

Synthesis of Crown Ethers for Use in Studies of Fluorescence

An Honors Thesis (Honors 499)

by

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Thesis Advisor: Dr. Lynn Sousa

A handwritten signature in black ink, appearing to read "Lynn Sousa", is written over a horizontal dashed line.

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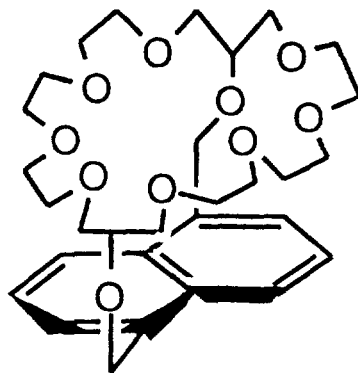
Date: May 8, 1997

Date of Graduation: May 10, 1997

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Abstract: The following thesis is an overview of the undergraduate research I performed during my last three semesters. I worked with Dr. Lynn Sousa in the area of Organic Chemistry, trying to synthesize, identify, and isolate a crown ether molecule that would be used in studies of fluorescence.

SYNTHESIS OF CROWN ETHERS
FOR USE IN STUDIES OF
FLUORESCENCE



Melissa Slater
Senior Honors Thesis

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Introduction

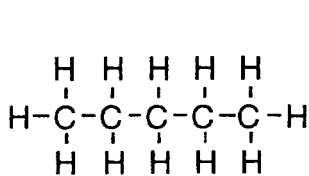
I began working with Dr. Sousa during the summer of 1996 as part of the chemistry department's Undergraduate Summer Research Program. It is a ten week program in which professors allow students to aid them with their independent research projects or develop one of their own. It gives students the opportunity to gain valuable experience in a number of areas in chemistry.

Dr. Sousa's main area of interest is Organic Chemistry. He had been working on a particular project for several years, and wanted a summer research student to continue with one particular idea he had been working on.

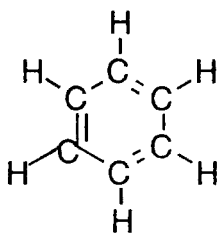
I was available for research the first five-week session of the program, and paired up with Dr. Sousa because he was also available then. We were both able to commit to the project on a part-time basis. Then in the fall of 1996 and spring of 1997 I continued doing research with Dr. Sousa part time. This thesis is an overview of what I have done.

Background

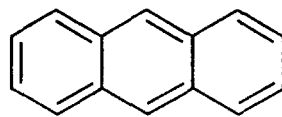
Organic Chemistry is the chemistry of carbon-containing compounds and their properties. It is also called the "chemistry of life" because all living things contain carbon and carbon-based chemistry. A feature unique to the element carbon is the fact that it can bond strongly to itself, creating straight and branched carbon chains and intricate ring structures up to great sizes:



pentane
C₅H₁₂

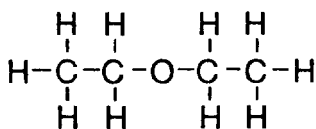


benzene
C₆H₆

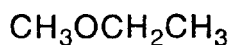


anthracene
C₁₄H₁₀

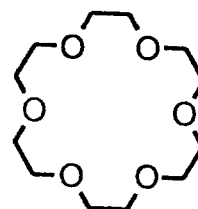
Many organic compounds have functional groups, or reactive areas on the molecule that give it specific properties. One common functional group in organic chemistry is called an ether, which consists of an oxygen atom that has a carbon on both sides.



diethyl ether



ethyl methyl ether



18-crown-6
1,4,7,10,13,16-hexaoxacyclo-octadecane

In 1967, Peterson first reported the existence of a new type of ether. Peterson's ether was actually a ring of ethers, the 18-crown-6 shown above. It was the first time a cyclic polyether of this type had been synthesized. Since then, a great amount of research has been dedicated to synthesizing and studying molecules of this type. A specific characteristic of crown ethers is their ability to complex or hold a positively-charged ion(cation) in their cavities. The attraction and complexation between the two is referred to as host-guest chemistry. The host crown can be one of several variations of the original 18-crown-6, and the guest is typically an alkali metal cation. Attaching a chromophore, a large ring structure that absorbs specific frequencies of light, causes the total molecule to fluoresce when energy is focused on it. Several studies have been devoted to the idea that heavier complexed cations such as cesium seem to quench fluorescence, while potassium does not stop the fluorescence.

In 1977, Dr. Sousa and his students first reported the observation of alkali-metal enhanced

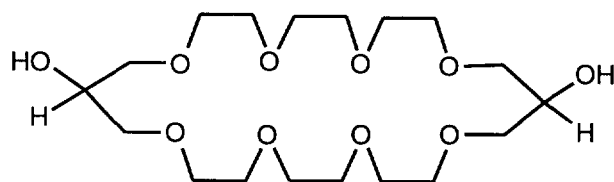
fluorescence of a 21-crown-6 attached to a naphalene chromophore. In 1981, they began to design and synthesize compounds that would display this type of cation-enhanced fluorescence.

