Syntheses of Multi-headed, Two-tailed, Anionic Surfactants as Topical Microbicides

By

Sheng Tu

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APPROVED:

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Gandour, Richard D. Advisor

_____ _____

Taylor, Larry T. Committee member Viers, Jimmy W. Committee member

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Department of Chemistry

Virginia Polytechnic Institute and State University

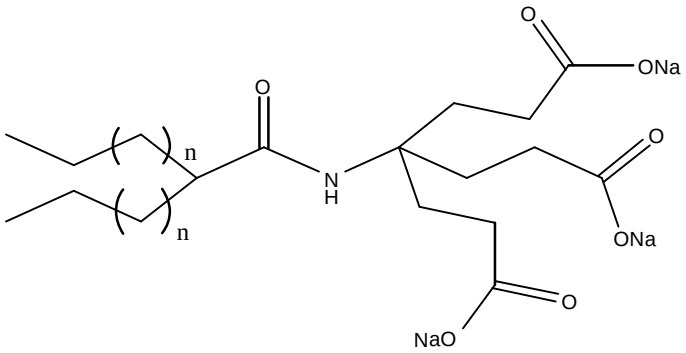
Blacksburg, VA 24061

Syntheses of Multi-headed, Two-tailed, Anionic Surfactants as Topical Microbicides

Sheng Tu

(Abstract)

The purpose of this research was to design and develop a facile synthesis of a series of multi-headed, two-tailed anionic surfactants (**3CAm1(n)$_2$**) as anti-HIV microbicides, and to compare the biological activities of these compounds to the activities of their straight-acyl chain derivatives.



3CAm1(n)$_2$

n=4,5,6,7,8

The synthesis requires coupling reaction of dialkylacetic acid ($R_2CHCOOH$) (R_2= n-C_6H_{13}, n-C_7H_{15}, n-C_8H_{17}, n-C_9H_{19}, n-$C_{10}H_{21}$) and Behera's amine ($H_2NC(CH_2CH_2COOtBu)_3$).

Commercially available diethyl malonate and straight chain alkyl bromide were selected to produce dialkylacetic acid. Sodium methoxide (MeONa) was used as a base to deprotonate the acidic protons of diethyl malonate. The monoalkylmalonate ($RCH(COOEt)_2$) was separated by vacuum distillation and then used as the starting material of the dialkylation step. This modification improved the yields of this reaction by about 10 percent from the routine procedure of introducing both alkyl groups in the same reaction.

The Behera's amine was prepared from the nitrotriester ($O_2NC(CH_2CH_2COOtBu)_3$) by Zn° reduction. The Behera's amine was then converted into an HCl salt by using a diluted HCl solution in 1:1 EtOH/H_2O. By doing so, lactam impurity can be separated by solid-liquid extraction in hexane with sonication. The Behera's amine HCl salt was then separated and converted back into Behera's amine by Et_3N in dry CH_2Cl_2.

Dialkylacetic acid was converted into its sodium salt by using aqueous NaOH solution; the sodium salt was then added to $SOCl_2$ to yield the acyl chloride ($R_2CHCOCl$). The coupling reaction of Behera's amine and acyl chloride was done in dry CH_2Cl_2 with 2.2 eq Et_3N under sonication to give crude ($R_2CHCONHC(CH_2CH_2COOtBu)_3$), which was identified by ^1H NMR.

The crude product from coupling reaction was treated with formic acid. The resulting product was the purified and isolated as a white solid by gravity column chromatography in 100:100:0.5 Hexane/EtOAc/AcOH. Five homologues ($R_2CHCONHC(CH_2CH_2COOH)_3$ R_2= n-C_6H_{13}, n-C_7H_{15}, n-C_8H_{17}, n-C_9H_{19}, n-$C_{10}H_{21}$) were produced by this method; all were fully characterized by ^1H and ^{13}C NMR, IR, and HRMS.

Future improvements can be achieved by replacing the carboxylate groups with the other anionic groups, such as sulfate and phosphate, or add making tri-tailed surfactants, and by, exploring other possible way to improve the biological activities.

Acknowledgments

I would like to thank my research advisor Dr. Ruchard Gandour for his guidance and kind help during the process of completing this work. I would also like to thank Dr. Larry Taylor and Dr. Jimmy Viers for serving on my advisory committee. I want to thank my groupmates Brett L. Kite, André A. Williams, Richard V. Macri, Winny Sugandhi for their help and participation in this research. Here I want to give a special thank to winny for the providing of starting material.

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CHAPTER 1

INTRODUCTION

I.1. RESEARCH OBJECTIVE

The research objective of this project is to synthesize a series of anionic surfactants (Figure 1.1) as potential topical microbicides with potential anti-HIV properties.

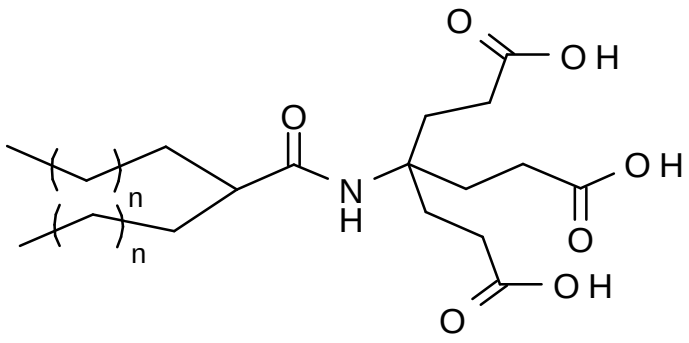


Figure 1.1 Target compounds
n=4,5,6,7,8

I.2 HIV/AIDS EPIDEMIC

Over 39 million people worldwide are living with human immunodeficiency virus (HIV), and over 3.1 million people died from AIDS in 2004.[1] The proportion of women infected with HIV has risen steadily in recent years from 41% in 1995 to 50% in 2002. It is probably the biggest infectious disease crisis modern humanity has ever faced.

I.2.1 Potential Protection Methods

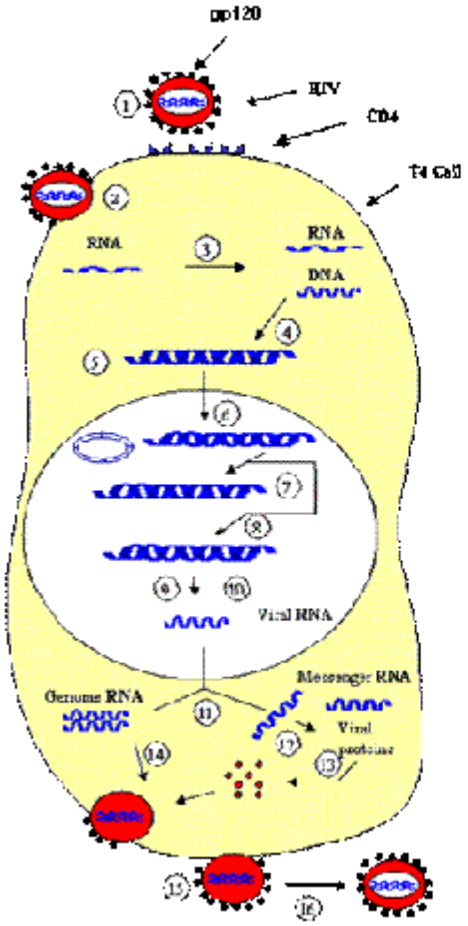
A vaginal topical agent is the primary technique whereby a woman can prevent both pregnancy and microbial infections transmitted by sexual intercourse.[2] To be effective, a vaginal prevention method must establish a barrier between the infectious organisms/spermatozoa and the woman's reproductive tract. Such a barrier might be physical (a female condom or cervical cap), chemical (a vaginal gel or other dosage form), or immunologic (a vaccine). Current technology for preventing transmission of genital herpes infections is limited to the male and female condoms.[3]

These methods have limited user acceptability; furthermore, the male condom does not provide coverage of the entire susceptible surface.

A transmission prevention technology that is being explored with increasing interest is a topical microbicide. Developing user-controlled, topical vaginal microbicides that provide protection against sexually transmitted infections (STIs) is a global priority.[4] These new agents must be safe, effective, acceptable, and affordable.

I.2.2 Infection of HIV

To learn more about strategies to prevent the spread of HIV, a brief review of the mechanism of HIV infection is useful. The HIV replication cycle[5] (Figure 1.2) starts with the attachment of the virus particle to a specific cell-surface receptor. This



1: Attachment
  a: CD4-gp120 Interaction
  b: Gp120-Chemokine
  c: Receptor Interaction
2: Viral Fusion/Uncoating
3: Reverse Transcription
4: RNaseH Degradation
5: Second Strand Synthesis
6: Migration to Nucleus
7: Interation
8: Latency
9: Early Transcription
10: Late Transcription
11: RNA Processing
12: Protein Synthesis
13: Protein Glycosylation
14: Assembly of Virion
15: Viral Budding
16: Virion Maturation

Figure 1.2 HIV life cycle
(Redrawn from figure in ref. 5)

receptor, CD4, will specifically interact with the viral glycoprotein gp120. After a life cycle, the HIV has been replicated.

Virtually all the compounds that are currently used, or under advanced clinical trial, for the treatment of HIV infections, belong to one of the following classes:[6] nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs); non-nucleoside reverse transcriptase inhibitors (NNRTIs); and protease inhibitors (PIs). In addition to the reverse transcriptase (step 3) and protease step (step 14), various other events in the HIV life cycle are potential targets for chemotherapeutic intervention: viral attachment (step 1); Viral entry (step 2); virus-cell fusion (step 3); viral assembly (step 14) and disassembly (step 2,3); proviral DNA integration (step 7); and viral mRNA transcription (step 9, 10). A few of these strategies have been applied to topical microbicides.

I.3 INTRODUCTION TO SURFACTANTS

The name "surfactant" comes from SURFace ACTive AgeNT.[7] Both detergents and soaps are classed as surfactants. A surfactant molecule has a "head" and a "tail". (Figure 1.3) The head is hydrophilic, which means that it is water loving; it is generally depicted as a circle. The tail is generally a long hydrocarbon chain and is hydrophobic, which means water-hating (therefore oil-loving). The tail may be depicted either as a straight line or a wavy tail.

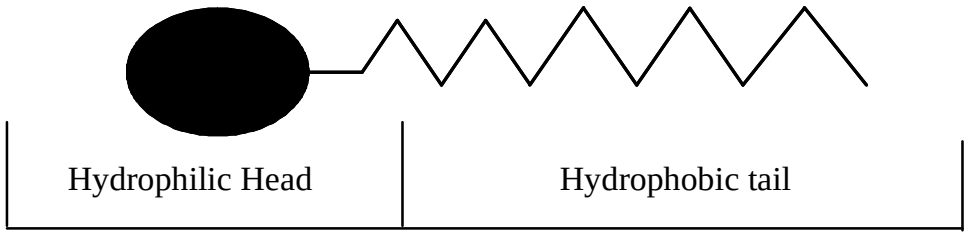
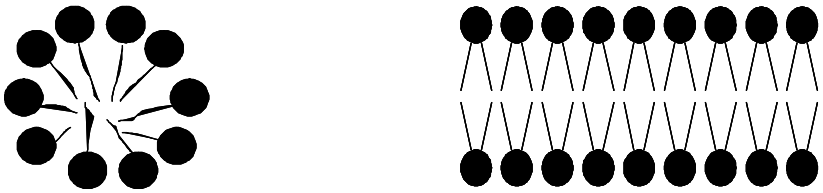


Figure 1.3 Structure of surfactant

Because of their unique structure, surfactants naturally adsorb at interfaces, which are defined as the boundaries between at least two immiscible phases. The adsorption of surfactants at an interface causes a decrease in the surface tension of water. However, when no other surfaces are present the surfactants will form these phases for themselves. The hydrocarbon chain will self assemble to form aggregates. Surfactants can form small aggregates called micelles, or large layer

structures called bilayers. (Figure 1.4) Surfactants with two hydrophobic tails tend to form bilayers.



Micelle Bilayer

Figure 1.4 Surfactants formed aggregates

I.3.1 Effect of Chain Length on the cmc (critical micelle concentration) of Surfactants

The length of the hydrocarbon chain directly affects the solubility and cmc of surfactants. Increases in the hydrocarbon chain length cause a decrease in the solubility of surfactants in aqueous solvents regardless of the head group.[8,9] The cmc decreases logarithmically with increasing hydrocarbon chain length (*m*). (Eqn. 1[9])

$$\text{Log}_{10}\,\text{cmc} = \text{A} - \text{B}m \qquad \text{Eqn. 1}$$

The A and B terms are constants for a homologous series of surfactants at a certain temperature. For a homologous series of the same chain length, A values are approximately constant. However, A values vary according to the number and types of head groups. Values of B are approximately equal to $\log_{10} 2$ for single chain surfactants. Values of B decrease for surfactants with two head groups. Eqn. 1 shows that the chain length, *m*, heavily affects cmc.

Wong et al.[10] made a homologous series of dihydroxylated cationic surfactants ADnX and studied the effect of chain length on the cmc. (Figure 1.5) The result showed nice linear relationship between chain length (n) and Log_{10} cmc.

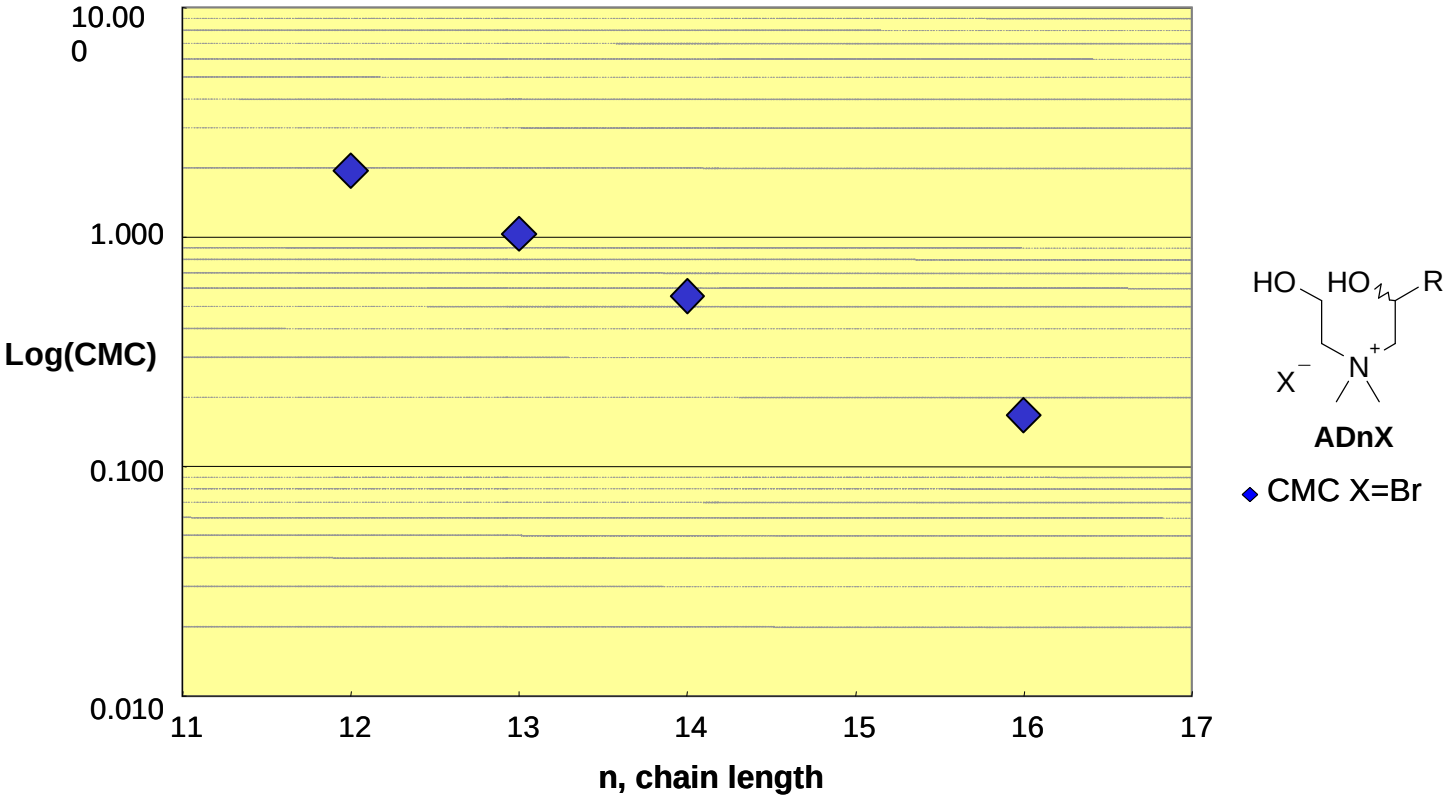
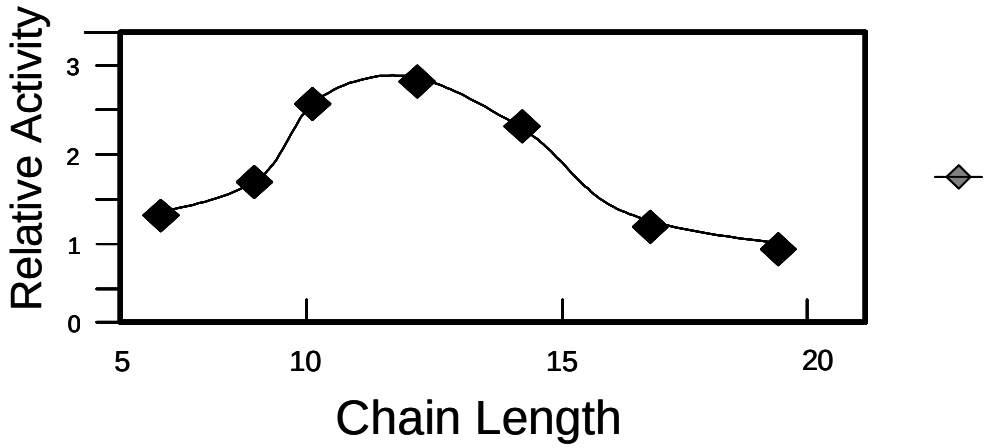


Figure 1.5 Effect of Chain Length on the cmc of ADnX

I.3.2 "Cut-off" effect

Biological activity of surfactants does not show a linear relationship with chain length. There is a phenomenon called the "cut-off effect". (Figure 1.6**)** The antimicrobial properties increase with hydrocarbon chain length. This is because long chains facilitate entry to the cell. On the other hand, the increased hydrocarbon chain tends to form micelles and decreases the solubility of surfactants in aqueous solvent. "Cut-off effect" limits the biological activity of long chain surfactants.[11-13]

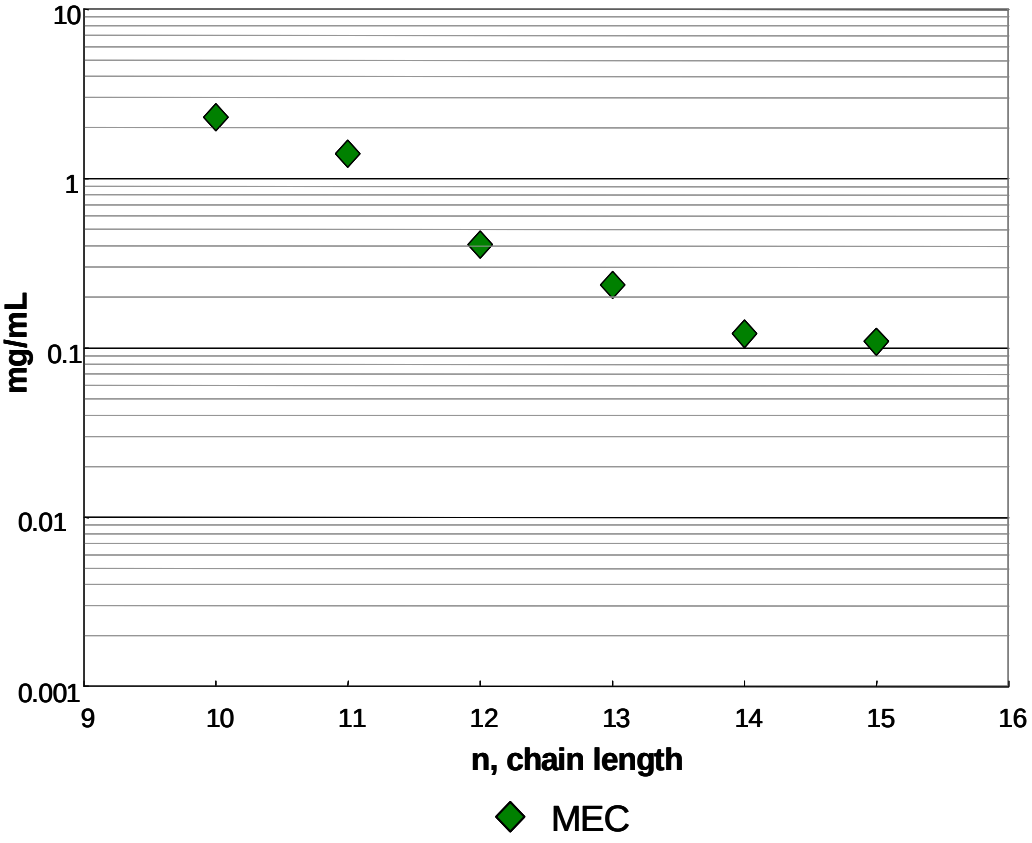
Figure 1.6 "Cut-off" effect

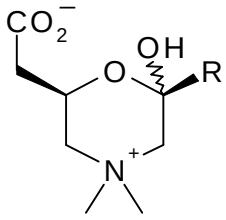


MIC is the minimum inhibition concentration

I.3.3 Chain Length and Biological Activity

Work done by the Gandour group illustrated that increased chain length correlates to increased spermicidal and anti-HIV activity.[10,14] A series of acylcarnitine analogues **Z-n** were synthesized with various chain lengths.[15] These compounds all showed in vitro activities as spermicidal, antifungal, and anti-HIV agent. (Figure 1.7) However, the spermicidal and antifungal activity became sporadic when the chain length was >15. This also correlated to a lower cmc value. The drop in spermicidal activity is attributed to decreased solubility of the longer chain surfactants.





Z-n

n= # of carbons
In alkyl chain

Figure 1.7 Effect of chain length on biological activity of **Z-n** compound
MEC (min. effective concn.) required to immotilize human sperm in 20 s.

I.3.4 Multiple Heads and the Solubility of Surfactants

The solubility of surfactants in water depends on the nature of the head group. Ionic groups do a much better job of making long hydrocarbons chains soluble than nonionic groups.[18] The solubility of different type of surfactants varies with pH (Table 1.1).[16] Hydrogen bonding helps the nonionic surfactants solubilize over a wide pH range. Zwitterionic surfactants have solubility over a wide pH range because they contain both cationic and anionic groups. Ammonium salts solubilize only in acidic solutions. This is because their positive charge comes from the proton. However, quaternary ammonium groups have a permanent positive charge and are soluble over the entire pH range in a variety of solvents. Anionic surfactants are soluble in slightly acidic to basic conditions.

Table 1.1 pH Solubility of Surfactants

Surfactant Type	pH Solubility
Anionic	5-14
Nonionic	3-12
Ammonium Salts	1-8
Quaternary Ammonium Salts	1-14
Zwitterionic	1-6, 8-14

I.3.5 Effect of Multiple Heads on the cmc of Surfactants

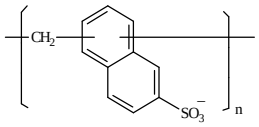
Changes in cmc values for various hydrophilic groups of the same chain length are generally small (Table 1.2).[17] There is a significant effect of the number of head groups on the cmc of surfactants. Shinoda et al. studied the relationship of $\log_{10}$cmc for a series of potassium alkane tricarboxylates as the chain length was increased.[9] When the cmc values of mono-, di-, and tri-carboxylates of equal chain length were compared, the cmc increased with the number of head groups. This meant that increasing the number of head groups made longer chains more soluble in aqueous solutions.

Table 1.2 cmc of surfactants with different headgroup.

Headgroups	CMC (moles/L)
$R_{12}COOK$	0.0125
$R_{12}SO_3K$	0.009
$R_{12}SO_3Na$	0.0081
R_{12}-$N(CH_3)_3Br$	0.016

I.4 POLYANIONS AS ANTI-HIV MICROBICIDES (ATTACHMENT INHIBITORS)

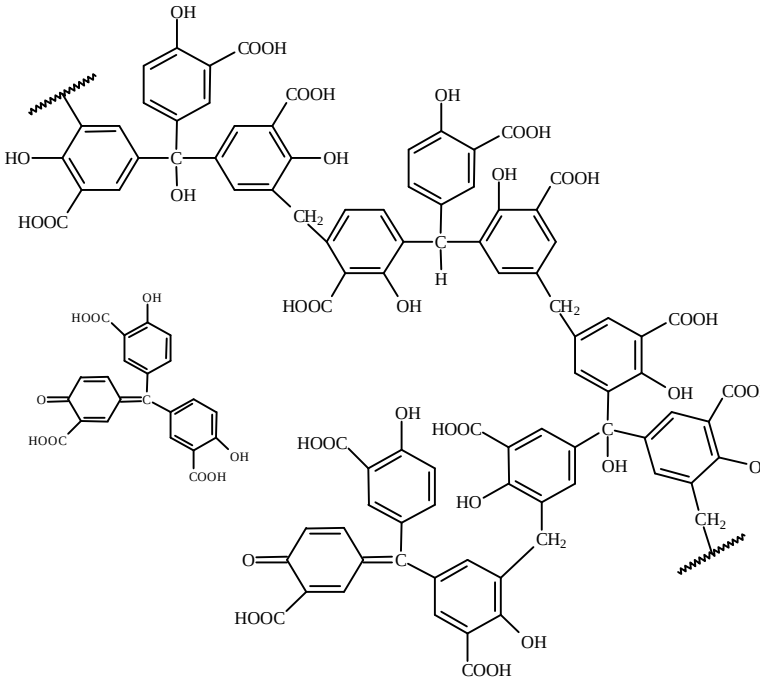
Polyanions can block HIV replication through interference with viral attachment to the cell surface. They may block HIV infection through both virus-to-cell and cell-to-cell contact when applied as a vaginal formulation. These compounds merit study as vaginal topical microbicides. Foremost among the polyanions that have been described as virus-cell binding inhibitors is a naphthalene sulfonate polymer (PRO 2000/5).[18] PRO 2000/5 gel can protect against genital herpes in a mouse model.[19] PRO 2000/5 gel is both safe and well tolerated as demonstrated in a phase 1 clinical trial.[20]



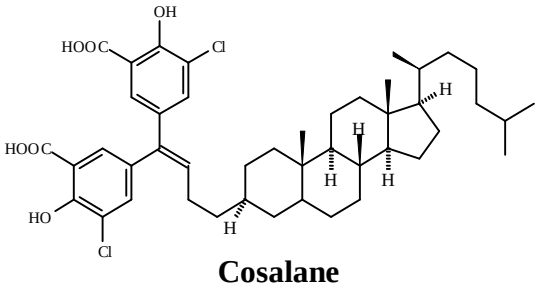
PRO 2000/5 n=21

I.4.1 Cosalane and Analogues

Cosalane was developed from ATA (aurintricarboxylic acid), which has anti-HIV activity.[21] ATA is a heterogeneous mixture of polymers. ATA is a potent inhibitor of cellular processes that depends on the binding of nucleic acids to proteins. ATA has not been developed as a drug because of difficulties in handling purification. ATA is not a single chemical, but a mixture of many different polymers; every batch tends to be different.

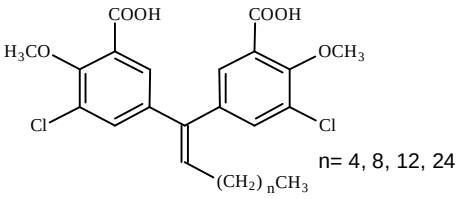


Separation of the low molecular weight molecules gives compounds with the least anti-HIV activity. Attaching a fragment of ATA to a cholestane moiety gives cosalane, which has a relatively low molecular weight but works as well as any of the ATA fractions. Cosalane is a unique anti-HIV agent. It was one of the most potent of the more than 70 ATA monomer analogs that have been prepared.



Cosalane

In order to define the role of the cholestane moiety in cosalane, a series of cosalane analogs were synthesized in which the cholestane ring system was replaced by normal alkenyl substituent having various chain length.[22] The potencies of the alkene congeners correlated positively with chain length and lipophilicity of the alkenes. The results indicated that the cholestane moiety serves as a lipophilic accessory appendage to escort the dichlorodisalicylmethane pharmacophore to a lipid environment. (Figure 1.8)



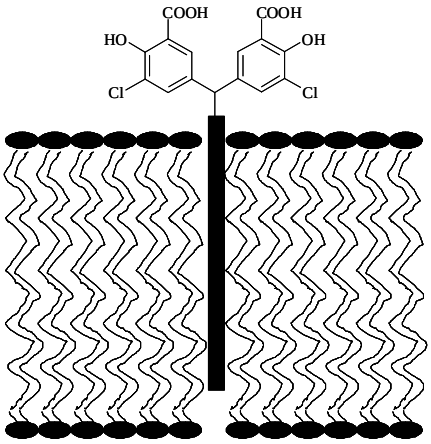


Figure 1.8. The Role of the Cholestanyl Moiety in Cosalane

Cosalane is a multi-headed anionic surfactant, which proved to be active against HIV. Based on the molecule of cosalane, multi-ion head with a hydrophobic tail, we are trying to make a tri-headed anionic surfactant, which is also active against HIV. (Figure 1.9)

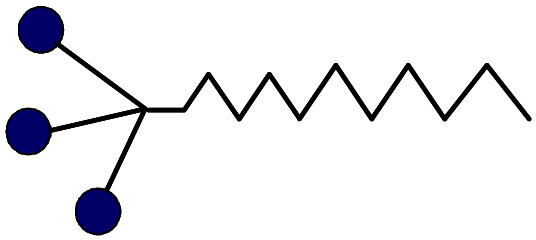
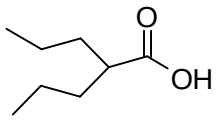


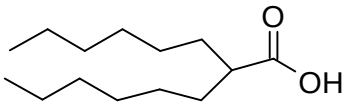
Figure 1.9. Surfactants with three anionic heads

I.5 BRANCHED-ACYL CHAIN COMPOUNDS

Branched-acyl chain compounds have been widely used as anticonvulsant drugs since the 1970s. One example is valproic acid. It is routinely used to treat epilepsy.[23] 2-Hexyloctanoic acid was found to have toxicity and growth-retarding activity against younger larvae of several species of mosquitoes.[24]



Valproic acid



2-Hexyloctanoic acid

A homologous series of Vitamin C derivatives were made and tested as topical prodrugs of ascorbic acid (AA) in a human living skin equivalent model.[25] Branched-acyl derivatives showed much higher stability in neutral solution and solubility to various solvents. (Figure 1.10)

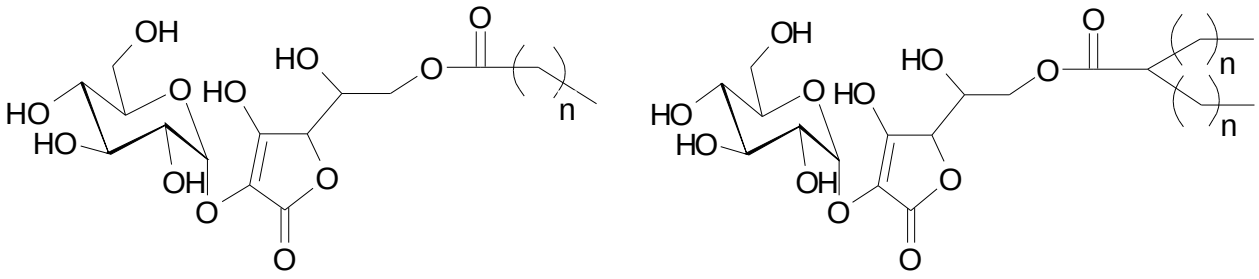


Figure 1.10. Vitamin C derivatives

As we described, multi-ionic head increases the aqueous solubility of the surfactants and increases the cmc. Multi-headed anionic surfactants such as cosalane showed high anti-HIV activity; Branched-acyl compounds showed higher biological activities, good permeability and less irritation in the human living skin equivalent model. We have designed a homologous of di-tailed, multi-headed anionic surfactants. (Figure 1.11) We shall make these surfactants and test their biological activities. We also will compare their biological activities with their straight-chain derivatives with the same number of carbons, which have been made by André Williams in our group.

