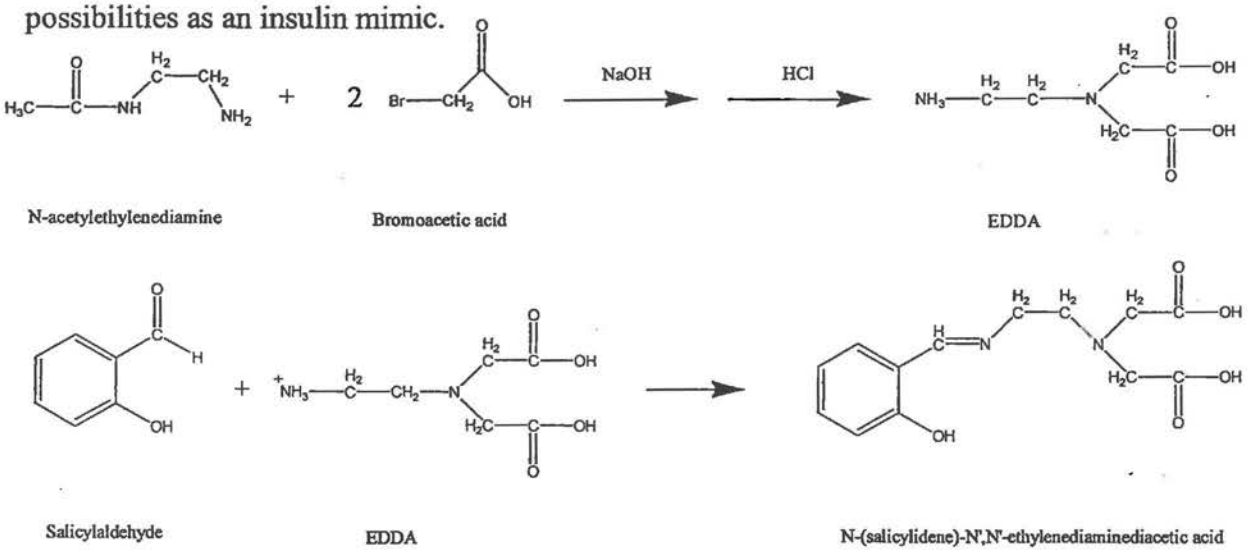


Figure 3: Synthesis of N-(salicylidene)-N',N'-ethylenediaminediacetic acid

When comparing the differences of N-(salicylidene)-N',N'-ethylenediaminediacetic acid to EDTA, the major difference can be seen that N-(salicylidene)-N',N'-ethylenediaminediacetic acid has a benzene ring with an –OH group attached. As stated previously, EDTA is a hexadentate ligand with six binding sites (four oxygen anions and two nitrogen with lone pairs of electrons). However, the deprotonation of the hydrogen atoms from the oxygen atoms in N-(salicylidene)-N',N'-ethylenediaminediacetic acid will result in a pentadentate ligand, in which N-(salicylidene)-N',N'-ethylenediaminediacetic acid has five binding sites (three oxygen anions and two nitrogen atoms with lone pairs of electrons) instead of the six binding sites in EDTA. Therefore, EDTA and N-(salicylidene)-N',N'-ethylenediaminediacetic acid are structurally different due to one less binding site in N-(salicylidene)-N',N'-ethylenediaminediacetic. This is significant because vanadium in the +5 oxidation state

(vanadium V) has the potential to bind to each site on N-(salicylidene)-N',N'-ethylenediaminediacetic. When vanadium (V) binds to EDTA, it binds to only five of the six available sites, leaving either a carboxylate group (-COO) or a nitrogen atom unbound in the vanadium-EDTA complex. However, vanadium (V) bound to N-(salicylidene)-N',N'-ethylenediaminediacetic provides the possibility of a tighter, more stable complex because each of the five binding sites in N-(salicylidene)-N',N'-ethylenediaminediacetic are bound to vanadium. The possibility of N-(salicylidene)-N',N'-ethylenediaminediacetic in complex with vanadium being more stable than the insulin mimic of the vanadium-EDTA complex makes it worthwhile to attempt an effective route of synthesis for N-(salicylidene)-N',N'-ethylenediaminediacetic.