Specifically, Dr. Sousa wanted to synthesize a chromophore-bearing crown ether that might have fluorescence sensitive to potassium ions. There are relatively few compounds with attached chromophores that display potassium-enhanced fluorescence specifically. The chromophore (flat ring structure) would form the flat bottom of the molecule, with the crown open and extending over the top as a “strap” or “jaw” that would hold the round cation in between the two. The resulting molecule would resemble a “basket”, with the chromophore bottom and the crown “handle”.

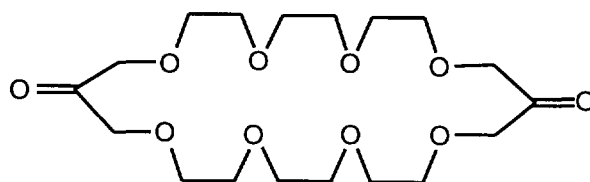
The purpose of creating a molecule of this type would be for biological studies and testing. If the molecule were to be found extremely specific in complexing potassium as opposed to other metal ions, the fluorescence given off would be a good measure of the presence or amount of potassium in a biological sample. The fluorescence of this molecule would provide an alternate method of looking at potassium concentrations.

While Dr. Sousa successfully synthesized and studied this single-strap molecule, in recent years he has given thought to the idea that where one strap binds the cation in place, two straps might be more effective in promoting potassium-enhanced fluorescence. The goal of my research was to synthesize, identify, and isolate a larger crown ether (to form a double strap), and attach it to a chromophore for use in fluorescence studies.

Focus and Primary Goal:

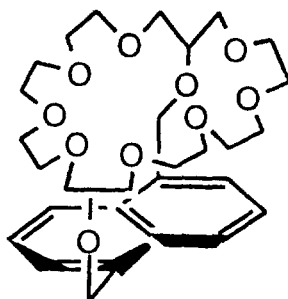


cis and trans
1,4,7,10,14,17,20,23-octaoxa-12,25-dihydroxycyclohexacosane



1,4,7,10,14,17,20,23-octaoxa-12,25-dioxocyclohexacosane

The focus of my research was these two crown ether compounds. The left molecule is the dihydroxy form of the crown, with alcohol end groups. The alcohol end groups are the part of the molecule that would allow the attachment to the chromophore to take place. When attached, the middle of the crown would bend upwards, forming the set of straps over the molecule:



The molecule to the right above is the diketone form of the crown, having the end two carbons double-bonded to oxygens. The diketone form would have to be reduced to the dihydroxy form before it could be attached. Since the reduction of the diketone could be done fairly easily, we pursued both types of the crown.

I. The Research Plan

Attempting to synthesize either crown ether involved several steps:

1. Envision the compound we wanted to make
2. Search the STN scientific computer databases to see if the compound had been made previously
3. Conduct a literature search for similar molecules and syntheses of similar molecules to use as guidelines for our reactions
4. Attempt to synthesize a crown using the literature precedence
5. Determine if the product of the reaction contains something that behaves like the crown

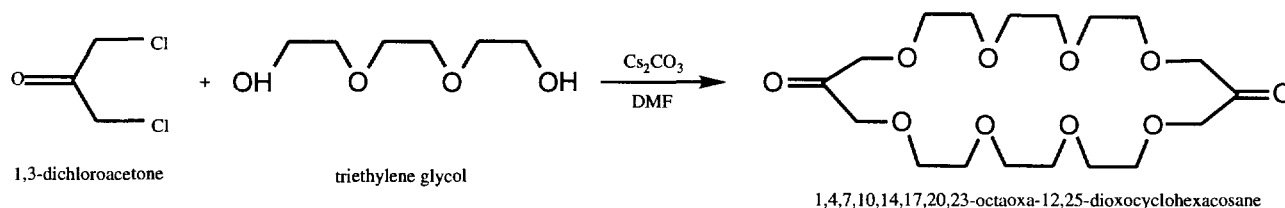
6. Attempt to separate the reaction product into molecules of different sizes and further isolate the desired crown
7. Determine and confirm which separate fraction contains the crown
8. Attach the crown to the chromophore for future study by Dr. Sousa

We began our research by conducting a computer search through STN, a worldwide scientific database that indexes molecules and scientific publications. After searching for the existence of both molecules, we found that neither had been previously made. If we had found one, our research would have consisted of synthesizing it according to the literature, then attaching it to the chromophore. However, since neither had been made our task was to be considerably larger.

The next step required an extensive search through the scientific literature. I needed to find reactions that resulted in molecules similar to the crown we were looking for. When I found something we might be able to apply, we would alter the procedure to favor the large type of product we desired. I spent several weeks looking for procedures, eventually finding a reaction that might be used in synthesizing each of the crown ethers.

