# THE DESIGN AND SYNTHESIS OF PEPTIDOMIMETIC HYBRIDS: EXPANDING SPIROLIGOMERS, PEPTOIDS AND PROLINE

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In Partial Fulfillment
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DOCTOR OF PHILOSOPHY

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by
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## ABSTRACT

Binding to protein surfaces or shallow grooves with synthetic molecules poses a unique challenge, since this inherently requires large areas to facilitate interactions. Peptoids have been shown to interact with proteins, and combinatorial libraries of peptoids have been proven to be effective in discovering new ligands for protein binding. Unfortunately, most peptoids are flexible and lack the surface area required to compete with larger protein interactions. To combat these problems, we have created spiroligomers that have a rigid backbone, exhibit functionality comparable to proteins, and are resistant to proteases. To facilitate the rapid installment of spiroligomers into peptoid subunits, we required a more streamlined approach for functionalization of spiroligomers. To this end we applied a single-pot alkylation method, with which we installed over 25 unique functional groups onto different spiroligomer hydantoins. These spiroligomer hydantoins are spirocycles that possesses two stereocenters, of which we have complete control, as well as a protected proline amino acid. These new proline amino acids (enhanced prolines) have been incorporated into peptides via Fmoc-SPPS. Finally, we have functionalized these enhanced proline residues with another functional group and a protected primary amine, which allow for their use in peptoid synthesis. We developed methods to tether multiple spiroligomers together utilizing a peptoid backbone, as well as being able to incorporate spiroligomers into peptoid macrocycles. These spiroligomer-peptoid hybrids are large, diverse, and preorganized structures that have a large potential interacting surface area for binding to protein surfaces or shallow grooves.

## ACKNOWLEDGMENTS

This work was a long time in the making, and as such, there are several people who deserve recognition for helping me along the way. I would like to first thank my advisor, Chris: Your support and drive to always have us performing the highest caliber of chemistry, and instilling the idea of purpose to our research, has led me to be a more thoughtful scientist. I will continue with these ideals throughout my career.

I could not have done any of this work without the rest of the Schafmeister Group. Conrad and Jae Eun, you both have been instrumental in the success of the group, and me in particular. Whether it was reading drafts of papers and presentations, or helping to "Chem-Support" the problems that naturally arise in lab, you have been an invaluable source of help throughout this journey. Along with the technical help you have both provided, the friendship we have shared throughout these years has been a wonderful experience. That goes for the rest of the Schafmeister Group as well: Kavitha, Henry, Fan, Steven, Melody, and all those who came before you – thank you for making it easier to come to lab every day. Long days (or weeks, or months, or years!) are easier with friends working by your side. Thank you all very much.

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- JD -

## TABLE OF CONTENTS

## LIST OF TABLES

Page

## LIST OF FIGURES

Page

## LIST OF ABBREVIATIONS

AcOH Acetic acid

Boc tert-Butoxycarbonyl

Alloc Allyloxycarbonyl

CANDO Computer-Aided Nanomaterial Design and Optimization

Cbz Carboxybenzyl

CNBr Cyanogen Bromide

DIC Diisopropylcarbodiimide

DCM Dichloromethane

DIPEA *N,N*-Diisoproylethylamine

DKP Diketopiperazine

DMAP 4-Methyldiaminopyridine

DMF *N,N*-Dimethylformamide

DMSO Dimethyl sulfoxide

EDC 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide

EtOAc Ethyl acetate

Fmoc 9-Fluorenylmethoxycarbonyl

HATU *O*-(7-azabenzotriazol-1-yl)*N,N,N',N'*-tetramethyluronium hexafluorophosphate

HBr Hydrobromic acid

HFIP Hexafluoroisopropanol

HOAt Hydroxyazabenzotriazole

HPLC High performance liquid chromatography

MALDI Matrix assisted laser desorption/ionization

MeCN Acetonitrile

MeOH Methanol

NMP *N*-Methyl-2-pyrrolidone

OBOC One-bead one-compound

PPI Polyproline Type I

PPII Polyproline Type II

PPIs Protein-protein interactions

PyAOP (7-Azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate

SPPS Solid-phase peptide-synthesis

*t*-Bu *tert*-Butyl

TEA Triethylamine

TFA Trifluoroacetic acid

THF Tetrahydrofuran

TIPS Triisopropylsilane

## GENERAL EXPERIMENTAL METHODS

- Anhydrous solvents including dichloromethane (DCM), *N,N*-dimethylformamide (DMF), *N*-methyl-2-pyrrolidone (NMP), methanol, and acetonitrile were purchased from Acros Organics. Reagent grade solvents including DCM, DMF, methanol, isopropanol (IPA), toluene, hexane, ethyl acetate, di-ethyl ether, and acetone were purchased from Fisher Scientific, as was sodium chloride, sodium bicarbonate, ammonium chloride, sodium sulfate and magnesium sulfate. Hydrobromic acid (33 wt% in glacial acetic acid), sodium cyanoborohydride, *N,N'*-diisopropylcarbodiimide (DIC), and ammonium carbonate were purchased from Acros Organics. Diisopropylethylamine (DIPEA), triethylamine ($Et_3N$), chromium (VI) oxide, benzyl chloroformate (Cbz-Cl), piperidine, 1,4-dioxane, potassium cyanide (KCN) and all aldehydes and ketones were purchased from Sigma-Aldrich. 1-Hydroxyl-7-azabenzotriazole (HOAt) and *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), and 9-Fluorenylmethyl N-succinimidyl carbonate (Fmoc-Osu) were purchased from Genscript. 4-Dimethylaminopyridine (DMAP) was purchased from EMD Millipore Co. (Novabiochem®). Natural/unnatural, protected/unprotected amino acids were purchased from EMD Millipore Co. (Novabiochem®) or Bachem. Resins for solid phase synthesis were purchased from Genscript or Rapp Polymere. Trifluoroacetic acid (TFA), di-tert-butyl dicarbonate ($Boc_2O$), (7-Azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyAOP), and all the electrophiles used in Chapter 2 were purchased from Oakwood Products or

Combiblocks. All compressed gases were purchased from Airgas, Inc. All chemicals were used as received, without further purification.

- Ultra-performance liquid chromatography - mass spectrometry (UPLC-MS) analysis was performed on an Agilent 1290 liquid chromatography system with a Supelco Ascentis® Express C18 column (2.7 μm packing, 2.1 x 50 mm) using a water-acetonitrile gradient solvent system containing 0.1 % formic acid at a flow rate of 1.0 mL/min, coupled to an Agilent 6120 single quadrupole mass spectrometer that utilizes electrospray ionization. UPLC grade solvents were purchased from Fisher Scientific.

- Nuclear magnetic resonance (NMR) experiments were performed on either a Bruker Avance 500 MHz instrument or a Bruker 400 MHz instrument. Deuterated solvents were purchased from Acros Organics.

- Analytical thin-layer chromatography (TLC) was performed on glass-backed, pre-coated silica gel plates (250μm thick) purchased from Sorbent Technologies. TLC plates were visualized by UV light and/or chemically stained.

- Normal-phase purifications were performed on an ISCO (Teledyne, Inc.) automated flash chromatography system using various sizes of pre-packed RediSep® silica gel columns or manually packed columns using silica gel (60Å porosity, 230 x 400 mesh particle size) purchased from Sorbent Technologies.

- Reverse-phase purifications were performed on an ISCO (Teledyne, Inc.) automated flash chromatography system using various sizes of pre-packed

RediSep® reverse phase columns using a water-acetonitrile gradient solvent system containing 0.1 % formic acid.

- Self-preparative reverse-phase HPLC purifications were performed on a Hewlett Packard 1100 Series liquid chromatography system with a Waters XTerra® Prep C18 column (10 μm packing, 10 x 150 mm) using a water-acetonitrile gradient solvent system containing 0.1 % formic acid at a flow rate of 5.0 mL/min. Peak collection was preformed manually.

- High-resolution mass spectrometry was performed on an Agilent 1260 liquid chromatography system with a Phenomenex Kinetex® C18 column (2.6 μm packing, 2.1 x 50 mm) using a water-acetonitrile gradient solvent system containing 0.1 % formic acid at a flow rate of 1.0 mL/min. For direct injection into the mass spectrometer, a 1:1 water-acetonitrile solvent system was used at a flow rate of 0.5 mL/min and the column was bypassed. This system is connected to an Agilent 6520 quadrupole time-of-flight (QTOF) mass spectrometer that utilizes electrospray ionization. UPLC grade solvents were purchased from Fisher Scientific.

- MALDI was performed on a Bruker Autoflex TOF/TOF mass spectrometer

- Optical rotations were measured on a Perkin-Elmer 341 polarimeter.

- Microwave reactions were carried out utilizing an Anton Paar Monowave 300

# CHAPTER 1

# INTRODUCTION

## 1.0 Nature's Inspiration: Peptides, Proteins, and amino acids

Proteins, the macromolecular building blocks of life, come in a variety of forms with a multitude of functions in nature. Some are used for signaling and stimulus response in signal transduction pathways, like hormones and their receptors such as G-protein coupled receptors, or receptor tyrosine kinases,[1-4] others such as polymerases for creating new molecules, others such as proteases for breaking down old molecules,[6] chaperonins for assisting in the folding and assembly of other proteins and protein structures,[7] and others such as ion channels or clathrin for molecular transport.[8,9]

Proteins are oligomers of α-amino acids that are connected through a peptide bond. The smallest structured protein is a Trp-Cage (PDB ID: 1L2Y) the sequence of which is a mere 20 amino acids (residues).[10] This is opposed to the human form of Titin, the largest known protein, that has over 34,000 residues.[12,13] These varying numbers of residues available to proteins leads to a variety of structures and functions.

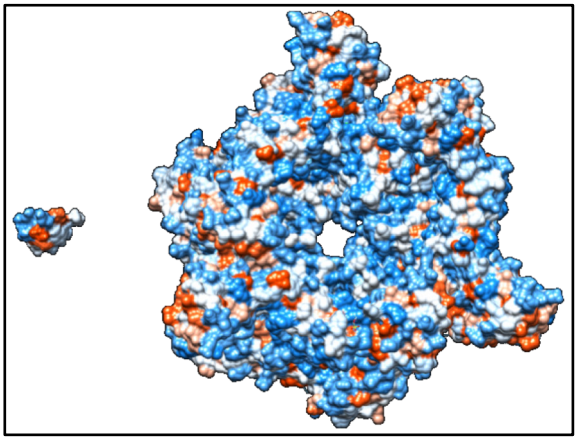

**Figure 1.1.** VDW surface size comparison of a Trp-cage (20 residues, PDB ID: 1L2Y) vs. DNA Helicase (454 residues, PDB ID: 4ESV)

Protein structure is hierarchical, and for the most part, proteins need structure in order to have function. The primary structure of a protein is its amino acid sequence (Figure 2A). Protein primary structures assemble into secondary structures (Figure 2B): α-helices (Red), β-sheets (Blue) and random coil (Beige), that form due to intrachain hydrogen bonds between the carbonyl of an amino acid residue and the hydrogen of a nearby amide. These secondary structures in turn assemble into a protein's tertiary structure; that is, the three-dimensional shape of a single protein (Figure 2C). Tertiary structure is usually dictated by sequestering the hydrophobic residues into the interior, while hydrophilic residues will mainly populate the exterior of the protein. Other factors that assist in the stabilization of a protein's tertiary structure include salt-bridges, disulfide bonds, side-chain hydrogen bonds, and even post-translational modification (modifications carried out on a protein by other proteins that affects the shape or function of the protein being modified.) Finally, multiple proteins (subunits) can interact together to form a quaternary structure (protein complex) that functions as a single unit (Figure 2D). Protein tertiary and quaternary structure is not

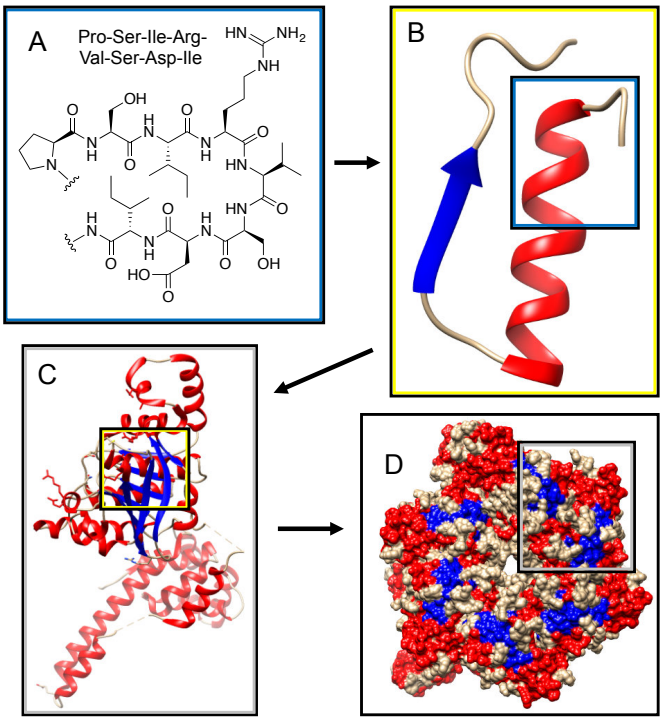


**Figure 1.2:** Protein structure from a primary sequence through a quaternary structure, utilizing DNA Helicase as an example (PDB: 4esv)

entirely rigid, though, as proteins can adopt different conformations in order to carry out their function. These conformational changes can be brought about by binding a substrate for catalysis or through interactions with other proteins.[14]

For decades now, scientists have been discovering and decoding numerous biologically important proteins, from hormones, receptors and neuropeptides to ion channels and enzymes. As they serve a plethora of functions in nature, proteins have tended to be the targets of therapeutics, which most drugs on the market would show.[15,16] There are two major classes of drugs on the market today, small molecules (drugs with a molecular weight <1,000 Da) and protein based therapeutics. Small molecule drugs are typically hydrophobic, have few to no stereocenters, bind into pockets or deep grooves on a protein, and are usually tailored to be membrane permeable. As they are relatively small, their potential surface area available for protein interactions will be as well. Protein based therapeutics, on the other hand, can contain many thousands of atoms. Their large size means that protein based therapeutics would have an advantage when targeting large surface interactions; however, these proteins are not generally cell permeable. This is important, as only about 3,000 of the approximately 25,000 proteins encoded in the human genome would contain a hydrophobic pocket that would be suitable for small molecule binding, yet studies also predict that less than 2,500 proteins are extracellular and able to be targeted by protein therapeutics.{Verdine:2007gd} Furthermore, attempting to interact with or disrupt protein-protein interactions (PPIs) with small molecules is a well-documented challenge.[17-19] Peptide based therapeutics also have several drawbacks. Peptides can lead to the development of immune responses, as well as possessing low metabolic stability due to rapid proteolysis and poor

gastrointestinal absorption. This means that they are usually degraded before they can even interact with their target.[20,21] Therefore, it is important to find molecules that can bridge this gap between small molecules and protein based therapeutics, these so called "undruggable" targets, that make up the majority of proteins in the human genome.

### 1.1 Mimicking Nature with Peptidomimetics and Foldamers

Due to the poor pharmacokinetics of peptides, much work has been devoted to developing peptidomimetics and foldamers, molecules that mimic the structure and function of peptides, but have much better pharmacokinetics than peptides. There are more peptidomimetics than can be discussed here, but some of the ones to be discussed are D-peptides, β-peptides, Stapled Peptides, Peptoids, and Spiroligomers.

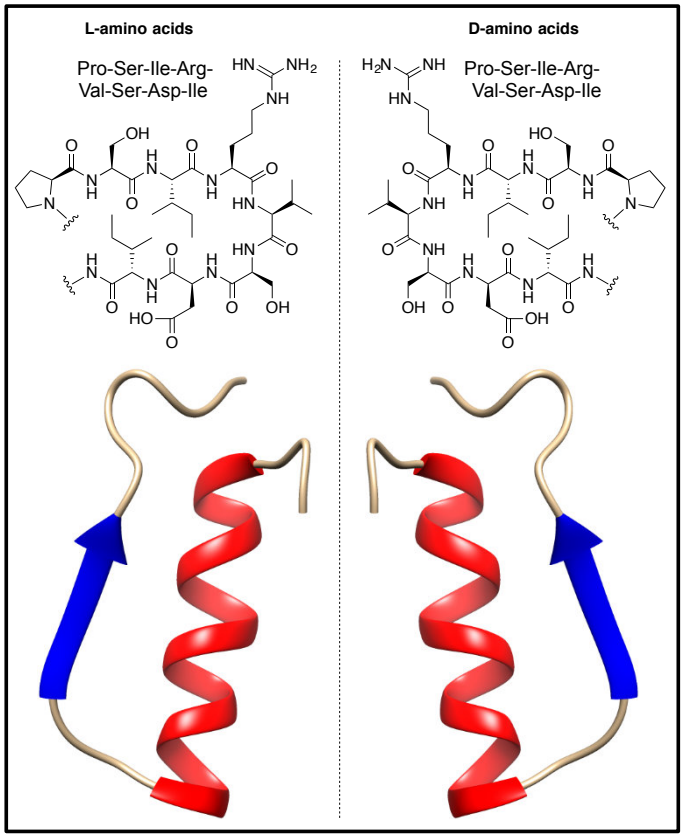


**Figure 1.3.** An L-amino acid sequence and partial peptide structure is shown vs. its mirror image D-amino acid sequence and structure

Peptides composed of D-amino acids, as opposed to the natural L-amino acids, were some of the first peptidomimetics to be explored, with work done contemporaneously with peptoids and β-peptides. A peptide consisting of only D-amino acids would be a mirror image of the peptide containing only L-amino acids.

Furthermore, a protein consisting of D-amino acids would have reciprocal substrate specificity compared to its L-amino acid counterpart, as shown by Milton et al.[22] D-amino acids and peptides rarely occur in nature, which makes them an attractive candidate as a peptidomimetic for drug design as the human body is not able to readily metabolize D-peptides. Not only are they resistant to proteases, some have also been shown to be orally bioavailable.[23] Conversely, D-peptides or peptides containing D-amino acids do run the risk of invoking an immune response

β-Peptides are proteolytically stable oligomers composed of β-amino acids, as opposed to the natural α-amino acids (one β-amino acid, β-alanine, is naturally occurring). β-amino acids insert an additional carbon between the carbonyl and the nitrogen of an α-amino acid. β-peptides still contain a secondary amide, and thus maintain the ability to form backbone hydrogen bonds.

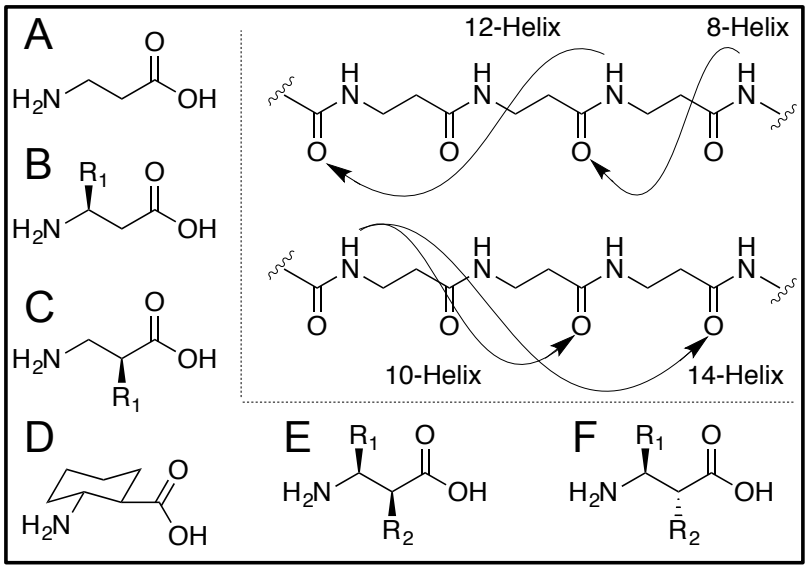


**Figure 1.4. β-peptide residues and helical types** (A) unsubstituted residue (B) $\beta^3$-residue (C) $\beta^2$-residue (D) $\beta^{2,3}$-residue cyclic, trans configuration (E) $\beta^{2,3}$-residue anti configuration (F) $\beta^{2,3}$-residue syn configuration (G) unsubstituted β-peptide highlighting the 8, 10, 12, or 14-helices

The ability for backbone hydrogen bonding allows for numerous helical conformations, such as the 8-Helix, 10-Helix, 12-Helix, 14-Helix, or 10/12-Helix, each of which is favored by different residues (the number preceding helix refers to the number of atoms

in the hydrogen bonded rings). For instance, the residues in Figure 4B and Figure 4D adopt 14-helix shapes,[24-26] whereas oligomers of trans-2-amino-cyclopentanecarboxylic acid (not shown) tend to form 12-helix structures.[26-28] β-peptides have been tested for numerous antibiotic and antimicrobial effects,[29,30] as well as their ability to bind α-peptidic receptors such as somatostatin.[29,31]

Stapled peptides are α-helical peptides that have been chemically braced via a macrocyclic bridge across a portion of the α-helix. They are synthesized via typical Fmoc-SPPS, followed by ring-closing metathesis (RCM) between two unnatural residues bearing olefins.[32,33] Schafmeister and Verdine employed two complementary chemical modifications to stabilize the α-helix, one being the hydrocarbon macrocyclic bridge and the other α-α-disubstitutions. These chemical modifications of a peptide stabilize a native α-helical shape. This confers a 5- to 5000-fold increase in target affinity, allows for cell-penetration via endocytosis, and affords resistance to proteases increasing *in vivo* half-life.[19] As such, stapled peptides have been deployed successfully to target numerous PPIs: reactivation of the p53 tumor suppressor pathway,[34] inhibition of the NOTCH transcription factor complex,[35] analysis and manipulation of BCL-2

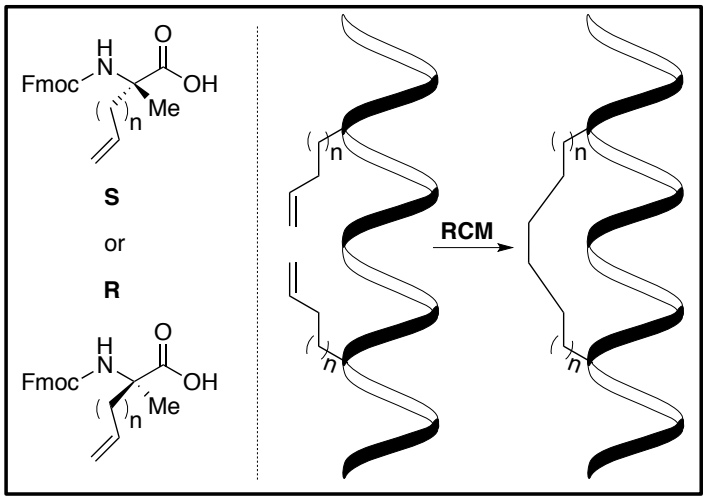


**Figure 1.5.** Residues utilized for peptide stapling, and a cartoon depicting a stapled peptide.

family interactions,[36] inhibitor of MCL-1 and sensitization to apoptosis,[37] and a potential HIV-1 inhibitor.[38]

Peptoids are oligomers of poly-N-substituted glycine, which means the side chain is attached to the nitrogen instead of the side chain being attached to the α-carbon as with α-amino acids. By moving the side chain from the α-carbon to the nitrogen (in relation to a typical peptide), the synthesis can be achieved via a submonomer approach utilizing *in situ* generated bromoacetic anhydride followed by a primary amine, which allows for an easy and efficient synthesis.[39] Moving the side chain from the α-carbon to the nitrogen, which removes the backbone chirality, as well as any inter- or intra-chain hydrogen bonding of the backbone, leads to increased flexibility of the backbone.[40-42] While this makes the synthesis of peptoids much easier than peptides, this means that peptoids are more reliant on their side chain functionality for inter- and intrachain interactions, as well as for any structural motifs that can be imparted by the limited chirality of the side chains. Most peptoids are synthesized using small, primary amines that are available for purchase from most chemical manufacturers. There are a large number of primary amines commercially available for peptoid synthesis, but relatively few of them contain chiral centers. Peptoids have been studied for decades, and

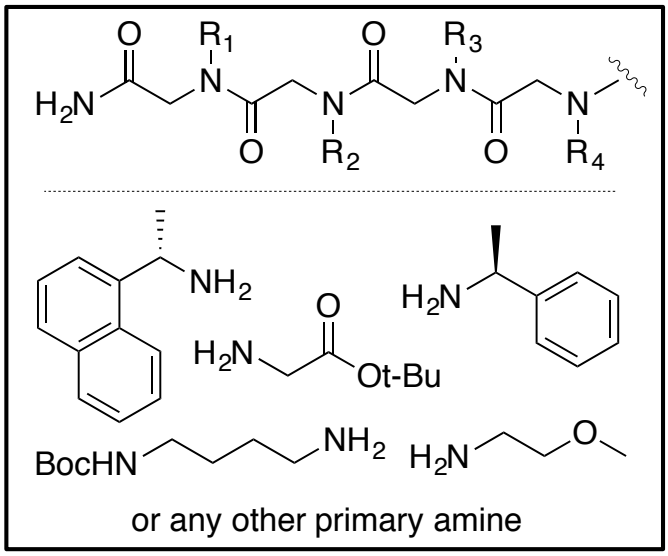


**Figure 1.6.** A theoretical peptoid and some stereotypical amines utilized for peptoid synthesis.

therefore have been utilized for a wide variety of purposes: Biologically active peptoids ranging from protein secondary structure mimetics[43-47] to antimicrobial agents[48-50] and other therapeutics;[51] nanomaterials such as nanotubes[52,53] and nanosheets;[54,55] and metal binding peptoids;[56] among many others as evidenced by these excellent reviews.[57-59]

One of the drawbacks with most peptidomimetics is the reduced ability for prediction of tertiary structure. Not knowing what shape a peptidomimetic oligomer will take after it is synthesized means a lot of work can be spent on developing molecules that, in the end, do not display functional groups in the correct orientation to mimic a groove or active site. In an attempt to solve this problem, the Schafmeister Group has developed Spiroligomers. Spiroligomers are peptidomimetic oligomers of bis-amino acids (Figure 1.7A) that are connected through a pair of amide bonds (Figure 1.7B-D), as

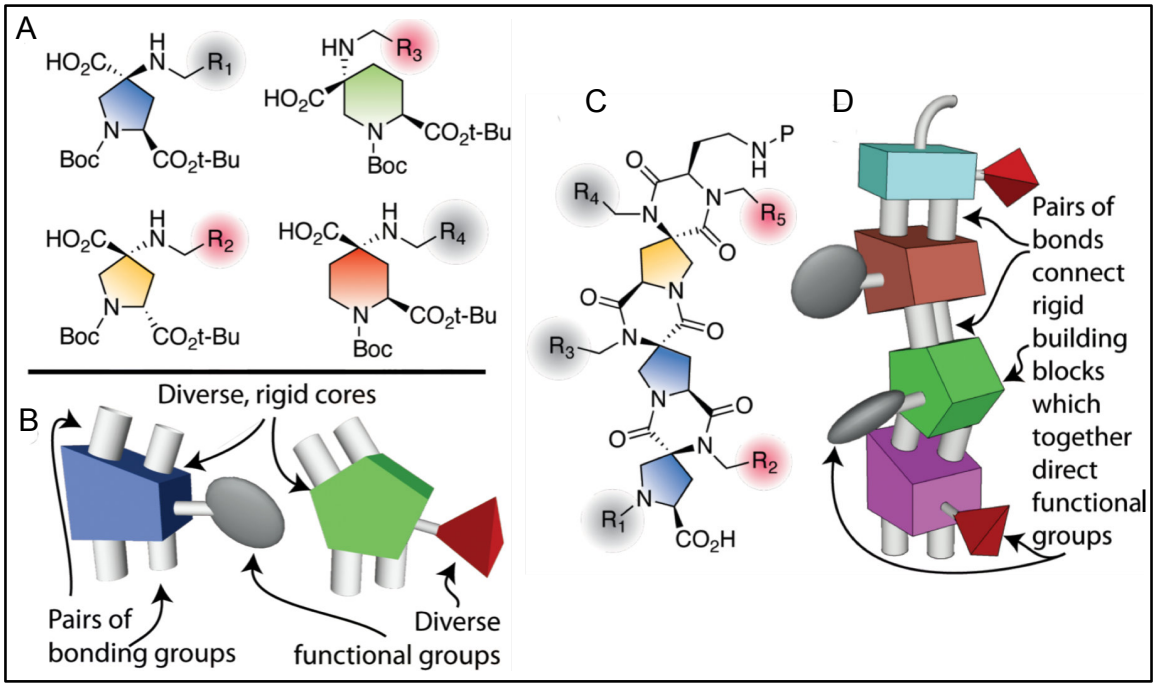


**Figure 1.7.** (A) Four bis-amino acid monomers are shown (B) Cartoon depiction of bis-amino acid monomers (C) A theoretical spiroligomer trimer (D) Cartoon showing that spiroligomers can be assembled to position functional groups in three-dimensional space

opposed to a single amide bond like many peptidomimetics. This means that spiroligomers are highly preorganized when it comes to their backbone, as their fused-spirocyclic structures do not allow any free rotation throughout the backbone.[60-63] Thus, unlike other peptidomimetics, spiroligomers do not require folding in order to have a defined structure. The structural backbone of spiroligomers allows for the preorganization of a large variety of functional groups in three-dimensional space, and is one of the most useful aspects of spiroligomers.

The most commonly used spiroligomer bis-amino acid building blocks are synthesized from *trans*-4-hydroxyproline on a 600 mmol scale (Figure 1.8).[60,64,65]

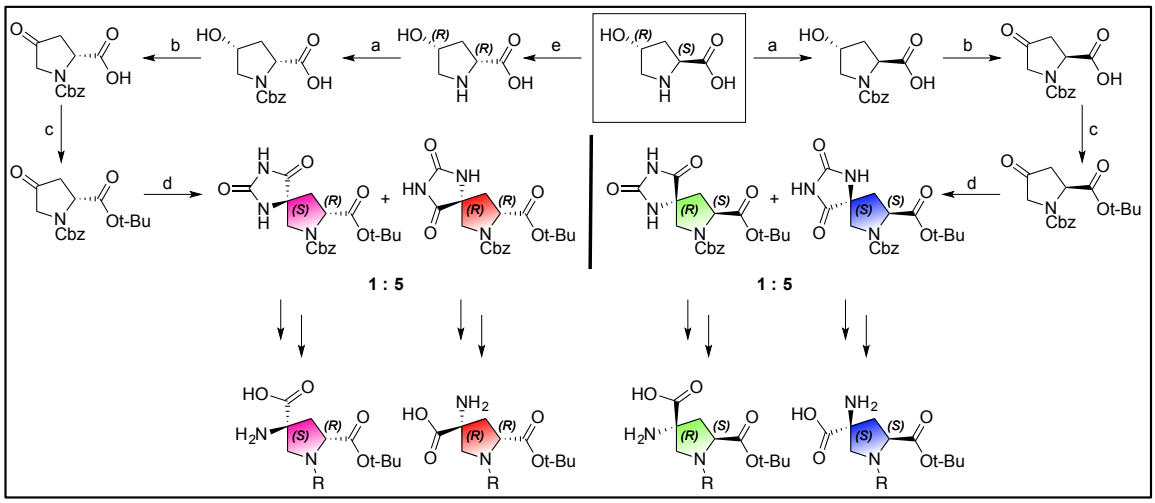


**Figure 1.8.** Synthesis of the four most commonly used spiroligomer monomers from a single *trans*-4-hydroxyproline feedstock. (a) Cbz-Cl, $NaHCO_3$, 1:10 dioxane/water (b) Jones reagents, acetone, 0 $^oC$ to rt; (c) Isobutylene, $H_2SO_4$ (cat.), $CH_2Cl_2$, 0 $^oC$ to rt; (d) $(NH_4)_2CO_3$, KCN, 1:1 EtOH/water, sealed flask; (e) 1. $AcOH/Ac_2O$, Reflux; 2. 2M HCl, reflux; 3. Recrystallization

Spiroligomers also benefit from having a large pool of functional groups that can be incorporated onto the spiroligomer backbone, as almost any aldehyde, ketone, alkyl halide, isocyanate, or amino acid can be used as a unique functional group.[66-68] Spiroligomers have been utilized for a variety of applications such as a transesterification

catalyst,[69] a proline-aldol catalyst,[70] an aromatic claisen rearrangement catalyst,[71] a spiroligomer that binds MDM2,[72] the formation of supramolecular metal binding complexes,[73] a donor-bridge-acceptor molecule that accelerates electron transfer in water,[74] a metal binding mechanical molecular actuator,[75] and molecular rulers.[76]

### 1.2 Enhancing Spiroligomers and Proline

With spiroligomers, we are attempting to bridge the gap between small molecule drugs and protein based therapeutics. One goal of this work was to facilitate a more rapid synthesis of spiroligomers, through the use of a new hydantoin alkylation strategy that allows for derivatives of 4-hydantoin-proline to be synthesized via a direct two-step alkylation method. This method is valuable in the development of applications of N,N'-disubstituted-hydantoin prolines by improving yields, reducing the time and number of steps required to synthesize these substituted molecules, and enabling late stage functionalization of spiroligomer termini. Over 20 unique electrophiles have been tested, highlighting the inherent versatility of this chemistry. These disubstituted amino acids have also been employed as unique proline derivatives for peptide synthesis. As they are more hindered than a traditional amino acid, these residues require either a standard reactor oven or a microwave synthesizer to help elevate the temperatures to achieve complete couplings. We have incorporated two different stereochemistries of enhanced prolines, each bearing two unique functional groups, into peptides.

These new enhanced proline derivatives can be further functionalized to bear another functional group as well as a protected primary amine. By installing this primary amine, we were able to incorporate several variants of spiroligomers into a new hybrid foldamer of spiroligomers and peptoids, which can be utilized for normal peptoid

synthesis or for peptoid macrocycles. Each spiroligomer is the size of a small molecule drug, so installing three or more spiroligomers onto a peptoid backbone will quickly build up the required surface area to enable interactions with protein surfaces and PPIs.

The contributions outlined herein have led to the development of chemistry that facilitates the rapid synthesis of spiroligomers, the synthesis of a new enhanced proline amino acid, and the synthesis of new peptidomimetic hybrids that are amalgams of both spiroligomer and peptoid. These advances should allow for the expedited discovery of new spiroligomers and spiroligomer-peptoid hybrids that could be used to target the undruggable targets.

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# CHAPTER 2

# ALKYLATION OF 4-HYDANTOIN-PROLINE DERIVATIVES

This chapter describes the synthesis of imidazolidine-2,4-dione (hydantoin) derivatives from a direct two-step alkylation method. This method is valuable in the development of applications of N,N'-disubstituted hydantoin bearing α-amino acids by improving yields, reducing the time and number of steps required to synthesize these substituted molecules, and enabling late stage functionalization of spiroligomer termini. Over 20 unique electrophiles have been tested, highlighting the inherent versatility of this chemistry, and this method has been applied for each subsequent chapter to facilitate the rapid synthesis of a variety of spiroligomers.

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## 2.0 Introduction

Substituted imidazolidine-2,4-dione (hydantoin) derivatives are currently being explored by many groups as biologically-active small molecules. Substituted hydantoins have been used for a wide array of applications, such as the anticonvulsant phenytoin,[1-4] antiproliferative agents,[1] and potential antitumor agents,[3,5,6] et al.[7,8] Similarly, the use of chiral, multifunctional, fused-ring spirocycles has been documented in the past, and a great deal of work is being done in this area.[1,9] In our lab, we have utilized both

diketopiperazines[10] and substituted hydantoins for the termini of spiroligomers,[11,12] and for creating bifunctional prolines with a variety of applications. These include a transesterification catalyst,[11] a proline-aldol catalyst,[13] and a template for metal complexes[12] (see Figure 2.1).

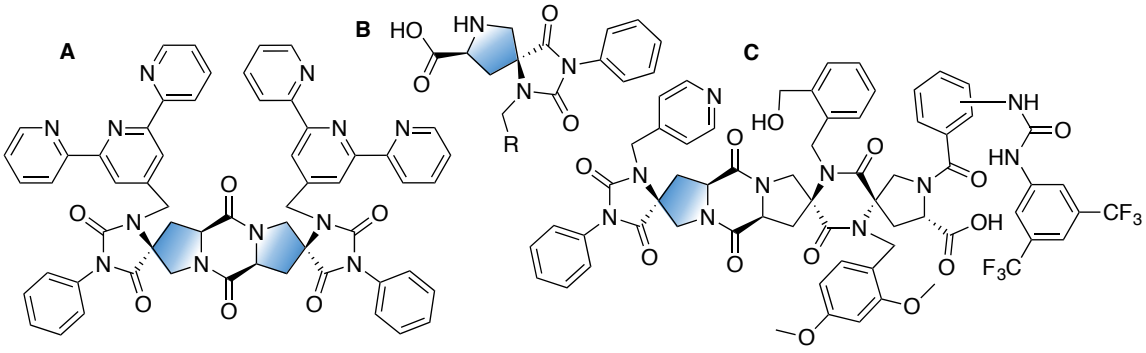
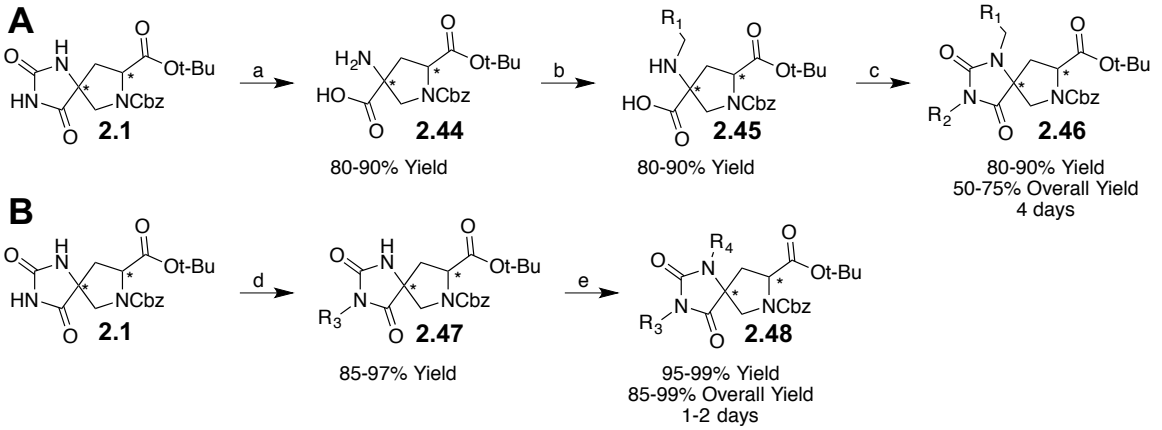



**Figure 2.1**. Three spiroligomers that we have synthesized utilizing substituted hydantoins on their termini. (A) A Mn/Zn binding scaffold; (B) a modified proline-aldol catalyst; (C) a transesterification catalyst.

We have also utilized the similar diketopiperazine scaffold for a donor-bridge-acceptor electron transfer model system[14], an aromatic Claisen rearrangement catalyst,[15] and as the end cap for a variety of spiroligomers.[10,16,17] The previous method for synthesizing hydantoin functionalized proline derivatives is shown in Scheme 2.1A involved synthesis of a protected 4-[imidazolidine-2,4-dione]-proline (aka 4-hydantoin-proline, **2.1**), followed by hydrolysis of the 4-[imidazolidine-2,4-dione] group and precipitation to give the bis amino-acid **2.44**. This was followed by a reductive amination to install a functional group on what would become the amide nitrogen of the hydantoin, followed by precipitation to give **2.45**. Finally, the hydantoin was reformed using a variety of isocyanates to make **2.46**. From the 4-hydantoin-proline, this involved a five-

step synthesis with modest yields (50-75%, dependent on the aldehyde and isocyanate utilized). In addition to the modest yields, there are a limited number of isocyanates available commercially for use in functionalization.




**Scheme 2.1** (a) i. $Boc_2O$, DMAP, THF ii. 2 M KOH; (b)i. MeOH, $R_1CHO$ ii. $NaCNBH_3$; (c) $R_2NCO$, TEA, THF; (d) $R_3$-X, DMF, $K_2CO_3$; (e) $R_4$-X, DMF, $K_2CO_3$

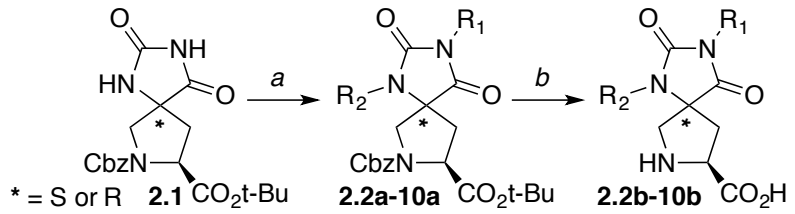
By developing a method for direct alkylation of a hydantoin (Scheme 2.1B), we have streamlined the synthesis of these bifunctionalized, hydantoin bearing spiroligomer building blocks and modified prolines. These building blocks can be obtained more rapidly and in higher isolated yields from a single pot reaction using the same starting material as our previous approach. By exploiting the differing reactivity between the imide and amide nitrogen of the hydantoin,[18] we have expanded this direct alkylation to enable a single-pot method for installing different side chains onto each nitrogen of the hydantoin. This alkylation method has fewer manipulations with higher isolated yields for each manipulation compared to our previous method for the synthesis of substituted 4-hydantoin-proline derivatives, and depending on the subsequent steps involved, does not require purification of the substituted intermediates. The loss in yield shown to make **2.47** in Scheme 2.1B comes from halting the reaction before unwanted dialkylation

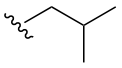
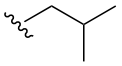
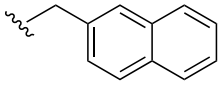
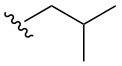
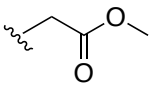
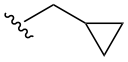
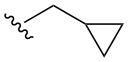
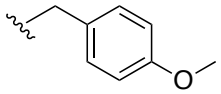
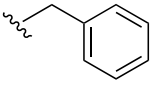
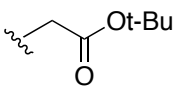
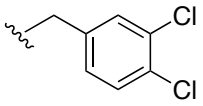
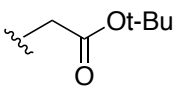
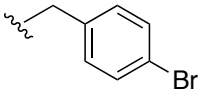
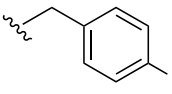
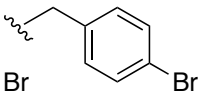
occurs, as the monoalkylated **2.47** is more easily separated from **2.1** and less easily separated from the dialkylated hyndantoin **2.48**. The separated starting material **2.1** can then be reused for other syntheses, thus negating the loss in yield. We have successfully attached alkyl, allyl and benzyl halides to both the imide and amide position of the hydantoin. The application of this alkylation method to the synthesis of spirocyclic ring systems allows the utilization of these highly diverse bifunctionalized 4-hydantoin-proline derivatives as small molecules or as the termini of larger spiroligomer structures.[19] Herein we detail the synthesis of 70 of the thousands of potential variants of these multi-substituted 4-hydantoin-proline derivatives. This chemistry will be applied throughout this thesis, highlighting its versatility for spiroligomer synthesis.

## 2.1 Alkylations to 4-Hydantoin Proline Derivatives

Starting from the known compound **2.1**, we were able to synthesize each difunctionalized hydantoin monomer in good to excellent yields in a one-pot synthesis, as shown in Scheme 2.2**.** It is worth noting that compound **2.1** is purified with a single column, and during this purification, unreactive impurities coelute with **2.1**. Further purification is unnecessary for the following alkylations, but to have the correct stoichiometry for an alkylation, the amount of inert impurities contained in a given batch of **2.1** must be determined. Using toluene or maleic acid as an internal standard, 1D NMR is used to quantitate the amount of hydantoin to the internal standard by integrating the standard relative to the hydantoin protons, and with this knowledge applied to determining the stoichiometry of subsequent reactions, high yields of monoalkylations are obtained. The hydantoin is typically 85% by weight of the product from the

chromatography. As noted previously, there is a differing reactivity between the imide and amide nitrogen of the hydantoin.[18] As shown in Figure 2.2, we confirmed that the first alkylation adds to the imide nitrogen of the hydantoin as opposed to the amide. This is exhibited by the benzylic protons coupling to both carbonyl carbons of the hydantoin in the HMBC, whereas the methyl protons couple to only one of the carbonyl carbons.




| Cmpd. # | * | $R_1$ | $R_2$ | % Yield |
|---|---|---|---|---|
| **2.2** | R |  |  | 97 |
| **2.3** | R |  |  | 97 |
| **2.4** | R |  |  | 97 |
| **2.5** | S |  |  | 94 |
| **2.6** | S |  |  | 94 |
| **2.7** | S |  |  | 93 |
| **2.8** | S |  |  | 92 |
| **2.9** | S |  |  | 92 |
| **2.10** | S |  |  | 89 |

**Scheme 2.2**. The general scheme for alkylation of the hydantoin, with representative examples highlighting stereochemical, side chain, and method diversity. a) i. DMF, $R_1$-X, $K_2CO_3$, time varies; ii. $R_2$-X, $K_2CO_3$, overnight; b) 1:1 DCM / (33% HBr / AcOH) to remove Cbz / t-Bu protecting groups. For compounds **2.8b** and **2.9b**, the tert-Butyl group on $R_1$ is also removed.

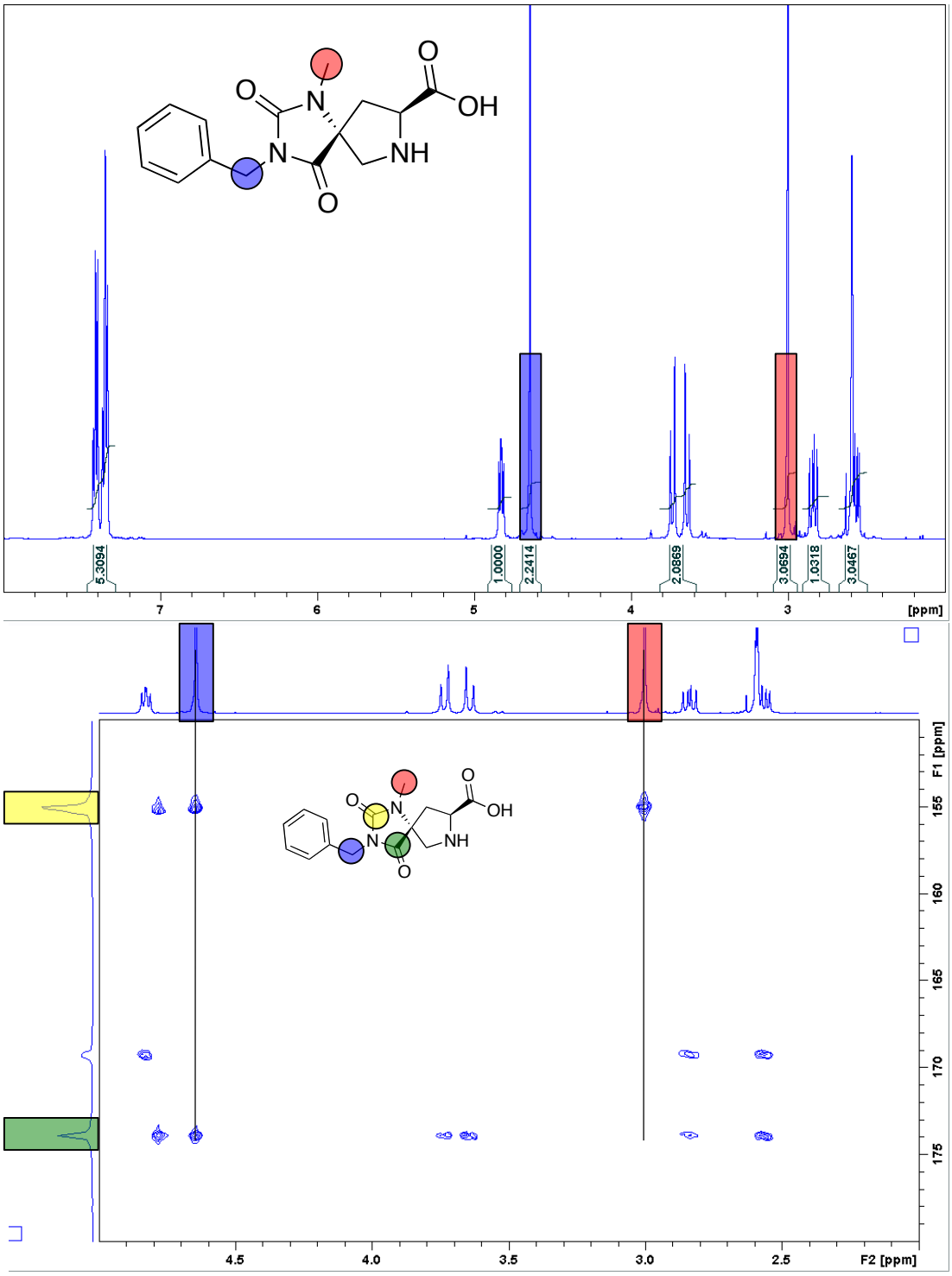
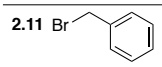
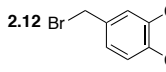
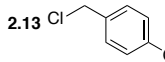
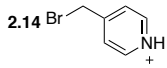
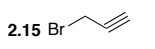
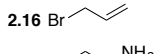
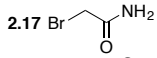
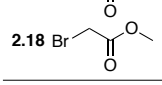
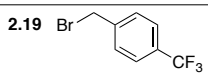
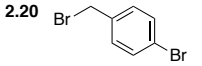
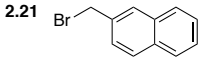
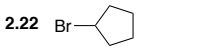
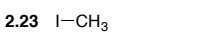
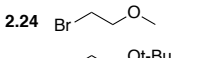
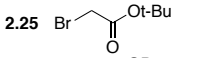
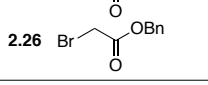
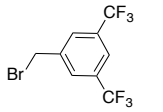
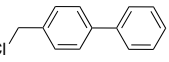
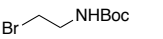
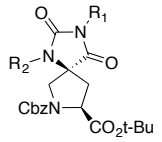



**Figure 2.2.** Verification of the order of addition to the hydantoin via NMR ($^{1}$H and HMBC). The benzylic protons (blue highlight) couple to both carbonyl carbons of the hydantoin, whereas the methyl protons (red highlight) only couples to one of the carbonyls (yellow)

In addition to the varying reactivities of the two nitrogens on the hydantoin, the conditions for the addition of the first functional group to the imide position are dependent on the functional group desired. For a dialkylation in which the desired side chains are identical, such as a 4-bromobenzyl addition to make **2.10**, we stirred overnight a mixture of known compound **2.1** in DMF (100 mM) with 2.2 eq of **2.20** (Table 2.1) and 2.5 equivalents of $K_2CO_3$. For dialkylations where different side chains are desired, and an allyl or benzyl moiety is desired on the imide, a slightly modified method was utilized. An example of this is compound **2.7**, wherein a mixture of **1** in DMF (100 mM) was reacted with 1.0 eq of **2.11** along with 1.5 equivalents of $K_2CO_3$. The reaction proceeded at room temperature for 2 hours, at which time 1.5 eq of **2.23** and 1.5 equivalents of $K_2CO_3$ were added to the reaction mixture, and stirred overnight. A third procedure was utilized when different side chains are desired, with one of the more sluggish electrophiles being installed on the imide position. An example is the synthesis of **2.3**, where a mixture of **1** in DMF (100 mM) was reacted with 2.0 eq of **2.30** along with 2.5 equivalents of $K_2CO_3$. The reaction proceeded at room temperature overnight, at which point 1.5 eq of **2.21** was added along with another 1.5 equivalents of $K_2CO_3$. The reaction was once again stirred overnight to yield the desired dialkylated product. It is worth mentioning that for **2.22**, **2.30**, and 2.**31**, alkylation of the amide was sluggish. After a full week (168 hours) there were still significant amounts of mono-alkylated species present.

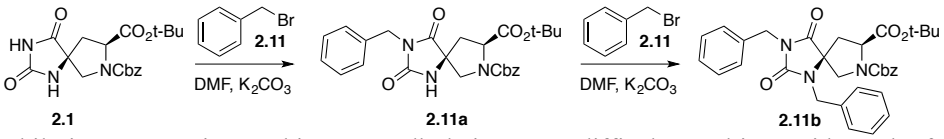
**Table 2.1.** Time required and percent yield for alkylating the imide or amide of the hydantoin for each electrophile tested. a) Mono alkylation of the hydantoin with **2.11-31** was performed. b) Dialkylation of both the imide and amide with **2.11-13**, **2.15-18**, **2.20**, **2.23-26**, and **2.28-29** was performed. Structures for these products can be found in the experimental section, but follow the example shown for **2.11a** and **2.11b**. NO means Not Obtained.

| Cmpd # | Electrophile | Time to alk. $R_1$ / $R_2$ | Yield % a / b |
|---|---|---|---|
| **2.11** |  | 2 h / 14 h | 93 / 99 |
| **2.12** |  | 2 h / 14 h | 87 / 99 |
| **2.13** |  | 6 h / 24 h | 91 / 99 |
| **2.14** |  | 2 h / ND | 90 / NO |
| **2.15** |  | 3 h / 14 h | 88 / 99 |
| **2.16** |  | 3 h / 14 h | 93 / 99 |
| **2.17** |  | 2 h / 14 h | 85* / 70 |
| **2.18** |  | 4 h / 24 h | 87 / 99 |
| **2.19** |  | 2 h / 14 h | 90 / NO |
| **2.20** |  | 2 h / 14 h | 89 / 99 |
| **2.21** |  | 2 h / 14 h | 85 / NO |
| **2.22** |  | 24 h / ND | 97 / NO |
| **2.23** |  | 2 h / 14 h | 85* / 99 |
| **2.24** |  | 14 h / 36 h | 95 / 99 |
| **2.25** |  | 6 h / 24 h | 92 / 99 |
| **2.26** |  | 6 h / 24 h | 89 / 99 |
| **2.27** |  | 2 h / 14 h | 90 / NO |
| **2.28** |  | 6 h / 24 h | 87 / 99 |
| **2.29** |  | 14 h / 36 h | 94 / 99 |
| **2.30** |  | 24 h / ND | 97 / NO |
| **2.31** |  | 24 h / ND | 86 / NO |



a) $R_1$ = Electrophile, $R_2$ = H

b) $R_1$ = $R_2$ = Electrophile




*Electrophile is very reactive, making monoalkylation more difficult to achieve without the formation of dialkylated products.

As shown in Table 2.1, the benzyl bromides **2.11-13**, **2.19-21**, and **2.27** alkylate the imide position in 2 hours, which holds true across all of the benzyl bromides tested, while the allyl bromides **2.15-16** require slightly longer (2.5-3 hours). The benzyl chlorides **2.13** and **2.28**, however, require more than twice the amount of time to fully alkylate the imide (from 5 to 7 hours depending on the electrophile), which is on par with the bromoacetates **2.18**, **2.25**, and **2.26** at 4-6 hours. As mentioned previously, the alkyl halides **2.22, 2.24**, and **2.29-31** are typically more sluggish, and require an overnight reaction to fully alkylate the imide position. The percent yields were calculated in two separate ways, depending on the functionality of the group added. If the functional group

would have no impact on the UV/Vis extinction coefficient, then the integrated ratios from an HPLC/MS UV chromatogram of the monoalkylated product versus any starting material or dialkylated product was used. If the added functionality would alter the extinction coefficient, we instead integrated the crude $^{1}$H NMR, comparing any leftover starting material to the product. Due to the Cbz protecting group on the proline nitrogen (a tertiary amide), at room temperature there are rotamers observed in all of the crude NMR spectra (**2.2b**-**10b**, **2.11**-**31a** or **b**). Most of the yields are excellent, and the "loss" of product usually stems from halting the reaction before any significant dialkylation can occur, since the monoalkylated product can be purified away from the starting material. If there is any undesired dialkylation, we can purify these side-products away as well. Once a monoalkylated product is synthesized, almost all of the groups that added to the amide position of the hydantoin did so quantitatively (complete conversion of starting material to product by HPLC and NMR). It should be noted that the hydantoin starting material often contains unreactive impurities (between 15-20% by weight), which is the main reason for undercutting the equivalents of electrophile added for allyl or benzyl halides. Most of the groups we tested have been used previously for a variety of applications: On spiroligomers, functional groups such as pyridines[11] have been used for catalysis; isobutyl, benzyl, and 3,4-dichlorobenzyl groups have been used on spiroligomers for protein binding,[17] while groups such as the trifluoromethyl benzyls are regularly used in small molecule drugs; and we can also install groups that are reactive towards other species such as the 4-bromobenzyl, propargyl, allyl, boronic esters, and protected amines or acid moieties. Having a number of interchangeable functional groups will allow us to tune the hydrophilicity/hydrophobicity of the molecule, without drastically altering the

steric bulk of the molecule. This interchangeability of a wide range of functional groups will be very beneficial for designing and synthesizing future spiroligomers.

Based on the current chemistry, we have synthesized a variety of combinations of the electrophiles added to the spiroligomer at either the imide or amide positions. Figure 2.3, below, shows the combinations we have synthesized thus far, as well as those we think are plausible or do not think are plausible combinations with the current chemistry. (NOTE: This table highlights combinations based on molecules synthesized for this chapter and for later chapters.) As shown in Figure 2.3, we have synthesized only 70 of the 594 possible combinations of functional groups, and this isn't taking into account the varied stereochemistry. If stereochemistry is included, the number of potential molecules increases to 2376 combinations of functional groups and stereocenters. Furthermore, there are hundreds of commercially available electrophiles, not just the 28 used here, meaning there are tens of thousands of potential molecules able to be synthesized with this method. Thus, there is much untapped design space that could still be explored with just the spiroligomer hydantoin alone.