Experimental Section

The first step of the synthesis was to form ethylenediaminediacetic acid (EDDA) (Figure 3) according to the method described by McLendon et.al. (1975). Four identical syntheses of EDDA on varying scales were carried out. The first synthesis was carried out on a 20% scale, the second and third syntheses on a 40% scale, and the fourth synthesis was carried out on a full scale. In each synthesis, the following steps were taken. Bromoacetic acid was dissolved in water. This solution was then combined with N-acetylethylenediamine, which had also been dissolved in water. The two solutions were then combined with each other and additional water was added. Next, 8 M (moles/liter) sodium hydroxide was combined with the bromoacetic acid N-acetylethylenediamine solution from a separatory funnel dropwise for about 30-45 minutes, while constantly stirring it with a magnetic stirrer. Then, 5 M sodium hydroxide was added, and this solution was then allowed to sit for at least 5 days, while the stirring

continued, and the protecting N-acetyl group was removed (amide hydrolysis) as a result of adding 5 M sodium hydroxide. The pH of the solution was then adjusted to about 4 by adding 20% hydrochloric acid solution. The first product in the synthesis, EDDA, was precipitated using absolute ethanol. After the product was obtained, it was analyzed by ^{1}H NMR (nuclear magnetic resonance) spectroscopy and ^{13}C NMR spectroscopy in order to determine if the structure of the compound was agreeable with what the expected structure is and for purity of the compound (by instruments in the Chemistry Department at the University of Tennessee, Chattanooga). Finally, the EDDA was complexed with vanadyl sulfate to form a vanadium-EDDA intermediate. The complex of EDDA-vanadyl sulfate was then placed in methanol in attempt to dissolve the complex.

Results

In the first procedure, which was a 20% scale, 0.126 grams of a white solid were obtained with a yield of 5.83%. In the second procedure, which was a 40% scale, 0.701 grams of a white solid were obtained giving a yield of 16.17%. In the third procedure, which was also a 40% scale, 3.397 grams of a white solid were obtained giving a yield of 78.52%. In the fourth procedure, which was full scale, 3.32 grams were obtained giving a yield of 30.7%, assuming that the product obtained was 100% EDDA. Only the products from the third and fourth syntheses of EDDA were analyzed, and only the analysis of the product from synthesis three is present in this paper. However, the analysis from synthesis four was very similar. The spectral data of the product (^{1}H NMR and ^{13}C NMR), which was presumed to be EDDA, is presented in Figures 4 and 5. The structure of EDDA is shown on Figure 4, and the hydrogen atoms in each respective environment are represented by the letter (a), (b), or (c). ^{1}H NMR data (Figure 4) showed

that hydrogen atoms in the product existed in three chemically distinct environments. The integrations, or relative areas of the peaks, showed that the three environments of hydrogen atoms existed in a 2:1:1 ratio. These peaks are represented as (a), (b), and (c) on Figure 4, respectively. The chemical shift in parts per million (ppm) showed that the hydrogen atoms represented by (a) showed the greatest chemical shift at 3.77 ppm. The other two environments of hydrogen atoms represented by (b) and (c) showed chemical shifts of about 3.5 ppm and 3.3 ppm. The peaks represented as (b) and (c) both showed a triplet pattern (i.e. three peaks). The triplet peak shows that the adjacent carbon has two hydrogen atoms attached to it. A duplet peak would show that the adjacent carbon has one hydrogen atom attached to it. Therefore, the neighboring carbon atoms of the hydrogen atoms represented by peaks (b) and (c) suggest that the neighboring carbon atoms have two hydrogen atoms attached. The peak represented by (a) is a singlet. The slight peak that is about halfway up represents an impurity. The single peak suggests that the neighboring carbon atom does not have any hydrogen atoms attached.
The large peak at about 4.7 ppm represents the hydrogen atoms in water (H_2O) because the solution of EDDA was dissolved in water. Any other small peaks in the spectrum represent impurities.

In Figure 5, the ^{13}C NMR spectrum and structure of EDDA are shown. Also, the differing chemical environments of the carbon atoms are represented as (a), (b), (c), and (d). The spectral data shows that the carbon atoms in this product exist in four separate chemical environments. The carbon atoms represented by peak (a) showed the greatest chemical shift at 171.1 ppm. The carbon atoms represented by peak (b) showed a chemical shift of 58.2 ppm. The carbon atoms represented by peak (c) showed a

chemical shift of 53.1 ppm, and the carbon atoms represented by peak (d) showed a chemical shift of 35.6 ppm.