II. Attempted Reaction #1

We decided to attempt to make the diketone form of the crown first. We modified a procedure that originally formed a smaller diketone crown, using triethylene glycol and 1,3-dichloroacetone in a 2:2 ratio according to the following reaction:

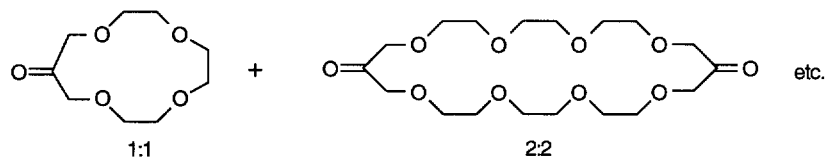


Experimental

A 250-ml round-bottom flask was fitted with a dry stopper, reflux condenser, and a gas inlet. Argon was run through the inlet and out the top of the reflux condenser. 1,3-Dichloroacetone (3.80g,30mmol) was weighed under the hood and quickly added to the flask, then 11.4g(35mmol) of Cs_2CO_3 was added. Triethylene glycol (4.51,30mmol) and 100 ml dry DMF(driedover sieves) were quickly added, creating a dark brown solution. The solution was stirred several minutes then heated by heating mantle to a gentle boil. The solution was boiled for approximately 48.5 hours, then removed from heat and stirring. The solution was a dark brown color with fine white sediment in it. After cooling, the sediment was separated by vacuum filtration, washed with 5x10ml portions of dichloromethane, then isolated and dried, resulting in 9.78g of solid. The liquid was transferred to a 250-ml round bottom flask and rotary evaporated to remove dichloromethane, and the DMF and remaining dichloromethane were removed by vacuum distillation at $\sim 28^\circ\text{C}$ and 1.33 mmHg. The result was a thick, dark brown oil only partially soluble in deuteriochloroform.

Reference used: J. Org. Chem. **1994**, 59, 2186-2196. by Gibson et al.

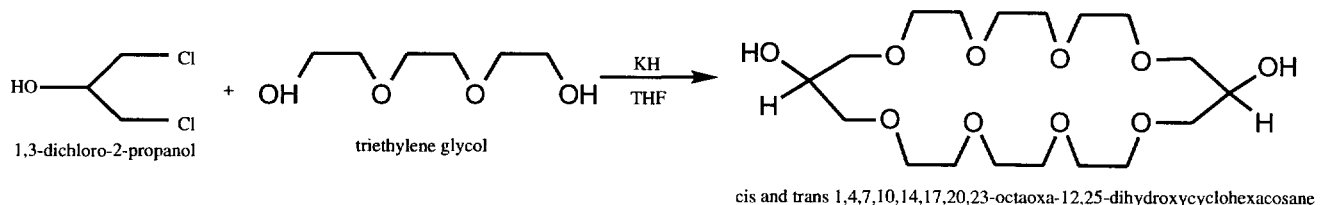
The difficulty in synthesizing crowns is the fact that many sizes form in a given reaction. The reaction would naturally tend to form the smaller, easier to form 1:1 product. While we may purposely use conditions favoring the formation of the larger crowns, it is highly probable that smaller ones form from different ratios.



To take a look at what different molecules the oil might contain, I first employed a technique called Thin-Layer Chromatography_a (see appendix). The resulting TLC's did not show the type of separation we had expected. In fact, it was difficult to tell if we had separation at all. The next step was to take a proton NMR_a to look for the presence of hydrogens on a molecule of our type. The spectrum did not indicate the correct ratio of hydrogens that would be found on our crown. After closely examining the mechanism of the reaction, we realized that it was possible for the starting materials to undergo the Favorski Rearrangement, which would result in a completely different product than desired. Since Dr. Sousa felt that the Favorski Rearrangemet was a definite threat to successfully synthesizing the diketone crown, we decided to move on to the dihydroxy crown.

III. Attempted Reaction #2

We then attempted to synthesize the dihydroxy form of the crown. With a more solid base in the literature, we followed a reaction that had resulted in a good amount of fairly large dihydroxy crown. Again, we altered the procedure to promote formation of a larger molecule, using 1,3-dichloro-2-propanol and triethylene glycol.



Experimental

Potassium hydride, KH (8.03g of 35% KH in oil suspension) was weighed and added to a three-necked, 500-ml round bottom flask. The slurry was washed with 4x30ml of dry THF. THF (100 ml) was added to the flask, and 4.81g(.032mol) of triethylene glycol in 50 ml dry THF was placed in a closed addition funnel on the middle neck of the flask. The triethylene glycol was added dropwise, with stirring, over several minutes, to produce a tan slurry. The flask was then heated to gentle reflux. 1,3-Dichloro-2-propanol (4.0g,.031mol) in 50 ml dry THF was added dropwise through the funnel. The slurry was kept at reflux(with stirring) under Argon for approximately 15 hours, resulting in a dark tan solution. Stirring was continued after removal of the heat source. After cooling, 5 ml methanol was added to destroy any remaining KH, then the reaction mixture was filtered through celite and washed with THF. The THF was rotary evaporated off to leave 9.62g of dark brown, thick oil. This oil was lighter and thinner than the previous reaction mixture, and also completely soluble in deuteriochloroform.