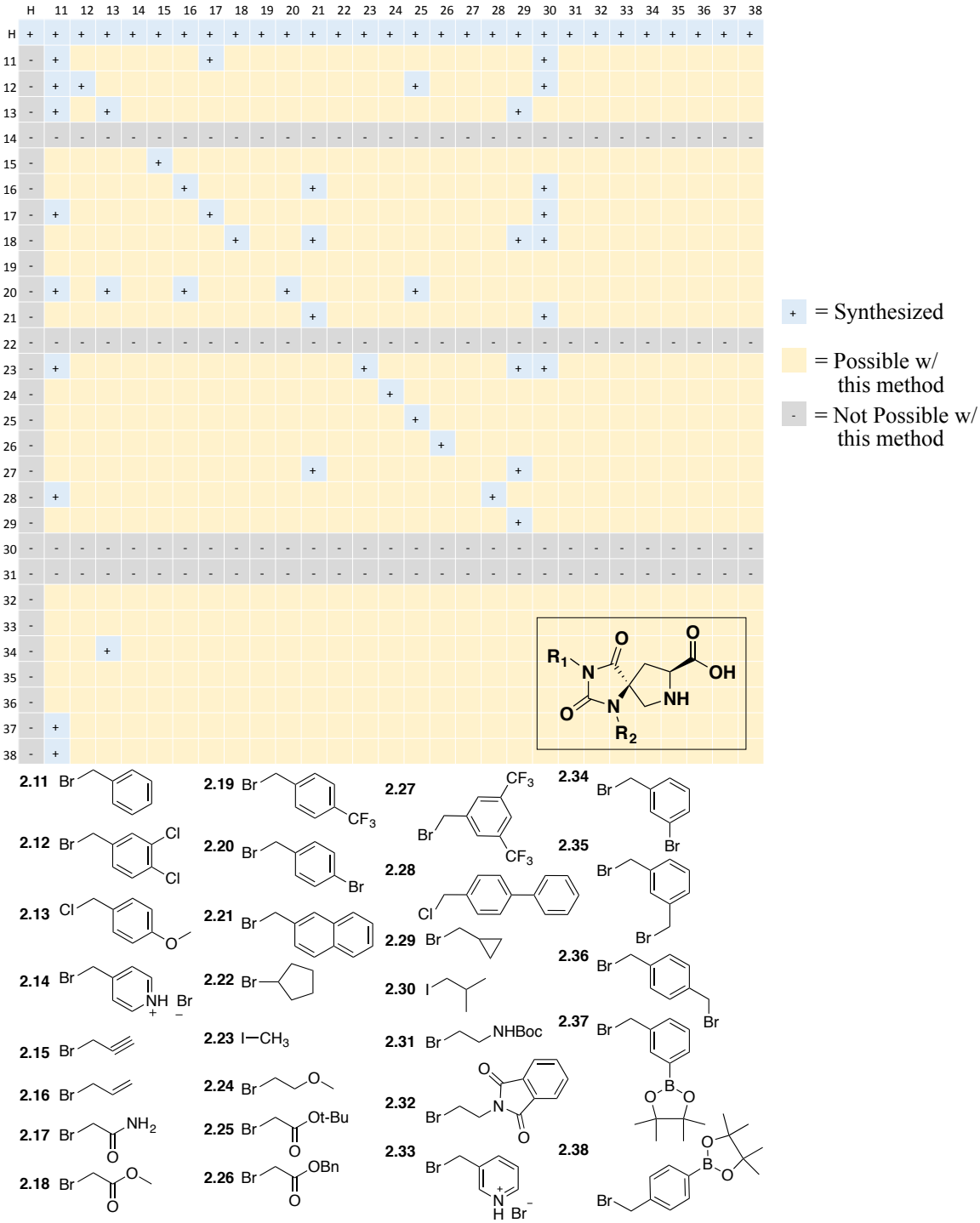
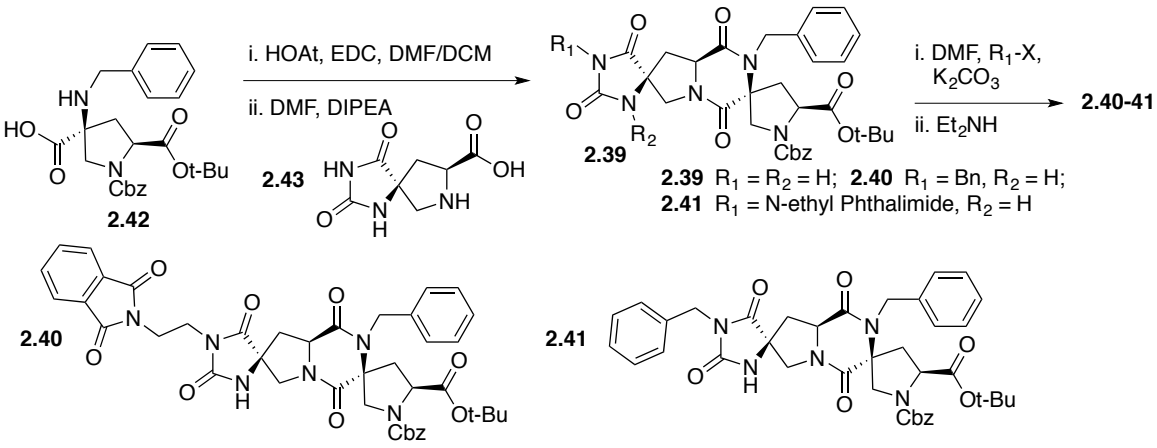



**Figure 2.3.** Electrophile list with $R_1/R_2$ combinations tested or thought to be plausible based on current chemistry

## 2.2 Alkylation of a Spiroligomer Dimer

The application of a direct alkylation to the hydantoin can be applied to any of the stereochemical configurations of the spirocyclic hydantoin. The different stereochemical configurations react similarly, in that neither the yield nor the time required to alkylate a given position depends on the stereochemistry of the starting material (data for the enantiomers not shown). This is important, as all possible configurations of stereocenters have been used previously.[10,11,16,17,19] On top of that, this strategy of hydantoin alkylation can be applied to a spiroligomer dimer, as shown in **Scheme 2.3**. We synthesized a




**Scheme 2.3.** Application of the alkylation strategy to a spiroligomer dimer **2.39** to make two alkylated dimers **2.40-41**

spiroligomer dimer, to which we applied our alkylation method. This demonstrates that we can implement our alkylation strategy as a final step in the synthesis of spiroligomers. This late stage terminus functionalization is valuable, as we typically synthesize a spiroligomer linearly starting from the hydantoin terminus.[11,17] It should be noted that, while not seen here, epimerization of the α-position of the diketopiperazine is theoretically possible. Therefore, one should take care when subjecting extended

spiroligomers to this chemistry. Heat or prolonged reaction times should also be avoided, as these could facilitate epimerization of the diketopiperazine. The ability to add more functional groups as a last step of a spiroligomer synthesis is a valuable application of this method to our work moving forward.

## 2.3 Conclusion

Utilization of a direct hydantoin alkylation has allowed for the rapid synthesis of highly-functionalized spiroligomer building blocks. This method has been adapted to allow for a one-pot, overnight reaction; thus reducing the time required for making functionalized hydantoin residues from four days to one or two days, reducing the number of steps from five to two, while increasing the isolated yields to 85-99%. Due to the fact that similar molecules have been used for a variety of applications, this approach should facilitate the discovery of new spiroligomer-based catalysts and ligands.

## 2.4 Experimental Details

HPLC-MS was carried out on a $C_{18}$ Column, Dimensions: 5.0 cm x 2.1 cm, 2.7 μm packing, coupled to a quadrupole mass spectrometer (ESI Source). HRMS was performed on a Q-ToF. NMR samples for compounds **2.2-7** were prepared by dissolving 75mg of each compound in DMSO-$d_6$, whereas samples for compounds **2.8-10** were prepared by dissolving 30 mg of each compound in DMSO-$d_6$. NMR experiments were performed on either a 400 or 500 MHz spectrometer as noted. Optical rotations were obtained at 20°C in DMSO or DCM using a polarimeter with a 1 mL cell using the sodium D line. The hydantoin starting material was synthesized with a known procedure, [10,20,21] and the halides were purchased from commercial sources.

To alkylate a hydantoin, the following three methods were used depending on the functionality desired. These reactions were carried out at scales ranging from 30 mg – 10 g (75 umol – 25 mmol) of **2.1**:

**General procedure 1: Synthesis of dialkylated hydantoins with the same side chain (compounds 2.10a and 2.11b-29b):**

To a stirred mixture of **2.1** in DMF (100 mM) was added 2.2 eq of allyl or benzyl halide along with 2.5 eq of $K_2CO_3$. The reaction proceeded at room temperature for 14 hours, at which time the reaction was treated with 1.0 eq of diethylamine for 1 hour to quench any leftover halide. Workup: The reaction was then diluted with four times the reaction volume of EtOAC and washed with water, saturated ammonium chloride solution, and brine. The organic layer was dried with $Na_2SO_4$, and concentrated *in vacuo* to yield a foamy off-white to yellow solid.

**General procedure 2: Synthesis of dialkylated hydantoins with different side chains, and an allyl, acetate, or benzyl moiety installed on the imide (compounds 2.7a-9a, and 2.11a-31a):**

To a stirred mixture of **2.1** in DMF (100 mM) was added 0.75-0.85 eq (equivalents dependent on salt content of the specific batch of 4-hydantoin-Z-Pro-OtBu, which can be determined utilizing a toluene standard**)** of allyl or benzyl halide along with 1.5 eq of $K_2CO_3$. The reaction proceeded at room temperature for 2-5 hours. For compounds **2.11a-31a**, proceed directly to the workup. For compounds **2.7a-9a**, 1.5 eq of another allyl or benzyl halide and 1.5 eq of $K_2CO_3$ was added to the reaction mixture and

stirred overnight. The reaction was then treated with 1.0 eq of diethylamine for 1 hour to quench any leftover halide. Workup: The reaction was diluted with four times the reaction volume of EtOAC and washed with water, saturated ammonium chloride solution, and brine. The organic layer was dried with $Na_2SO_4$, and concentrated *in vacuo* to yield a foamy off-white to yellow solid.

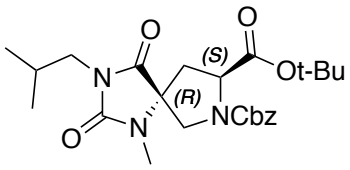
**General procedure 3: Synthesis of dialkylated hydantoins with different side chains, and an alkyl halide on the imide position (compounds 2.2a-6a):**

To a stirred mixture of **2.1** in DMF (100 mM) was added 1.5 eq of alkyl halide along with 2.5 equivalents of $K_2CO_3$. The reaction proceeded at room temperature for 14-24 hours, at which point 1.5 eq of an allyl or benzyl halide was added along with another 1.5 equivalents of $K_2CO_3$. The reaction was stirred overnight, and was then treated with 2.0 eq of diethylamine for 1 hour to quench any leftover halide. Workup: The reaction was diluted with four times the reaction volume of EtOAC and washed with water, saturated ammonium chloride solution, and brine. The organic layer was dried with $Na_2SO_4$, and concentrated *in vacuo* to yield a foamy off-white to yellow solid.

**General procedure 4: Deprotection and purification of the amino acids:**

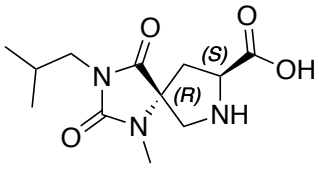
To a stirred mixture of the substituted hydantoin in DCM (200mM) was added an equal volume of 33% HBr in acetic acid. The reaction was stirred for 30 minutes, diluted with toluene (1/4 the total volume) and the solvent removed by rotary evaporation. The product was purified by reverse phase flash chromatography with a gradient of 5-95% $H_2O$/ACN (0.1% formic acid additive).

**2.2a 7-benzyl 8-(*tert*-butyl) (5*R*,8*S*)-3-isobutyl-1-methyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**



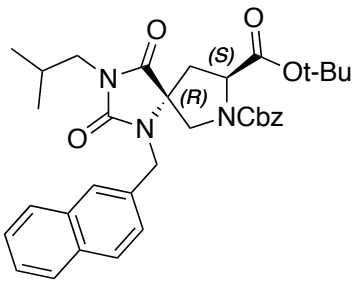

Using procedure 3, 111 mg (97%) was recovered; $^{1}$H NMR (500 MHz, $CDCl_3$) 0.88 (6H, dd, *J* = 3.3, 6.7), 1.43 (9H, s, rotameric), 2.01-2.08 (m, 1H, *J* = 7.1), 2.46-2.52 (1H, m), 2.53-2.61 (1H, m), 2.91 (3H, s, rotameric), 3.32 (2H, dd, *J* = 7.5, 9.4), 3.81-3.94 (2H, m), 4.55 (1H, dd, rotameric, *J* = 7.6, 9.0), 5.11-5.20 (2H, m), 7.30-7.36 (5H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 19.9, 25.7, 27.3, 27.9, 37.6, 38.6, 46.5, 53.8, 59.0, 67.7, 68.6, 82.5, 128.1, 128.3, 128.5, 136.1, 153.8, 155.3, 169.7, 172.4; $[\alpha]_D^{20}$ +12.6 (*c* 11.1, DCM); HRMS-ESI: m/z calcd for $C_{24}H_{33}N_3O_6K$ $(M+K)^+$ 498.2001, found 498.2010

**2.2b - (5*R*,8*S*)-3-isobutyl-1-methyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxylic acid**



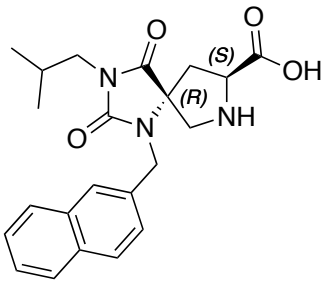

Using procedure 4 starting with **2.2a**, 61 mg (91%) was recovered; $^{1}$H NMR (400 MHz, DMSO-$d_6$) - 0.81 (6H, d, *J* = 6.85), 1.92 (1H, m, *J* = 6.85), 2.20 (1H, dd, *J* = 14.2, 8.80), 2.54 (1H, dd, *J* = 14.2, 8.3), 2.90 (3H, s), 3.16 (2H, d, *J* = 7.35) 3.35 (2H, dd, *J* = 16.2, 12.7), 4.07 (1H, t, *J* = 8.56); $^{13}$C NMR (100 MHz, DMSO-$d_6$) 19.8, 25.3, 27.0, 37.6, 45.6, 51.7, 60.5, 69.2, 154.9, 171.8, 174.5; $[\alpha]_D^{20}$ +8.4 (*c* 6.1, DMSO); HRMS-ESI: m/z calcd for $C_{12}H_{19}N_3O_4$ $(M+H)^+$ 270.1448, found 270.1451

**2.3a - 7-benzyl 8-(*tert*-butyl) (5*R*,8*S*)-3-isobutyl-1-(naphthalen-2-ylmethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**




Using procedure 3, 139 mg (97%) was recovered; $^{1}$H NMR (500 MHz, DMSO-$d_6$, 65 °C) 0.91 (6H, d, rotameric, $J$ = 6.6), 1.36 (9H, s, rotameric), 2.06 (1H, sep, $J$ = 7.0), 2.30 (2H, dd, $J$ = 7.3, 14.5), 2.7 (2H, m), 3.34 (2H, d, $J$ = 6.9), 3.50 (1H, m), 3.57-3.81 (1H, m), 4.06-4.32 (1H, m), 4.46-4.52 (1H, m), 4.69-4.90 (3H, m), 6.93 (1H, s, broad), 7.22-7.35 (4H, m), 7.42 (1H, dd, $J$ = 1.6, 8.5), 7.49 (2H, m), 7.74 (1H, s), 7.84-7.88 (3H, m); $^{13}$C NMR (125 MHz, DMSO-$d_6$, rotamers present) 19.9, 27.5, 37.4, 43.3, 46.1, 54.0, 58.9, 65.9, 68.9, 81.4, 125.0, 125.5, 125.9, 126.2, 127.3, 127.6, 127.8,, 127.9, 128.1, 128.3, 132.3, 132.8, 135.1, 136.2, 152.4, 155.8, 170.1, 172.1; $[\alpha]_D^{20}$ +3.9 (*c* 13.9, DCM); HRMS-ESI: m/z calcd for $C_{34}H_{39}N_3O_6K$ $(M+K)^+$ 624.2470, found 624.2484

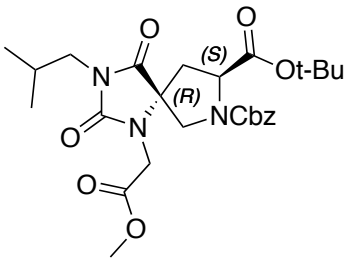
**2.3b - (5*R*,8*S*)-3-isobutyl-1-(naphthalen-2-ylmethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxylic acid**




Using procedure 4 starting from **2.3a**, 81 mg (86%) was recovered; $^{1}$H NMR (400 MHz, DMSO-$d_6$) 0.67 (6H, d, $J$ = 6.8), 2.00 (1H, m, $J$ = 6.85), 2.17 (1H, dd, $J$ = 14.2, 7.58), 2.30 (1H, dd, $J$ = 13.9, 8.80), 3.15 (1H, d, $J$ = 12.0), 3.30 (3H, m), 3.83 (1H, t, $J$ = 8.1), 4.79 (2H, dd, $J$ = 11.7), 7.49 (3H, m), 7.88 (4H, m); $^{13}$C NMR (100 MHz, DMSO-$d_6$) 19.9, 27.0, 38.1, 43.0, 45.7, 53.1, 59.9, 69.9, 125.3, 125.4, 126.0, 126.3, 127.6, 127.7, 128.3, 132.2, 132.9, 135.9, 155.8, 172.7, 172.7, 175.0; $[\alpha]_D^{20}$

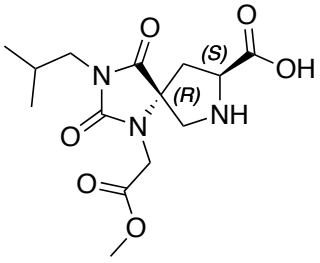
+6.9 (*c* 8.1, DMSO); HRMS-ESI: m/z calcd for $C_{22}H_{25}N_3O_4$ $(M+H)^+$ 396.1918, found 396.1894

**2.4a - 7-benzyl 8-(*tert*-butyl) (5*R*,8*S*)-3-isobutyl-1-(2-methoxy-2-oxoethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**




Using procedure 3, 125 mg (97%) was recovered; $^1$H NMR (500 MHz, $CDCl_3$) 0.90 (6H, dd, *J* = 3.3, 6.7), 1.42 (9H, s, rotameric), 2.07 (1H, m, *J* = 7.1), 2.56 (1H, td, *J* = 7.3, 14.3), 2.61-2.71 (1H, m), 3.36 (2H, dd, *J* = 7.6, 9.4), 3.69 (3H, s, rotameric), 3.85 (1H, d, rotameric, *J* = 6.0), 3.96-4.01 (1H, m), 4.02-4.13 (2H, m), 4.37-4.45 (1H, m), 5.09-5.18 (2H, m), 7.30-7.36 (5H, m); $^{13}$C NMR (125 MHz, CDCl3, rotamers present) 19.9, 27.2, 27.9, 38.0, 39.0, 41.8, 46.6, 52.7, 52.4, 58.6, 67.7, 68.9, 82.6, 128.1, 12.3, 128.6, 136.0, 153.8, 155.6, 168.9, 169.5, 171.9; $[\alpha]_D^{20}$ +2.0 (*c* 12.5, DCM); HRMS-ESI: m/z calcd for $C_{26}H_{35}N_3O_8K$ $(M+K)^+$ 556.2056, found 556.2049

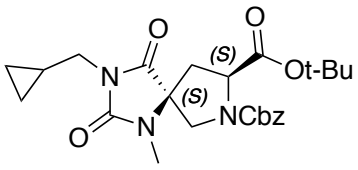
**2.4b - (5*R*,8*S*)-3-isobutyl-1-(2-methoxy-2-oxoethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxylic acid**




Using procedure 4 starting with **2.4a**, 72 mg (92%) was recovered; $^1$H NMR (400 MHz, DMSO-$d_6$) 1H – 0.81 (6H, d, *J* = 6.85), 1.92 (1H, m, *J* = 6.85), 2.23 (1H, dd, *J* = 14.2, 6.85), 2.47 (1H, dd, *J* = 14.2, 9.05), 3.20 (4H, m), 3.68 (3H, s), 3.97 (1H, dd, *J* = 9.2, 6.7), 4.23 (2H, q, *J* = 18.2) $^{13}$C NMR (100 MHz, DMSO-$d_6$) 19.7, 26.9, 37.6, 41.5, 45.5, 52.2, 53.2, 59.3, 69.3, 155.3, 169.7, 173.1, 174.7; $[\alpha]_D^{20}$ +4.9

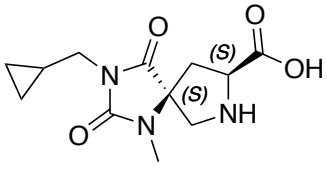
(*c* 7.2, DMSO); HRMS-ESI: m/z calcd for $C_{14}H_{21}N_3O_6$ $(M+H)^+$ 328.1503, found 328.1505

**2.5a - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-3-(cyclopropylmethyl)-1-methyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**




Using procedure 3, 107 mg (93%) was recovered; $^1$H NMR (500 MHz, $CDCl_3$) 0.29-0.32 (2H, m), 0.47-0.48 (2H, m), 1.12 (1H, m), 1.33 (9H, s, rotameric), 2.18-2.24 (1H, m), 2.59-2.65 (1H, m), 2.91 (3H, s), 3.33-3.35 (2H, m), 3.68 (1H, d, rotameric, *J* = 6.0), 3.91 (1H, d, rotameric, *J* = 11.8), 4.55-4.60 (1H, m), 5.07-5.18 (2H, m), 7.28-7.32 (5H, m); $^{13}$C NMR (125 MHz, CDCl3, rotamers present) 3.7, 10.0, 24.9, 27.8, 36.0, 43.7, 50.2, 58.7, 66.2, 67.6, 82.21, 127.9, 128.1, 128.5, 136.0, 154.1, 155.5, 170.8, 174.6; $[\alpha]_D^{20}$ -3.2 (*c* 10.7, DCM) ; HRMS-ESI: m/z calcd for $C_{24}H_{31}N_3O_6K$ $(M+K)^+$ 496.1844, found 496.1841

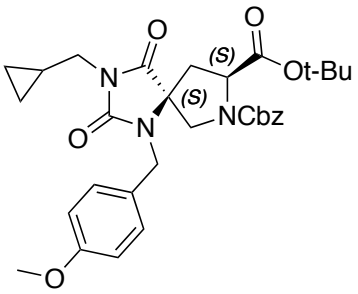
**2.5b - (5*S*,8*S*)-3-(cyclopropylmethyl)-1-methyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxylic acid**




Using procedure 4 starting from **2.5a**, 72 mg (90%) was recovered; $^1$H NMR (400 MHz, DMSO-$d_6$) 0.25 (2H, m) 0.43 (2H, m), 1.02 (1H, m), 2.25 (1H, dd, *J* = 13.9, 9.78), 2.36 (1H, dd, *J* =13.9, 7.83), 2.86 (3H, s), 3.22 (2H, d, *J* = 7.09), 3.26 (1H, d, *J* = 13.0), 3.44 (1H, d, *J* = 13.0), 3.99 (1H, dd, *J* = 9.78, 7.83) $^{13}$C NMR (100 MHz, DMSO-$d_6$) 3.5, 9.9,

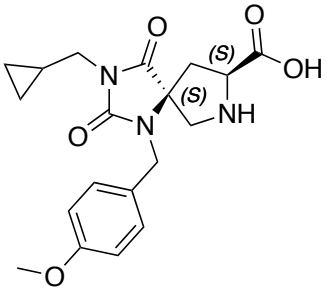
24.8, 35.8, 42.8, 49.3, 60.7, 68.3, 154.9, 170.4, 174.9; $[\alpha]_D^{20}$ +2.5 (*c* 7.2, DMSO) ; HRMS-ESI: m/z calcd for $C_{12}H_{17}N_3O_4$ $(M+H)^+$ 268.1292, found 268.1281

**2.6a - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-3-(cyclopropylmethyl)-1-(4-methoxybenzyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**




Using procedure 3, 132 mg (94%) was recovered; $^1$H NMR (500 MHz, $CDCl_3$) 0.33-0.36 (2H, m), 0.50-0.51(2H, m), 1.17 (1H, m), 1.30 (9H, s, rotameric), 2.15 (1H, dd, *J* = 8.1, 13.7), 2.36-2.50 (1H, m), 3.40 (2H, m), 3.62 (1H, d, *J* = 11.6), 3.72-3.76 (3H, s, rotameric), 3.78-3.82 (1H, m), 4.49-4.59 (3H, m), 5.05-5.17 (2H, m), 6.83 (2H, d, rotameric, *J* = 8.1), 7.23-7.34 (7H, m); $^{13}$C NMR (125 MHz, CDCl3, rotamers present) 3.7, 10.0, 27.7, 35.7, 42.5, 43.6, 49.8, 55.2, 58.2, 64.8, 67.5, 82.0, 113.8, 114.3, 127.81, 128.4, 128.9, 129.4, 130.04, 133.4, 136.0, 156.0, 159.3, 170.9, 174.5; $[\alpha]_D^{20}$ -1.1 (*c* 13.2, DCM); HRMS-ESI: m/z calcd for $C_{31}H_{37}N_3O_7K$ $(M+K)^+$ 602.2263, found 602.2267

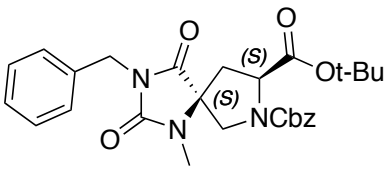
**2.6b - (5*S*,8*S*)-3-(cyclopropylmethyl)-1-(4-methoxybenzyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxylic acid**




Using procedure 4 starting from **2.6a**, 68 mg (78%) was recovered; $^1$H NMR (400 MHz, DMSO-$d_6$) 0.28 (2H, m), 0.46 (2H, m), 1.08 (1H, m), 2.13 (1H, dd, *J* = 13.7, 10.3), 2.31 (1H, dd, *J* = 13.7, 7.40), 3.18 (1H, d, *J* = 12.7), 3.26 (1H, d, *J* = 13.3), 3.30 (2H, d, *J* = 7.00), 3.73 (3H, s), 3.91 (1H, dd, 10.4, 7.40), 4.54 (2H,

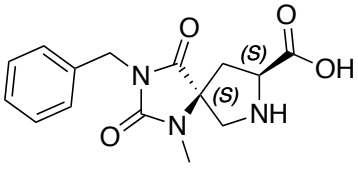
m), 6.89 (2H, d, $J$ = 8.56), 7.27 (2H, d, $J$ = 8.56) $^{13}$C NMR (100 MHz, DMSO-$d_6$) 3.5, 9.9, 36.5, 37.1, 41.7, 42.8, 50.0, 55.0, 60.5, 69.0, 99.2, 113.5, 113.9, 127.9, 128.5, 129.9, 155.5, 158.4, 170.6, 175.2; $[\alpha]_D^{20}$ -2.6° (*c* 6.8, DMSO); HRMS-ESI: m/z calcd for $C_{19}H_{23}N_3O_5$ $(M+H)^+$ 374.1710, found 374.1701

**2.7a - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-3-benzyl-1-methyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**




Using procedure 2, 114 mg (93%) was recovered; $^1$H NMR (500 MHz, $CDCl_3$) 1.33 (9H, s, rotameric), 2.16-2.22 (1H, m), 2.55-2.64 (1H, m), 2.90 (3H, s, rotameric), 3.66 (1H, t, rotameric, $J$ = 10.8), 3.90 (1H, d, rotameric, $J$ = 11.7), 4.55-4.60 (1H, m), 4.60-4.67 (2H, m) 5.08-5.19 (2H, m), 7.26-7.37 (10H, m); $^{13}$C NMR (125 MHz, CDCl3, rotamers present) 25.0, 27.9, 29.7, 35.9, 42.6, 50.1, 58.6, 66.2, 67.6, 82.4, 127.9, 128.0, 128.4, 128.5, 128.8, 135.7, 154.1, 155.1, 170.1, 174.2; $[\alpha]_D^{20}$ -1.9 (*c* 11.4, DCM); HRMS-ESI: m/z calcd for $C_{27}H_{31}N_3O_6K$ $(M+K)^+$ 532.1844, found 532.1848

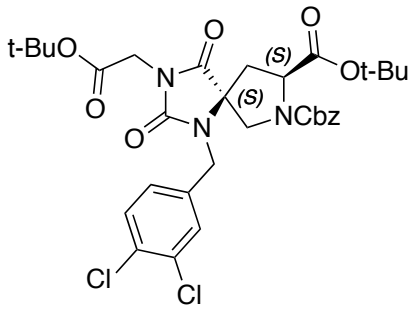
**2.7b - (5*S*,8*S*)-3-benzyl-1-methyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxylic acid**




Using procedure 4 starting from **2.7a**, 65 mg (93%) was recovered; $^1$H NMR (400 MHz, DMSO-$d_6$) 2.24 (1H, dd, $J$ = 13.9, 9.30), 2.39 (1H, dd, $J$ =13.9 7.90), 2.86 (3H, s), 3.23 (1H, d, $J$ = 13.0), 3.43 (1H, d, $J$ = 13.0), 3.94 (1H, dd, $J$ = 9.30, 8.01), 4.55 (2H, s), 7.27 (5H, m) $^{13}$C NMR (100 MHz, DMSO-$d_6$) 25.2, 36.3, 42.0, 50.2, 61.0, 69.0, 127.6,

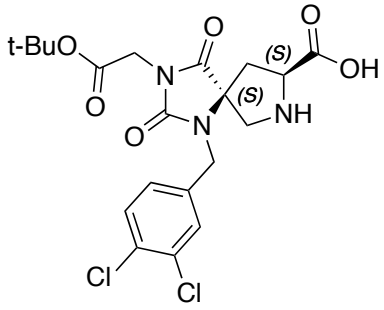
127.8, 128.9, 136.7, 155.0, 170.9, 175.3; $[\alpha]_D^{20}$ -4.2° ( *(c* 6.5, DMSO); HRMS-ESI: m/z calcd for $C_{15}H_{17}N_3O_4$ $(M+H)^+$ 304.1292, found 304.1289

**2.8a - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-3-(2-(*tert*-butoxy)-2-oxoethyl)-1-(3,4-dichlorobenzyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**




Using procedure 2, 148 mg (90%) was recovered; $^1$H NMR (500 MHz, $CDCl_3$) 1.47 (18H, s, rotameric), 2.14 (1H, dd, *J* = 7.0, 14.0) 2.59-2.72 (1H, m) 3.59 (1H, d, rotameric, *J* = 10.2), 3.91 (1H, d, rotameric, *J* = 11.0), 4.19 (2H, s), 4.50 (1H, dd, rotameric, *J* = 7.1, 9.1), 4.55-4.63 (2H, m), 5.05-5.17 (2H, m) 7.18-7.24 (1H, m) 7.27-7.41 (6H, m), 7.43-7.49 (1H, m); $^{13}$C NMR (125 MHz, CDCl3, rotamers present) 27.9, 29.7, 36.6, 40.5, 42.0, 46.8, 56.5, 66.9, 67.7, 68.3, 83.3, 126.0, 127.5, 127.9, 128.1, 128.5, 130.1, 130.9, 132.1, 133.0, 135.9, 154.0, 155.2, 165.7, 170.9, 173.7; $[\alpha]_D^{20}$ +2.3 ( *(c* 14.8, DCM); HRMS-ESI: m/z calcd for $C_{32}H_{37}Cl_2N_3O_8Na$ $(M+Na)^+$ 684.1850, found 684.1873

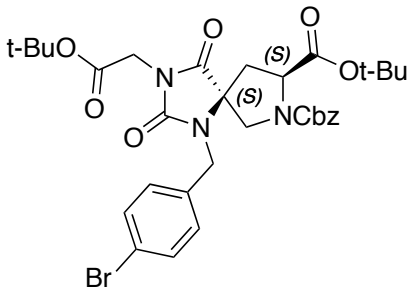
**2.8b - (5*S*,8*S*)-3-(carboxymethyl)-1-(3,4-dichlorobenzyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxylic acid**




Using procedure 4 starting from **2.8a**, 70 mg (76%) was recovered; $^1$H NMR (400 MHz, DMSO-$d_6$) 2.11 (1H, dd, *J* = 13.6, 10.3), 2.35 (1H, dd, *J* = 13.6, 7.30), 3.23 (2H, m), 3.93 (1H, dd, *J* = 10.3, 7.30), 4.11 (2H, s), 4.64 (2H,

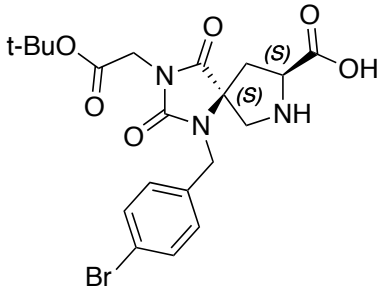
m), 7.30 (1H, m), 7.59 (2H, m) $^{13}$C NMR (100 MHz, DMSO-$d_6$) 40.0, 60.1, 69.1, 127.3, 129.0, 129.8, 130.7, 131.1, 139.1, 155.0, 168.7, 170.5, 174.7; $[\alpha]_D^{20}$ -5.5° ( *(c* 7.0, DMSO); HRMS-ESI: m/z calcd for $C_{16}H_{15}Cl_2N_3O_6$ $(M+H)^+$ 416.0411, found 416.0416

**2.9a - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-1-(4-bromobenzyl)-3-(2-(*tert*-butoxy)-2-oxoethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**




Using procedure 2, 153 mg (91%) was recovered; $^1$H NMR (500 MHz, $CDCl_3$) 1.47 (18H, s, rotameric) 2.12 (1H, dd, rotameric, *J* = 7.6, 13.9), 2.51-2.63 (1H, m), 3.57-3.61 (1H, m), 3.89 (1H, d, rotameric, *J* = 11.6), 4.18 (2H, s), 4.50 (1H, dd, *J* = 7.6, 8.9), 4.58 (2H, s), 5.06-5.17 (2H, m), 7.19-7.25 (2H, m), 7.29-7.36 (5H, m), 7.43 (2H, m); $^{13}$C NMR (125 MHz, CDCl3, rotamers present) 28.0, 36.3, 40.4, 42.5, 50.1, 58.1, 66.7, 67.7, 83.2, 121.9, 127.8, 128.1, 128.4, 129.1, 129.3, 132.1, 135.9, 154.0, 155.2, 165.7, 170.9, 173.9; $[\alpha]_D^{20}$ -2.9 ( *(c* 15.3, DCM); HRMS-ESI: m/z calcd for $C_{32}H_{38}BrN_3O_8K$ $(M+K)^+$ 710.1474, found 710.1489

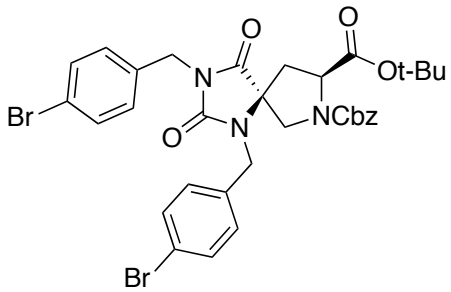
**2.9b - (5*S*,8*S*)-1-(4-bromobenzyl)-3-(carboxymethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxylic acid**




Using procedure 4 starting from **2.9a**, 81 mg (78%) was recovered; $^1$H NMR (400 MHz, DMSO-$d_6$) 2.13 (1H, dd, *J* = 13.6, 10.6), 2.35 (1H, dd, *J* = 13.6, 7.35), 3.17 (1H, d, *J* = 12.9), 3.22 (1H, d, *J* = 12.9) 3.97 (1H, dd, *J* = 10.6, 7.35), 4.11 (2H, s), 4.60 (2H, m), 7.26 (2H, d, *J* = 8.56), 7.52 (2H, d, *J* = 8.56); $^{13}$C

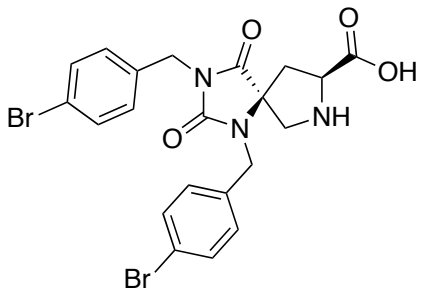
NMR (100 MHz, DMSO-$d_6$) 36.0, 40.1, 41.7, 49.5, 60.1, 68.9, 120.2, 129.1, 131.4, 137.3, 155.0, 168.7, 170.3, 174.7 [α]D = -4.6° ( *(c* 8.1, DMSO); HRMS-ESI: m/z calcd for $C_{16}H_{17}BrN_3O_6$ $(M+H)^+$ 426.0295, found 426.0299

**2.10a - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-1,3-bis(4-bromobenzyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**




Using procedure 1, 2.54 g (98%) was recovered; $^1$H NMR (500 MHz, $CDCl_3$) 1.28 (9H, s, rotameric), 2.08 (1H, dd, rotameric, *J* = 7.5, 13.9), 2.39-2.55 (1H, m), 3.53-3.59 (1H, m), 3.79 (1H, d, rotameric, *J* = 11.4), 4.48 (1H, dd, rotameric, *J* = 7.6, 8.8), 4.52 (2H, s, rotameric), 4.62 (2H, s), 5.08-5.15 (2H, m), 7.16-7.26 (3H, m), 7.27-7.47 (10H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 27.7, 36.2, 42.6, 50.2, 58.2, 66.6, 67.6, 82.3, 122.1, 122.3, 127.9, 128.1, 128.4, 128.5, 129.3, 129.5, 130.4, 132.0, 132.1, 134.5, 135.9, 154.0, 155.4, 170.8, 173.8; $[\alpha]_D^{20}$ +3.5 (*c* 10.0, DCM); HRMS-ESI: m/z calcd for $C_{33}H_{33}Br_2N_3O_6K$ $(M+K)^+$ 764.0368, found 764.0385

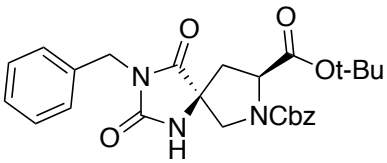
**2.10b - (5*S*,8*S*)-1,3-bis(4-bromobenzyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxylic acid**




Using procedure 4 starting with 100 mg of **2.10a**, 61 mg (83%) was recovered; $^1$H NMR (400 MHz, DMSO-$d_6$) 2.08 (1H, dd, *J* = 13.7, 10.0), 2.39 (1H,

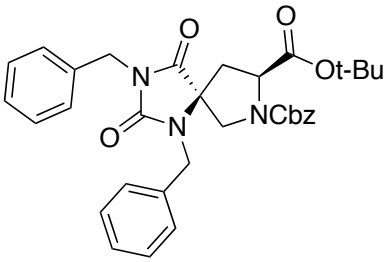
dd, *J* = 13.7, 7.45), 3.22 (2H, m), 3.87 (1H, dd, *J* = 10.0, 7.45), 4.58 (4H, m), 7.26 (4H, m) 7.54 (4H, m) $^{13}$C NMR (100 MHz, DMSO-*d*$_6$) 41.3, 41.9, 60.4, 69.3, 120.3, 120.7, 129.3, 129.6, 131.3, 131.5, 135.7, 137.4, 155.2, 170.7, 175.0; $[\alpha]_D^{20}$ -4.0° (*c* 6.1, DMSO); HRMS-ESI: m/z calcd for $C_{21}H_{19}Br_2N_3O_4$ $(M+H)^+$ 535.9815, found 535.9822

**2.11a - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-3-benzyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**




Using procedure 2, 45 mg (93%) was recovered; $^1$H NMR (500 MHz, $CDCl_3$) 1.28 (9H, s, rotameric), 1.97 (1H, dd, rotameric, *J* = 5.9, 13.8), 2.66-2.75 (1H, m), 3.72-3.87 (1H, m), 4.39 (1H, d, rotameric, *J* = 9.6), 4.60 (2H, d, *J* = 8.3), 5.02-5.12 (2H, m), 6.66 (1H, s, rotameric), 7.20-7.33 (10H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 27.9, 39.2, 40.0, 42.6, 56.5, 58.9, 65.2, 66.2, 67.6, 83.3, 127.9, 128.0, 128.2, 128.5, 128.6, 128.7, 135.7, 135.9, 153.6, 155.0, 172.2, 172.7; $[\alpha]_D^{20}$ -10.0 (*c* 4.5, DCM); HRMS-ESI: m/z calcd for $C_{26}H_{29}N_3O_6K$ $(M+K)^+$ 518.1687, found 518.1688

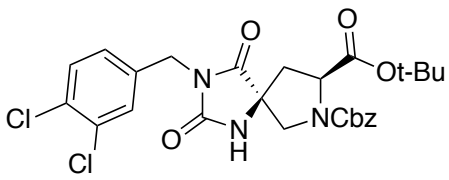
**2.11b - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-1,3-dibenzyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**




Using procedure 1, 56 mg (99%) was recovered; $^1$H NMR (500 MHz, $CDCl_3$) 1.29 (9H, s, rotameric), 2.12 (1H, dd, rotameric, *J* = 8.1, 13.9), 2.37-2.44 (1H, m), 3.62 (1H, d, *J* = 11.5), 3.71-3.84 (1H, m, rotameric), 4.52 (1H, t, *J* = 8.1), 4.55-4.66 (2H, m), 4.71 (2H, s, rotameric), 5.08-5.21 (2H, m), 7.27-

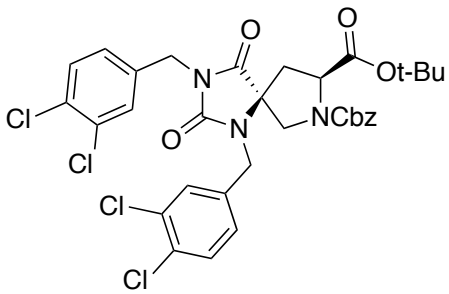
7.42 (15H, m); $^{13}$C NMR (125 MHz, $CDCl_3$) 27.6, 29.6, 35.8, 42.6, 49.8, 58.2, 65.0, 65.5, 67.4, 81.9, 126.8, 127.4, 127.7, 127.9, 128.0, 128.3, 128.4, 128.7, 128.8, 135.7, 135.9, 136.9, 153.9, 155.5, 170.6, 174.0; $[\alpha]_D^{20}$ +3.0 (*c* 5.6, DCM); HRMS-ESI: m/z calcd for $C_{33}H_{35}N_3O_6Na$ $(M+Na)^+$ 592.2418, found 592.2429

**2.12a - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-3-(3,4-dichlorobenzyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**




Using procedure 2, 47 mg (87%) was recovered; $^1$H NMR (500 MHz, $CDCl_3$) 1.32 (9H, s, rotameric), 2.03 (1H, dd, rotameric, *J* = 5.5, 13.8), 2.76 (1H, ddd, *J* = 9.6, 13.8, 19.7), 3.78-3.91 (2H, m), 4.44 (1H, dd, rotameric, *J* = 9.6, 16.9), 4.57 (2H, d, *J* = 9.4), 5.06-5.17 (2H, m), 6.80 (1H, s, rotameric), 7.21-7.47 (8H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 27.7, 38.9, 39.8, 41.3, 56.3, 58.7, 65.1, 66.1, 67.4, 83.3, 127.7, 127.9, 128.0, 128.3, 128.4, 130.6, 132.2, 132.6, 135.5, 136.6, 153.4, 154.4, 171.2, 172.6; $[\alpha]_D^{20}$ -8.6 (*c* 4.7, DCM); HRMS-ESI: m/z calcd for $C_{26}H_{27}Cl_2N_3O_6K$ $(M+K)^+$ 586.0908, found 586.0928

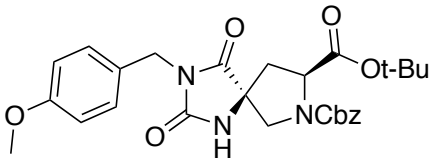
**2.12b - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-1,3-bis(3,4-dichlorobenzyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**




Using procedure 1, 70 mg (99%) was recovered; $^1$H NMR (500 MHz, $CDCl_3$) 1.30 (9H, s, rotameric) , 2.09 (1H, dd, *J* = 7.1, 14.1), 2.53-2.63 (1H, m), 3.57

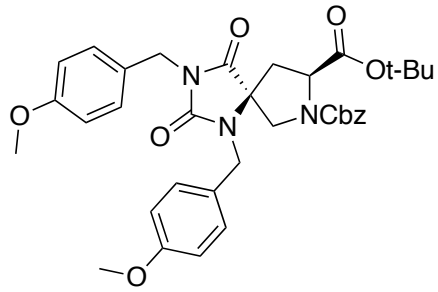
(1H, d, rotameric, *J* = 9.2), 3.72-3.82 (1H, m), 4.49 (1H, dd, *J* = 7.1, 9.2), 4.62 (4H, s, rotameric), 5.09 (2H, m), 7.16-7.49 (11H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 27.7, 36.5, 42.1, 50.6, 58.2, 63.5, 66.7, 67.7, 68.2, 82.5, 126.0, 126.9, 127.2, 127.5, 127.9, 128.0, 128.5, 129.5, 130.3, 130.6, 130.9, 132.2, 132.5, 132.8, 133.0, 135.5, 135.8, 137.1, 141.5, 153.9, 155.3, 170.8, 173.5; $[\alpha]_D^{20}$ +2.8 (*c* 7.0, DCM); HRMS-ESI: m/z calcd for $C_{33}H_{31}Cl_4N_3O_6K$ $(M+K)^+$ 744.0599, found 744.0577

**2.13a - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-3-(4-methoxybenzyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**



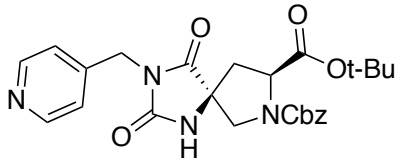

Using procedure 2, 46 mg (91%) was recovered; $^1$H NMR (400 MHz, $CDCl_3$) 1.32 (9H, s, rotameric), 2.01 (1H, d, *J* = 14.0), 2.77 (1H, ddd, *J* = 9.8, 13.4, 17.6), 3.78 (4H, m), 3.83-3.91 (1H, m), 4.43 (1H, dd, *J* = 9.1, 14.9), 4.60 (1H, d *J* = 8.8), 5.06-5.19 (2H, m), 6.63 (1H, s, rotameric), 6.85 (1H, d, rotameric, *J* = 8.8), 7.31-7.34 (7H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 27.9, 39.2, 42.1, 55.2, 56.3, 58.9, 65.3, 66.2, 67.6, 83.3, 114.0, 127.9, 128.0, 128.3, 128.5, 130.1, 135.9, 154.0, 154.9, 159.4, 172.0, 172.6; $[\alpha]_D^{20}$ -3.4 (*c* 4.6, DCM); HRMS-ESI: m/z calcd for $C_{27}H_{31}N_3O_7K$ $(M+K)^+$ 548.1794, found 548.1787

**2.13b - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-1,3-bis(4-methoxybenzyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**



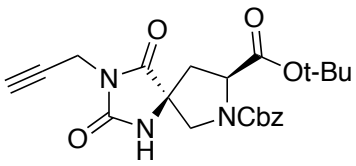
Using procedure 1, 62 mg (99%) was recovered; $^{1}$H NMR (500 MHz, $CDCl_3$) 1.3 (9H, s, rotameric), 2.12 (1H, dd, *J* = 8.1, 13.7), 2.31-2.42 (1H, m), 3.60 (1H, d, *J* = 11.6), 3.76-3.80 (7H, m), 4.49-4.56 (3H, m), 4.62 (2H, s, rotameric), 5.06-5.18 (2H, m), 6.82-6.89 (4H, m), 7.22-7.36 (9H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 14.2, 27.8, 19.7, 35.9, 42.6, 46.3, 49.8, 55.2, 58.3, 64.8, 66.3, 67.5, 82.0, 113.9, 114.3, 127.8, 128.0, 128.4, 129.0, 129.2, 130.1, 136.1, 155.6, 159.4, 170.8, 174.1; $[\alpha]_D^{20}$ +2.1 (*c* 6.2, DCM); HRMS-ESI: m/z calcd for $C_{31}H_{31}N_3O_8K$ $(M-(t\text{-}Bu)+K)^+$ 612.1748, found 612.1766

**2.14a - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-2,4-dioxo-3-(pyridin-4-ylmethyl)-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**



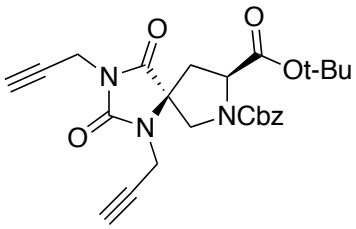
Using procedure 2, 43 mg (90%) was recovered; $^{1}$H NMR (400 MHz, $CDCl_3$) 1.50 (9H, s, rotameric), 2.03-2.06 (1H, m), 2.81 (1H, ddd, *J* = 9.8, 13.9, 18.5), 3.82-3.95 (2H, m), 4.41-4.48 (1H, m), 4.64 (2H, d, *J* = 9.5), 5.08-5.21 (2H, m), 6.86 (1H, s, rotameric), 7.24-7.26 (2H, m), 7.32-7.35 (5H, m), 8.57-8.59 (2H, m) ;$^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 27.9, 29.7, 39.2, 40.0, 41.4, 56.3, 58.9, 65.6, 66.6, 67.8, 83.7, 124.3, 127.9, 128.0, 128.1, 128.3, 128.4, 128.5, 128.6, 135.7, 146.9, 153.6, 154.1, 172.3, 172.6; [α]D = -1.6 (*c* 4.3, DCM); HRMS-ESI: m/z calcd for $C_{25}H_{28}N_4O_6K$ $(M+K)^+$ 519.1640, found 519.1641

**2.15a - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-2,4-dioxo-3-(prop-2-yn-1-yl)-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**



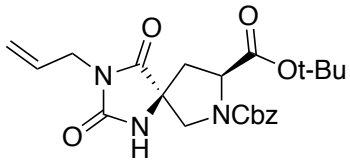

Using procedure 2, 37 mg (88%) was recovered; $^{1}$H NMR (500 MHz, $CDCl_3$) 1.32 (9H, s, rotameric), 2.05 (1H, dd, *J* = 8.3, 14.0), 2.25 (1H, dt, *J* = 2.5, 3.6, 5.1), 2.78 (1H, ddd, rotameric, *J* = 9.7, 14.0, 21.4), 3.81-3.93 (2H, m), 4.26 (2H, d, rotameric, *J* = 2.5), 4.44 (1H, dd, rotameric, *J* = 9.6, 17.6), 5.06-5.17 (2H, m), 6.83 (1H, s, rotameric), 7.28-7.35 (5H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 27.5, 27.9, 38.9, 39.8, 56.2, 58.7, 65.2, 66.2, 67.5, 71.9, 76.4, 83.3, 127.8, 128.1, 128.3, 135.7, 153.4, 153.8, 171.4, 172.6; $[\alpha]_D^{20}$ + 2.7 (*c* 3.7, DCM); HRMS-ESI: m/z calcd for $C_{22}H_{25}N_3O_6K$ $(M+K)^+$ 466.1375, found 466.1376

**2.15b - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-2,4-dioxo-1,3-di(prop-2-yn-1-yl)-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**



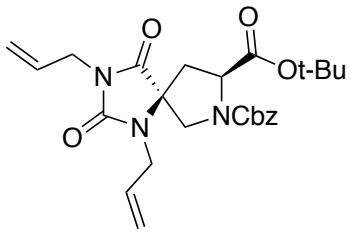

Using procedure 1, 45 mg (98%) was recovered; $^{1}$H NMR (500 MHz, $CDCl_3$) 1.35 (9H, s, rotameric), 2.25 (2H, s, rotameric), 2.47-2.54 (1H, m), 2.61-2.68 (1H, m), 3.87-3.99 (2H, m), 4.21 (1H, d, rotameric, *J* = 2.6), 4.23- 4.27 (3H, m), 4.57 (1H, t, *J* = 7.6), 5.08-5.18 (2H, m), 7.28-7.35 (5H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 27.8, 28.3, 29.0, 35.9, 50.4, 58.4, 66.6, 67.6, 72.2, 73.2, 76.3, 78.0, 82.4, 127.9, 128.1, 128.4, 135.9, 153.7, 154.0, 170.8, 172.9; $[\alpha]_D^{20}$ +3.5 (*c* 4.5, DCM); HRMS-ESI: m/z calcd for $C_{25}H_{27}N_3O_6K$ $(M+K)^+$ 504.1531, found 504.1543

**2.16a - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-3-allyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**



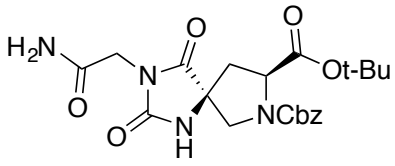

Using procedure 2, 40 mg (93%) was recovered; $^{1}$H NMR (500 MHz, $CDCl_3$) 1.32 (9H, s, rotameric), 2.02 (1H, dd, *J* = 6.6, 13.8), 2.75 (1H, ddd, *J* = 9.8, 13.8, 22.1), 3.86 (2H, m), 4.09 (2H, m), 4.44 (1H, dd, rotameric, *J* = 9.6, 18.3), 5.06-5.15 (2H, m), 5.18-5.21 (2H, m), 5.77-5.82 (1H, m), 6.74 (1H, s, rotameric), 7.29-7.33 (5H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 27.5, 39.0, 39.9, 40.8, 56.4, 58.7, 65.0, 66.0, 67.5, 83.0, 118.2, 127.7, 128.1, 128.3, 130.6, 135.8, 153.8, 154.6, 172.1, 172.6; $[\alpha]_D^{20}$ +3.9 (*c* 4.0, DCM); HRMS-ESI: m/z calcd for $C_{22}H_{27}N_3O_6K$ $(M+K)^+$ 468.1531, found 468.1520

**2.16b - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-1,3-diallyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**



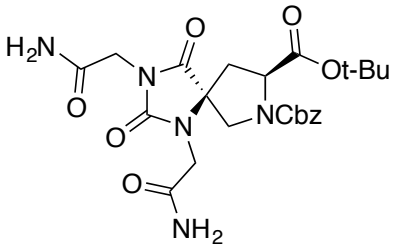

Using procedure 1, 46 mg (99%) was recovered; $^{1}$H NMR (500 MHz, $CDCl_3$) 1.32 (9H, s, rotameric), 2.26-2.30 (1H, m), 2.51-2.55 (1H, m), 3.74 (1H, d, *J* = 11.8), 3.89 (1H, d, rotameric, *J* = 10.7), 4.00 (2H, dt, rotameric, *J* = 1.5, 5.5), 4.10 (2H, d, *J* = 5.3) 4.55 (1H, t, *J* = 8.4), 5.08-5.23 (6H, m), 5.77-5.87 (2H, m), 7.29-7.35 (5H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 27.4, 29.2, 35.8, 40.5, 41.6, 50.0, 58.0, 66.0, 67.1, 81.7, 117.5, 118.0, 127.4, 127.6, 128.0, 130.4, 132.5, 135.6, 153.7, 154.5, 170.4, 173.6; $[\alpha]_D^{20}$ +2.4 (*c* 4.6, DCM); HRMS-ESI: m/z calcd for $C_{25}H_{31}N_3O_6Na$ $(M+Na)^+$ 492.2105, found 492.2119

**2.17a - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-3-(2-amino-2-oxoethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**




Using procedure 2, 37 mg (85%) was recovered; $^{1}$H NMR (500 MHz, $CDCl_3$) 1.32 (9H, s, rotameric), 2.15 (1H, t, *J* = 13.7), 2.75 (1H, ddd, *J* = 9.5, 13.7, 16.7), 3.82-3.92 (2H, m), 4.15 (2H, s), 4.43 (1H, td, *J* = 2.6, 9.5), 5.06-5.16 (2H, m), 6.34 (1H, s, rotameric), 6.74 (1H, s, rotameric), 6.95 (1H, s, rotameric), 7.27-7.33 (5H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 27.6, 27.9, 38.8, 39.6, 40.6, 55.6, 58.7, 65.2, 66.3, 67.5, 83.0, 127.7, 128.0, 128.3, 135.7, 153.6, 155.0, 168.5, 172.2, 172.6; $[\alpha]_D^{20}$ -2.4 (*c* 3.7, DCM); HRMS-ESI: m/z calcd for $C_{21}H_{26}N_4O_7K$ $(M+K)^+$ 485.1433, found 485.1444

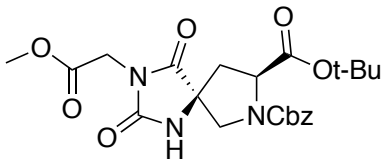
**2.17b - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-1,3-bis(2-amino-2-oxoethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**




Using procedure 1, 35 mg (70%) was recovered; $^{1}$H NMR (500 MHz, $CDCl_3$) 1.29 (9H, s, rotameric), 2.28-2.33 (1H, m), 2.68-2.78 (1H, m), 3.68 (1H, d, *J* = 11.3), 3.90-3.99 (3H, m), 4.11-4.16 (2H, m), 4.49 (1H, t, *J* = 8.2), 5.02-5.15 (2H, m), 6.67 (2H, s, broad), 7.13 (2H, s, broad), 7.27-7.36 (5H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 27.7, 36.2, 40.7, 41.9, 50.8, 58.3, 65.2, 66.9, 67.7, 82.5, 127.9, 128.2, 128.5, 135.8, 154.5, 156.1, 169.5, 171.1, 171.3, 174.4; $[\alpha]_D^{20}$

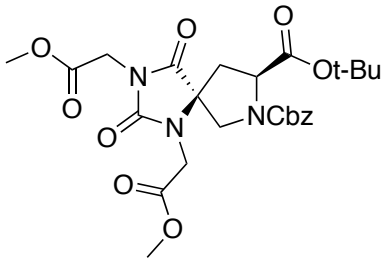
+2.0 (*c* 3.5, DCM); HRMS-ESI: m/z calcd for $C_{23}H_{29}N_5O_8K$ $(M+K)^+$ 542.1648, found 542.1653

**2.18a - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-3-(2-methoxy-2-oxoethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**




Using procedure 2, 40 mg (87%) was recovered; $^1$H NMR (500 MHz, $CDCl_3$) 1.32 (9H, s, rotameric), 2.08-2.13 (1H, m), 2.74-2.83 (1H, m), 3.73 (3H, s, rotameric), 3.87-3.92 (2H, m), 4.25 (2H, d, *J* = 6.7), 4.43 (1H, ddd, *J* = 1.5, 9.5, 11.0), 5.06-5.17 (2H, m), 6.85 (1H, s, rotameric), 7.28-7.34 (5H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 27.5, 38.9, 39.3, 39.8, 52.6, 55.9, 58.7, 65.3, 66.3, 67.5, 83.1, 127.7, 128.0, 128.4, 135.7, 153.4, 154.0, 167.1, 172.1, 172.6; $[\alpha]_D^{20}$ +2.6 (*c* 4.0, DCM); HRMS-ESI: m/z calcd for $C_{22}H_{27}N_3O_8K$ $(M+K)^+$ 500.1430, found 500.1436