The EDDA-vanadyl sulfate complex formed a bright blue solid. When the solid from this complex was placed in methanol, it did not dissolve. The insolubility of the vanadium-EDDA complex prevented the second step of the synthesis (Figure 3) in which EDDA was to react with salicylaldehyde to form N-(salicylidene)-N',N'-ethylenediaminediacetic acid.

Discussion

When analyzing the results from the project, successful results can be seen while unsuccessful results can be seen as well. It can be seen (Figure 3) that the first step in the proposed two-step synthesis (bromoacetic acid + N-acetylethylenediamine → EDDA) proceeded as expected. It took several tries to develop a good technique to synthesize the EDDA in step one of the two-step synthesis. As can be seen in the results, the first two syntheses yielded low percent yields (5.83% and 16.17%). However, synthesis was much better with a yield of 78.52%. This percent yield was higher than the published yield of McLendon et.al. (1975), which was 70%. The fourth synthesis dropped lower again to 30.7%.

The yield from synthesis three gave the highest percent because of one critical factor. If several days were allowed to pass between each part of the reaction, a much higher yield was observed. For example, once bromoacetic acid was added to N-acetylethylenediamine (Figure 3), it was allowed to stir for two days before the next step. After two days, 5 M sodium hydroxide was added in a process known as amide hydrolysis to remove the N-acetyl protecting group to yield EDDA. At least five days

were allowed for the sodium hydroxide to remove the protecting N-acetyl group. This step was the most critical in allowing for enough time for the reaction to proceed. Once complete amide hydrolysis had occurred, greater precipitation of EDDA was obtained after lowering the pH to 4 and adding absolute ethanol. However, in the fourth synthesis, amide hydrolysis was allowed to proceed for about one month. The excessive length may have resulted in the formation of an equilibrium, which allowed the reverse reaction to occur in which the N-acetyl protecting group to reattach to EDDA, and this significantly lowered the percent yield. The percent yield for each synthesis is based on the assumption that the product obtained was 100% EDDA. However, that is not likely the case. When looking at the spectral data (Figures 4 and 5), small peaks can be observed, and these peaks are not part of the spectral data for EDDA. Thus, impurities were present in the EDDA that was synthesized. However, based on the small size of the peaks, the impurities existed in very small amounts. Nonetheless, the percent yields that are listed actually are slightly lower because some of the mass of the product was not EDDA.

Although some impurities existed in EDDA, ^{1}H NMR and ^{13}C NMR clarify that the product obtained in the first step of the synthesis was EDDA. ^{1}H NMR (Figure 4) shows that the hydrogen atoms in the product obtained exist in three chemically distinct environments. The structure of EDDA (Figure 4) shows that the hydrogen atoms exist in three chemically distinct environments, represented by (a), (b), and (c). Also, the hydrogen atoms in the three environments of EDDA exist in a 4:2:2 ratio or 2:1:1, and the ^{1}H NMR shows a 20.8:11.09:12.71 ratio for peaks (a), (b), and (c). Based on the purity of the product, this is roughly a 2:1:1 ratio. Also, the chemical shift in ppm shows

that the hydrogen atoms represented by peak (a) demonstrated the greatest chemical shift. This was expected because these hydrogen atoms are attached to a carbon atom that is adjacent to a carboxyl (-COOH) group. It is well known in organic chemistry that hydrogen atoms attached to a carbon adjacent to a –COOH group display a high chemical shift (6). Also, the hydrogen atoms represented in peaks (b) and (c) display chemical shifts in relation to the proximity that they have to the –COOH group. Therefore, the data from ^{1}H NMR supports the product as being EDDA. However, ^{1}H NMR cannot be the final answer in determination of the product.

^{13}C NMR allows for characterization of the carbon atoms in the product. Each peak in ^{13}C NMR represents carbon atoms in a chemically distinct environment, and it can clearly be seen (Figure 5) that four distinct environments are found in the product. It can be seen that the two carbon atoms represented by (a) in the structure of EDDA (Figure 5), and which are also represented by peak (a), display a chemical shift of 171.1 ppm. These two carbon atoms are carboxyl (–COOH) carbons. Carboxyl carbons are known to have chemical shifts between 170-185 ppm. Therefore, peak (a) in the ^{13}C NMR data is characteristic of the carboxyl carbon of EDDA. Also, peaks (b), (c), and (d) display chemical shifts in relation each carbon represented by the peak has in proximity to the carboxyl group. The closer the carbon atom is to the carboxyl group, the greater the chemical shift it displays. The data from ^{13}C NMR is also supportive of the product being EDDA. The four chemically distinct carbon environments, as well as peak (a) displaying the chemical shift of a carboxyl group, suggest that the product is EDDA. When combining the data from ^{1}H NMR and ^{13}C NMR, it can be quite certain that the product synthesized in step of the proposed two-step synthesis was EDDA.