Reference used: Liebigs Ann. Chem. **1985**, 210-213 by Ulrich et al.

The result of this reaction was a lighter, thinner oil than previously obtained in the diketone synthesis. Silica Gel TLC_a now indicated that we had created a mixture of different molecules.

The molecules tended to travel at two rough speeds. To separate the reaction mixture into molecules of different size, I employed a technique called column chromatography_a. I then studied the fractions collected from the alumina column separation for signs of the the crown. Again, TLC's of several fractions over time showed evidence that we had two main sizes of compounds. The faster-moving molecules were evident in the beginning fractions, and a slower-moving spot mainly appeared in the later fractions.

Proton and Carbon NMR_a were used to study the makeup of the molecules in the main groups of fractions. NMR MS-22-1a is an example proton NMR spectrum of an early fraction, fraction 8, believed to contain the bulk of the desired compound. The peak that would be attributed to hydrogens of crown compounds is where it was expected. The corresponding carbon NMR, spectrum also showed evidence of a crown. While there is clear evidence that triethylene

glycol, one of the starting materials, still remains, there is also a new peak that cannot be attributed to anything but the possible formation of the new crown.

NMR's MS-22-3a and MS-22-3b are again proton and carbon NMR's, respectively, this time for a later fraction. You can see that there is more triethylene glycol (see NMR MS-17-5) in this fraction, and also evidence of the new peak. MS-22-3b also shows what is called an Attached Proton Test(APT)_a. Looking at the target crown ether, we would hope to see all peaks pointing up except one. The signal from the carbon with the -OH and H group attached would point down. On the upper spectrum of MS-22-3b, that is exactly what we see. While this is not clear proof of our crown, it is promising.

Close to the end of the semester, I attempted another separation of the original reaction mixture, this time using a silica gel column. However, the results of the separation were not very clear. After examining TLC and NMR data, it appears that most of the component of interest did not leave the column as expected.

IV. Conclusion

We are unable to say if we have obtained the dihydroxy crown. The separation by the alumina column seemed successful, and the NMR spectra were promising. However, more research needs to be done before it can be confirmed either way.

Doing research has been a rewarding experience for me. I have learned a lot of valuable techniques in the lab, as well as organization and how to manage my time efficiently. I am glad I was able to be a part of the undergraduate research program. I feel I have learned many valuable things I can apply to my future.

Appendix

Attached Proton Test (APT) - a test that can be run on the NMR that looks at the number of hydrogens attached to the carbons in your molecule. If the peak is pointing up, the carbon that peak represents has either 0 or 2 attached hydrogens. If the peak is pointing down, the carbon has 1 or 3 hydrogens.

Column Chromatography - method of separation involving an open glass tube with a valve at the bottom. The column is packed with alumina or silica gel, then a layer of sample is introduced at the top. Solvent is added through the top and allowed to run out the bottom, taking different components of the original mixture through with it. The speed of the different molecules depends on their polarity and size, so by taking several fractions off the bottom of the column it is possible to separate your mixture.

Nuclear Magnetic Resonance (NMR) - an instrument used to qualitatively analyze organic samples. The spectrum and integration obtained can suggest the number and ratio of different hydrogen and carbon types in your sample.

Thin Layer Chromatography - a method of separation involving a thin layer of alumina or silica gel on a glass or plastic plate. A small drop of the sample is spotted onto the plate, and the plate is then set upright in a shallow amount of solvent in an airtight container. Because the components of the mixture have different polarities and solubilities, they are carried up the plate and spread differently as the solvent front rises. It is used to separate and visualize the different components of the mixture.