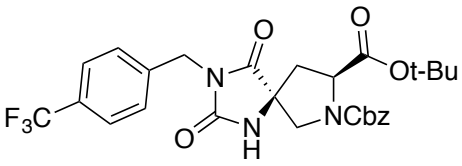
**2.18b - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-1,3-bis(2-methoxy-2-oxoethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**




Using procedure 1, 52 mg (99%) was recovered; $^1$H NMR (500 MHz, $CDCl_3$) 1.30 (9H, s, rotameric), 2.30 (1H, dd, *J* = 6.2, 14.3), 2.79 (1H, dd, *J* = 9.5, 14.3), 3.68-3.73 (1H, m), 3.73 (3H, s), 3.74 (3H, s), 3.93-4.04 (1H, m), 4.17-4.23 (2H, m), 4.27 (2H, s), 4.49 (1H, dd, *J* = 6.2, 9.4), 5.08-5.15 (2H, m), 7.29-7.35 (5H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 27.4, 29.3, 36.1, 36.9, 39.2, 40.7, 51.3, 52.2, 52.4, 57.9, 58.5, 66.7, 67.2, 82.0, 127.5, 127.7, 128.0, 135.5, 153.6,

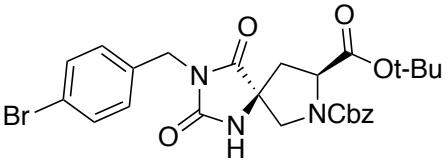
154.3, 166.7, 168.6, 170.8, 173.1; $[\alpha]_D^{20}$ +2.6 (*c* 5.2, DCM); HRMS-ESI: m/z calcd for $C_{25}H_{31}N_3O_{10}K$ $(M+K)^+$ 572.1641, found 572.1648

**2.19a - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-2,4-dioxo-3-(4-(trifluoromethyl)benzyl)-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**




Using procedure 2, 49 mg (90%) was recovered; $^1$H NMR (400 MHz, $CDCl_3$) 1.33 (9H, s, rotameric), 2.00 (1H, d, *J* = 13.6), 2.78 (1H, ddd, *J* = 9.9, 13.7, 17.6), 3.80-3.94 (2H, m), 4.43 (1H, dd, *J* = 9.9, 15.3), 4.71 (2H, d, *J* = 9.1), 5.07-5.19 (2H, m), 6.79 (1H, s, rotameric), 7.32-7.35 (5H, m), 7.50 (2H, d, *J* = 8.3), 7.58 (2H, d, rotameric, *J* = 8.3); $^{13}$C NMR (100 MHz, $CDCl_3$, rotamers present) 28.3, 39.6, 40.4, 42.4, 56.7, 59.3, 65.8, 66.8, 68.1, 83.9, 126.1, 126.2, 128.3, 128.5, 128.7, 128.9, 129.4, 130.9, 136.2, 139.8, 154.9, 172.4, 173.3; $[\alpha]_D^{20}$ -1.7 (*c* 4.9, DCM); HRMS-ESI: m/z calcd for $C_{27}H_{28}F_3N_3O_6K$ $(M+K)^+$ 586.1562, found 586.1575

**2.20a - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-3-(4-bromobenzyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**

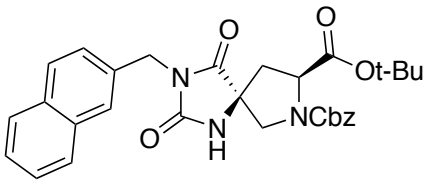



Using procedure 2, 49 mg (89%) was recovered; $^1$H NMR (400 MHz, $CDCl_3$) 1.33 (9H, s, rotameric), 2.02 (1H, d, rotameric, *J* = 13.4), 2.77 (1H, ddd, rotameric, *J* = 9.7, 13.9, 17.3), 3.78-3.92 (2H, m), 4.43 (1H, dd, *J* = 9.8, 15.2), 4.61 (1H, d, *J* = 8.9), 5.07-5.21 (2H, m), 6.75 (1H, s, rotameric), 7.25-7.27 (2H, m), 7.33-

7.35 (5H, m), 7.43-7.51 (2H, m); $^{13}$C NMR (100 MHz, $CDCl_3$) 28.1, 30.1, 39.6, 42.4, 56.7, 59.3, 65.7, 66.7, 68.1, 83.9, 122.6, 128.3, 138.4, 128.6, 129.0, 130.9, 132.3, 135.0, 154.4, 155.1, 172.6, 173.2; $[\alpha]_D^{20}$ -2.4 (*c* 4.9, DCM); HRMS-ESI: m/z calcd for $C_{26}H_{28}BrN_3O_6K$ $(M+K)^+$ 596.0793, found 596.0802

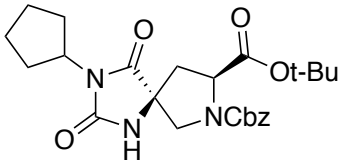
**2.20b - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-1,3-bis(4-bromobenzyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate – <u>SAME AS 10A</u>**

**2.21a - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-3-(naphthalen-2-ylmethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**



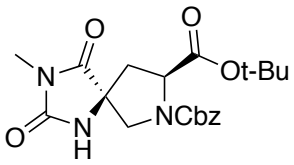

Using procedure 2, 44 mg (89%) was recovered; $^1$H NMR (400 MHz, $CDCl_3$) 1.32 (9H, s, rotameric), 2.02 (1H, d, *J* = 13.9), 2.78 (1H, 9.8, 13.9, 16.7), 3.79-3.94 (2H, m), 4.43 (1H, dd, *J* = 9.0, 16.4), 4.83 (2H, d, *J* = 8.4), 5.06-5.18 (2H, m), 6.73 (1H, s, rotameric), 7.32-7.36 (5H, m), 7.44-7.51 (3H, m), 7.79-7.85 (4H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 27.7, 29.7, 39.2, 40.1, 42.8, 56.7, 59.4, 65.3, 66.3, 67.7, 53.5, 126.2, 126.3, 126.4, 127.7, 127.8, 127.9, 128.0, 128.3, 128.5, 128.6, 133.0, 133.2, 135.8, 153.5, 154.9, 172.1, 172.9; $[\alpha]_D^{20}$ -3.3 (*c* 4.4, DCM); HRMS-ESI: m/z calcd for $C_{30}H_{31}N_3O_6K$ $(M+K)^+$ 568.1844, found 568.1853

**2.22a - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-3-cyclopentyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**



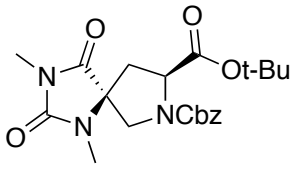
Using procedure 2, 43 mg (97%) was recovered; $^{1}$H NMR (500 MHz, $CDCl_3$) 0.79-0.88 (1H, m), 1.32 (9H, s, rotameric), 1.55 (2H, m) 1.85 (4H, m), 1.98-2.02 (2H??, m), 2.75 (1H, ddd, *J* = 9.8, 13.7, 21.6), 3.76-3.89 (2H, m), 4.37-4.46 (2H, m), 5.07-5.17 (2H, m), 6.62 (1H, s, rotameric), 7.29-7.35 (5H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 25.1, 27.8, 29.2, 39.4, 40.3, 52.1, 56.44, 59.6, 64.5, 65.6, 67.7, 83.4, 128.1, 128.3, 128.6, 136.1, 153.7, 155.4, 172.4, 172.9; $[\alpha]_D^{20}$ -1.8 (*c* 4.3, DCM); HRMS-ESI: m/z calcd for $C_{24}H_{31}N_3O_6Na$ $(M+Na)^+$ 480.2105, found 480.2122

**2.23a - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-3-methyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**



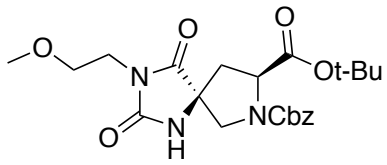
Using procedure 2, 34 mg (85%) was recovered; $^{1}$H NMR (500 MHz, $CDCl_3$) 1.31 (9H, s, rotameric), 2.02 (1H, dd, rotameric, *J* = 4.9, 13.9), 2.76 (1H, ddd, *J* = 9.9, 13.7, 21.5), 3.01 (3H, s, rotameric), 3.78-3.90 (2H, m), 4.43 (1H, dd, *J* = 9.7, 19.7), 5.06-5.17 (2H, m), 6.73 (1H, s, rotameric), 7.27-7.34 (5H, m) ; $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 24.8, 27.7, 39.0, 39.9, 56.3, 58.7, 65.1, 66.1, 67.5, 83.0, 127.8, 128.1, 128.4, 135.7, 153.4, 155.2, 172.4, 172.6; $[\alpha]_D^{20}$ +2.0 (*c* 3.4, DCM); HRMS-ESI: m/z calcd for $C_{20}H_{25}N_3O_6K$ $(M+K)^+$ 442.1375, found 442.1365

**2.23b - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-1,3-dimethyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**



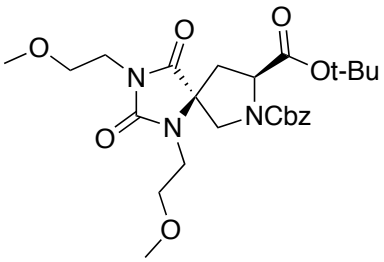

Using procedure 1, 41 mg (99%) was recovered; $^{1}$H NMR (500 MHz, $CDCl_3$) 1.33 (9H, s, rotameric), 2.17-2.22 (1H, m), 2.62 (1H, dd, *J* = 9.2, 13.8), 2.91 (3H, s, rotameric), 3.01 (3H, s, rotameric), 3.65-3.68 (1H, m), 3.85 (1H, d, rotameric, *J* = 11.9), 4.54-4.60 (1H, m), 5.07-5.18 (2H, m), 7.28-7.35 (5H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 24.6, 27.6, 29.3, 34.9, 35.6, 49.8, 58.2, 58.8, 66.0, 67.2, 81.9, 127.5, 127.6, 128.0, 135.6, 153.7, 155.1, 170.4, 174.2; $[\alpha]_D^{20}$ -4.0 (*c* 4.1, DCM); HRMS-ESI: m/z calcd for $C_{21}H_{27}N_3O_6K$ $(M+K)^+$ 456.1531, found 456.1555

**2.24a - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-3-(2-methoxyethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**



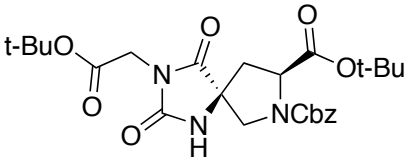

Using procedure 2, 42 mg (95%) was recovered; $^{1}$H NMR (500 MHz, $CDCl_3$) 1.32 (9H, s, rotameric), 2.04 (1H, dd, *J* = 7.5, 13.9), 2.76 (1H, ddd, *J* = 9.8, 13.9, 21.8), 3.31 (3H, s, rotameric), 3.53-3.56 (2H, m), 3.67-3.71 (2H, m), 3.78-3.91 (2H, m), 4.44 (1H, 9.2, 17.1), 5.06-5.16 (2H, m), 6.71 (1H, s, rotameric), 7.28-7.32 (5H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 27.7, 29.5, 38.1, 39.0, 56.0, 58.4, 58.8, 64.9, 65.9, 67.4, 68.6, 83.0, 127.7, 128.0, 128.3, 135.7, 153.4, 155.0, 172.4, 172.6; $[\alpha]_D^{20}$ +1.9 (*c* 4.2, DCM); HRMS-ESI: m/z calcd for $C_{22}H_{29}N_3O_7Na$ $(M+Na)^+$ 470.1898, found 470.1925

**2.24b - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-1,3-bis(2-methoxyethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**



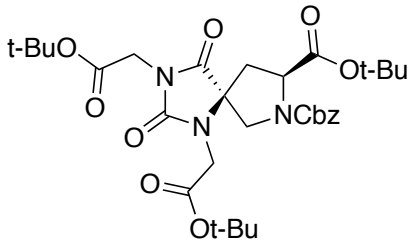
Using procedure 1, 49 mg (99%) was recovered; $^{1}$H NMR (500 MHz, $CDCl_3$) 1.32 (9H, s, rotameric), 2.39-2.43 (1H, m), 2.51-2.57 (1H, m), 3.28 (3H, s), 3.31 (3H, s), 3.45-3.47 (2H, m), 3.50-3.59 (4H, m), 3.66-3.68 (2H, m), 3.73 (1H, d, rotameric $J$ = 11.9), 3.88 (1H, d, rotameric, $J$ = 11.8), 4.53 (1H, t, $J$ = 7.8), 5.06-5.16 (2H, m), 7.27-7.33 (5H, m); $^{13}C$ NMR (125 MHz, $CDCl_3$, rotamers present) 27.8, 29.6, 36.3, 38.1, 40.5, 50.9, 58.6, 58.7, 66.4, 67.5, 68.7, 82.0, 127.8, 128.0, 128.4, 136.1, 154.1, 155.5, 171.0, 174.8; $[\alpha]_D^{20}$ -2.9 (c 4.9, DCM); HRMS-ESI: m/z calcd for $C_{25}H_{35}N_3O_8H$ $(M+H)^+$ 506.2497, found 506.2492

**2.25a - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-3-(2-(*tert*-butoxy)-2-oxoethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**



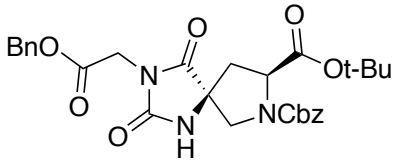
Using procedure 2, 46 mg (92%) was recovered; $^{1}$H NMR (500 MHz, $CDCl_3$) 1.43 (18H, s, rotameric), 2.06-2.11 (1H, m), 2.79 (1H, ddd, $J$ = 9.7, 13.9, 23.1), 3.85-3.91 (2H, m), 4.13 (2H, d, $J$ = 6.4), 4.43 (1H, dd, $J$ = 9.6, 16.5), 5.06-5.14 (2H, m), 6.79 (1H, s, rotameric), 7.27-7.32 (5H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 27.8, 39.0, 40.2, 56.2, 58.7, 65.3, 66.3, 67.5, 82.8, 83.2, 127.7, 128.0, 128.3, 135.8, 153.4, 154.2, 165.5, 172.1, 172.4; $[\alpha]_D^{20}$ +1.4 (*c* 4.6, DCM); HRMS-ESI: m/z calcd for $C_{25}H_{33}N_3O_8Na$ $(M+Na)^+$ 526.2160, found 526.2181

**2.25b - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-1,3-bis(2-(*tert*-butoxy)-2-oxoethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**



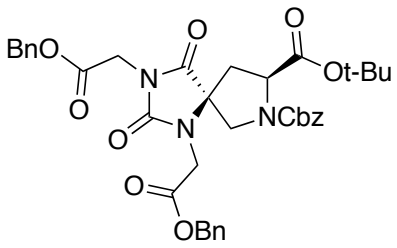

Using procedure 1, 60 mg (99%) was recovered; $^{1}$H NMR (500 MHz, $CDCl_3$) 1.44 (27H, s, rotameric), 2.16 (1H, dd, *J* = 7.3, 14.1), 2.68-2.72 (1H, m), 3.60-3.66 (1H, m), 4.01 (3H, s, broad), 4.13 (2H, s), 4.51 (1H, dd, *J* = 7.3, 8.8), 5.09-5.15 (2H, m), 7.27-7.34 (5H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 27.7, 27.9, 28.0, 36.2, 36.9, 40.4, 41.7, 50.8, 58.3, 60.5, 66.4, 67.5, 82.2, 83.0, 127.8, 128.0, 128.4, 136.0, 154.85, 160.0, 165.5, 167.3, 171.1, 173.8; $[\alpha]_D^{20}$ -2.2 (*c* 6.0, DCM); HRMS-ESI: m/z calcd for $C_{31}H_{43}N_3O_{10}K$ $(M+K)^+$ 656.2580, found 656.2578

**2.26a - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-3-(2-(benzyloxy)-2-oxoethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**



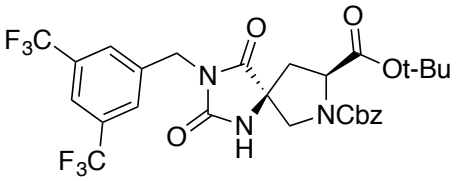

Using procedure 2, 4.73 g (89%) was recovered; $^{1}$H NMR (500 MHz, $CDCl_3$) 1.35 (9H, s, rotameric), 2.06 (1H, dd, *J* = 7.7, 13.9), 2.76 (1H, ddd, rotameric, *J* = 9.6, 13.9, 21.6), 3.84-3.94 (2H, m), 4.31 (2H, d, rotameric *J* = 5.8), 4.45 (1H, dd, rotameric, *J* = 9.6, 18.5) 5.08-5.14 (2H, m), 5.15-5.19 (2H, m), 6.86 (1H, s, rotameric), 7.28-7.38 (10H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 27.5, 27.8, 31.3, 36.3, 38.9, 39.5, 55.8, 58.7, 65.3, 66.3, 67.5, 83.2, 127.7, 127.9, 128.1, 128.2, 128.3, 128.5, 134.6, 135.7, 153.4, 153.9, 166.5, 171.9, 172.6; $[\alpha]_D^{20}$ +2.8 (*c* 10.0, DCM); HRMS-ESI: m/z calcd for $C_{28}H_{31}N_3O_8Na$ $(M+Na)^+$ 560.2003, found 560.2022

**2.26b - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-1,3-bis(2-(benzyloxy)-2-oxoethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**



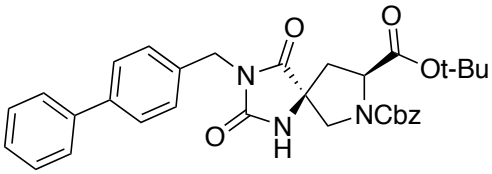

Using procedure 1, 68 mg (99%) was recovered; $^{1}$H NMR (500 MHz, $CDCl_3$) 1.32 (9H, s, rotameric), 2.28 (1H, dd, *J* = 6.4, 14.3), 2.73 (1H, dd, *J* = 9.4, 14.3), 3.71 (1H, d, *J* = 12.4), 3.93-4.03 (1H, m), 4.19-4.29 (2H, m), 4.34 (2H, s), 4.51 (1H, dd, *J* = 6.6, 9.0), 5.10-5.24 (6H, m), 7.32-7.36 (15H, m); $^{13}$C NMR (125 MHz, $CDCl_3$) 26.7, 27.8, 29.6, 36.3, 39.7, 41.2, 51.4, 58.7, 60.5, 65.0, 67.0, 64.5, 68.0, 82.3, 126.8, 127.8, 128.0, 128.2, 128.3, 128.4, 128.5, 134.6, 134.7, 135.8, 153.8, 154.5, 166.4, 168.3, 169.4, 171.0, 173.4; $[\alpha]_D^{20}$ + 2.1 (*c* 6.8, DCM); HRMS-ESI: m/z calcd for $C_{37}H_{39}N_3O_{10}Na$ $(M+Na)^+$ 708.2528, found 708.2524

**2.27a - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-3-(3,5-bis(trifluoromethyl)benzyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**



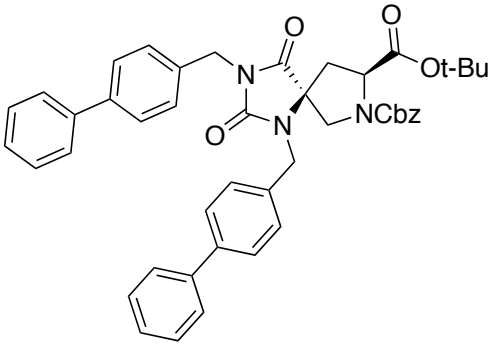

Using procedure 2, 55 mg (90%) was recovered; $^{1}$H NMR (400 MHz, $CDCl_3$) 1.33 (9H, s, rotameric), 2.05 (1H, d, *J* = 13.7), 2.81 (1H, ddd, *J* = 9.8, 13.9, 17.6), 3.82-3.95 (2H, m), 4.46 (1H, dd, *J* = 9.3, 14.9), 4.74-4.77 (2H, m), 5.08-5.19 (2H, m), 6.85 (1H, s, rotameric), 7.31-7.37 (5H, m), 7.83-7.87 (3H, m); $^{13}$C NMR (100 MHz, $CDCl_3$, rotamers present) 28.1, 40.4, 42.1, 57.1, 59.2, 65.9, 66.9, 68.1, 83.9, 122.0, 124.8, 128.4, 128.5, 128.7, 128.9, 129.4, 132.4, 136.2, 138.3, 153.9, 154.6, 172.3, 173.1; $[\alpha]_D^{20}$ -4.3 (*c* 5.5, DCM); HRMS-ESI: m/z calcd for $C_{28}H_{27}F_6N_3O_6K$ $(M+K)^+$ 654.1436, found 654.1443

**2.28a - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-3-([1,1'-biphenyl]-4-ylmethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**




Using procedure 2, 48 mg (87%) was recovered; $^1$H NMR (400 MHz, $CDCl_3$) 1.33 (9H, s, rotameric), 2.05 (1H, d, *J* = 14.3), 2.80 (1H, ddd, *J* = 9.8, 13.7, 17.6), 3.80-3.95 (2H, m), 4.45 (1H, dd, *J* = 8.9, 15.8), 4.71 (2H, d, *J* = 9.0), 5.01-5.19 (2H, m), 6.74 (1H, s, rotameric), 7.30-7.35 (6H, m), 7.40-7.46 (4H, m), 7.53-7.59 (4H, m); $^{13}$C NMR (100 MHz, $CDCl_3$, rotamers present) 27.7, 29.7, 39.2, 40.0, 42.3, 46.0, 56.3, 59.4, 65.3, 66.3, 67.6, 83.4, 127.0, 127.4, 127.5, 127.9, 128.0, 128.2, 128.5, 128.7, 129.0, 129.1, 134.6, 135.8, 140.5, 141.0, 154.0, 154.8, 172.1, 172.8; $[\alpha]_D^{20}$ -4.2 (*c* 4.8, DCM); HRMS-ESI: m/z calcd for $C_{32}H_{33}N_3O_6K$ $(M+K)^+$ 594.2001, found 594.2003

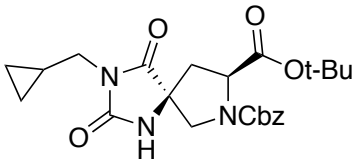
**2.28b - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-1,3-bis([1,1'-biphenyl]-4-ylmethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**




Using procedure 1, 71 mg (99%) was recovered; $^1$H NMR (500 MHz, $CDCl_3$) 1.32 (9H, s, rotameric), 2.22 (1H, dd, *J* = 7.8, 13.8), 2.47-2.56 (1H, m), 3.72-3.81 (1H, m), 3.87-3.94 (1H, m), 4.59 (1H, t, *J* = 8.3), 4.66-4.79 (4H, m), 5.12-5.23 (2H, m), 7.27-7.63 (23H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 27.7, 29.7, 36.0, 72.8, 45.8, 50.0, 55.4, 58.2,

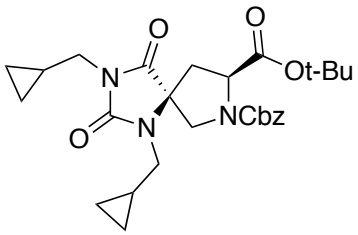
66.5, 67.5, 67.7, 82.2, 127.0, 127.1, 127.2, 127.3, 127.4, 127.5, 127.6, 127.8, 127.9, 128.0, 128.1, 128.4, 128.8, 128.9, 129.0, 131.3, 134.7, 136.0, 139.9, 140.3, 140.5, 141.0, 154.0, 155.7, 170.8, 174.1; $[\alpha]_D^{20}$ +2.8 (*c* 7.1, DCM); HRMS-ESI: m/z calcd for $C_{45}H_{43}N_3O_6K$ $(M+K)^+$ 760.2783, found 760.2793

**2.29a - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-3-(cyclopropylmethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**




Using procedure 2, 41 mg (94%) was recovered; $^1$H NMR (500 MHz, $CDCl_3$) 0.30-0.33 (2H, m), 0.46-0.49 (2H, m), 1.12-1.15 (1H, m), 1.33 (9H, s, rotameric), 2.04 (1H, dd, *J* = 7.1, 13.9), 2.78 (1H, ddd, *J* = 9.6, 13.7, 22.8), 3.35 (1H, dd, *J* = 7.5, 9.2), 3.80-3.92 (2H, m), 4.45 (1H, dd, *J* = 9.3, 18.1), 5.07-5.17 (2H, m), 6.68 (1H, s, rotameric), 7.29-7.35 (5H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 3.6, 9.9, 27.8, 39.9, 43.5, 56.1, 58.8, 65.0, 66.0, 67.5, 83.0, 127.8, 128.0, 128.3, 135.7, 153.4, 155.2, 172.4, 172.6; $[\alpha]_D^{20}$ -1.8 (*c* 4.1, DCM); HRMS-ESI: m/z calcd for $C_{23}H_{29}N_3O_6Na$ $(M+Na)^+$ 466.1949, found 466.1959

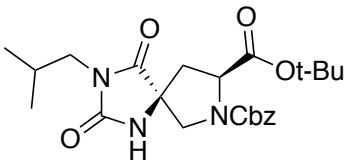
**2.29b - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-1,3-bis(cyclopropylmethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**




Using procedure 1, 49 mg (99%) was recovered; $^1$H NMR (500 MHz, $CDCl_3$) 0.31-0.38 (4H, m), 0.48 (2H, d, *J* = 8.1) 0.56 (2H, m), 1.02-1.05 (1H, m), 1.13 (1H, m), 1.32 (9H, s,

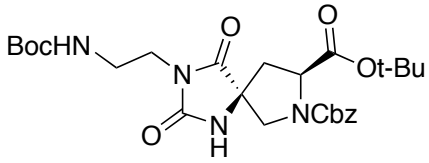
rotameric), 2.29-2.31 (1H, m), 2.58-2.61 (1H, m), 3.17-3.20 (1H, m), 3.26 (1H, d, rotameric, *J* = 7.1), 3.35 (2H, d, *J* = 6.8), 3.78-3.95 (2H, m), 4.57 (1H, t, *J* = 8.2), 5.07-5.17 (2H, m), ; $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 3.6, 4.9, 9.8, 11.0, 27.7, 29.5, 36.3, 43.5, 44.5, 50.8, 58.4, 66.0, 67.1, 67.4, 82.0, 127.7, 127.9, 128.3, 135.9, 154.1, 155.7, 170.9, 174.5; $[\alpha]_D^{20}$ + 4.2 (*c* 4.9, DCM); HRMS-ESI: m/z calcd for $C_{27}H_{35}N_3O_6Na$ $(M+Na)^+$ 520.2418, found 520.2432

**2.30a - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-3-isobutyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**



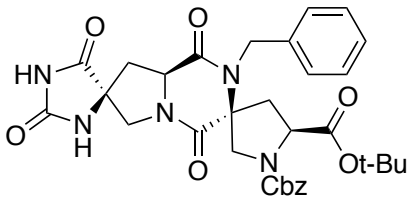

Using procedure 2, 43 mg (97%) was recovered; $^1$H NMR (500 MHz, $CDCl_3$) 0.88 (6H, d, rotameric, *J* = 6.7), 1.33 (9H, s, rotameric), 1.99-2.09 (2H, m), 2.78 (1H, ddd, *J* = 9.6, 13.6, 22.9), 3.31 (2H, t, *J* = 7.8), 3.78-3.92 (2H, m), 4.44 (1H, dd, *J* = 9.0, 17.7), 5.07-5.18 (2H, m), 6.63 (1H, s, rotameric), 7.28-7.35 (5H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 19.8, 27.1, 27.8, 29.2, 40.0, 46.0, 56.6, 58.8, 64.9, 65.9, 67.5, 83.0, 127.8, 128.1, 128.4, 135.8, 153.5, 155.3, 172.4, 172.6; $[\alpha]_D^{20}$ +4.5 (*c* 4.3, DCM); HRMS-ESI: m/z calcd for $C_{23}H_{31}N_3O_6Na$ $(M+Na)^+$ 468.2105, found 468.2116

**2.31a - 7-benzyl 8-(*tert*-butyl) (5*S*,8*S*)-3-(2-((*tert*-butoxycarbonyl)amino)ethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**




Using procedure 2, 46 mg (86%) was recovered; $^{1}$H NMR (500 MHz, $CDCl_3$) 1.36 (9H, s, rotameric), 1.45 (18H, s, rotameric), 2.14 (1H, t, *J* = 14.8), 2.82 (1H, ddd, *J* = 9.7, 13.9, 22.0), 3.34-3.47 (2H, m), 3.62-3.65 (2H, m), 3.89-3.96 (2H, m), 4.46 (1H, dd, *J* = 9.5, 13.8), 5.11-5.20 (3H, m), 6.77 (1H, s), 7.35 (5H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 14.0, 20.8, 27.5, 28.2, 29.5, 38.5, 38.8, 39.5, 39.7, 56.2, 58.7, 64.9, 65.9, 67.4, 79.1, 83.0, 127.8, 128.0, 128.3, 135.7, 153.4, 155.2, 156.2, 172.5, 172.6; $[\alpha]_D^{20}$ +2.1 (*c* 4.6, DCM); HRMS-ESI: m/z calcd for $C_{26}H_{36}N_4O_8Na$ $(M+Na)^+$ 555.2425, found 555.2422

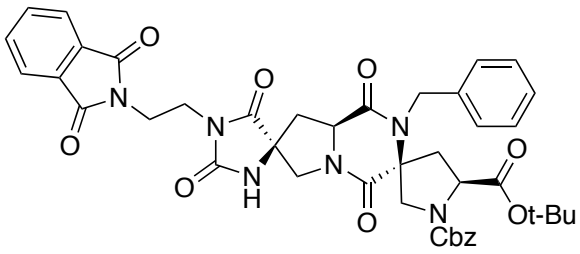
**2.39 - 1''-benzyl 5''-(*tert*-butyl) (3'*S*,4*S*,5''*S*,8a'*S*)-2'-benzyl-1',2,4',5-tetraoxotetrahydro-4'*H*,6'*H*-dispiro[imidazolidine-4,7'-pyrrolo[1,2-*a*]pyrazine-3',3''-pyrrolidine]-1'',5''-dicarboxylate**




This new spiroligomer dimer was synthesized via the following known procedure.[16] 1.09 g of **2.42** was preactivated with 1.63 g (6 eq) of 1-hydroxy-7-azabenzotriazole (HOAt) and 400 mg (1.05 eq) of N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) in 30 mL of anhydrous 2:1 DCM/DMF for 1.5 h, after which 400 mg of **2.43** and 1.05 mL of DIPEA dissolved in 10 mL of DMF were added to the reaction and stirred overnight. In the morning, 985 mg (2.5 eq) of EDC-HCl were added and the reaction stirred for another 4 hours. The reaction was diluted with EtOAc, and washed with saturated solutions of $NH_4Cl$, $NaHCO_3$, and brine, dried over $Na_2SO_4$, and rotovapped. The foamy solid was then purified via normal phase flash

chromatography to afford 952 mg (77% yield) of **2.39**; $^{1}$H NMR (500 MHz, DMSO-$d_6$) 1.26 (9H, s, rotameric), 2.22 (1H, ddd, *J* = 8.3, 13.3, 20.9), 2.43 (1H, dd *J* = 8.8, 13.4), 2.66 (1H, dd, *J* = 8, 13.3), 2.85 (1H, td, *J* = 8.3, 13.3), 3.55 (1H, d, rotameric, *J* = 11.4), 3.69 (1H, d, *J* = 12.5), 3.77 (1H, d, *J* = 12.5), 3.96 (1H, dd), 4.03 (1H, dt, 8.3, 22.0), 4.54 (1H, dd, *J* = 8.6, 16.7), 4.83-4.90 (2H, m), 4.98-5.06 (2H, m), 7.20-7.35 (11H, m), 8.33 (1H, s); $^{13}$C NMR (125 MHz, DMSO-$d_6$, rotamers present) 27.8, 28.0, 38.2, 38.8, 45.8, 50.6, 51.2, 52.8, 57.2, 57.8, 65.2, 66.6, 66.9, 67.6, 81.8, 81.6, 118.5, 126.6, 127.0, 127.6, 127.8, 128.3, 128.7, 128.8, 129.1, 134.8, 136.8, 137.2, 138.2, 153.8, 154.3, 156.8, 165.1, 170.2, 170.5, 177.3; HRMS-ESI: m/z calcd for $C_{32}H_{35}N_5O_8Na$ $(M+Na)^+$ 640.2378, found 640.2385

**2.40 - 1''-benzyl 5''-(*tert*-butyl) (3'*S*,4*S*,5''*S*,8a'*S*)-2'-benzyl-1-(2-(1,3-dioxoisoindolin-2-yl)ethyl)-1',2,4',5-tetraoxotetrahydro-4'*H*,6'*H*-dispiro[imidazolidine-4,7'-pyrrolo[1,2-*a*]pyrazine-3',3''-pyrrolidine]-1'',5''-dicarboxylate**




Using procedure 3, 59 mg (87%) was recovered; $^{1}$H NMR (500 MHz, $CDCl_3$) 2.17 (1H, m), 2.45 (1H, dd, *J* = 8.2, 13.5), 2.59 (1H, dd, *J* = 8.4, 13.5), 2.80-2.86 (1H, m), 3.51-3.60 (2H, m), 3.64-3.66 (2H, m), 3.79-3.83 (3H, m), 3.92-4.03 (2H, m), 4.55 (1H, dd, *J* = 6.5, 16.6), 4.77-4.88 (2H, m), 4.97-5.07 (2H, m), 7.21-7.36 (10H, m), 7.80-7.88 (4H, m), 8.78 (1H, s); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 27.7, 29.7, 36.0, 38.1, 39.5, 46.2, 51.0, 52.1, 57.4, 57.6, 62.9, 67.0, 67.6, 82.2, 82.4, 126.5,

127.6, 127.9, 128.4, 129.0, 131.8, 134.3, 136.1, 136.7, 154.0, 155.7, 165.3, 168.4, 170.2, 175.1; HRMS-ESI: m/z calcd for $C_{42}H_{42}N_6O_{10}K$ $(M+K)^+$ 829.2594, found 829.2590

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# CHAPTER 3

# SYNTHESIS OF ENHANCED PROLINE DERIVATIVES, AND THEIR INCOPORATION INTO SHORT PEPTIDES

## 3.0 Introduction

Amino acids and proteins are the building blocks of life, with much work devoted to understanding the structure and function of different amino acid sequences and proteins in general. As such, work into mimicking amino acids and peptides with unnatural amino acids or peptidomimetics has been an ongoing process for many decades. Significant work has been done to mimic the structural and functional diversity of nature using a variety of peptidomimetics, from beta-amino acids[1,2] and stapled peptides[3-6] to peptoids[7-10] and spiroligomers. [11,12] Each of these peptidomimetics has advantages and drawbacks, whether it's peptoids flexible backbone and lack of stereochemistry, or the difficulty of designing peptidomimetics to adopt a desired shape.

One amino acid that has received significant focus is proline, as it is prevalent across a wide array of natural and synthetic structures,[13-17] with nonproteinogenic proline being used for conformationally rigid peptides, angiotensin converting enzyme inhibitors,[13] and asymmetric synthesis[15-17] among many other applications.[17]

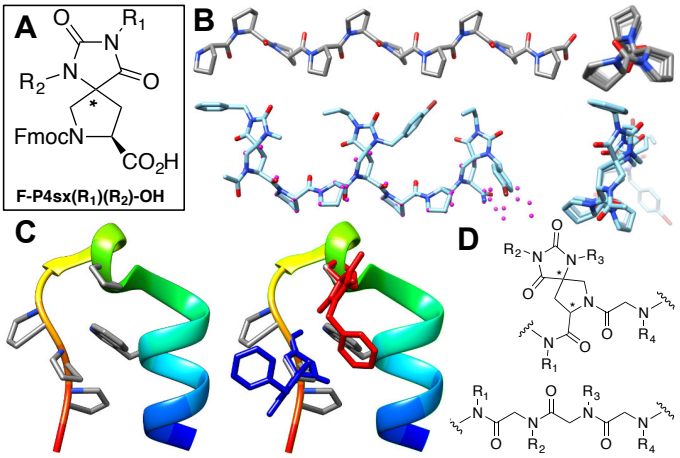


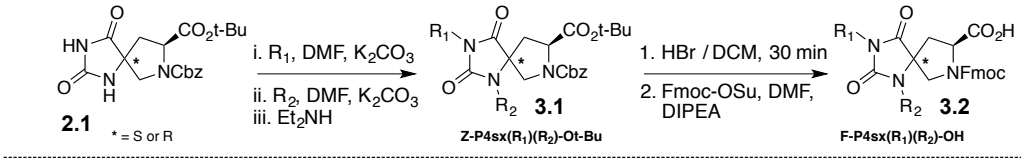

**Figure 3.1.** Possible uses for enhanced proline derivatives (A) Theoretical enhanced proline residue (B) Polyproline II (PPII) helices (C) Designed peptides like a Trp-Cage (D) Adding structure to peptoid backbones.

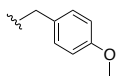
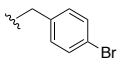
Our goal was to introduce a new conformationally constrained residue that could be readily incorporated into multiple peptidomimetic platforms. We wanted the synthesis of this new peptidomimetic to be relatively straight forward, able to be carried out on multigram scale, and with minimal purifications required. Many of the nonproteinogenic prolines mentioned above are functionalized at the 4-position of the pyrrolidine ring,[14, 17] so by utilizing the chemistry described in Chapter 2,[18] and with a simple protecting group swap, we were able to synthesize a new peptidomimetic residue built off of a 4-substituted proline. These enhanced proline residues are synthesized on multigram scale in a 3-4 step synthesis from our readily available hydantoin starting material **2.1**, with a single purification after the final reaction. These enhanced proline residues have two stereocenters, of which we have total control, as well as two points of functionalization. These residues have the potential to be incorporated into any peptidomimetic that can utilize solid-phase peptide-synthesis (SPPS). Given their rigid core, and the enormous diversity afforded by incorporating functional groups through a simple alkylation reaction, these residues could be very useful in the development of peptidomimetics with new properties. A particularly valuable feature of these monomers is that the two functional groups on the monomers are incorporated on stereochemically pure building blocks after the stereochemistry of the monomers is set. This allows us to synthesize large quantities of a stereochemically pure, unfunctionalized building block, and then later create functionalized versions of them using well behaved chemistry. This functional group/stereochemistry decoupling avoids a difficulty that occurs in the synthesis of many other unnatural building blocks, where stereochemistry and functionality are introduced at the same time, resulting in mixtures of stereoisomers that are difficult to separate. With the wide range of potential applications, proline will continue to be a hot-topic of study for the foreseeable future. The development of these

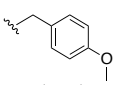
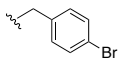
new enhanced-proline derivatives will provide a unique residue to be utilized for new peptidomimetic syntheses, while also providing a fresh scaffold for past applications.

### 3.1 Synthesis of Enhanced Proline Residues

Starting from a stereochemically pure hydantoin **2.1** and utilizing the alkylation strategy discussed in Chapter 2, we synthesize mono- or difunctionalized proline amino acids (Cbz-Pro4($R_1$)($R_2$)-O*t*-Bu or Boc-Pro4($R_1$)($R_2$)-O*t*-Bu) on large scales (up to 10 g of starting material **2.1**) as shown in Scheme 3.1. Deprotection of the proline protecting groups is readily achieved, followed by reprotection of the pyrrolidine nitrogen with Fmoc succinimide to make molecules like **3.3-3.12**.




| | Name | Stereo-chem. | $R_1$ | $R_2$ | % Yield |
|---|---|---|---|---|---|
| **3.3** | F-P4sr(H)(H)-OH | R | H | H | 61 |
| **3.4** | F-P4sr(Meo)(H)-OH | R |  | H | 60 |
| **3.5** | F-P4sr(Bn)(Me)-OH | R |  | $CH_3$ | 73 |
| **3.6** | F-P4sr(All)(4BrBn)-OH | R |  |  | 56 |

| | Name | Stereo-chem. | $R_1$ | $R_2$ | % Yield |
|---|---|---|---|---|---|
| **3.7** | F-P4ss(H)(H)-OH | S | H | H | 55 |
| **3.8** | F-P4ss(H)(Meo)-OH | S |  | H | 63 |
| **3.9** | F-P4ss(Bn)(Me)-OH | S |  | $CH_3$ | 78 |
| **3.10** | F-P4ss(All)(4BrBn)-OH | S |  |  | 58 |
| **3.11** | F-P4ss(BnAc)(BnAc)-OH | S |  |  | 49** |
| **3.12** | F-P4ss(BnAc)(H)-OH | S | H |  | 49** |

**Scheme 3.1.** Synthesis of enhanced proline residues **3.3-3.12** from the common 4-hydantoin proline starting material (compound **2.1**)

At this point, the Fmoc-Pro4($R_1$)($R_2$)-OH amino acids **3.3-3.12** were purified by reverse-phase flash-chromatography, as shown in Figure 3.2. It is possible to separate the enhanced prolines from other impurities, as shown in Figure 3.2A for **3.5**, and it is also possible to separate a mono functionalized proline **3.12** from a difunctionalized proline

**3.11** as shown in Figure 3.2B. Figure 3.2C shows an HPLC chromatogram for the purified amino acid **3.5** shown in Figure 3.2A, proving that these purifications work exceedingly well for removing any impurities. The fractions can then be lyophilized, or the acetonitrile evaporated and the compound extracted from the aqueous mixture with ethyl acetate. This synthesis over 3-4 steps yields the enhanced proline amino acids **3.3-3.12** in moderate to good overall yields (49-78%) from **2.1**.

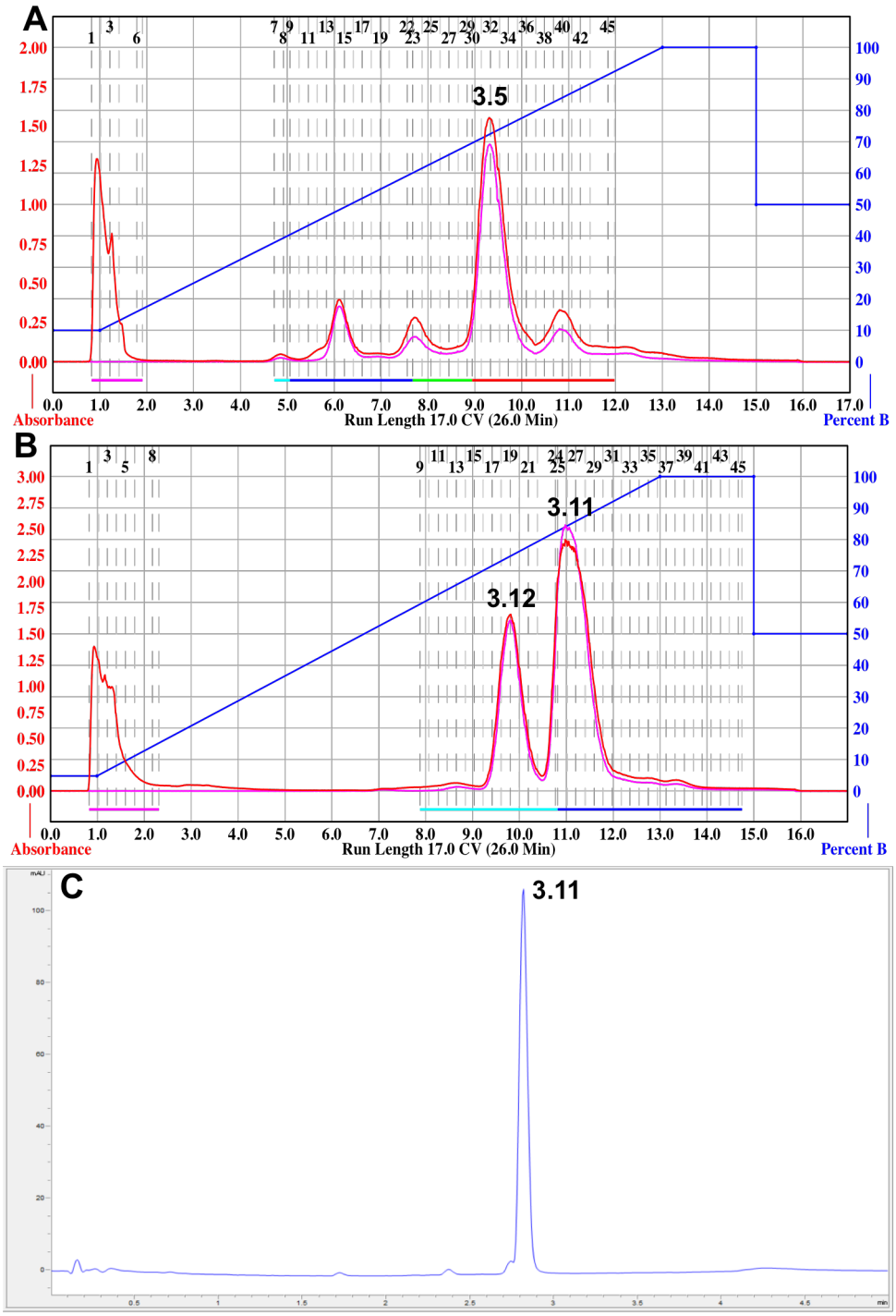


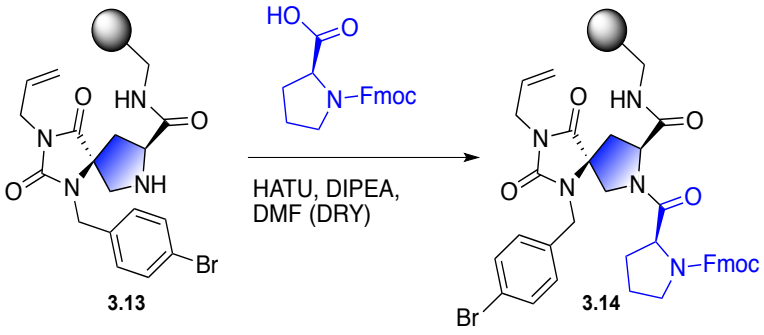

**Figure 3.2.** (A, B) Reverse Phase flash chromatography for **3.15** and a mix of **3.11 and 3.12**, 5%-95% ACN in $H_2O$ with 0.1% formic acid modifier (C) HPLC chromatogram of **3.11**

## 3.2 Coupling Reactions Involving Enhanced Proline Residues

The enhanced proline derivatives were then evaluated for their ability to undergo peptide synthesis. Compound **3.10** was loaded onto rink amide resin, followed by a quantitative Fmoc release to determine the amount of resin bound amino acid. A normal Fmoc-Pro-OH was then coupled to the resin bound enhanced proline residue (Scheme

**Table 3.1.** Coupling Fmoc-Pro-OH to an enhanced proline residue at RT and elevated temperatures

| Trial | Eq | Res | Time | Temp (℃) | 2x Coup. | SM ?? | HPLC Est. % Comp. |
|---|---|---|---|---|---|---|---|
| 1 | 2 | Pro | O/N | RT | No | Yes | 50 |
| 2 | 5 | Pro | 1h | RT | No | Yes | 60 |
| 3 | 2 | Pro | 1h | RT | Yes | Yes | 25 |
| 4 | 3 | Pro | 1h | RT | Yes | Yes | 50 |
| 5 | 4 | Pro | 1h | RT | Yes | Yes | 50 |
| 6 | 2 | Pro | 1h | 50 | No | Yes* | >98 |
| 7 | 2 | Pro | 2h | 50 | No | Yes* | >98 |
| 8 | 2 | Pro | 1h | 50 | Yes | No | 100 |
| 9 | 2 | Pro | 2h | 50 | Yes | No | 100 |
| 10 | 3 | Pro | 1h | 50 | No | Yes* | >98 |
| 11 | 3 | Pro | 2h | 50 | No | No | 100 |
| 12 | 3 | Pro | 1h | 50 | Yes | No | 100 |
| 13 | 3 | Pro | 2h | 50 | Yes | No | 100 |
| 14 | 4 | Pro | 1h | 50 | Yes | No | 100 |
| 15 | 4 | Pro | 2h | 50 | Yes | No | 100 |
| 16 | 5 | Pro | 2h | 50 | No | No | 100 |
| 17 | 5 | Pro | 1h | 50 | Yes | No | 100 |
| 18 | 5 | Pro | 2h | 50 | Yes | No | 100 |




**Scheme 3.2.** Coupling the proteogenic Fmoc-Pro4-OH to an enhanced amino acid on Rink Amide resin.

3.2) to synthesize the dipeptide **3.14**. Unfortunately, regardless of the number of equivalents or with the use of double couplings (Table 3.1, Trials 1-5), quantitative coupling to the resin bound enhanced amino acid could not be achieved. Trials 2-4 are shown in Figure 3.3A, where a majority of the starting material **3.13** is still present as opposed to the desired dipeptide **3.14**. Our hypothesis is that this must be due to the steric strain associated with the $R_2$ group on the amide of the hydantoin, as shown in Figure 3.3B. This $R_2$ group, as shown in previous studies,[19] can have a steric blocking effect. By carrying out the coupling reactions at higher temperatures, quantitative couplings with proline could be achieved (Table 3.1, Trials 6-18).

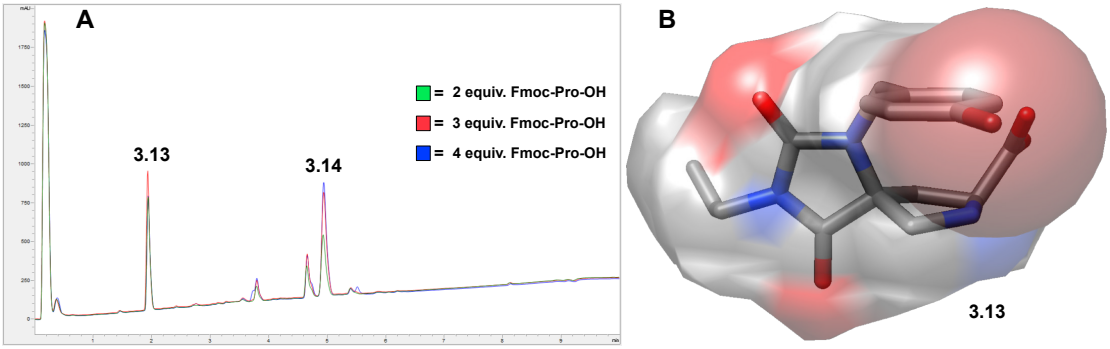



**Figure 3.3.** (A) HPLC chromatogram showing incomplete coupling of Trials 2-4 from Table 3.1, as indicated by the large amount of starting material (**3.13)** versus product (**3.14**) (B) Three-dimensional model of **3.13** with solvent-excluded surface (image generated with Chimera)

An HPLC-MS chromatogram of a complete coupling to form dipeptide **3.14**, with no leftover **3.13**, is shown in Figure 3.4A and 3.4B. To facilitate more rapid synthesis of peptides incorporating these enhanced proline residues, we utilized a microwave reactor based on protocols set forth in Gellman, et al[2] (Table 3.2, Trials 19-24.) With a hold temperature of 60 °C and a ramp time of 3 min (to ensure the sample was not heated too rapidly causing the polypropylene reactor to melt), hold times of 2, 4, and 6 min were employed, which all returned similar results of near quantitative couplings (starting

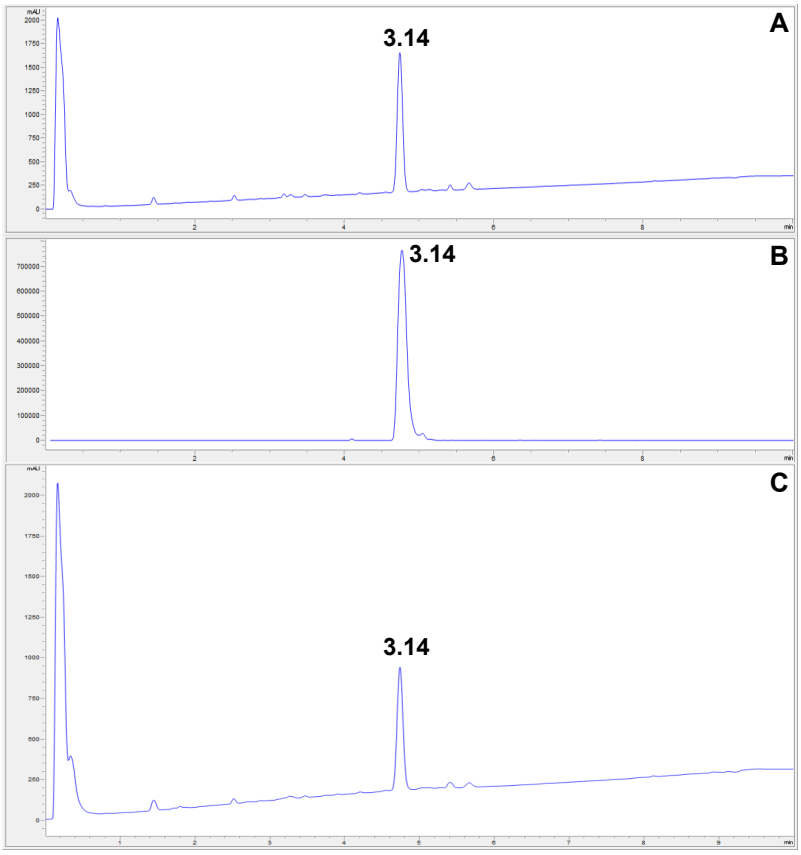


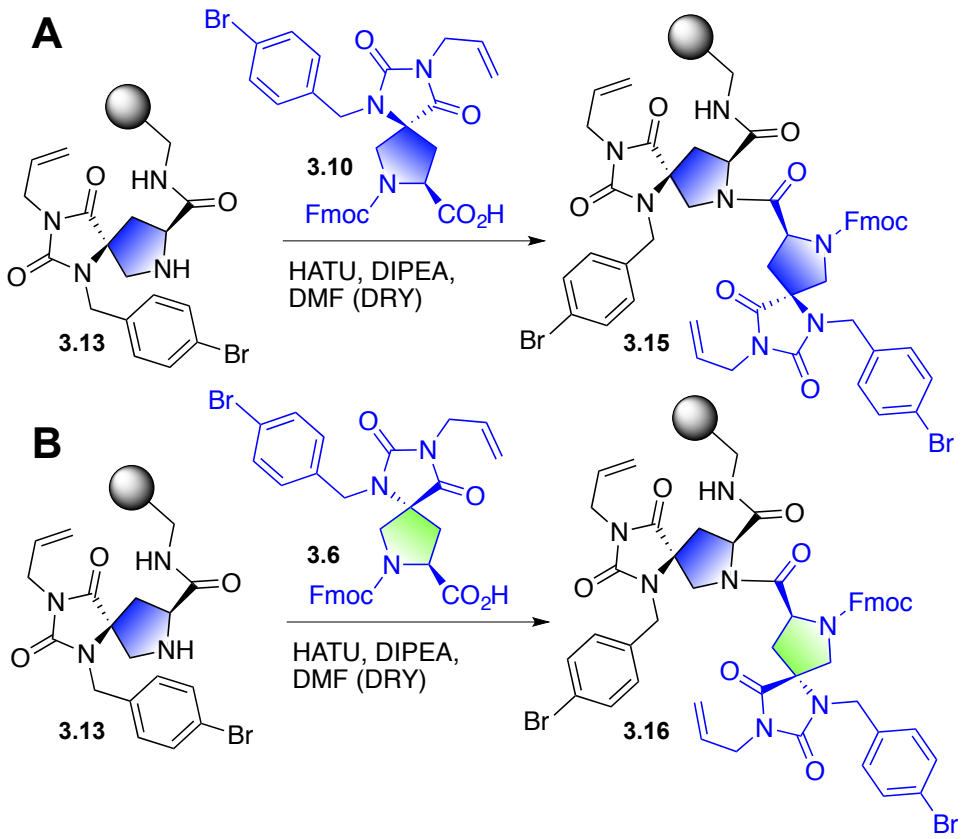

Figure 3.4. (A, B) HPLC-MS chromatogram of a successful proline-to-enhanced proline coupling reaction performed in a conventional oven reactor (C) HPLC chromatogram of the same coupling performed in a microwave synthesis reactor

material **3.13** was only found via mass and not the UV, indicating yields > 95%.)

We next wanted to determine whether enhanced prolines could be coupled sequentially (Scheme 3.3A). Following the protocol established for coupling a proline to an enhanced proline via microwave irradiation, we attempted to synthesize the dipeptide **3.15**. Unfortunately, we again ran into a problem with the first reaction (Table 3.2, Trial 25), where the results mirrored those of room temperature couplings of regular Fmoc-Pro-OH to an enhanced proline derivative (Table 3.1, Trials1-5). However, by employing compressed air cooling during the hold time, we were able to successfully couple two enhanced proline residues with the same stereochemistry (Table 3.2, Trials 26-28) to synthesize dipeptide **3.15**, and we were also able to couple two enhanced proline residues with varied stereochemistry (Table 3.2, Trials 29-31) to synthesize dipeptide **3.16** shown in Scheme 3.3B.

**Table 3.2.** Coupling Fmoc-Pro-OH or an enhanced proline residue to an enhanced proline residue using a microwave synthesis reactor

| Trial | Res. | Ramp (min) | Hold (min) | Temp (℃) | Hold Cool | 2x Coup | SM ?? | HPLC Est. % Comp. |
|---|---|---|---|---|---|---|---|---|
| 19 | Pro | 3 | 2 | 60 | No | No | Yes | 95 |
| 20 | Pro | 3 | 4 | 60 | No | No | Yes | 95 |
| 21 | Pro | 3 | 6 | 60 | No | No | Yes | 95 |
| 22 | Pro | 3 | 2 | 60 | No | Yes | Yes* | >98 |
| 23 | Pro | 3 | 4 | 60 | No | Yes | Yes* | >98 |
| 24 | Pro | 3 | 6 | 60 | No | Yes | Yes* | >98 |
| 25 | Pro-4 | 3 | 4 | 60 | No | Yes | Yes | 50 |
| 26 | Pro-4 | 3 | 4 | 60 | Yes | Yes | Yes* | >98 |
| 27 | Pro-4 | 3 | 8 | 60 | Yes | Yes | Yes* | >98 |
| 28 | Pro-4 | 3 | 10 | 60 | Yes | Yes | Yes* | >98 |
| 29 | Pro-4 | 3 | 4 | 60 | Yes | Yes | Yes* | >98 |
| 30 | Pro-4 | 3 | 8 | 60 | Yes | Yes | Yes* | >98 |
| 31 | Pro-4 | 3 | 10 | 60 | Yes | Yes | Yes* | >98 |




Scheme 3.3. (A) Coupling an enhanced amino acid **3.10** with identical stereochemistry to one on resin to synthesize a dipeptide **3.15** (B) Coupling an enhanced amino acid **3.6** with varied stereochemistry to one on resin to synthesize a dipeptide **3.16**

### 3.3 Synthesis of Short Peptides Containing Enhanced Proline Derivatives

With these techniques successfully debugged, we set about making peptides that incorporated enhanced proline residues. Our first attempts at incorporating these residues were based off Wennemers' work[20-23] on poly-proline helices that also incorporated unique proline residues. Loading the resin with an enhanced proline residue **3.10,** followed by couplings of two normal proline residues showed no hints of any problems; however, after the addition of the second enhanced proline residue **3.10**, we noticed in the test cleavage that the peak that corresponded to the expected 4-mer peptide **3.17** was broadening, as shown in Figure 3.5 below.