After EDDA was synthesized, it was quite easily complexed with vanadyl sulfate to form a vanadium-EDDA complex. It was hoped that this complex would dissolve in methanol so that it could react in solution with salicylaldehyde to form N-(salicylidene)-N',N'-ethylenediaminediacetic acid. However, the vanadium-EDDA complex would not dissolve in methanol. This left little hope for continuation of the synthesis because methanol is the most polar protic solvent besides water. However, water cannot be used as a solvent in the reaction with salicylaldehyde because water destroys the product of the reaction by hydrolysis. Therefore, the conclusion of the attempted synthesis was that the proposal for the two-step synthesis of N-(salicylidene)-N',N'-ethylenediaminediacetic acid does not work, and that other routes of synthesis must be developed. Further research should be conducted, and already an alternate pathway is being proposed.

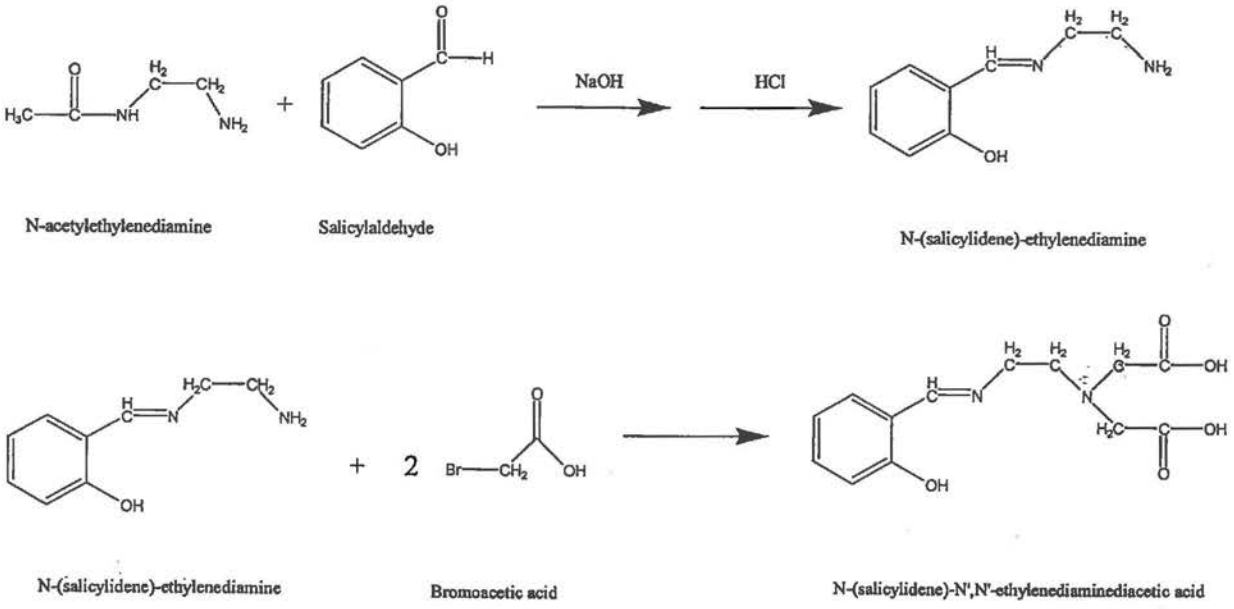


Figure 6: New Proposal for Synthesis of N-(salicylidene)-N',N'-ethylenediaminediacetic acid

The proposed synthesis (Figure 6) now consists of N-acetylethylenediamine reacting with salicylaldehyde to form an intermediate. This intermediate will then be reacted with bromoacetic acid, after removal of the protecting group, to form N-(salicylidene)-N',N'-ethylenediaminediacetic acid. It is hoped that this will provide a better alternative to the route that was described in this paper.