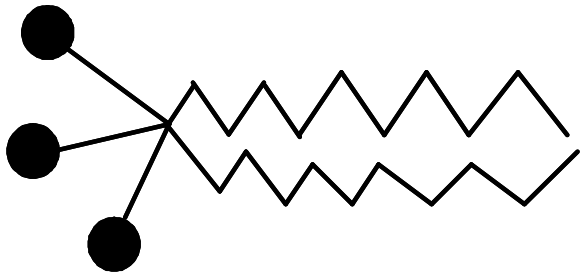
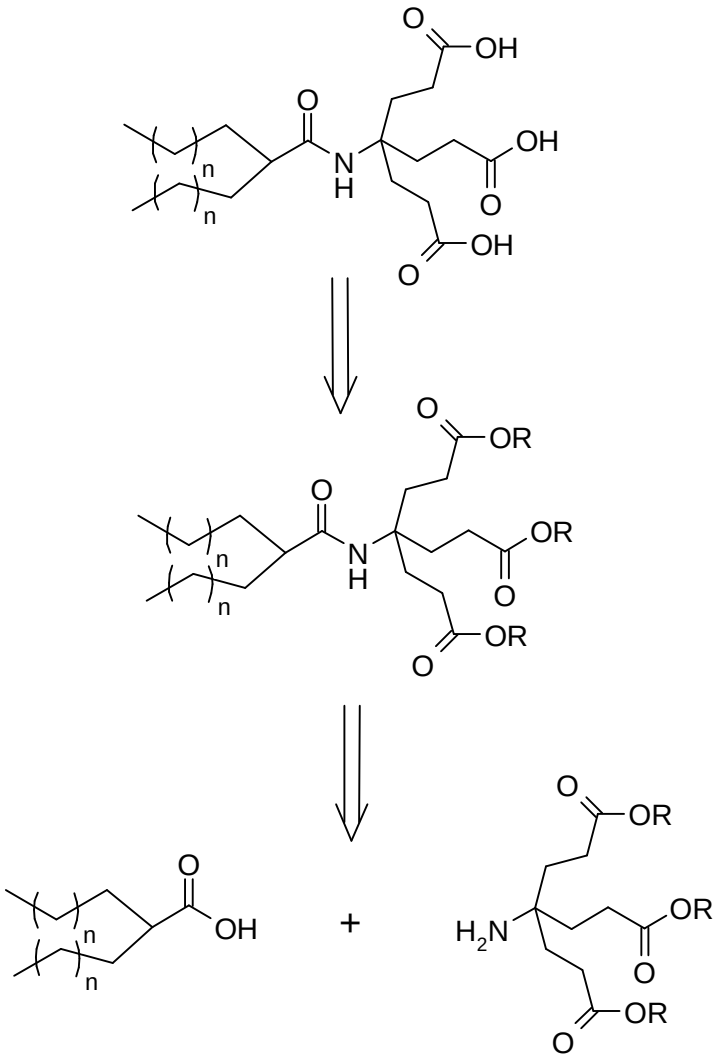


Figure 1.11. Di-tailed, multi-headed anionic surfactant

I.6 PROPOSED SYNTHESIS

The synthesis is rationalized from the disconnection approach shown below (Figure 1.12). The main approach behind this synthesis is to form the amide bond.



Dialkylacetic acid **Behera's amine**

Figure 1.12. Proposed synthesis

The syntheses of the dialkylacetic acid can be accomplished from commercially available malonate ester and straight chain alkyl bromides;(Figure 1.13) Behera's amine, a primary amine attached to a tertiary carbon, can be prepared from its nitro derivatives(Figure 1.14); The coupling reaction of dialkylacetic acid and Behera's amine will be investigated.

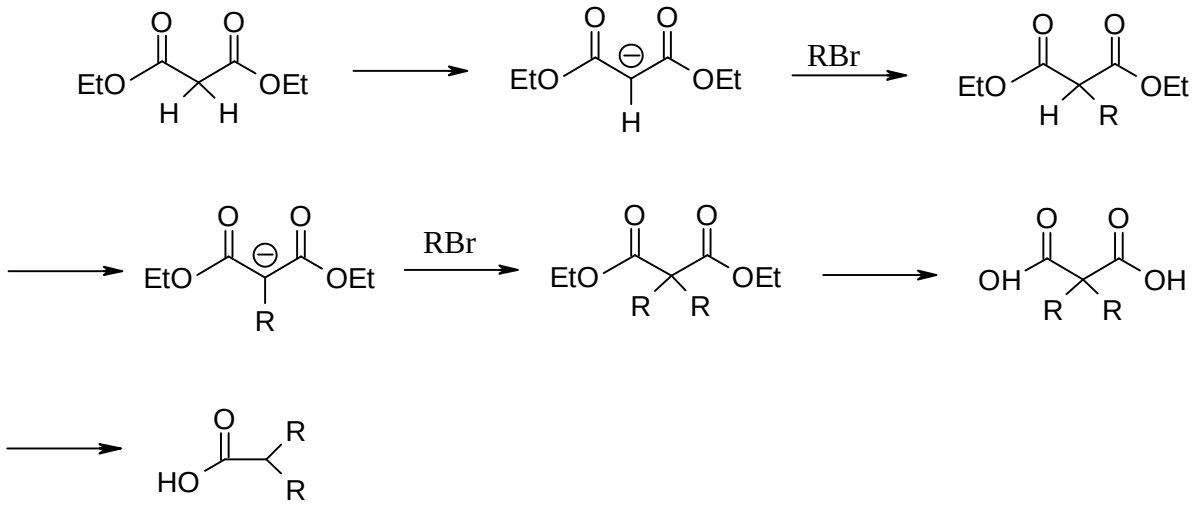


Figure 1.13 Preparation of dialkylacetic acids

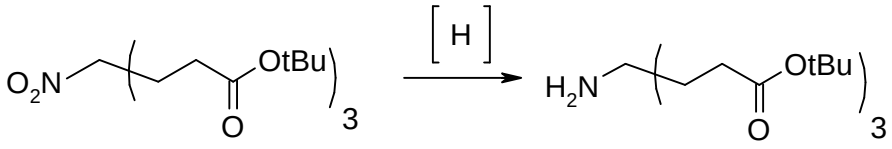


Figure 1.14 Preparation of Behera's amine

I.7 SUMMARY

Topical vaginal microbicides are very important anti-HIV agents. We introduced some of the developing topical microbicides. Then, we introduced properties of surfactants. After that, the anti-HIV activity of polyanionic surfactants and development of cosalane, a polyanion with hydrophobic tail, were discussed. After the exploration, we realized that the role of the long side chain in the cosalane molecule is to target the substance more effectively to the surface of viruses and of cells. The biological properties of branched-acyl chain compounds were then discussed. We found that branched-acyl compounds have high biological activities, good permeability and low irritation in the "human-living-skin-equivalent" model. Based on the above analysis, we designed a homologous series of di-tailed, multi-headed anionic surfactants, which were expected to contain the anti-HIV properties of cosalane but less irritating to human tissue.

In the synthesis part, we proposed a disconnection on these di-tailed, multi-headed anionic surfactants and strategies on making the moieties.

CHAPTER 2
RESULTS AND DISCUSSION

II.1 GENERAL

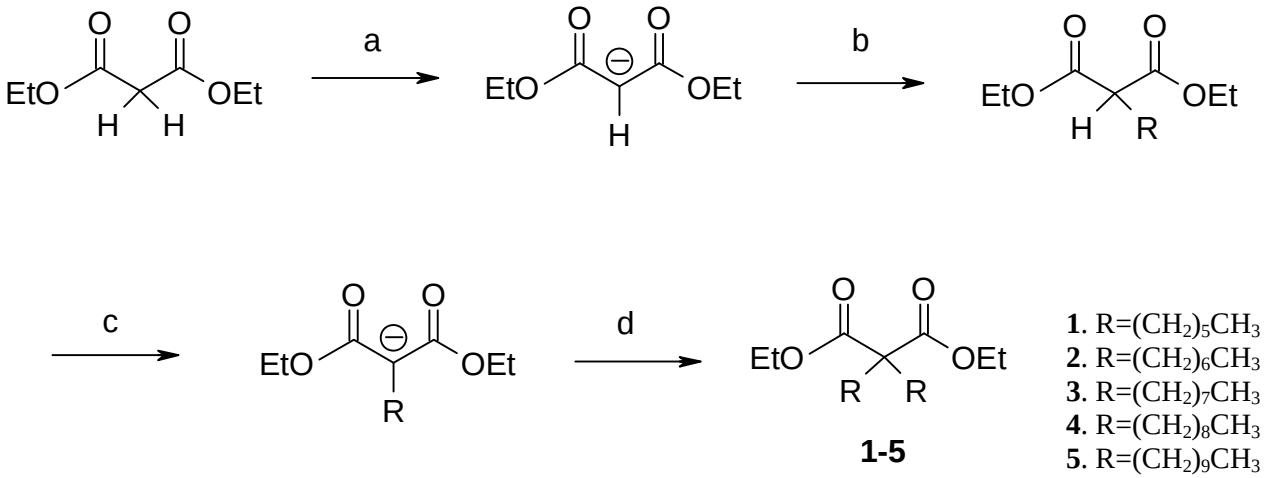
As described in the introduction, dialkylacetic acid was required for the synthesis of the tri-headed, di-tailed surfactants. Attempts on the syntheses of the dialkylacetic acid were made with commercially available malonate ester and straight chain alkyl bromides. The preparation of Behera's amine and the investigation of the coupling reaction of dialkylacetic acid and Behera's amine were also discussed. Sonication was applied as an energy source to the syntheses.

II.2 PREPARATION OF A HOMOLOGOUS SERIES OF SYMMATRICAL DIALKYLACETIC ACID

The syntheses of the dialkylacetic acids were initiated with the commercially available malonate ester and straight chain alkyl bromides with different chain length. This is a four-step synthesis. The first two steps are the adding of two straight chain substituents. The α-proton of malonate ester was removed by a base, which gives the reactive enolate. Then the straight chain alkyl bromides were used to alkylate the malonate ester. After that, acid or base catalyzed hydrolysis of both esters gives the parent dicarboxylic acid, a dialkylmalonic acid. Finally, loss of CO_2 (decarboxylation) gives the dialkyl acetic acid.

II.2.1 Preparation of Symmetrically Dialkylmalonate esters(1–5)

The synthesis of dialkylmalonate esters is described in Scheme 2.1. The α-protons of malonate ester molecule are relatively acidic (pK_a=13).[26] They can be removed under basic environment. This property is used to alkylate malonate esters in organic synthesis. Sodium ethoxide in ethanol was used to deprotonate the acidic protons. Straight-chain alkyl bromides were used to perform the attachment of the first straight alkyl chain. After isolating the alkylmalonate esters, the second alkyl chain substituent was attached by the same method. The yields are given in Table 2.1.

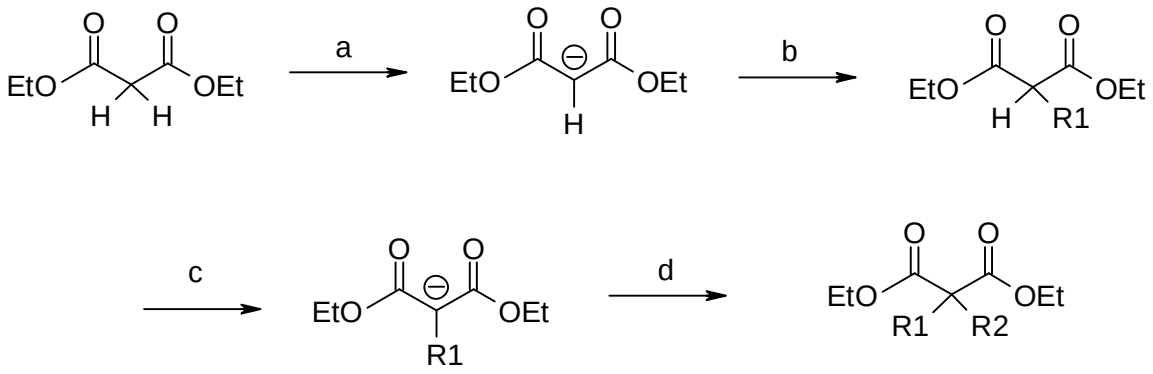


Scheme 2.1 Syntheses of symmetrical dialkylmalonate esters (a) Na^0/Absolute ethanol, 30 min; (b) RBr, reflux 12 h; (c) Na^0/Absolute ethanol, 30 min; (d) RBr, reflux 24 h.

Table 2.1 Isolated yields in the syntheses of symmetrical dialkylmalonate esters

Compound	Step 1, yield (%)	Step 2, yield (%)	Overall yield (%)
1	58.3	61.6	35.9
2	62.8	80.6	50.6
3	58.6	58.5	34.3
4	81.6	73.2	59.7
5	73.3	68.0	49.8

My synthesis closely follows that of Hwang et al.,[24] who reported the synthesis of a series of dialkylmalonate esters. (Scheme 2.2) With a 50–70% yield for each alkylation, this procedure provides a good way to prepare these dialkylmalonate esters.

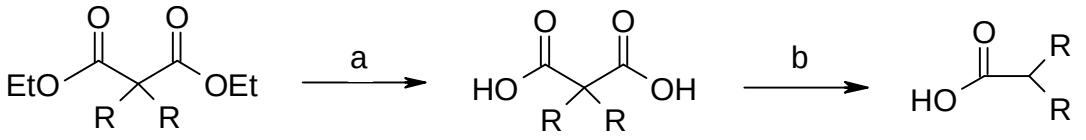


Scheme 2.2 Hwang et al's syntheses of dialkylmalonate esters
(a) Na^0/Absolute ethanol, 30 min; (b) R_1Br, reflux, 5 h;
(c) Na^0/Absolute ethanol, 30 min; (d) R_2Br, reflux, 5 h.
Carbon # of R1: 2, 4, 6
Carbon # of R2: 6, 8, 10, 12, 14, 16, 18, 20

We applied Hwang et al.'s procedure[24] on my synthesis of dioctylmalonate ester (**3**) and got an overall 34.3 % yields for both alkylations. In Hwang et al.'s procedure, the longest chain length of the first substituent is only 6. As we can expect, as the chain length of the first substituent increases, the addition of the second alkyl chain will become increasingly difficult. In my syntheses, (Scheme 2.1) this procedure gives good yields on all five dialkylmalonate esters.(Table 2.1)

II.2.2 Preparation of a homologous series of symmetrical dialkylacetic acid

The preparation of dialkylacetic acid (Scheme 2.3) followed Hwang et al.'s method.[24] To prepare the dialkylacetic acids, the previously made dialkyl malonate esters were hydrolyzed in refluxing 50% aqueous potassium hydroxide solution. The reaction mixture was worked up to give a dialkylmalonic acid. The dialkylmalonic acid was then decarboxylated at 180 °C for 4 hours. Pure dialkylacetic acids as a white solids were isolated in around 80% yield.(Table 2.2) These compounds were characterized by ^{1}H NMR and agreed with reported melting points.[27-29]



6–**10**

6. R=$(CH_2)_5CH_3$
7. R=$(CH_2)_6CH_3$
8. R=$(CH_2)_7CH_3$
9. R=$(CH_2)_8CH_3$
10. R=$(CH_2)_9CH_3$

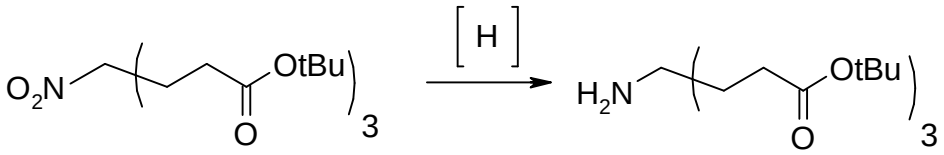
Scheme 2.3 Syntheses of symmetrical dialkylacetic acids (a) 50% KOH/H_2O, Reflux, 8-12 h; (b) 180 °C, 4 h.

Table 2.2 Isolated yields in the syntheses of symmetrical dialkylacetic acids

Compound	Overall yield (%)
6	81.0
7	80.5
8	81.0
9	80.0
10	80.0

II.3. INVESTIGATION OF THE PREPARATION OF BEHERA'S AMINE 12

As we mentioned earlier, the Behera's amine is a primary amine attached to a tertiary carbon. The preparation of Behera's amine was done by reducing the nitrotriester **11** (Scheme 2.4), which was made previously by group member Winny Sugandhi



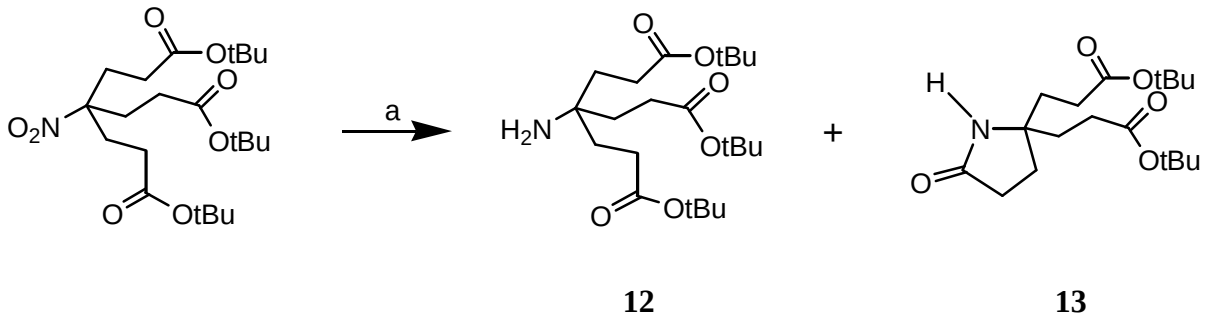
11 **12**

Scheme 2.4 Reduction of nitrotriesters

II.3.1. Preparation of Behera's amine 12 with Raney Ni/ Dihydrogen

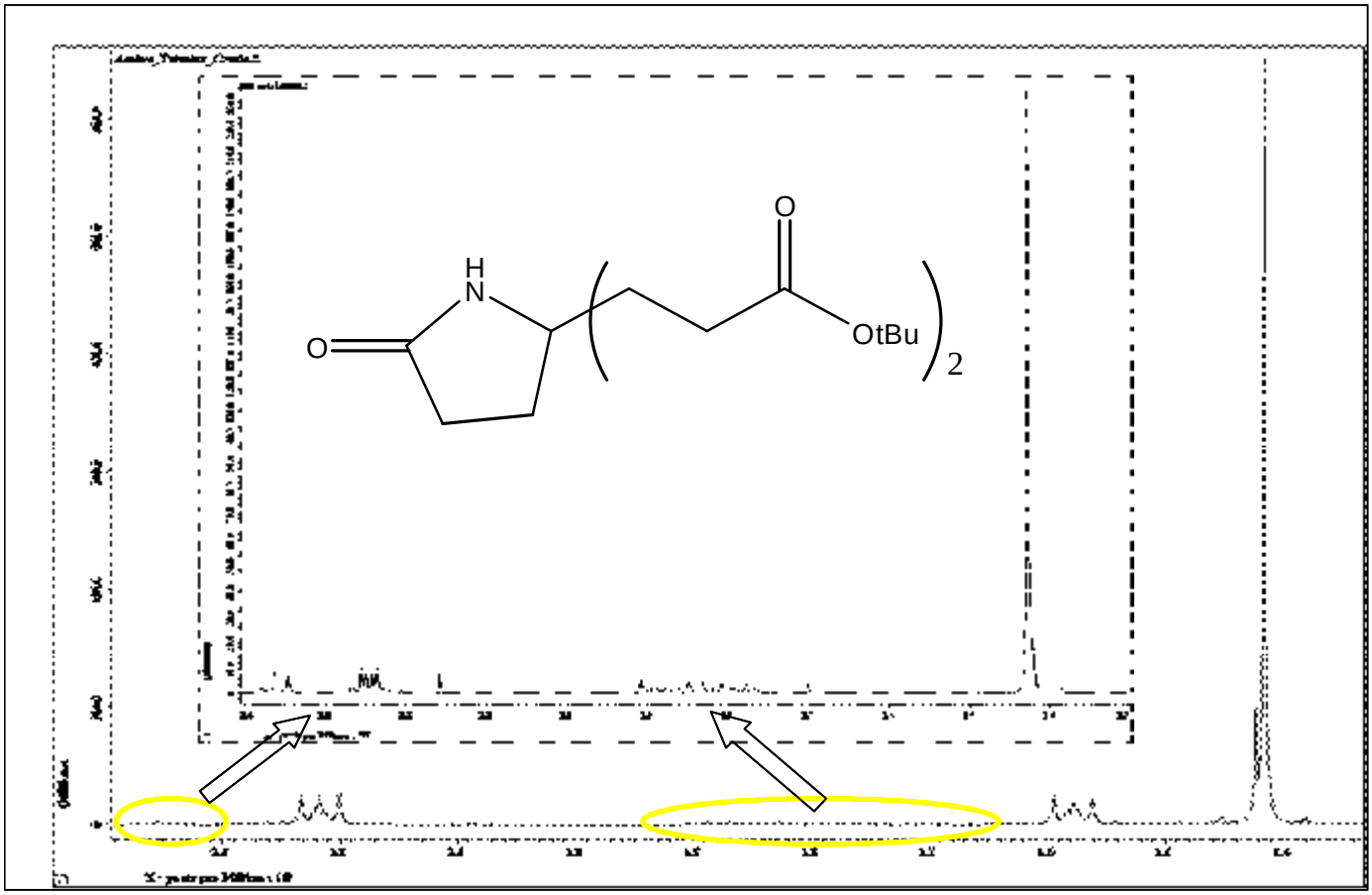
Behera's amine **12** can be prepared from the hydrogenation of **11** with Raney Ni and dihydrogen as described by Newkome et al.[30,31] The nitrotriester **11** is slighlty soluble in the solvent (absolute ethanol) used for hydrogenation. As hydrogenation proceeds, there are two signs for monitoring the completion of the reaction: (a) the depression of the dihydrogen pressure; (b) the disappearance of the solid, because **12** is soluble in absolute ethanol. This procedure normally gives a high yield (~98%).

Even though this procedure provides high yields, there are two problems with it: (a) the size of the hydrogenator limits the scale of this reaction. The largest scale that had been applied in our lab was 20 g of **11**; (b) Behera's amine **12** can cyclize under the above conditions, to afford the 5-membered ring lactam **13**.[30] (Scheme 2.5) Problem (b) can lead to underestimating the purity of Behera's amine.



Scheme 2.5 Lactam **13** formation (a) Raney Ni/H_2, Absolute ethanol, 35 °C, 24 h.

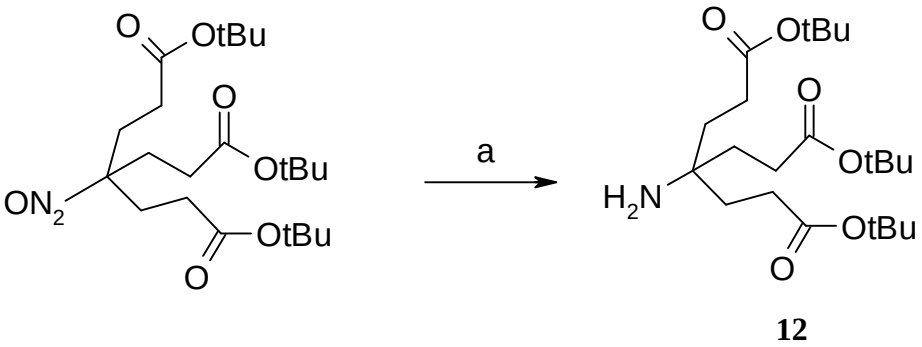
Figure 2.1 ^{1}H NMR spectra of Behera's amine and lactam
(NMR spectra of crude Behera's amine is on the bottom)



The NMR signals of lactam are spread out and relatively small (Figure 2.1). That means even with a large amount of lactam, NMR spectra of Behera's amine will still looks clean. This property makes it hard to estimate the amount of the lactam in the mixture. Due to the very similar physical properties, it is also extremely difficult to remove the lactam from Behera's amine. Consequently, I have spent considerable effort in both developing new procedures for large scale synthesis and purification of Behera's amine.

II.3.2 Preparation of Behera's amine 12 with Zn^0 reduction procedure

Try to solve the scale problem mentioned above, Mr. Brett L. Kite in our group developed a Zn reduction procedure on a relatively small scale.(Scheme 2.6)Zn metal was used as the electron source in this reduction reaction. The complete disappearance of Zn metal is the sign of the reaction completion. I did the scale-up work on this procedure and successfully finished a 40-grams scale reaction in 3 hours. This procedure should be scalable to larger quantities. This procedure solved our scale problem and gives about 98% crude yield but still did not avoid the lactam formation.



12

Scheme 2.6 Preparation of Behera's amine **12** with Zn^0 reduction procedure
(a) Zn^0, 1:2(2eq:4eq) HCl/AcOH added dropwise in ice bath, dry CH_2Cl_2, 4 h.

The adding of the acid mixture has to be performed under low temperature (ice bath). Otherwise, a large amount of lactam can be seen in the ^1H NMR of the crude product. Another possible source of the lactam could be the usage of 4M NaOH solution in the workup. The $ZnCl_2$ formed in the reaction has an unexpected high solubility in organic solvents such as methanol. It is very difficult to separate the $ZnCl_2$ salt from the formed Behera's amine in the reaction mixture. The purpose of using concentrated (4M) NaOH solution is to neutralize the extra acid and convert Zn ion into $Zn(OH)_2$, which is a non-organic or water soluble white solid that can be easily removed by vacuum filtration. To decrease the lactam formation, we tried to lower the concentration of the NaOH solution used in the workup to 1M. After the workup, we got a crude yield higher than 100%, which means that the $ZnCl_2$ salt was not totally removed from the reaction mixture. Because a high concentration of NaOH solution is necessary, we decided to focus on the purification of the crude Behera's amine.

II.3.3. Attempted purification of Behera's amine 12

As mentioned earlier, the purification of Behera's amine **12** contains lactam **13** is extremely difficult with regular methods such as recrystalization. More than five different solvent systems were tried in the recrystalization of crude Behra's amine. Solubility tests of both Behera's amine **12** and lactam **13** were done before choosing the solvent system. Table 2.3 showed the result of the solubility tests.

Table 2.3. Solubility of Behera's amine and Lactam in different solvents (all measured under room temperature)

	Behera's amine	Lactam
Hexane	Slightly soluble	Slightly soluble
Toluene	Soluble	Soluble
Acetonitrile	Soluble	Soluble
Ether	Slightly soluble	Slightly soluble
EtOAc	Soluble	Soluble
MeOH	Soluble	Soluble
THF	Soluble	Soluble
Acetone	Soluble	Soluble
AcOH	Soluble	Soluble

The first solvent system we tried was pure hexane. White crystals precipitated out after the recrystallization. The NMR spectrum showed that the white crystals still contains lactam. The lactam stays with the Behera's amine even after two more recrystallizations. One possible reason is that there is a very strong interaction between the Behera's amine **12** and the lactam **13**, which keeps the lactam with Behera's amine when the Behera's amine crystallize out of the solution.

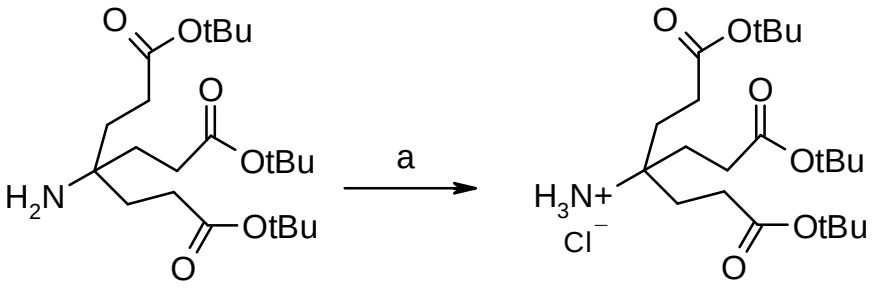
We have also tried to use a good–poor solvent system in the recrystallization. We dissolved the crude Behera's amine **12** in very small amount hot acetic acid and then added cold hexane into the solution dropwise till it turned to cloudy. The cloudy solution was left under room temperature without

disturbing until white crystals formed out. Unfortunately, we got the same result that we obtained in the hexane recrystallization.

More than 5 other different solvent systems were tried based on the solubility test and none of them worked.

II.3.4. Preparation of Behera's amine HCl salt 14

To find a promising purification procedure to remove the lactam, we converted the Behera's amine **12** into its HCl salt **14** (Scheme 2.7). We found out that it is possible to remove the lactam **13** from the Behera's amine HCl salt **14**. Solid–liquid extraction of crude Behera's amine HCl salt in hexane with sonication gives "NMR-clean" **14**. It is because the lactam **13** is soluble in hexane and the Behera's amine HCl salt **14** is not. Because the Behera's amine **12** can be oxidized by the oxygen in the air, the HCl salt of Behera's amine **14** also provides a stable storage form for Behera's amine.



12 **14**

Scheme 2.7 Preparation of Behera's amine HCl salt **14**
(a) Add conc. HCl until pH=1, 1:1 EtOH/H2O.

II.3.5. Attempted preparation of Behera's amine 12 from Behera's amine HCl salt 14 without lactam 13 formation

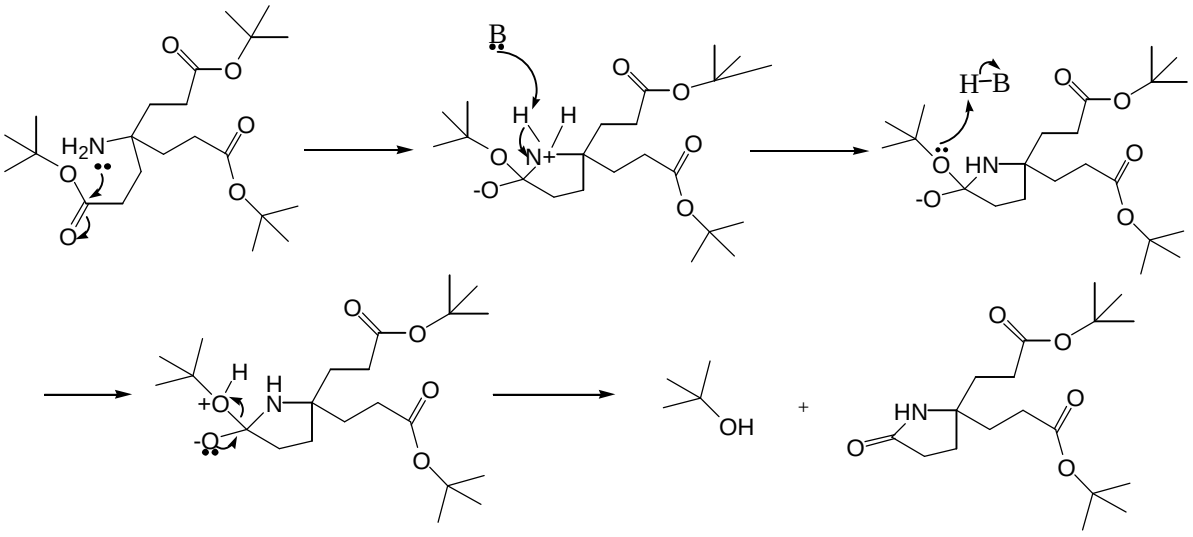
After successfully removing the lactam **13** impurity from Behera's amine HCl salt **14**, the next question is: how can we convert **14** back into the Behera's amine **12** without generating any lactam **13**?

We need a base to remove the proton from Behera's amine HCl salt. We first tried several inorganic bases in different solvents (as shown in Table 2.4.) and none of these attempts worked without forming lactam **13**.