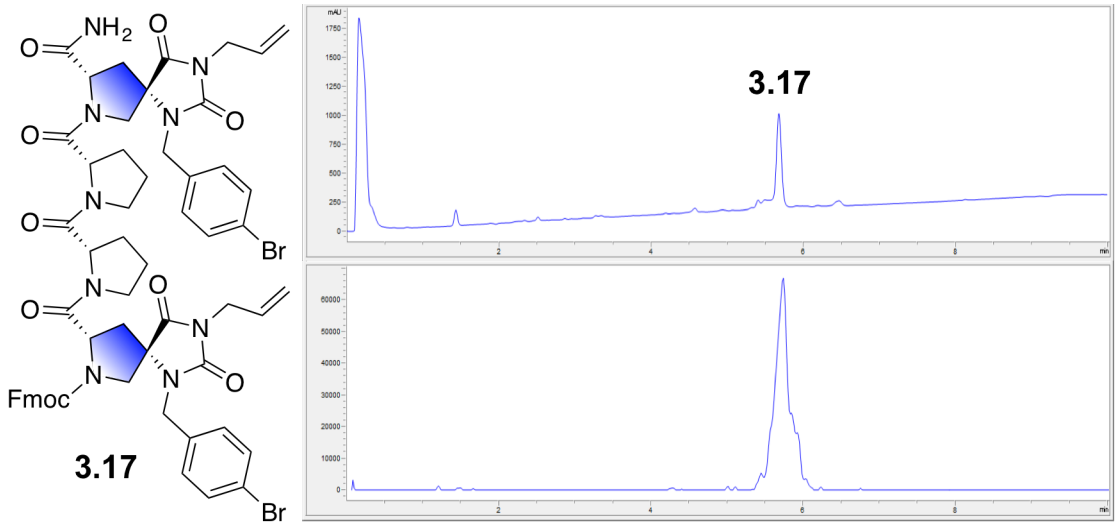



**Figure 3.5.** HPLC-MS test cleavage of a peptide 4-mer **3.17** incorporating two proline residues between two enhanced proline residues. Peak is showing broadening

Taking this material forward and coupling another two prolines results in 6-mer peptide **3.18**, shown in Figure 3.6. The peak corresponding to this peptide is showing increased broadening when compared to peptide 4-mer **3.17**. One final enhanced proline residue **3.09** was then coupled to this peptide, Fmoc deprotected, and cleaved from the resin, resulting in the 7-mer peptide **3.19** shown in Figure 3.7.

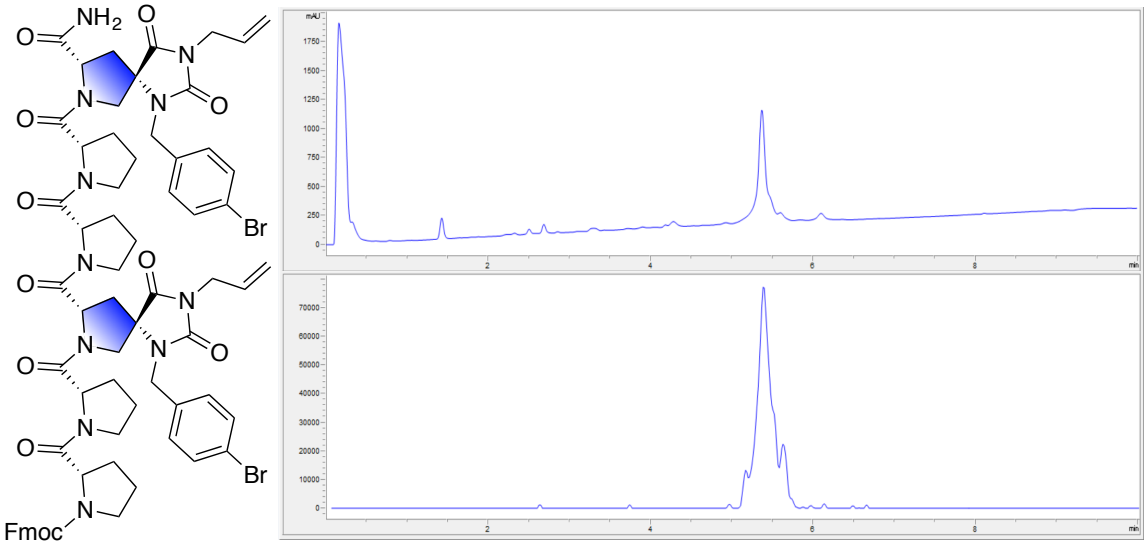



**Figure 3.6.** HPLC-MS test cleavage of a peptide 6-mer incorporating two enhanced proline and four proline residues. The bottom peak is showing increased broadening when compared to the peptide 4-mer.

Figure 3.7A shows the HPLC of the peptide at 30 °C, the same temperature as the preceding HPLC-MS chromatograms. The peak for **3.19** is very broad, with what appear to be multiple shoulders with masses that all correspond to the expected mass of **3.19**, which was very unexpected. We injected the material onto our LCMS, but with the column heater turned up to 50 °C (Figure 3.7B) and then 65 °C (Figure 3.7C), which resulted in the peaks coalescing into a single peak. This suggests that these peptides we synthesized, **3.17-3.19**, can readily adopt multiple slowly interconverting conformations

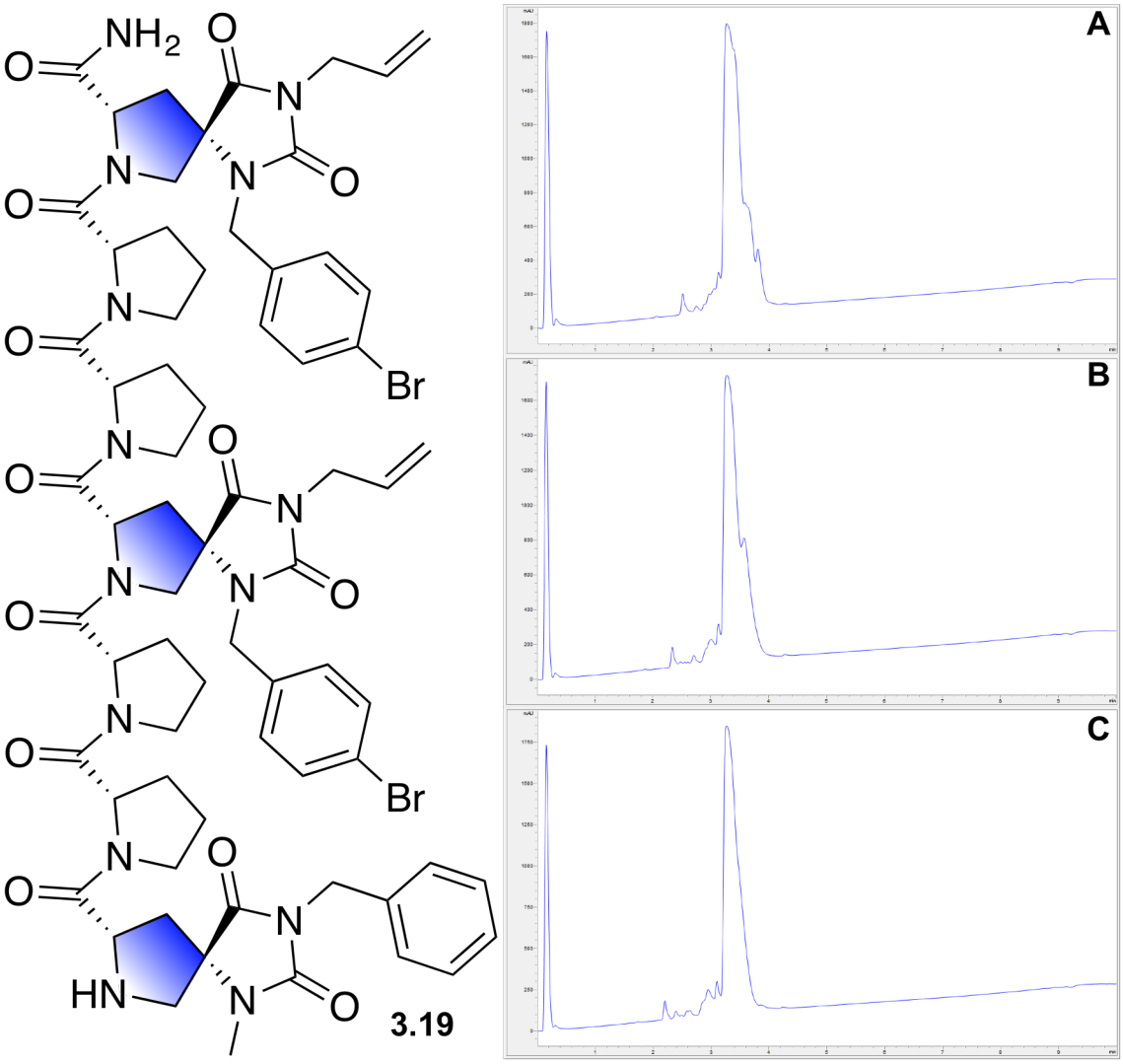



**Figure 3.7.** Peptide **3.19**, a 7-mer incorporating three enhanced-proline and four proline residues (A) HPLC-MS of **3.19** at 30 °C (B) HPLC-MS of **3.19** at 50 °C (C) HPLC-MS of **3.19** at 65 °C

at room temperature. We went ahead and purified peptide **3.19** on a semi-prep scale, collecting the front of the main peak, the peak tip, and successive peaks separately. These samples were left overnight, and then injected into an HPLC-MS. Each sample, after overnight equilibration, looked identical to the parent chromatogram for **3.19**, strongly suggesting that we had one molecule, but multiple conformations at room temperature. With this knowledge, we decided to model these peptides that contain enhanced prolines. Models of the enhanced proline containing peptides were generated by Christian Schafmeister with CANDO (Computer-Aided Nanomaterial Design and Optimization). Figure 3.8 shows the peptide **3.19** modeled to reflect either a PPII helix (A and B) or a PPI helix (C and D). If the backbone of the peptide containing the enhanced proline derivatives is modeled to conform to the coordinates expected for either the PPI or PPII helices, as shown by overlapping with the traditional residues (purple spheres), then it appears that in either model, there will be some form of steric clash between different parts of the peptide. In the PPII helix, it appears that the side chain in the $R_2$ position cannot freely rotate without coming into steric contact with the proline backbone. In the case of the PPI helix, it seems that both the $R_1$ and $R_2$ functional groups will be sterically hindered. While this initially seems like a detrimental aspect of these molecules, if more modeling is done, it could be possible to find the right side chains and stereochemistry of the hydantoin to facilitate a strictly PPI or PPII helix. Furthermore, it might also be possible to force a peptide to adopt a tight turn by sterically blocking helical potentials. Further modeling work should be done to determine the best stereochemical and functional groups to facilitate a number of unique three-dimensional structures.

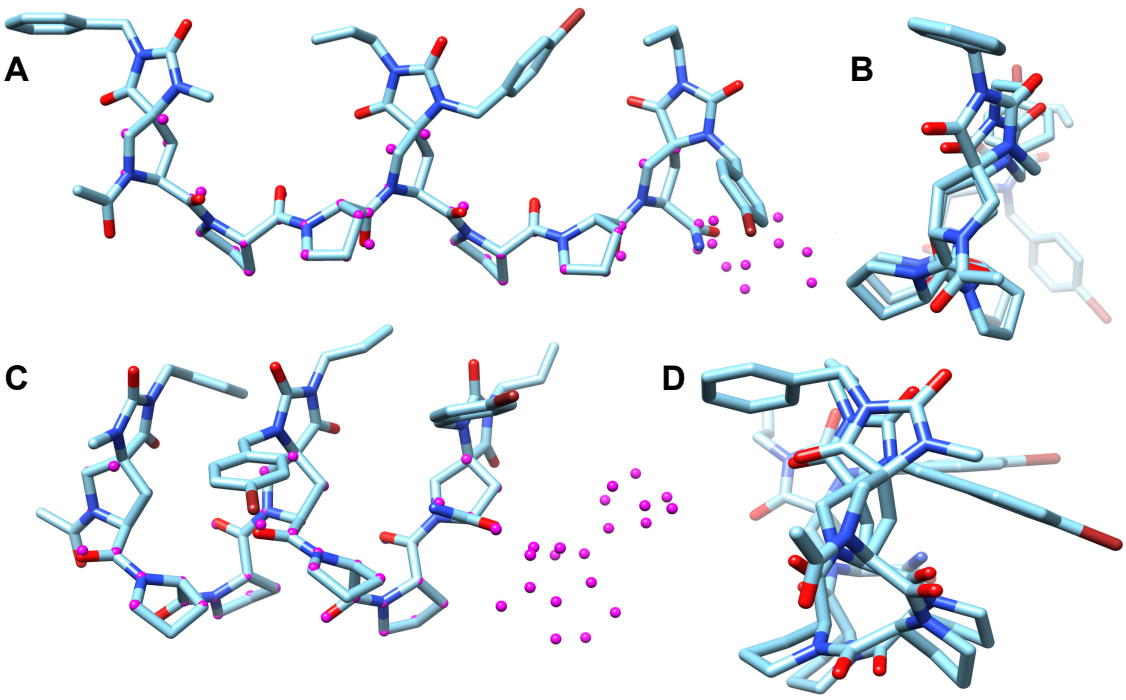



**Figure 3.8.** (A) Side view of the peptide 7-mer **3.19** containing three enhanced prolines modeled to conform to PPII backbone angles (B) Top view of the PPII model (C) Side view of the peptide 7mer containing three enhanced prolines modeled to conform to PPI backbone angles (D) Top view of the PPI model

## 3.4 Conclusion

We have successfully synthesized Fmoc protected enhanced proline residues from the stereochemically pure proline hydantoin **2.1**, in modest to good yields. The proline hydantoin **2.1** is an intermediate in bis-amino acid synthesis used in the synthesis of spiroligomers and we are able to synthesize all stereoisomers on hundreds of gram scales. These Fmoc protected amino acids are easily purified by reverse phase flash chromatography, and can either be lyophilized or extracted from the aqueous solution. Enhanced proline derivatives can be readily incorporated into Fmoc-SPPS, requiring only mild heat or the use of a microwave synthesizer to ensure complete couplings. Due to the bulky side chains, when multiple enhanced prolines are incorporated into peptides, the resulting peptides do not adopt a single conformation at room temperature but appear to

exist in multiple, slowly interconverting species involving different permutations of amide rotamers.

Further work with enhanced proline residues should include exhaustive modeling searches to discover unique molecules to enforce desired structures, and to ensure that the molecule should be able to adopt a single conformation at room or physiological temperatures. Interesting molecules that could be synthesized could include PPI and PPII helices. Another interesting avenue could be to model what could happen to an enhanced proline, or multiple enhanced prolines, if they were incorporated into a designed peptide such as the Trp-Cage, which utilizes four proline residues to sequester the Trp residue in the interior of the peptide. Utilizing the proline for coupling could also be a potential connection point to incorporate larger spiroligomer dimers, trimers, or larger into peptides or other macromolecules. Another application for these enhanced prolines could be their incorporation into peptoids, as the stereochemistry and steric effects could help to enforce more structure into the peptoid backbone.

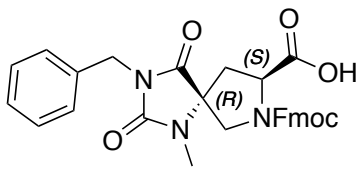
## 3.5 Experimental Details

***General Procedure 3.1 – Synthesis of enhanced proline derivatives from 2.1***

To a stirred mixture of **2.1** in DMF (100 mM) was added 0.75-0.88 equiv (equivalents dependent on salt content of the specific batch of **3.1**, which can be determined utilizing an internal standard**)** of a halide along with 1.5 equiv of $K_2CO_3$. The reaction proceeded at room temperature for 2-24 hours, at which time 1.5 equiv of an allyl or benzyl halide and 1.5 equiv of $K_2CO_3$ was added to the reaction mixture and stirred overnight. The reaction was diluted with four times the reaction volume of EtOAC and washed with water, saturated ammonium chloride solution, and brine. The organic layer was dried

with $Na_2SO_4$, and concentrated in vacuo to yield a foamy off-white to yellow solid. This compound was then deprotected using a 1:1 mixture of DCM/(33% HBr in AcOH) for 30 minutes, rotovapped, and again put on a high vacuum pump overnight. The deprotected amino acid was dissolved in DMF and free based with 3 equiv of DIPEA, after which 1.1 equiv of Fmoc-OSu was added to the reaction. This was stirred for 2 hours, and the progress checked via HPLC-MS. Upon completion, the reaction was diluted with EtOAc, washed with ammonium chloride and brine, dried with $Na_2SO_4$, concentrated in vacuo to yield a dark yellow solid, which was then purified by reverse phase flash chromatography (5-95% acetonitrile in water, 0.1% formic acid). The fractions were combined, the acetonitrile removed in vacuo, and the product extracted from the residual aqueous mixture with EtOAc, which was then rinsed with brine and dried over $Na_2SO_4$. The EtOAc was removed in vacuo, and the compound put on a high vacuum pump overnight to yield a white solid.

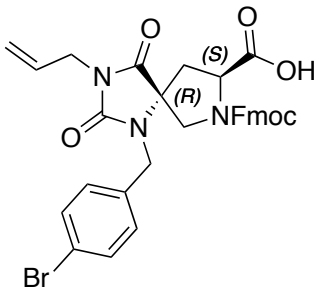
**Enhanced Proline 3.5** Using general procedure 3.1, 900 mg **2.1** (2S, 4R), 0.75 equiv benzyl bromide, then 1.25 equiv iodomethane, 73% purified yield




$^1$H NMR (500 MHz, $CDCl_3$, rotamers present) 2.46 (1H, dd, *J* = 14.5, 9.3), 2.62 (1H, dd, *J* = 14.6, 7.0), 2.76 (3H, s, 3.56 (1H, d, rotameric to 3.82 ppm, *J* = 11.6), 3.77 (1H, d, *J* = 11.9), 4.21 (1H, t, *J* = 6.3), 4.33 (1H, m), 4.52 (2H, m), 4.62 (2H, s), 4.66 (1H, dd, *J* = 9.2, 7.0), 7.31 (9H, m), 7.52 (2H, m), 7.71 (2H, m) ; $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 14.2, 25.43, 25.46, 25.55, 36.7, 37.9, 42.9, 43.0, 46.3, 47.1, 47.2, 53.1,

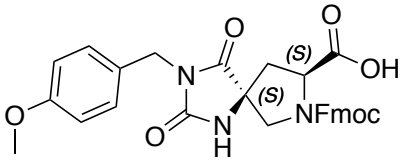
53.3, 57.8, 58.4, 67.5, 67.80, 67.82, 68.6, 72.9, 119.9, 120.00, 120.04, 120.2, 124.7, 124.8, 125.2, 127.1, 127.2, 127.4, 127.74, 127.77, 127.83, 127.87, 128.07, 128.14, 128.19, 128.4, 128.6, 128.76, 128.78, 135.5, 135.6, 141.20, 141.23, 141.27, 141.37, 141.42, 142.4, 143.25, 143.33, 143.6, 143.7, 153.7, 154.4, 154.69, 154.71, 168.7, 171.8, 172.2, 173.4, 174.1; HRMS-ESI: m/z calcd for $C_{30}H_{27}N_3O_6Na$ $(M+Na)^+$ 548.1792, found 548.1799

**Enhanced Proline 3.6** Using general procedure 3.1, 900 mg **2.1** (2S, 4R), 0.75 equiv allyl bromide, then 1.25 equiv 4-bromobenzyl bromide, 56% purified yield



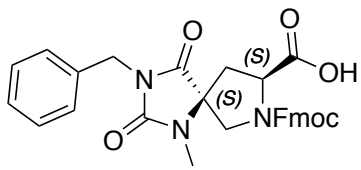

$^1$H NMR (500 MHz, $CDCl_3$, rotamers present) 2.39 (1H, ddd, $J$ = 14.6, 9.2, 1.2), 2.60(1H, dd, $J$ = 14.6, 7.6), 3.45 (1H, dd, rotameric to 3.83, $J$ = 11.6, 1.2), 3.74 (1H, m), 4.11 (5H, m), 4.39 (1H, m), 4.54 (2H, m), 5.22 (2H, m), 5.84 (1H, m), 7.03 (2H, d, $J$ = 8.2), 7.35 (6H, m), 7.48 (2H, m), 7.73 (2H, t, $J$ = 8.4); $^{13}$C NMR (125 MHz, $CDCl_3$ or DMSO-$d_6$, rotamers present) 14.2, 37.5, 41.7, 43.5, 46.9, 47.0, 54.1, 58.3, 67.8, 69.2, 118.57, 118.59, 119.99, 120.04, 120.06, 122.1, 124.95, 124.99, 127.0, 127.10, 127.14, 127.16, 127.21, 127.6, 127.8, 127.9, 128.9, 129.0, 130.5, 130.6, 132.0, 132.1, 135.6, 141.29, 141.3, 143.3, 143.4, 153.9, 155.4, 171.2, 173.4; HRMS-ESI: m/z calcd for $C_{32}H_{28}BrN_3O_6Na$ $(M+Na)^+$ 652.1024, found 652.1029

**Enhanced Proline 3.8** Using general procedure 3.1, 1.50 g **2.1** (2S, 4S), 0.88 equiv 4-methoxybenzylchloride, recovered 1.35 g pure, 63% yield




$^1$H NMR (500 MHz, $CDCl_3$, rotamers present) 2.18 (1H, d, *J* = 14.0), 2.85 (1H, dd, *J* = 9.8, 4.3), 3.78 (3H, s), 3.92 (2H, m), 4.19 (1H, m), 4.36 (2H, m, rotameric to 4.49), 4.62 (2H, m, rotameric to 4.53), 6.85 (2H, dd, *J* = 10.4, 8.9), 7.28 (6H, m), 7.49 (1H, d, *J* = 7.5), 7.57 (1H, d, *J* = 8.2), 7.70 (2H, m), 8.45 (1H, s); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 31.5, 36.6, 39.7, 40.8, 42.27, 42.31, 46.9, 47.1, 55.2, 55.3, 55.6, 56.4, 57.7, 58.3, 66.0, 67.1, 68.10, 68.16, 114.0, 114.2, 119.94, 119.96, 119.98, 124.9, 125.00, 125.05, 125.08, 127.0, 127.08, 127.11, 127.2, 127.5, 127.6, 127.72, 127.75, 127.76, 128.9, 130.03, 130.05, 130.2, 141.17, 141.22, 141.24, 141.3, 143.5, 143.7, 153.5, 154.0, 158.0, 158.2, 159.5, 159.6, 162.8, 172.1, 172.2, 176.3, 176.4; HRMS-ESI: m/z calcd for $C_{30}H_{27}N_3O_7Na$ $(M+Na)^+$ 564.1741, found 564.1755

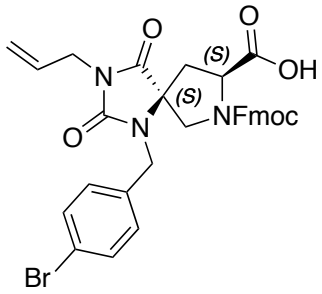
**Enhanced Proline 3.9** Using general procedure 3.1, 1.50 g **2.1** (2S, 4S), 0.88 equiv benzyl bromide, then 1.25 equiv iodomethane, recovered 1.60 g pure, 76% yield




$^1$H NMR (500 MHz, $CDCl_3$ rotamers present) 2.36 (1H, dd, *J* = 13.9, 7.5), 2.54 (1H, dd, *J* = 13.7, 8.9) 2.67 (4H, m), 3.59 (1H, d, *J* = 11.6), 3.78 (1H, d, *J* = 11.6), 4.17 (1H, t, *J* = 7.0), 4.37 (1H, d, *J* = 7.0), 4.44 (1H, dd, rotameric, *J* = 10.7, 7.0), 4.66 (2H, ),

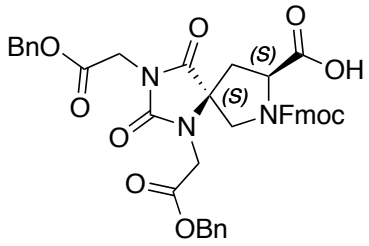
4.72 (1H, m), 7.31 (9H, m), 7.50 (2H, t, $J$ = 7.2), 7.72 (2H, m); $^{13}$C NMR (125 MHz, $CDCl_3$ or DMSO-$d_6$, rotamers present) 24.9, 25.0, 24.2, 31.8, 34.3, 35.6, 36.9, 42.6, 42.7, 46.9, 47.1, 49.6, 49.8, 57.8, 58.5, 66.1, 67.0, 68.3, 68.4, 119.9, 120.1, 124.9, 125.0, 125.09, 125.13, 127.1, 127.7, 127.80, 127.84, 128.11, 128.15, 128.45. 128.50, 128.57, 128.8, 135.7, 141.23, 141.25, 143.4, 143.5, 143.6, 155.1, 155.2, 163.5, 173.7, 174.0, 174.6; HRMS-ESI: m/z calcd for $C_{30}H_{27}N_3O_6Na$ $(M+Na)^+$ 548.1792, found 548.1784

**Enhanced Proline 3.10** Using general procedure 3.1, 1.50 g **2.1** (2S, 4S), 0.88 equiv allyl bromide, then 1.25 equiv 4-bromobenzyl bromide, recovered 1.42 g pure, 56% yield



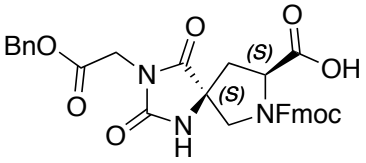

$^1$H NMR (500 MHz, $CDCl_3$, rotamers present) 2.39 (1H, dd, $J$ = 13.6, 7.8), 2.52 (1H, dd, $J$ = 13.7, 8.9), 3.40 (1H, d, $J$ = 11.8), 3.64 (1H, d, $J$ = 11.7), 4.18 (3H, m), 4.32 (1H, m), 4.40 (2H, m), 4.52 (1H, m), 4.67 (2H, m), 5.24 (2H, m), 5.87 (1H, m), 7.13 (2H, m), 7.26 (2H, m), 7.44 (6H, m), 7.72 (2H, m); $^{13}$C NMR (125 MHz, $CDCl_3$ or DMSO-$d_6$, rotamers present) 14.2, 34.6, 41.3, 42.5, 46.8, 50.1, 58.1, 67.1, 68.1, 68.4, 118.7, 120.1, 122.1, 124.8, 124.9, 127.14, 127.17, 127.8, 127.9, 129.0, 129.2, 129.7, 130.6, 130.8, 131.7, 132.2, 135.9, 136.5, 141.22, 141.25, 143.3, 143.4, 155.4, 155.37, 155.39, 155.45, 173.5, 173.9; HRMS-ESI: m/z calcd for $C_{32}H_{28}BrN_3O_6Na$ $(M+Na)^+$ 652.1024, found 652.1024

**Enhanced Proline 3.11** Using general procedure 3.1, 2.33 g **2.1** (2S, 4S, Boc instead of Cbz), 1.3 equiv benzyl bromoacetate, recovered 1.59 g pure, 49% yield




$^1$H NMR (500 MHz, $CDCl_3$, rotamers present) 2.35 (1H, dd, $J$ = 13.0, 7.5), 2.51 (1H, m), 3.56 (1H, d, $J$ = 11.3), 3.84 (4H, m), 4.15 (5H, m), 4.43 (1H, t, $J$ = 7.9), 5.02 (4H, m), 7.06 (2H, m), 7.23 (14H, m), 7.54 (2H, dd, $J$ = 13.6, 7.5); $^{13}$C NMR (125 MHz, $CDCl_3$ or DMSO-$d_6$, rotamers present) 35.7, 39.5, 41.0, 46.6, 50.5, 60.7, 67.4, 67.6, 67.9, 119.8, 125.1, 125.2, 127.0, 127.11, 127.6, 128.1, 128.28, 128.33, 128.50, 128.52, 128.59, 134.8, 135.0, 140.99, 141.03, 143.5, 143.7, 157.7, 155.6, 166.7, 169.1, 174.0, 177.7; HRMS-ESI: m/z calcd for $C_{40}H_{35}N_3O_{10}Na$ $(M+Na)^+$ 740.2215, found 740.2219

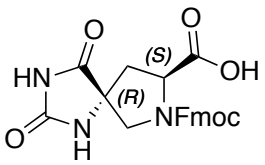
**Enhanced Proline 3.12** Synthesized with **3.11**, recovered 590 mg pure, 49% yield




$^1$H NMR (500 MHz, $CDCl_3$ or DMSO-$d_6$) 2.26 (1H, d, $J$ = 13.7), 2.87 (1H, td, $J$ = 13.6, 9.8), 3.98 (2H, d, $J$ = 5.8), 4.20 (1H, m), 4.36 (4H, m), 4.55 (1H, m), 5.19 (2H, d, $J$ = 9.2), 7.28 (2H, m), 7.34 (7H, m), 7.51 (1H, d, $J$ = 7.3), 7.58 (1H, t, $J$ = 8.5), 7.71 (2H, m), 8.68 (1H, s); $^{13}$C NMR (125 MHz, $CDCl_3$ or DMSO-$d_6$, rotamers present) 21.1, 39.7, 39.88, 39.9, 40.8, 46.9, 47.1, 55.7, 56.3, 57.6, 58.2, 60.4, 66.4, 67.5, 67.95, 67.98, 68.1, 68.2, 119.98, 119.99, 120.01, 124.93, 124.94, 125.05, 125.11, 127.07, 127.10, 127.13, 127.2,

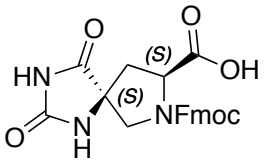
127.7, 127.75, 127.79, 128.42, 127.44, 127.7, 128.8, 134.6, 141.2, 141.25, 141.34, 143.4, 143.5, 143.7, 143.8, 153.5, 156.0, 166.4, 172.0, 172.1, 176.3, 176.4; HRMS-ESI: m/z calcd for $C_{31}H_{27}N_3O_8Na$ $(M+Na)^+$ 592.1690, found 592.1702

**Enhanced Proline 3.3**




2.0 g (5.14 mmol) of **2.1** (2S, 4R) was deprotected using a 1:1 mixture of DCM/(33% HBr in AcOH) for 30 minutes, rotovapped, and put on a high vacuum pump overnight. The deprotected amino acid was dissolved in DMF and free based with 3 equiv of DIPEA, after which 1.1 equiv of Fmoc-OSu was added to the reaction. This was stirred for 2 hours, and the progress checked via HPLC-MS. Upon completion, the reaction was diluted with EtOAc, washed with ammonium chloride and brine, dried with $Na_2SO_4$, concentrated in vacuo to yield a dark yellow solid, which was then purified by reverse phase flash chromatography (5-95% acetonitrile in water, 0.1% formic acid). The fractions were combined, the acetonitrile removed in vacuo, and the product extracted from the residual aqueous mixture with EtOAc, which was then rinsed with brine and dried over $Na_2SO_4$. The EtOAc was removed in vacuo, and the compound put on a high vacuum pump overnight to yield a white solid in 61% purified yield; HRMS-ESI: m/z calcd for $C_{22}H_{19}N_3O_6Na$ $(M+Na)^+$ 444.1166, found 444.1158

**Enhanced Proline 3.7**




4.0 g (10.3 mmol) of **2.1** (2S, 4S) was deprotected using a 1:1 mixture of DCM/(33% HBr in AcOH) for 30 minutes, rotovapped, and put on a high vacuum pump overnight. The deprotected amino acid was dissolved in DMF and free based with 3 equiv of DIPEA, after which 1.1 equiv of Fmoc-OSu was added to the reaction. This was stirred for 2 hours, and the progress checked via HPLC-MS. Upon completion, the reaction was diluted with EtOAc, washed with ammonium chloride and brine, dried with $Na_2SO_4$, concentrated in vacuo to yield a dark yellow solid, which was then purified by reverse phase flash chromatography (5-95% acetonitrile in water, 0.1% formic acid). The fractions were combined, the acetonitrile removed in vacuo, and the product extracted from the residual aqueous mixture with EtOAc, which was then rinsed with brine and dried over $Na_2SO_4$. The EtOAc was removed in vacuo, and the compound put on a high vacuum pump overnight to yield a white solid in 55% purified yield; HRMS-ESI: m/z calcd $C_{22}H_{19}N_3O_6Na$ $(M+Na)^+$ 444.1166, found 444.1169

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# CHAPTER 4

# DEVELOPMENT OF SPIROLIGOMER-PEPTOID HYBRIDS

## 4.0 Introduction

Spiroligomers and peptoids are both peptidomimetic oligomers that are synthesized from readily available building blocks, with spiroligomers being synthesized from a unique set of bis-amino acids monomers,[1-3] while peptoids are typically synthesized from bromoacetic acid and a primary amine via a submonomer method.[4] Peptoids have been studied for decades and have been utilized for a wide variety of purposes: Biologically active peptoids ranging from protein secondary structure mimetics[5-9] to antimicrobial agents[10-12] and other therapeutics;[13] nanomaterials such as nanotubes[14,15] and nanosheets;[16,17] and metal binding peptoids;[18] among many others as evidenced by these excellent reviews.[19-21] For peptoids, by moving the side chain from the α-carbon to the nitrogen (in relation to a typical peptide), the synthesis can be achieved via a submonomer approach, which allows for an easy and efficient synthesis. Compared to peptides, the side chain is moved from the α-carbon to the backbone nitrogen, which removes the backbone chirality, and any inter- or intrachain hydrogen bonding of the backbone, thus leading to increased flexibility of the backbone.[22-24] While this makes the synthesis of peptoids much easier than peptides, this means that peptoids are more reliant on their side chain functionality for inter- and intra-chain interactions, as well as for any structural motifs that can be imparted by the limited chirality of the side chains.

To alleviate the problems associated with flexibility, Kirshenbaum,[25] and many others, have developed a macrocyclization strategy for the peptoid core.[11,12,26-28] Peptoid

macrocycles can be highly ordered, as evidenced by the numerous crystal structures in the peptoid databank.[25,29] These macrocycles range from only three residues in the peptoid core,[26] to six, eight, or more residues.[25] Peptoid containing macrocycles have been utilized for many differing applications, from protein inhibitors[30] or antimicrobials,[11] to antifreeze agents.[31]

Spiroligomers, on the other hand, are good at preorganizing functional groups, as the fused-spirocyclic structures do not allow any free rotation throughout the backbone.[1,32-34] Spiroligomers also benefit from having a large pool of side chains to utilize, as almost any aldehyde, ketone, alkyl halide, isocyanate, or amino acid can be incorporated as a unique functional group.[35-37] Spiroligomers, like peptoids, have been utilized for a variety of applications such as a transesterification catalyst,[38] a proline-aldol catalyst,[39] an aromatic claisen rearrangement catalyst,[40] a spiroligomer that binds MDM2,[41] the formation of supramolecular metal binding complexes,[42] a donor-bridge-acceptor molecule that accelerates electron transfer in water,[43] a metal binding mechanical molecular actuator,[44] and molecular rulers.[45]

It is worth noting that to date, no group has utilized a large, structurally diverse primary amine for peptoid synthesis. Larger side chains have been incorporated into peptoids in the past; however, these larger groups were incorporated with a click reaction, after the displacement step with a relatively small primary amine. Here, we are investigating linking spiroligomers through a peptoid backbone. To achieve this, we need only to incorporate a protected free amine at some point in the spiroligomer synthesis. We can then incorporate any number of unique functional groups as side chains on the spiroligomer.

As shown in Figure 4.1A, a linear peptoid 15-mer would not be very difficult to assemble, as peptoids as long as 50mers have been synthesized previously, but it will lack a lot of side chain structural-complexity. In comparison, a spiroligomer-peptoid hybrid (Figure 4.1B) would only be a peptoid 5-mer, but would incorporate 15 functional groups, as well as 10 stereocenters, of which we have total control. If one were to incorporate a spiroligomer trimer into a hybrid (Figure 4.1C), 15 functional groups could be incorporated with just three spiroligomer trimers, which would also incorporate 18 stereocenters. Each of these functionalized spiroligomers approximates the structural complexity of a small molecule, fused ring, natural product containing two, four, six or more fused rings and

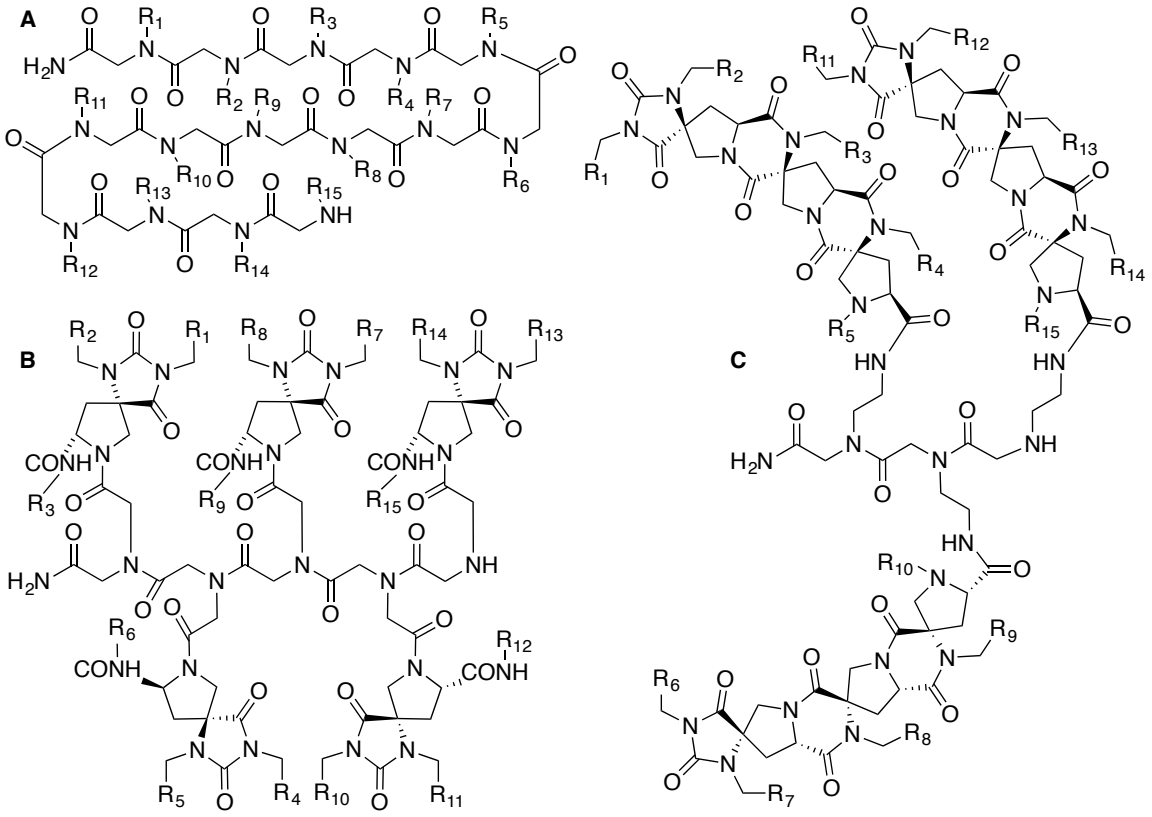
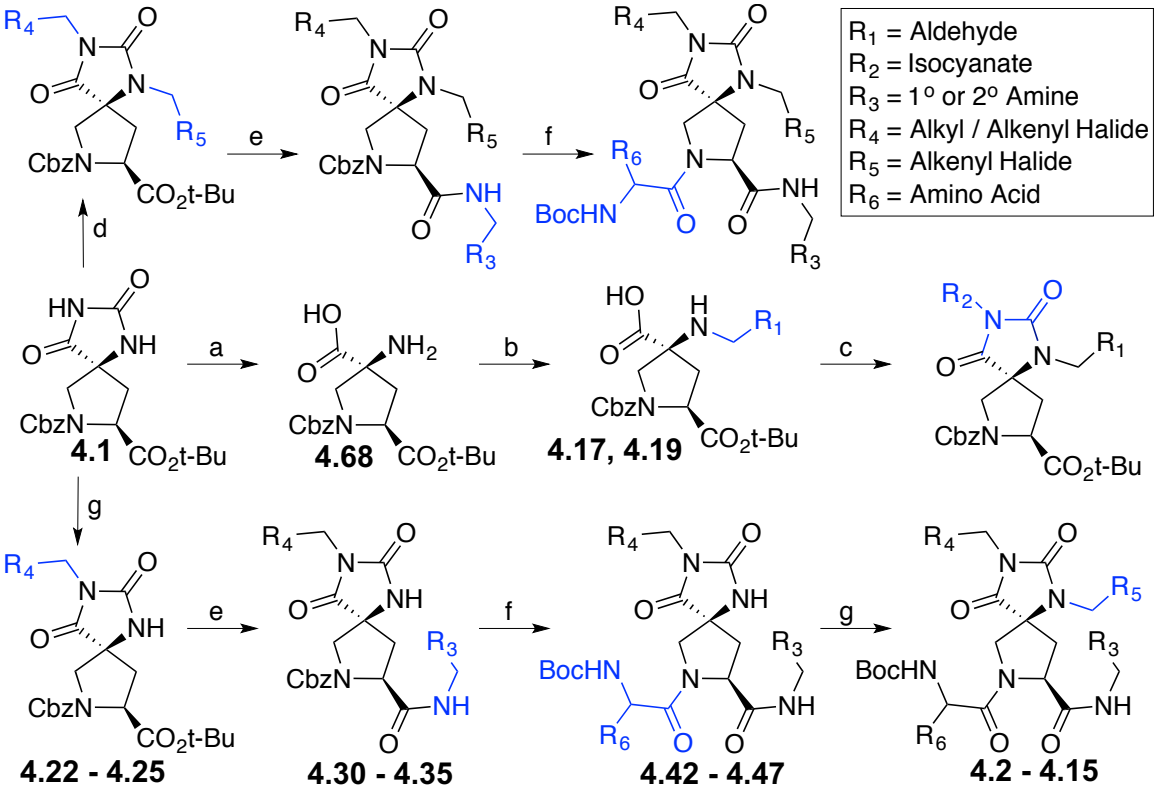



**Figure 4.1.** (A) A theoretical peptoid 15-mer (B) A theoretical spiroligomer-peptoid hybrid 15-mer, incorporating five spiroligomers which each contain three functional groups and two stereocenters (15 total groups, 10 total stereocenters) (C) A different spiroligomer-peptoid hybrid 15-mer, incorporating three spiroligomers which each contain five functional groups and six stereocenters (15 total groups, 18 total stereocenters)

several functional groups in precise three-dimensional constellations. Three to five of these spiroligomer domains can be displayed in close proximity on a peptoid chain provide a molecule with a great deal of preorganization that can bury a lot of surface area and yet display flexibility between the flexible domains to accommodate different protein surfaces. One could imagine molecules like these binding to multiple shallow grooves on a protein surface through large numbers of non-covalent contacts. By incorporating the structural rigidity and stereochemical diversity of spiroligomers with the modular linking provided by peptoid synthesis, this should facilitate the rapid discovery of new molecules of interest for catalysis, disrupting protein-protein interactions, and exploring large surface area host-guest interactions.

## 4.1 Synthesis of Spiroligomer Primary Amines




**Scheme 4.1**: A general scheme showing three of the many different pathways to spiroligomer primary amines. a) i. THF, $Boc_2O$, DMAP, ii. 2 M KOH; b) i. MeOH, $R_1CHO$; ii. $NaCNBH_3$; c) THF, $R_2NCO$, TEA; d) i. DMF, $K_2CO_3$, $R_4$-X; ii. $K_2CO_3$, $R_5$-X; iii. $Et_2NH$; e) 1. TFA; 2. i. HOAT, EDCI, DMF, DCM; ii. $R_3$-$NH_2$; f) 1. 1:1 HBr/DCM; 2. DMF, DCM, DIPEA, Boc-AA-OAt; g) DMF, $K_2CO_3$, $R_4$-X or $R_5$-X

To facilitate the synthesis of spiroligomer-peptoid hybrids, we must first synthesize a variety of spiroligomers containing primary amines. The building blocks of spiroligomers are the 4 stereoisomers of the proline hydantoin intermediate in spiroligomer bisamino acid synthesis that we routinely synthesize on a 600 mmol scale (230g of mixed diastereomers, access to all 4 stereoisomers).[2,3] As shown in Scheme 4.1, we can hydrolyze the hydantoin **4.1** to form an amino acidat the four position of the proline ring, giving the bis-amino acid **4.48**. We can then functionalize this bis-amino acid bye reductively alkylating any one of a variety of aldehydes to make a functionalized bis-amino acid **4.49**. This amino acid can then react with an isocyanate to form a functionalized hydantoin **4.50**.[38,39] Alternatively,

we have recently developed chemistry which allows us to directly functionalize the hydantoin by exploiting the varying reactivities between the imide and amide nitrogen atoms of the hydantoin.[37] As we have shown, we can install a variety of functional groups onto the hydantoin **4.1** via direct alkylation with either alkyl, alkenyl, or aryl halides to make **4.22-4.25**. We then remove the carboxylic acid protecting group with TFA, and couple a variety of primary amines to make **4.30-4.35**, followed by removal of the proline Cbz protecting group and coupling an N-Boc protected amino acid to make **4.42-4.27**. Finally, we performed another alkylation, this time on the amide of the hydantoin, to form the final spiroligomer amines **4.2-4.15**, shown in Figure 4.2. Spiroligomers similar in size and functional group display to these amines have been used previously by our group as small, organic catalysts.[39,40]

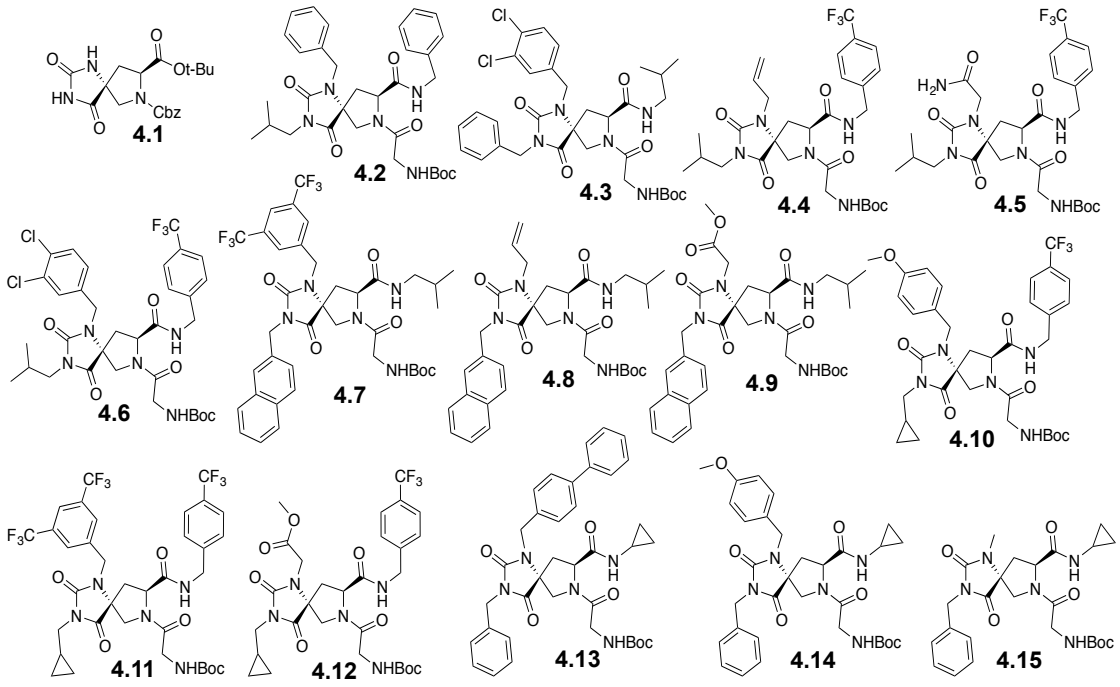



**Figure 4.2**. Spiroligomer hydantoin starting material and spiroligomer amines utilized for synthesis of spiroligomer-peptoid hybrid

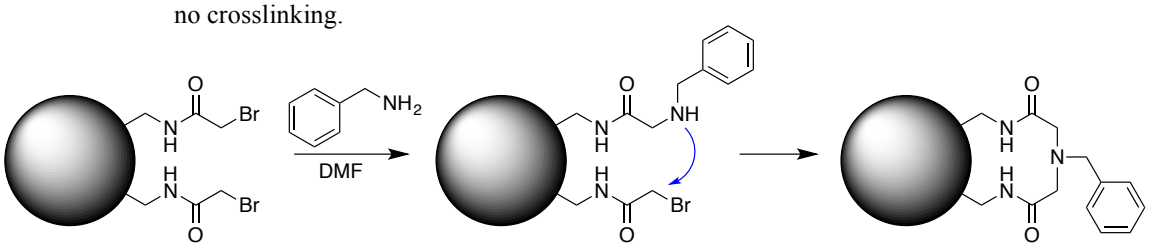
## 4.2 Testing the Limits of Peptoid Chemistry

Since we put a significant amount of work into synthesizing our primary amines, we did not want to waste them by throwing away large excesses of the amines, as is typically done in peptoid couplings. As such, we decided to run several trials to verify the minimum concentration of amine required for peptoid chemistry to function reliably. We utilized commercially available benzylamine to obtain the baseline conditions for peptoid chemistry shown in Table 1, below. We started on Rink Amide polystyrene resin (loading 0.63 mmol/g), and using the original submonomer synthesis[4,19] as a benchmark, we ramped down to 3 equivalents with one equivalent of exogenous base (DIPEA), at a concentration of 150 mM of benzylamine in DMF. Any further decrease in equivalents or concentration resulted in significant crosslinking (C-L) of the resin, whereby a secondary amine on resin would react with another bromoacetate on resin before an amine in solution could react, thereby terminating the sequence (Shown in Scheme 4.2). When we moved over to a Rink Amide Tentagel® resin (loading 0.37 mmol/g), we found that we could lower the required concentration to 125 mM, but the number of equivalents required could not be reduced. Finally, switching to a 2-Cl-Trityl Chloride polystyrene resin (loading 1.1 mmol/g) required a marked increase in both the equivalents of benzylamine and thus the concentration of benzylamine the solution (5 and 250 mM, respectively) in order to ensure complete coupling of the amine without significant resin cross-linking. These couplings were all allowed to proceed overnight; however, it is worth noting that recently, we have reduced the time down to 4 hours for the coupling step without any reduction in yield.

**Table 4.1. Minimum equivalents required for peptoid couplings on various resins**

| Exp. | Equiv Amine | Equiv Base | Conc. (mM) | Res. Load. (mmol/g) | Products | % C-L UV Est. |
|---|---|---|---|---|---|---|
| 1 | 20 | 0 | 2000 | 0.63 | Good | < 1% |
| 2 | 3 | 2 | 150 | 0.63 | Side Rxn | - |
| 3 | 3 | 1 | 150 | 0.63 | Min. C-L | < 2% |
| 4 | 3 | 1 | 10 | 0.63 | C-L | 6-8% |
| 5 | 3 | 1 | 30 | 0.63 | C-L | 6-8% |
| 6 | 3 | 1 | 100 | 0.63 | C-L | 2-3% |
| 7 | 2 | 1 | 100 | 0.63 | C-L | 2-3% |
| 8 | 2 | 1 | 150 | 0.63 | C-L | 2-3% |
| 9 | 3 | 1 | 125 | 0.37 | Min. C-L | < 2% |
| 10 | 3 | 1 | 75 | 0.37 | C-L | 6-8% |
| 11 | 3 | 1 | 150 | 1.1 | C-L | 8-10% |
| 12 | 5 | 1 | 250 | 1.1 | Min. C-L | < 2% |

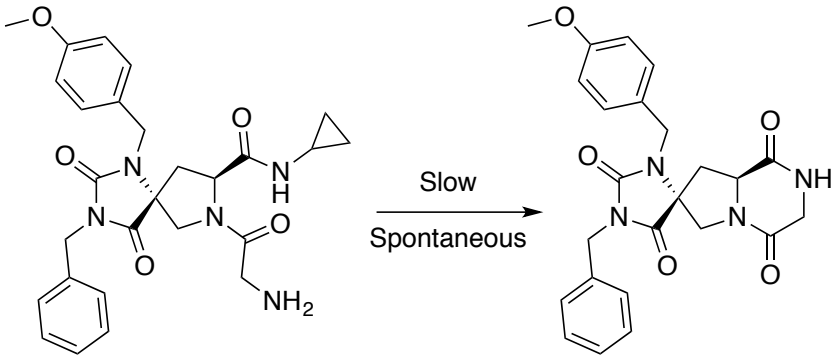
C-L = crosslinked products, explained in Scheme 4.2. Good indicates no crosslinking.




**Scheme 4.2**. Benzylamine is reacted with the resin; however, insufficient concentration of amine in solution allows for an amine on resin to displace a neighboring bromine. This results in a crosslinked resin, and the termination of a peptoid sequence.

Having determined the requirements of peptoid chemistry at sub molar concentrations, we proceeded to synthesize several variants of spiroligomer-peptoid hybrids. Any primary amine we use would first need to be Boc deprotected, which was achieved with a 50% TFA / DCM mix, followed by a quick concentration *in vacuo*. The amine was reconstituted in EtOAc [200 mM], followed by two washes with an equal volume of 1 M KOH to free-base, which were back extracted with EtOAc. The protected

amines are stable at room temperature for several years; however, if the free amine is left on the bench or in solution, it is possible to have slow, spontaneous formation of a diketopiperazine (DKP) between the glycine and the proline (Scheme 4.3). Therefore, the amine was concentrated under reduced pressure to yield a foamy solid, which is stable for over a year (dry, -20 °C). It is worth noting that although the free amines can undergo the spontaneous DKP formation, the amines can be reprotected with di-*tert*-butyl dicarbonate (Boc anhydride) after their use as primary amines for a peptoid coupling reaction. The



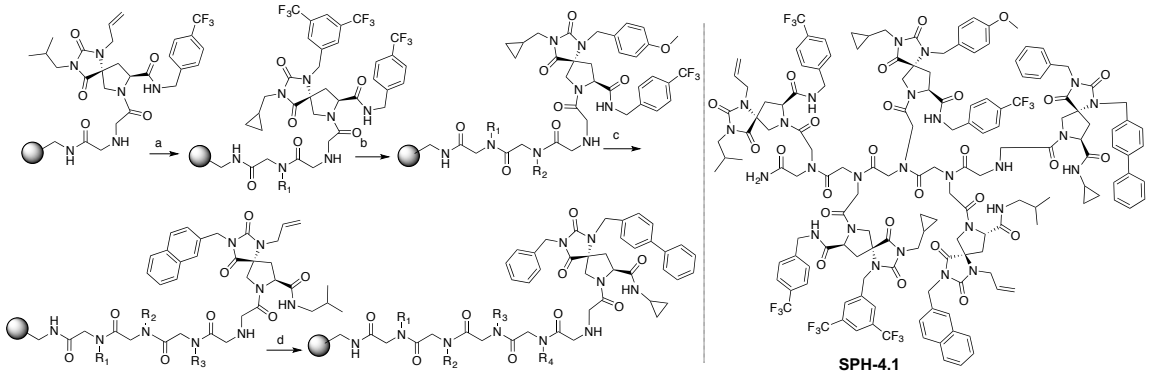

**Scheme 4.3.** Spontaneous DKP formation from spiroligomer free amines in solution.

amine should then be repurified to remove any DKP side-products and any excess Boc anhydride.

## 4.3 Synthesis of Spiroligomer Peptoid Hybrids

Scheme 4.4 shows the synthesis of spiroligomer-peptoid hybrid 1 (**SPH1**). We started with rink amide resin, which we treated with a solution of bromoacetic acid and DIC in DMF for 30 minutes. We then reacted the deprotected spiroligomer amine **4.4** to displace the bromine on resin. Then, following standard peptoid synthesis as described in Scheme 4.4, we were able to synthesize **SPH-4.1**. This spiroligomer-peptoid hybrid proves

that we can make a hybrid that incorporates 15 functional groups and ten stereocenters on rigid spiroligomer side chains.




**Scheme 4.4.** Peptoid synthesis of the spiroligomer-peptoid hybrid, **SPH-4.1** (a) i. Bromoacetic acid, DIC, DMF; ii. DMF, 150 mM **4.11**; (b) i. Bromoacetic acid, DIC, DMF; ii. DMF, 150 mM **4.12**; (c) i. Bromoacetic acid, DIC, DMF; ii. DMF, 150 mM **4.8**; (d) i. Bromoacetic acid, DIC, DMF; ii. DMF, 150 mM **4.13**

To test the fidelity of a spiroligomer-peptoid hybrid with increasing chain length, we synthesized both a 9-mer (**SPH-4.2**) and a 12-mer (**SPH-4.3**) incorporating 3 and 6 stereotypical amines, respectively, without any loss in coupling yields or efficiency (Figure 4.3). These two hybrids also added another spiroligomer when compared to the 5-mer that we had previously synthesized. Other spiroligomer-peptoid hybrids that we have synthesized include custom linkers incorporating propargyl (post-peptoid synthesis modification potential) or bromobenzyl side chains (the bromobenzyl group provides an easily identified mass spectroscopy signature due to the natural isotopic abundance of bromine). Installation of a c-terminal methionine residue to facilitate CNBr mediated cleavage from resin has been tested on various spiroligomer-peptoid hybrids. These types of hybrids would be most beneficial to those interested in on-resin screening for compounds, as it allows for the deprotection of any side chains, while leaving the peptoid still attached to the resin. Furthermore, this could also be used for on-bead degradation

(such as an Edman Degradation) followed by sequencing, to enable split-pool library synthesis of spiroligomer peptoid hybrids.