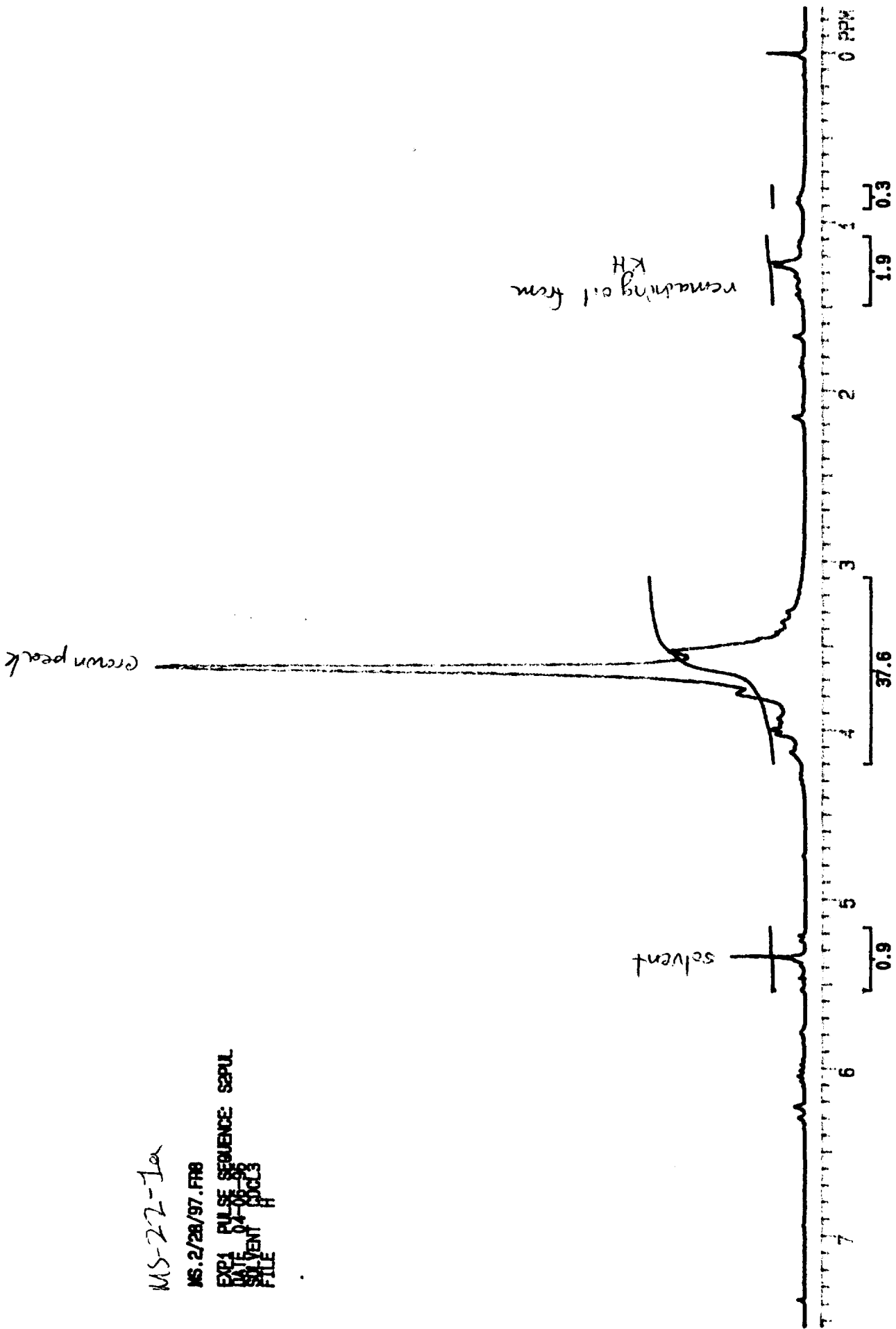
References

- Bloomfield, Molly M. Chemistry and the Living Organism, 2nd Ed; John Wiley & Sons: New York, 1980.
- Braun, Robert D. Introduction to Instrumental Analysis; McGraw-Hill: New York, 1987.
- Cram, Donald J; Trueblood, Kenneth N. Concept, Structure, and Binding in Complexation. In Host Guest Complex Chemistry: Macrocycles; Vogtle, F; Weber, E., Ed.; Springer-Verlag: Berlin, 1985.
- Ebbing, Darrel D. General Chemistry; Houghton-Mifflin Company: Boston, 1984.
- Skoog, Douglas A. Principles of Instrumental Analysis; Harcourt Brace: Orlando, 1992.
- Zumdahl, Steven S. Chemistry, 2nd Ed; D.C. Heath and Company: Lexington, MA, 1989.