Table 2.4 Recovery of Behera's amine from HCl salt **14** with inorganic bases

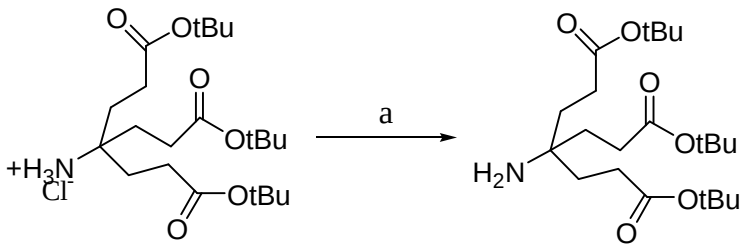
Solvent	Base	Lactam formation
1:1 EtOH/H_2O	Na_2CO_3	Yes
H_2O	$NaHCO_3$	Yes
EtOH	$NaHCO_3$	Yes
EtOAc	NaOH	Yes
EtOAc	Na_2CO_3	Yes

We looked back to the mechanism of the lactam formation (Scheme 2.8) and tried to find the reason. The mechanism showed that the Behera's amine needs one molecule of base B and a proton source to finish the formation of lactam. This means excess amount of base and a protic solvent should be avoided in this reaction.



Scheme 2.8 Mechanism of lactam formation

To avoid any extra base and proton source, we used 1 equivalent of triethylamine in dry methylene chloride. In this case, Behera's amine HCl salt **14** was converted back into pure amine **12** without lactam **13** formation (Scheme 2.9).



14 **12**

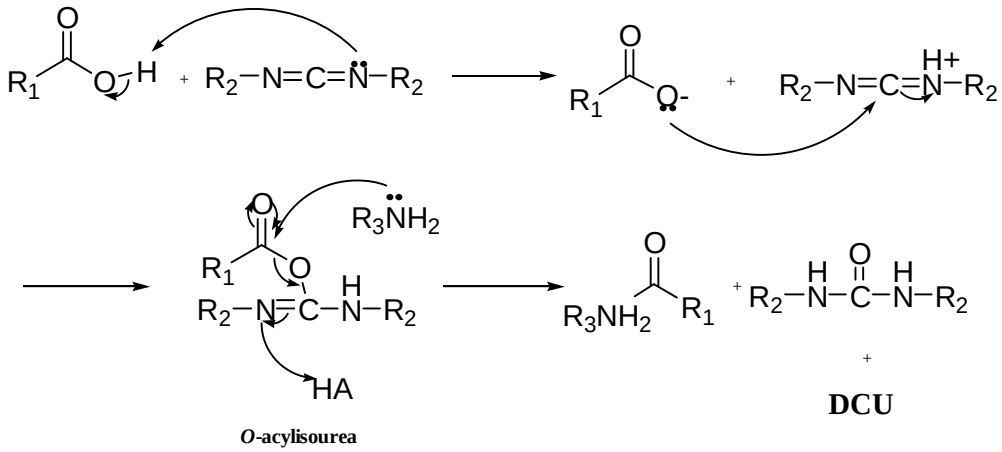
Scheme 2.9 Preparation of Behera's amine **12** from Behera's amine HCl salt **14** without lactam **13** formation
(a) 1eq Et_3N added dropwise, dry CH_2Cl_2.

II.4. INVESTIGATION OF COUPLING REACTION OF DIALKYLACETIC ACID AND BEHERA'S AMINE

After successfully making the dialkylacetic acid **6**–**10** and the Behera's amine **12**, our next goal is to find a high yield, effective coupling reaction to construct the amide bond. DCC is one of the most commonly used coupling reagents in formation of amides and has been used with Behera's amime.[30,32] We picked the DCC coupling as our first trial. We also tried to use acyl chloride in our coupling reactions, which has also been used with Behera's amine,[30,33] and finally got a satisfactory procedure.

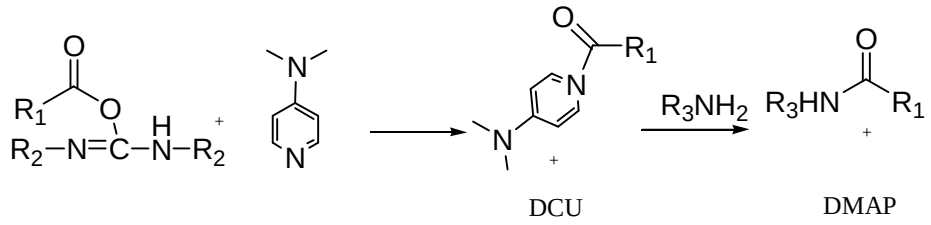
II.4.1. Investigation of DCC coupling of dialkylacetic acid and Behera's amine.

Dicyclohexylcarbodiimide (DCC) has been known and well studied as a reagent for formation of amide bond for a long time. The mechanism starts by a proton transfer, followed by addition of the carboxylate to form the *O*-acylisourea(Scheme 2.10). This is the most reactive species that can attack the amino group to give the corresponding amide. The activity of DCC coupling reaction is dependent on the solvent. If the reaction is carried in solvent such as $CHCl_3$ or CH_2Cl_2, the formation of *O*-acylisourea occurs immediately. If the reaction is carried in a more polar solvent such as DMF, some byproducts formed.



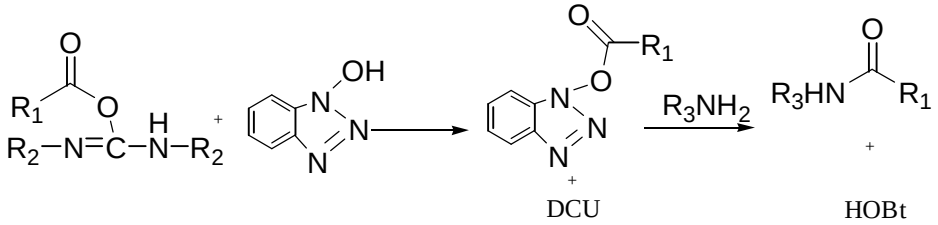
Scheme 2.10 Mechanism of DCC coupling reaction
R_2: cyclohexyl

4-Dimethylaminopyridine (DMAP) is one of most commonly used catalyst for DCC coupling. By forming a more reactive intermediate than *O*-acylisourea, the DMAP helps the reaction go faster(Scheme 2.11).



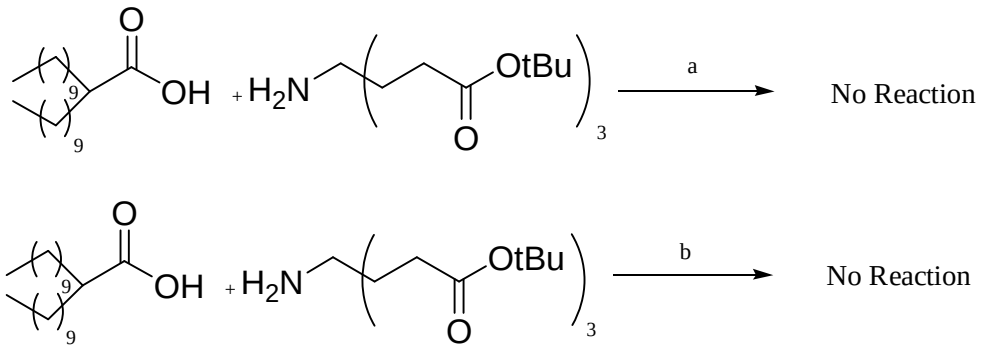
Scheme 2.11 DMAP-catalyzed DCC coupling

1-Hydroxybenzotriazole (HOBt) is another widely used catalyst for DCC coupling. The mechanism of HOBt catalysis is similar to the DMAP. It also forms a more reactive intermediate to help the reaction go faster.



Scheme 2.12 HOBt-catalyzed DCC coupling

We tried DCC coupling reactions with both DMAP and HOBt with dinonylacetic acid **9** in dry methylene chloride (See Figure 2.13). Both DMAP-catalyzed and HOBt-catalyzed DCC coupling did not give the desired product. The statement was made based on the ^{1}H NMR spectrum. Figure 2.2 showed the ^{1}H NMR data of Behera's amine and expected H^1 NMR data of desired amide. Simply by looking at the chemical shift change of three methylene groups 2(triplet), we can tell if the coupling reaction works or not.



Scheme 2.13 DCC coupling reactions with both DMAP and HOBt with dinonylacetic acid **9** (a) DCC, DMAP, dry CH_2Cl_2, rt, 24 h. (b) DCC, HOBt, dry CH_2Cl_2, rt, 3 d.

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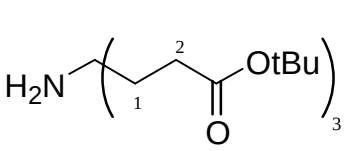
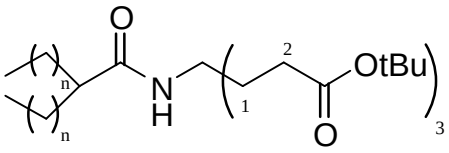




^{1}H NMR	δ (ppm)	^{1}H NMR	δ (ppm)
1	1.62-1.66	1	1.95-1.98
2	2.18-2.21	2	2.18-2.21

Figure 2.2. ^{1}H NMR data of Behera's amine and expected H^1 NMR data (in CD_3Cl) of desired amide

We ran a series of DCC coupling reactions (Table 2.5) in dry CH_2Cl_2 under different conditions to investigate the possible reason of failure. Trial 1 with no catalyst basically had no reaction at all. Trial 2–4 have the same amount of catalyst DMAP but different a reaction time, from 1–3 days. Unfortunately, none of these four DCC coupling reactions gave the desired product

We also tried to change the adding sequence of reagents. Because DCU is not soluble in cold CH_2Cl_2, it will precipitate out and the solution will turn cloudy once it formed. In trial 2, the reaction mixture did not turn cloudy until DCC, DMAP and carboxylic acid were all added. Based on the mechanism of DMAP catalyzed DCC coupling, this means the reactive intermediate formed and DCU was kicked out. We can get the same conclusion from Trial 3 and 4. Apparently, the failure of amine attack is the reason of the failure of whole reaction. One possible explanation is that the Behera's amine, which is a primary amine attached to a tertiary carbon, is too bulky to attack the intermediate and give the corresponding amide.

Table 2.5 DCC coupling reaction in dry CH_2Cl_2 under different conditions

Catalyst	Reaction time	Adding sequence	Observation
N/A	3 days	N/A	Large amount leftover SM
DMAP	1 day	Acid-DCC-DMAP-Amine	Cloudy after added DMAP
DMAP	2 days	DMAP-DCC-Acid-Amine	Cloudy after added acid
DMAP	3 days	Amine-DCC-DMAP-Acid	Cloudy after added acid

II.4.2. Investigation of coupling reaction with acyl chloride

Acyl chlorides are very reactive molecules. They are normally made from the corresponding carboxylic acid. They have been widely used in coupling reactions with amine. This procedure normally gives high yield. The disadvantage of this procedure is the difficulty of purification.

II.4.2.1 Preparation of a homologous series of acyl chloride (15–19)

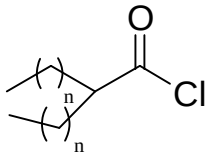
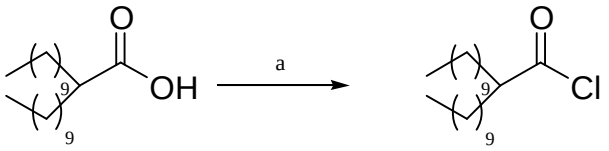


Figure 2.3 A homologous series of acyl chloride **15**–**19**
n= 5,6,7,8,9

The most commonly used procedure for making acyl chloride from carboxylic acid is the thionyl chloride procedure. The biggest disadvantage of this procedure is that the thionyl chloride generates large amount of HCl gas when exposed to air and is hard to handle.



10 **19**

Reaction does not go to completion

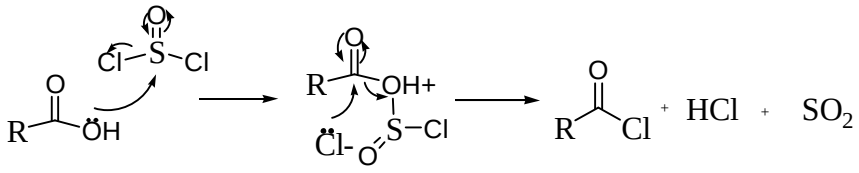
Scheme 2.14 Regular thionyl chloride procedure
(a) $SOCl_2$, 80 °C, 3 h.

We applied the thionyl chloride procedure on the didecylacetic acid **10** and the reaction does not go to completion (Scheme 2.14). This conclusion was made based on the H^1 NMR spectrum of the product. Figure 2.4 showed the H^1 NMR data of the didecylacetic acid and corresponding acyl chloride.

^{1}H NMR	δ(ppm)	^{1}H NMR	δ(ppm)
1	2.29-2.36	1	2.71-2.78

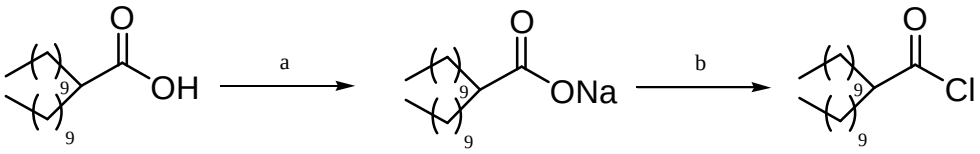
Figure 2.4 ^{1}H NMR data (in CD_3Cl) of didecylacetic acid and corresponding acyl chloride

The proton signal of methine group in the acid appears around 2.3 ppm and the chemical shift changes to around 2.7 ppm after being converted into acyl chloride. We observed both of these two signals in the product with an about 1:1 ratio, which means we were getting a mixture and the reaction did not go to completion.



Scheme 2.15 Mechanism of thionyl chloride reaction

In the mechanism of the thionyl chloride reaction, the carboxylic acid attacks the thionyl chloride as a nucleophile. Because carboxylate is a much better nucleophile than carboxlic acid, we should be able to improve the yield of this reaction by converting carboxylic acid into the carboxylate. Another advantage of using carboxylate is that the formed metal chloride salt will precipitate out of the solution, which can accelerate the reaction. We applied this idea on didecylacetic acid **10** and the reaction went to completion. This conclusion was proved by the full disappearance of the methine proton signal around 2.3 ppm. The optimized procedure requires a shorter reaction time and lower temperature.



10 **19**

Scheme 2.16 Synthesis of acyl chloride using sodium carboxylate
(a) $NaOH/H_2O$, (b) $SOCl_2$, 25 °C, 5 min.

II.4.2.2. Preparation of a homologous series of amides by coupling reaction with acyl chloride and Behera's amine (20-24)

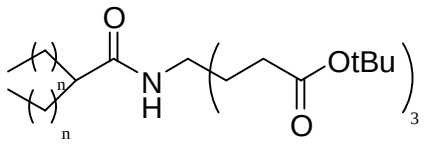
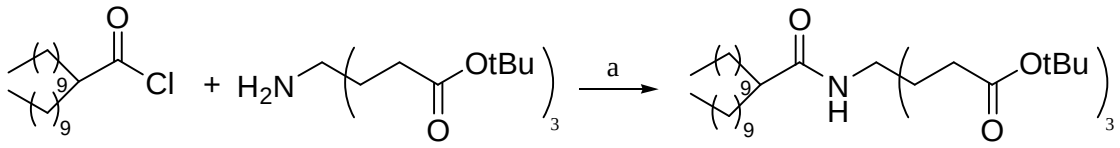


Figure 2.5 A homologous series of amides **20**–**24**
n= 5,6,7,8,9

The crude acyl chloride **15**–**19** was used in the coupling reaction with Behera's amine **12**. As an example, the coupling reaction of **19** and **12** in dry CH_2Cl_2 with sonication was demonstrated below.



19 **24**

Scheme 2.17 Coupling reaction of acyl chloride **19** and Behera's amine **12**
(a) Et_3N, dry CH_2Cl_2, rt, (10min sonication +5 min pulp)×12 , stirring, 24 h.

The formation of the amide was demonstrated by the proton signal of the methylene group appears around 1.92 ppm in H^1 NMR spectra.

II.5. FORMOLYSIS AND THE PURIFICATION OF A HOMOLOGOUS SERIES OF DI-TAILED TRIACIDS (25–29)

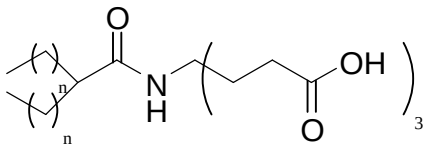
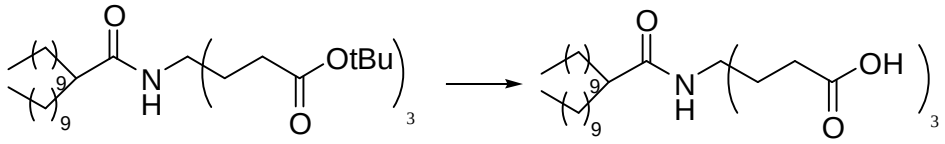


Figure 2.6 A homologous series of di-tailed triacids **25–29**
n= 5,6,7,8,9

After the amide was made, the next step is the removal of the *tert*-butyl group from the heads. Formolysis was used in this step to hydrolyze the *tert*-butyl esters, which gives the di-tailed triacid as the final product. This procedure gives very high crude yields.

II.5.1. Formolysis of Tri-esters



24 **29**

Scheme 2.18 Formolysis reaction
(a) Formic acid, rt, 8 h.

The crude product from the coupling reaction was dissolved in formic acid and stirred for 8 hours under rt. Evaporation of formic acid gives the crude triacids.

II.5.2 Purification of di-tailed triacid

Because the final product has never been made before, we could not find any known purification method for these triacids in a literature search. We tried several different techniques and eventually were able to get clean triacid by using column chromatography.

The first solvent system we tried was pure chloroform. White crystals precipitated out after the recrystallization. The NMR spectrum showed that the white crystal still contains impurity. The impurity stays even after two more recrystallizations.

We then tried hexane-acetic acid solvent system. This solvent system was successfully applied on the recrystallization of mono-tailed triacid. Unfortunately, the di-tailed triacid could not crystallize in this solvent system.

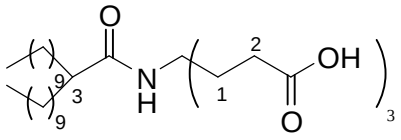
Column chromatography is one of the most fundamental separation techniques in organic chemistry. The disadvantages of this technique are relatively long time, low yield, and the usage of large amount of solvent.

At first, we used 1:1 hexane:EtOAc solvent system on the purification of didecyltriacid. The triacid gives a long tail on the TLC plate under this solvent system. We tried to raise the EtOAc ratio up to 80% (1:4 hexane:EtOAc) and still could not solve this problem. The tailing disappeared after about 0.1% acetic acid was added into 1:1 hexane:EtOAc mixture. By using this new solvent system, we were able to separate pure didecyltriacid with around 80% yield. This solvent system works great on the separation of dinonyltriacid and dioctylacid. We were getting tailing again when applied it to the separation of diheptyl and dihexyltriacid. Better purification methods still need to be discovered for these two tri-acids.

II.6. CHARACTERAZATION OF DI-TAILED TRIACIDS

All di-tailed triacids were fully characterized after isolation. Techniques used in the characterization include ^{1}H and ^{13}C NMR, IR, melting point, and high resolution mass spectrum.

Due to the very low solubility in chloroform, the ^{1}H NMR of triacids was obtained in d_6-DMSO instead of d_3-CH_3Cl. Figure 2.7 shows the data of **29.**



^{1}H NMR	δ (ppm)
1	2.09-2.13
2	1.81-1.84
3	2.15-2.20

Figure 2.7 ^{1}H NMR data (in d_6-DMSO) of didecyltriacid **29**

The other four triacids have similar ^{1}H NMR spectrum. The only distinguish difference is the integration.

The ^{13}C NMR spectra of all five triacids contained more peaks than expected. Figure 2.8 shows the structures of dihexyltriacid **25** and diheptyltriacid **26.** The 10 CH_2 carbons on the two tails of **25** (1a–5a, 1b–5b) appeared as 11 peaks in the ^{13}C NMR. However, the 12 CH_2 carbons on the two tails in the molecule **26** (1a–6a, 1b–6b) appeared 12 peaks. One possibility is that the new added CH_2 carbons 6a and 6b are magnetically equivalent.

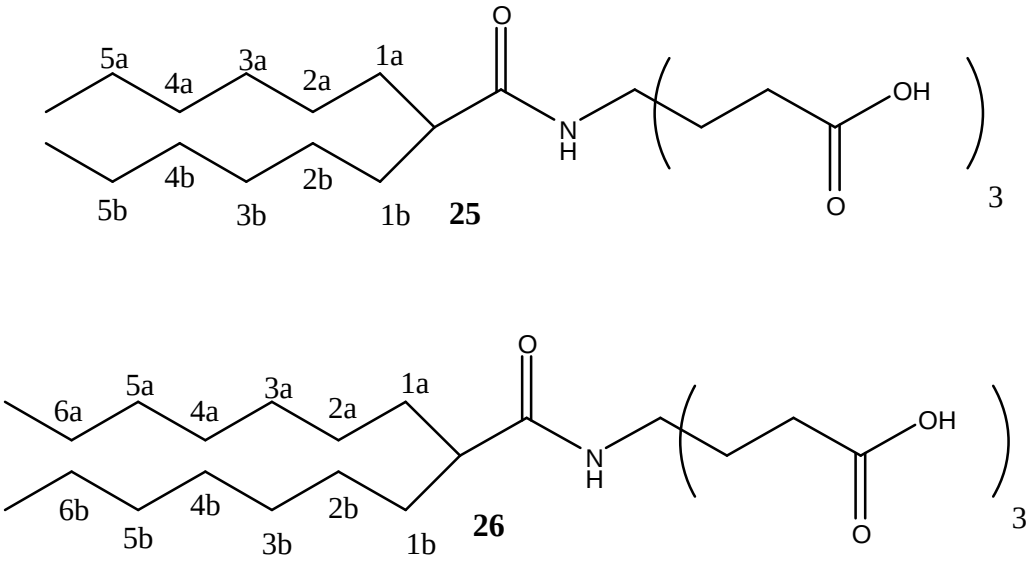


Figure 2.8 Carbon assignments of dihexyltriacid **25** and diheptyltriacid **26**

From the ^{13}C data of all five triacids, we noticed that each time when a pair of CH_2 carbons were added, only one more peak was observed.

The IR spectra of all five triacids contained the C=O stretch for carboxylic acid (1700–1710 cm^{-1}) and amide (1650–1640 cm^{-1})

The melting point showed an interesting tendency: The longer the tail, the lower the melting point (mp (°C): 184.8-185.7(C10); 174.6–175.4(C9); 164.8–165.3(C8); 155.0–155.8(C7); 144.8–145.7(C6)). This phenomenon may due to the packing of the long alkyl chain.

The high resolution mass spectra showed the M+1 ion peak of all five triacids, which proved that these compounds are the compounds that we planned to make.

II.7 CONCLUSION

We successfully synthesized a homologous series of di-tailed triacids ($R_2CHCONHC(CH_2CH_2COOH)_3$ R_2= n-C_6H_{13}, n-C_7H_{15}, n-C_8H_{17}, n-C_9H_{19}, n-$C_{10}H_{21}$). The biggest difficulty we faced in this synthesis was in the coupling reaction of dialkylacetic acid ($R_2CHCOOH$) and Behera's ammine ($H_2NC(CH_2CH_2COOtBu)_3$). DCC, one of the most commonly used coupling reagent, was applied on our starting materials and could not get any desired product. The acyl chloride ($R_2CHCOCl$) procedure is another commonly used method and it works well for our reaction.

We overcome two disadvantages for making the acyl chloride with thionyl chloride ($SOCl_2$): (a) the thionyl chloride is a smoky liquid, it generates large amount of HCl gas, which is very harmful to human body, when it reacts with carboxylic acid is used; (b) the resulting product acyl chloride is hard to purify. We modified the routine acyl chloride method by making the sodium salt of dialkylacetic acid. The modified procedures gave higher yields and allow the reaction undergo under much lower temperature and shorter time, it also reduced the amount of HCl gas by forming NaCl.

The preparation of Behera's amine is another problem we faced during the synthesis. Behera's amine can be prepared from the hydrogenation of nitrotriester with Raney Ni and dihydrogen as described by Newkome. There are two problems with it: (a) the size of the hydrogenator limits the scale of this reaction. The largest scale that had been applied in our lab was 20 g scale; (b) Behera's amine cyclizes under the above conditions, to afford the 5-membered ring lactam. The scale problem was solved the development of a Zn reduction procedure. I did the scale-up work on this procedure and successfully finished a 40-grams scale reaction in 3 hours. This procedure should be scalable to larger quantities. This procedure solved our scale problem and gives about 98% crude yield but still did not avoid the lactam formation.

Try to separate the lactam impurity in the Behera's amine, we converted the Behera's amine into its HCl salt. We found out that it is possible to remove the lactam from the Behera's amine HCl salt. Solid–liquid extraction of crude Behera's amine HCl salt in hexane with sonication gives "NMR-clean" Behera's amine HCl salt. It is because the lactam is soluble in hexane and the Behera's amine HCl salt is not. Because the Behera's amine can be oxidized by the

oxygen in the air, the HCl salt of Behera's amine also provides a stable storage form for Behera's amine.

After the coupling reaction and formolysis, the purification of triacid is the third issue. Flash column chromatograph gives very low yield and takes a very long time and large amount of solvent. A better purification method also needs to be developed.

CHAPTER 3
EXPERIMENTAL

III.1 INSTRUMENTATION

The ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded with a JEOL spectrometer at 500 and 125 Mhz, respectively. Chemical shifts are shown in δ (parts per million, ppm) from the standard tetramethylsilane. The solvent used in the NMR spectroscopy was deuterated chloroform unless otherwise specified. Resonances are reported in the order of the value of δ followed by the number of protons. The abbreviations used are listed as the following: s=singlet; d=doublet; t=triplet; q=quartet; dd=doublet of doublets; dt=doublet of triplets; m=multiplet, b=broad;

Infrared (IR) spectra were obtained with a MIDAC FT-IR spectrometer. The positions of the absorptions are shown with reciprocal centimeter (cm^{-1}). The abbreviations used are listed as the following: s=strong; m=medium; w=weak; br=broad; sh=sharp.

HRMS data were obtained on a dual-sector mass spectrometer in FAB mode with 2-nitrobenzylalcohol as the proton donor. They are shown as m/z (relative height)

Melting points were recorded with a VWR digital melting point apparatus. They are reported in °C. The low temperature melting point was measured as the appearance of the first drop of liquid.

III.2 GENERAL PROCEDURE

Thin layer chromatography (TLC) was performed on silica gel plates made by EMD chemicals, Inc. 1:9 PMA (phosphomolybdic acid) ethanol solution was used as indicator. Flash chromatography for separation and purification was performed with silica gel made by Aldrich. Drying of organic solvent solutions was performed with anhydrous magnesium sulfate unless other specified. Dichloromethane was dried with calcium hydride. Toluene was dried with 4A molecular sieves.

III.3 EXPERIMENTAL PROCEDURES

III.3.1 General Procedure of the preparation of dialkylmalonate diesters (1-5)

1. Sodium metal (3.2 g, 140 mmol) was added into a three-neck-round bottom flask containing absolute ethanol (50 mL) with stirring. Malonate ester (44.8 g, 280 mmol) was added when all the sodium was dissolved. The reaction mixture was heated up and kept under reflux for about 60 min. Alkyl bromide (140 mmol) was then added into the reaction mixture dropwise. The resulting solution was heated under reflux for 5 h. The solution was cooled to room temperature. The cool solution was then concentrated by rotatory evaporation. Enough water was added until two clear layers formed. The resulting solution was extracted with ether (3×50 mL). The collected ether solution was then washed with 2M HCl solution (3×50 mL) and distilled water (3×50 mL). Magnesium sulfate anhydrous was used to dry the resulting solution. The dried solution was then concentrated with a rotatory evaporator to yield light yellow oil. The alkylmalonate diester was vacuumed distilled and collected under around 140 °C as a colorless oil.

2. Sodium metal (1.886 g, 82 mmol) was added into a three-neck-round bottom flask containing absolute ethanol (50 mL) with stirring. Alkylmalonate diester (82 mmol) was added when all the sodium as dissolved. The resulting solution was then heated and kept under reflux for about 60 minutes. Alkyl bromide (68 mmol) was added into the reaction mixture dropwise. The reaction mixture was heated under reflux for 18 hours. The solution was cooled to room temperature after reflux. The cool solution was then concentrated by rotatory evaporation. The residue was extracted with ether (3×50 mL). Filtration separated the white solid from the ether solution. The ether solution was then concentrated by rotatory evaporation to yield light yellow oil. The dialkylmalonate diester was vacuumed distilled in a bulb-to-bulb apparatus and collected at 140 –180 °C as colorless oil.

Diethyl dihexylmalonate (1) Vacuum distilled to give a colorless oil (35.9%): TLC (1:1 Hexane/CH_3Cl) R_f= 0.5; bp: 140 °C, 0.5 torr (lit.[34] bp 136–137 °C, 0.8 torr)

Diethyl diheptylmalonate (2) Vacuum distilled to give a colorless oil (50.6%): TLC (1:1 Hexane/CH_3Cl) R_f= 0.5; bp: 150 °C, 0.5 torr (lit.[28] bp 178–180 °C, 3 torr)

Diethyl dioctylmalonate (3) Vacuum distilled to give a colorless oil (34.3%): TLC (1:1 Hexane/CH_3Cl) R_f= 0.5; bp: 160 °C, 0.5 torr (lit.[28] bp 192–195 °C, 3 torr)

Diethyl dinonylmalonate (4) Vacuum distilled to give a colorless oil (34.3%): TLC (1:1 Hexane/CH_3Cl) R_f= 0.5; bp: 170 °C, 0.5 torr (no lit. data)

Diethyl didecylmalonate (5) Vacuum distilled to give a colorless oil (34.3%): TLC (1:1 Hexane/CH_3Cl) R_f= 0.5; bp: 180 °C, 0.5 torr (lit.[35] bp 196–198 °C, 0.2 torr)

III.3.2 General Procedure of the preparation of dialkylacetic acid (6-10)

Dialkylmalonate diester (50 mmol) was added into a 250 mL reaction flask containing 50:50 wt KOH:H_2O solution (200 mL). The reaction mixture was heated and kept under reflux for 8 hours. Enough water (~ 200 mL) was added to dissolve the white solid formed after the reflux. The resulting solution was then washed with ether (50 mL) once before concentrated HCl was added dropwise until no more solid formed. Ether (3×50 mL) was used to extract the reaction solution and concentrated by rotatory evaporation, which yields yellow solid. The yellow solid was transferred into a 250 mL reaction flask and heated to 180 °C for 3 hours. The cooled down residue was then washed with ether (3×50 mL). The collected ether solution was concentrated to yield light yellow oil. The yellow oil was vacuumed distilled in a bulb-to-bulb apparatus to give colorless oil or a white solid, which solidifies on cooling below rt. The ^{1}H NMR spectrum indicated the presence of impurities, which were removed by washing with acetone (3×25 mL).

2-Hexyloctanoic acid (6) Colorless oil (81%): mp 13.6–14.0 °C; (lit. [27] mp 13 °C); ^{1}H NMR (500 MHz) δ 0.87 (6H, t, *J* = 7 Hz), 1.26 (16H, b), 1.46 (2H, m), 1.61 (2H, m), 2.33 (1H, m); (lit.[24] 60 MHz ^{1}H NMR)

2-Heptylnonanoic acid (7) Colorless oil (80.5%): mp 26.5–27 °C; (lit.[28] mp 26–27 °C); ^{1}H NMR (500 MHz) δ 0.87 (6H, t, *J* = 7 Hz), 1.26 (20H, b), 1.46 (2H, m), 1.61 (2H, m), 2.33 (1H, m); (lit.[24] 60 MHz ^{1}H NMR)

2-Octyldecanoic acid (8) White solid (81.0%): mp 35–35.6 °C; (lit. [28] mp 35–36 °C); ^{1}H NMR (500 MHz) δ 0.87 (6H, t, *J* = 7 Hz), 1.26 (24H, b), 1.46 (2H, m), 1.61 (2H, m), 2.34 (1H, m) (lit.[24] 60 MHz ^{1}H NMR)

2-Nonylundecanoic acid (9) White solid (80.0%): mp 47–47.5 °C; (lit.[29] mp 47–47.5 °C); ^{1}H NMR (500 MHz) δ 0.87 (6H, t, *J* = 7 Hz), 1.26 (28H, b), 1.46 (2H, m), 1.61 (2H, m), 2.33 (1H, m) (lit.[24] 60 MHz ^{1}H NMR);

2-Decyldodecanoic acid (10) White solid (80.0%): mp 54–54.8 °C; (lit.[29] mp 54 °C); ^{1}H NMR (500 MHz) δ 0.87 (6H, t, *J* = 7 Hz), 1.26 (32H, b), 1.46 (2H, m), 1.61 (2H, m), 2.33 (1H, m) (lit.[24] 60 MHz ^{1}H NMR);

III.3.3 Preparation of Behera's amine (12)

III.3.1. Zn reduction

Nitrotriester (20 g, 44.88 mmol) was added into a 250 mL reaction flask in ice bath containing methanol (140 mL). Zn dust (8.8g, 134.6 mmol) was then added into the reaction mixture. HCl (8 mL, 89.76 mmol) and HOAc (16 mL, 179.52 mmol) were mixed together and added slowly into the reaction mixture through a dropping funnel. The ice bath was then removed. The reaction mixture was stirred at rt for 3 hours. The resulting solution was concentrated with a rotatory evaporator. The residue was dissolved in EtOAc(200 mL). 4M NaOH aqueous solution (12.8 g NaOH in 80 mL water) was mixed with the EtOAc solution in a separation funnel. After shaking, the mixture solution was left without disturbing until two layers formed. The top organic layer was collected and dried with magnesium sulfate. The dried solution was then concentrated with rotatory evaporation to yield 18.38 g of a light yellow solid (98% yield).