We have developed these molecules towards the development of split-pool synthesis of a one-bead-one-compound (OBOC) libraries of spiroligomer/peptoid libraries. Before we can screen a library, however, we needed to develop methodology to sequence the hits that would be pulled from the resin pool. Unfortunately, our initial attempts to sequence our molecules via MALDI were fruitless. As evidenced by figure 4.4a and 4.4b, which shows two separate single bead cleavages, we were unable to decode the final two residues of any sequence from an OBOC library. We believe this is due to the spiroligomer amines being able to cleave at the proline-glycine linkage. This made the mass range for

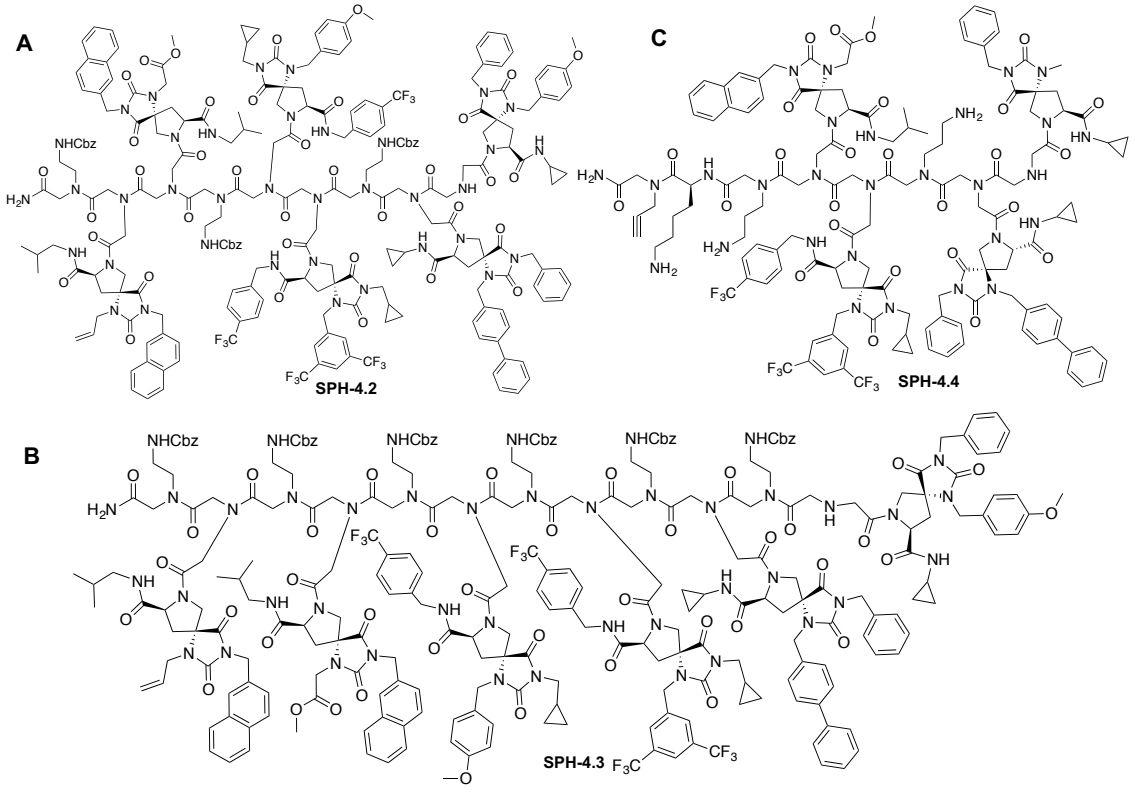



Figure 4.3. Three spiroligomer-peptoid hybrids of varying length, functional groups, and resin linker. (a) **SPH-4.2**, a peptoid 9-mer incorporating 5 spiroligomers (b**) SPH-4.3**, a peptoid 12-mer incorporating 5 spiroligomers (c) **SPH-4.4**, a hybrid peptoid containing 8 residues, four of which are spiroligomers.

where we expected to find the second to last residue (from 2500-2800 Da) hidden by other proline-glycine fragmentation.

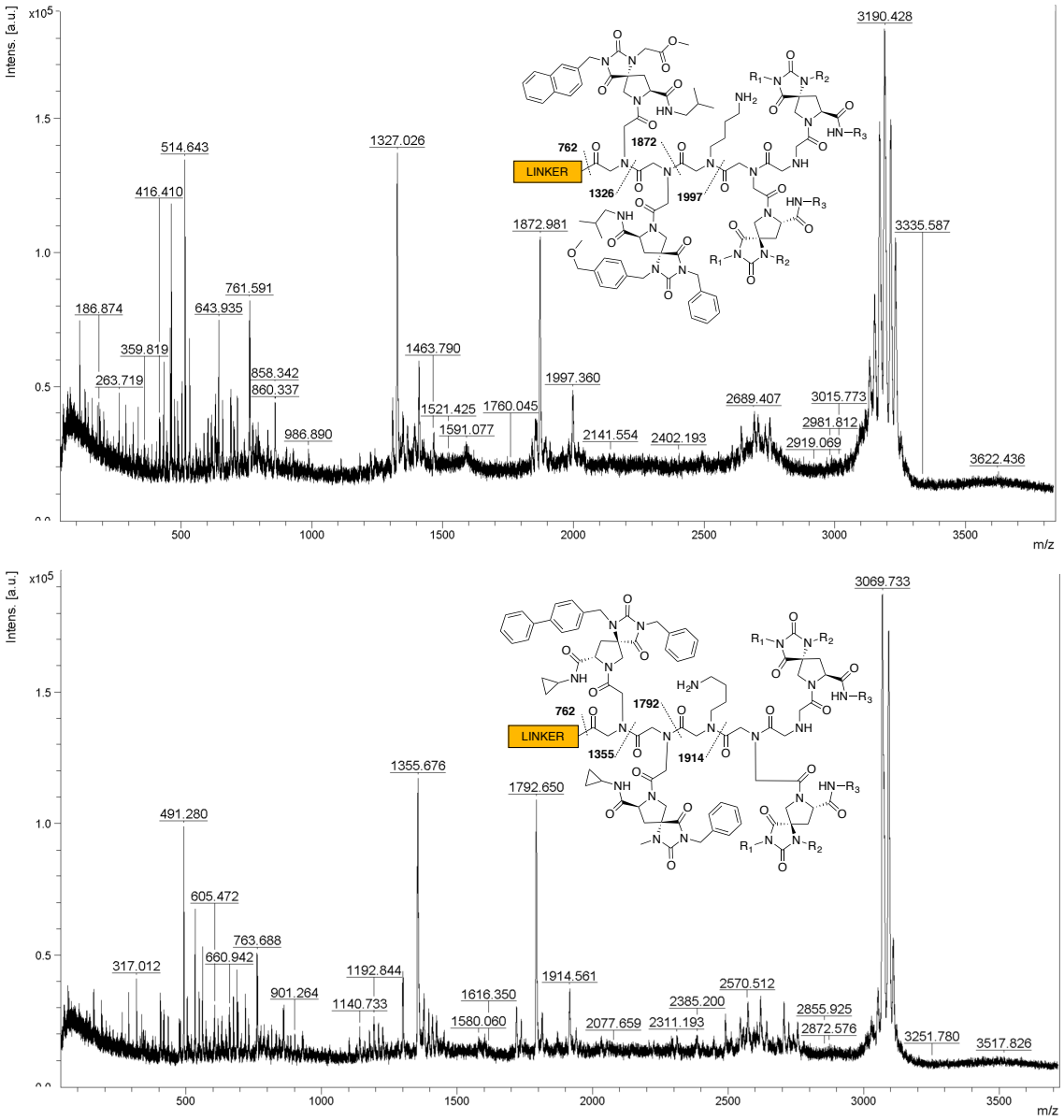



**Figure 4.4.** Single bead cleavages and MALDI MS/MS sequencing, highlighting the problems with sequencing the final two residues of a spiroligomer-peptoid hybrid using MALDI.

To address this, we made a change by adding a terminus modifier that incorporated a free amine as well as a separate mass tag (3,4-dichlorobenzylamine) that could alleviate this problem in either of two separate ways. The hope was that these two additional residues

would move any proline-glycine dissociations outside of the mass range of the final two residues. Furthermore, by adding the separate mass tag, if this linker didn't help de-clutter the mass range for where the expected second to last residue would be found, we would still be able to sequence the molecule by using this unique mass tag to differentiate the terminal end from the bromine mass tag at the starting linker. Two spiroligomer hybrids with this terminus modifier are shown in Figure 4.5, below.

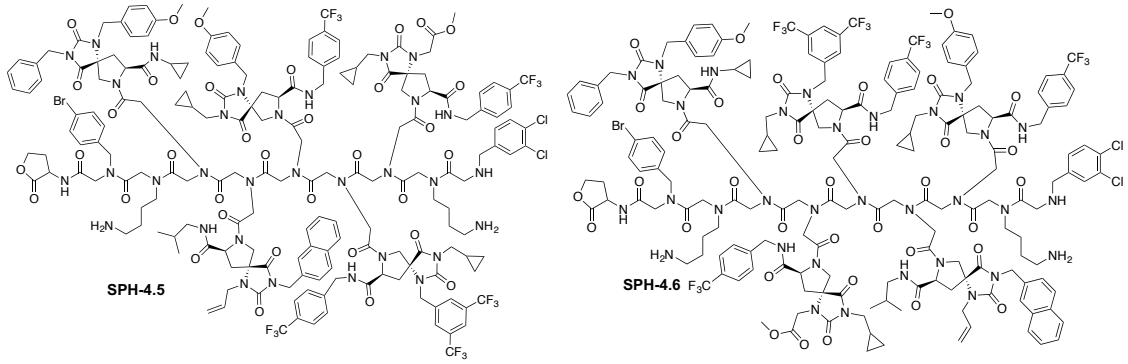
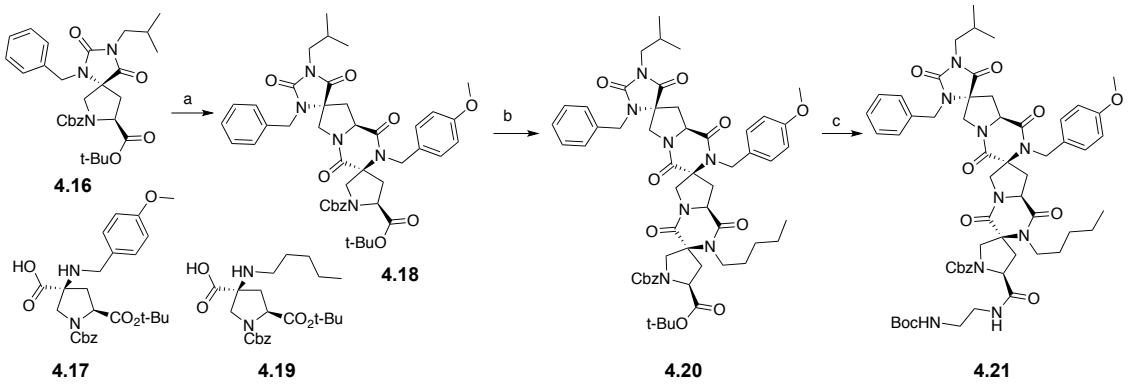



**Figure 4.5**. Two spiroligomer-peptoid hybrids, **SPH-4.5** and **SPH-4.6**, with the new terminus modifier.

Finally, we have synthesized a spiroligomer-peptoid hybrid containing an extended spiroligomer trimer, as opposed to the monomeric units we have shown up to this point. Spiroligomer trimers have been used previously for catalysis and for protein-protein interactions, so incorporating a large trimer onto a peptoid backbone is a crucial step to the continued synthesis of larger structures. The trimer was synthesized from three bis-amino acids, as shown in Scheme 4.5. A Cbz and *t*-Bu protected, dialkylated proline hydantoin **4.16** was treated with 1:1 DCM/(33% HBr/AcOH) for 30 minutes, after which the solvent was removed *in vacuo*, and the molecule left on a high vacuum pump overnight. The following morning, Bis-amino acid **4.17** was preactivated under inert conditions with EDC and HOAt in a 1:1 mix of dry DMF/DCM for 1.5 h. Deprotected compound **4.16** was then




**Scheme 4.5**. Synthesis of the spiroligomer trimer **4.21** (a) i. 1:1 DCM/(33% HBr/AcOH); ii. (Preactivated **4.17**: HOAT, EDC, 1:1 dry DMF/DCM, 1.5 h), DMF, DIPEA; (b) i. 1:1 DCM/(33% HBr/AcOH); ii. (Preactivated **4.19**: HOAT, EDC, 1:1 dry DMF/DCM, 1.5 h), DMF, DIPEA; (c) i. 95:4:1 TFA/$H_2O$/TIPS, 1 h; ii. EDC, HOAT, 1:1 dry DMF/DCM, 1.5 h; iii. N-boc-ethylenediamine

dissolved into a minimal amount of dry DMF then added to the preactivated bis-amino acid along with DIPEA. After stirring overnight, more EDC was added to the reaction to close the diketopiperazine ring to afford a spiroligomer dimer **4.18**. The preceding steps were followed to make the spiroligomer trimer **4.20**, except that **4.19** was substituted for **4.17** at the preactivation step. Spiroligomer **4.20** can then be treated with a 95:4:1 TFA/$H_2O$/TIPS mixture to remove the *t*-Bu protecting group, then following the removal of the solvent, this spiroligomer is preactivated in the same manner as the bis-amino acids, to which we added N-Boc-ethylenediamine to give the spiroligomer trimer **4.21**, which was utilized to make **SPH-4.7** shown in Figure 4.6. This spiroligomer trimer is the largest amine utilized for the displacement step of a peptoid synthesis, containing five unique functional groups and six stereocenters, of which we have total control. This means the spiroligomer-peptoid hybrid trimer contains 15 functional groups and 18 stereocenters in total. These large spiroligomer trimers, like the smaller monomers shown previously, can be used sequentially, or interspersed with smaller units, indicating that they would make excellent candidate amines for future work. Furthermore, large spiroligomer trimers preorganize

many more groups than the spiroligomer monomer amines or regular peptoid amines, thus reducing any entropic penalty that would be associated when binding a protein.

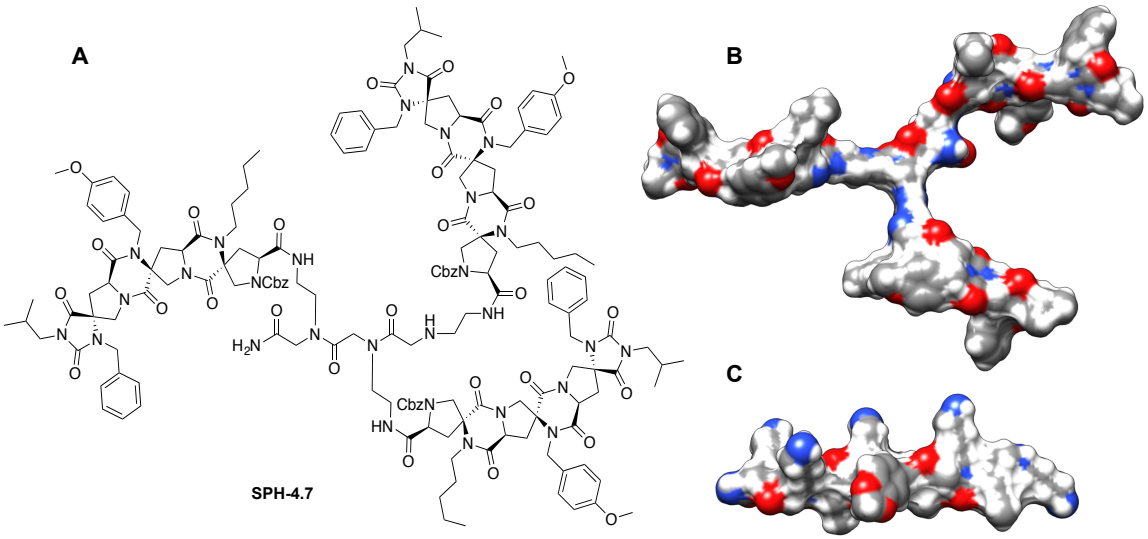
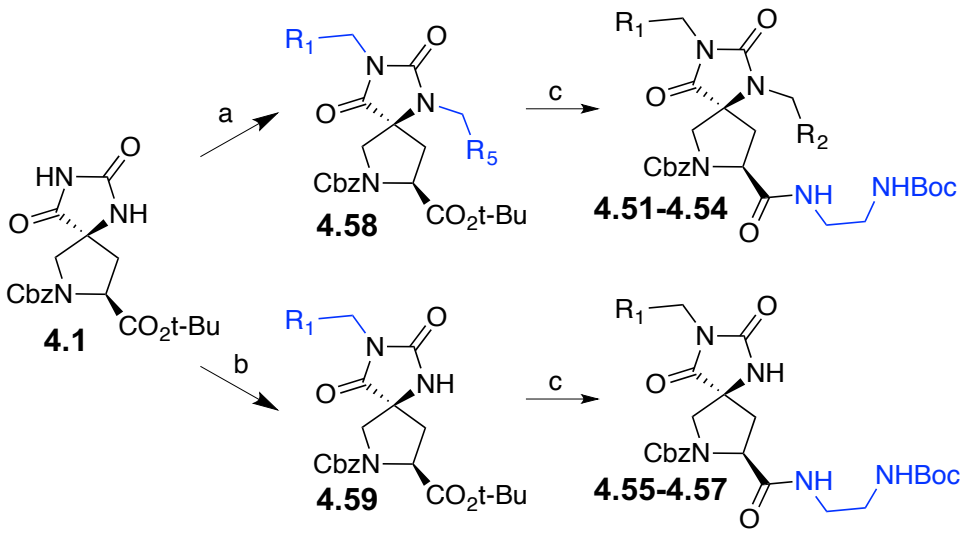



**Figure 4.6.** A spiroligomer-peptoid hybrid **SPH4.7** synthesized with spiroligomer trimers. (A) chemdraw of **SPH-4.7** (B) Cando model of **SPH4.7**, shown with solvent-excluded surface (C) A peptoid 10-mer with solvent-excluded surface

### 4.4 Synthesis of an Amphiphilic Spiroligomer-Peptoid Hybrid-Macrocycle

For synthesis of spiroligomer-peptoid hybrid macrocycles, we chose a different type of spiroligomer amine when compared to **4.2-4.15**, the synthesis of which is shown in Scheme 4.6. This amine linkage can be readily reprotected, which means that we can utilize large excesses of the spiroligomer amine and then recover the spiroligomer. The seven spiroligomer amines **4.51-4.57** utilized for spiroligomer-peptoid hybrid-macrocycle synthesis are shown in Figure 4.7.




**Scheme 4.6.** Synthesis of spiroligomer primary amines for use in spiroligomer-peptoid hybrid-macrocycles. a) i. DMF, $K_2CO_3$, $R_1$-X; ii. $K_2CO_3$, $R_2$-X; iii. $Et_2NH$; b) DMF, $K_2CO_3$, $R_1$-X; c) 1. TFA; 2. i. HOAT, EDCI, DMF, DCM; ii. N-Boc-ethylenediamine

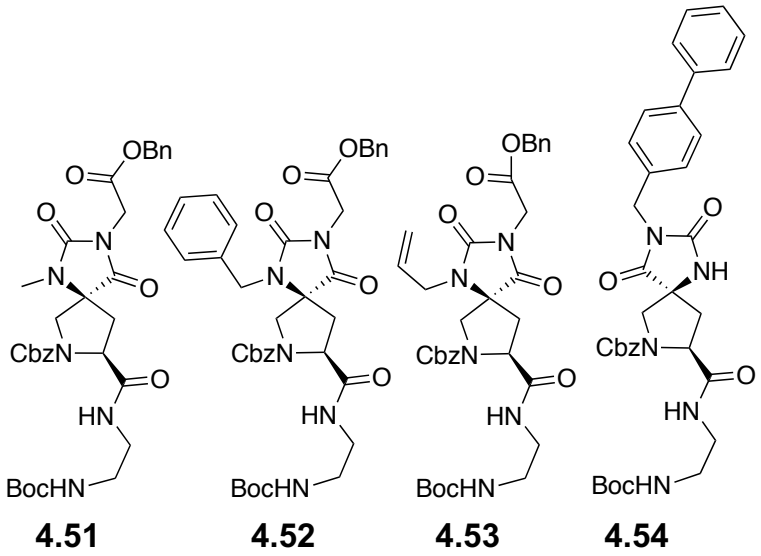
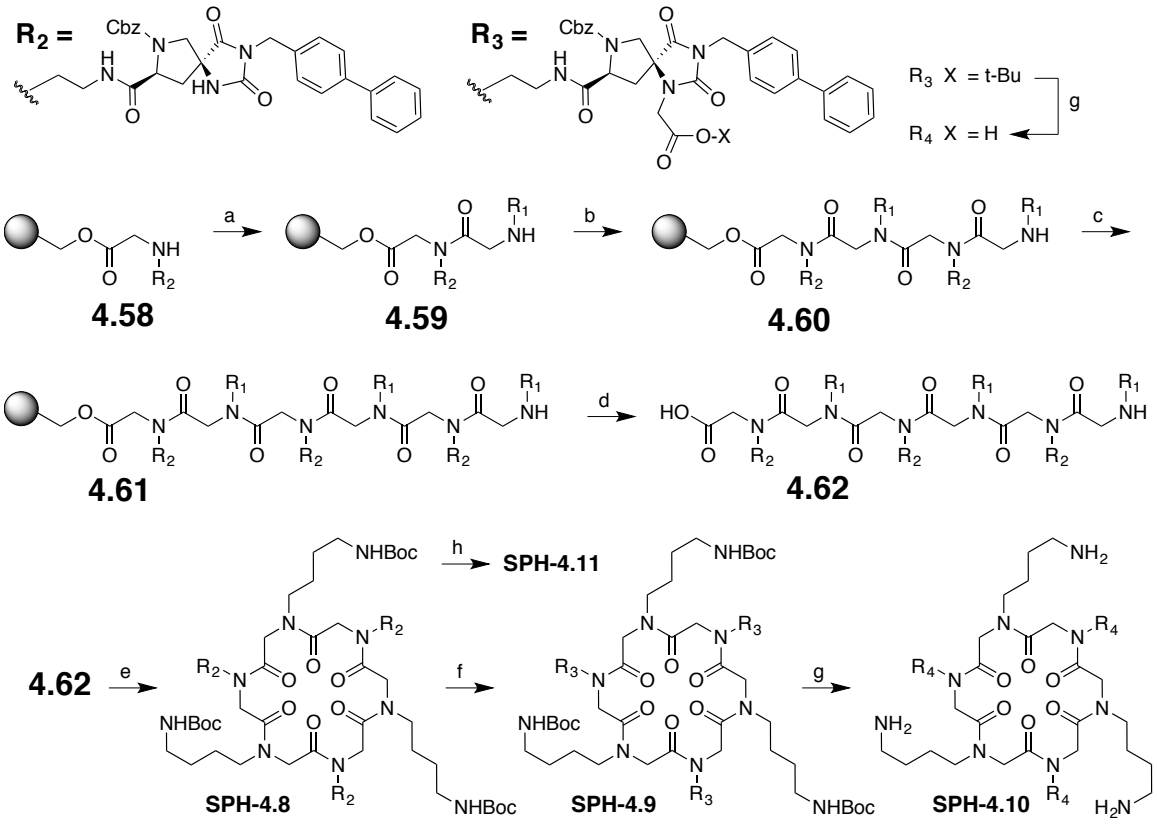



**Figure 4.7.** Spiroligomer amines synthesized for inclusion into spiroligomer-peptoid hybrid-macrocycles

Following the work of Shin,[25] we set about synthesizing the first spiroligomer-peptoid macrocycles. We started with 2-Cl-Trityl Chloride resin loaded with bromoacetic acid, then utilized **4.54** as our amine to displace the bromine. Then, following normal peptoid synthesis with alternating amines shown in Scheme 4.7, below, we were able to

synthesize **4.61** successfully. Due to the fact that this peptoid chain contains protecting groups that are quite acid labile, a very mild resin cleavage method was required. Utilizing a 30% hexafluoroisopropanol (HFIP) in DCM solution, we were able to successfully cleave **4.62** from the resin. To facilitate macrocyclization as opposed to polymerization, infinite dilution conditions were employed. PyAOP and DIPEA were dissolved in a DMF solution in which the final concentration would amount to 5 mM with respect to the amount of **4.62** present. **4.62** was dissolved in a minimal amount of DCM, and then slowly added dropwise to the stirred coupling reagent and base to afford the spiroligomer-peptoid hybrid-macrocycle **SPH-4.8**. This method afforded good results, with 88% yield of the purified macrocycle (reverse phase flash chromatography, 5-100% acetonitrile, 0.1% formic acid.)




**Scheme 4.7.** Synthesis of **SPH-4.8** and **SPH-4.9** with subsequent deprotetions to **SPH-4.11** and **SPH-4.10**, respectively. (a) i. Bromoacetic acid, DIC, DMF; ii. 1 M N-Boc-1,4-butanediamine in DMF (b) i. Bromoacetic acid, DIC, DMF; ii. 500 mM **4.54** in DMF, 1 equiv DIPEA iii. Bromoacetic acid, DIC, DMF; iv. 1 M N-Boc-1,4-butanediamine in DMF (c) i. Bromoacetic acid, DIC, DMF; ii. 500 mM **4.54** in DMF, 1 equiv DIPEA iii. Bromoacetic acid, DIC, DMF; iv. 1 M N-Boc-1,4-butanediamine in DMF (d) 30% HFIP in DCM (e) 3 equiv PyAOP, 6 equiv DIPEA, 5 mM final concentration of **5.30** in DMF (f) DMF, $K_2CO_3$, *tert*-Butyl bromoacetate (g) 95:4:1 TFA/Water/TIPS (h) 1:1 TFA/DCM

With the ability to alkylate the hydantoin, we thought it would be beneficial to test out the alkylation chemistry on a peptoid macrocycle, as a late stage functionalization could prove useful down the road. We also wanted to synthesize a macrocycle that contained carboxylic acids to attempt the binding of various metals, so the installation of three *t*-Bu protected acetate groups via alkylation seemed like a good choice for a test of the alkylation chemistry on a macrocycle. As the reaction was carried out on microscale, the amount of $K_2CO_3$ used is not sufficient to allow for grinding by the stir bar, so the 3.6 equiv of $K_2CO_3$

was first ground with mortar and pestle, and then added to a 50 mM solution of **SPH-4.8** in DMF followed by 3.3 equiv of *t*-Bu bromoacetate. After the reaction was allowed to stir overnight, the desired product **SPHM-4.9** was obtained in 96% yield. Both **SPHM-4.8** and **SPHM-4.9** were deprotected, the former with 1:1 TFA/DCM for 10 min to remove the Boc groups and afford **SPHM-4.11**, while the latter was deprotected with 95:4:1 TFA/$H_2O$/TIPS solution to remove both the Boc and *t*-Bu groups to afford **SPHM-4.10**, which is shown in Figure 4.8.

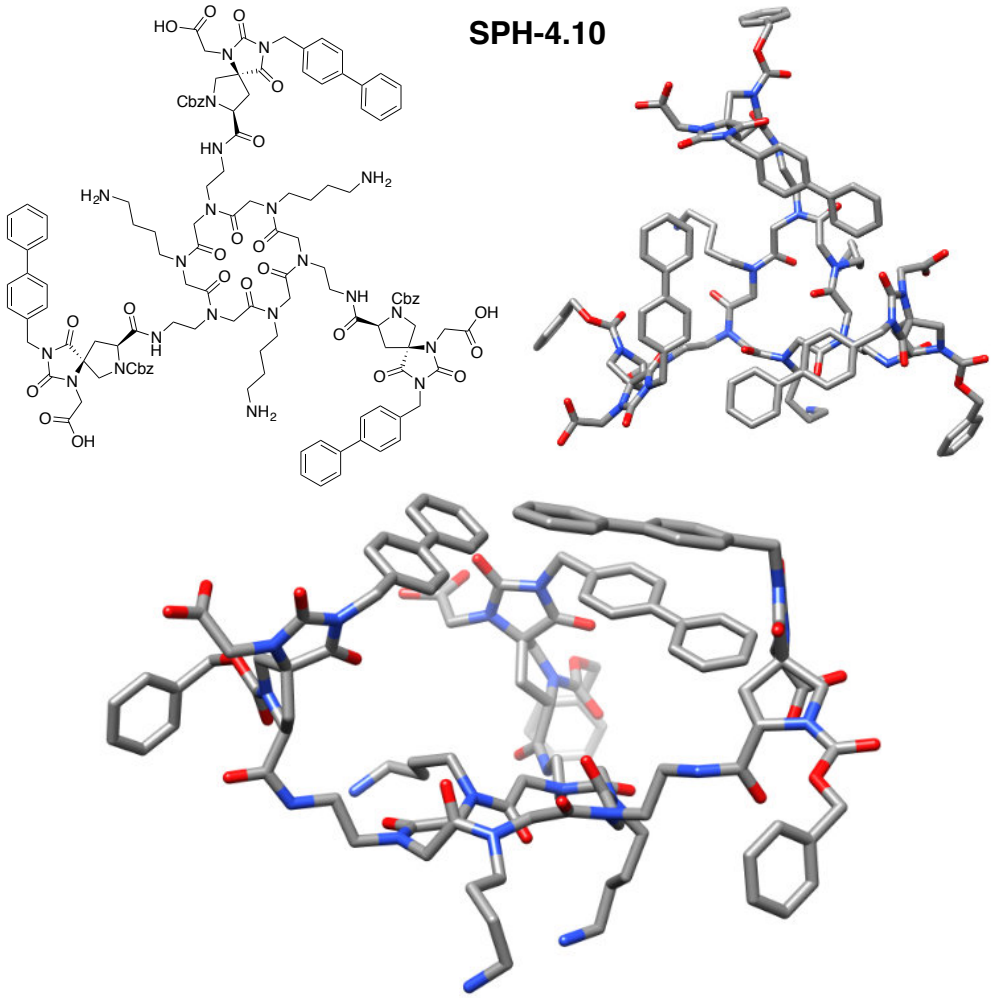
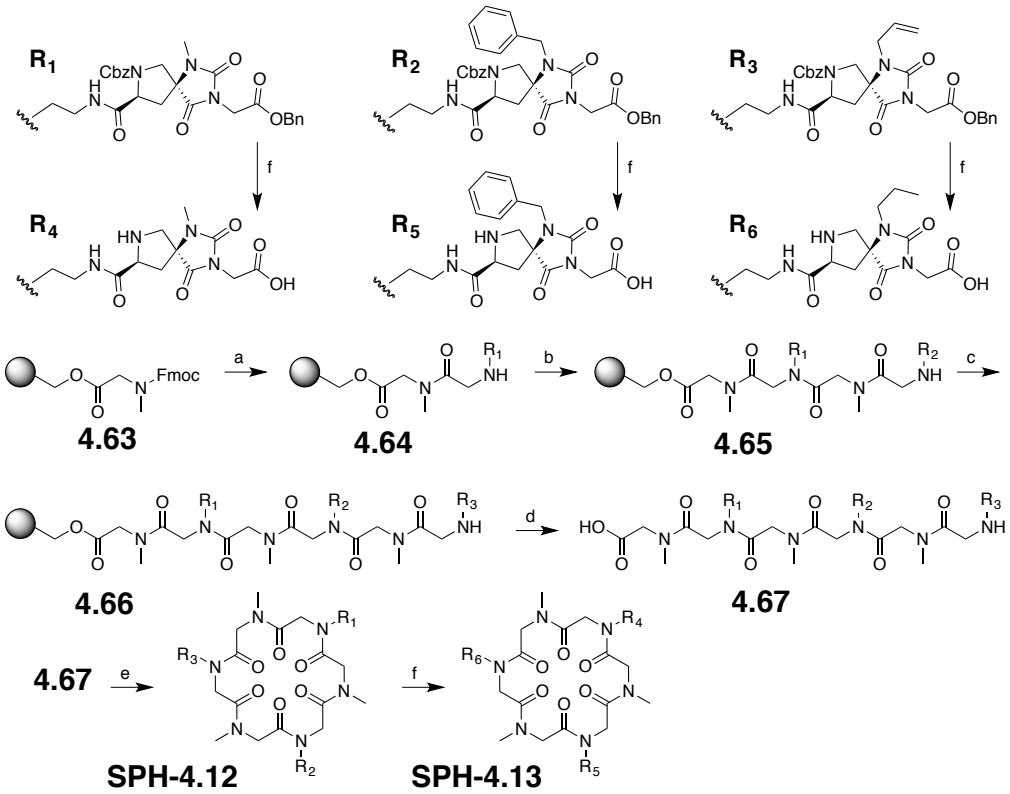



**Figure 4.8.** A large, amphiphilic spiroligomer-peptoid hybrid-macrocycle, **SPH-4.10** (A) Chemdraw of **SPH-4.10** (B) CANDO generated model* of **SPH-4.10**viewed from the top (C) Same model* as B, viewed from the side. *Rotatable bond torsions were adjusted using Chimera.

## 4.5 Synthesis of an Asymmetric Water-Soluble Hybrid Macrocycle

With the ability to successfully synthesize symmetric hybrid macrocycles, we next wanted to tackle asymmetric macrocycles. We also designed the molecule such that it would be water soluble, with the potential to fold and form a small hydrophobic core. As the overall goal of the project was to create asymmetric pockets for ligand or metal binding, this was a logical step to take. Once again utilizing the alkylation chemistry, we installed benzyl protected carboxylic acids on the imide position of the hydantoin, followed by either a methyl, benzyl, or allyl functional group to make three unique amines **4.51-4.53**, which would be quite hydrophilic when deprotected.




**Scheme 4.8.** Synthesis of **SPH-4.12** with subsequent deprotection to form **SPH-4.13** (a) i. 20% Piperidine in DMF; ii. Bromoacetic acid, DIC, DMF; iii. 500 mM **4.51** in DMF (b) i. Fmoc-Sar-OH, HATU, DIPEA, NMP; ii. 20% Piperidine in DMF; iii. Bromoacetic acid, DIC, DMF; iv. 500 mM **4.52** in DMF, 1 equiv DIPEA (c) i. Fmoc-Sar-OH, HATU, DIPEA, NMP; ii. 20% Piperidine in DMF; iii. Bromoacetic acid, DIC, DMF; iv. 500 mM **4.53** in DMF, 1 equiv DIPEA (d) 30% HFIP in DCM (e) 3 equiv PyAOP, 6 equiv DIPEA, 5 mM final concentration of **4.67** in DMF (f) 1:1 $H_2O$/THF with 1% formic acid, Pd/C, $H_{2(g)}$

Instead of loading the 2-Cl-Trityl Chloride resin with bromoacetic acid, we instead loaded Fmoc-Sarcosine, as shown in Scheme 4.8. The first step after loading was removal of the Fmoc protecting group, followed by stringent rinsing. We can then use standard peptoid submonomer chemistry to couple our first amine (**4.51**) to the resin. After the coupling is completed, another Fmoc-Sarcosine can be coupled to the resin using standard Fmoc-SPPS conditions. Utilizing these two methods, the three amines **4.51-4.53** were incorporated successfully onto a peptoid core **4.66**, which was cleaved from the resin utilizing 30% HFIP in DCM to afford peptoid **4.67**. The peptoid was then able to be macrocyclized to form **SPH-4.12** with the same conditions as the amphiphilic macrocycle **SPH-4.8**.

We then set about determining how best to deprotect the macrocycle. The hope was that we could simply utilize a 1:1 mix of DCM/(33% HBr in AcOH) solution, as opposed to hydrogenation, since that would also saturate the allyl double bond. Unfortunately, the HBr method does not completely remove the benzyl groups from the carboxylic acids, as the macrocycle with one or two benzyl groups still present were found as the two products via HPLC-MS, while the fully deprotected version was not found. While disheartened that this method was ineffective for complete removal of the protecting groups, we pressed on with a simple Pd/C in THF hydrogenation method. Unexpectedly, this method also hit a snag, as several side products were formed during the reaction. However, by performing the hydrogenation in a 1:1 $H_2O$/THF mixture with a 1% formic acid additive to ensure that all of the amines and carboxylic acids were protonated after protecting group removal, we were able to isolate the deprotected macrocycle **SPH-4.13**. This macrocycle is soluble in water, as 36 mg was dissolved in 0.500 mL of water for purification by reverse phase flash

chromatography. Unfortunately, only 7mg was recovered from the purification. This is most likely due to a dehydration that occurred between the workup and purification of the molecule, as shown by HPLC-MS. A full chemdraw and CANDO generated model of **SPH-4.13** is shown in Figure 4.9.

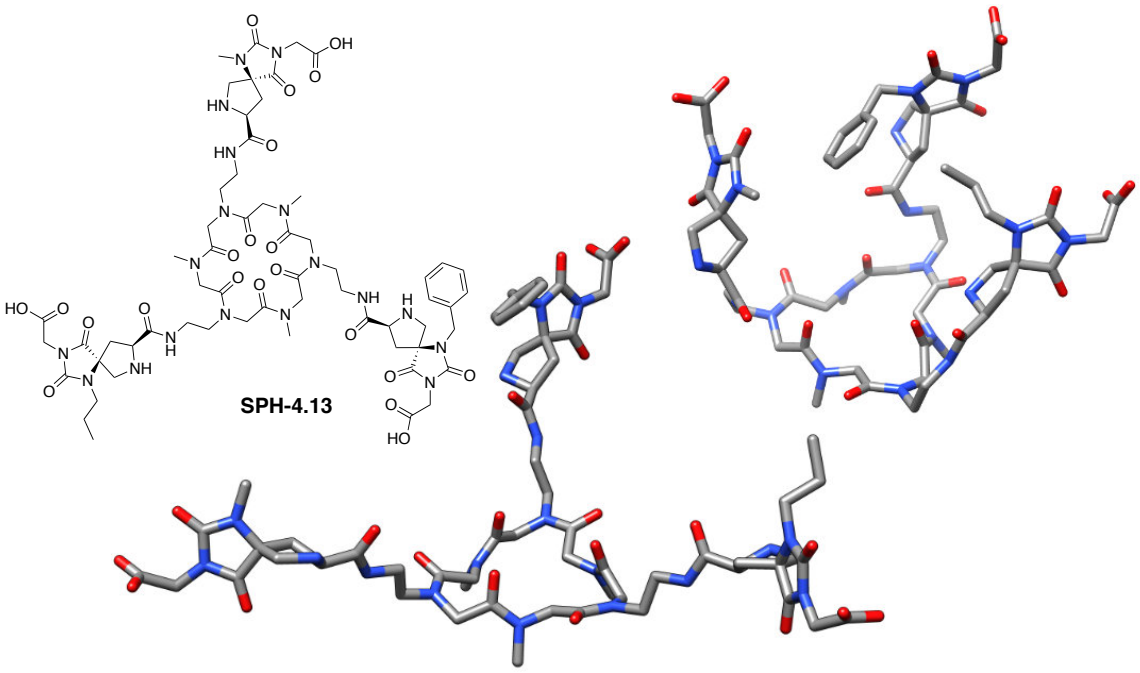



**Figure 4.9.** A large, hydrophilic spiroligomer-pepeotid hybrid-macrocycle, **SPH-4.13** (A) Chemdraw of **SPH-4.13** (B) CANDO generated model* of **SPH-4.13** (C) Same model as B (without bonds rotated), viewed from a different angle. *Rotatable bond torsions were adjusted using Chimera

## 4.6 Conclusion

We have successfully synthesized over 20 new spiroligomers containing three to five functional groups, two to six stereocenters, and a protected primary amine, which we have been able to incorporate into peptoids of varying lengths, such as peptoids **SPH-4.1-SPH-4.4**. This is the first reported synthesis which utilized large amines during the displacement step of peptoid synthesis, of particular note would be the use of a spiroligomer trimer **4.21** to make the hybrid **SPH-4.7**. We have begun developing ways to

sequence a library of spiroligomer-peptoid hybrids, and we are currently developing a new peptoid terminus modifier. Now that we can synthesize these new hybrid peptidomimetics, further work on straight chain spiroligomer peptoid hybrids can be focused on developing spiroligomers that display the appropriate side chains for new host-guest interactions and protein-protein interactions.

We have also successfully synthesized three unique spiroligomer-peptoid hybrid-macrocycles. **SPH-4.8**, which could be alkylated to install functional groups after macrocyclization to make the amphiphilic macrocycle **SPH-4.10**, or **SPH-4.8** could simply be deprotected to give a slightly less hydrophilic macrocycle **SPH-4.11.** We were also able to synthesize a spiroligomer-peptoid hybrid-macrocycle **SPH-4.13** from **SPH-4.12** via an acid modified Pd/C hydrogenation. This macrocycle proved to be water soluble, as 36 mg was readily dissolved in 0.500 mL of water.

Future work will entail testing of the macrocycles for ligand or metal binding. It would also be interesting to test **SPH-4.13** for the ability to create a hydrophobic core via NMR experiments; however, the multiple rotatable bonds in the connecting chains could create too much disorder for this to occur. Further work for the alkylation chemistry would be the synthesis of bridged macrocycles through a dialkylation. Finally, work on shortening the connector by attaching through a glycine, or even through enhanced proline derivatives could enable some unique structures for metal binding or catalysis. This would naturally require remodeling of the compounds to ensure proper orientation of the functional groups on each spiroligomer residue.

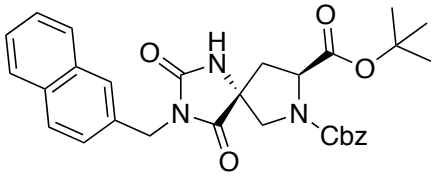
## 4.7 Experimental Details

**General Method 1: Mono alkylation of P4srZBHyd**

To a stirred mixture of **4.1** in DMF [100 mM] was added 0.75-0.85 equiv of alkyl, allyl, or benzyl halide (equiv dependent on salt content of the specific batch of 4-hydantoin-Z-Pro-OtBu, which can be determined utilizing an internal standard) along with 1.5 equiv of $K_2CO_3$. The reaction proceeded at room temperature for 2-24 h, and the progress checked via LCMS. The reaction was diluted with four times the reaction volume of EtOAc and washed with water, saturated ammonium chloride solution, and brine. The organic layer was dried with $Na_2SO_4$, and concentrated *in vacuo* to yield **4.22-4.25** as foamy off-white to yellow solids.

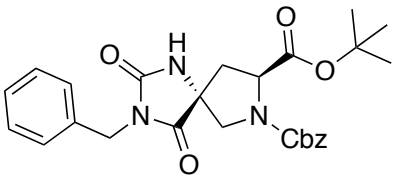
**Compound 4.22 - 7-benzyl 8-(*tert*-butyl) (5*R*,8*S*)-3-(naphthalen-2-ylmethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**

General procedure 1 was utilized, with 7.5 mmol of **4.1** (2S, 4R) and 6 mmol of 2-(bromomethyl)naphthalene (0.80 equiv). Recovered yield was quantitative and the product used without purification



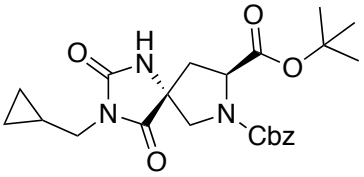

$^1$H NMR (500 MHz, $CDCl_3$) 1.42 (9H, s, rotameric), 2.36 (1H, m), 2.48 (1H, m), 3.81 (2H, m), 4.45 (1H, m), 4.78 (1H, s, rotameric), 5.07 (2H, m), 7.25-7.84 (13H), 9.08 (1H, s, rotameric); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 27.8, 34.1, 39.6, 40.6, 42.8, 55.7, 58.4, 65.9, 66.8, 67.6, 82.4, 125.8, 126.2, 126.3, 126.4, 126.6, 126.8, 127.6, 127.8, 127.9, 128.0, 128.1, 128.2, 128.5, 128.6, 128.7, 132.9, 133.0, 133.2, 135.1, 135.8, 136.0, 154, 7, 156.3, 169.8, 172.9; HRMS-ESI: m/z calcd for $C_{30}H_{31}N_3O_6K$ $(M+K)^+$ 568.1844, found 568.1840

**Compound 4.23 - 7-benzyl 8-(*tert*-butyl) (5*R*,8*S*)-3-benzyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate** General procedure 1 was utilized, with 15 mmol of **4.1** (2S, 4R) and 12 mmol of benzyl bromide (0.80 equiv). Recovered yield was quantitative and the product used without purification




$^1$H NMR (500 MHz, $CDCl_3$) 1.43 (9H, s, rotameric), 2.31 (1H, dd, rotameric, J = 14.5, 8.1), 2.49 (1H, ddd, J = 18.0, 13.4, 9.2), 3.84 (2H, m), 4.43 (1H, dt, J = 24.4, 8.6), 4.63 (2H, s), 5.08 (2H, m), 7.30 (11H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 27.9, 39.7, 40.7, 42.5, 55.7, 58.9, 65.5, 66.2, 67.6, 82.4, 127.7, 127.9, 128.1, 128.2, 128.5, 128.6, 128.8, 135.7, 136.0, 154.5, 155.9, 169.7, 171.7; HRMS-ESI: m/z calcd for $C_{26}H_{29}N_3O_6K$ $(M+K)^+$ 518.1688, found 518.1699

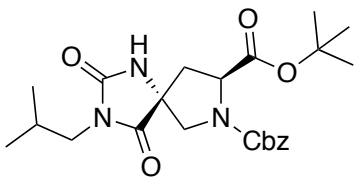
**Compound 4.24 - 7-benzyl 8-(*tert*-butyl) (5*R*,8*S*)-3-(cyclopropylmethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate** General procedure 1 was utilized, with 7.5 mmol of **4.1** (2S, 4R) and 6 mmol of (bromomethyl)cyclopropane (0.80 equiv). Recovered yield was quantitative and the product used without purification




$^1$H NMR (500 MHz, $CDCl_3$) 0.33 (2H, d, J = 5.8), 0.49 (2H, d, J = 7.8), 1.13 (1H, m), 1.45 (9H, s, rotameric), 2.40 (1H, dd, rotameric, J = 14.3, 8.2), 2.54 (1H, ddd, J = 18.2, 13.4, 9.0), 3.35 (2H, dd, J = 7.2, 3.5), 3.81 (2H, m), 4.47 (1H, dt, J = 24.8, 8.3), 5.11 (2H, m), 7.32 (6H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 3.7, 10.2, 27.9, 40.7, 43.7, 55.8, 58.9, 66.1, 67.6, 82.4, 127.9, 128.2, 128.5, 136.0, 154.5, 156.8, 169.7, 172.2; HRMS-ESI: m/z calcd for $C_{23}H_{29}N_3O_6K$ $(M+K)^+$ 482.1688, found 482.1693

**Compound 4.25 - 7-benzyl 8-(*tert*-butyl) (5*R*,8*S*)-3-isobutyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate** – General procedure 1 was utilized, with 15

mmol of **4.1** (2S, 4R) and 18 mmol of 1-iodo-2-methylpropane (1.2 equiv). Recovered yield was quantitative and the product used without purification

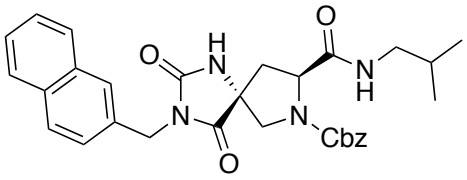



$^{1}$H NMR (500 MHz, $CDCl_3$) 0.88 (6H, d, $J$ = 6.7), 1.45 (9H, s, rotameric), 2.05 (1H, m), 2.38 (1H, m), 2.53 (1H, ddd, $J$ = 18.2, 13.3, 9.2), 3.31 (2H, dd, $J$ = 7.3, 3.1), 3.78 (2H, m), 4.48 (1H, dt, $J$ = 24.4, 8.6), 5.11 (2H, m), 7.35 (6H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 20.9, 27.8, 27.9, 39.8, 40.8, 46.1, 55.8, 56.0, 58.4, 58.9, 65.2, 65.9, 67.6, 82.4, 127.8, 127.9, 128.2, 128.5, 135.9, 136.0, 154.2, 154.5, 156.6, 156.8, 169.7, 169.9, 172.2, 172.4; HRMS-ESI: m/z calcd for $C_{23}H_{31}N_3O_6K$ $(M+K)^+$ 484.1844, found 484.1861

**General Method 2: Deprotection of C2 t-Bu group and coupling**

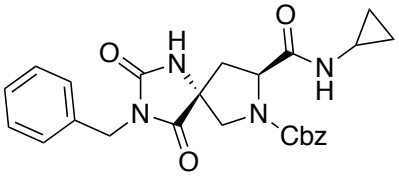
Each of **4.22-4.25** was placed in a RB flask and then treated with 95% TFA / TIPS for 1 h, with the reaction progress checked via LCMS. Upon successful deprotection of the *tert*-butyl group, the solvent was removed *in vacuo*, and placed on a high vacuum pump overnight. To an inert atmosphere RB flask containing the free acid in dry DMF/DCM (1:1 ratio, [100 mM]) was added 1.5 equiv of 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 3 equiv of 1-Hydroxy-7-azabenzotriazole (HOAT), then stirred for 1 h, at which time 3 equiv of an amine and 2 equiv of DIPEA**,** were added, and the reaction proceeded for 2 h, at which point the progress was checked via LCMS. Upon completion, the reaction was diluted with four times the reaction volume of EtOAc and washed with saturated ammonium chloride solution, saturated sodium bicarbonate solution, and brine. The organic layer was then dried with $Na_2SO_4$, and concentrated *in vacuo* to yield **4.30-4.35** as foamy yellow solids.