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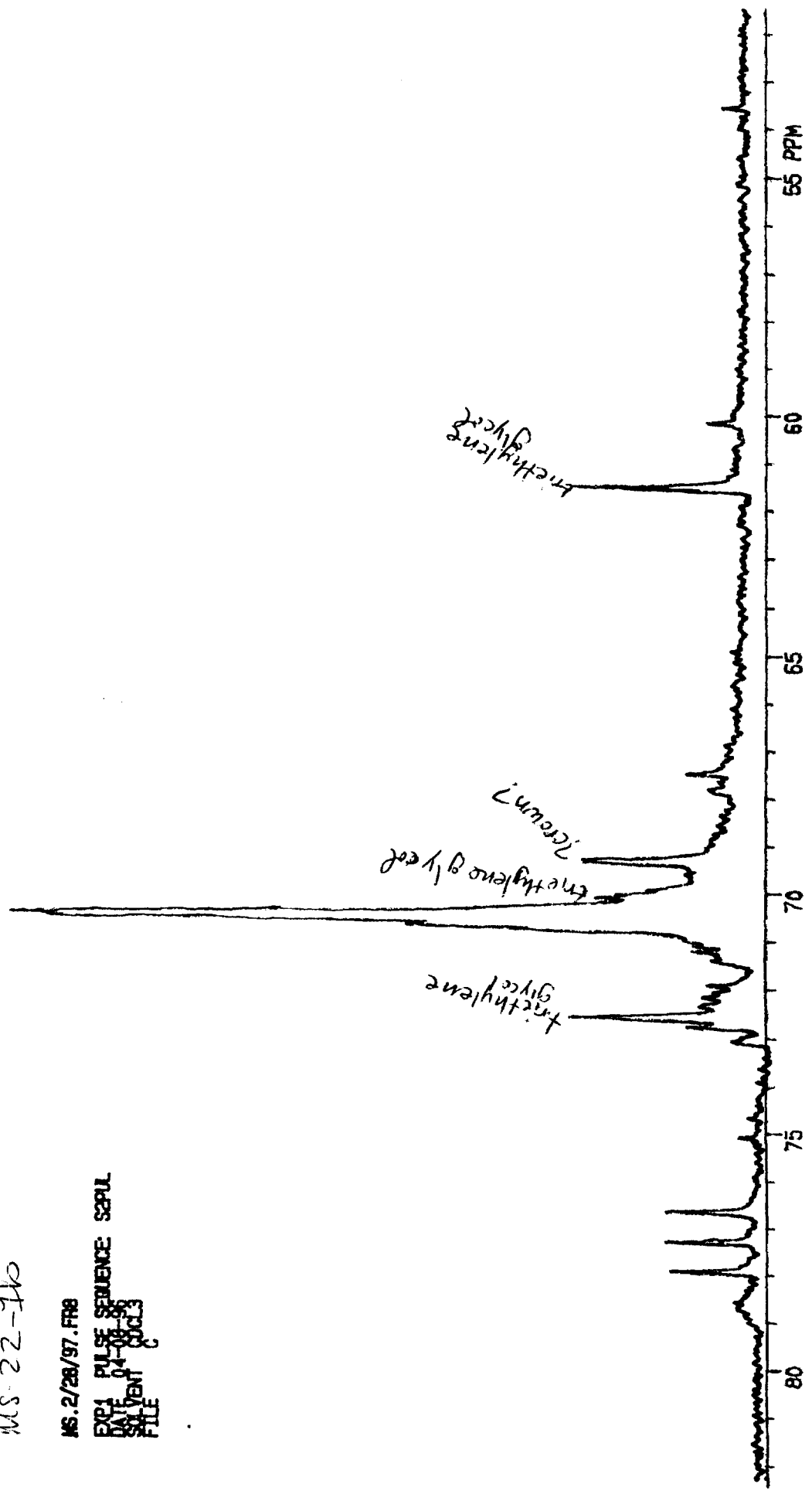
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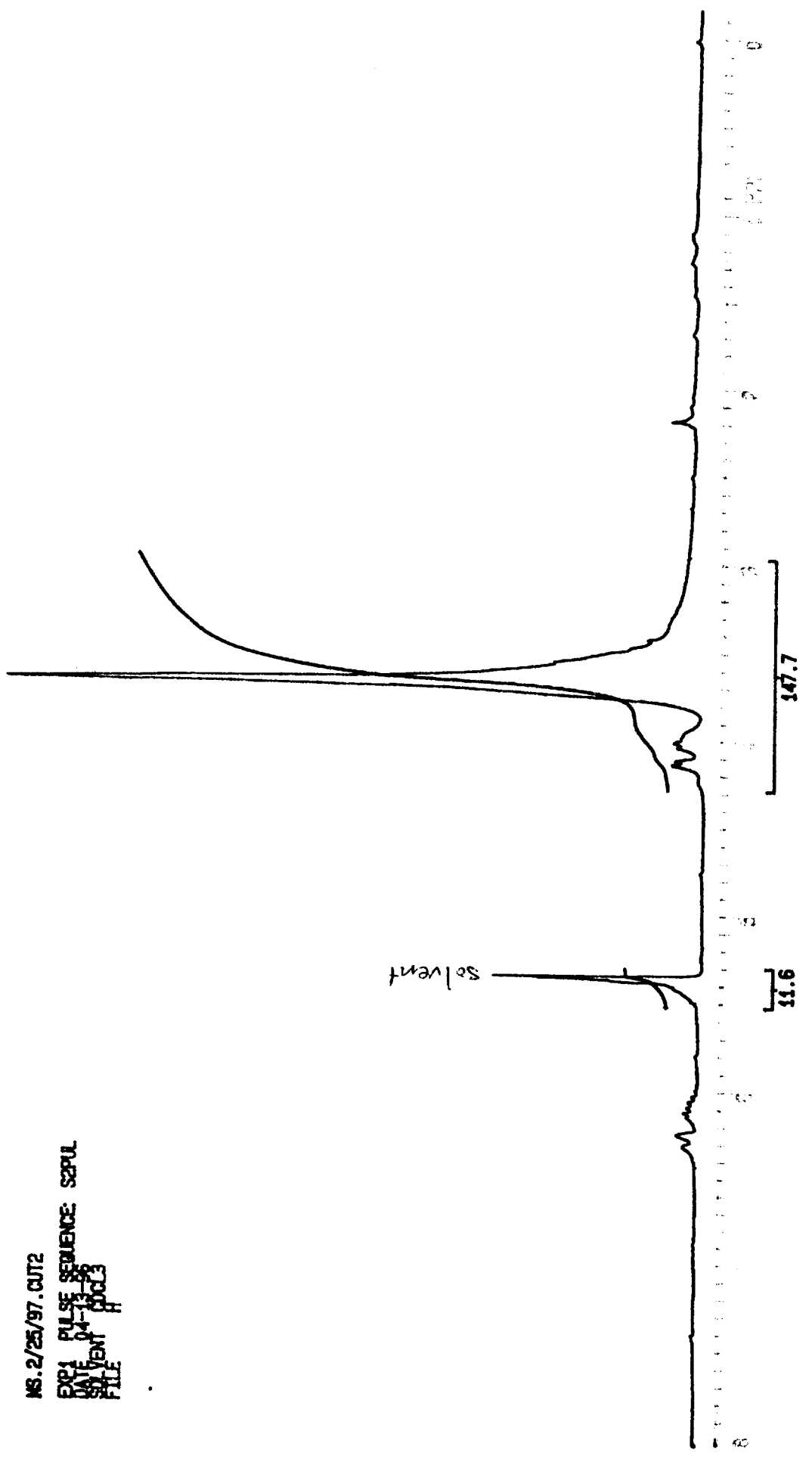