4-Amino-4-(2-*tert*-butoxycarbonylethyl)-heptanedioic acid di-*tert*-butyl ester (12) ^{1}H NMR (500 MHz) δ 1.42 (9H, s), 1.59 (6H, m), 2.17 (6H, m); (lit. [30] 60 MHz ^{1}H NMR)

III.3.2. Preparation of Behea's amine HCl salt (14)

Crude Behera's amine (5 g) was dissolved in ethanol (100 mL). The solution turned to cloudy and solid precipitated out when water (100 mL) was added. Concentrated HCl (3 mL) was then added into the cloudy solution until all solid dissolved. The resulting solution was extracted with chloroform (2×200 mL). The collected organic solution was dried with magnesium sulfate and then concentrated to give 5.12 g of a white solid. The white solid was put in a 100-mL round bottom flask containing 50 mL hexane. After being sonicated for 30 minutes, the mixture was filtered to give 4.44 g of a white solid.

4-Amino-4-(2-*tert*-butoxycarbonylethyl)-heptanedioic acid di-*tert*-butyl ester HCl salt (14) ^{1}H NMR (500 MHz) δ 1.43 (9H, s), 2.06 (6H, m), 2.49 (6H, m); 11.19 (4H, b);

III.3.3. Preparation of Behera's amine 12 from Behera's amine HCl 14 salt

Behera's amine HCl salt **14** (0.678 g, 1.5 mmol) was added into a 50 mL round bottom flask containing dichloromethane (20 mL) in an ice bath. A dichloromethane (5 mL) solution containing triethylamine(0.152 g, 1.5 mmol) was then added into the flask dropwise over 5 minutes. The resulting solution was left undisturbed in a refrigerator (~ 2 °C) for 10 hours. Following vacuum filtration, 0.828 g of a white solid is isolated.

4-Amino-4-(2-*tert*-butoxycarbonylethyl)-heptanedioic acid di-*tert*-butyl ester (12) ^{1}H NMR (500 MHz) δ 1.42 (9H, s), 1.59 (6H, m), 2.17 (6H, m); (lit. [30] 60 MHz ^{1}H NMR)

III.3.4 General procedure of the preparation of acyl chloride (15–19)

Dialkylacetic acid (7.0 mmol) was added into a 250-mL beaker containing distilled water (50 mL) and NaOH (0.84 g, 21.0 mmol). The resulting water solution was heated to boiling. After the water was gone, the white solid left in the beaker was collected and put in an oven for 10 minutes to remove the water. The dried white solid was then added into a 100-mL reaction flask containing thionyl chloride (50 mL) in small portions over 5 minutes. After stirring for another 30 minutes, the resulting solution was concentrated by rotatory evaporation The residue was

dissolved in dry toluene (50 mL) and filtered. The filtrate was concentrated to yield light yellow solid as the product, which was used without further purification.(~98% crude yield)

2-Hexyloctanoyl chloride (15) ^{1}H NMR (500 MHz) δ 0.88 (6H, t, *J* = 7 Hz), 1.27 (16H, b), 1.53 (2H, m), 1.73 (2H, m), 2.74 (1H, m).

2-Heptylnonanoyl chloride (16) ^{1}H NMR (500 MHz) δ 0.87 (6H, t, *J* = 7 Hz), 1.27 (20H, b), 1.54 (2H, m), 1.72 (2H, m), 2.73 (1H, m).

2-Octyldecanoyl chloride (17) ^{1}H NMR (500 MHz) δ 0.88 (6H, t, *J* = 7 Hz), 1.27 (24H, b), 1.53 (2H, m), 1.73 (2H, m), 2.74 (1H, m).

2-Nonylundecanoyl chloride (18) ^{1}H NMR (500 MHz) δ 0.88 (6H, t, *J* = 7 Hz). 1.26 (28H, b), 1.54 (2H, m), 1.72 (2H, m), 2.74 (1H, m).

2-Decyldodecanoyl chloride (19) ^{1}H NMR (500 MHz) δ 0.88 (6H, t, *J* = 7 Hz), 1.27 (32H, b), 1.53 (2H, m), 1.73 (2H, m), 2.74 (1H, m).

III.3.5 General procedure of preparation of the triestersamides (20–24)

Acyl chloride (2.78 mmol) was added into a 50-mL reaction flask containing dry CH_2Cl_2 (10 mL). Behera's amine (1.155 g, 2.78 mmol) was added in portions. The reaction mixture was then sonicated for 10 min. Triethylamine (0.617 g, 6.12 mmol, 2.2 eq) was then added dropwise. After 12 × (10 min sonication + 5 min rest), the resulting solution was stirred for 24 h. The reaction mixture was filtered and, then, the filtrate washed with diluted HCl solution (50 mL). The organic layer was collected and dried with magnesium sulfate. The resulting solution was filtered and concentrated to yield light yellow solid as the product.

Di-*tert*-butyl-4-(2-*tert*-butoxycarbonylethyl)-4-(2-hexyloctanoylamino)octanedioate (20) ^{1}H NMR (500 MHz) δ 0.89–0.85 (t, 6H, *J*=7 Hz), 1.29–1.21 (b, 16H), 1.43 (s, 9H), 1.98–1.94 (m, 6H), 2.24–2.19 (m, 6H).

Di-*tert*-butyl-4-(2-*tert*-butoxycarbonylethyl)-4-(2-heptylnonanoylamino)heptanedioate (21) ^{1}H NMR δ 0.89–0.85 (t, 6H, *J*=7 Hz), 1.29–1.21 (b, 20H), 1.43 (s, 9H), 1.98–1.94 (m, 6H), 2.24–2.19 (m, 6H).

Di-*tert*-butyl-4-(2-*tert*-butoxycarbonylethyl)-4-(2-octyldecanoylamino)heptanedioate (22) ^{1}H NMR δ 0.89–0.85 (t, 6H, *J*=7 Hz), 1.29–1.21 (b, 24H), 1.43 (s, 9H), 1.98–1.94 (m, 6H), 2.24–2.19 (m, 6H).

Di-*tert*-butyl-4-(2-*tert*-butoxycarbonylethyl)-4-(2-nonylundecanoylamino)heptanedioate (23) ^{1}H NMR δ 0.89–0.85 (t, 6H, *J*=7 Hz), 1.29–1.21 (b, 28H), 1.43 (s, 9H), 1.98–1.94 (m, 6H), 2.24–2.19 (m, 6H).

Di-*tert*-butyl-4-(2-*tert*-butoxycarbonylethyl)-4-(2-decyldodecanoylamino)heptanedioate (24) ^{1}H NMR δ 0.89–0.85 (t, 6H, *J*=7 Hz), 1.29–1.21 (b, 32H), 1.43 (s, 9H), 1.98–1.94 (m, 6H), 2.24–2.19 (m, 6H).

III.3.6 General procedure of the preparation of di-tailed triacids (25–29)

Amide (3 mmol) was added into a 50-mL reaction flask containing formic acid (20 mL). The reaction mixture was stirred for 8 hours under rt and then concentrated to yield crude di-tailed triacids as light yellow solid. The crude product was then chromatographed (SiO_2), eluting with 1:1:0.1 hexane:EtOAc:AcOH to give the di-tailed triacids. (20–80%). The melting points decrease as the chain length increases. This might due to more alkyl chain packing between the triacids with longer alkyl chain.

4-(2-Carboxyethyl)-4-(2-hexyloctanoylamino)heptanedioic acid (25) The general procedure described above afforded a white solid in ~ 10% yield; mp 184.8–185.7 °C; ^{1}H NMR (500 MHz, d_6-DMSO) δ 0.87–0.81 (t, 6H), 1.30–1.12 (b, 16H), 1.43–1.32 (b, 4H), 1.89–1.79 (m, 6H), 2.14–2.08 (m, 6H), 2.21–2.15 (m, 1H).; ^{13}C NMR (125 MHz, d_6-Acetone) δ 13.52, 22.52, 27.62, 27.82, 28.55, 28.70, 28.85, 29.01, 29.17, 29.32, 29.47, 29.50, 31.70, 33.27, 47.27, 57.16, 173.8, 175.1; IR: 1709, 1622, 1554 cm^{-1}. HRMS: calcd for $C_{38}H_{71}NO_7$ calcd 458.3118, found 458.3098.

4-(2-Carboxyethyl)-4-(2-heptylnonanoylamino)heptanedioic acid (26) The general procedure described above afforded a white solid in ~ 10% yield; mp 174.6–175.4 °C; ^{1}H NMR (500 MHz, d_6-DMSO) δ 0.87–0.81 (t, 6H), 1.30–1.12 (b, 20H), 1.43–1.32 (b, 4H), 1.89–1.79 (m, 6H), 2.14–2.08 (m, 6H), 2.21–2.15 (m, 1H); ^{13}C NMR (in d_6-Acetone) δ 13.54, 22.5, 27.68, 27.83, 28.56, 28.71, 28.87, 29.02, 29.17, 29.33, 29.48, 29.52, 29.62, 31.79, 33.26, 47.27, 57.18, 173.9, 175.2; IR: 1708, 1622, 1554 cm^{-1}. HRMS: calcd for $C_{26}H_{47}NO_7$ 486.3431, found 486.3445.

4-(2-Carboxyethyl)-4-(2octyl-decanoylamino)heptanedioic acid (27) The general procedure described above afforded a white solid in ~ 40% yield; mp 164.8–165.3 °C; ^{1}H NMR (500 MHz, d_6-DMSO) 0.87–0.81 (t, 6H), 1.30–1.12 (b, 24H), 1.43–1.32 (b, 4H), 1.89–1.79 (m, 6H), 2.14–2.08 (m, 6H), 2.21–2.15 (b, 1H); ^{13}C NMR (in d_6-Acetone) δ 13.56, 22.50, 27.68, 27.85, 28.56, 28.87, 29.03, 29.18, 29.25, 29.33, 29.47, 29.53, 29.68, 31.82, 33.25, 47.26, 57.19, 173.9, 175.2; IR: 1708, 1623, 1554 cm^{-1}. HRMS: for $C_{28}H_{51}NO_7$ calcd 514.3744, found 514.3761.

4-(2-Carboxyethyl)-4-(2-nonylundecanoylamino)heptanedioic acid (28) The general procedure described above afforded a white solid in ~ %40 yield; mp 155.0–155.8 °C; ^{1}H NMR (500 MHz, d_6-DMSO) 0.87–0.81 (t, 6H), 1.30–1.12 (b, 28H), 1.43–1.32 (b, 4H), 1.89–1.79 (m, 6H), 2.14–2.08 (m, 6H), 2.21–2.15 (m, 1H); ^{13}C NMR (in d_6-Acetone) δ 13.55, 22.50, 27.68, 27.85, 28.57, 28.72, 28.87, 29.03, 29.19, 29.26, 29.34, 29.52, 29.63, 29.68, 31.81, 33.24, 47.26, 57.21, 174.0, 175.3; IR: 1710, 1624, 1560 cm^{-1}. HRMS: for $C_{30}H_{55}NO_7$ calcd 542.4057, found 542.4055.

4-(2-Carboxyethyl)-4-(2-decyldodecanoylamino)heptanedioic acid (29) The general procedure described above afforded a white solid in ~ 60%; mp 144.8–145.7 °C; ^{1}H NMR (500 MHz, d_6-DMSO) 0.87–0.81 (t, 6H), 1.30–1.12 (b, 32H), 1.43–1.32 (b, 4H), 1.89–1.79 (m, 6H), 2.14–2.08 (m, 6H), 2.21–2.15 (m, 1H); ^{13}C NMR (in d_6-Acetone) δ 13.55, 22.51, 27.67, 27.85, 28.57, 28.72, 28.87, 29.02, 29.18, 29.26, 29.34, 29.51, 29.55, 29.59, 29.67, 31.84, 33.23, 47.24, 57.19, 173.9, 175.3; IR: 1703, 1645, 1536 cm^{-1}. HRMS: for $C_{32}H_{59}NO_7$ calcd 570.4370, found 570.4374.

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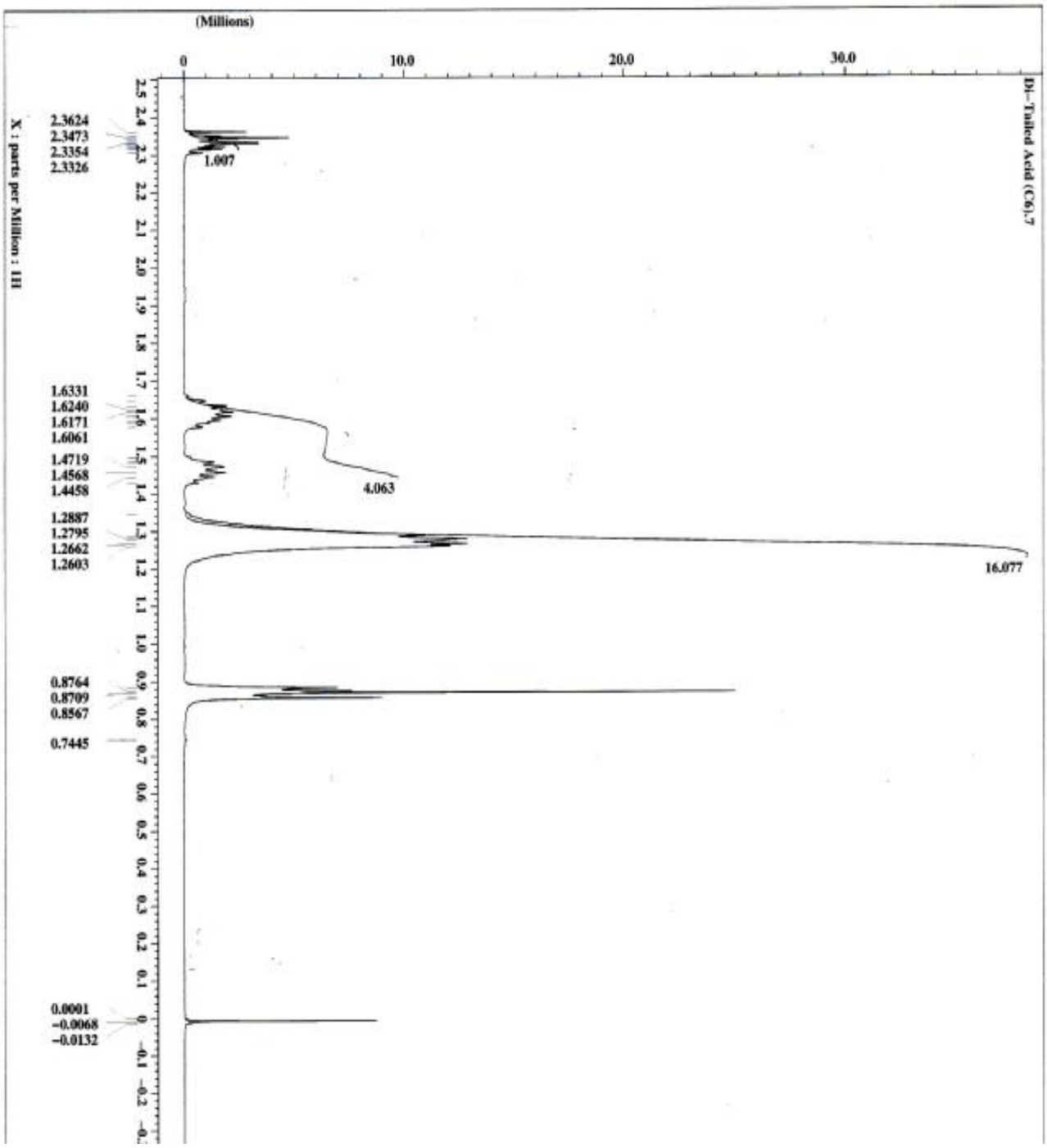
Appendix A