**Compound 4.30 benzyl (5*R*,8*S*)-8-(isobutylcarbamoyl)-3-(naphthalen-2-ylmethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7-carboxylate** – General Procedure 4.2 was used on compound **4.22** with 22.5 mmol HOAt, 11.25 mmol EDC, 22.5 mmol Isobutylamine, 15 mmol DIPEA, and 75 mL of 1:1 DMF/DCM - Recovered yield was quantitative and the product used without purification




$^1$H NMR (500 MHz, $CDCl_3$) 0.82 (6H, d, $J$ = 6.4), 1.89 (1H, m), 2.25 (1H, m), 2.64 (1H, m), 2.99 (1H, m), 3.05 (1H, sep, $J$ = 6.5), 3.68 (1H, m), 3.84 (1H, d, $J$ = 11.3), 4.59 (1H, m), 4.78 (2H, s, rotameric), 5.05 (2H, m), 7.02 (1H, m), 7.21 (5H, m), 7.44 (3H, m), 7.66 (1H, m), 7.78 (4H, m); $^{13}$C NMR (125 MHz, DMSO-$d_6$, rotamers present) 20.0, 28.0, 28.1, 40.6, 41.5, 41.7, 46.0, 56.1, 56.5, 58.7, , 59.2, 65.1, 65.8, 66.2, 125.4, 125.5, 125.7, 125.8, 126.0, 126.3, 127.1, 127.5, 127.6, 127.7, 127.8, 128.2, 128.3, 132.2, 132.8, 134.0, 136.5, 136.6, 153.5, 152.7, 155.4, 155.5, 170.4, 170.7, 172.4; HRMS-ESI: m/z calcd for $C_{30}H_{32}N_4O_5H$ $(M+H)^+$ 529.2445, found 529.2446

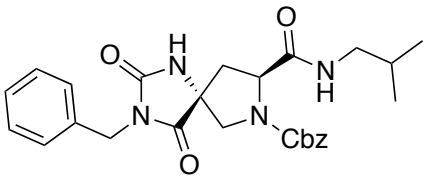
**Compound 4.31 - benzyl (5*R*,8*S*)-3-benzyl-8-(cyclopropylcarbamoyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7-carboxylate** - General Procedure 4.2 was used on compound **4.23**, with 22.5 mmol HOAt, 11.25 mmol EDC, 22.5 mmol cyclopropylamine, 15 mmol DIPEA, and 75 mL of 1:1 DMF/DCM. Recovered yield was quantitative and the product used without purification




$^1$H NMR (500 MHz, $CDCl_3$) 0.30 (1H, m), 0.41 (1H, M), 0.58 (2H, dd, rotameric, $J$ = 23.2, 7.1), 2.21 (1H, m), 2.33 (1H, m), 2.60 (1H, m), 3.64 (1H, dd, rotameric, $J$ = 15.5, 11.4), 3.81 (1H, d, $J$ = 11.3), 4.30 (1H, m), 4.55 (2H, d, $J$ = 12.5), 5.05 (2H, m), 7.30 (10H, m), 8.20 (1H, m), 9.16 (1H, m); $^{13}$C NMR (125 MHz, DMSO-$d_6$, rotamers present) 5.4, 5.5, 5.6, 5.7, 22.3, 40.4, 41.3, 41.4, 56.0, 56.4, 58.6, 59.0, 65.1, 65.7, 66.2, 127.1, 127.2,

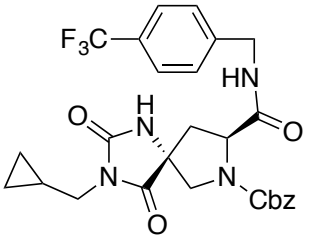
127.4, 127.5, 127.7, 127.8, 128.2, 128.4, 128.5, 128.6, 136.5, 136.6, 153.4, 153.6, 155.3, 155.4, 171.5, 171.8, 172.3; HRMS-ESI: m/z calcd for $C_{25}H_{26}N_4O_5Na$ $(M+Na)^+$ 485.1795, found 485.1790

**Compound 4.32 - benzyl (5*R*,8*S*)-3-benzyl-8-(isobutylcarbamoyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7-carboxylate** - General Procedure 4.2 was used on compound **4.23**, with 22.5 mmol HOAt, 11.25 mmol EDC, 22.5 mmol Isobutylamine, 15 mmol DIPEA, and 75 mL of 1:1 DMF/DCM. Recovered yield was quantitative and the product used without purification




$^1$H NMR (500 MHz, $CDCl_3$) 0.76 (6H, d, rotameric, *J* = 6.7) 1.62 (1H, sep, rotameric, *J* = 6.7), 2.22 (1H, dt, *J* = 12.8, 10.0), 2.38 (1H, m), 2.87 (2H, m), 3.66 (1H, dd, *J* = 17.0, 11.5), 3.84 (1H, d, rotameric, *J* = 11.5), 4.43 (1H, dd, rotameric, *J* = 10.0, 7.5), 4.56 (2H, d, *J* = 11.9), 5.05 (1H, d, *J* = 7.6), 5.07 (1H, d, *J* = 5.2), 7.30 (10H, m), 8.14 (1H, t, rotameric, *J* = 5.9), 9.22 (1H, s, rotameric); $^{13}$C NMR (125 MHz, DMSO-$d_6$, rotamers present) 19.42, 27.4, 27.5, 40.0, 40.9, 45.4, 55.5, 55.9, 58.1, 58.6, 64.5, 65.1, 65.6, 126.5, 126.6, 126.8, 126.9, 127.0, 127.2, 127.6, 127.8, 128.0, 135.9, 136.0, 152.9, 153.1, 154.8, 154.9, 169.8, 170.1, 171.8; HRMS-ESI: m/z calcd for $C_{26}H_{30}N_4O_5Na$ $(M+Na)^+$ 501.2108, found 501.2110

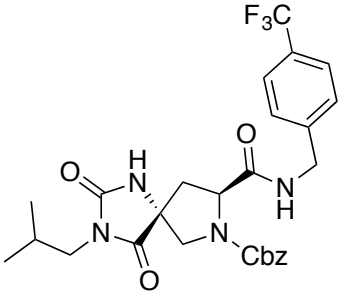
**Compound 4.33 - benzyl (5*R*,8*S*)-3-(cyclopropylmethyl)-2,4-dioxo-8-((4-(trifluoromethyl)benzyl)carbamoyl)-1,3,7-triazaspiro[4.4]nonane-7-carboxylate**
General Procedure 4.2 was used on compound **4.24**, with 22.5 mmol HOAt, 11.25 mmol EDC, 22.5 mmol 4-(trifluoromethyl)benzylamine, 15 mmol DIPEA, and 75 mL of 1:1 DMF/DCM. Recovered yield was quantitative and the product used without purification



$^1$H NMR (500 MHz, $CDCl_3$) 0.25 (2H, m), 0.43 (2H, m), 1.04 (1H, m), 2.26 (1H, ddd, *J* = 13.0, 10.0, 7.2), 2.38 (1H, m), 3.23 (2H, dd, *J* = 11.0, 7.3), 3.67 (1H, d, rotameric, *J* = 11.2), 4.33 (1H, dd, *J* = 12.5, 5.8), 4.40 (1H, t, *J* = 6.1), 4.50 (1H, dd, rotameric, *J* = 9.9, 7.5), 5.07 (2H, m), 7.34 (6H, m), 7.50 (2H, m), 7.65 (1H, d, *J* = 7.9), 8.83 (1H, m), 9.10 (1H, s, rotameric); $^{13}$C NMR (125 MHz, DMSO-$d_6$, rotamers present) 3.4, 9.9, 40.3, 41.3, 41.7, 42.5, 56.1, 56.4, 58.9, 59.4, 64.9, 65.5, 66.3, 123.2, 125.0, 127.0, 127.3, 127.5, 127.6, 127.8, 128.2, 128.3, 136.5, 144.0, 153.7, 155.6, 155.7, 170.9, 171.1, 172.3; HRMS-ESI: m/z calcd for $C_{27}H_{27}F_3N_4O_5Na$ $(M+Na)^+$ 567.1826, found 567.1827

**Compound 4.34 - benzyl (5*R*,8*S*)-3-isobutyl-2,4-dioxo-8-((4-(trifluoromethyl)benzyl)carbamoyl)-1,3,7-triazaspiro[4.4]nonane-7-carboxylate**

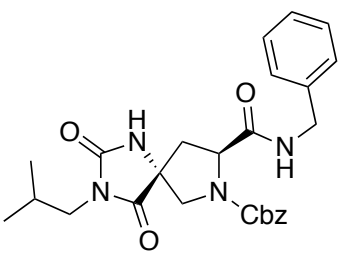
General Procedure 4.2 was used on compound **4.25**, with 22.5 mmol HOAt, 11.25 mmol EDC, 22.5 mmol 4-(trifluoromethyl)benzylamine, 15 mmol DIPEA, and 75 mL of 1:1 DMF/DCM. Recovered yield was quantitative and the product used without purification



$^1$H NMR (500 MHz, $CDCl_3$) 0.83 (6H, d, *J* = 6.8), 1.94 (1H, m), 2.26 (1H, ddd, *J* = 13.0, 10.1, 8.1), 2.41 (1H, ddd, rotameric, *J* = 20.6, 13.1, 7.3), 3.18 (2H, dd, *J* = 10.2, 7.5), 3.65 (1H, d, rotameric, *J* = 11.4), 3.82 (1H, d, rotameric, *J* = 11.2), 4.33 (1H, dd, *J* = 10.8, 6.0), 4.40 (1H, t, *J* = 5.6), 4.48 (1H, dd, rotameric, *J* = 9.9, 7.5), 5.07 (2H, M), 7.34 (6H, M), 7.51 (2H, M), 7.66 (1H, d, rotameric, *J* = 8.2), 8.83 (1H, m), 9.10 (1H, s, rotameric); $^{13}$C NMR (125 MHz, DMSO-$d_6$, rotamers present) 19.8, 26.9, 40.4, 41.4, 41.7, 41.8, 45.3, 56.5, 58.9, 59.4, 64.8, 65.4, 66.3, 123.2, 123.3, 125.0, 125.1, 125.4, 127.0, 127.3, 127.6, 127.7, 127.9, 128.3, 128.4, 136.5,

136.6, 144.1, 144.3, 153.7, 153.8, 155.7, 155.8, 171.0, 171.2, 172.6; HRMS-ESI: m/z calcd for $C_{27}H_{29}F_3N_4O_5Na$ $(M+Na)^+$ 569.1982, found 569.1986

**Compound 4.35 - benzyl (5*R*,8*S*)-8-(benzylcarbamoyl)-3-isobutyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7-carboxylate** - General Procedure 4.2 was used on compound **4.25**, with 22.5 mmol HOAt, 11.25 mmol EDC, 22.5 mmol benzylamine, 15 mmol DIPEA, and 75 mL of 1:1 DMF/DCM. Recovered yield was quantitative and the product used without purification

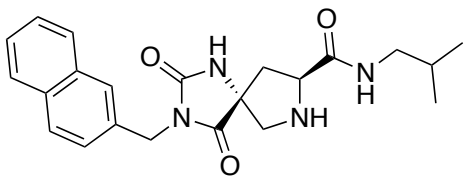



$^1$H NMR (500 MHz, DMSO-$d_6$) 0.82 (6H, dd, $J$ = 6.7, 4.6), 1.94 (1H, m), 2.24 (1H, m), 2.39 (1H, m), 3.18 (2H, dd, $J$ = 10.4, 7.3), 3.66 (1H, m), 3.81 (1H, m), 4.27 (2H, m), 4.46 (1H, ddd, $J$ = 17.5, 8.9, 7.5), 5.07 (2H, m), 7.20 (10H, m), 8.72 (1H, m), 9.10 (1H, s, rotameric); $^{13}$C NMR (125 MHz, DMSO-$d_6$, rotamers present) 19.9, 26.9, 40.5, 41.4, 42.0, 42.1, 45.3, 56.5, 58.9, 59.3, 64.8, 65.4, 66.3, 126.7, 127.0, 127.1, 127.6, 127.7, 127.9, 128.2, 128.3, 128.4, 136.6, 139.2, 153.6, 153.7, 155.7, 155.8, 170.7, 170.9, 172.6; HRMS-ESI: m/z calcd for $C_{26}H_{30}N_4O_5Na$ $(M+Na)^+$ 501.2108, found 501.2113

**General Method 3: Deprotection of Proline Cbz group**

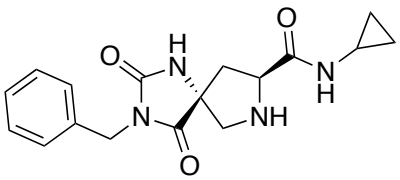
Each of **4.30-4.35** was added to a RB flask and reacted with 1:1 DCM/(33% HBr in AcOH) for 30 min, and the reaction progress checked via LCMS. Upon successful deprotection of the Cbz group, the solvent was removed *in vacuo* with the aid of toluene, and placed on a high vacuum pump overnight to afford **4.36-4.41**

**Compound 4.36 - (5*R*,8*S*)-*N*-isobutyl-3-(naphthalen-2-ylmethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxamide**



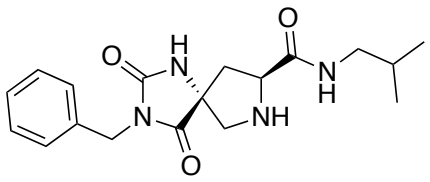
$^1$H NMR (500 MHz, DMSO-$d_6$) 08.4 (6H, d, $J$ = 6.7), 1.72 (1H, m), 2.23 (1H, dd, $J$ = 13.4, 11.9), 2.69 (1H, dd, $J$ = 13.4, 6.7), 2.93 (1H, m), 3.02 (1H, m), 3.53 (1H, d, $J$ = 12.8), 3.67 (1H, d, $J$ = 12.8), 4.53 (1H, dd, $J$ = 11.6, 6.7), 4.72 (2H, m), 7.41 (2H, m), 7.50 (1H, m), 7.76 (1H, m), 7.90 (3H, m), 8.66 (1H, m), 9.01 (1H, s), 9.31 (1H, s), 9.91 (1H, s); $^{13}$C NMR (125 MHz, DMSO-$d_6$, rotamers present) 20.0, 27.9, 34.6, 40.7, 42.0, 46.4, 48.6, 52.5, 55.0, 59.3, 65.7, 125.6, 125.9, 126.1, 126.4, 127.6, 127.7, 128.3, 128.7, 129.3, 132.3, 132.8, 133.8, 155.2, 165.9, 172.7; HRMS-ESI: m/z calcd for $C_{22}H_{26}N_4O_3H$ $(M+H)^+$ 395.2078, found 395.2084

**Compound 4.37 (5*R*,8*S*)-3-benzyl-*N*-cyclopropyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxamide**



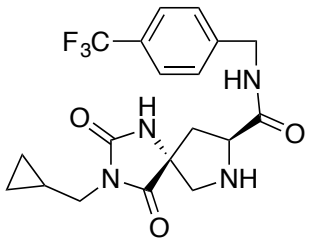
$^1$H NMR (500 MHz, DMSO-$d_6$) 0.47 (2H, m), 0.66 (2H, m), 2.21 (1H, dd, $J$ = 13.4, 11.6), 2.56 (1H, dd, $J$ = 13.3, 6.9), 2.7 (1H, tq, $J$ = 7.3, 3.9) 3.49 (1H, d, $J$ = 13.1), 3.62 (1H, d, $J$ = 12.8), 4.42 (1H, dd, $J$ = 11.6, 6.7), 4.55 (2H, s), 7.28 (5H, m), 8.73 (1H, d, $J$ = 4.3), 8.96 (1H, s), 9.30 (1H, s), 9.88 (1H, s); $^{13}$C NMR (125 MHz, DMSO-$d_6$,) 5.6, 21.1, 22.6, 40.5, 41.7, 48.6, 52.5, 59.2, 65.5, 127.3, 127.5, 128.6, 136.2, 150.1, 166.9, 172.6; HRMS-ESI: m/z calcd for $C_{17}H_{20}N_4O_3H$ $(M+H)^+$ 329.1608, found 329.1602

**Compound 4.38 (5*R*,8*S*)-3-benzyl-*N*-isobutyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxamide**



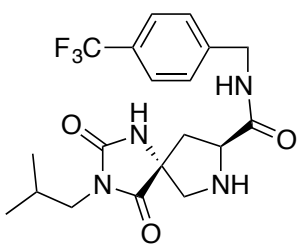
$^1$H NMR (500 MHz, DMSO-$d_6$) 0.86 (6H, d, $J$ = 7.6), 1.74 (1H, m), 2.22 (1H, dd, $J$ = 13.4, 11.6), 2.69 (1H, dd, $J$ = 13.4, 7.0), 2.95 (1H, m), 3.03 (1H, m), 3.54 (1H, d, $J$ = 12.8), 3.66 (1H, d, $J$ = 13.1), 4.57 (2H, s), 4.58 (1H, dd, obscured, $J$ = 11.7, 6.7), 7.31 (5H, m), 8.70 (1H, t, $J$ = 5.8), 9.01 (1H, s), 9.29 (1H, s), 9.96 (1H, s); $^{13}$C NMR (125 MHz, DMSO-$d_6$) 20.0, 20.1, 27.9, 35.6, 40.7, 41.7, 46.4, 48.6, 52.4, 59.3, 65.6, 127.3, 127.5, 128.3, 128.6, 136.2, 155.1, 165.9, 172.7; HRMS-ESI: m/z calcd for $C_{18}H_{24}N_4O_3H$ $(M+H)^+$ 345.1921, found 345.1920

**Compound 4.39 - (5*R*,8*S*)-3-(cyclopropylmethyl)-2,4-dioxo-*N*-(4-(trifluoromethyl)benzyl)-1,3,7-triazaspiro[4.4]nonane-8-carboxamide**



$^1$H NMR (500 MHz, DMSO-$d_6$) 0.24 (2H, m), 0.43 (2H, m), 1.02 (1H, m), 2.27 (1H, dd, $J$ = 13.4, 11.6), 2.67 (1H, dd, $J$ = 13.3, 6.9), 3.22 (2H, d, $J$ = 7.0), 3.49 (1H, d, $J$ = 13.1), 3.62 (1H, d, $J$ = 13.1), 4.46 (2H, m), 4.65 (1H, dd, $J$ = 11.4, 6.9), 7.51 (2H, d, $J$ = 8.2), 7.69 (2H, d, $J$ = 7.9), 8.89 (1H, s), 9.36 (1H, t, $J$ = 6.0), 9.46 (1H, s), 9.80 (1H, s); $^{13}$C NMR (125 MHz, DMSO-$d_6$) 3.5, 9.8, 40.4, 42.1, 42.8, 48.6, 52.5, 59.4, 65.4, 125.3, 125.4, 127.7, 127.9, 128.1, 143.3, 155.4, 166.3, 172.7; HRMS-ESI: m/z calcd for $C_{19}H_{21}F_3N_4O_3H$ $(M+H)^+$ 411.1639, found 411.1638

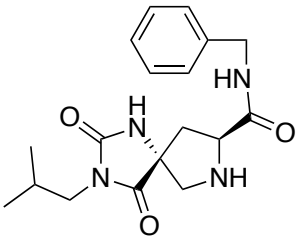
**Compound 4.40 - (5*R*,8*S*)-3-isobutyl-2,4-dioxo-*N*-(4-(trifluoromethyl)benzyl)-1,3,7-triazaspiro[4.4]nonane-8-carboxamide**



$^1$H NMR (500 MHz, $CDCl_3$) 0.77 (6H, d, $J$ = 6.4), 1.88 (1H, m), 2.49 (1H, m), 2.83, 1H, m), 3.16 (2H, m), 3.29 (1H, m), 3.91 (2H, m), 4.36 (1H, d, $J$ = 11.0), 4.56 (1H, d, $J$ = 9.2), 5.36 (1H, m), 7.35 (2H, d, $J$ = 7.2), 7.47 (2H, d, $J$ = 7.6), 8.31 (1H, m),

8.68 (1H, m), 9.45 (1H, m); $^{13}$C NMR (125 MHz, $CDCl_3$) 19.8, 27.3, 33.6, 43.4, 46.5, 60.2, 65.9, 122.9, 125.0, 125.5, 127.7, 127.9, 128.8, 129.0, 129.6, 129.8, 141.2, 156.3, 167.3, 172.2; HRMS-ESI: m/z calcd for $C_{18}H_{24}N_4O_3H$ $(M+H)^+$ 413.1795, found 413.1794

**Compound 4.41 (5*R*,8*S*)-*N*-benzyl-3-isobutyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxamide**



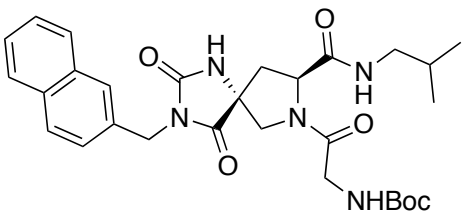
$^1$H NMR (500 MHz, DMSO-$d_6$) 0.82 (6H, d, $J$ = 6.7), 1.92 (1H, m), 2.24 (1H, dd, $J$ = 13.3, 11.7), 2.65 (1H, dd, $J$ = 13.3, 6.9), 3.17 (2H, d, $J$ = 7.3), 3.49 (1H, d, $J$ = 13.1), 3.61 (1H, d, $J$ = 12.8), 4.36 (2H, dd, $J$ = 5.8, 3.1), 4.60 (1H, dd, $J$ = 11.6, 7.0), 7.29 (6H, m), 8.87 (1H, s), 9.21 (1H, t, $J$ = 5.8); $^{13}$C NMR (125 MHz, DMSO-$d_6$) 19.8, 26.9, 34.6, 40.5, 42.5, 45.5, 52.5, 59.3, 65.3, 127.1, 127.3, 128.4, 128.7, 129.3, 138.3, 155.4, 166.0, 172.9; HRMS-ESI: m/z calcd for $C_{18}H_{24}N_4O_3H$ $(M+H)^+$ 345.1921, found 345.1921

**General Method 4.4: Coupling of Boc-Gly-OH to 4.36-4.41**

To an inert atmosphere RB flask containing 2 equiv of Boc-Gly-OH in dry DMF/DCM (1:1 ratio, [200 mM]) was added 2 equiv of 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 4 equiv of 1-Hydroxy-7-azabenzotriazole (HOAT) and stirred for 1 h. Afterwards, one of **4.36-4.41** in DMF [200 mM] and 4 equiv of diisopropylethylamine were added and the reaction stirred for another 2 h, at which point the progress was checked via LCMS. Upon completion, the reaction was diluted with four times the reaction volume of EtOAc and washed with saturated ammonium chloride solution, saturated sodium bicarbonate solution, and brine. The organic layer was then dried with $Na_2SO_4$, and concentrated *in vacuo* to yield **4.42-4.47** as foamy dark-yellow

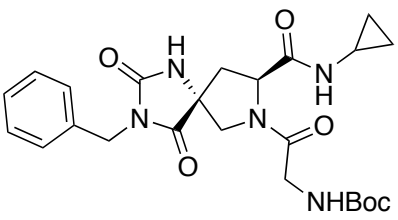
solids. These compounds were then purified by normal phase flash chromatography (0-5% MeOH in DCM) to yield off-white foamy solids.

**Compound 4.42 - *tert*-butyl (2-((5*R*,8*S*)-8-(isobutylcarbamoyl)-3-(naphthalen-2-ylmethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate** - General procedure 4.4 was used with 15 mmol Boc-Gly-OH, 30 mmol HOAt, 15 mmol EDC, 30 mmol DIPEA, compound **4.36**. Purified yield 3.23 g (78%)




$^1$H NMR (500 MHz, $CDCl_3$) 0.80 (6H, m), 1.21 (9H, s, rotameric), 1.65 (1H, m), 2.24 (1H, dd, *J* = 12.8, 7.6), 2.47 (2H, m), 3.24 (1H, m), 3.73 (1H, dd, *J* = 17.1, 4.6), 3.95 (1H, m), 4.08 (1H, dd, *J* = 10.4), 4.43 (1H, m), 4.69 (1H, d, *J* = 14.6), 4.79 (1H, m), 4.82 (1H, d, *J* = 14.6), 5.77 (1H, m), 7.32 (1H, m), 7.46 (3H, m), 7.76 (4H, m), 8.18 (1H, s); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 20.0, 20.1, 25.3, 28.1, 28.3, 28.4, 40.9, 43.0, 46.7, 54.5, 59.7, 64.3, 66.5, 79.6, 126.4, 126.5, 127.7, 127.8, 128.1, 128.6, 132.9, 133.0, 133.1, 155.5, 156.5, 169.6, 171.5; HRMS-ESI: m/z calcd for $C_{29}H_{37}N_5O_6Na$ $(M+Na)^+$ 574.2636, found 574.2633

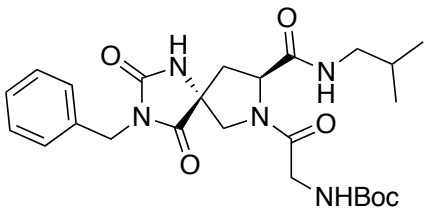
**Compound 4.43 - *tert*-butyl (2-((5*R*,8*S*)-3-benzyl-8-(cyclopropylcarbamoyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate** - General procedure 4.4 was used with 15 mmol Boc-Gly-OH, 30 mmol HOAt, 15 mmol EDC, 30 mmol DIPEA, compound **4.37** - Purified yield 2.94 g (81%)




$^1$H NMR (500 MHz, $CDCl_3$, rotamers present) 0.44 (1H, m), 0.52 (2H, m), 0.67 (1H, m), 1.31 (9H, s, rotameric), 2.17 (1H, m), 2.43 (1H, t, *J* = 11.7), 2.55 (1H, m), 3.76 (1H, dd, *J* = 17.1, 5.5), 3.84 (1H, d, *J* = 10.4), 4.06 (1H, d, *J* = 10.4), 4.22 (1H, m), 4.58 (1H, d, *J* = 14.6), 4.65 (1H, d, *J* = 14.6), 4.67 (1H, m), 5.80

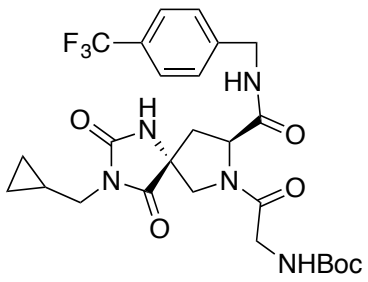
(1H, s), 7.30 (5H, m), 7.45 (1H, m), 8.21 (1H, s); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 5.9, 6.7, 22.6, 28.2, 40.3, 42.7, 43.2, 54.9, 59.7, 66.4, 79.8, 128.1, 128.6, 128.8, 135.7, 155.6, 156.6, 171.1, 171.5; HRMS-ESI: m/z calcd for $C_{24}H_{31}N_5O_6$ Na $(M+Na)^+$ 508.2167, found 508.2165

**Compound 4.44 - *tert*-butyl (2-((5*R*,8*S*)-3-benzyl-8-(isobutylcarbamoyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate** - General procedure 4.4 was used with 15 mmol Boc-Gly-OH, 30 mmol HOAt, 15 mmol EDC, 30 mmol DIPEA, compound **4.38**. Purified yield 2.87 g (78%)



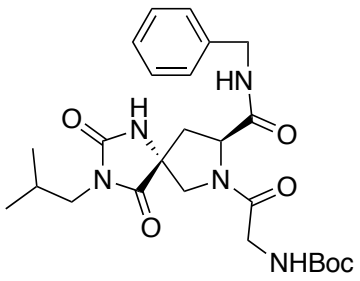

$^1$H NMR (500 MHz, $CDCl_3$) 0.85 (6H, m), 1.29 (9H, s, rotameric), 1.70 (1H, m), 2.22 (1H, dd, $J$ = 12.5, 7.6), 2.45 (1H, t, $J$ = 11.9), 2.47 (1H, m), 3.27 (1H, m), 3.75 (1H, dd, $J$ = 17.1, 4.6), 3.99 (1H, m), 4.08 (1H, d, $J$ = 9.8), 4.49 (1H, m), 4.55 (1H, d, $J$ = 14.6), 4.67 (1H, d, $J$ = 14.6), 4.82 (1H, dd, $J$ = 10.1, 8.2), 5.79 (1H, m), 7.31 (6H, m), 8.17 (1H, s); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 20.1, 20.2, 25.3, 28.2, 28.3, 28.4, 41.0, 42.8, 43.1, 46.7, 54.4, 59.7, 60.4, 66.5, 79.6, 128.2, 128.7, 128.8, 135.7, 155.5, 156.5, 169.5, 171.4; HRMS-ESI: m/z calcd for $C_{25}H_{35}N_5O_6$ Na $(M+Na)^+$ 524.2480, found 524.2487

**Compound 4.45 - *tert*-butyl (2-((5*R*,8*S*)-3-benzyl-8-(isobutylcarbamoyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate** - General procedure 4.4 was used with 15 mmol Boc-Gly-OH, 30 mmol HOAt, 15 mmol EDC, 30 mmol DIPEA, compound **4.39 –** Purified yield 3.64 g (86% Yield)



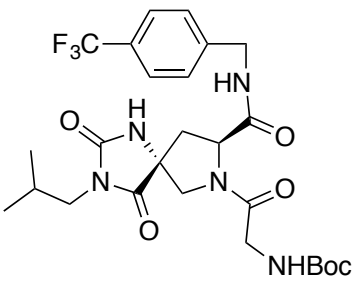
$^1$H NMR (500 MHz, $CDCl_3$) 0.25 (2H, d, $J$ = 4.6), 0.44 (2H, m), 1.04 (1H, m), 1.33 (9H, s, rotameric), 2.07 (1H, m), 2.45 (1H, t, $J$ = 11.9), 3.22 (1H, dd, $J$ = 14.0, 7.3), 3.33 (1H, dd, $J$ = 14.0, 7.0), 3.83 (1H, dd, $J$ = 17.1, 5.2), 3.92 (1H, m), 4.11 (1H, m), 4.34 (2H, m), 4.62 (1H, dd, $J$ = 15.4, 6.6), 4.89 (1H, m), 5.82 (1H, s), 7.42 (2H, d, $J$ = 8.2), 7.54 (2H, d, $J$, = 8.2), 8.08 (1H, s), 8.30 (1H, s); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 3.6, 4.1, 10.1, 21.1, 25.3, 28.2, 42.9, 43.2, 43.8, 54.8, 60.1, 60.4, 66.3, 79.9, 123.0, 125.2, 125.4, 125.5, 127.6, 129.4, 129.7, 142.2, 156.1, 156.6, 170.3, 171.5; HRMS-ESI: m/z calcd for $C_{26}H_{32}F_3N_5O_6$ Na $(M+Na)^+$ 590.2197, found 590.2196

**Compound 4.46 - *tert*-butyl (2-((5*R*,8*S*)-3-isobutyl-2,4-dioxo-8-((4-(trifluoromethyl)benzyl)carbamoyl)-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate** - General procedure 4.4 was used with 30 mmol Boc-Gly-OH, 60 mmol HOAt, 30 mmol EDC, 60 mmol DIPEA, compound **4.40** - Purified yield 6.82 g (91%)



$^1$H NMR (500 MHz, $CDCl_3$) 0.82 (6H, d, rotameric, $J$ = 6.7), 1.33 (9H, s, rotameric), 1.96 (1H, m), 2.02 (1H, m), 2.44 (1H, t, $J$ = 11.9), 3.12 (1H, dd, $J$ = 13.1, 8.2), 3.29 (1H, dd, $J$ = 13.4, 6.7), 3.78 (1H, dd, $J$ = 17.1, 5.2), 3.92 (1H, m), 4.07 (1H, m), 4.17 (1H, m), 4.56 (1H, dd, $J$ = 15.1, 7.2), 4.86 (1H, m), 5.81 (1H, s), 7.26 (5H, m), 7.85 (1H, m), 8.36 (1H, s); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 19.7, 19.8, 25.3, 27.1, 28.2, 40.7, 43.1, 43.3, 45.8, 54.5, 59.9, 66.1, 79.7, 127.3, 127.3, 128.5, 138.0, 156.0, 156.5, 169.9, 170.7, 171.7; HRMS-ESI: m/z calcd for $C_{25}H_{35}N_5O_6$ Na $(M+Na)^+$ 524.2480, found 524.2487

**Compound 4.47 - *tert*-butyl (2-((5*R*,8*S*)-8-(benzylcarbamoyl)-3-isobutyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate** - General procedure 4.4 was used with 15 mmol Boc-Gly-OH, 30 mmol HOAt, 15 mmol EDC, 30 mmol DIPEA, compound **4.41** – Purified Yield 3.50 (82%)

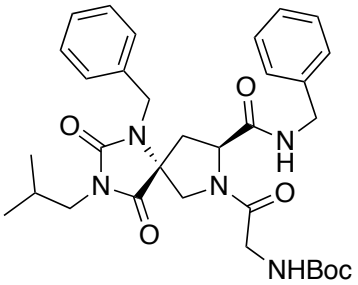



$^1$H NMR (500 MHz, $CDCl_3$) 0.84 (6H, d, rotameric, *J* = 6.7), 1.32 (9H, s), 2.00 (2H, m), 2.16 (1H, s), 2.46 (1H, t, *J* = 11.9), 3.17 (1H, dd, *J* = 13.1, 7.9), 3.31 (1H, dd, *J* = 13.4, 7.0), 3.81 (1H, dd, *J* = 16.9, 5.2), 4.08 (1H, d, *J* = 10.7), 4.32 (2H, m), 4.57 (1H, dd, *J* = 15.6, 6.4), 4.88 (1H, m), 5.72 (1H, s), 7.4 (2H, d, *J* = 8.2), 7.54 (2H, d, *J* = 8.2), 7.99 (1H, m), 8.30 (1H, s); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 19.7, 19.8, 27.2, 28.2, 31.0, 40.3, 42.9, 43.3, 46.0, 54.9, 60.1, 66.1, 80.0, 123.0, 125.1, 125.4, 125.5, 127.5, 129.5, 129.7, 142.2, 156.1, 156.6, 170.4, 171.5; HRMS-ESI: m/z calcd for $C_{26}H_{34}F_3N_5O_6$ Na $(M+Na)^+$ 592.2353, found 592.2354

**General Method 45: Alkylation of 4.42-4.47 to Synthesize 4.2-4.15**

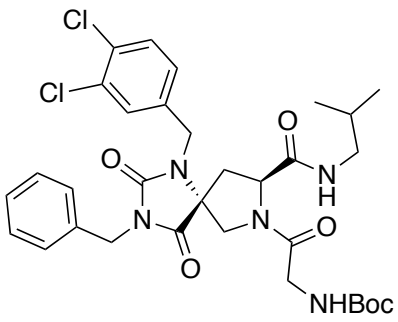
To a stirred mixture of one of **4.42-4.47** in DMF [100 mM] was added 1.05 equiv of allyl or benzyl halide along with 1.5 equiv of $K_2CO_3$. The reaction proceeded at room temperature for 14-24 h, and the progress checked via LCMS. The reaction was diluted with four times the reaction volume of EtOAc and washed with water, saturated ammonium chloride solution, and brine. The organic layer was dried with $Na_2SO_4$, and concentrated *in vacuo* to yield **4.2-4.15** as foamy off-white to yellow solids.

**Spiroligomer Amine 4.2 - *tert*-butyl (2-((5*R*,8*S*)-1-benzyl-8-(benzylcarbamoyl)-3-isobutyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate** - General Procedure 4.5 was used on compound **4.46**, with 1.05 equiv benzyl bromide.




$^1$H NMR (500 MHz, $CDCl_3$) 0.94 (6H, d, *J* = 6.7), 1.39 (9H, s, rotameric), 2.16 (1H, m), 2.36 (1H, dd, *J* = 14.5, 9.0), 2.44 (1H, dd, *J* = 17.1, 4.3), 3.03 (1H, dd, *J* = 14.6, 8.5), 3.27 (1H, dd, *J* = 16.9, 5.3), 3.4 (1H, t, *J* = 3.5), 3.44 (2H, d, rotameric, *J* = 7.6), 3.80 (1H, d, *J* = 11.6), 4.05 (1H, d, *J* = 16.2), 4.37 (1H, d, rotameric, *J* = 5.5), 4.46 (1H, d, rotameric, *J* = 6.4), 4.76 (1H, t, *J* = 8.7), 5.00 (1H, t, *J* = 4.4), 5.04 (1H, d, *J* = 16.2), 7.07 (1H, t, *J* = 5.8), 7.18 (2H, d, *J* = 7.3), 7.25 (3H, m), 7.33 (5H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 19.9, 20.0, 21.0, 24.8, 24.9, 27.3, 28.3, 36.5, 42.7, 43.7, 44.1, 46.8, 54.6, 60.0, 63.8, 69.3, 80.0, 80.8, 120.7, 127.1, 127.4, 127.5, 128.3, 128.7, 129.1, 129.3, 137.0, 137.8, 151.3, 156.1, 168.3, 169.0, 171.5 ;HRMS-ESI: m/z calcd for $C_{32}H_{41}N_5O_6Na$ $(M+Na)^+$ 614.2949, found 614.2955

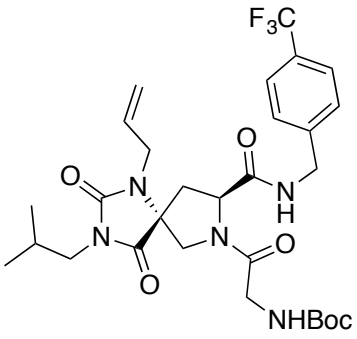
**Spiroligomer Amine 4.3 - *tert*-butyl (2-((5*R*,8*S*)-3-benzyl-1-(3,4-dichlorobenzyl)-8-(isobutylcarbamoyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate** - General Procedure 4.5 was used on compound **4.44**, with 1.05 equiv 3,4-dichlorobenzyl bromide




$^1$H NMR (500 MHz, $CDCl_3$) 0.87 (6H, d, *J* = 6.7), 1.43 (9H, s, rotameric), 1.75 (1H, m), 2.24 (1H, ddd, *J* = 14.7, 9.1, 1.2), 2.96 (1H, dd, *J* = 17.1, 4.6), 3.05 (3H, m), 3.45 (1H, dd, *J* = 17.1, 5.2), 3.52 (1H, d, *J* = 11.6), 3.84 (1H, d, *J* = 11.9), 4.21 (1H, d, *J* = 16.2), 4.63 (1H, t, *J* = 8.5), 4.75 (3H, m), 5.14 (1H, t, *J* = 4.7), 6.70 (1H, t, *J* = 5.5), 7.06 (1H, d, *J* = 6.4), 7.36 (7H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 20.1,

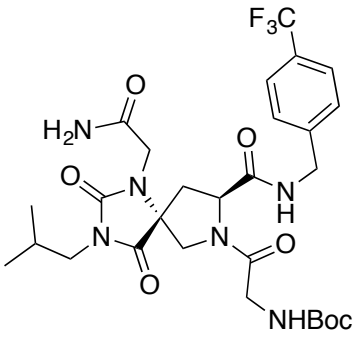
28.3, 28.4, 36.0, 43.1, 43.2, 43.3, 47.2, 54.5, 59.7, 69.5, 80.2, 126.6, 128.3, 128.6, 128.7, 128.9, 129.1, 129.2, 130.0, 131.1, 132.6, 133.3, 135.2, 135.4, 137.2, 151.7, 155.7, 168.2, 168.6, 170.9 ;HRMS-ESI: m/z calcd for $C_{32}H_{39}Cl_2N_5O_6Na$ $(M+Na)^+$ 682.2170, found 682.2161

**Spiroligomer Amine 4.4 - *tert*-butyl (2-((5*R*,8*S*)-1-allyl-3-isobutyl-2,4-dioxo-8-((4-(trifluoromethyl)benzyl)carbamoyl)-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate** - General Procedure 4.5 was used on compound **4.47**, with 1.05 equiv allyl bromide



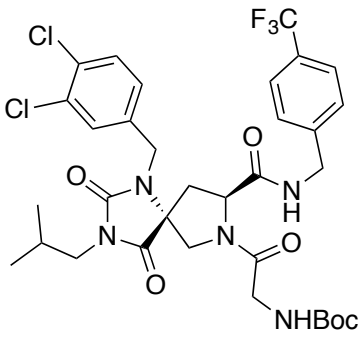

$^1$H NMR (500 MHz, $CDCl_3$) 0.80 (6H, d, *J* = 6.7), 1.39 (9H, s, rotameric), 2.07 (1H, m), 2.44 (1H, dd, *J* = 14.3, 9.5), 2.95 (1H, dd, *J* = 14.5, 7.5), 3.36 (2H, d, rotameric, *J* = 7.3), 3.82 (4H, m), 3.97 (1H, d, *J* = 1.16), 4.14 (1H, dd, rotameric, *J* = 16.5, 4.9), 4.46 (1H, d, rotameric, *J* = 5.8), 4.53 (1H, d, rotameric, *J* = 6.1), 4.83 (1H, t, *J* = 8.2), 5.18 (2H, m), 5.32 (1H, t, *J* = 4.6), 5.84 (1H, dddd, *J* = 16.9, 10.5, 6.5, 5.2), 7.32 (1H, t, *J* = 6.0), 7.40 (2H, d, *J* = 8.2), 7.57 (2H, d, *J* = 7.9); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 19.9, 27.3, 28.2, 36.1, 43.1, 43.6, 46.6, 54.3, 60.2, 68.8, 80.5, 118.1, 125.5, 125.6, 127.7, 133.4, 142.1, 156.3, 156.0, 168.7, 169.5, 172.1; HRMS-ESI: m/z calcd for $C_{29}H_{38}F_3N_5O_6Na$ $(M+Na)^+$ 632.2666, found 632.2655

**Spiroligomer Amine 4.5 - *tert*-butyl (2-((5*R*,8*S*)-1-(2-amino-2-oxoethyl)-3-isobutyl-2,4-dioxo-8-((4-(trifluoromethyl)benzyl)carbamoyl)-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate** - General Procedure 4.5 was used on compound **4.47**, with 1.05 equiv 4-(trifluoromethyl)benzyl bromide



$^1$H $CDCl_3$ (500 MHz, $CDCl_3$) 0.87 (6H, t, $J$ = 6.7), 1.31 (9H, s, rotameric), 2.02 (1H, tq, $J$ = 13.4, 6.6), 2.16 (1H, m), 2.47 (1H, dd, $J$ = 13.3, 9.6), 3.32 (2H, d, $J$ = 7.3), 3.81 (4H, m), 4.19 (1H, d, $J$ = 11.3), 4.42 (3H, m), 4.83 (1H, m), 5.88 (1H, s), 6.62 (1H, s), 7.36 (2H, d, $J$ = 7.6), 7.51 (2H, d, $J$ = 7.9), 7.83 (1H, s, rotameric), 8.23 (1H, s, rotameric); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 3.6, 9.9, 19.8, 25.3, 27.3, 28.2, 28.3, 37.2, 42.6, 42.9, 43.7, 46.8, 54.9, 60.1, 64.4, 69.1, 80.7, 122.0, 122.9, 125.1, 125.4, 127.5, 127.7, 129.6, 155.8, 157.0, 170.0, 170.3, 170.9; HRMS-ESI: m/z calcd for $C_{28}H_{37}F_3N_6O_7Na$ $(M+Na)^+$ 649.2568, found 649.2566

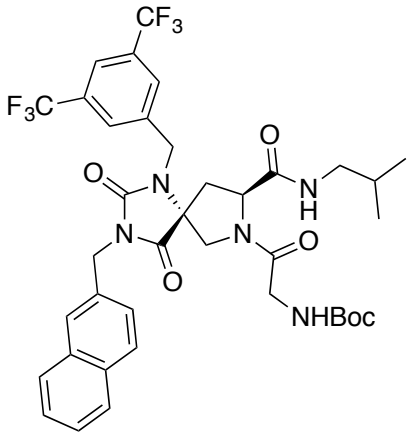
**Spiroligomer Amine 4.6 - *tert*-butyl (2-((5*R*,8*S*)-1-(3,4-dichlorobenzyl)-3-isobutyl-2,4-dioxo-8-((4-(trifluoromethyl)benzyl)carbamoyl)-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate** - General Procedure 4.5 was used on compound **4.47**, with 1.05 equiv 3,4-Dichlorobenzyl bromide



$^1$H NMR (500 MHz, $CDCl_3$) 0.93 (6H, d, $J$ = 7.0), 1.37 (9H, s, rotameric), 2.12 (1H, m), 2.35 (1H, dd, $J$ = 14.2, 9.6), 2.52 (1H, q, $J$ = 7.3), 2.98 (1H, m), 3.43 (3H, m), 3.53 (1H, m), 3.90 (1H, d, $J$ = 11.6), 4.23 (1H, d, $J$ = 16.5), 4.41 (1H, d, rotameric, $J$ = 5.8), 4.50 (1H, d, rotameric, $J$ = 6.1), 4.74 (1H, t, $J$ = 8.4), 4.82 (1H, d, $J$ = 16.2), 5.17 (1H, t, $J$ = 4.9), 7.09 (1H, dd, $J$ = 8.2, 1.8), 7.34 (4H, m), 7.44 (1H, m), 7.56 (1H, d, $J$ = 7.9); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 19.9, 27.3, 28.2, 28.3, 36.3, 43.0, 43.1, 43.2, 46.8, 46.9, 54.5, 56.5, 59.9, 69.2, 80.5, 123.0, 125.6, 126.6, 127.6, 128.1, 129.2, 130.1, 130.6,

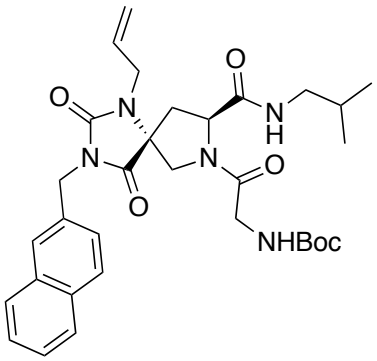
131.2, 135.6, 133.3, 137.4, 142.0, 156.0, 156.1, 168.5, 169.2, 171.5; HRMS-ESI: m/z calcd for $C_{33}H_{38}Cl_2F_3N_5O_6Na$ $(M+Na)^+$ 750.2043, found 750.2042

**Spiroligomer Amine 4.7 - *tert*-butyl (2-((5*R*,8*S*)-1-(3,5-bis(trifluoromethyl)benzyl)-8-(isobutylcarbamoyl)-3-(naphthalen-2-ylmethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate** - General Procedure 4.5 was used on compound **4.42**, with 1.05 equiv 3,5-bis(trifluoromethyl)benzyl bromide



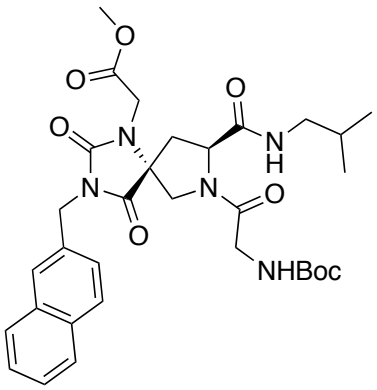

$^1$H NMR (500 MHz, $CDCl_3$) 0.85 (6H, d, *J* = 6.7), 1.41 (9H, s, rotameric), 1.73 (1H, dquin, *J* = 13.5, 6.8), 2.09 (1H, dd, *J* = 14.5, 9.0), 3.01 (2H, m), 3.12 (1H, dd, *J* = 14.8, 7.8), 3.19 (1H, dd, *J* = 17.1, 5.2), 3.42 (1H, dd, *J* = 17.1, 4.9), 3.64 (1H, d, *J* = 11.6), 3.88 (1H, d, *J* = 11.9) 4.53 (1H, t, *J* = 8.2), 4.61 (1H, d, *J* = 16.8), 4.78 (1H, d, *J* = 16.5), 4.93 (2H, m), 5.14 (1H, t, *J* = 4.7), 6.71 (1H, t, *J* = 5.8), 7.50 (3H, m), 7.82 (7H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 20.0, 20.1, 28.2, 28.3, 35.8, 43.0, 43.5, 43.6, 47.2, 54.3, 59.4, 69.5, 80.3, 122.3, 122.9, 124.0, 126.1, 126.4, 126.5, 127.5, 127.7, 128.0, 128.9, 132.4, 132.6, 132.7, 133.1, 133.3, 139.9, 155.8, 156.0, 168.3, 168.4, 170.9; HRMS-ESI: m/z calcd for $C_{38}H_{41}F_6N_5O_6Na$ $(M+Na)^+$ 800.2853, found 800.2864

**Spiroligomer Amine 4.8 - *tert*-butyl (2-((5*R*,8*S*)-1-allyl-8-(isobutylcarbamoyl)-3-(naphthalen-2-ylmethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate** - General Procedure 4.5 was used on compound **4.42**, with 1.05 equiv allyl bromide



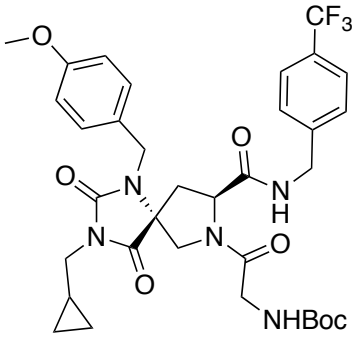

$^1$H NMR (500 MHz, $CDCl_3$) 0.89 (6H, d, *J* = 6.7), 1.42 (9H, s, rotameric), 1.78 (1H, m), 2.34 (1H, dd, *J* = 14.5, 9.0), 3.06 (3H, m), 3.73 (2H, m), 3.84 (2H, m), 3.91 (1H, d, *J* = 11.6), 4.10 (1H, dd, *J* = 16.5, 5.2), 4.72 (1H, t, *J* = 8.4), 4.84 (2H, m), 5.15 (2H, m), 5.30 (1H, t, *J* = 4.4), 5.81 (1H, ddd, *J* = 16.5, 11.6, 6.3), 6.73 (1H, t, *J* = 5.8), 7.48 (3H, m), 7.82 (4H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 20.1, 20.2, 28.3, 28.4, 36.1, 43.3, 43.6, 47.2, 54.4, 60.1, 69.2, 80.2, 118.2, 126.3, 126.4, 127.7, 128.0, 128.7, 133.0, 133.2, 133.3, 154.9, 155.8, 168.3, 168.9, 171.5; HRMS-ESI: m/z calcd for $C_{32}H_{41}N_5O_6Na$ $(M+Na)^+$ 614.2949, found 614.2951

**Spiroligomer Amine 4.9 - methyl 2-((5*R*,8*S*)-7-((*tert*-butoxycarbonyl)glycyl)-8-(isobutylcarbamoyl)-3-(naphthalen-2-ylmethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-1-yl)acetate** - General Procedure 4.5 was used on compound **4.42**, with 1.05 equiv methyl-bromoacetate



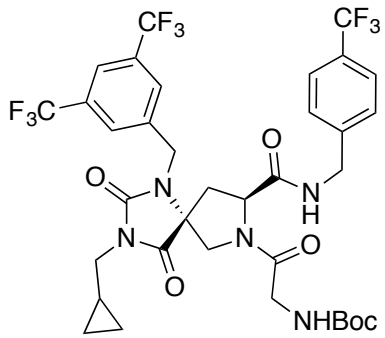

$^1$H NMR (500 MHz, $CDCl_3$) 0.88 (6H, d, *J* = 6.7), 1.41 (9H, s), 1.78 (1H, m), 2.41 (1H, dd, *J* = 14.5, 9.0), 2.99 (1H, m), 3.07 (2H, m), 3.73 (3H, s), 3.77 (2H, m), 3.90 (2H, m), 4.09 (1H, m), 4.34 (1H, d, *J* = 18.3), 4.68 (1H, t, *J* = 8.5), 4.86 (2H, m), 5.24 (1H, t, *J* = 4.3), 6.76 (1H, t, *J* = 5.8), 7.48 (3H, m), 7.82 (4H, m),; $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 20.1, 20.2, 28.3, 28.4, 36.0, 41.9, 43.4, 47.1, 52.9, 54.5, 59.6, 69.4, 80.2, 126.2, 126.3, 126.4, 127.7, 127.9, 128.1, 128.8, 132.7, 133.0, 133.2, 155.2, 155.9, 168.8, 168.9, 169.4, 170.9; HRMS-ESI: m/z calcd for $C_{32}H_{41}N_5O_8Na$ $(M+Na)^+$ 646.2847, found 646.2848

**Spiroligomer Amine 4.10 - *tert*-butyl (2-((5*R*,8*S*)-3-(cyclopropylmethyl)-1-(4-methoxybenzyl)-2,4-dioxo-8-((4-(trifluoromethyl)benzyl)carbamoyl)-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate** - General Procedure 4.5 was used on compound **4.45**, with 1.05 equiv 4-methoxybenzyl chloride



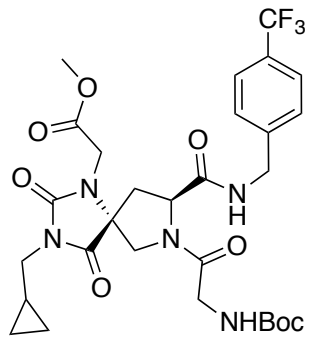

$^1$H NMR (500 MHz, $CDCl_3$) 0.39 (2H, m), 0.55 (2H, m), 1.21 (1H, m), 1.35 (9H, s), 2.45 (1H, dd, *J* = 14.5, 9.3), 2.63 (1H, dd, *J* = 16.8, 4.3), 2.97 (1H, dd, *J* = 14.6, 7.9), 3.34 (1H, dd, *J* = 16.8, 5.5), 3.47 (3H, m), 3.78 (3H, s), 3.83 (1H, d, *J* = 11.6), 4.05 (1H, d, *J* = 15.9), 4.42 (1H, d, rotameric, *J* = 5.8), 4.51 (1H, d, rotameric, *J* = 6.1), 4.81 (1H, t, *J* = 8.5), 4.97 (1H, d, *J* = 16.2), 5.04 (1H, t, *J* = 4.7), 6.87 (2H, d, *J* = 8.5), 7.13 (2H, d, *J* = 8.5), 7.29 (1H, t, *J* = 5.8), 7.37 (2H, d, *J* = 8.2), 7.56 (2H, d, *J* = 8.2); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present)3.8, 3.9, 10.1, 28.2, 28.3, 36.3, 43.0, 43.1, 43.5, 44.4, 54.6, 55.4, 60.2, 69.4, 80.2, 114.4, 125.5, 125.6, 127.6, 128.5, 128.8, 142.1, 155.8, 155.9, 159.5, 168.5, 169.5, 171.7; HRMS-ESI: m/z calcd for $C_{34}H_{40}F_3N_5O_7Na$ $(M+Na)^+$ 710.2772, found 710.2778

**Spiroligomer Amine 4.11 - *tert*-butyl (2-((5*R*,8*S*)-1-(3,5-bis(trifluoromethyl)benzyl)-3-(cyclopropylmethyl)-2,4-dioxo-8-((4-(trifluoromethyl)benzyl)carbamoyl)-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate** - General Procedure 4.5 was used on compound **4.45**, with 1.05 equiv 3,5-bis(trifluoromethyl)benzyl bromide



$^1$H NMR (500 MHz, $CDCl_3$) 0.39 (2H, m), 0.56 (2H, m), 1.22 (1H, m), 1.38 (9H, s, rotameric), 2.22 (1H, dd, *J* = 14.3, 9.2), 3.04 (1H, dd, *J* = 14.6, 7.3), 3.21 (1H, dd, *J* = 16.8, 5.2), 3.46 (1H, dd, *J* = 16.9, 5.2) 3.49 (2H, d, *J* = 7.3), 3.67 (1H, d, *J* = 11.6), 3.93 (1H, d, *J* = 11.9), 4.42 (1H, d, rotameric, *J* = 5.8), 4.47 (1H, d, rotameric, *J* = 6.1), 4.60 (1H, d, *J* = 16.8), 4.66 (1H, t, *J* = 8.2), 4.83 (1H, d, *J* = 16.8), 5.16 (1H, t, *J* = 5.0), 7.29 (1H, t, *J* = 5.5), 7.35 (2H, d, rotameric, *J* = 7.9), 7.56 (2H, d, *J* = 7.9), 7.72 (2H, s, rotameric), 7.84 (1H, s); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 3.8, 10.0, 28.1, 35.9, 43.0, 43.2, 43.3, 44.6, 54.2, 59.6, 69.3, 80.5, 121.8, 122.3, 123.0, 124.0, 125.5, 125.6, 127.4, 127.6, 128.2, 132.4, 132.7, 140.0, 142.0, 156.0, 156.3, 168.6, 169.0, 171.3; HRMS-ESI: m/z calcd for $C_{xxx}H_{xx}F_xN_xO_xNa$ $(M+Na)^+$ 748.2540, found 748.2548

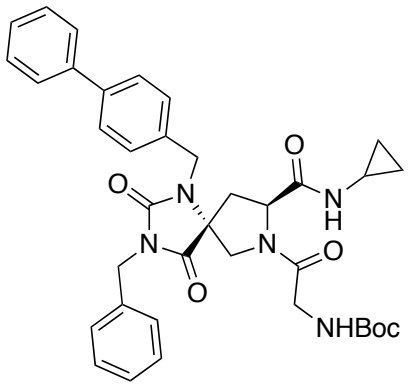
**Spiroligomer Amine 4.12 methyl 2-((5*R*,8*S*)-7-((*tert*-butoxycarbonyl)glycyl)-3-(cyclopropylmethyl)-2,4-dioxo-8-((4-(trifluoromethyl)benzyl)carbamoyl)-1,3,7-triazaspiro[4.4]nonan-1-yl)acetate** - General Procedure 4.5 was used on compound **4.45**, with 1.05 equiv methyl bromoacetate



$^1$H NMR (500 MHz, $CDCl_3$) 0.35 (2H, m), 0.52 (2H, m), 1.15 (1H, m), 1.36 (9H, s, rotameric), 2.50 (1H, dd, *J* = 14.3, 9.2), 2.94 (1H, dd, *J* = 14.6, 7.6), 3.42 (2H, d, *J* = 7.3), 3.72 (1H, m), 3.72 (3H, s), 3.83 (3H, m), 3.95 (1H, d, *J* = 11.6), 4.15 (1H, *J* = 11.3), 4.40 (1H, d, *J* = 18.3), 4.46 (1H, d, rotameric, *J* = 5.8), 4.52 (1H, d, rotameric, *J* = 6.1), 4.79 (1H, t, *J* = 8.5), 5.28 (1H, t, *J* = 5.0), 7.39 (2H, d, *J* = 7.9), 7.57 (2H, d, *J* = 8.2); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 3.8, 3.9, 10.0, 28.2, 35.9, 41.8, 43.1, 43.6, 44.4, 52.9, 54.5, 59.7, 69.1, 80.5, 123.6,

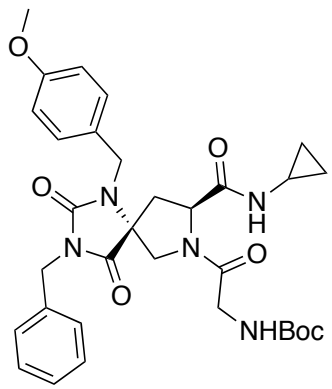
125.2, 125.5, 125.6, 127.6, 128.2, 142.1, 155.5, 156.2, 169.2, 169.5, 169.6, 171.4; HRMS-ESI: m/z calcd for $C_{29}H_{36}F_3N_5O_8Na$ $(M+Na)^+$ 662.2408, found 662.2415

**Spiroligomer Amine 4.13 - *tert*-butyl (2-((5*R*,8*S*)-1-([1,1'-biphenyl]-4-ylmethyl)-3-benzyl-8-(cyclopropylcarbamoyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate** - General Procedure 4.5 was used on compound **4.43**, with 1.05 equiv 4-(bromomethyl)biphenyl



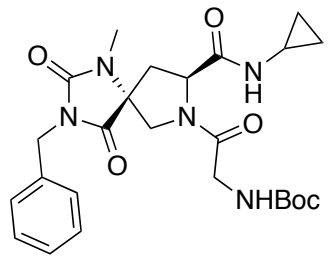

$^1$H NMR (500 MHz, $CDCl_3$) 0.50 (2H, m), 0.71 (2H, m), 1.34 (9H, s, rotameric), 2.38 (1H, dd, *J* = 14.2, 9.0), 2.58 (1H, dd, *J* = 16.8, 4.3), 2.66 (1H, tq, *J* = 7.2, 3.7), 2.96 (1H, dd, *J* = 14.4, 8.6), 3.32 (1H, dd, *J* = 16.8, 5.5), 3.45 (1H, d, *J* = 11.6), 3.79 (1H, d, *J* = 11.3), 4.13 (1H, d, *J* = 16.2), 4.68 (1H, t, *J* = 8.5), 4.76 (2H, m), 4.96 (1H, t, *J* = 4.9), 5.01 (1H, d, *J* = 16.2), 6.84 (1H, m), 7.25 (2H, d, *J* = 7.9), 7.35 (4H, m), 7.42 (4H, m), 7.55 (4H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 6.3, 6.4, 22.8, 28.2, 28.3, 36.3, 43.0, 43.3, 43.8, 54.5, 60.0, 69.5, 80.1, 126.8, 127.0, 127.7, 128.2, 128.5, 128.6, 128.7, 128.9, 135.6, 135.7, 140.0, 141.3, 155.6, 155.7, 168.2, 170.4, 171.2; HRMS-ESI: m/z calcd for $C_{29}H_{36}F_3N_5O_8Na$ $(M+Na)^+$ 674.2949, found 674.2935

**Spiroligomer Amine 4.14 - *tert*-butyl (2-((5*R*,8*S*)-3-benzyl-8-(cyclopropylcarbamoyl)-1-(4-methoxybenzyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate** - General Procedure 4.5 was used on compound **4.43**, with 1.05 equiv 4-methoxybenzyl chloride



$^1$H NMR (500 MHz, $CDCl_3$) 0.50 (2H, m), 0.71 (2H, m), 1.42 (9H, s, rotameric), 2.33 (1H, dd, *J* = 14.5, 9.0), 2.61 (1H, dd, *J* = 16.8, 4.3), 2.67 (1H, tq, *J* = 7.2, 3.7), 2.93 (1H, dd, *J* = 14.7, 8.2), 3.31 (1H, dd, *J* = 16.8, 5.2), 3.39 (1H, d, *J* = 11.6), 3.76 (3H, s) 3.77 (1H, m), 4.03 (1H, d, *J* = 15.9), 4.65 (1H, t, *J* = 8.5), 4.73 (2H, m), 4.91 (1H, d, *J* = 16.2), 5.08 (1H, t, *J* = 4.6), 6.84 (3H, m), 7.09 (2H, d, *J* = 8.5), 7.34 (3H, m), 7.4 (2H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 6.3, 6.4, 22.8, 28.3, 2=36.2, 43.0, 43.2, 43.5, 54.4, 55.3, 60.0, 69.5, 80.0, 114.4, 128.2, 128.5, 128.7, 128.9, 135.6, 155.6, 159.5, 168.2, 170.4, 171.3; HRMS-ESI: m/z calcd for $C_{32}H_{39}N_5O_7Na$ $(M+Na)^+$ 628.2742, found 628.2734

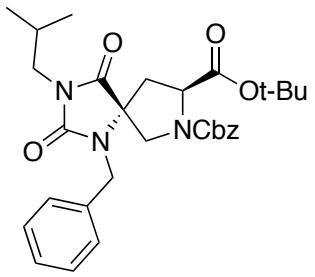
**Spiroligomer Amine 4.15 - *tert*-butyl (2-((5*R*,8*S*)-3-benzyl-8-(cyclopropylcarbamoyl)-1-methyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate** - General Procedure 4.5 was used on compound **4.43**, with 1.05 equiv iodomethane



$^1$H NMR (500 MHz, $CDCl_3$, rotamers present) 0.54 (2H, m), 0.75 (2H, m), 1.43 (9H, s), 2.35 (1H, dd, *J* = 14.3, 9.2), 2.66 (1H, d, *J* = 7.3), 2.70 (1H, tq, *J* = 7.2, 3.7), 2.89 (3H, s), 3.76 (2H, m), 3.90 (2H, m), 1.94 (2H, s), 4.72 (1H, m), 5.40 (1H, t, *J* = 4.7)6.83 (1H, m), 7.32 (5H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 6.3, 6.4, 22.8, 25.7, 28.3, 35.2, 42.9, 43.4, 53.3, 59.8, 68.7, 80.3, 128.1, 128.4, 128.6, 128.8, 135.6, 154.8, 156.0, 168.6, 170.5, 171.6; HRMS-ESI: m/z calcd for $C_{25}H_{33}N_5O_6Na$ $(M+Na)^+$ 522.2323, found 522.2325

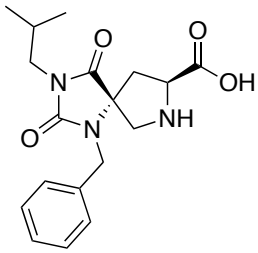
## Synthesis of the spiroligomer trimer 4.21

**Compound 4.16 7-benzyl 8-(tert-butyl) (5R,8S)-1-benzyl-3-isobutyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate**



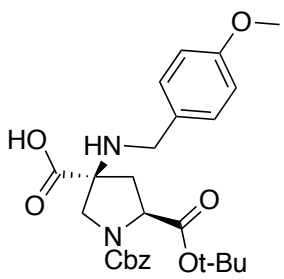

To a stirred mixture of 5.0 mmol **4.1** (2S, 4R) in DMF [100 mM] was added 1.1 equiv of isobutyl iodide along with 2.0 equiv of $K_2CO_3$. The reaction proceeded at room temperature for 24 h, and the progress checked via LCMS. After completion, 1.0 equiv of benzyl bromide was added and the reaction stirred for another 24 hours. The reaction was diluted with four times the reaction volume of EtOAc and washed with water, saturated ammonium chloride solution, and brine. The organic layer was dried with $Na_2SO_4$, and concentrated *in vacuo* to yield **4.16** as a foamy off-white to yellow solid; $^1$H NMR (500 MHz, $CDCl_3$); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present); HRMS-ESI: m/z calcd for $C_{30}H_{39}N_3O_6$ Na $(M+Na)^+$ 558.2575, found 558.2574

**Compound 4.48 - (5*R*,8*S*)-1-benzyl-3-isobutyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxylic acid**



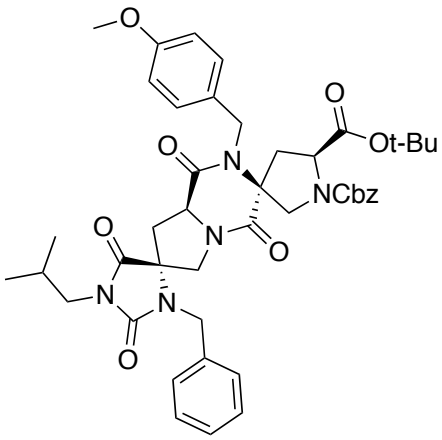

Compound **4.43** was reacted with 25 mL of 1:1 DCM/(33% HBr in AcOH) for 30 min, and the reaction progress checked via LCMS. Upon successful deprotection of the Cbz group, the solvent was removed *in vacuo* with the aid of toluene, and placed on a high vacuum pump overnight to afford **4.48**; $^1$H NMR (500 MHz, $CDCl_3$) ; $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) ; HRMS-ESI: m/z calcd for $C_{18}H_{24}N_3O_4H$ $(M+H)^+$ 346.1761, found 346.1764