Despite the failure of the reported method to synthesize the proposed insulin mimic, this research allows for different pathways to be attempted that will ultimately lead to the successful synthesis of N-(salicylidene)-N',N'-ethylenediaminediacetic acid. It is now known that the route that was attempted in this synthesis is not a suitable pathway because EDDA could not be dissolve in a suitable solvent to continue the synthesis. Now, further routes can be attempted until the final product is obtained. Once the final product is obtained, its usefulness as an insulin mimic can be studied. This could lead to a better understanding of the relationship that exists between the structure-activity relationship of N-(salicylidene)-N',N'-ethylenediaminediacetic acid in complex with vanadium with five binding sites as opposed to the other complexes of vanadium-EDTA (and structural analogs) with six binding sites and the efficacy of these complexes. Greater understanding of these relationships will be useful in treating patients suffering from diabetes.

Figure 4: ^{1}H NMR

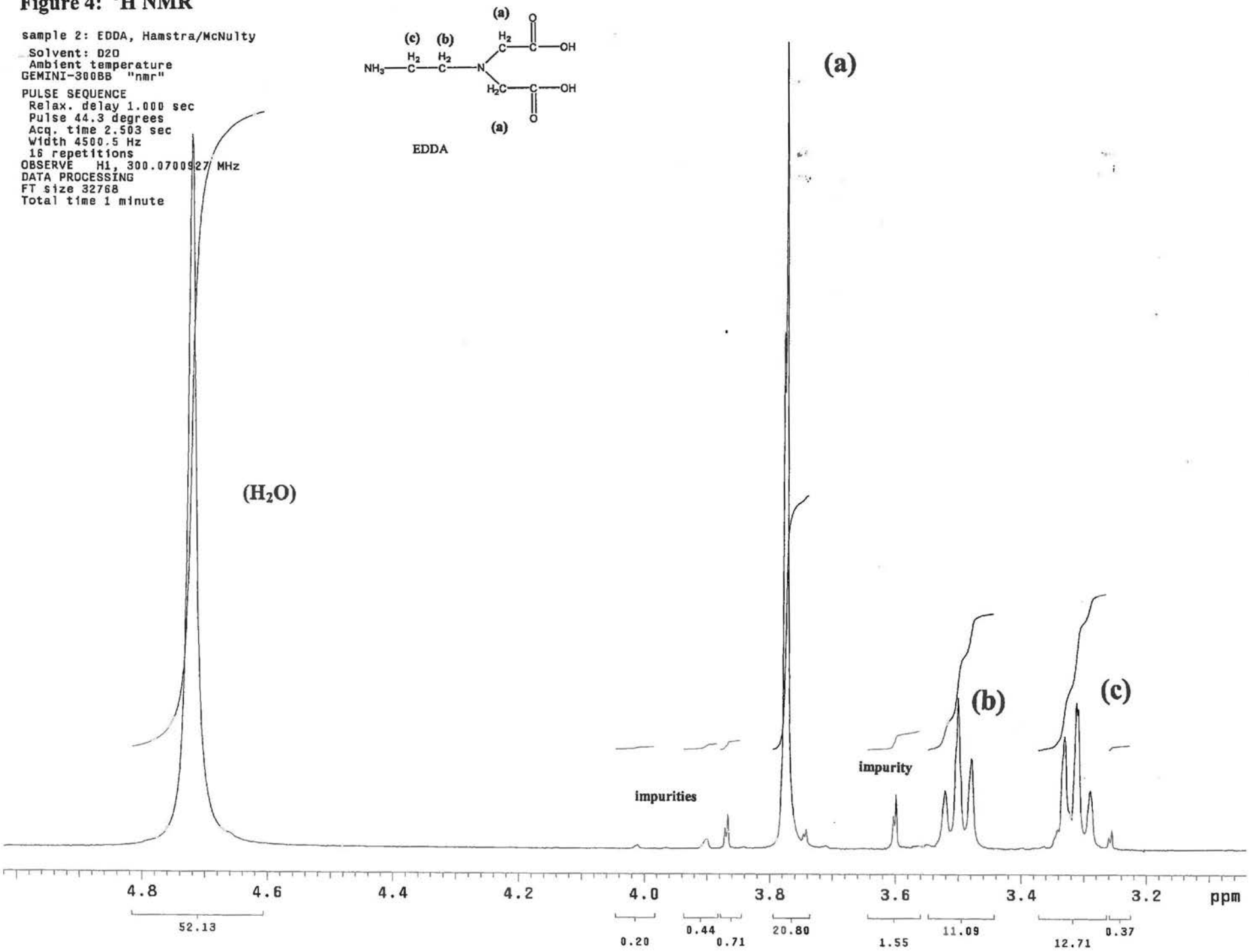
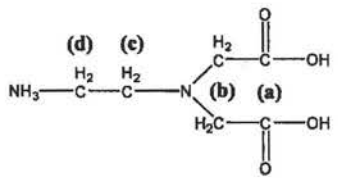