NMR Spectrum

^{1}H Spectrum

^{13}C Spectrum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2.3326
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1.6331
1.6240
1.6171
1.6061
1.4719
1.4568
1.4458
4.063
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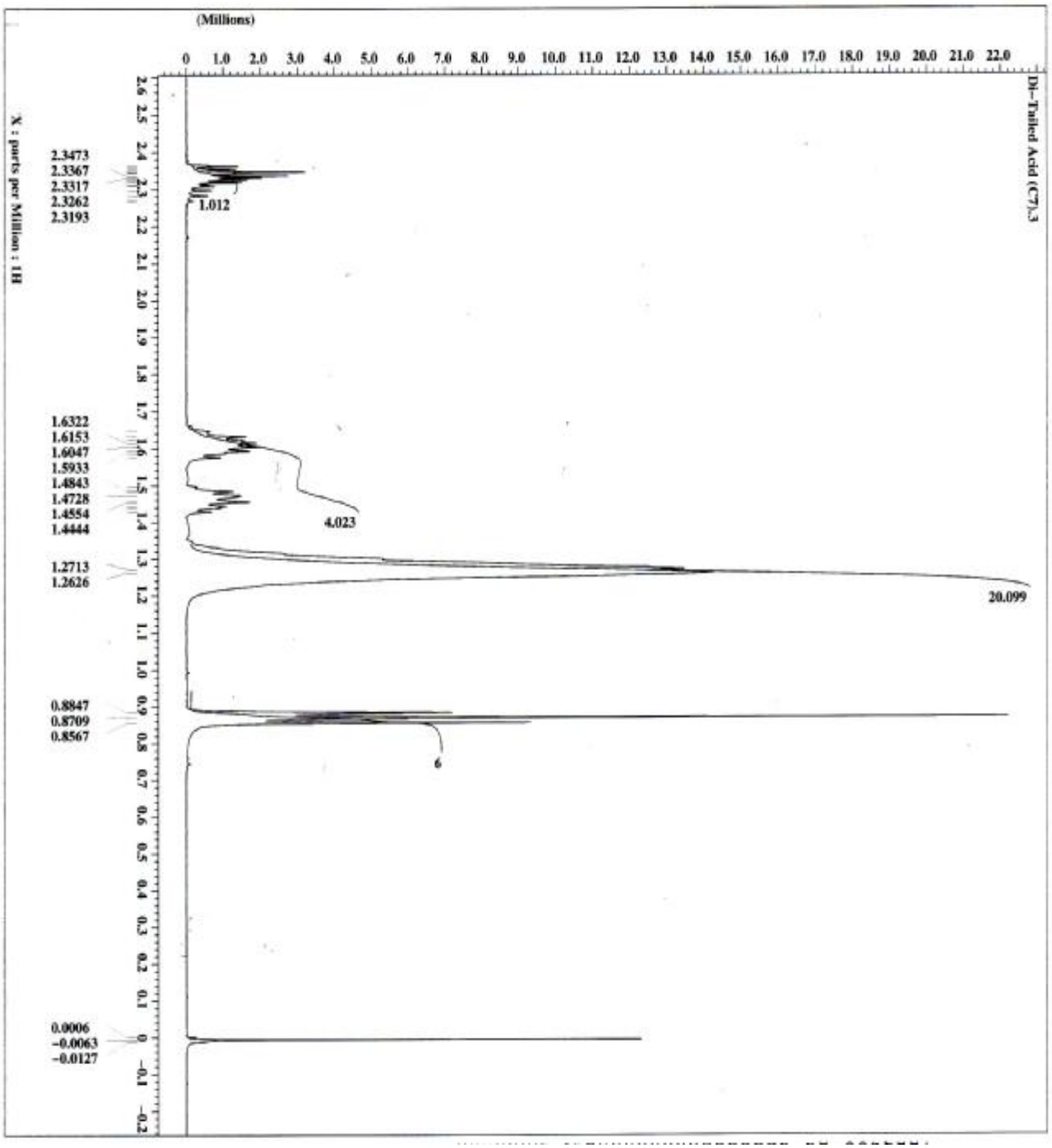
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Di-Tailed Acid (C7).3
X : parts per Million : 1H
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2.3367
2.3317
2.3262
2.3193
1.012
1.6322
1.6153
1.6047
1.5933
1.4843
1.4728
1.4554
1.4444
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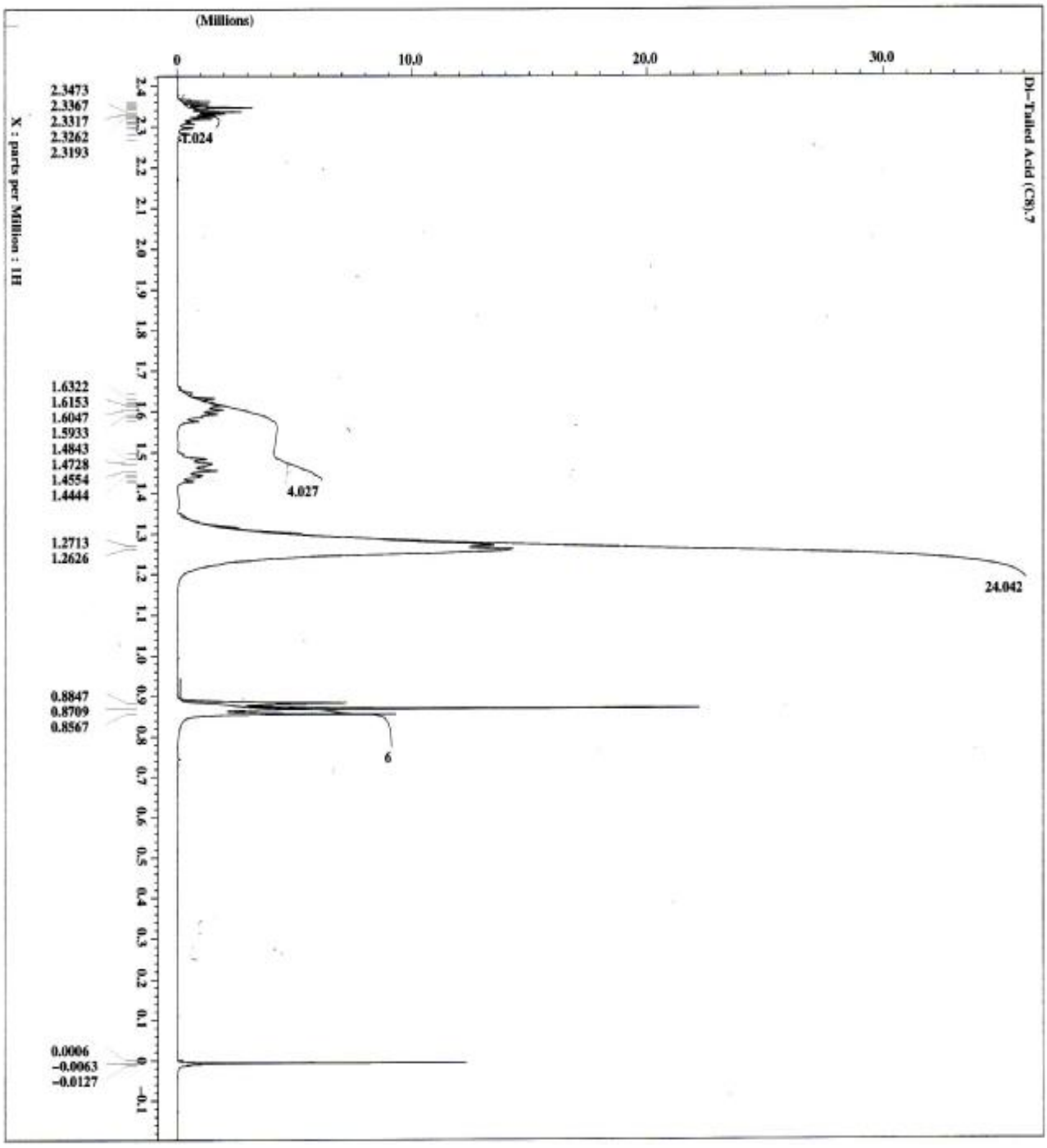
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2.3317
2.3262
2.3193
1.6322
1.6153
1.6047
1.5933
1.4843
1.4728
1.4554
1.4444
1.2713
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0.8709
0.8567
0.0006
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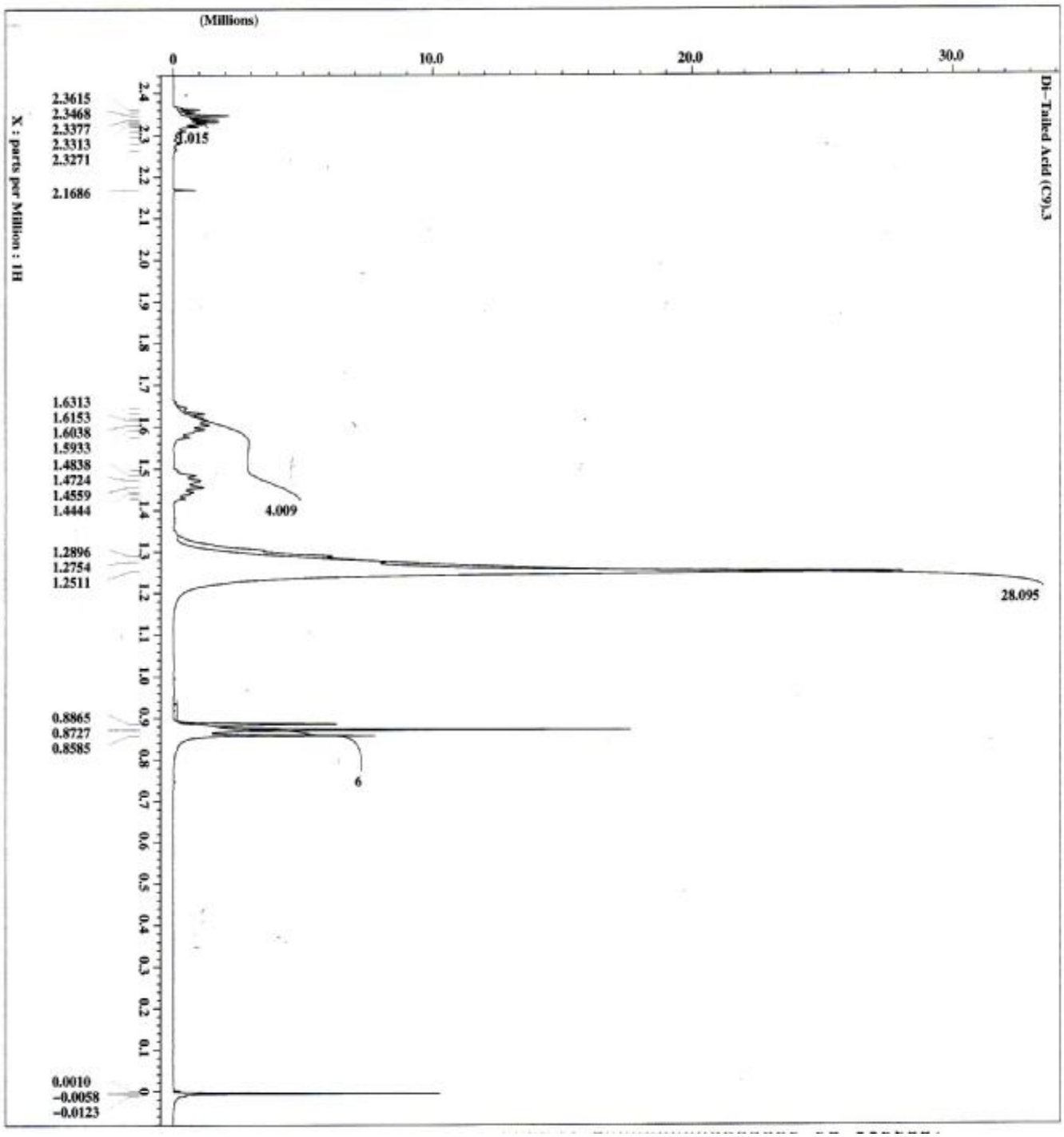
7.024
4.027
24.042
6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2.1686
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1.6153
1.6038
1.5933
1.4838
1.4724
1.4559
1.4444
1.2896
1.2754
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-0.0123
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4.009
28.095
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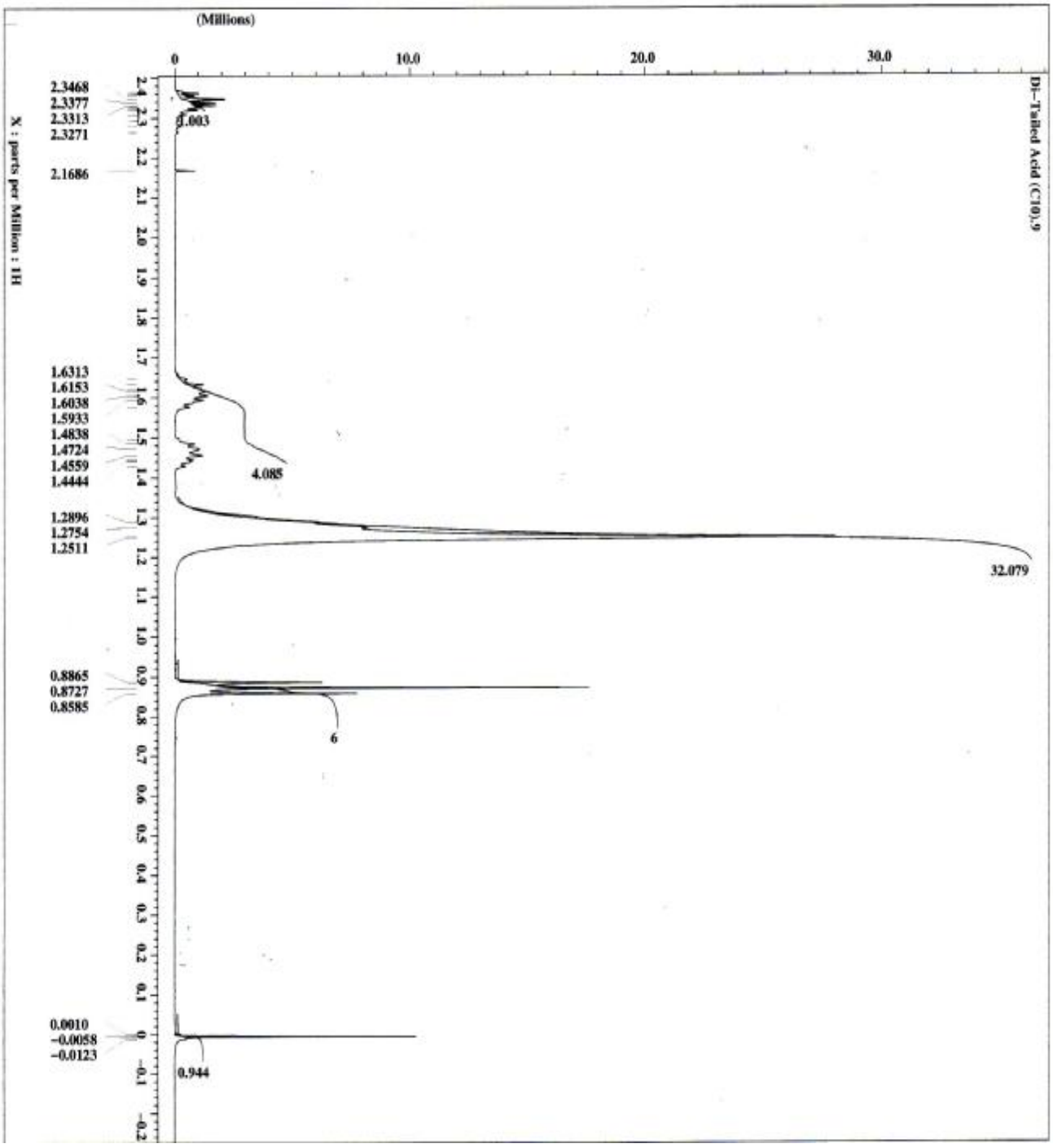
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2.3271
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1.6313
1.6153
1.6038
1.5933
1.4838
1.4724
1.4559
1.4444
1.2896
1.2754
1.2511
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6
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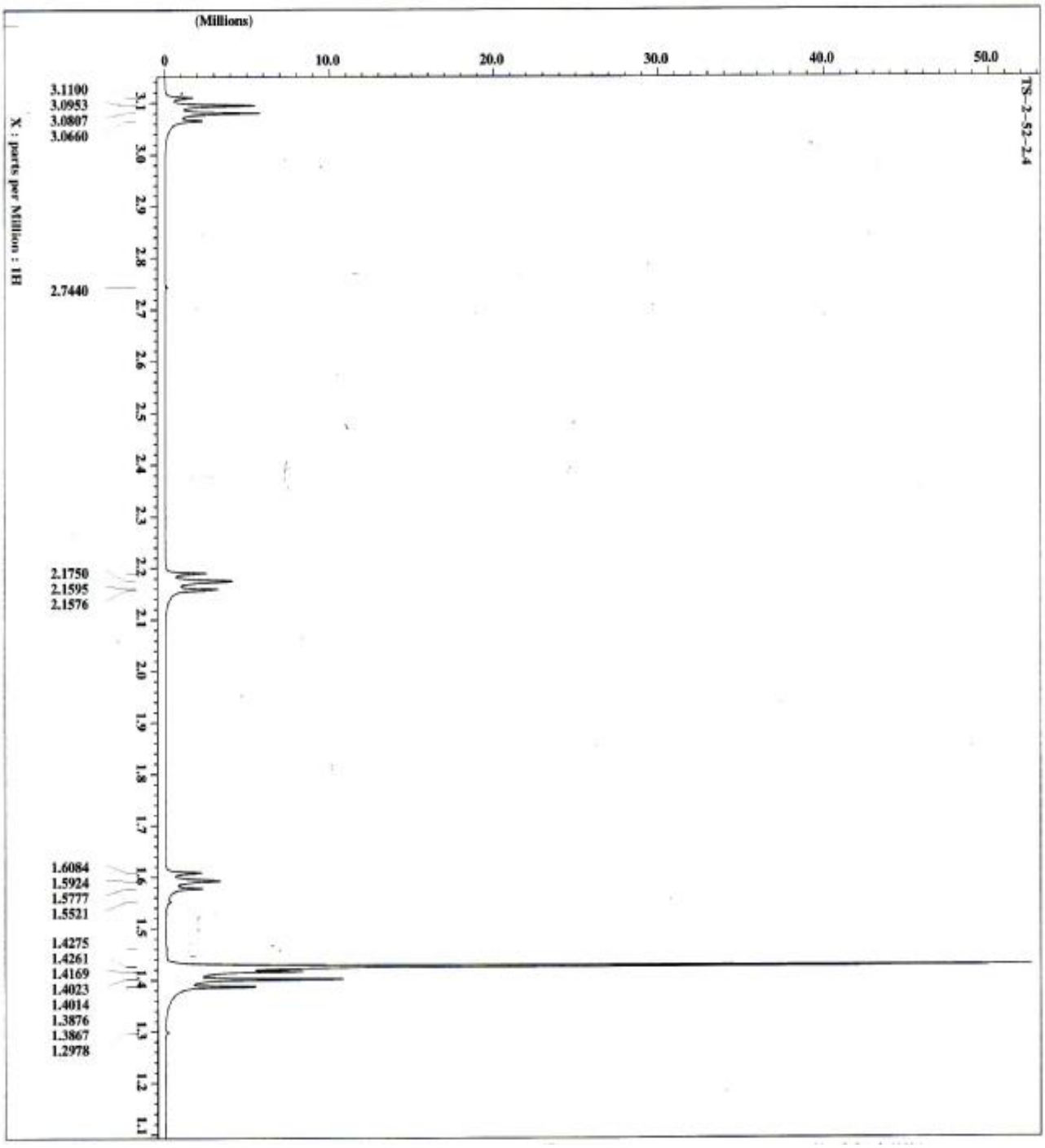
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3.0660
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2.1576
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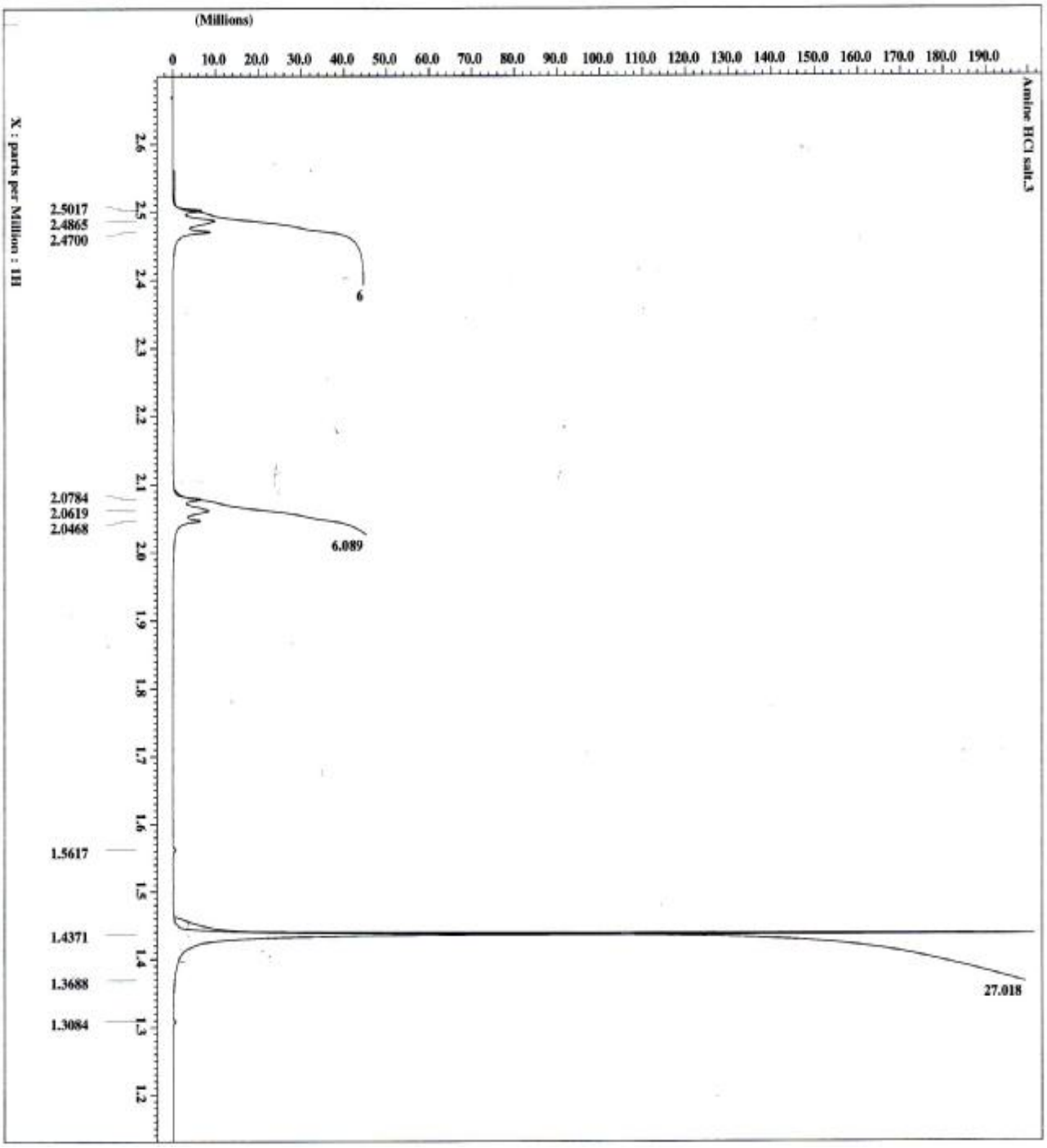
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Amine HCl salt.3
X : parts per Million : 1H
2.6 2.5 2.4 2.3 2.2 2.1 2.0 1.9 1.8 1.7 1.6 1.5 1.4 1.3 1.2
2.5017
2.4865
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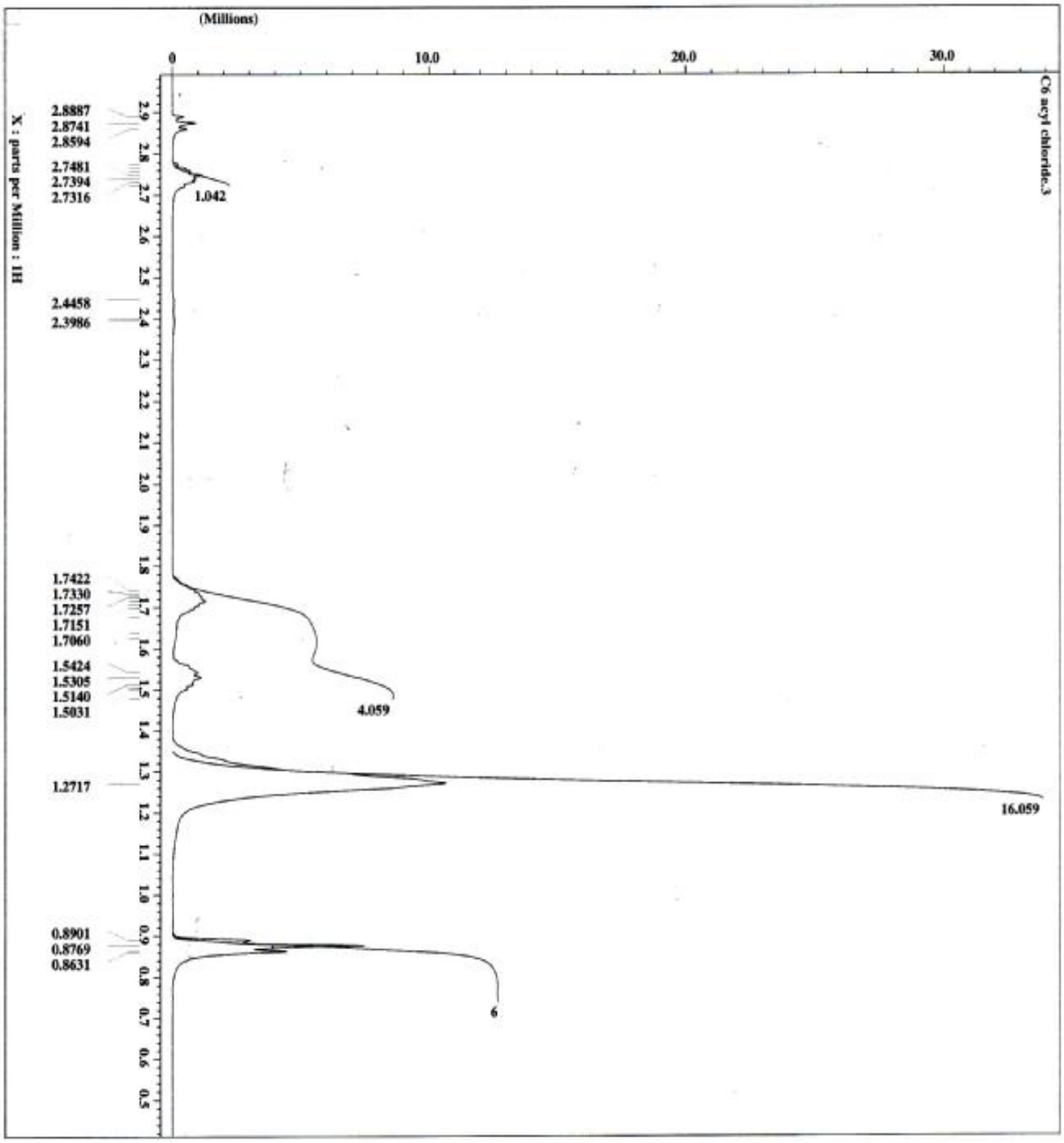
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1.7257
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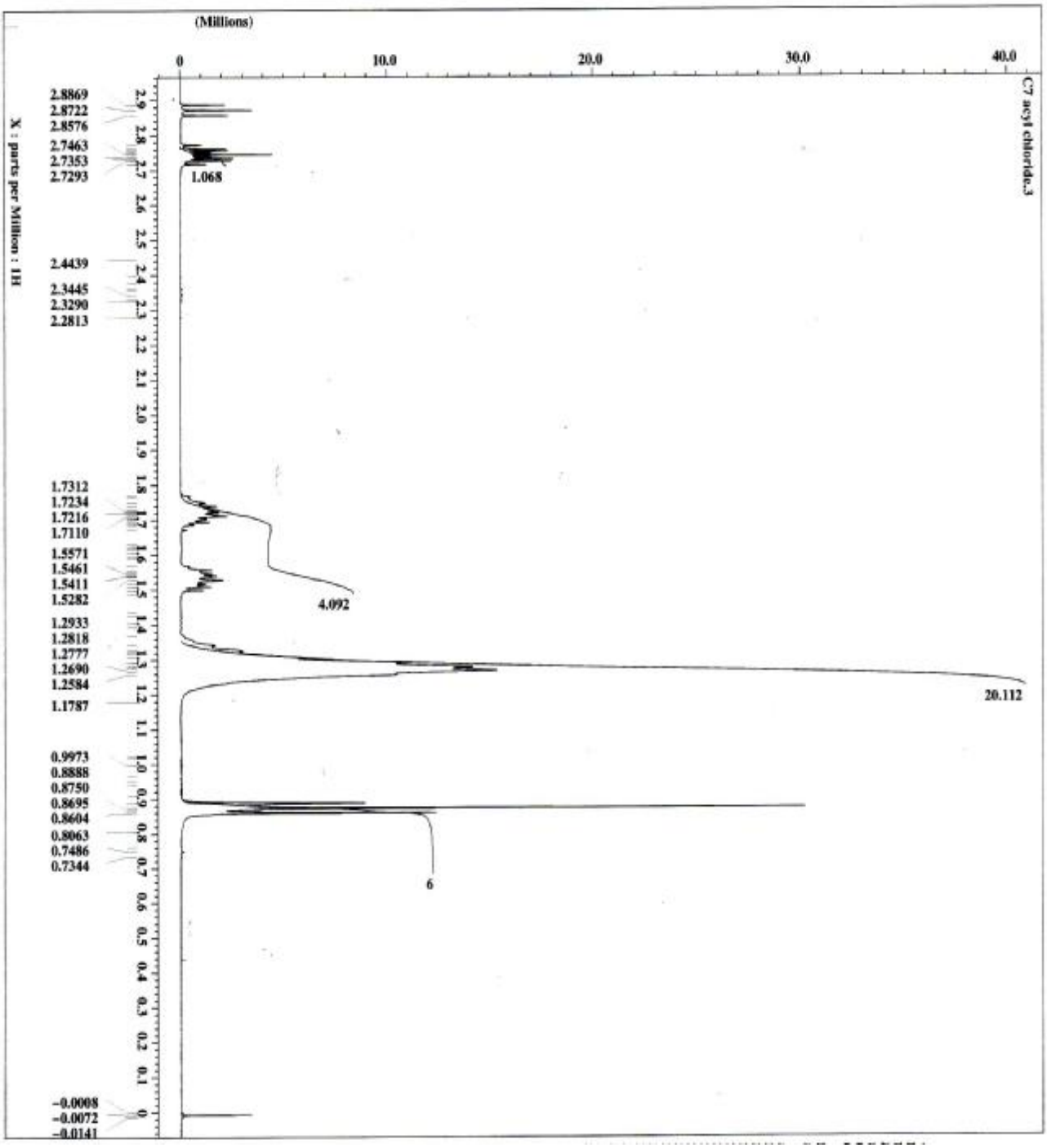
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2.7353
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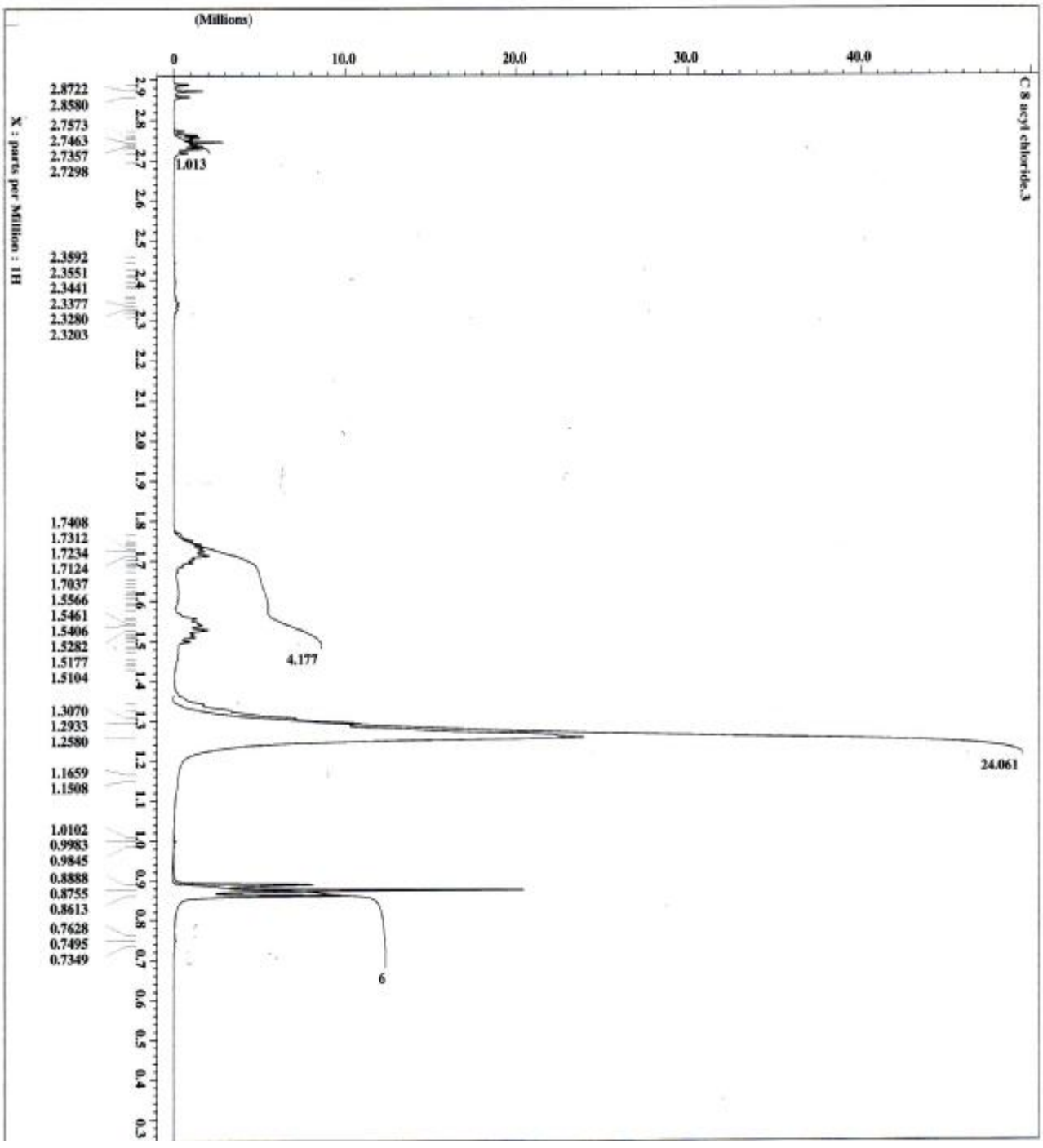
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30.0
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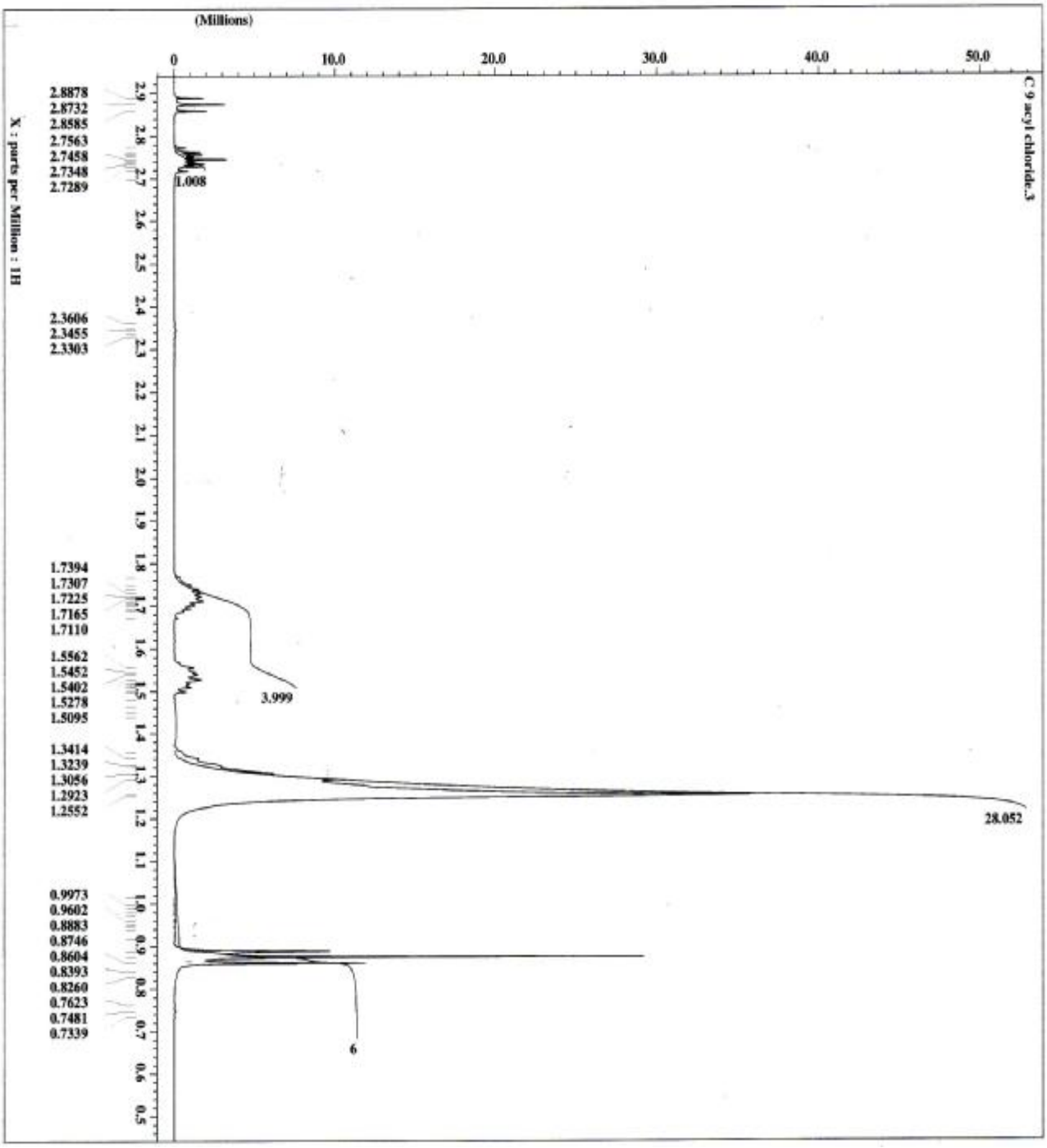
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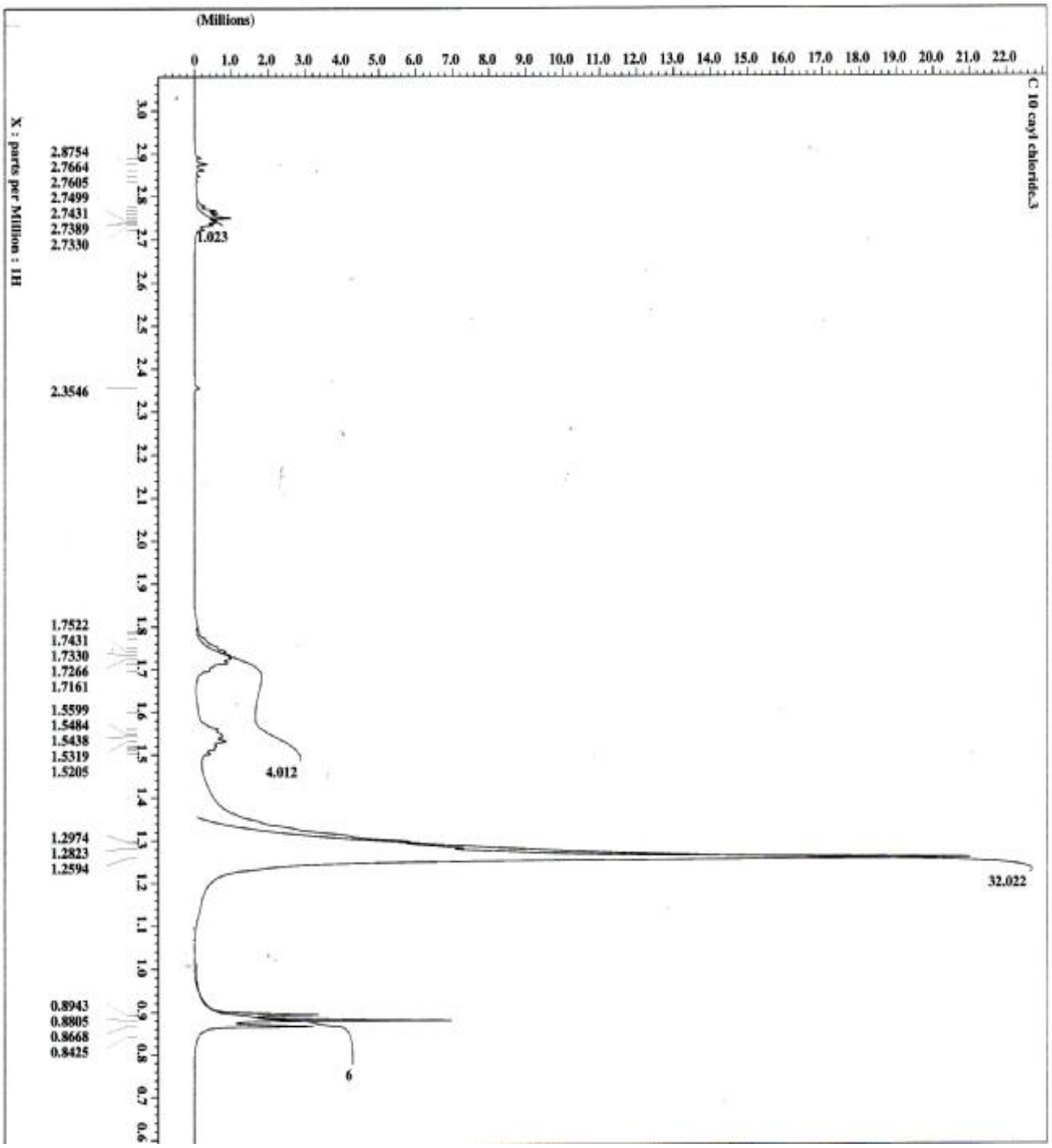
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C 10 cayl chloride.3
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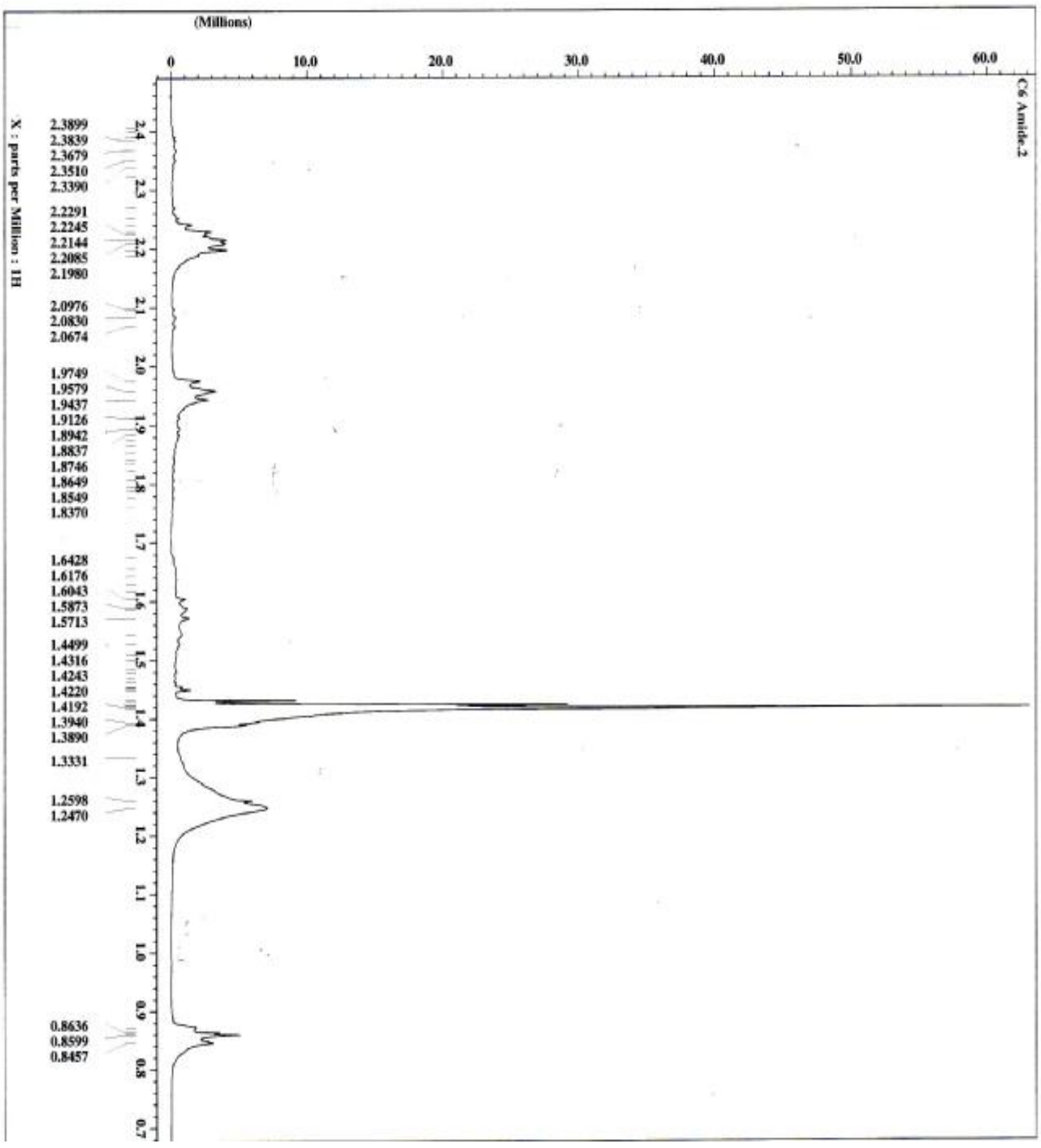
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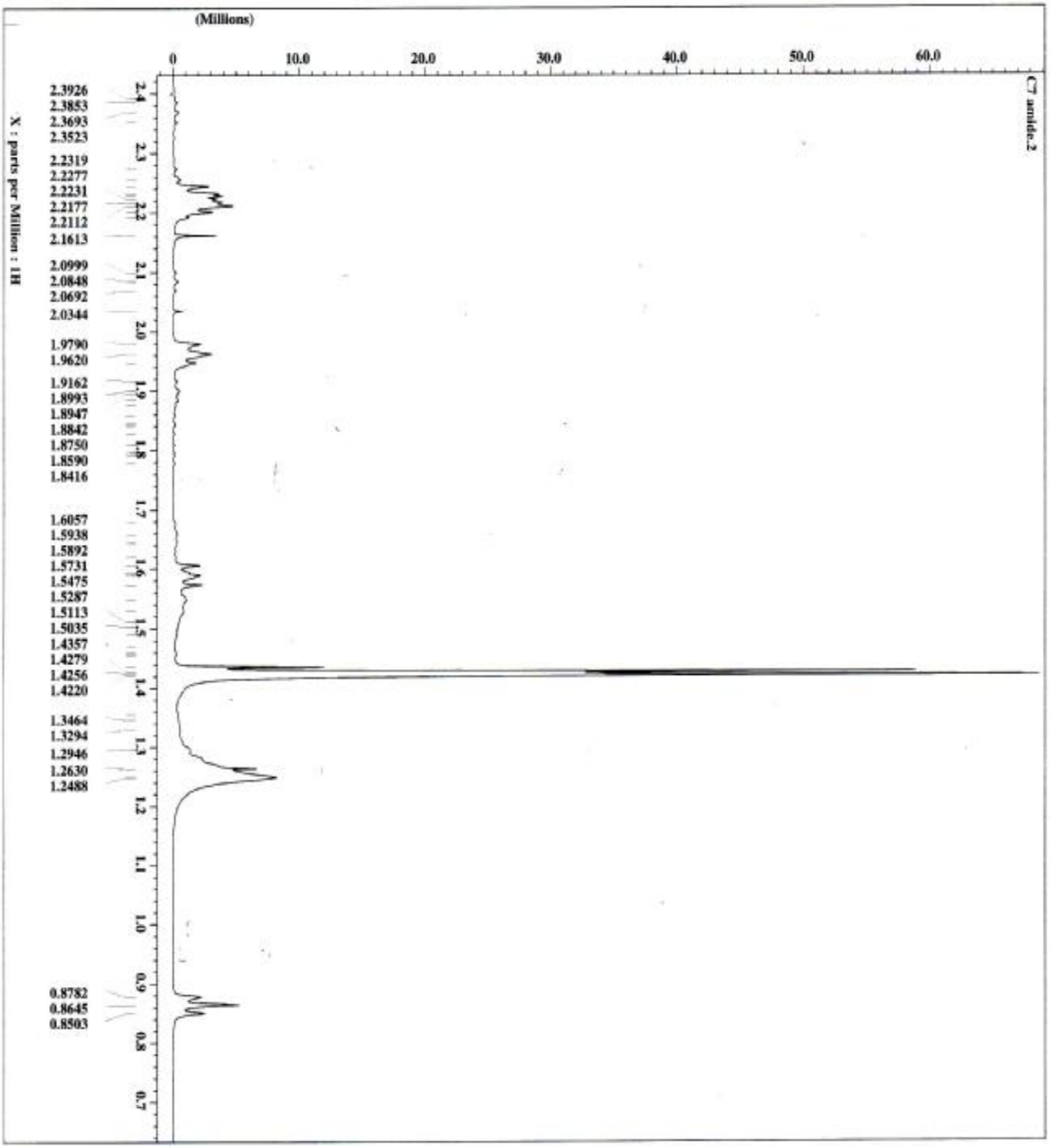
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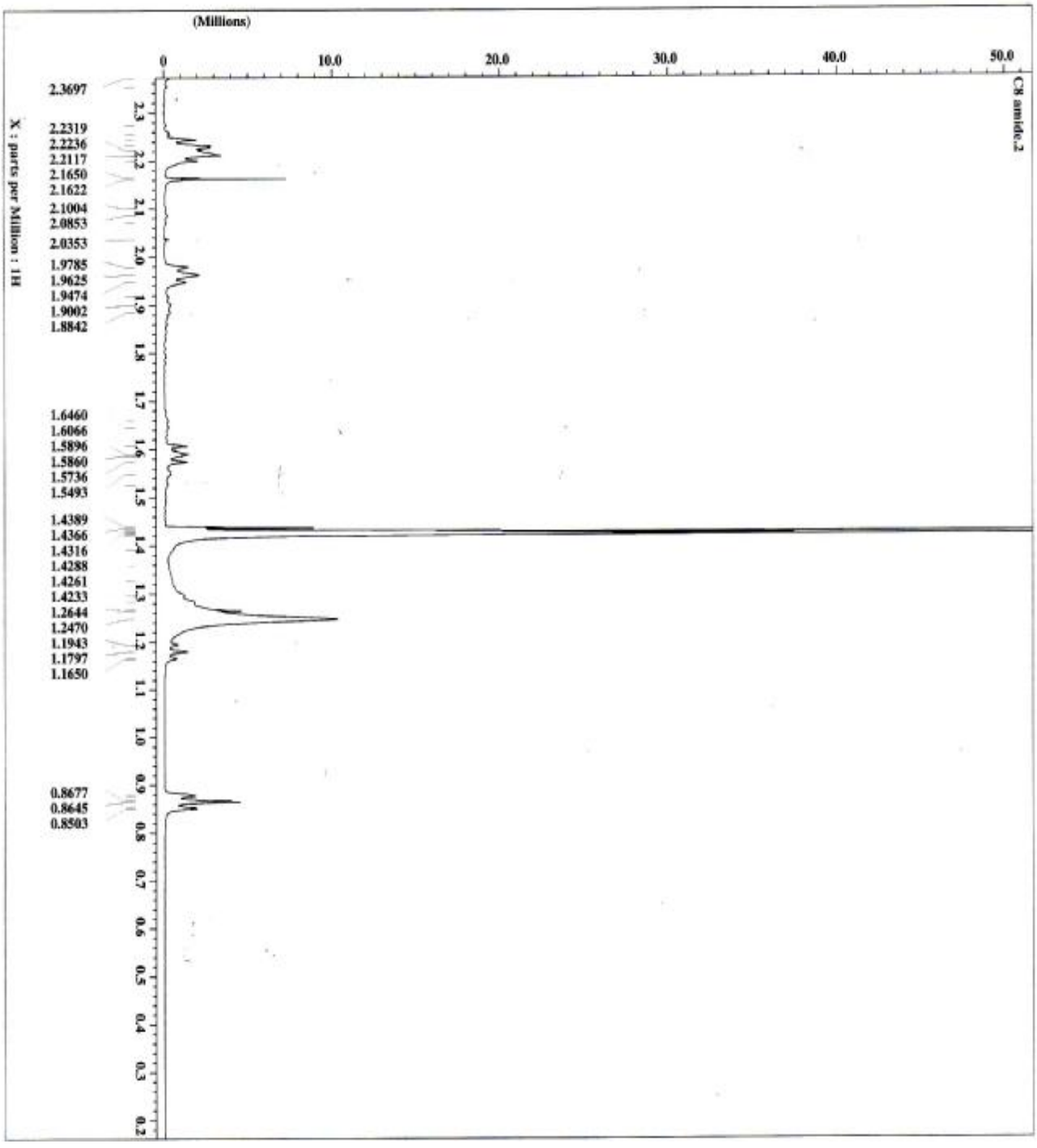
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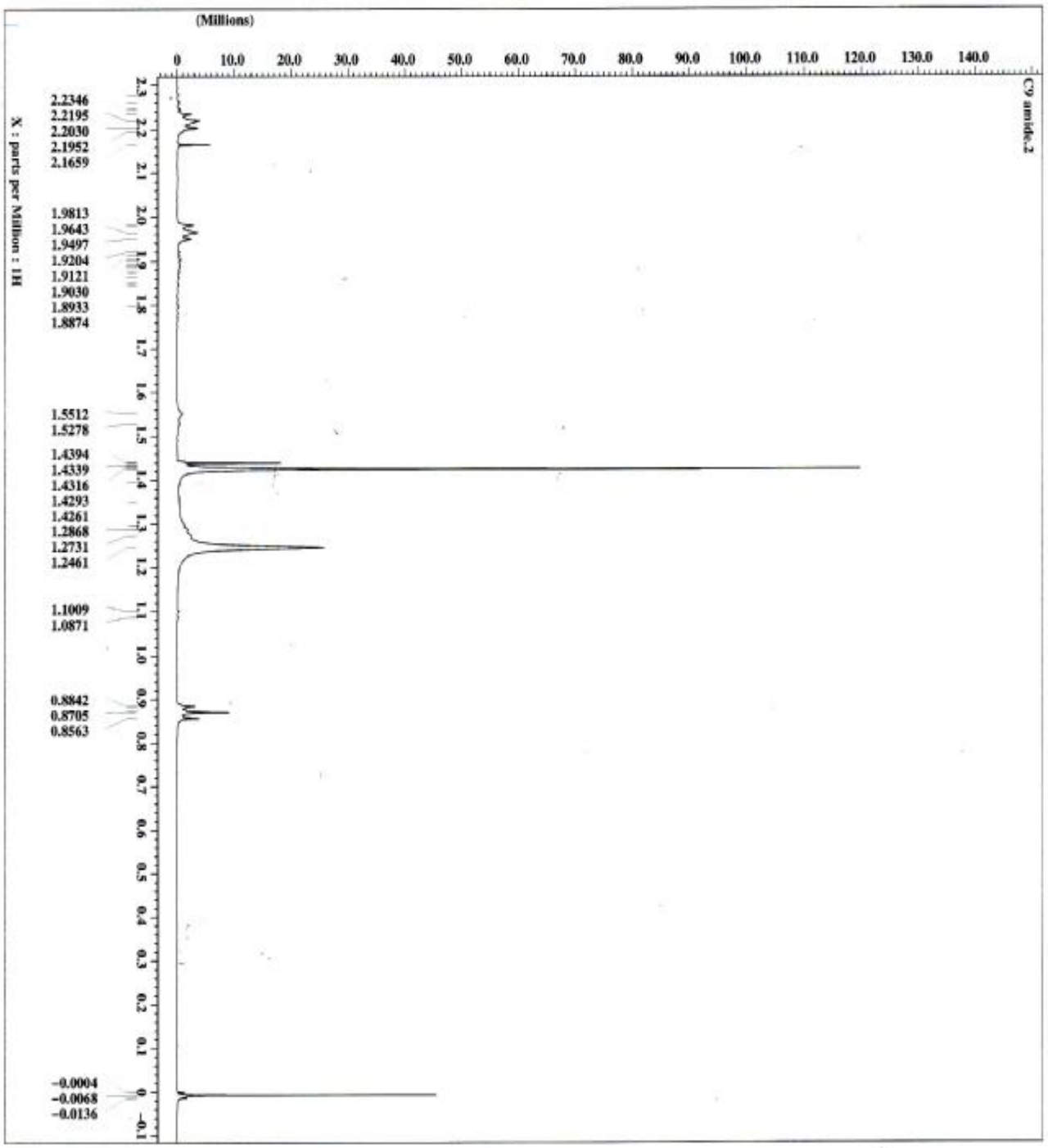
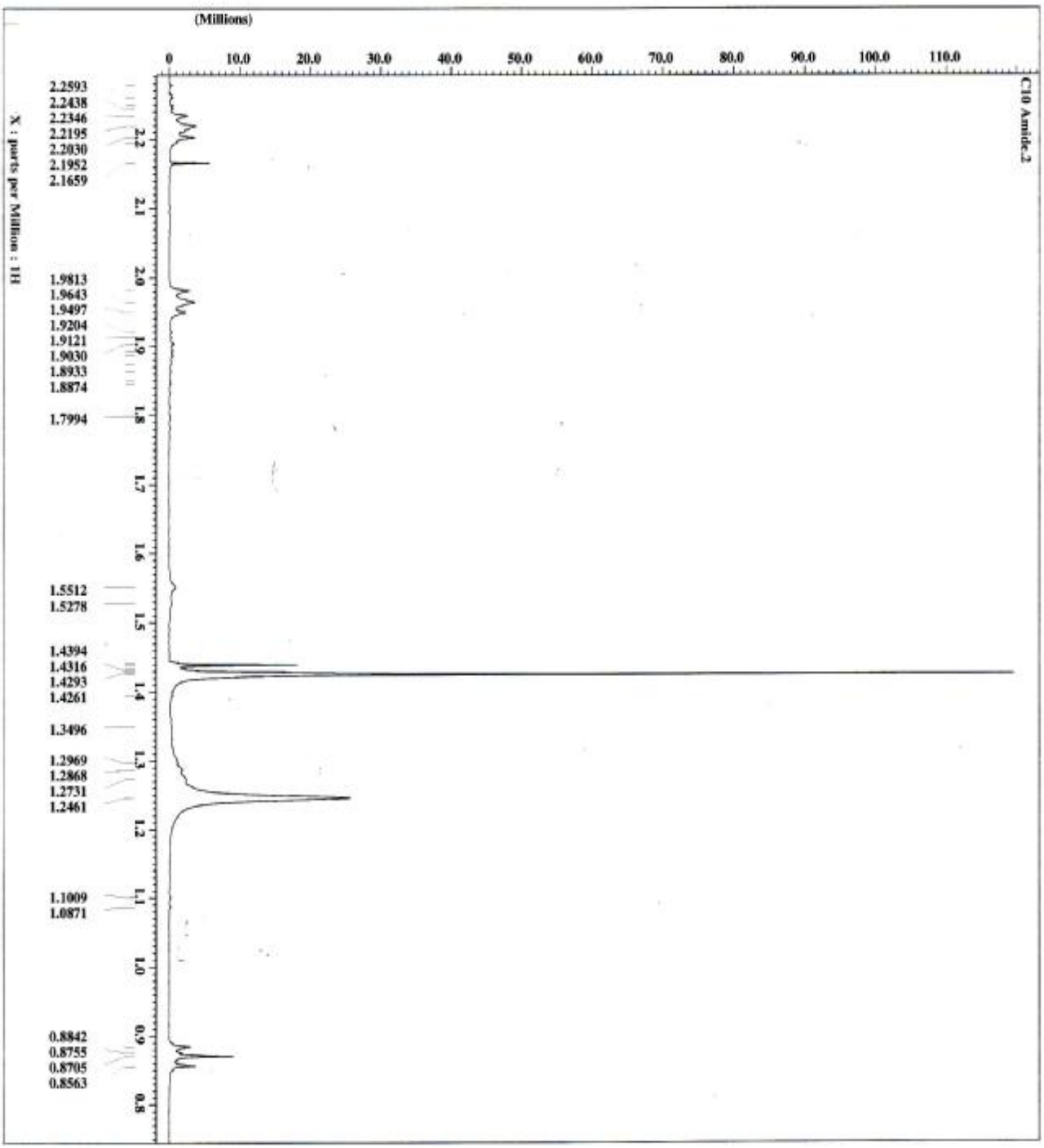
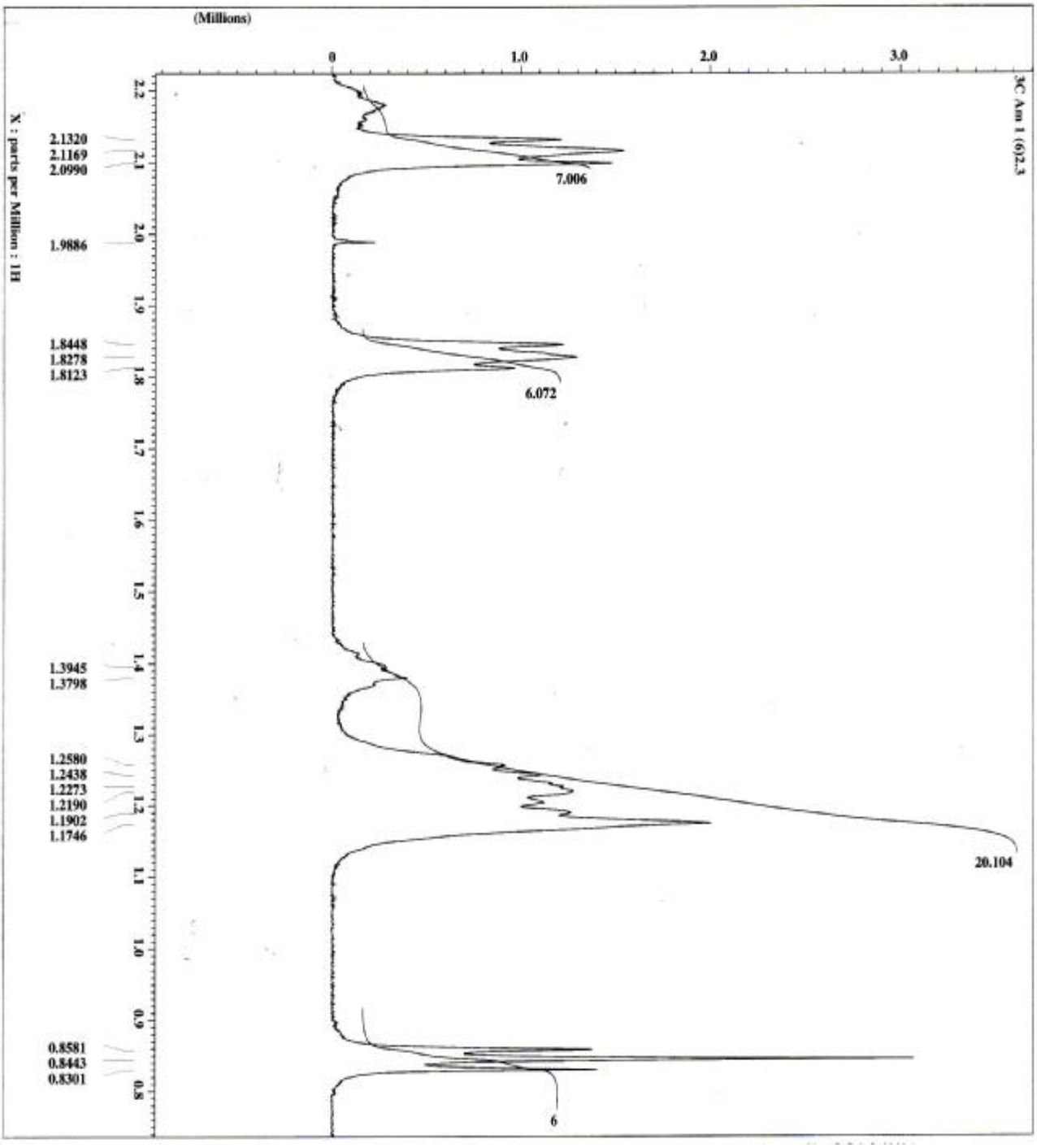
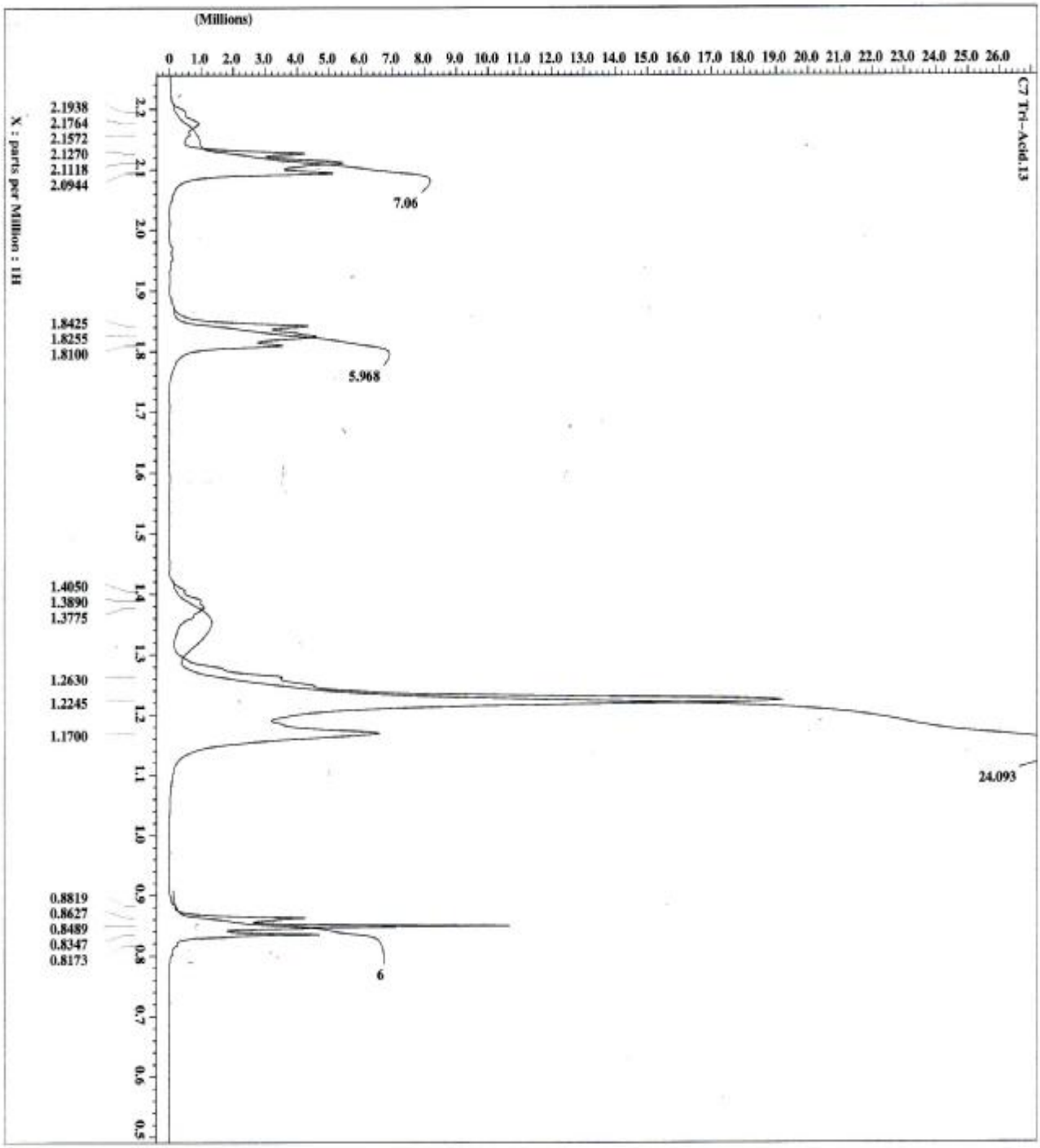
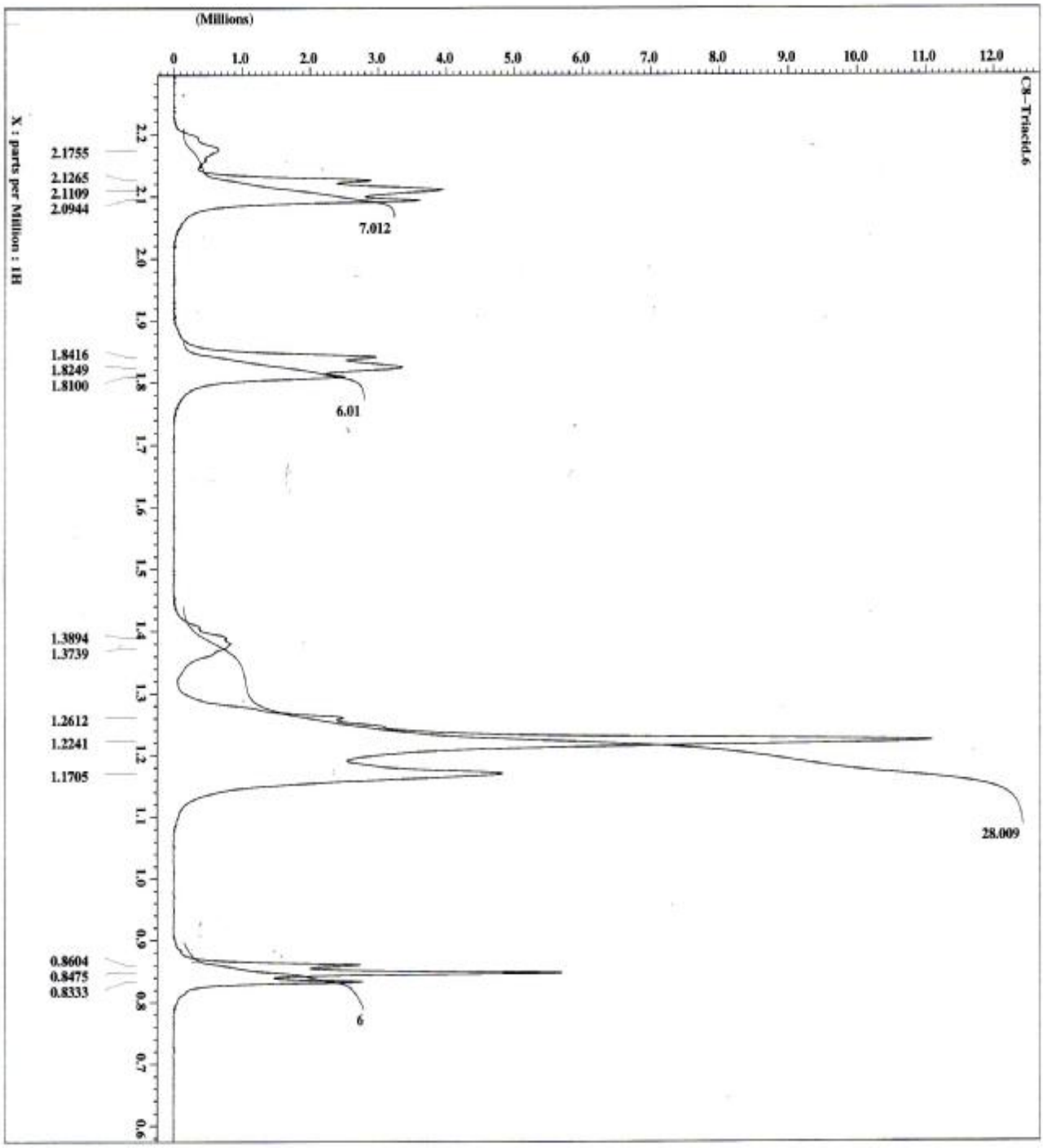
0.8645
0.8503