**Compound 4.17 (3S,5S)-1-((benzyloxy)carbonyl)-5-(tert-butoxycarbonyl)-3-((4-methoxybenzyl)amino)pyrrolidine-3-carboxylic acid**



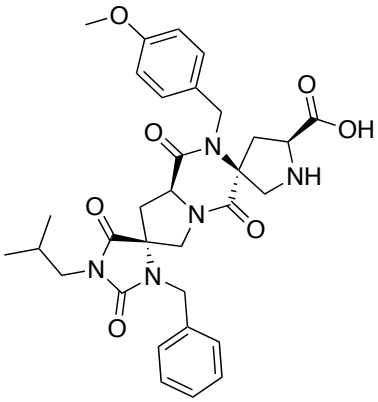

13 mmol of compound **4.68** was dissolved in 100 mL of MeOH along with 10 mmol of *p*-anisaldehyde and stirred for 1 hour, after which 14 mmol of $NaCNBH_3$ was added and reacted for 3 hours. After completion, the solvent was removed *in vacuo*, the resulting solid was redissolved in DI $H_2O$, and the amino acid precipitated upon neutralization with dropwise addition of 2M HCl. Precipitate was collected by vacuum filtration and dried on a lyophilizer (3.72 g (7.68 mmol) recovered, 77% yield) $^1$H NMR (500 MHz, DMSO-$d_6$) 1.28 (9H, s, rotameric), 1.43 (1H, d, rotameric, $J$ = 3.4), 2.09 (1H, ddd, $J$ = 18.1, 12.6, 7.4), 2.65 (1H, ddd, $J$ = 20.9, 12.2, 8.2), 3.46 (1H, t, $J$ = 10.2), 3.71 (5H, m), 3.92 (1H, dd, $J$ = 10.5, 8.9), 4.22 (1H, t, rotameric, $J$ = 7.8), 5.06 (2H, m), 6.87 (2H, m), 7.32 (8H, m); $^{13}$C NMR (125 MHz, DMSO-$d_6$, rotamers present) 27.9, 28.1, 28.1, 28.2, 37.5, 38.3, 48.3, 53.9, 54.5, 55.5, 59.4, 59.7, 63.0, 66.5, 66.7, 67.0, 67.9, 80.5, 81.0, 81.2, 114.0, 127.8, 127.9, 128.2, 128.3, 128.4, 128.7, 128.8, 128.9, 130.4, 130.5, 137.0, 137.3, 154.0, 154.3, 159.1, 159.2, 170.9, 171.3, 173.2, 173.3; HRMS-ESI: m/z calcd for $C_{26}H_{33}N_2O_7H$ $(M+Na)^+$ 485.2282, found 485.2287

**Compound 4.18 - 1''-benzyl 5''-(tert-butyl) (3'S,4R,5''S,8a'S)-3-benzyl-1-isobutyl-2'-(4-methoxybenzyl)-1',2,4',5-tetraoxotetrahydro-4'H,6'H-dispiro[imidazolidine-4,7'-pyrrolo[1,2-a]pyrazine-3',3''-pyrrolidine]-1'',5''-dicarboxylate**



700 mg of **4.17** was preactivated with 979 mg (6 eq) of 1-hydroxy-7-azabenzotriazole (HOAt) and 253 mg (1.1 eq) of N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) in 21 mL of anhydrous 2:1 DCM/DMF for 1.5 h, after which all of **4.48** and 0.63 mL of DIPEA dissolved in 7 mL of DMF were added to the reaction and stirred overnight. In the morning, 575 mg (2.5 eq) of EDC-HCl were added and the reaction stirred for another 4 hours. The reaction was diluted with EtOAc, and washed with saturated solutions of $NH_4Cl$, $NaHCO_3$, and brine, dried over $Na_2SO_4$, and rotovapped. The foamy solid was then purified via normal phase flash chromatography to afford 478 mg (46% yield) of **4.18**; $^1$H NMR (500 MHz, $CDCl_3$); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present); HRMS-ESI: m/z calcd for $C_{44}H_{51}N_5O_9$ Na $(M+Na)^+$ 816.3579, found 816.3589

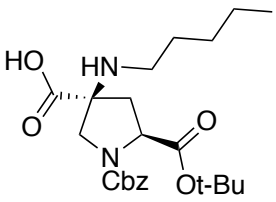
**Compound 4.49 - (3'S,4R,5''S,8a'S)-3-benzyl-1-isobutyl-2'-(4-methoxybenzyl)-1',2,4',5-tetraoxotetrahydro-4'H,6'H-dispiro[imidazolidine-4,7'-pyrrolo[1,2-a]pyrazine-3',3''-pyrrolidine]-5''-carboxylic acid**



**4.18** was treated with 1:1 DCM/(33% HBr/AcOH) for 30 minutes, after which the solvent was removed *in vacuo*, and the molecule left on a high vacuum pump overnight to give the deprotected spiroligomer dimer ; $^1$H NMR (500 MHz, DMSO-$d_6$) 0.88 (6H, dd, $J$ = 6.7, 1.8), 1.99 (1H, dquin, $J$ = 13.7, 7.0), 2.14 (1H, t, $J$ = 12.8), 2.60 (2H, m), 2.79 (1H, dd, $J$ = 13.4, 6.7), 3.27 (2H, d, $J$ = 7.3), 3.42 (3H, m), 3.53 (1H, dd, $J$ = 11.9, 6.7), 3.72 (3H, s), 3.94 (1H, d, $J$ = 12.8), 4.43 (1H, d, $J$ = 16.8), 4.62

(1H, d, *J* = 16.5), 4.81 (2H, m), 4.96 (1H, d, *J* = 16.5), 6.86 (2H, d, *J* = 8.8), 7.17 (2H, d, *J* = 8.5), 7.28 (1H, m), 7.39 (4H, m); $^{13}$C NMR (125 MHz, DMSO-$d_6$, rotamers present) 20.3, 20.4, 27.5, 35.6, 38.3, 42.9, 44.8, 46.2, 48.1, 51.3, 55.5, 57.0, 59.6, 65.7, 69.5, 114.4, 127.4, 127.9, 129.2, 130.2, 138.4, 156.3, 158.7, 165.5, 168.3, 168.8, 174.8; HRMS-ESI: m/z calcd for $C_{32}H_{37}N_5O_7H$ $(M+H)^+$ 626.2585, found 626.2587

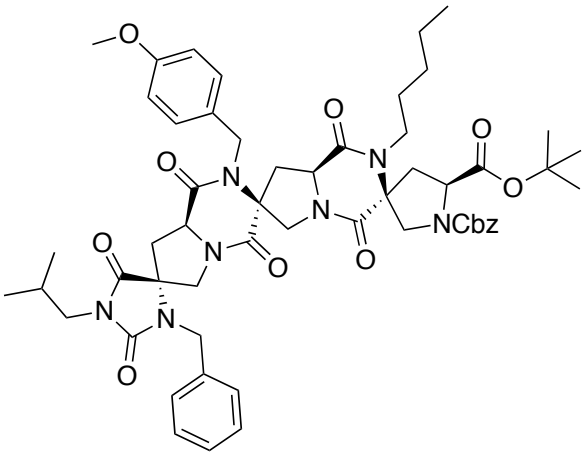
**Compound 4.19 - (3S,5S)-1-((benzyloxy)carbonyl)-5-(tert-butoxycarbonyl)-3-(pentylamino)pyrrolidine-3-carboxylic acid**




13 mmol of compound **4.68** was dissolved in 100 mL of MeOH along with 10 mmol of valeraldehyde and stirred for 1 hour, after which 14 mmol of $NaCNBH_3$ was added and reacted for 3 hours. After completion, the solvent was removed *in vacuo*, the resulting solid was redissolved in DI $H_2O$, and the amino acid precipitated upon neutralization with dropwise addition of 2M HCl. Precipitate was collected by vacuum filtration and dried on a lyophilizer (3.56 g (8.21 mmol) recovered, 82% yield); $^1$H NMR (500 MHz, DMSO-$d_6$) 0.84 (4H, m), 1.29 (16H, m), 1.46 (1H, m), 2.04 (1H, ddd, rotameric, *J* = 17.1, 12.2, 7.9), 2.62 (2H, m), 3.44 (1H, m), 3.87 (1H, dd, *J* = 10.1, 3.7), 4.28 (1H, t, rotameric, *J* = 8.3), 5.04 (2H, m), 7.33 (5H, m); $^{13}$C NMR (125 MHz, DMSO-$d_6$, rotamers present) 13.8, 13.9, 14.0, 14.3, 19.0, 21.0, 21.8, 22.1, 27.2, 27.3, 27.4, 27.6, 27.7, 27.8, 28.3, 31.5, 31.7, 36.2, 36.9, 44.3, 53.5, 59.0, 59.4, 66.1, 66.2, 67.0, 80.5, 80.7, 127.3, 127.4, 127.7, 127.8, 128.2, 128.3, 128.4, 136.5, 136.8, 153.5, 153.8, 170.5, 170.9, 171.0; HRMS-ESI: m/z calcd for $C_{23}H_{35}N_2O_6H$ $(M+H)^+$ 435.2490 , found 435.2481

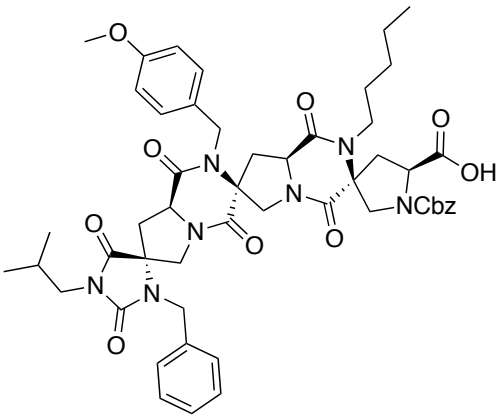
**Compound 4.20 - 1'''-benzyl 5'''-(tert-butyl) (3'S,3''S,4R,5'''S,8a'S,8a''S)-3-benzyl-1-isobutyl-2'-(4-methoxybenzyl)-1',1'',2,4',4'',5-hexaoxo-2''-pentyloctahydro-**

**4'H,4''H,6'H,6''H-trispiro[imidazolidine-4,7'-pyrrolo[1,2-a]pyrazine-3',7''-pyrrolo[1,2-a]pyrazine-3'',3'''-pyrrolidine]-1''',5'''-dicarboxylate**



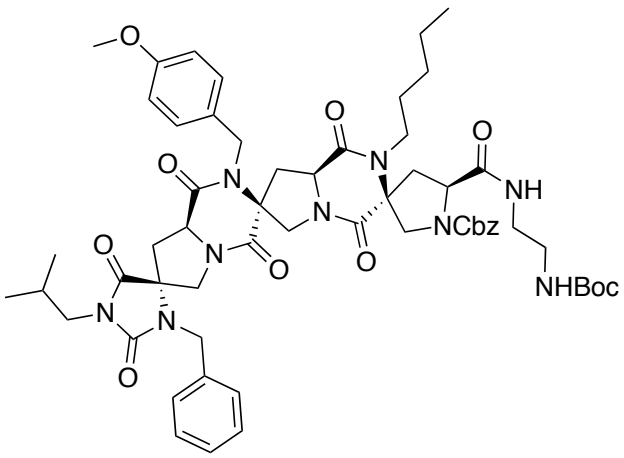

370 mg (1.2 equiv) of **4.19** was preactivated with 563 mg (6 equiv) of HOAt and 143 mg (1.1 equiv) of EDC in 15 mL of anhydrous 2:1 DCM/DMF for 1.5 h, after which 420 mg of **4.18** and 366 uL of DIPEA dissolved in 5 mL of DMF were added to the reaction and stirred overnight. In the morning, 330 mg (2.5 eq) of EDC-HCl were added and the reaction stirred for another 4 hours. The reaction was diluted with EtOAc, and washed with saturated solutions of $NH_4Cl$, $NaHCO_3$, and brine, dried over $Na_2SO_4$, and rotovapped. The foamy solid was then purified via normal phase flash chromatography to afford 148 mg (21% yield) of **4.20**; $^1$H NMR (500 MHz, $CDCl_3$, rotamers present) 0.92 (9H, m), 1.29 (10H, m), 1.46 (4H, m), 1.59 (1H, m), 1.98 (1H, m), 2.13 (2H, m), 2.20 (1H, m), 2.61 (3H, m), 2.85 (1H, dd, *J* = 14.3, 7.3), 3.12 (1H, m), 3.38 (1H, d, *J* = 12.8), 3.42 (2H, d, *J* = 7.6), 3.64 (1H, m), 3.75 (3H, m), 3.82 (1H, d, *J* = 11.6), 4.05 (4H, m), 4.25 (2H, m), 4.37 (2H, m), 4.56 (1H, d, *J* = 16.2), 4.83 (1H, d, *J* = 16.5), 4.94 (1H, m), 5.06 (1H, d, *J* = 12.2), 5.19 (2H, m), 6.82 (2H, m), 7.12 (2H, m), 7.33 (10H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 13.9, 14.0, 20.0, 22.2, 22.3, 22.6, 27.4, 27.7, 27.9, 29.0, 29.3, 36.2, 38.7, 39.7, 43.4, 43.7, 45.8, 46.6, 49.5, 51.1, 51.7, 55.2, 55.9, 57.4, 57.7, 65.4, 66.5, 67.5, 67.5, 82.1, 114.5, 127.1, 127.7, 127.9, 128.35, 128.46, 128.48, 128.52, , 128.9, 129.4, 136.0, 136.5, 154.1, 156.3, 159.1, 164.7, 165.5, 166.6, 167.1, 170.5, 173.6; HRMS-ESI: m/z calcd for $C_{55}H_{67}N_7O_{11}Na$ $(M+Na)^+$ 1024.4791, found 1024.4784

**Compound 4.50 - (3'S,3''S,4R,5'''S,8a'S,8a''S)-3-benzyl-1'''-((benzyloxy)carbonyl)-1-isobutyl-2'-(4-methoxybenzyl)-1',1'',2,4',4'',5-hexaoxo-2''-pentyloctahydro-4'H,4''H,6'H,6''H-trispiro[imidazolidine-4,7'-pyrrolo[1,2-a]pyrazine-3',7''-pyrrolo[1,2-a]pyrazine-3'',3'''-pyrrolidine]-5'''-carboxylic acid**




Spiroligomer trimer **4.20** was treated with a 95:4:1 TFA/$H_2O$/TIPS mixture to remove the *t*-Bu protecting group and afford compound **4.50;** $^1$H NMR (500 MHz, $CDCl_3$) 0.85 (3H, m), 0.93 (6H, d, *J* = 6.6), 1.26 (6H, m), 1.56 (1H, m), 2.12 (1H, tt, *J* = 13.7, 6.9), 2.22 (2H, m), 2.55 (1H, m), 2.65 (2H, m), 2.80 (1H, dd, *J* = 13.2, 6.6), 3.06 (1H, t, *J* = 11.2), 3.40 (3H, m), 3.63 (1H, m), 3.74 (3H, m), 3.85 (1H, m), 4.02 (3H, m), 4.18 (1H, m), 4.26 (1H, m), 4.37 (3H, m), 4.59 (1H, m), 4.70 (1H, m), 4.91 (1H, m), 5.14 (2H, m), 6.80 (2H, m), 7.09 (2H, d, *J* = 8.2), 7.32 (10 H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 10.2, 11.7, 12.2, 13.9, 14.1, 17.3, 17.6, 19.4, 19.94, 19.96, 22.17, 22.22, 27.4, 28.9, 29.3, 36.1, 38.0, 38.2, 43.6, 45.9, 46.6, 49.5, 51.5, 55.2, 56.0, 57.3, 57.6, 65.5, 66.6, 67.4, 68.1, 114.4, 127.1, 127.2, 127.4, 127.6, 127.67, 127.72, 128.2, 128.3, 128.4, 128.6, 128.9, 129.3, 135.8, 136.6, 155.6, 156.4, 159.1, 164.5, 165.6, 167.3, 167.6, 173.8**;** HRMS-ESI: m/z calcd for $C_{51}H_{59}N_7O_{11}Na$ $(M+Na)^+$ 968.4165, found 968.4133

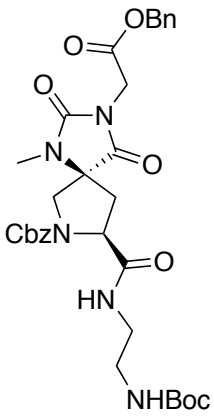
**Compound** 4.21 benzyl (3'S,3"S,4R,5'"S,8a'S,8a"S)-3-benzyl-5'"-((2-((tert-butoxycarbonyl)amino)ethyl)carbamoyl)-1-isobutyl-2'-(4-methoxybenzyl)-1',1",2,4',4",5-hexaoxo-2"-pentyloctahydro-4'H,4"H,6'H,6"H-trispiro[imidazolidine-4,7'-pyrrolo[1,2-a]pyrazine-3',7"-pyrrolo[1,2-a]pyrazine-3",3'"-pyrrolidine]-1'"-carboxylate




All of **4.50** was preactivated with 82 mg (4 eq) of HOAt and 400 mg (2 eq) of EDC in 6.0 mL of anhydrous 2:1 DCM/DMF for 1.5 h, after which 143 uL of N-Boc-Ethylenediamine was added to the reaction and stirred overnight. The reaction was diluted with EtOAc, and washed with saturated solutions of $NH_4Cl$, $NaHCO_3$, and brine, dried over $Na_2SO_4$, and rotovapped. The foamy solid was then purified via normal phase flash chromatography to afford 127 mg (46% yield) of **4.21**; $^1$H NMR (500 MHz, $CDCl_3$) 0.87 (3H, t, *J* = 6.8), 0.92 (6H, d, *J* = 6.6), 1.30 (6H, m), 1.40 (9H, s), 1.57 (1H, m), 2.11 (1H, dt, *J* = 13.7, 7.0), 2.18 (1H, dd, *J* = 12.9, 11.3), 2.40 (1H, dd, *J* = 11.7, 7.3), 2.57 (3H, m), 2.82 (1H, dd, *J* = 14.0, 7.7), 3.12 (5H, m), 3.37 (4H, m), 3.68 (5H, m), 4.01 (3H, m), 4.10 (1H, m), 4.23 (2H, d, *J* = 16.4), 4.35 (2H, m), 4.56 (1H, d, *J* = 16.1), 4.77 (1H, d, *J* = 16.4), 4.93 (1H, m), 5.17 (2H, m), 6.79 (2H, t, *J* = 9.5), 7.08 (2H, d, *J* = 8.2), 7.18 (1H, s, broad), 7.32 (10H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 14.0, 20.0, 22.3, 25.3, 28.4, 28.9, 19.4, 36.3, 38.6, 40.2, 43.4, 43.8, 45.8, 46.6, 49.4, 50.9, 51.8, 55.2, 55.9, 57.5, 58.9, 65.5, 66.6, 67.4, 67.9, 79.3, 114.4, 127.1, 127.2, 127.7, 127.9, 128.2, 128.49, 128.54, 128.9, 129.4, 136.0, 136.6, 156.0, 156.3, 159.1, 165.1, 165.3, 166.7, 167.3, 170.7, 173.6; HRMS-ESI: m/z calcd for $C_{58}H_{73}N_9O_{12}H$ $(M+H)^+$ 1088.5451, found 1088.5495

## Macrocycle Amine Synthesis

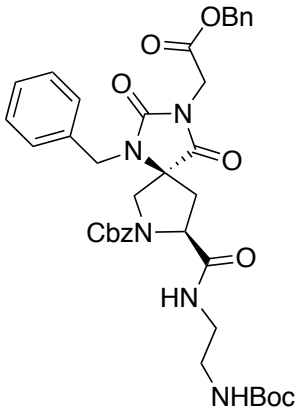
**Spiroligomer Amine 4.51 - benzyl (5*S*,8*S*)-3-(2-(benzyloxy)-2-oxoethyl)-8-((2-((*tert*-butoxycarbonyl)amino)ethyl)carbamoyl)-1-methyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7-carboxylate**




To a stirred mixture of 6.66 mmol of **4.1** (2S, 4S) in DMF [100 mM] was added 0.88 equiv of benzyl bromoacetate along with 2.5 equiv of $K_2CO_3$. The reaction proceeded at room temperature for 12 h, at which time 8 mmol of iodomethane was added and the reaction stirred overnight. The reaction was diluted with four times the reaction volume of EtOAc and washed with water, saturated ammonium chloride solution, and brine. The organic layer was dried with $Na_2SO_4$, and concentrated *in vacuo*. The dialkylated hydantoin was then treated with 95:4:1 TFA/$H_2O$/TIPS for 1 h, the solvent again removed *in vacuo*, and put on high vacuum overnight. The free acid was dissolved in dry DMF/DCM (1:1 ratio, [100 mM]), to which was added 1.5 equiv of 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 3 equiv of 1-Hydroxy-7-azabenzotriazole (HOAT), then stirred for 1 h, at which time 3 equiv of N-Boc-ethylenediamine and 2 equiv DIPEA were added and stirred overnight. The reaction was diluted with EtOAc, and washed with saturated solutions of $NH_4Cl$, $NaHCO_3$, and brine, dried over $Na_2SO_4$, and rotovapped. The foamy solid was then purified via normal phase flash chromatography to afford 3.85 g (6.03 mmol) of purified **4.51**.; $^1$H NMR (500 MHz, $CDCl_3$) 1.43(9H, s), 1.95 (1H, m), 2.39 (1H, m), 2.69 (1H, dd, $J$ = 12.4, 5.6), 2.96 (3H, s), 3.26 (4H, m), 3.60 (1H, d, $J$ = 11.6), 3.95 (1H, m), 4.29 (2H, s), 4.58 (1H, t, $J$ = 7.6), 5.15 (4H, m), 7.12 (1H, s), 7.33 (10H, m); $^{13}$C NMR (125 MHz, $CDCl_3$ rotamers present) 25.0, 25.3, 28.4, 31.5, 33.3, 36.5, 39.6, 40.1, 40.5, 49.5, 59.6, 67.3, 67.7,

68.0, 79.5, 128.0, 128.3, 128.4, 128.6, 128.67, 128.69, 134.8, 154.5, 166.8, 170.9, 174.4; HRMS-ESI: m/z calcd for $C_{32}H_{39}N_5O_9H$ $(M+H)^+$ 638.2821, found 638.2829

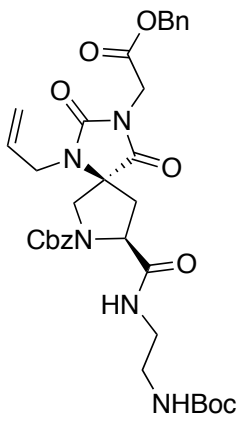
**Spiroligomer Amine 4.52benzyl (5*S*,8*S*)-1-benzyl-3-(2-(benzyloxy)-2-oxoethyl)-8-((2-((*tert*-butoxycarbonyl)amino)ethyl)carbamoyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7-carboxylate**




To a stirred mixture of 6.66 mmol of **4.1** (2S, 4S) in DMF [100 mM] was added 0.88 equiv of benzyl bromoacetate along with 2.5 equiv of $K_2CO_3$. The reaction proceeded at room temperature for 12 h, at which time 8 mmol of benzyl bromide was added and the reaction stirred overnight. The reaction was diluted with four times the reaction volume of EtOAc and washed with water, saturated ammonium chloride solution, and brine. The organic layer was dried with $Na_2SO_4$, and concentrated *in vacuo*. The dialkylated hydantoin was then treated with 95:4:1 TFA/$H_2O$/TIPS for 1 h, the solvent again removed *in vacuo*, and put on high vacuum overnight. The free acid was dissolved in dry DMF/DCM (1:1 ratio, [100 mM]), to which was added 1.5 equiv of 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 3 equiv of 1-Hydroxy-7-azabenzotriazole (HOAT), then stirred for 1 h, at which time 3 equiv of N-Boc-ethylenediamine and 2 equiv DIPEA were added and stirred overnight. The reaction was diluted with EtOAc, and washed with saturated solutions of $NH_4Cl$, $NaHCO_3$, and brine, dried over $Na_2SO_4$, and rotovapped. The foamy solid was then purified via normal phase flash chromatography to afford 4.15 g (5.81 mmol) of purified **4.52**.; $^1H$ NMR (500 MHz, $CDCl_3$) 1.42 (9H, s), 1.88 (1H, m), 2.27 (1H, m), 2.69 (1H, m), 3.03 (1H) 3.25 (2H, m), 3.39 (2H, m), 3.93 (1H, m), 4.34 (2H, s), 4.52 (1H, t, $J$ = 7.9), 4.68 (2H, m),

5.06 (1H, m), 5.12 (1H, m), 5.18 (2H, m), 7.00 (1H, s), 7.30 (15H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 28.4, 31.4, 36.5, 39.7, 40.1, 40.5, 42.6, 43.0, 49.9, 59.2, 67.6, 67.80, 67.83, 127.0, 127.3, 127.6, 127.8, 127.9, 128.1, 128.2, 128.4, 128.5, 128.64, 128.67, 128.71, 128.8, 129.0, 134.7, 135.8, 155.0, 166.7, 170.8, 174.4; HRMS-ESI: m/z calcd for $C_{38}H_{43}N_5O_9H$ $(M+H)^+$ 714.3134, found 714.3152

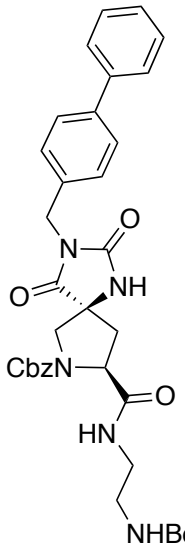
**Spiroligomer 4.53 benzyl (5*S*,8*S*)-1-allyl-3-(2-(benzyloxy)-2-oxoethyl)-8-((2-((*tert*-butoxycarbonyl)amino)ethyl)carbamoyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7-carboxylate**




To a stirred mixture of 6.66 mmol of **4.1** (2S, 4S) in DMF [100 mM] was added 0.88 equiv of benzyl bromoacetate along with 2.5 equiv of $K_2CO_3$. The reaction proceeded at room temperature for 12 h, at which time 8 mmol of allyl bromide was added and the reaction stirred overnight. The reaction was diluted with four times the reaction volume of EtOAc and washed with water, saturated ammonium chloride solution, and brine. The organic layer was dried with $Na_2SO_4$, and concentrated *in vacuo*. The dialkylated hydantoin was then treated with 95:4:1 TFA/$H_2O$/TIPS for 1 h, the solvent again removed *in vacuo*, and put on high vacuum overnight. The free acid was dissolved in dry DMF/DCM (1:1 ratio, [100 mM]), to which was added 1.5 equiv of 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 3 equiv of 1-Hydroxy-7-azabenzotriazole (HOAT), then stirred for 1 h, at which time 3 equiv of N-Boc-ethylenediamine and 2 equiv DIPEA were added and stirred overnight. The reaction was diluted with EtOAc, and washed with saturated solutions of $NH_4Cl$, $NaHCO_3$, and brine, dried over $Na_2SO_4$, and rotovapped. The foamy solid was then purified via normal phase flash chromatography to

afford 3.97 g (5.98 mmol) of purified **4.53**.; $^{1}$H NMR (500 MHz, $CDCl_3$) 1.43 (9H, s), 1.90 (1H, m), 2.32 (1H, dd, *J* = 11.9, 8.2), 2.72 (1H, dd, *J* = 12.4, 7.5), 3.19 (1H, m), 3.27 (2H, m), 3.43 (1H, m), 3.62 (1H, m), 3.90 (1H, d, rotameric, *J* = 11.6), 4.05 (2H, m), 4.3 (2H, s, rotameric), 4.55 (1H, t, *J* = 7.8), 5.17 (6H, m), 5.81 (1H, m), 7.02 (1h, s), 7.33 (10H, m); $^{13}$C NMR (125 MHz, $CDCl_3$, rotamers present) 28.4, 33.6, 39.6, 40.1, 40.5, 40.88, 40.89, 49.9, 59.3, 67.4, 67.8, 68.0, 79.5, 117.8, 118.3, 128.0, 128.3, 128.4, 128.6, 128.69, 128.70, 132.6, 134.7, 135.8, 154.4, 155.5, 166.7, 170.8, 174.4; HRMS-ESI: m/z calcd for $C_{34}H_{41}N_5O_9H$ $(M+H)^+$ 664.2977, found 664.2969

**Spiroligomer Amine 4.54 benzyl (5*S*,8*S*)-3-([1,1'-biphenyl]-4-ylmethyl)-8-((2-((*tert*-butoxycarbonyl)amino)ethyl)carbamoyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7-carboxylate**

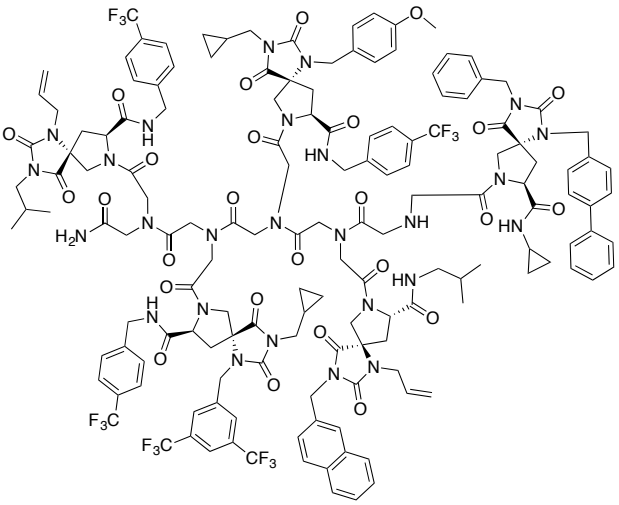



To a stirred mixture of 10.6 mmol of **4.1** (2S, 4S) in DMF [100 mM] was added 0.88 equiv of 4-(bromomethyl)biphenyl along with 1.5 equiv of $K_2CO_3$. The reaction proceeded at room temperature for 12 h, at which time the reaction was diluted with four times the reaction volume of EtOAc and washed with water, saturated ammonium chloride solution, and brine. The organic layer was dried with $Na_2SO_4$, and concentrated *in vacuo*. The dialkylated hydantoin was then treated with 95:4:1 TFA/$H_2O$/TIPS for 1 h, the solvent again removed *in vacuo*, and put on high vacuum overnight. The free acid was dissolved in dry DMF/DCM (1:1 ratio, [100 mM]), to which was added 1.5 equiv of 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 3 equiv of 1-Hydroxy-7-azabenzotriazole (HOAT), then stirred for 1 h, at which time 3 equiv of N-Boc-ethylenediamine and 2 equiv DIPEA were added and stirred overnight. The reaction was diluted with EtOAc, and washed with saturated solutions of $NH_4Cl$, $NaHCO_3$, and brine, dried over $Na_2SO_4$, and

rotovapped. The foamy solid was then purified via normal phase flash chromatography to afford 5.45 g (8.49 mmol) of purified **4.52**.$^{1}$H NMR (500 MHz, $CDCl_3$ or DMSO-$d_6$) $^{13}$C NMR (125 MHz, $CDCl_3$ or DMSO-$d_6$, rotamers present) HRMS-ESI: m/z calcd for $C_{35}H_{40}F_3N_5O_7H$ $(M+H)^+$ 642.2922, found 642.2934

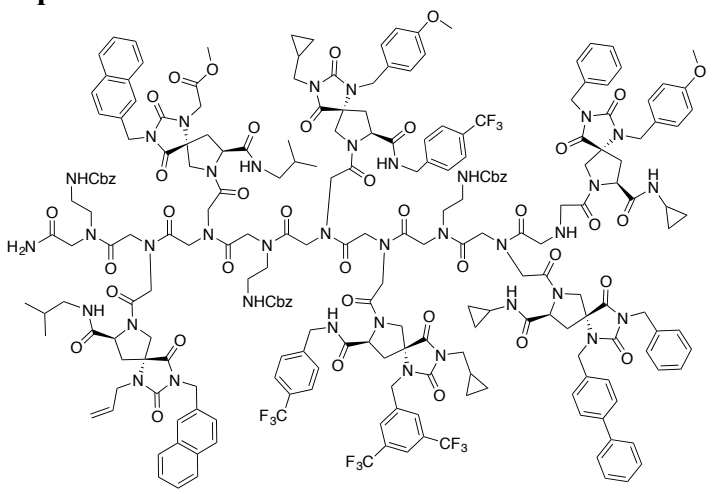
## Peptoid Synthesis

### Peptoid SPH-4.1



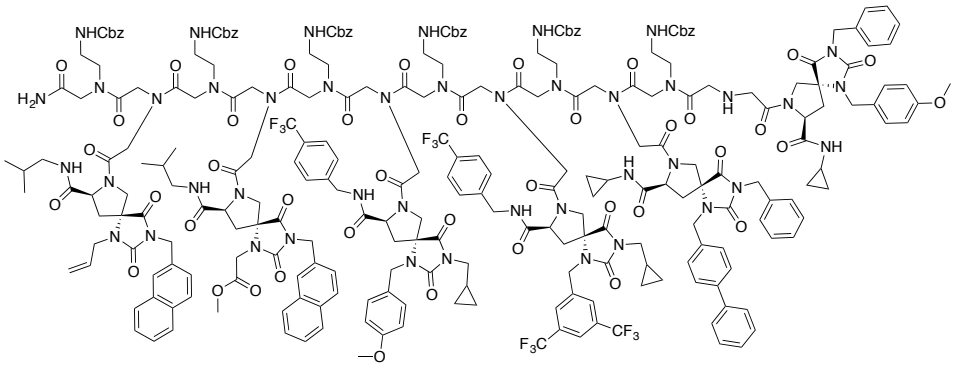
54 mg of Rink amide resin was swelled with DCM, and then treated with 20% Piperidine in DMF 2x15 min, and subsequently rinsed with DCM and DMF. 800 uL of 1.3 M Bromoacetic acid in DMF was reacted with 200 uL of DIC for 5 minutes, added to the resin, and allowed to react for 30 min. The resin was drained and washed repeatedly with DCM and DMF, after which 150 mM **4.11** (3 equiv, previously deprotected) in DMF was added to the resin and stirred overnight. The bromoacetic acid addition and subsequent amine additions were repeated for amines **4.12, 4.8,** and **4.13**. After the final amine addition, the resin was rinsed with DMF and DCM, and the resin treated with neat TFA to cleave the peptoid from the resin. HRMS-ESI: $C_{152}H_{161}F_{15}N_{26}O_{26}$ Target mass: 3049.1676; Found: 3049.1543

**Peptoid SPH-4.2**



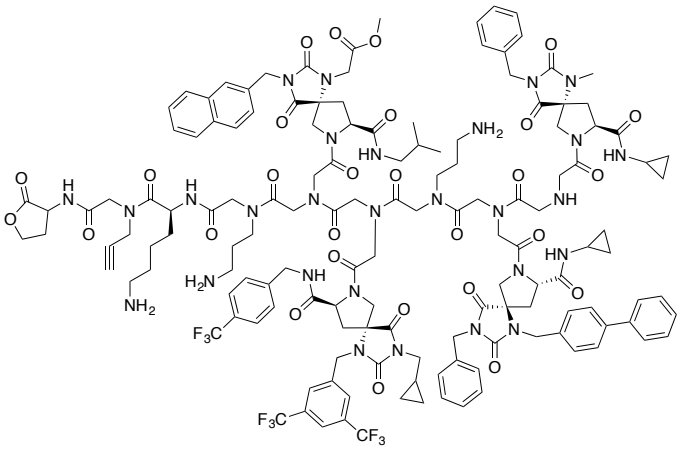
60 mg of Rink amide resin was swelled with DCM, and then treated with 20% Piperidine in DMF 2x15 min, and subsequently rinsed with DCM and DMF. 800 uL of 1.3 M Bromoacetic acid in DMF was reacted with 200 uL of DIC for 5 minutes, added to the resin, and allowed to react for 30 min. The resin was drained and washed repeatedly with DCM and DMF, after which 150 mM **4.8** (3 equiv, previously deprotected) in DMF was added to the resin and stirred overnight. In the morning, the resin was rinsed with DMF and DCM, and then the next round of peptoid coupling proceeded as the first, except with the use amine **4.9** (3 equiv, previously deprotected). Another round of peptoid coupling follows, with the use of N-(Z)-Ethylenediamine-HCl (10 equiv) as the amine for 1 h (10 equiv of DIPEA was used to freebase the amine prior to addition). The bromoacetic acid addition and subsequent amine additions were repeated for amines **4.10, 4.11, 4.13,** and **4.14**, with the N-(Z)-Ethylenediamine-HCl amine residue after every two spiroligomer amine additions. After the final amine addition, the resin was rinsed with DMF and DCM, and the resin treated with neat TFA to cleave the peptoid from the resin. HRMS-ESI: $C_{220}H_{235}F_{12}N_{37}O_{43}$ Target Mass: 4310.7148; Found: 4310.6912

**Peptoid SPH-4.3**



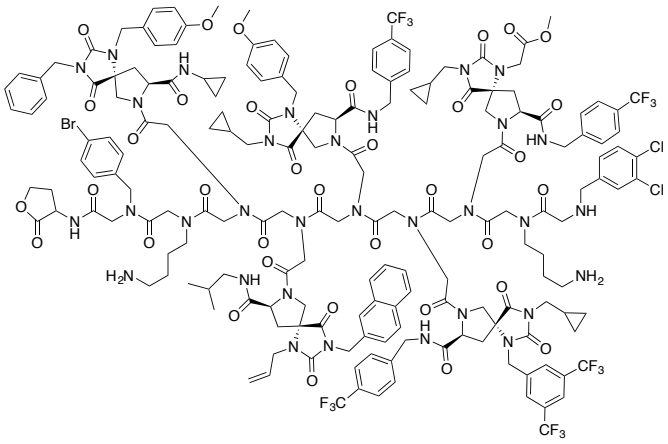
60 mg of Rink amide resin was swelled with DCM, and then treated with 20% Piperidine in DMF 2x15 min, and subsequently rinsed with DCM and DMF. 800 uL of 1.3 M Bromoacetic acid in DMF was reacted with 200 uL of DIC for 5 minutes, added to the resin, and allowed to react for 30 min. The resin was drained and washed repeatedly with DCM and DMF, after which 150 mM **4.8** (3 equiv, previously deprotected) in DMF was added to the resin and stirred overnight. In the morning, the resin was rinsed with DMF and DCM, and then the next round of peptoid coupling proceeded as the first, except with the use of N-(Z)-Ethylenediamine-HCl (10 equiv) as the amine for 1 h (10 equiv of DIPEA was used to freebase the amine prior to addition). The bromoacetic acid addition and subsequent amine additions were repeated for amines **4.9, 4.10, 4.11, 4.13,** and **4.14**, with the N-(Z)-Ethylenediamine-HCl amine residue after each spiroligomer amine addition. After the final amine addition, the resin was rinsed with DMF and DCM, and the resin treated with neat TFA to cleave the peptoid from the resin. HRMS-ESI: for $C_{256}H_{277}F_{12}N_{43}O_{52}$ Target Mass: 5013.0161; Found 5012.9707

**Peptoid SPH-4.4 -**



100 mg of Tentagel-$NH_2$ (S) resin was rinsed multiple times with DCM and DMF. 3 equiv of Fmoc-Met-OH and HATU in 0.6 mL of NMP with 6 equiv of DIPEA were then added to the resin and stirred for 1 hour (Standard SPPS). The resin was treated with 20% piperidine in DMF 2x15 min, and rinsed with DMF and DCM. 800 uL of 1.3 M Bromoacetic acid in DMF was reacted with 200 uL of DIC for 5 minutes, added to the resin, and allowed to react for 30 min. The resin was drained and washed repeatedly with DCM and DMF, after which 0.3 mL of a 1M solution of propargylamine was added to the resin and stirred for 1 h (standard peptoid coupling). Fmoc-Lys(Boc)-OH was coupled to the resin using standard SPPS, Fmoc-deprotected, and followed by an N-substituted, boc protected ornithine derivative (N-Orn-Boc) with standard peptoid coupling. The resin was drained and washed repeatedly with DCM and DMF, after which 150 mM **4.9** (3 equiv, previously deprotected) in DMF was added to the resin and stirred overnight. This was repeated for addition of amine **4.11**. Standard peptoid coupling was used to add another (N-Orn-Boc), and finally two more round of spiroligomer peptoid reactions using amines **4.13** and **4.15**. The resin was washed repeatedly with DMF and DCM, then treated with 1:1 TFA/DCM to remove the Boc protecting groups. The resin was washed exhaustively with water, then treated with a 7:3 Formic Acid/$H_2O$ mixture containing 30 mg of cyanogen bromide to cleave the peptoid from the resin. HRMS-ESI for $C_{142}H_{163}F_9N_{28}O_{28}$ Target Mass: 2879.2048; Found: 2879.1845

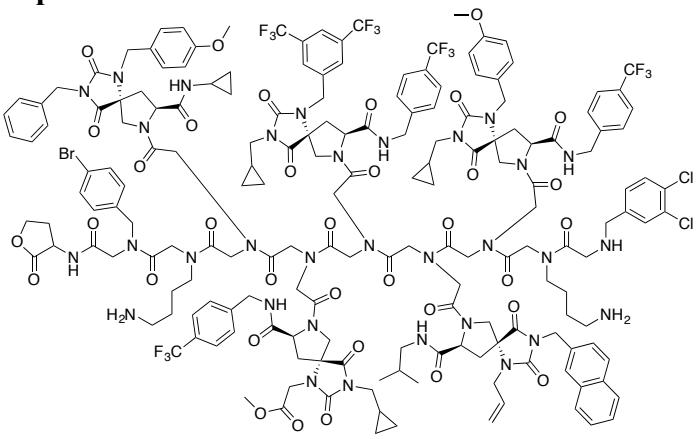
**Peptoid SPH-4.5**



35 mg of Tentagel-$NH_2$ (S) resin was rinsed multiple times with DCM and DMF. 3 equiv of Fmoc-Met-OH and HATU in 0.6 mL of NMP with 6 equiv of DIPEA were then added to the resin and stirred for 1 hour. The resin was treated with 20% piperidine in DMF 2x15 min, and rinsed with DMF and DCM. 400 uL of 1.3 M Bromoacetic acid in DMF was reacted with 100 uL of DIC for 5 minutes, added to the resin, and allowed to react for 30 min. The resin was drained and washed repeatedly with DCM and DMF, after which 0.2 mL of a 1M solution of 4-bromobenzylamine was added to the resin and stirred for 1 h (standard peptoid coupling). Another round of standard peptoid coupling was used to add N-Boc-1,4-diaminobutane. 400 uL of 1.3 M Bromoacetic acid in DMF was reacted with 100 uL of DIC for 5 minutes, added to the resin, and allowed to react for 30 min. The resin was drained and washed repeatedly with DCM and DMF, after which 150 mM **4.14** (3 equiv, previously deprotected) in DMF was added to the resin and stirred overnight (standard spiroligomer-peptoid synthesis). This standard spiroligomer-peptoid synthesis was repeated for addition of amines **4.8, 4.10, 4.11,** and **4.12**. Standard peptoid coupling was used to add another N-Boc-1,4-diaminobutane, followed by a 3,4-dichlorobenzylamine residue. The resin was washed repeatedly with DMF and DCM, then treated with 1:1 TFA/DCM to remove the Boc protecting groups. The resin was washed exhaustively with water, then treated with a 7:3 Formic Acid/$H_2O$ mixture containing 30 mg of cyanogen bromide to cleave the peptoid from the resin, which was purified with

reverse phase flash chromatography (5-100% acetonitrile in water, 0.1% formic acid modifier), to yield 3.7 mg of purified peptoid **SPH-4.5.** HRMS-ESI for $C_{181}H_{198}BrCl_2F_{15}N_{32}O_{35}$ Target Mass: 3813.3018, Found: 3813.3135

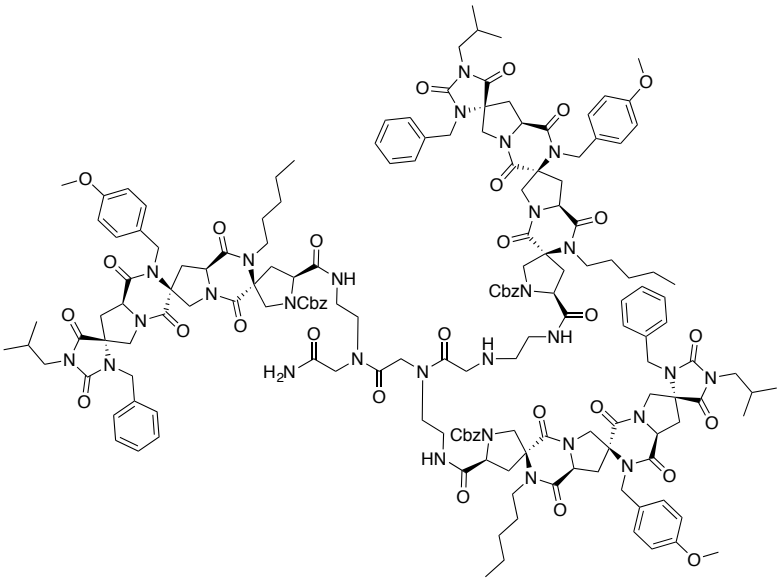
**Peptoid SPH-4.6**



35 mg of Tentagel-$NH_2$ (S) resin was rinsed multiple times with DCM and DMF. 3 equiv of Fmoc-Met-OH and HATU in 0.6 mL of NMP with 6 equiv of DIPEA were then added to the resin and stirred for 1 hour. The resin was treated with 20% piperidine in DMF 2x15 min, and rinsed with DMF and DCM. 400 uL of 1.3 M Bromoacetic acid in DMF was reacted with 100 uL of DIC for 5 minutes, added to the resin, and allowed to react for 30 min. The resin was drained and washed repeatedly with DCM and DMF, after which 0.2 mL of a 1M solution of 4-bromobenzylamine was added to the resin and stirred for 1 h (standard peptoid coupling). Another round of standard peptoid coupling was used to add N-Boc-1,4-diaminobutane. 400 uL of 1.3 M Bromoacetic acid in DMF was reacted with 100 uL of DIC for 5 minutes, added to the resin, and allowed to react for 30 min. The resin was drained and washed repeatedly with DCM and DMF, after which 150 mM **4.14** (3 equiv, previously deprotected) in DMF was added to the resin and stirred overnight (standard spiroligomer-peptoid synthesis). This standard spiroligomer-peptoid synthesis was repeated for addition of amines **4.12, 4.11, 4.8** and **4.10**. Standard peptoid coupling was used to add another N-Boc-1,4-diaminobutane, followed by a 3,4-dichlorobenzylamine residue. The resin was washed repeatedly with DMF and DCM, then treated with 1:1 TFA/DCM to remove the Boc protecting groups.

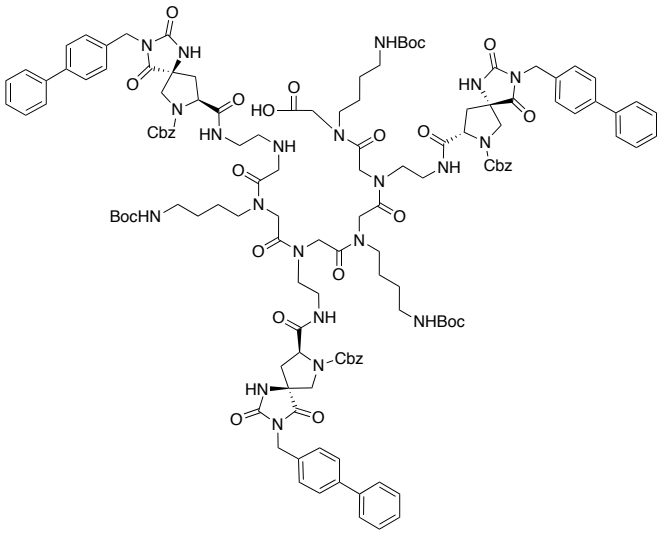
The resin was washed exhaustively with water, then treated with a 7:3 Formic Acid/$H_2O$ mixture containing 30 mg of cyanogen bromide to cleave the peptoid from the resin. This was purified with reverse phase flash chromatography (5-100% acetonitrile in water, 0.1% formic acid modifier), to yield 3.9 mg of purified peptoid **SPH-4.6.** HRMS-ESI for $C_{181}H_{198}BrCl_2F_{15}N_{32}O_{35}$ Target Mass: 3813.3018, Found: 3813.2888

**Peptoid SPH-4.7**



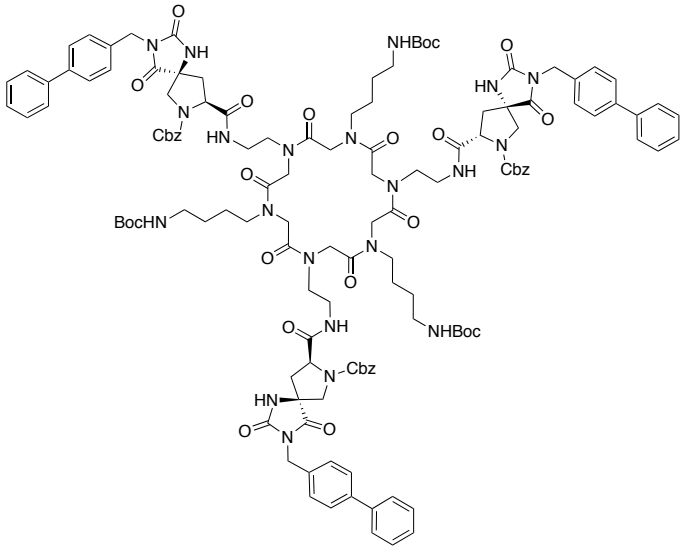
15 mg of Rink amide resin was swelled with DCM, and then treated with 20% Piperidine in DMF 2x15 min, and subsequently rinsed with DCM and DMF. 400 uL of 1.3 M Bromoacetic acid in DMF was reacted with 100 uL of DIC for 5 minutes, added to the resin, and allowed to react for 30 min. The resin was drained and washed repeatedly with DCM and DMF, after which 150 mM **4.21** (3 equiv, previously deprotected) in DMF was added to the resin and stirred overnight. The resin was drained into a small vial containing 1.05 equiv of $Boc_2O$ (relative to the mmol of amine) to reprotect the spiroligomer trimer. This peptoid coupling was repeated with amine **4.21** two more times, the resin washed exhaustively with DMF and DCM, then treated with neat TFA, followed by 1:1 TFA/DCM, and finally DCM to give 7.5 mg of crude **SPH-4.7**. HRMS-ESI for $C_{165}H_{198}N_{28}O_{33}$ Target Mass: 3099.4676; Found: 3099.4803

**Synthesis of Peptoid 4.62**



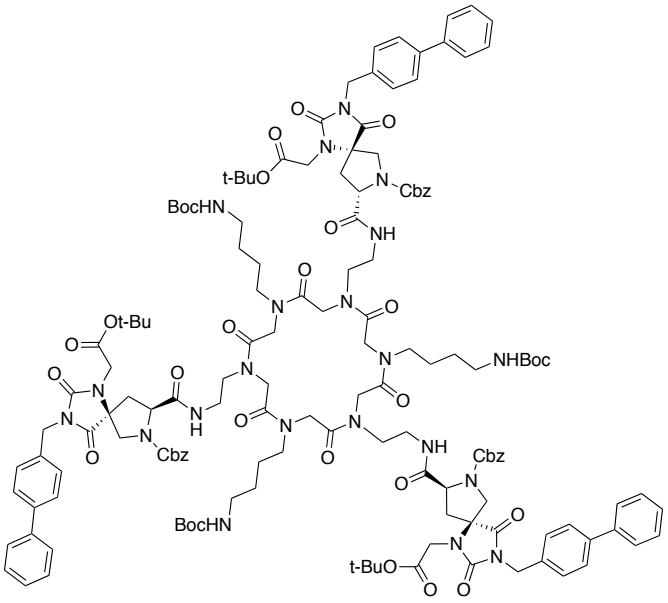

125 mg 2-Cl-Trityl Chloride resin was reacted with 0.138 M bromoacetic acid in DCM for 30 min to load the bromoacetic acid on the resin, which was subsequently washed with DCM and DMF. 10 equiv of Amine **4.57** in DMF (500 mM) was then added to the resin and stirred for 2 h. The resin was drained and rinsed once with DMF into a round bottom flask containing 1.05 equiv of $Boc_2O$ (relative to the amount of amine) to reprotect the primary amine. The resin was then rinsed repeatedly with DMF and DCM to the waste stream. 1600 uL of 1.3 M Bromoacetic acid in DMF was reacted with 400 uL of DIC for 5 minutes, added to the resin, and allowed to react for 30 min. The resin was drained and washed repeatedly with DCM and DMF, after which 10 equiv of N-Boc-1,4-diaminobutane (1 M) was added to the resin, and stirred for one hour. These two steps were repeated two more times to give the peptoid 6-mer **4.62**, which was cleaved from the resin with 30% HFIP in DCM and then purified by reverse phase flash chromatography (0-100% acetonitrile in water, 0.1% formic acid modifier), purified yield 198 mg. HRMS-ESI for $C_{129}H_{155}N_{21}O_{28}$ Target Mass: 2446.1350; Found 2446.1397

**Macrocyclization of 4.62 to give peptoid macrocycle SPH-4.8**



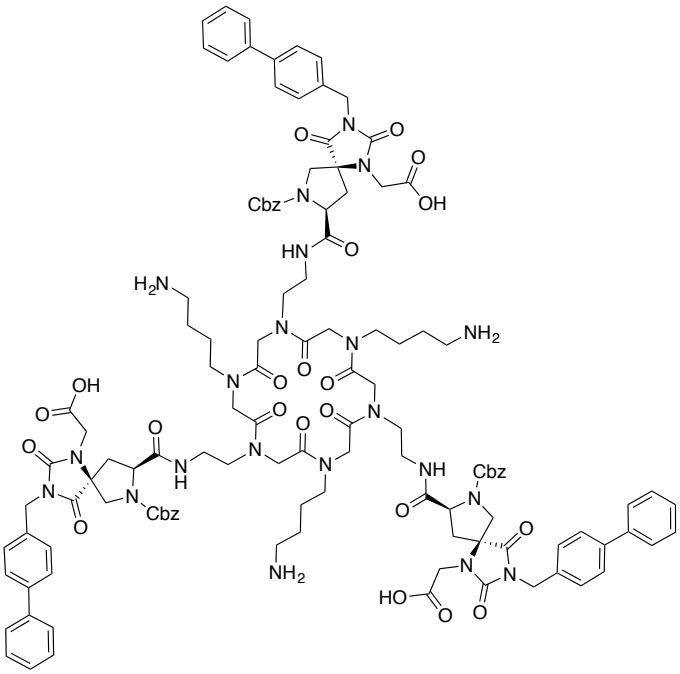
In a RB flask, 109 mg of PyAOP was dissolved in 12 mL of dry DMF along with 72.8 uL of DIPEA. 170 mg of Peptoid **4.62** was dissolved in 2 mL of DCM, and added to the coupling reagent dropwise. The reaction was allowed to proceed at room temperature for 1 h, at which time the reaction was diluted with EtOAc, and washed with saturated solutions of $NH_4Cl$, $NaHCO_3$, and brine, dried over $Na_2SO_4$, and the solvent removed *in vacuo*. The macrocycle was purified by reverse phase flash chromatography (0-100% acetonitrile in water, 0.1% formic acid modifier), purified yield 149 mg. HRMS-ESI for $C_{129}H_{155}N_{21}O_{28}$ Target Mass: 2428.1245; Found 2428.1231

**Peptoid macrocycle SPH-4.9**



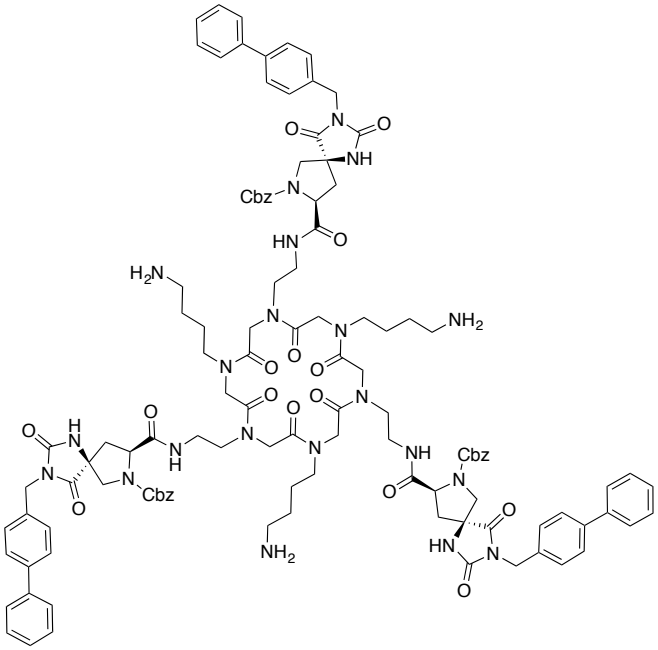

In a 25 mL RB flask, 40 mg of **SPH-4.8** was dissolved in 5 mL of DMF, to which 8.3 mg of ground $K_2CO_3$ and 7.97 uL *tert*-butyl bromoacetate. The reaction was stirred overnight, then analyzed via HPLC-MS, which showed complete conversion to the product. The reaction was diluted with EtOAc, and washed with saturated solutions of $NH_4Cl$ and brine, dried over $Na_2SO_4$, and the solvent removed *in vacuo*. 43 mg recovered (96% yield) HRMS-ESI for $C_{147}H_{183}N_{21}O_{33}$ Target Mass: 2770.3287; Found 2770.3294

**Peptoid macrocycle SPH-4.10**



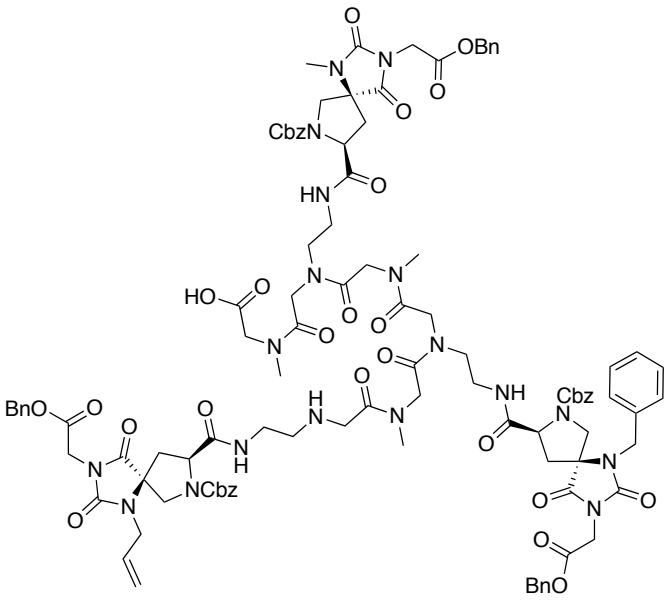
43 mg of **SPH-4.9** was treated with 95:4:1 TFA/$H_2O$/TIPS for 1.0 h to remove both the Boc and *t*-Bu protecting groups, at which time the solvent was removed *in vacuo* and the molecule placed on a high vacuum pump. **SPH-10** was recovered in quantitative yield. HRMS-ESI for $C_{120}H_{135}N_{21}O_{27}$ Target Mass: 2301.9836; Found 2301.9902

**Peptoid Macrocycle SPH-4.11**



32 mg of **SPH-4.8** was treated with 1:1 TFA/DCM for 15 min to remove the Boc protecting groups, at which time the solvent was removed *in vacuo* and placed on a high vacuum pump. **SPH-11** was recovered in quantitative yield. HRMS-ESI for $C_{114}H_{129}N_{21}O_{21}$ Target Mass: 2127.9672; Found 2127.9577

**Synthesis of Peptoid 4.67**

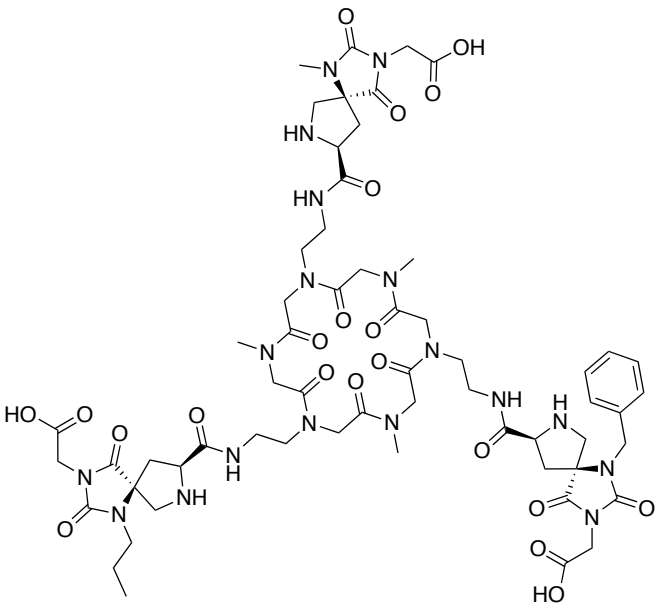


140 mg of 2-Cl-Trityl Chloride resin was reacted with Fmoc-Sar-OH and DIPEA in DCM for 30 min to load the Fmoc-Sar-OH on the resin, which was subsequently deprotected with 20% Piperidine in DMF, and washed with DCM and DMF. 1600 uL of 1.3 M Bromoacetic acid in DMF was reacted with 400 uL of DIC for 5 minutes, added to the resin, and allowed to react for 30 min. The resin was drained and washed repeatedly with DCM and DMF, after which 10 equiv of Amine **4.51** in DMF (500 mM) was then added to the resin and stirred for 2 h. The resin was drained and rinsed once with DMF into a round bottom flask containing 1.05 equiv of $Boc_2O$ (relative to the amount of amine) to reprotect the primary amine. The resin was then rinsed repeatedly with DMF and DCM to the waste stream. Fmoc-Sar-OH was then coupled to the reaction utilizing standard SPPS protocols. The steps from Fmoc-Deprotection through the coupling of the primary amine to the resin were repeated two more times (using amines **4.52** and then **4.53**) to give the peptoid 6-mer **4.62**, which was cleaved from the resin with 30% HFIP in DCM, and then purified by reverse phase flash chromatography (0-100% acetonitrile in water, 0.1% formic acid modifier), purified yield 130 mg. HRMS-ESI for $C_{104}H_{116}N_{18}O_{28}$ Target Mass: 2064.8206; Found 2064.8119

**Macrocyclization of 4.67 to give peptoid macrocycle SPH-4.12**

In a RB flask, 41 mg of PyAOP was dissolved in 5.2 mL of dry DMF along with 27.0 uL of DIPEA. 54 mg of Peptoid **4.62** was dissolved in 1.0 mL of DCM, and added to the coupling reagent dropwise. The reaction was allowed to proceed at room temperature for 1 h, at which time the reaction was diluted with EtOAc, and washed with saturated solutions of $NH_4Cl$, $NaHCO_3$, and brine, dried over $Na_2SO_4$, and the solvent removed *in vacuo*. The macrocycle was purified by reverse phase flash chromatography (0-100% acetonitrile in water, 0.1% formic acid modifier), purified yield 49 mg. HRMS-ESI for $C_{104}H_{114}N_{18}O_{27}$ Target Mass: 2046.8101; Found 2046.8170

**Peptoid macrocycle SPH-4.13**



8 mg of Pd/C was added to a 25 mL egg shaped flask, followed by 1 mL of THF and 2.5 mL of water. **SPH-4.12** was dissolved in 1.5 mL of THF, and added to the flask, followed by 50 uL of formic acid. A $H_{2(g)}$ balloon was fitted to a three way valve, and attached to the flask. The valve was then hooked up to vacuum, and the flask purged repeatedly to ensure complete removal of any air. The reaction was stirred overnight, and then analyzed via HPLC-MS, which showed complete conversion to the product. The reaction was filtered through a small bed of celite, and the THF removed *in vacuo*. 1 mL of acetonitrile was added to the flask, which was then frozen with liquid nitrogen and lyophilized to give 36 mg of crude **SPH-4.13** as a white powder. **SPH-4.13** was then purified by reverse phase flash chromatography (0-100% acetonitrile in water, 0.1% formic acid modifier), Purified yield 7 mg. HRMS-ESI calcd for $C_{59}H_{80}N_{18}O_{21}H$ $(M+H)^+$ Target Mass: 1377.5818; Found 1377.5884

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acs.joc.6b01773–29.