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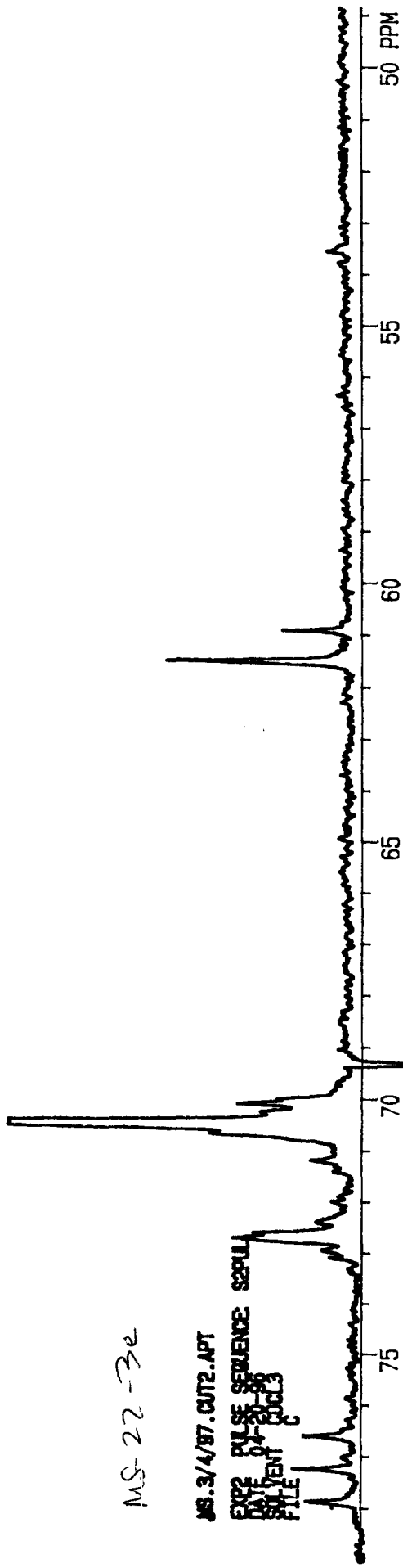
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