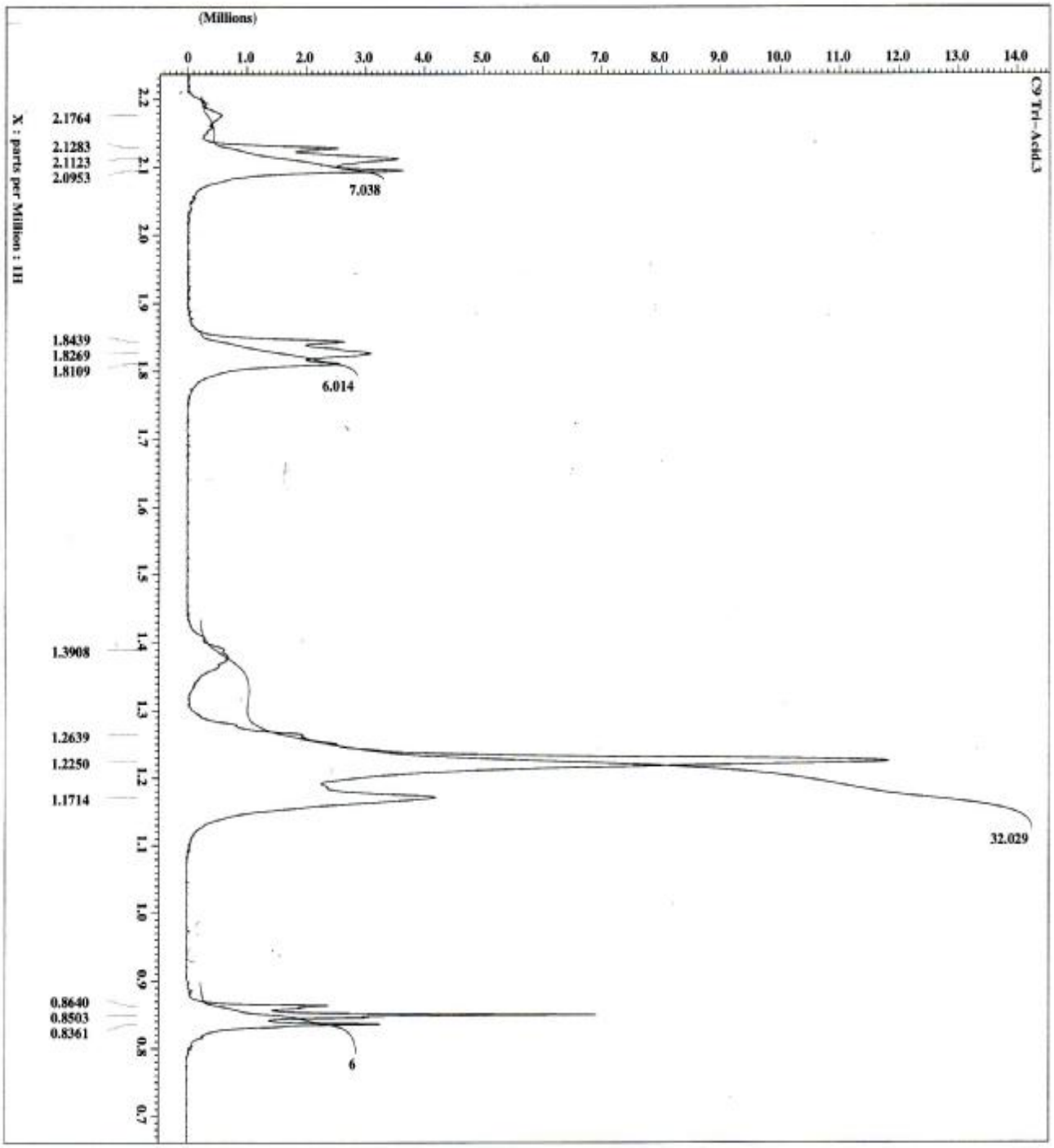

(Millions)
0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
110.0
120.0
130.0
140.0
C9 amide-2
X : parts per Million : 1H
2.2346
2.2195
2.2030
2.1952
2.1659
1.9813
1.9643
1.9497
1.9204
1.9121
1.9030
1.8933
1.8874
1.5512
1.5278
1.4394
1.4339
1.4316
1.4293
1.4261
1.2868
1.2731
1.2461
1.1009
1.0871
0.8842
0.8705
0.8563
−0.0004
−0.0068
−0.0136

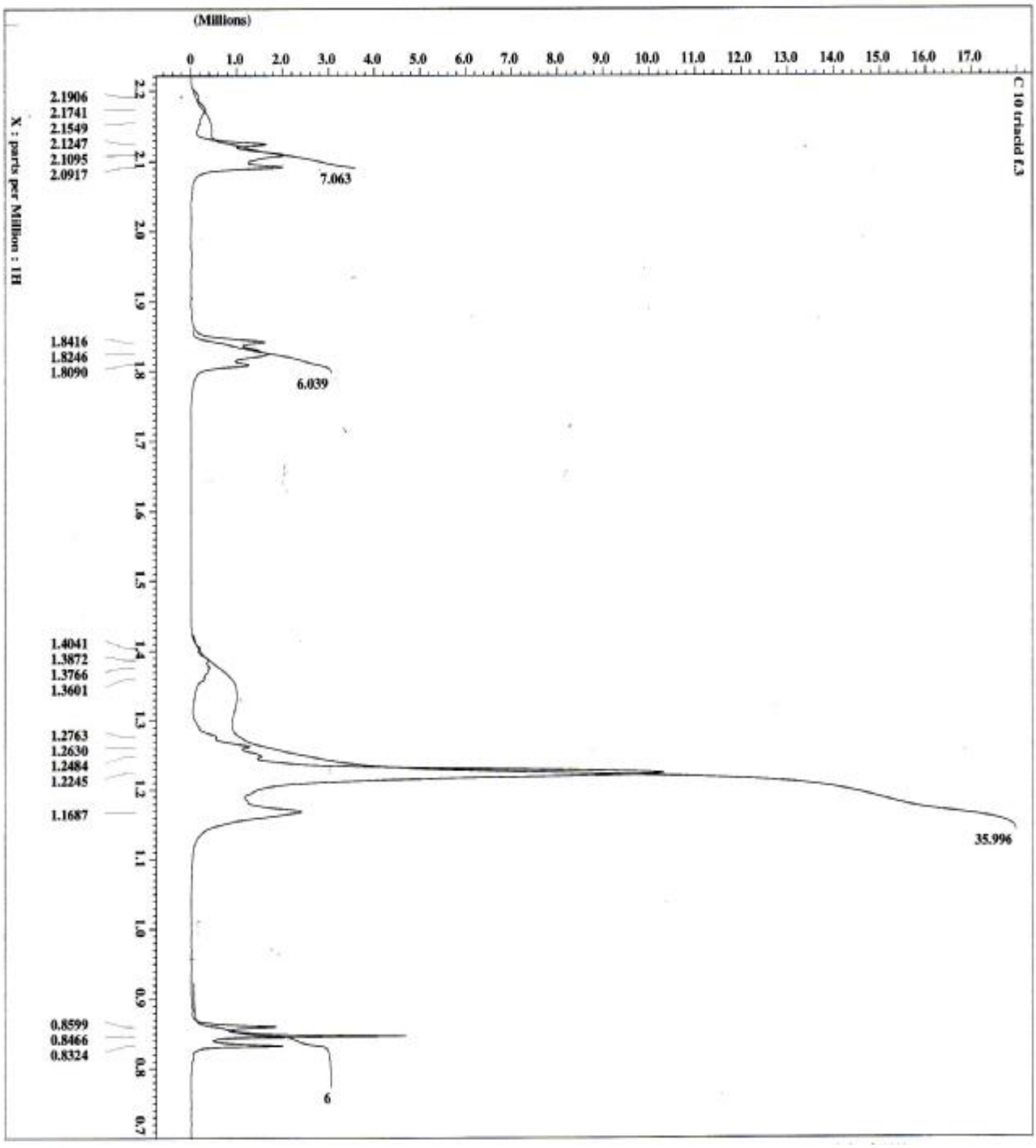

(Millions)
0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
110.0
C10 Amide.2
X : parts per Million : 1H
2.2593
2.2438
2.2346
2.2195
2.2030
2.1952
2.1659
1.9813
1.9643
1.9497
1.9204
1.9121
1.9030
1.8933
1.8874
1.7994
1.5512
1.5278
1.4394
1.4316
1.4293
1.4261
1.3496
1.2969
1.2868
1.2731
1.2461
1.1009
1.0871
0.8842
0.8755
0.8705
0.8563