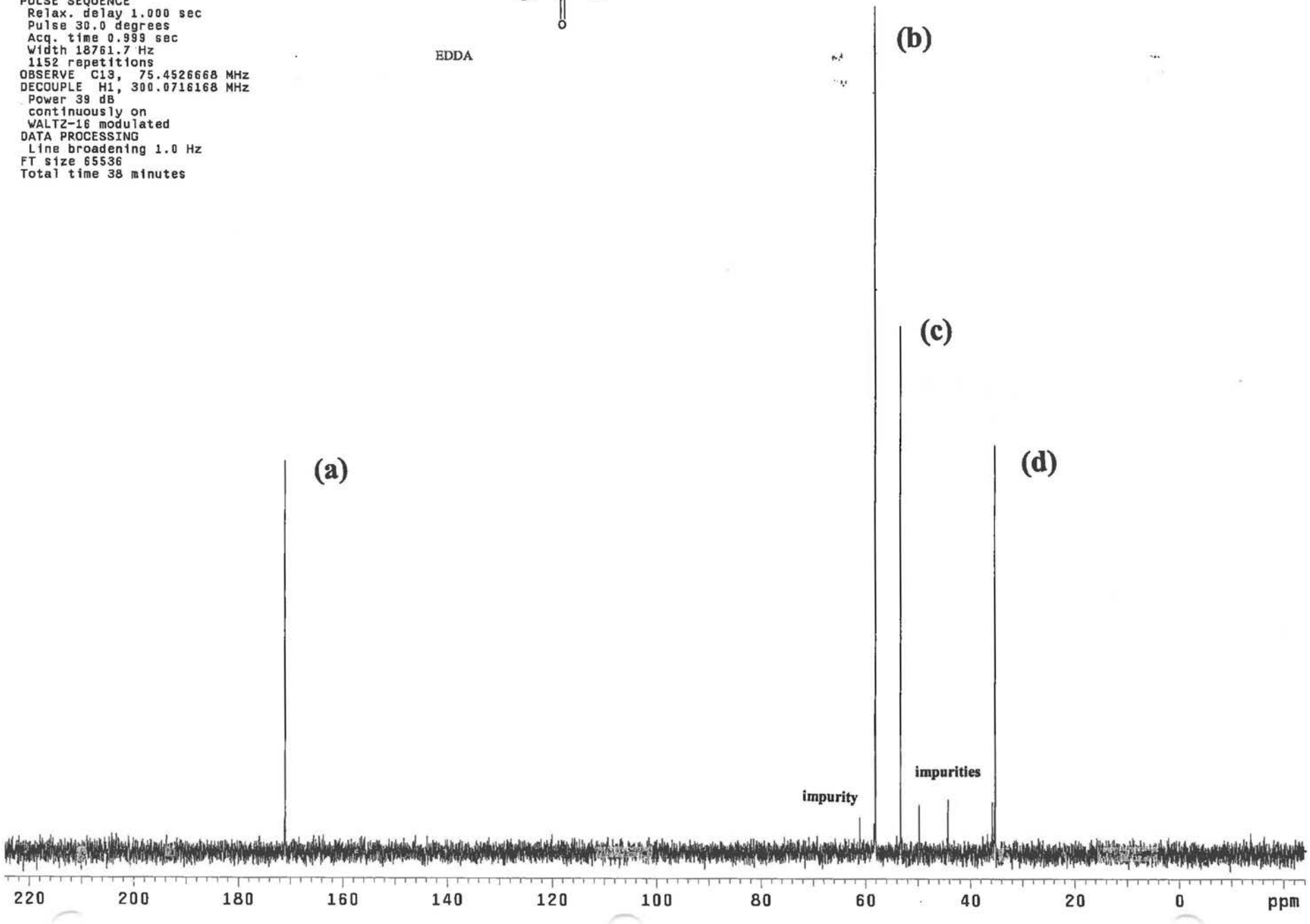
Figure 5: ^{13}C NMR

sample 2: EDDA, Hamstra/McNulty
Solvent: D2O
Ambient temperature
GEMINI-300BB "nmr"

PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 30.0 degrees
Acq. time 0.999 sec
Width 18761.7 Hz
1152 repetitions
OBSERVE C13, 75.4526668 MHz
DECOUPLE H1, 300.0716168 MHz
Power 39 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 38 minutes



EDDA



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SOUTHERN SCHOLARS SENIOR PROJECT

Name: Norman McNulty Date: 10/5/99 Major: Biochemistry

SENIOR PROJECT

A significant scholarly project, involving research, writing, or special performance, appropriate to the major in question, is ordinarily completed the senior year. The project is expected to be of sufficiently high quality to warrant a grade of A and to justify public presentation.

Under the guidance of a faculty advisor, the Senior Project should be an original work, should use primary sources when applicable, should have a table of contents and works cited page, should give convincing evidence to support a strong thesis, and should use the methods and writing style appropriate to the discipline.

The completed project, to be turned in in duplicate, must be approved by the Honors Committee in consultation with the student's supervising professor three weeks prior to graduation. Please include the advisor's name on the title page. The 2-3 hours of credit for this project is done as directed study or in a research class.

Keeping in mind the above senior project description, please describe in as much detail as you can the project you will undertake. You may attach a separate sheet if you wish:

Please see separate sheet

Signature of faculty advisor Brent Hamaker Expected date of completion 3/28/2000

Approval to be signed by faculty advisor when completed:

This project has been completed as planned: YES

This in an "A" project: YES

This project is worth 2-3 hours of credit: YES

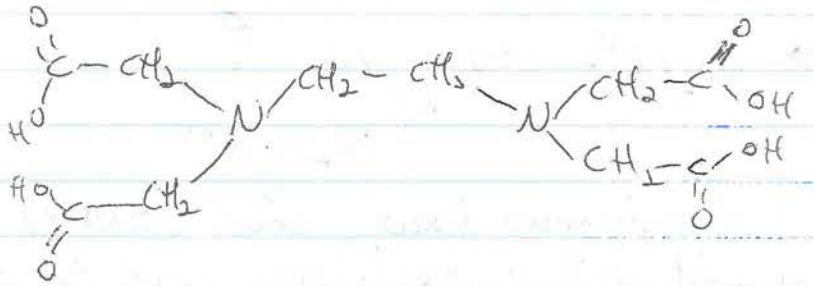
Advisor's Final Signature Brent Hamaker

Chair, Honors Committee _____ Date Approved: _____

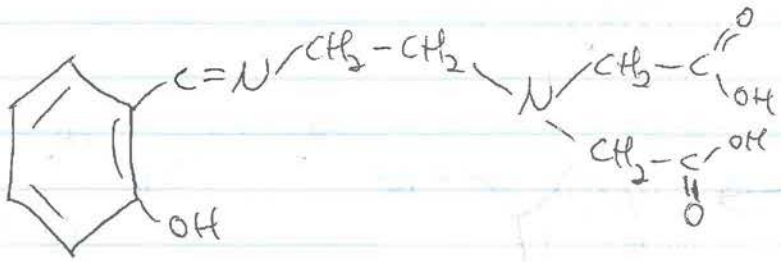
Dear Advisor, please write your final evaluation on the project on the reverse side of this page. Comment on the characteristics that make this "A" quality work.

Project Outline:

Ethylene diamine tetra acetic acid (EDTA) is a well-known organic compound that chelates divalent cations. EDTA has the following structure:



For this research project, a very similar compound will be attempted to be synthesized, and it is believed to be an original compound. It has the following structure:

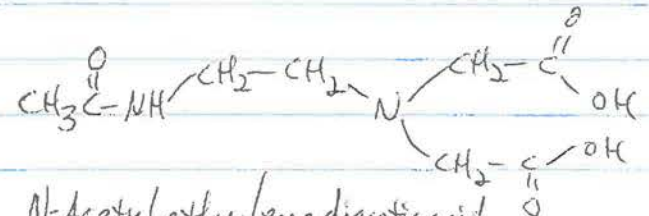


The multi-step synthesis will occur as follows:

Step 1) 

N-Acetylethylene diamine

Bromoacetic acid



N-Acetylethylenediaceticacid

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