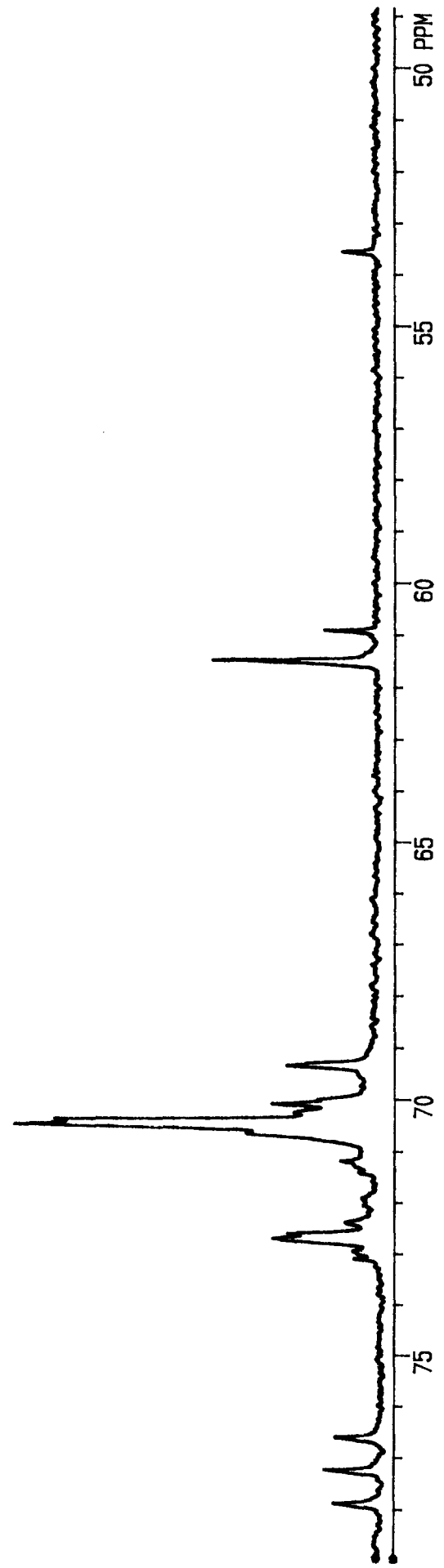
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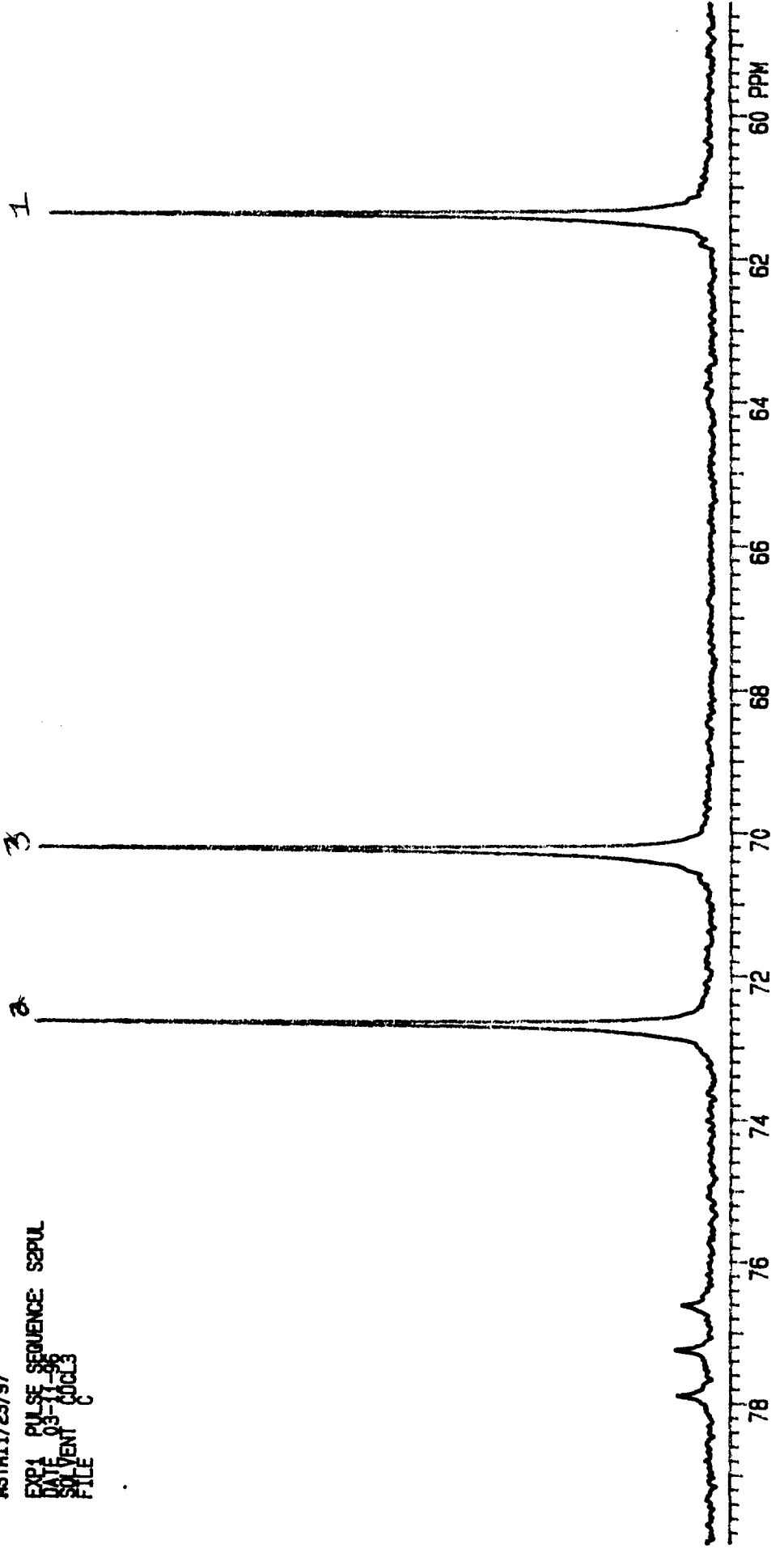
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