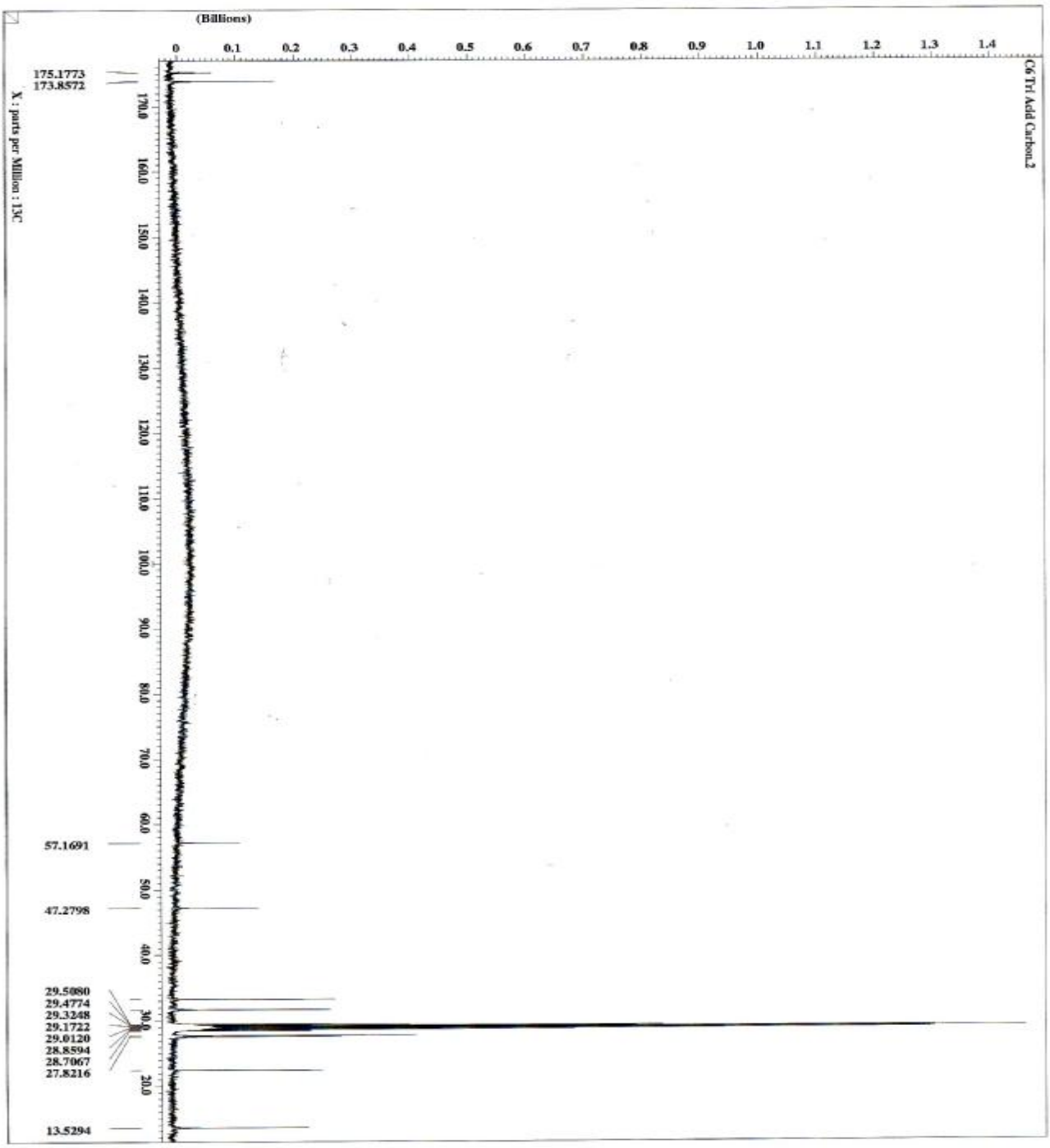

(Millions)
0
1.0
2.0
3.0
3C Am 1 (6)2.3
X : parts per Million : 1H
2.1320
2.1169
2.0990
7.006
1.9886
1.8448
1.8278
1.8123
6.072
1.3945
1.3798
1.2580
1.2438
1.2273
1.2190
1.1902
1.1746
20.104
0.8581
0.8443
0.8301
6

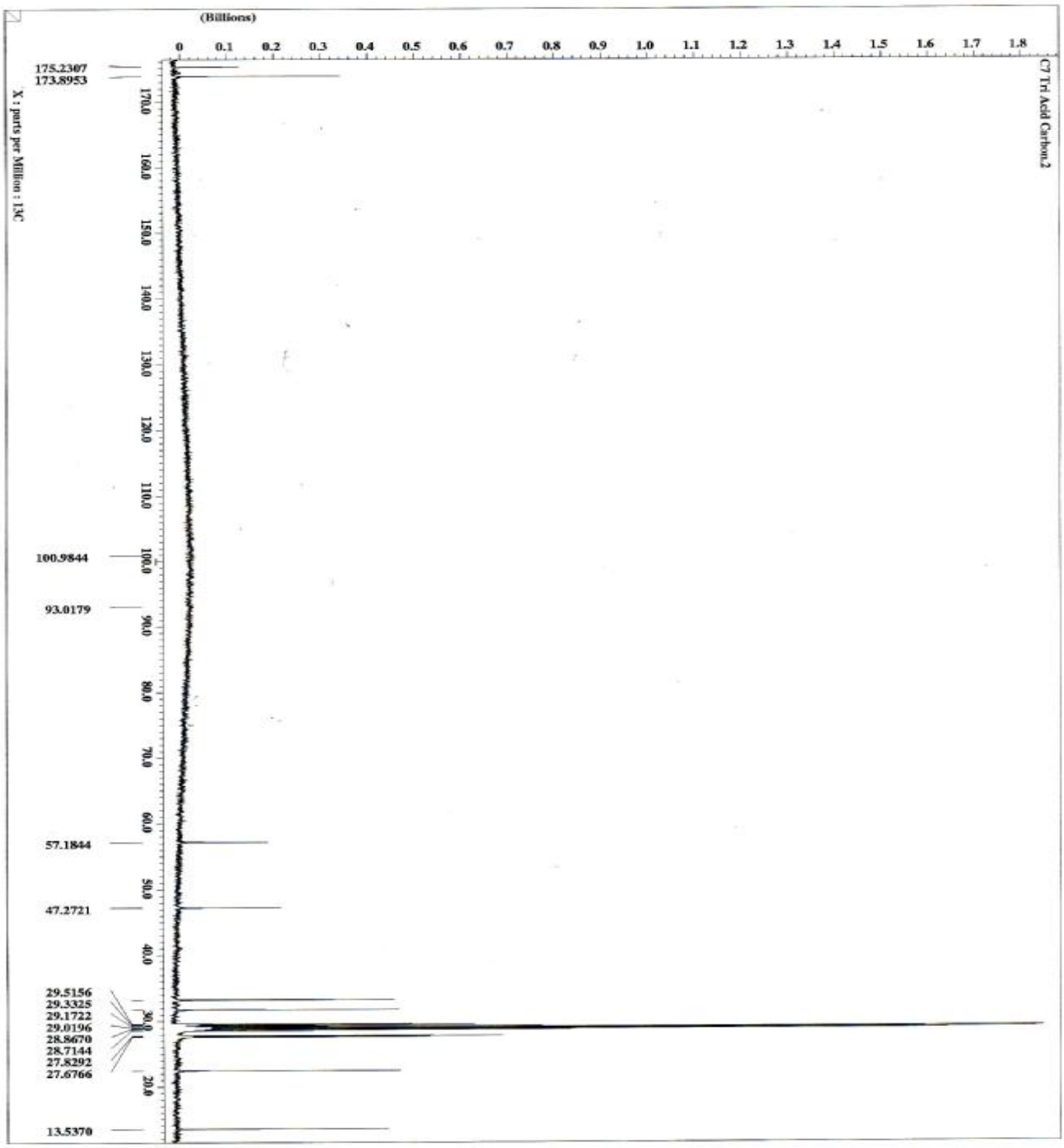

(Millions)
0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0 13.0 14.0 15.0 16.0 17.0 18.0 19.0 20.0 21.0 22.0 23.0 24.0 25.0 26.0
C7 Tri-Acid.13
X : parts per Million : 1H
2.1938
2.1764
2.1572
2.1270
2.1118
2.0944
7.06
1.8425
1.8255
1.8100
5.968
1.4050
1.3890
1.3775
1.2630
1.2245
1.1700
24.093
0.8819
0.8627
0.8489
0.8347
0.8173
6

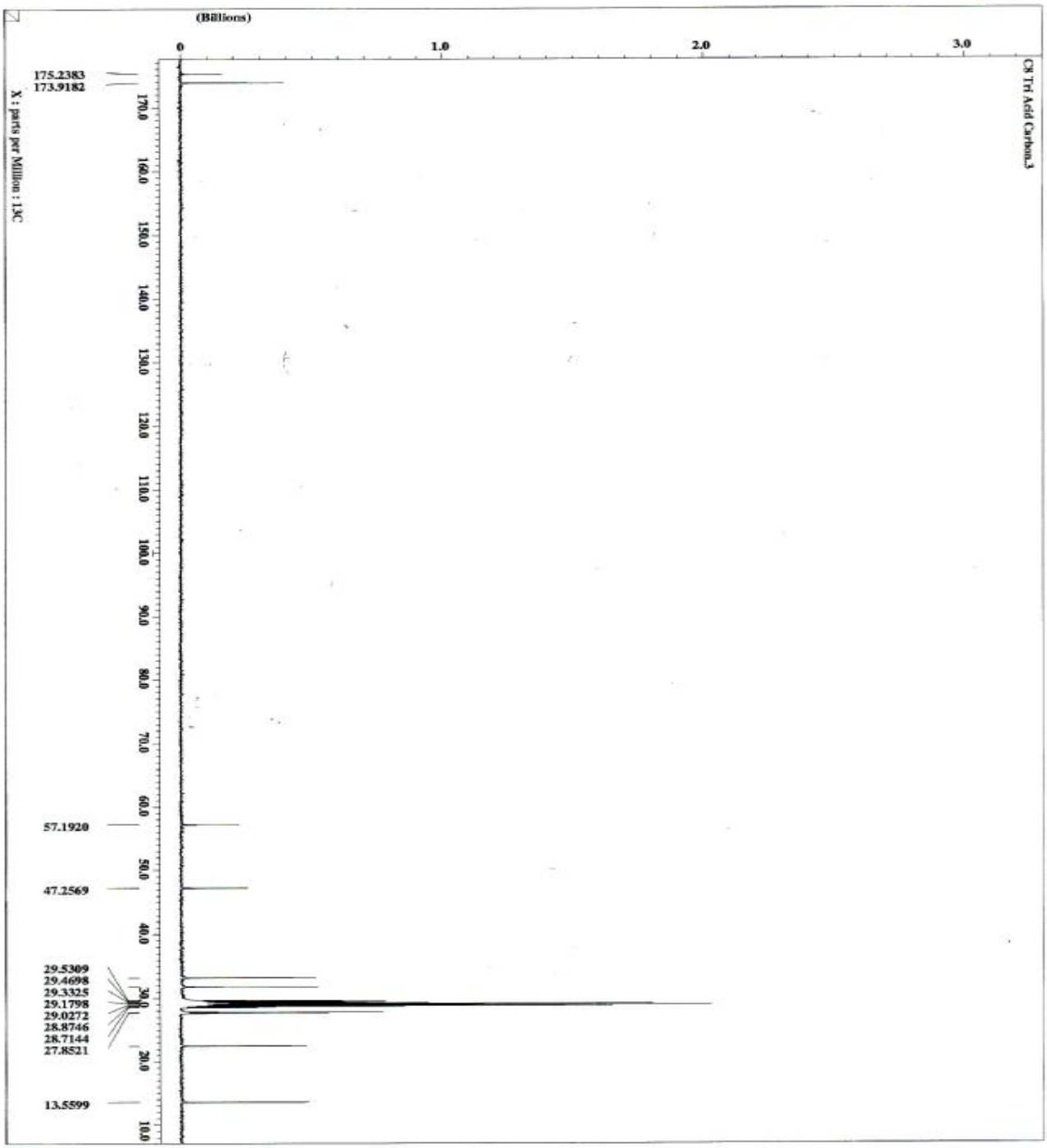

(Millions)
0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
C8-Triacid.6
X : parts per Million : 1H
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
2.1755
2.1265
2.1109
2.0944
7.012
1.8416
1.8249
1.8100
6.01
1.3894
1.3739
1.2612
1.2241
1.1705
28.009
0.8604
0.8475
0.8333
6

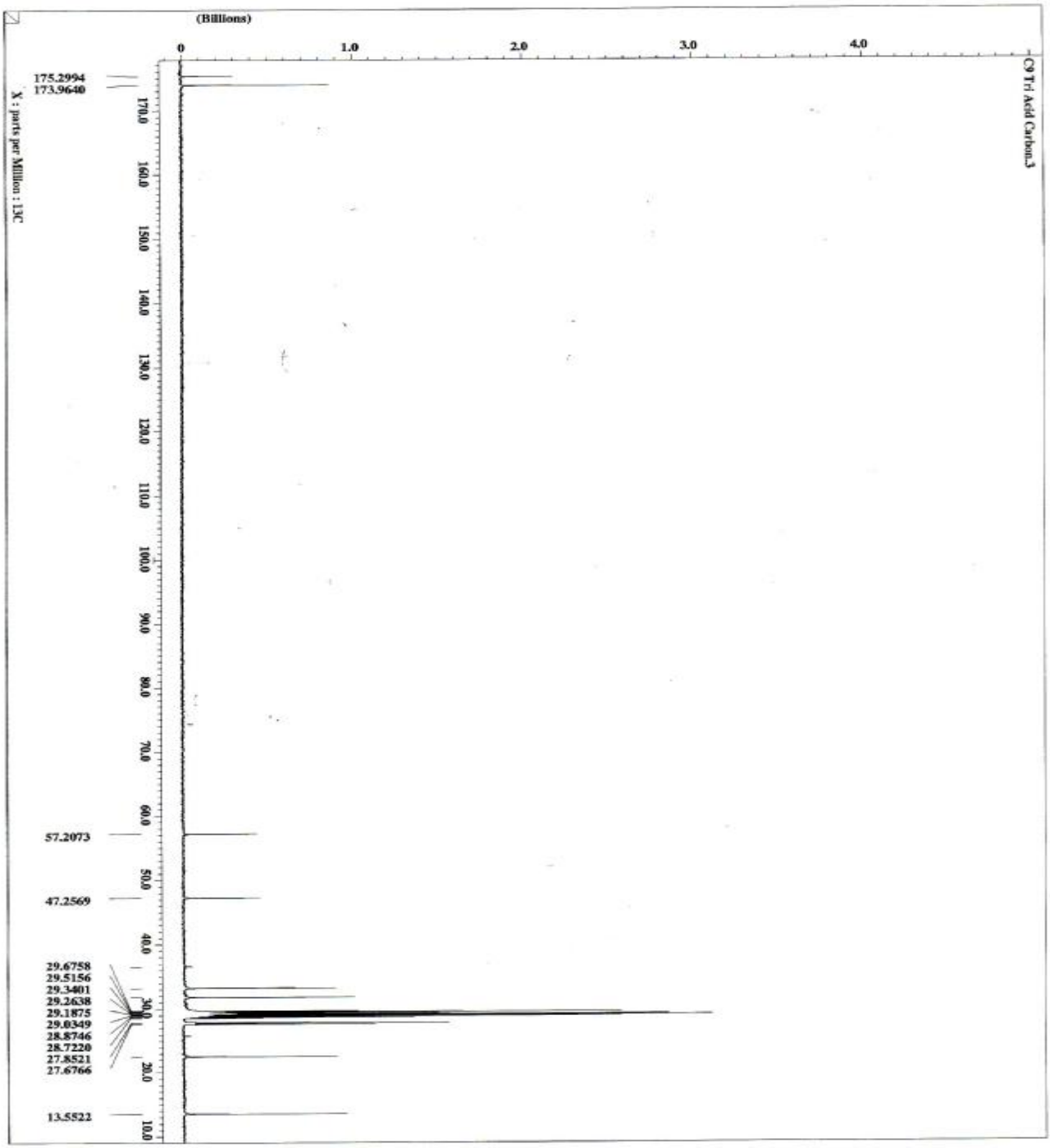

(Millions)
0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
C9-Tri-Acid3
X : parts per Million : 1H
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
2.1764
2.1283
2.1123
2.0953
1.8439
1.8269
1.8109
1.3908
1.2639
1.2250
1.1714
0.8640
0.8503
0.8361
7.038
6.014
32.029
6

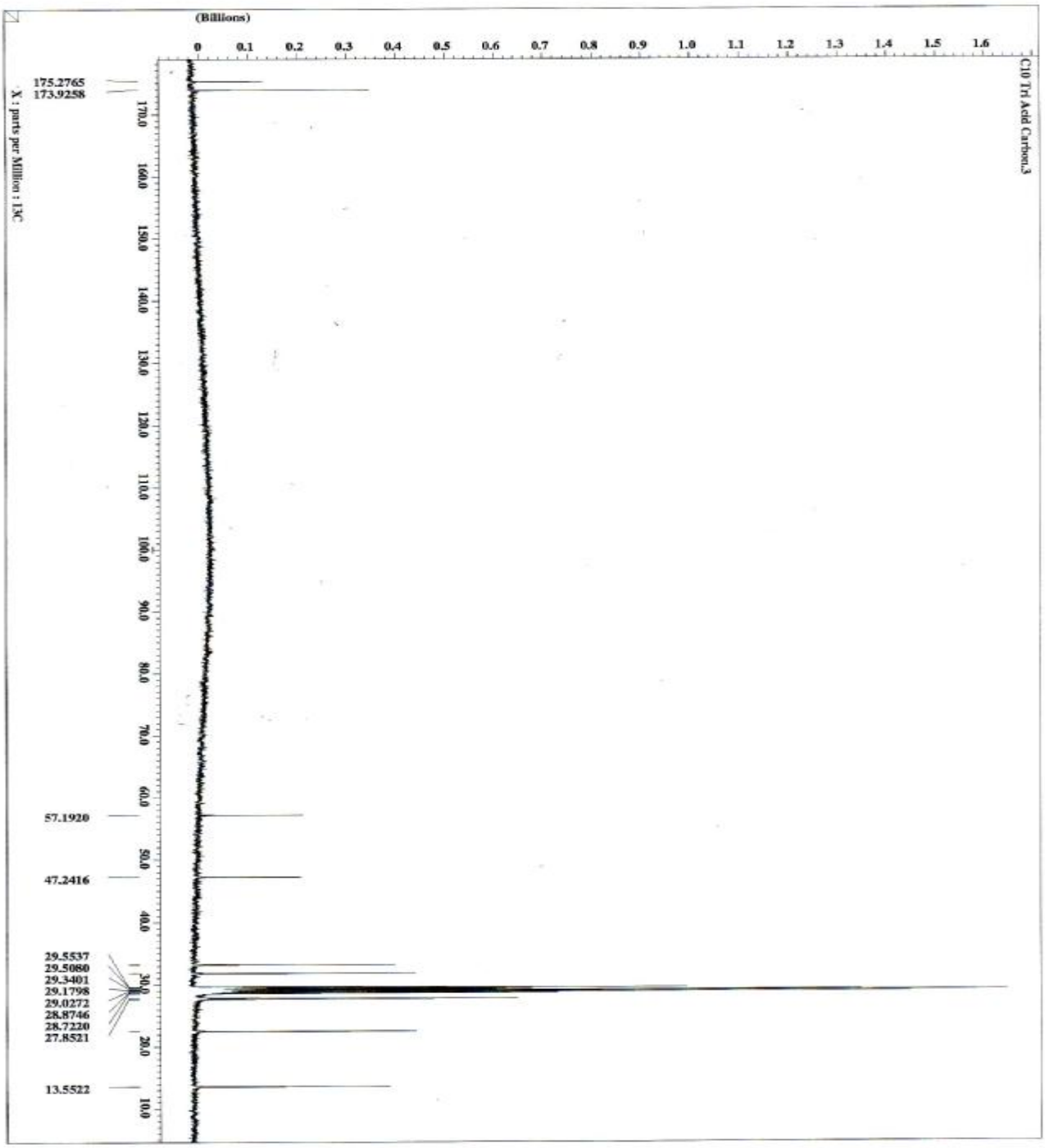

(Millions)
0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0 13.0 14.0 15.0 16.0 17.0
X : parts per Million : 1H
C 10 triacid f.3
2.1906
2.1741
2.1549
2.1247
2.1095
2.0917
7.063
1.8416
1.8246
1.8090
6.039
1.4041
1.3872
1.3766
1.3601
1.2763
1.2630
1.2484
1.2245
1.1687
35.996
0.8599
0.8466
0.8324
6


(Billions)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
1.3
1.4
C6 Trf Acid Carbon.2
X : parts per Million : 13C
175.1773
173.8572
57.1691
47.2798
29.5080
29.4774
29.3248
29.1722
29.0120
28.8594
28.7067
27.8216
13.5294


(Billions)
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1 1.2 1.3 1.4 1.5 1.6 1.7 1.8
C7 Tri Acid Carbon.2
X : parts per Million : 13C
175.2307
173.8953
100.9844
93.0179
57.1844
47.2721
29.5156
29.3325
29.1722
29.0196
28.8670
28.7144
27.8292
27.6766
13.5370


(Billions)
0
1.0
2.0
3.0
C8 TH Acid Carbon.3
X : parts per Million : 13C
175.2383
173.9182
57.1920
47.2569
29.5309
29.4698
29.3325
29.1798
29.0272
28.8746
28.7144
27.8521
13.5599

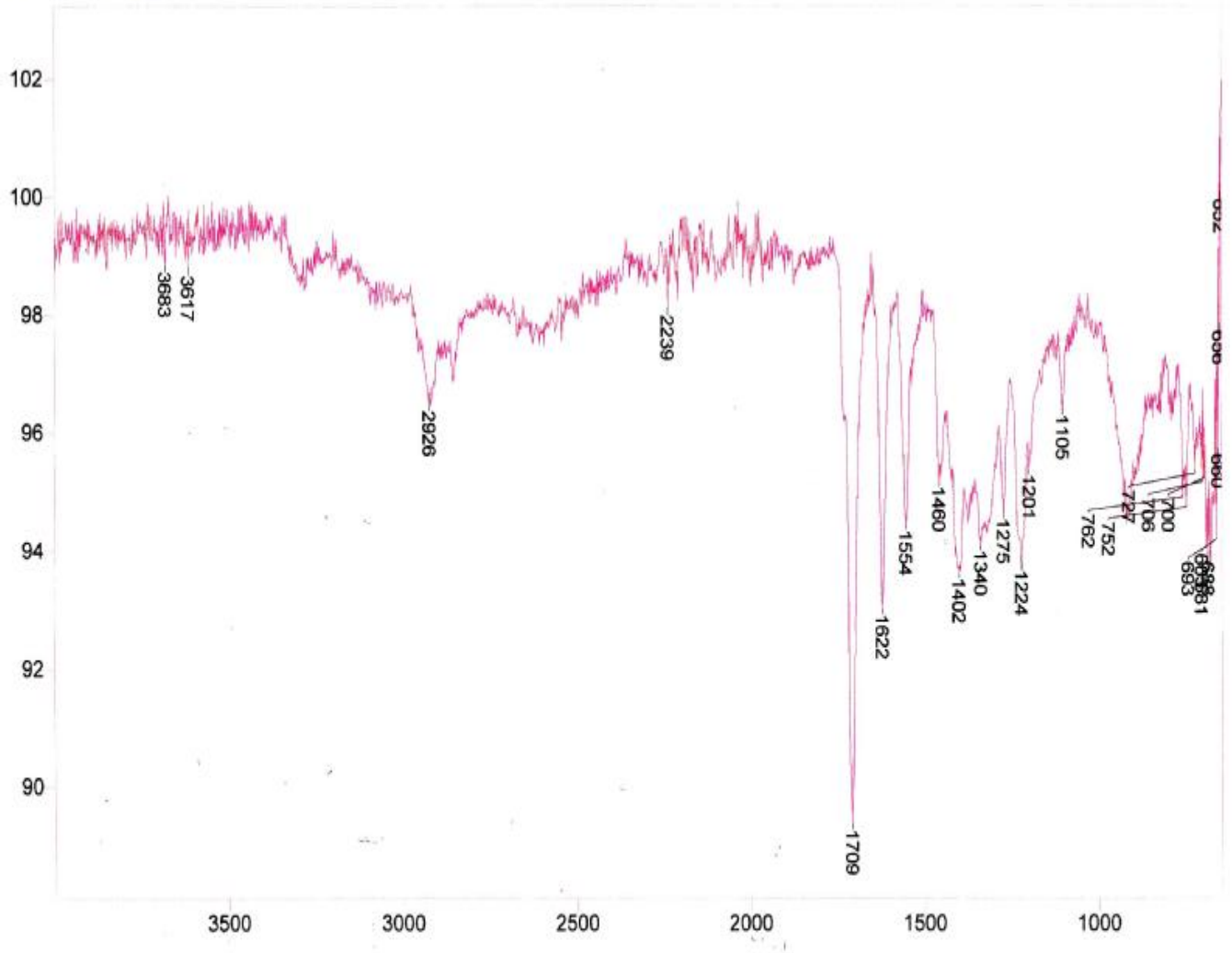

(Billions)
0
1.0
2.0
3.0
4.0
C9 Tri Acid Carbon.3
X : parts per Million : 13C
175.2994
173.9640
57.2073
47.2569
29.6758
29.5156
29.3401
29.2638
29.1875
29.0349
28.8746
28.7220
27.8521
27.6766
13.5522


(Billions)
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1 1.2 1.3 1.4 1.5 1.6
C10 Tri Acid Carbon.3
X : parts per Million : 13C
175.2765
173.9258
57.1920
47.2416
29.5537
29.5080
29.3401
29.1798
29.0272
28.8746
28.7220
27.8521
13.5522
170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0

Appendix B

IR Spectrum

3C AmICOD$_2$

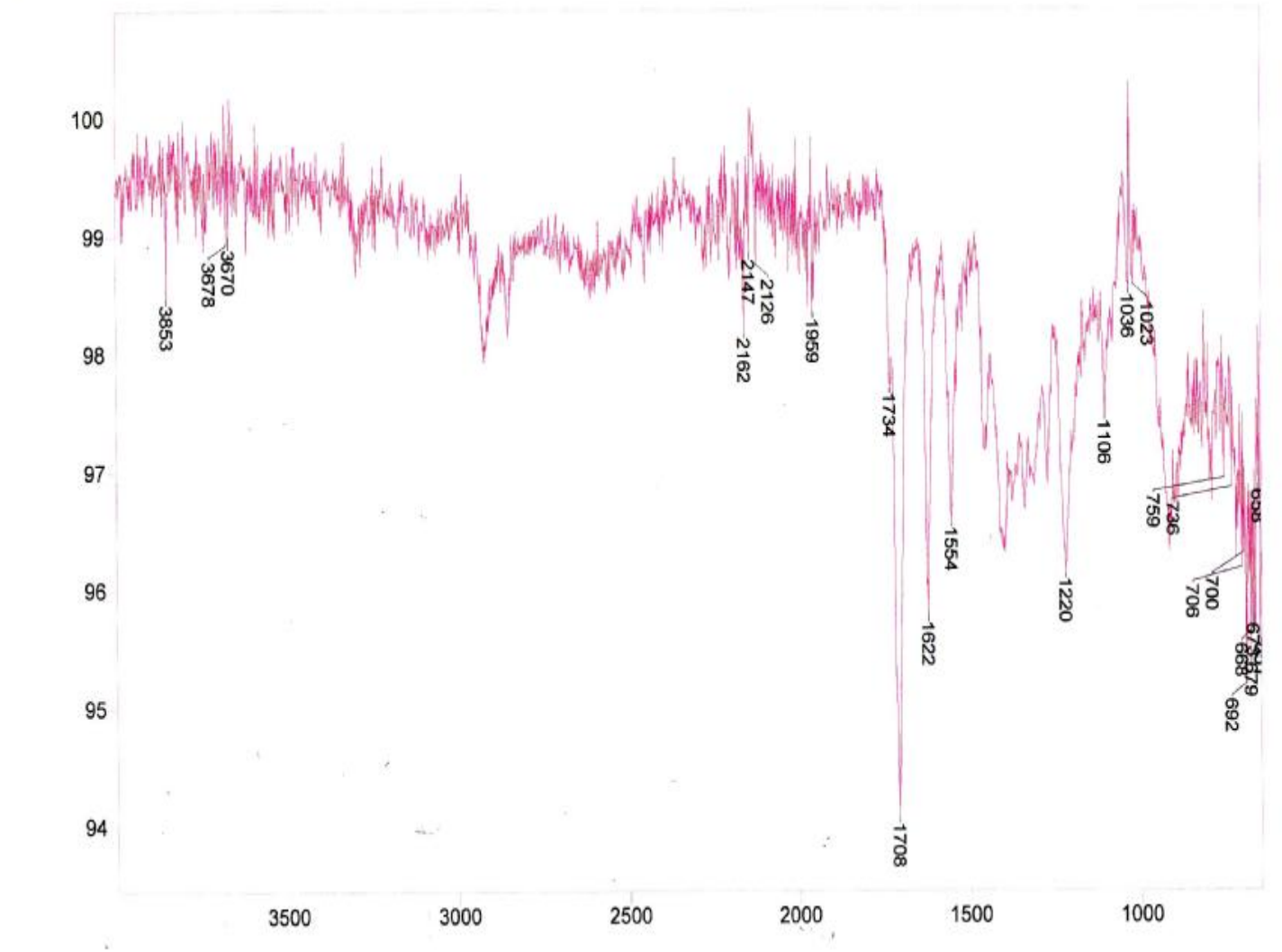


Transmittance / Wavenumber (cm-1)

Paged Z-Zoom SCROLL

File # 1 = C6TRIACIDS

8/22/04 16:41 PM Res=4 cm-1

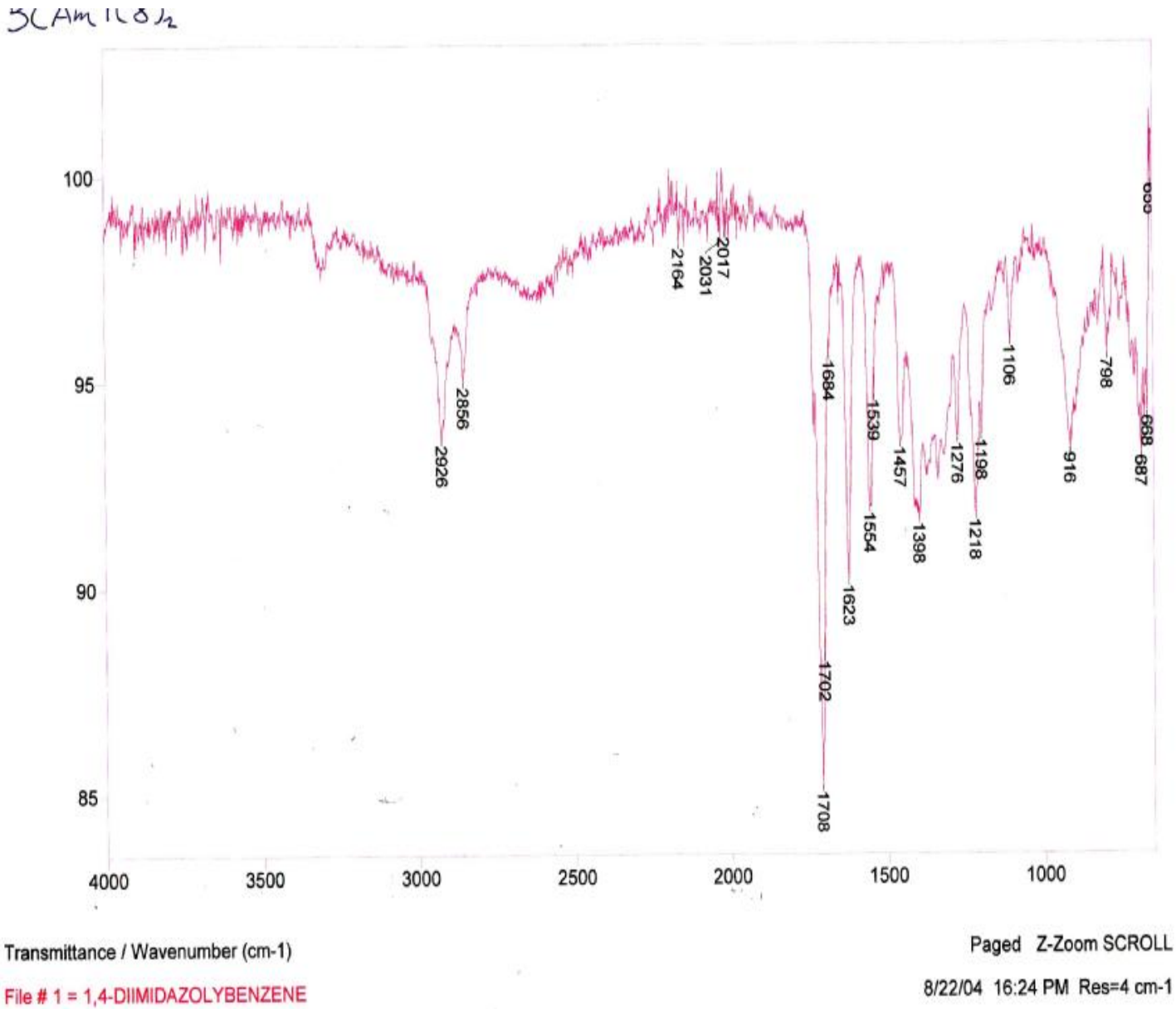


Transmittance / Wavenumber (cm-1)

Paged Z-Zoom SCROLL

File # 2 = C7TRIACIDS

8/22/04 16:29 PM Res=4 cm-1

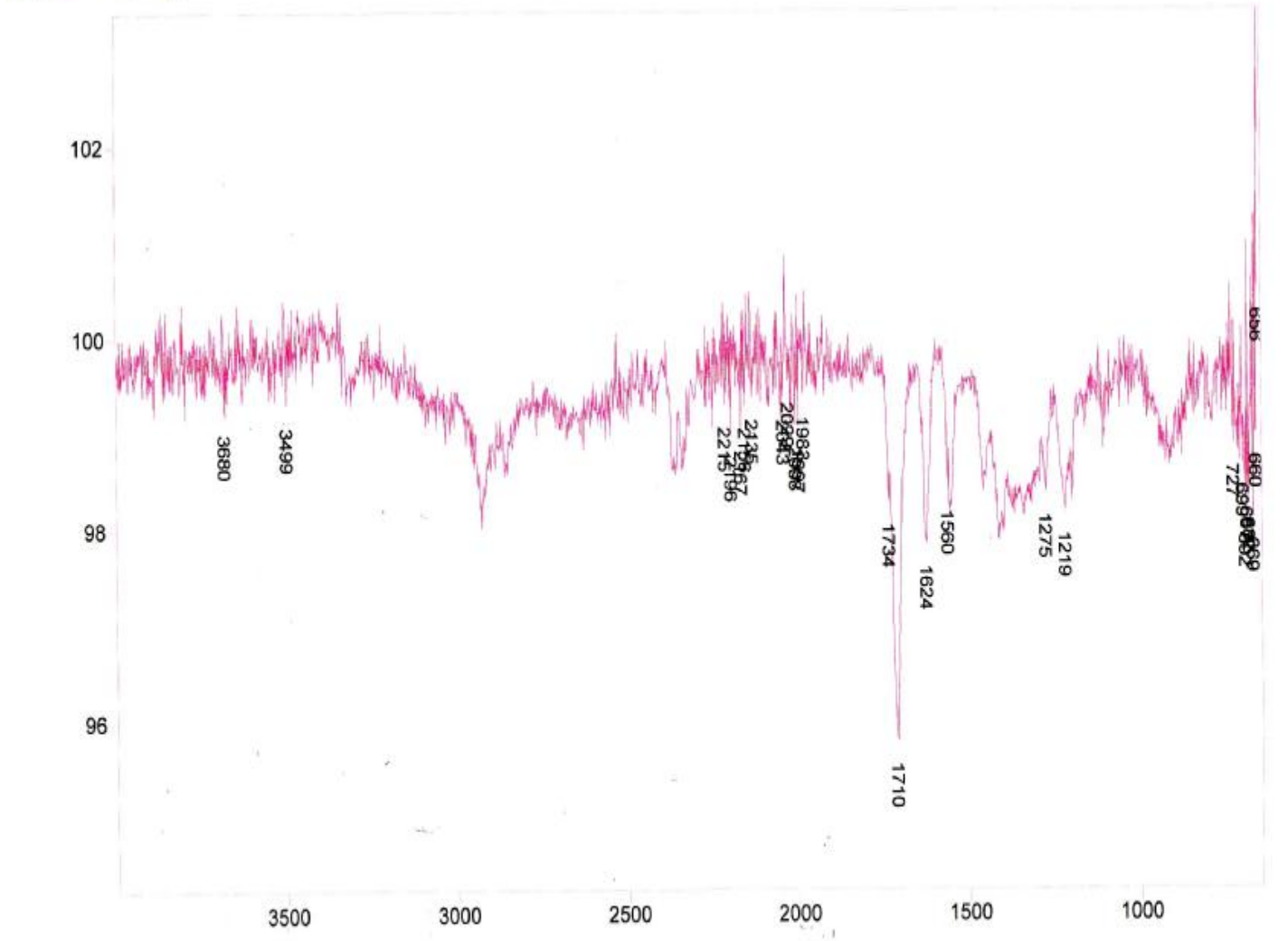


Transmittance / Wavenumber (cm-1)

File # 1 = 1,4-DIIMIDAZOLYBENZENE

Paged Z-Zoom SCROLL

8/22/04 16:24 PM Res=4 cm-1



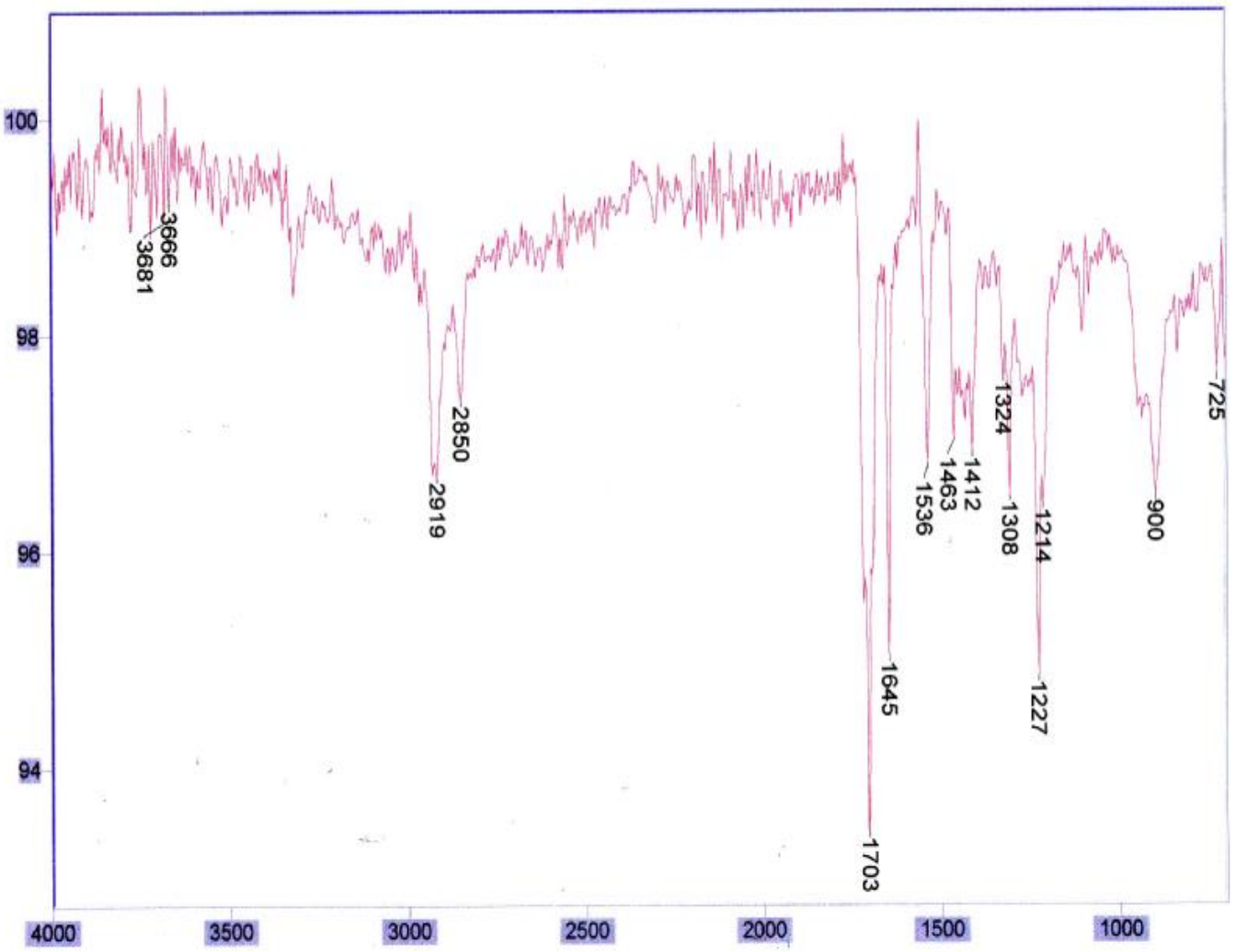
Transmittance / Wavenumber (cm-1)

File # 1 - 1,4 DIIMIDAZOLYLBENZENE

Paged Z-Zoom SCROLL

8/22/04 16:12 PM Res=4 cm-1

3CAm1C(O)2



Transmittance / Wavenumber (cm-1)

File # 1 = GANDOUR

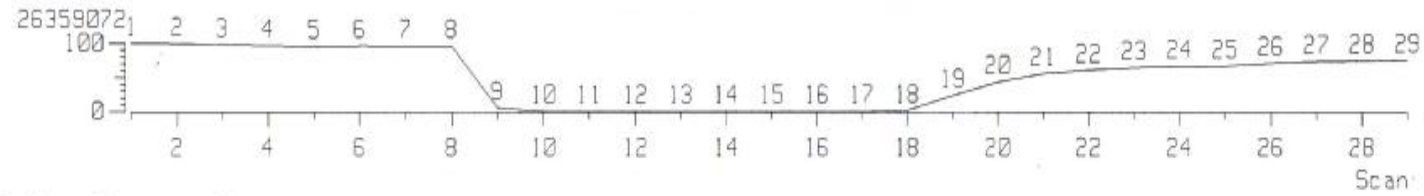
Paged Y-Zoom CURSOR

7/27/04 11:57 PM Res=8 cm-1

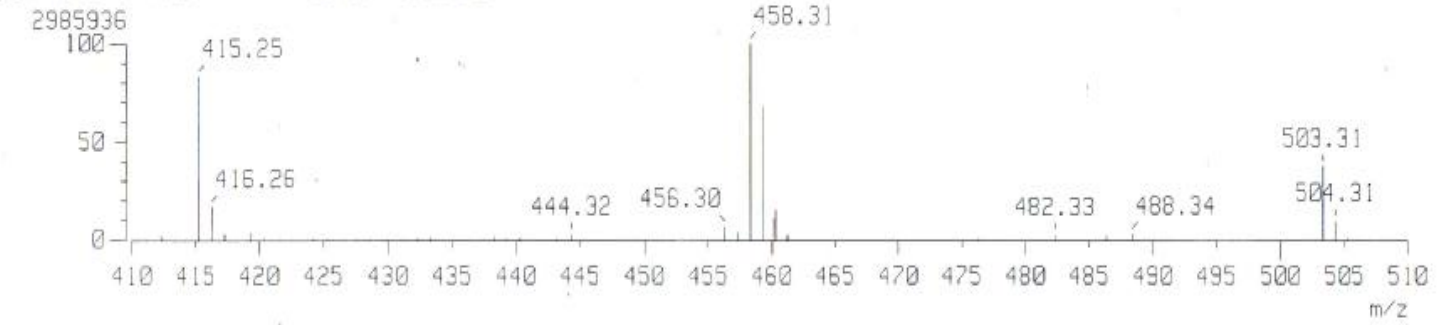
Appendix C

Mass Spectrum

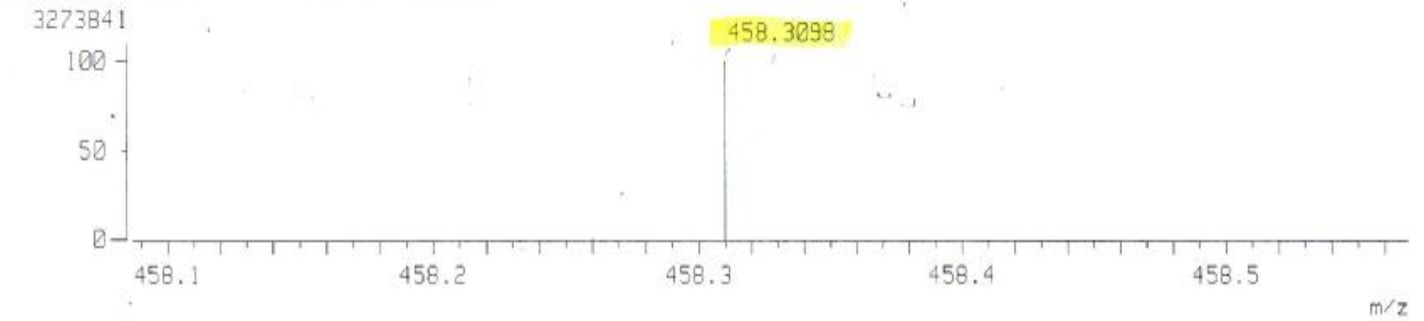
[TIC]
Data : BB01028-001 Date : 20-Jul-104 10:09
Sample: C6-tri acid 20Jul04
Note : S. Tu C24H43NO7 NBA PEG
Inlet : Direct Ion Mode : FAB+
Ion Species : Normal Ion [EF-Linear]
TIC Range : m/z 410 to 510 Output RT Range : 0.00 to 5.60 min



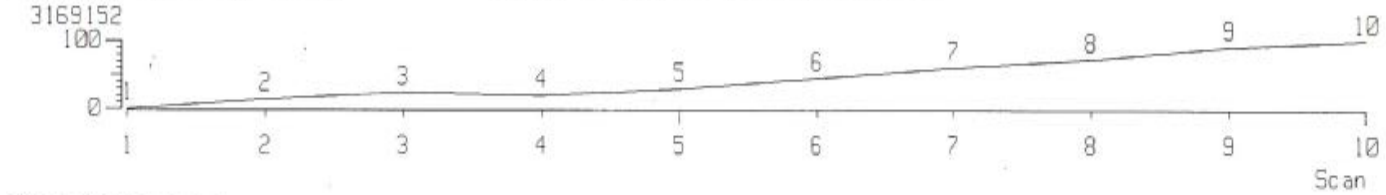
[Mass Spectrum]
RT : 4.70 min Scan# : (21,28) Temp : 52.5 deg.C
Ion Mode : FAB+ Int. : 35.60



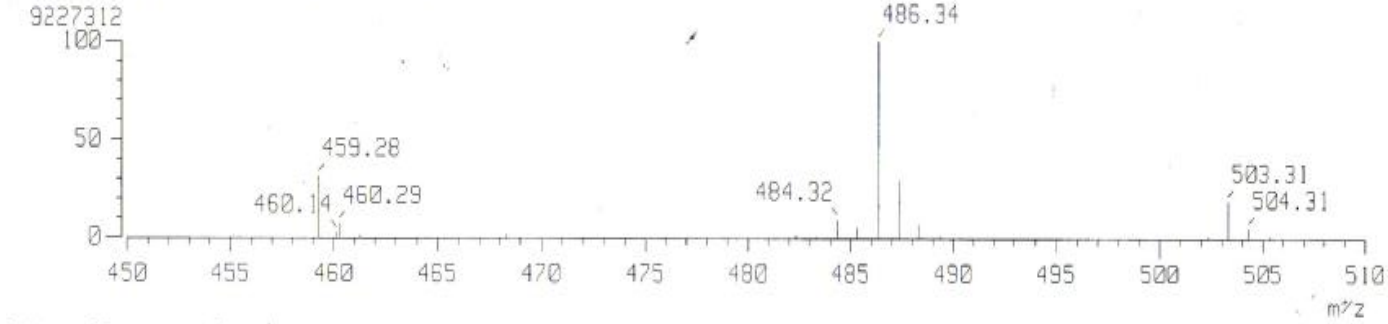
[Mass Spectrum]
RT : 4.70 min Scan# : (21,28) Temp : 52.5 deg.C
Ion Mode : FAB+ Int. : 35.60



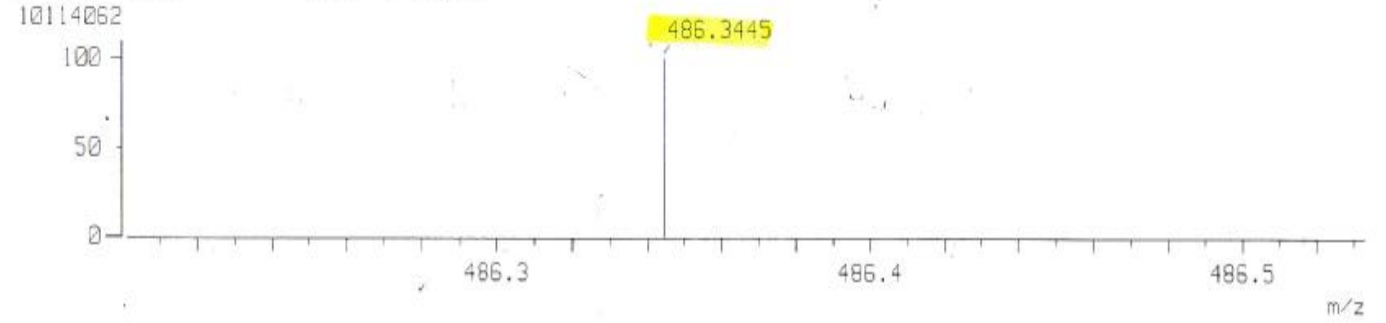
[TIC]
Data : BB01028-002 Date : 20-Jul-104 10:33
Sample: C7-tri acid 20Jul04
Note : S. Tu C26H47N07 NBA PEG
Inlet : Direct Ion Mode : FAB+
Ion Species : Normal Ion [EF-Linear]
TIC Range : m/z 450 to 510 Output RT Range : 0.00 to 1.80 min



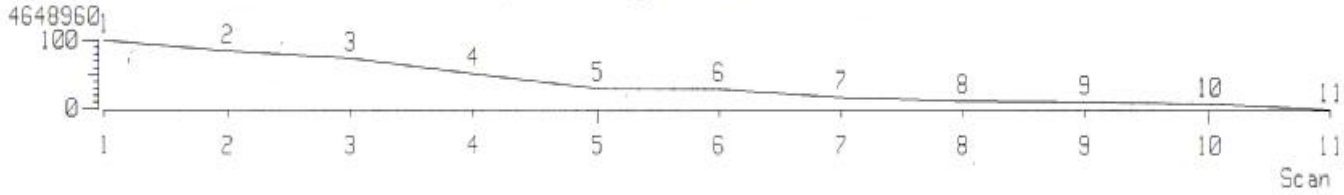
[Mass Spectrum]
RT : 0.90 min Scan# : (2,9) Temp : 52.7 deg.C
Ion Mode : FAB+ Int. : 110.00



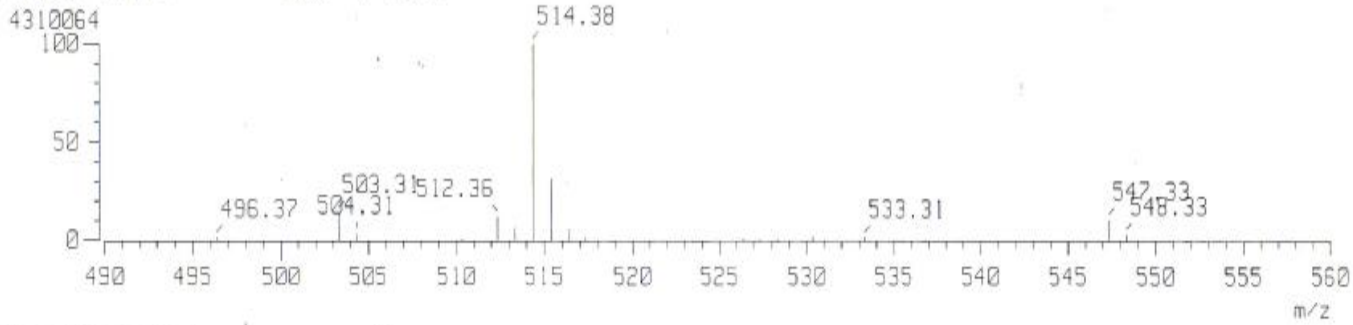
[Mass Spectrum]
RT : 0.90 min Scan# : (2,9) Temp : 52.7 deg.C
Ion Mode : FAB+ Int. : 110.00



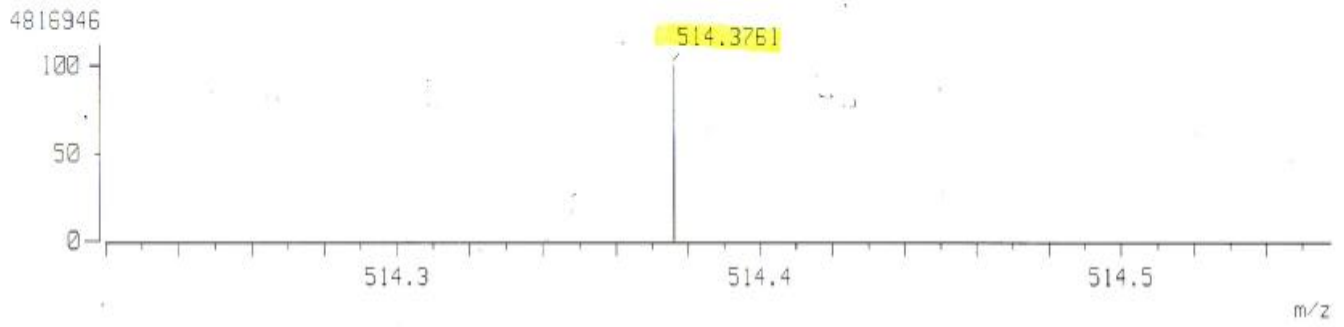
[TIC]
Data : BB01028-003 Date : 20-Jul-104 13:47
Sample: C8-tri acid 20Jul04
Note : S. Tu C28H51NO7 NBA PEG
Inlet : Direct Ion Mode : FAB+
Ion Species : Normal Ion [EF-Linear]
TIC Range : m/z 490 to 560 Output RT Range : 0.00 to 2.00 min



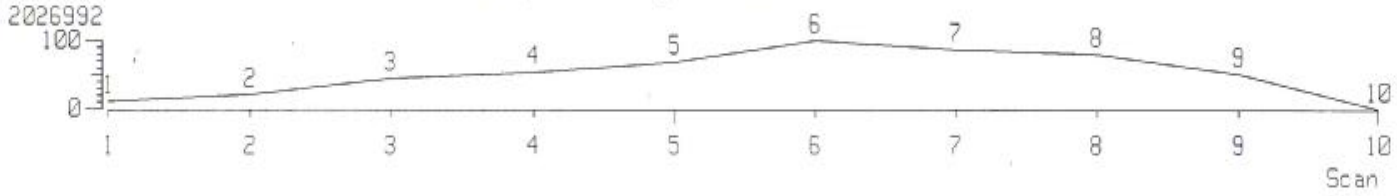
[Mass Spectrum]
RT : 0.90 min Scan# : (3,8) Temp : 53.7 deg.C
Ion Mode : FAB+ Int. : 68.51



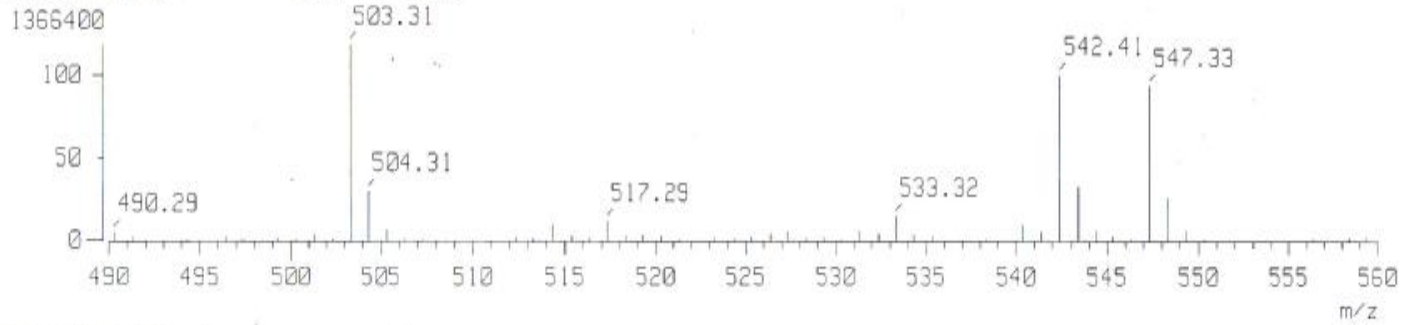
[Mass Spectrum]
RT : 0.90 min Scan# : (3,8) Temp : 53.7 deg.C
Ion Mode : FAB+ Int. : 68.51



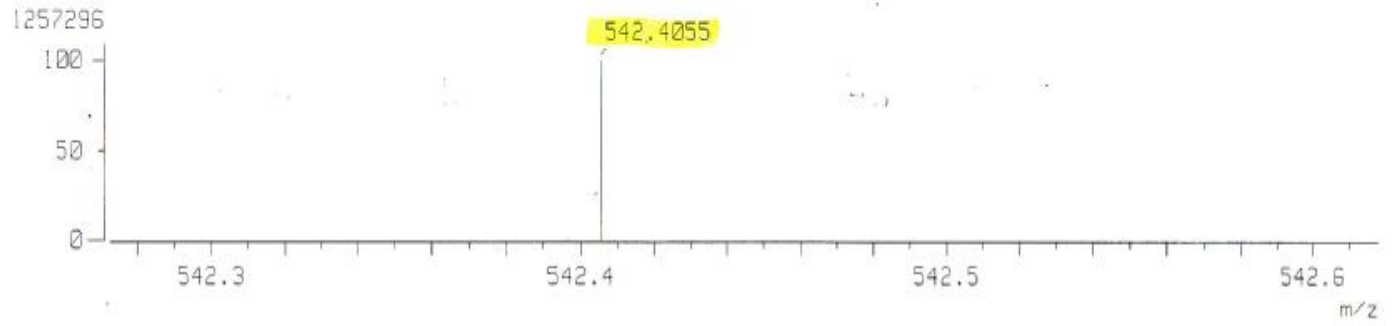
[TIC]
Data : BB01028-004 Date : 20-Jul-104 14:06
Sample: C9-tri acid 20Jul04
Note : S. Tu C30H55N07 NBA PEG
Inlet : Direct Ion Mode : FAB+
Ion Species : Normal Ion [EF-Linear]
TIC Range : m/z 490 to 560 Output RT Range : 0.00 to 1.80 min



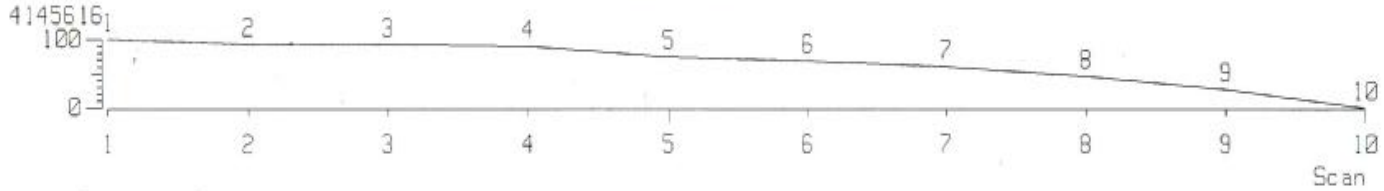
[Mass Spectrum]
RT : 0.60 min Scan# : (2,6) Temp : 53.9 deg.C
Ion Mode : FAB+ Int. : 21.98



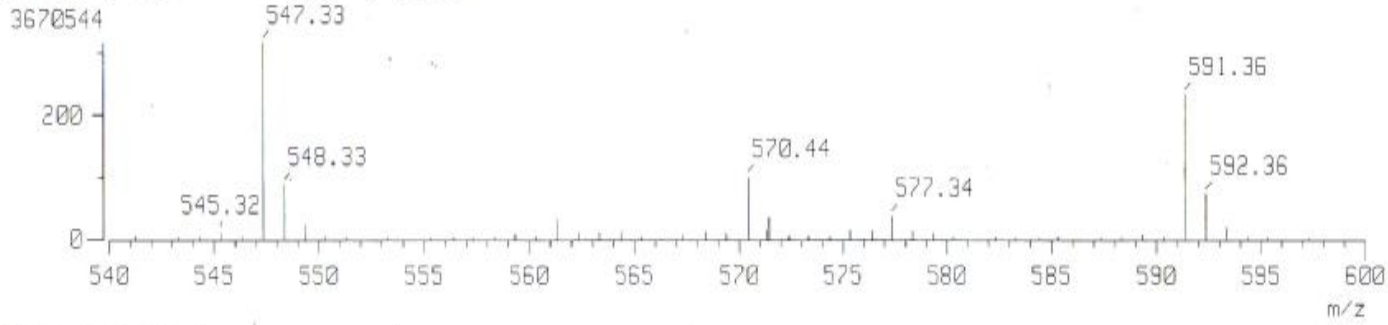
[Mass Spectrum]
RT : 0.60 min Scan# : (2,6) Temp : 53.9 deg.C
Ion Mode : FAB+ Int. : 21.98



[TIC]
Data : BB01028-005 Date : 20-Jul-104 14:24
Sample: C10-tri acid 20Jul04
Note : S. Tu C32H59NO7 NBA PEG
Inlet : Direct Ion Mode : FAB+
Ion Species : Normal Ion [EF-Linear]
TIC Range : m/z 540 to 600 Output RT Range : 0.00 to 1.80 min



[Mass Spectrum]
RT : 0.90 min Scan# : (2,9) Temp : 54.1 deg.C
Ion Mode : FAB+ Int. : 13.84



[Mass Spectrum]
RT : 0.90 min Scan# : (2,9) Temp : 54.1 deg.C
Ion Mode : FAB+ Int. : 13.84