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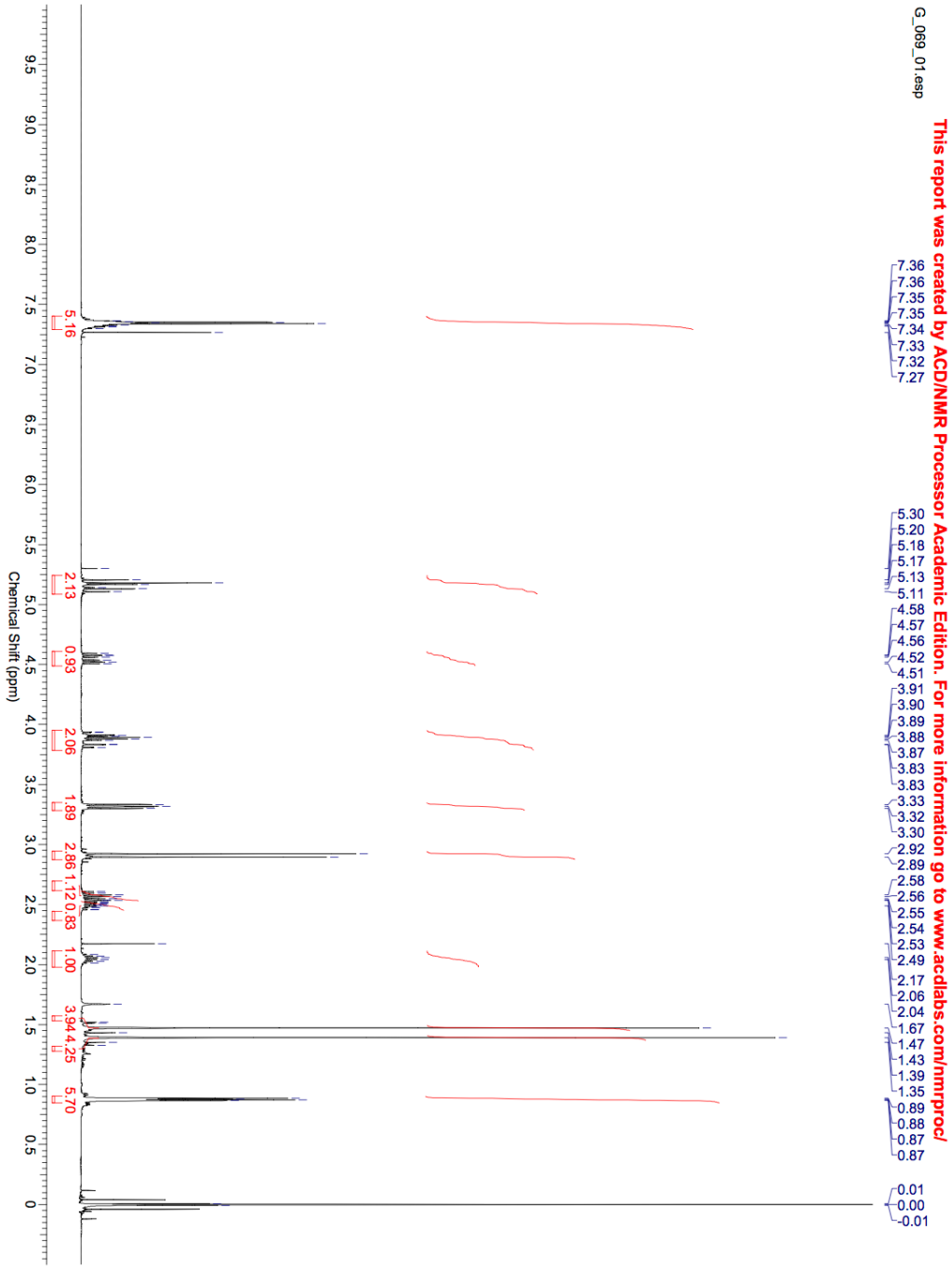
# APPENDIX A

## CHAPTER 2 NMR SPECTRA AND HPLC DATA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This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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7.35
7.35
7.34
7.33
7.32
7.27
5.30
5.20
5.18
5.17
5.13
5.11
4.58
4.57
4.56
4.52
4.51
3.91
3.90
3.89
3.88
3.87
3.83
3.83
3.33
3.32
3.30
2.92
2.89
2.58
2.56
2.55
2.54
2.53
2.49
2.17
2.06
2.04
1.67
1.47
1.43
1.39
1.35
0.89
0.88
0.87
0.87
0.01
0.00
-0.01
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2.06
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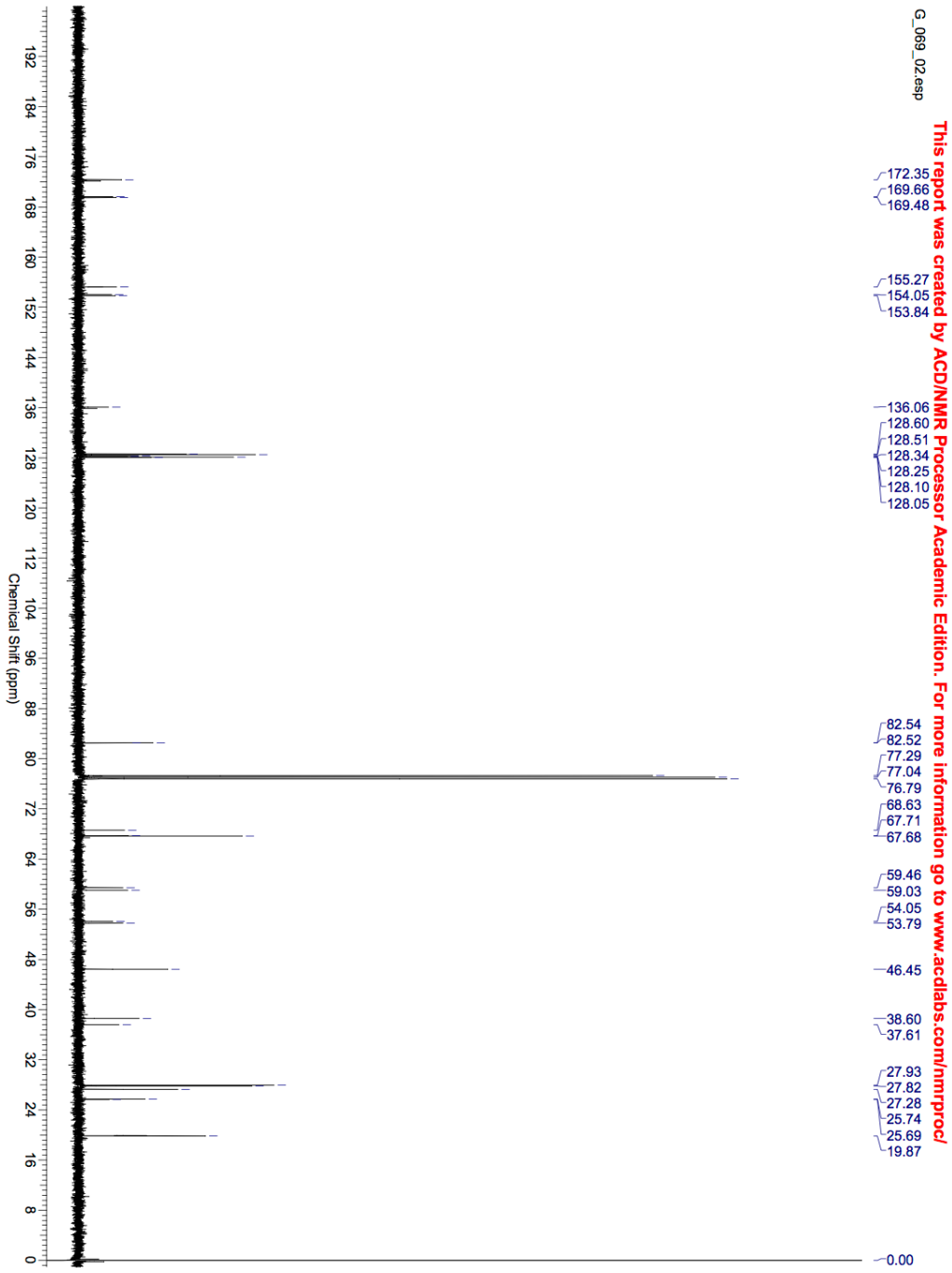
1.12
0.83
1.00
3.94
4.25
5.70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155.27
154.05
153.84
136.06
128.60
128.51
128.34
128.25
128.10
128.05
82.54
82.52
77.29
77.04
76.79
68.63
67.71
67.68
59.46
59.03
54.05
53.79
46.45
38.60
37.61
27.93
27.82
27.28
25.74
25.69
19.87
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
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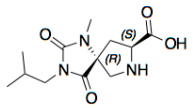
88
80
72
64
56
48
40
32
24
16
8
0

**2 – HPLC @ 254 nm**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NH
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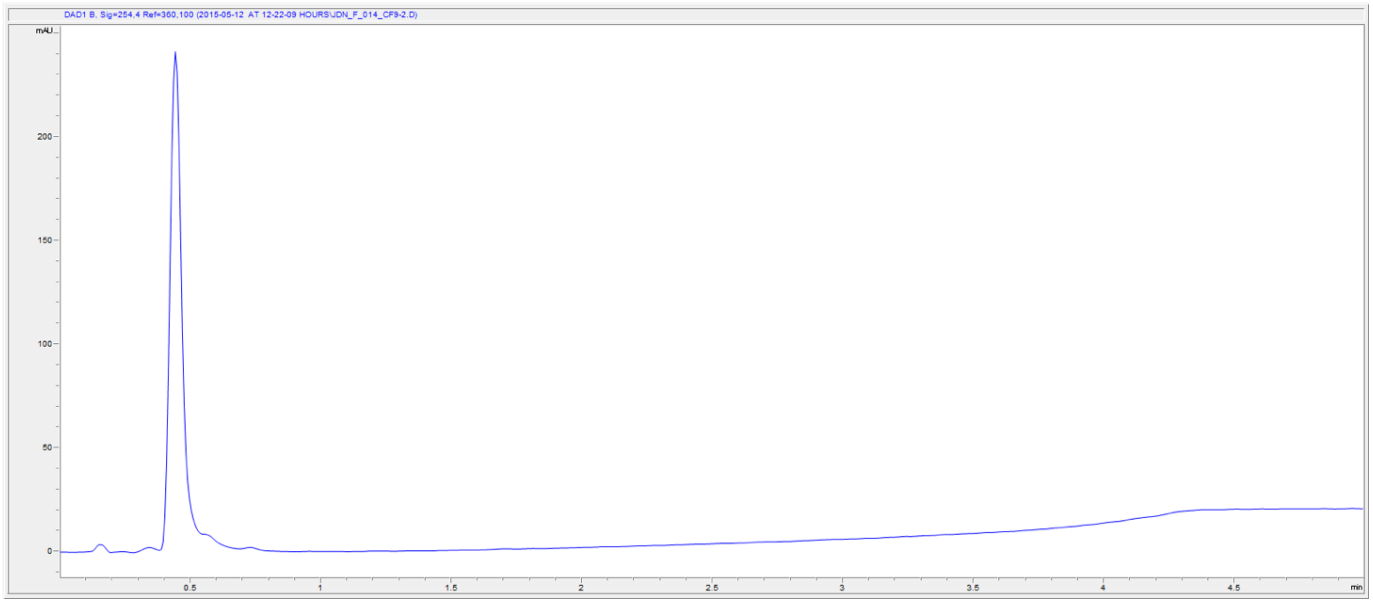
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100
50
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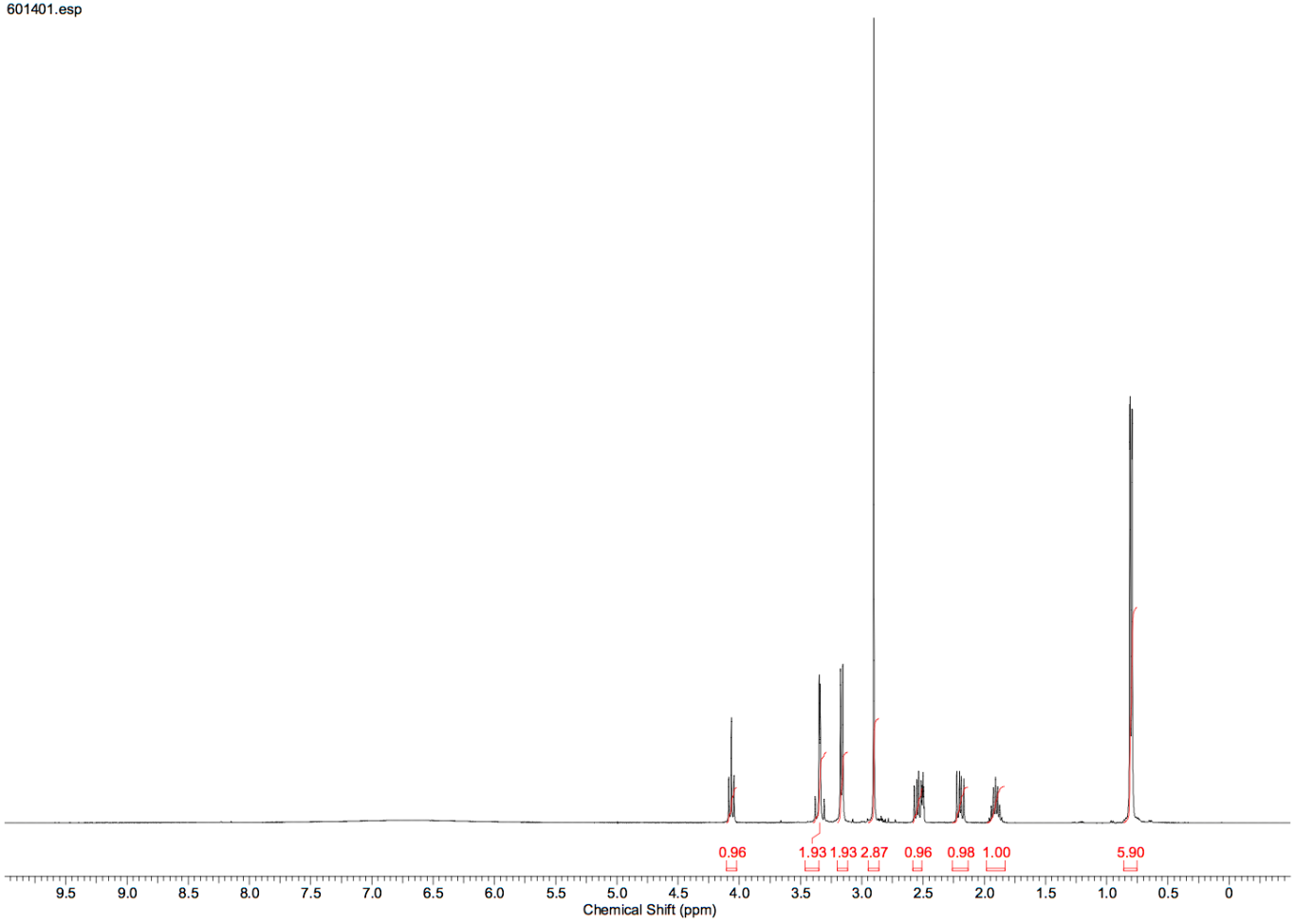
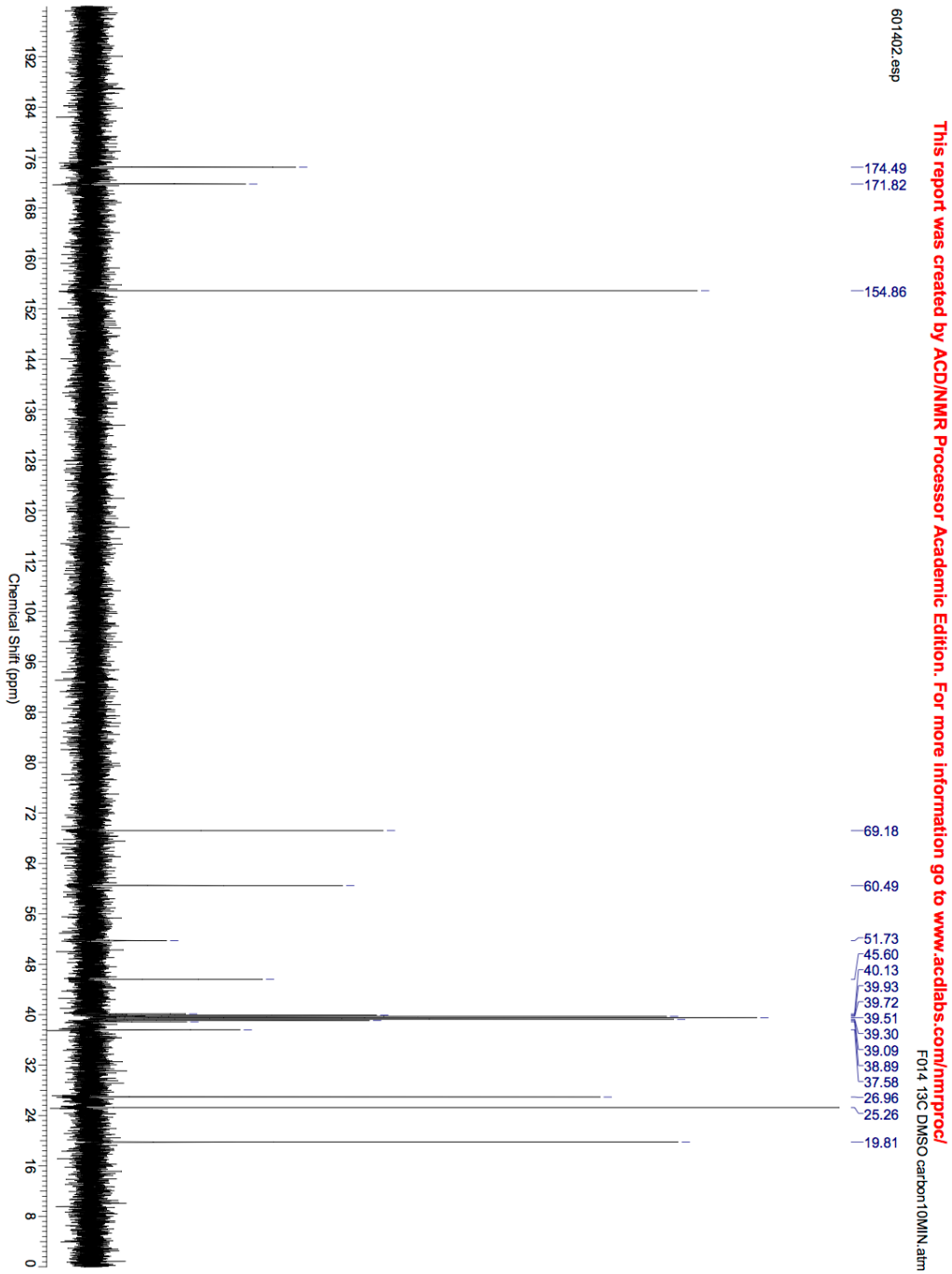
1.5
2
2.5
3
3.5
4
4.5
min

F014 1H DMSO proton.atm

601401.esp

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154.86
69.18
60.49
51.73
45.60
40.13
39.93
39.72
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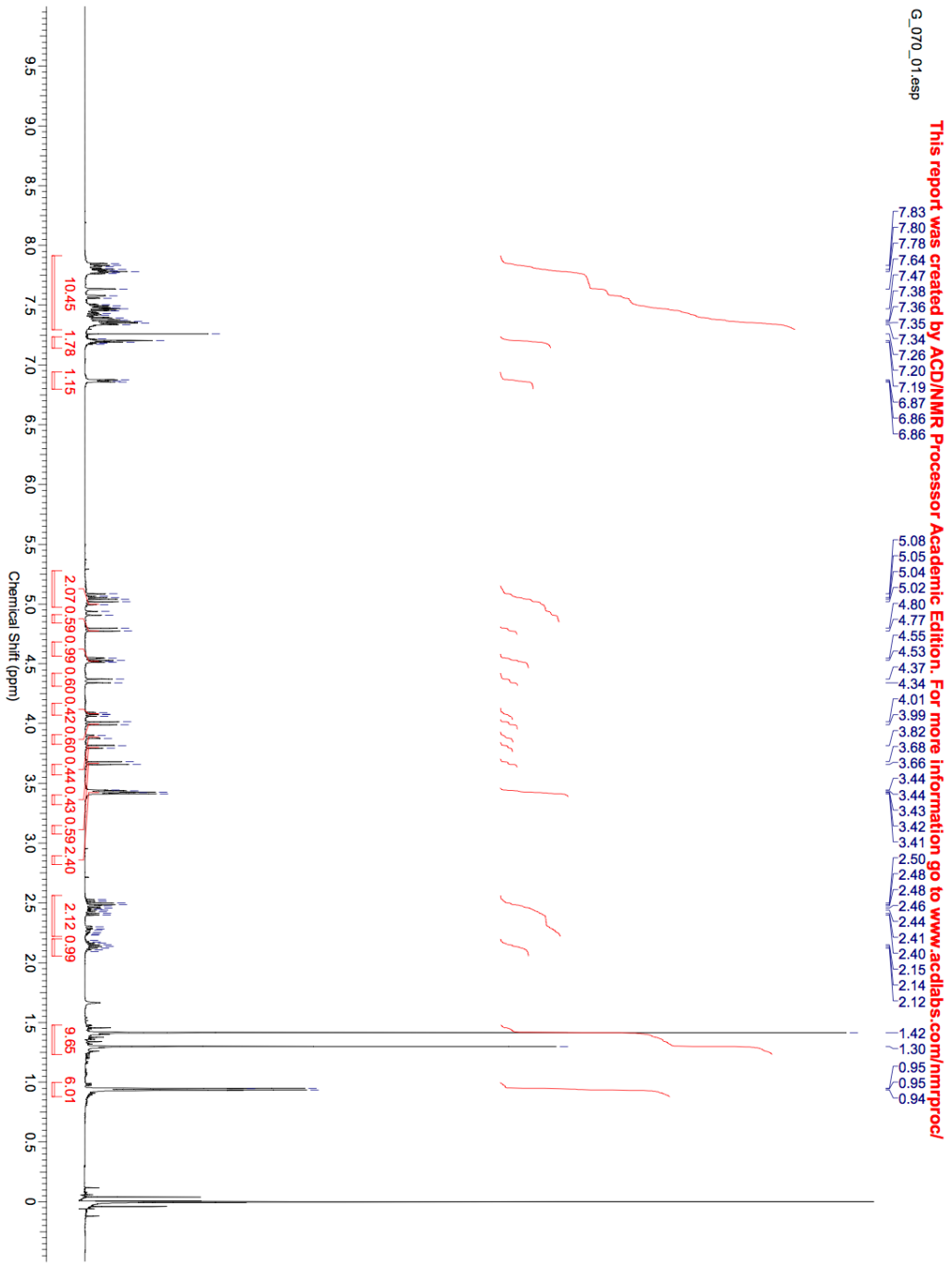
39.30
39.09
38.89
37.58
26.96
25.26
19.81
Chemical Shift (ppm)
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
F014 13C DMSO carbon10MIN.atm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7.26
7.20
7.19
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6.86
6.86
5.08
5.05
5.04
5.02
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4.77
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4.37
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4.01
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3.44
3.43
3.42
3.41
2.50
2.48
2.48
2.46
2.44
2.41
2.40
2.15
2.14
2.12
1.42
1.30
0.95
0.95
0.94
10.45
1.78
1.15
2.07
0.59
0.98
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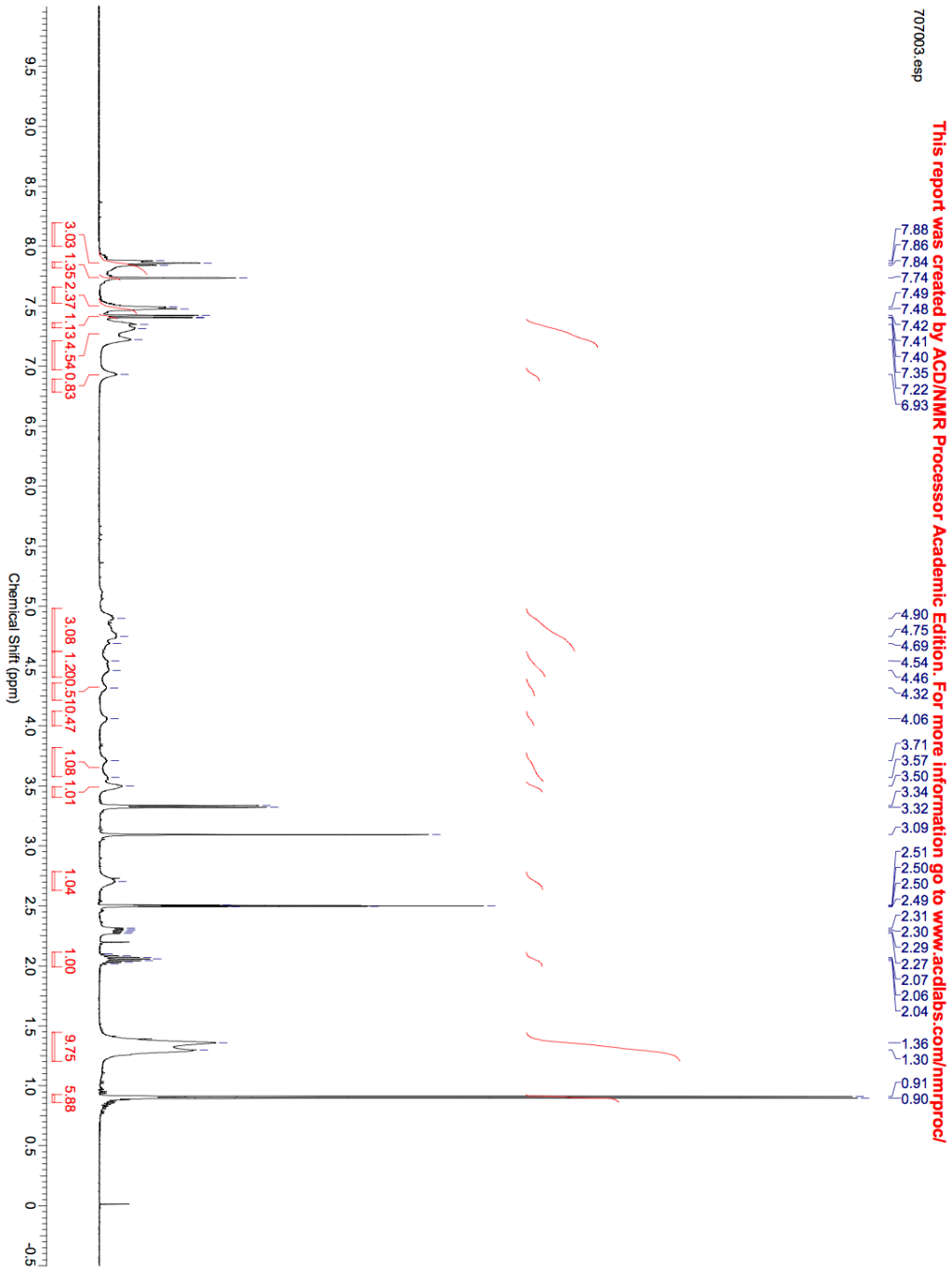
6.01
Chemical Shift (ppm)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7.22
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2.50
2.50
2.49
2.31
2.30
2.29
2.27
2.07
2.06
2.04
1.36
1.30
0.91
0.90
3.03
1.35
2.37
1.13
4.54
0.83
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1.08
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1.04
1.00
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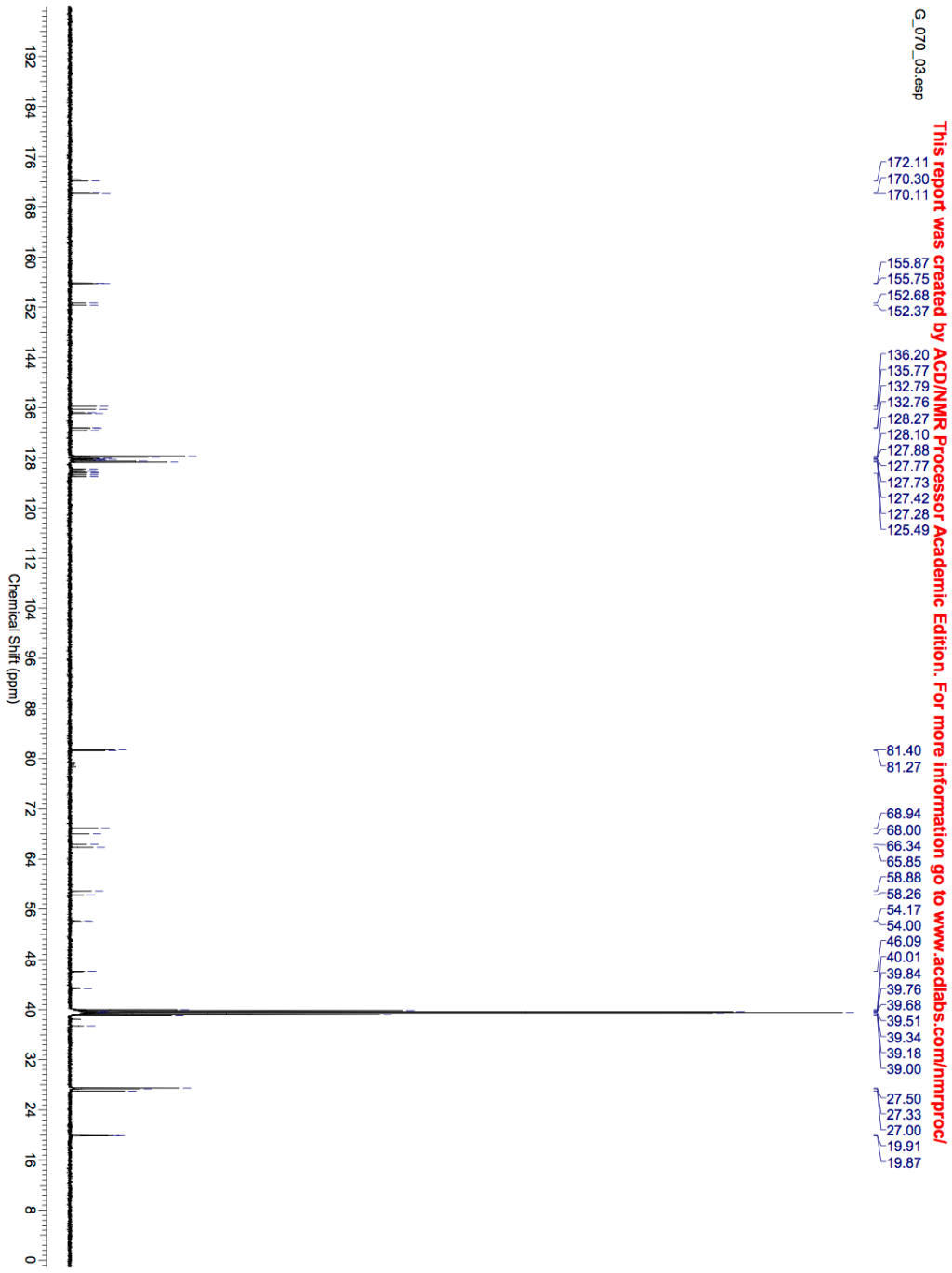
5.88
Chemical Shift (ppm)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152.37
136.20
135.77
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128.27
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40.01
39.84
39.76
39.68
39.51
39.34
39.18
39.00
27.50
27.33
27.00
19.91
19.87
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
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80
72
64
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32
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8
0

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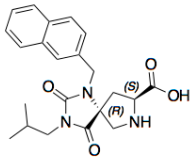
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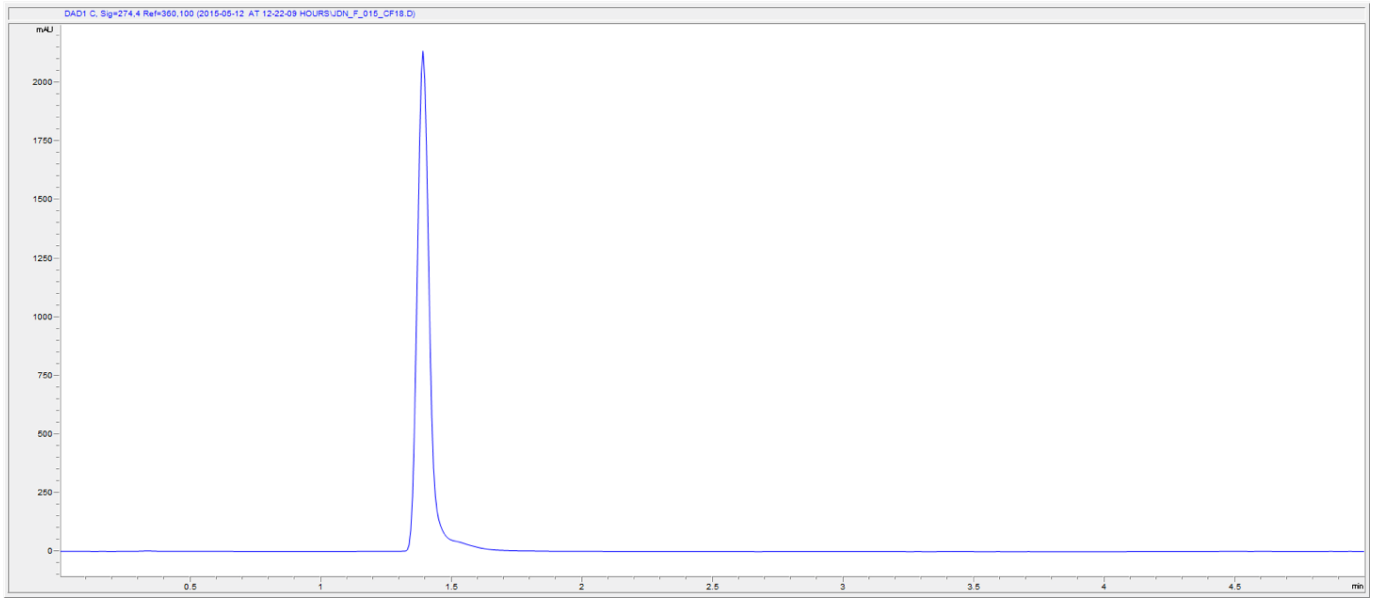
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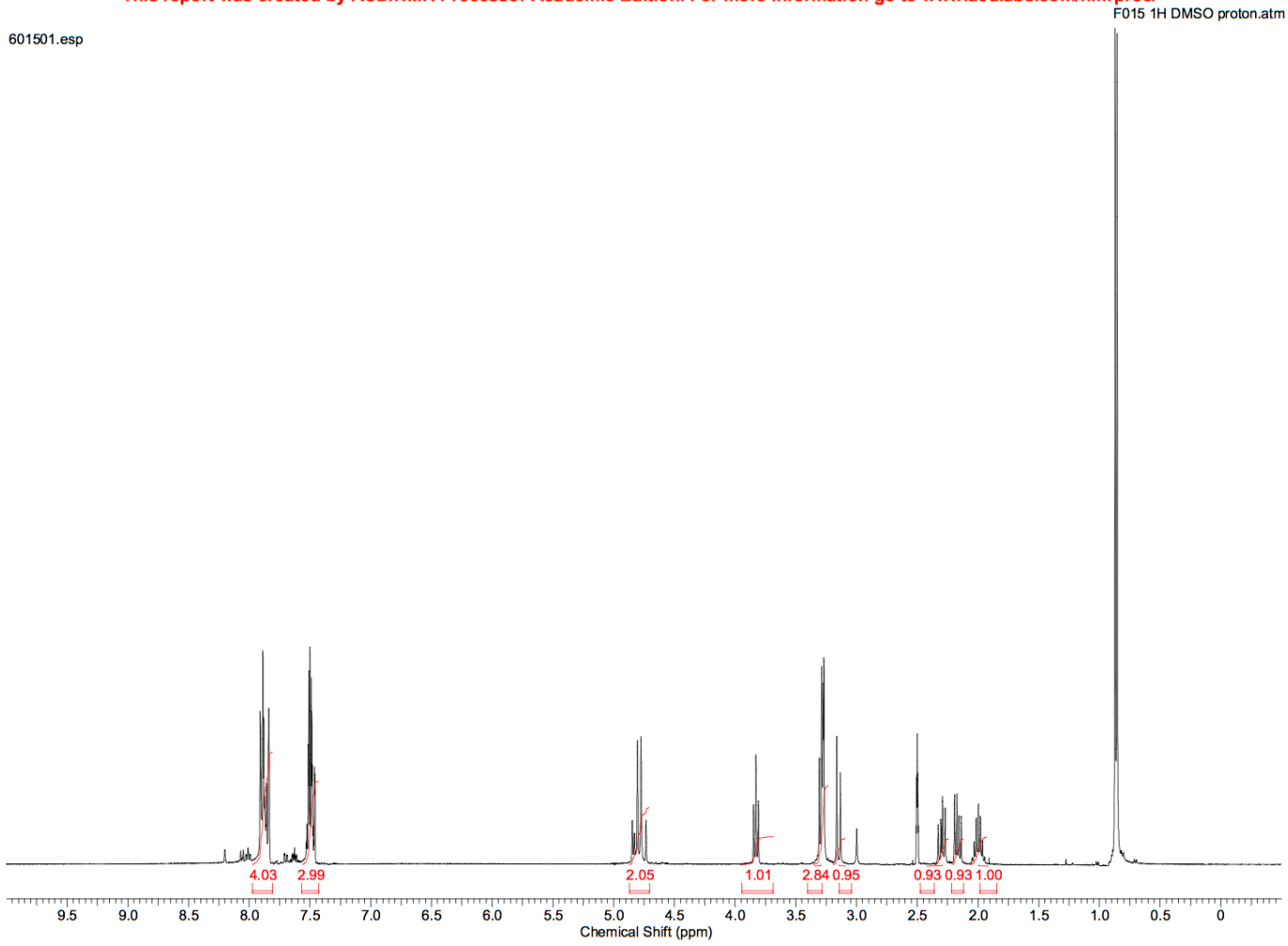
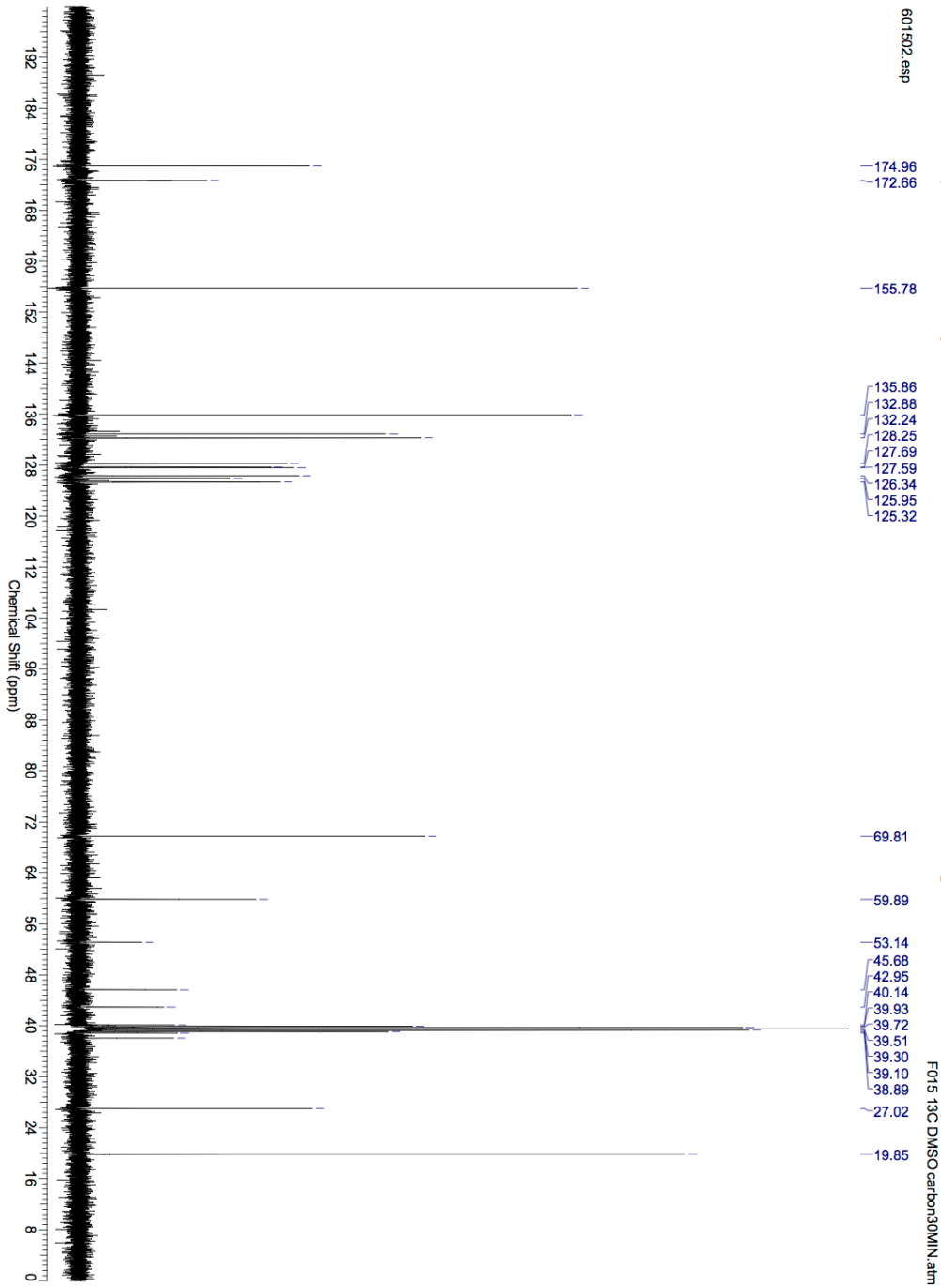
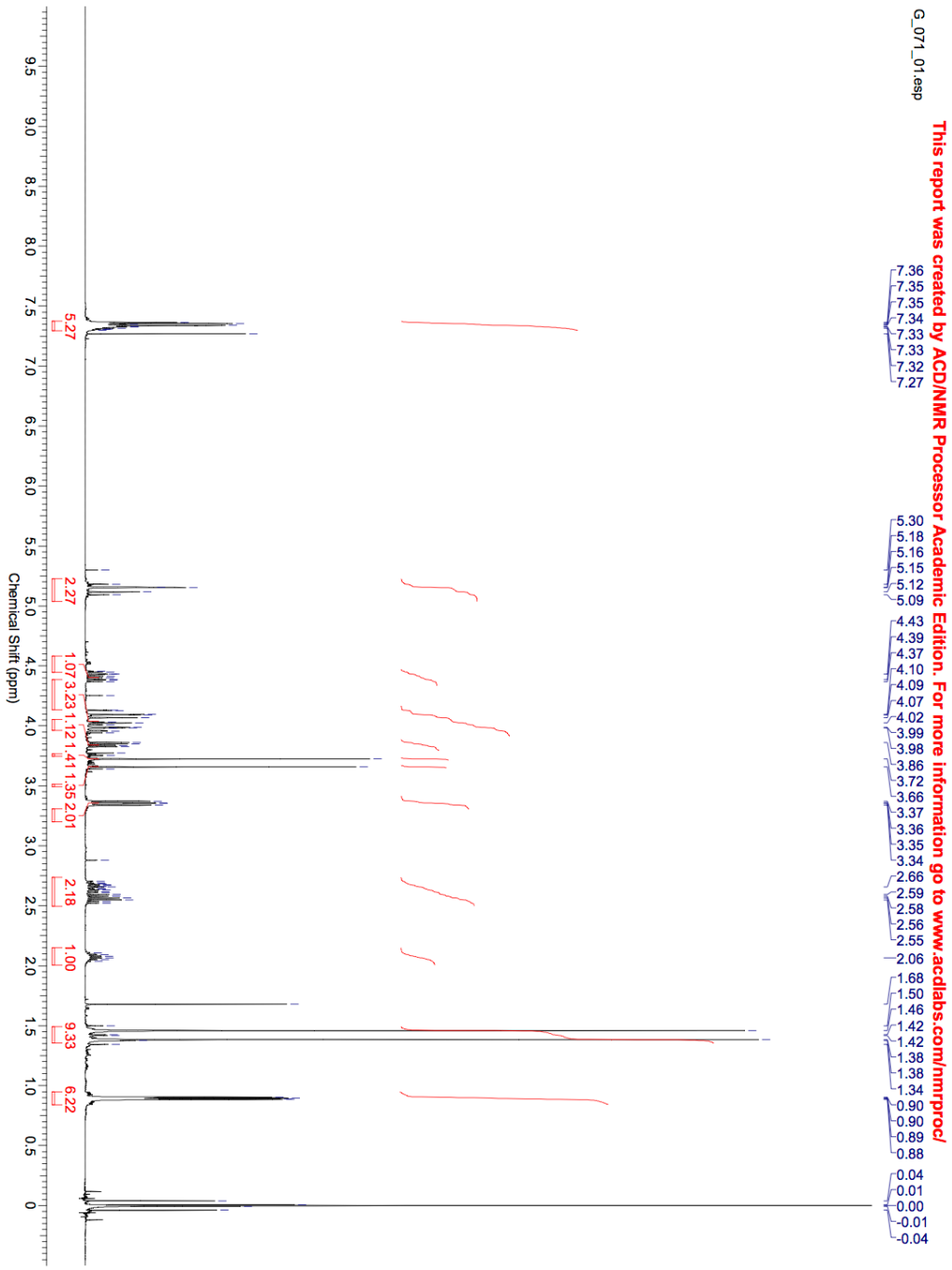
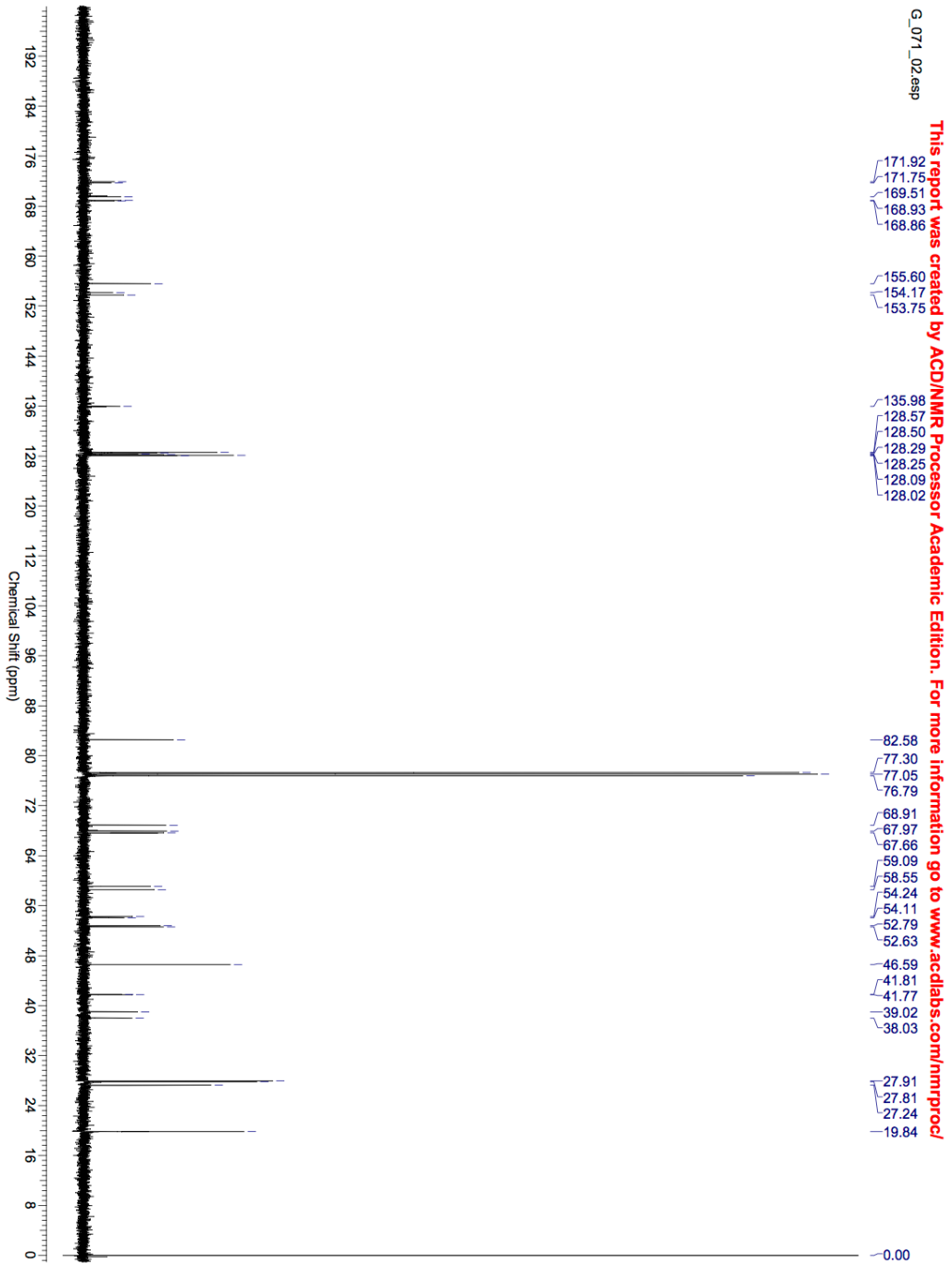
750
500
250
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
min

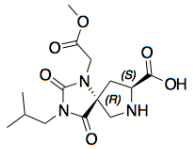


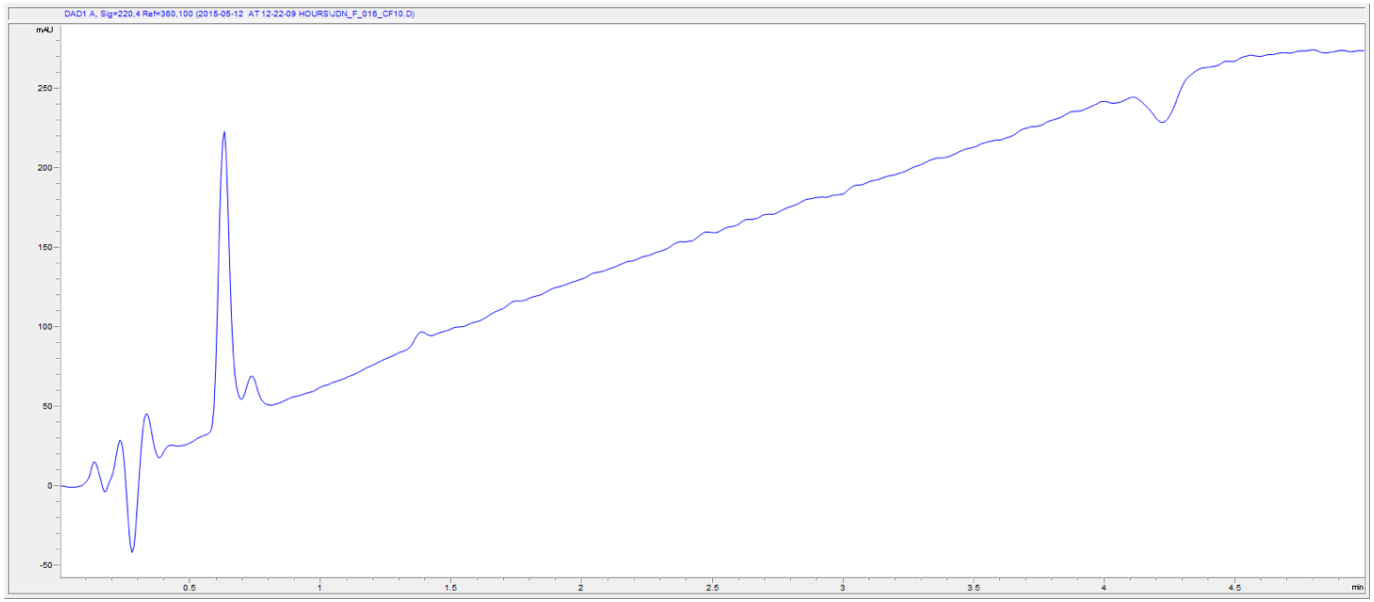

601502.esp
174.96
172.66
155.78
135.86
132.88
132.24
128.25
127.69
127.59
126.34
125.95
125.32
69.81
59.89
53.14
45.68
42.95
40.14
39.93
39.72
39.51
39.30
39.10
38.89
27.02
19.85
Chemical Shift (ppm)
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
F015 13C DMSO carbon30MIN.atm

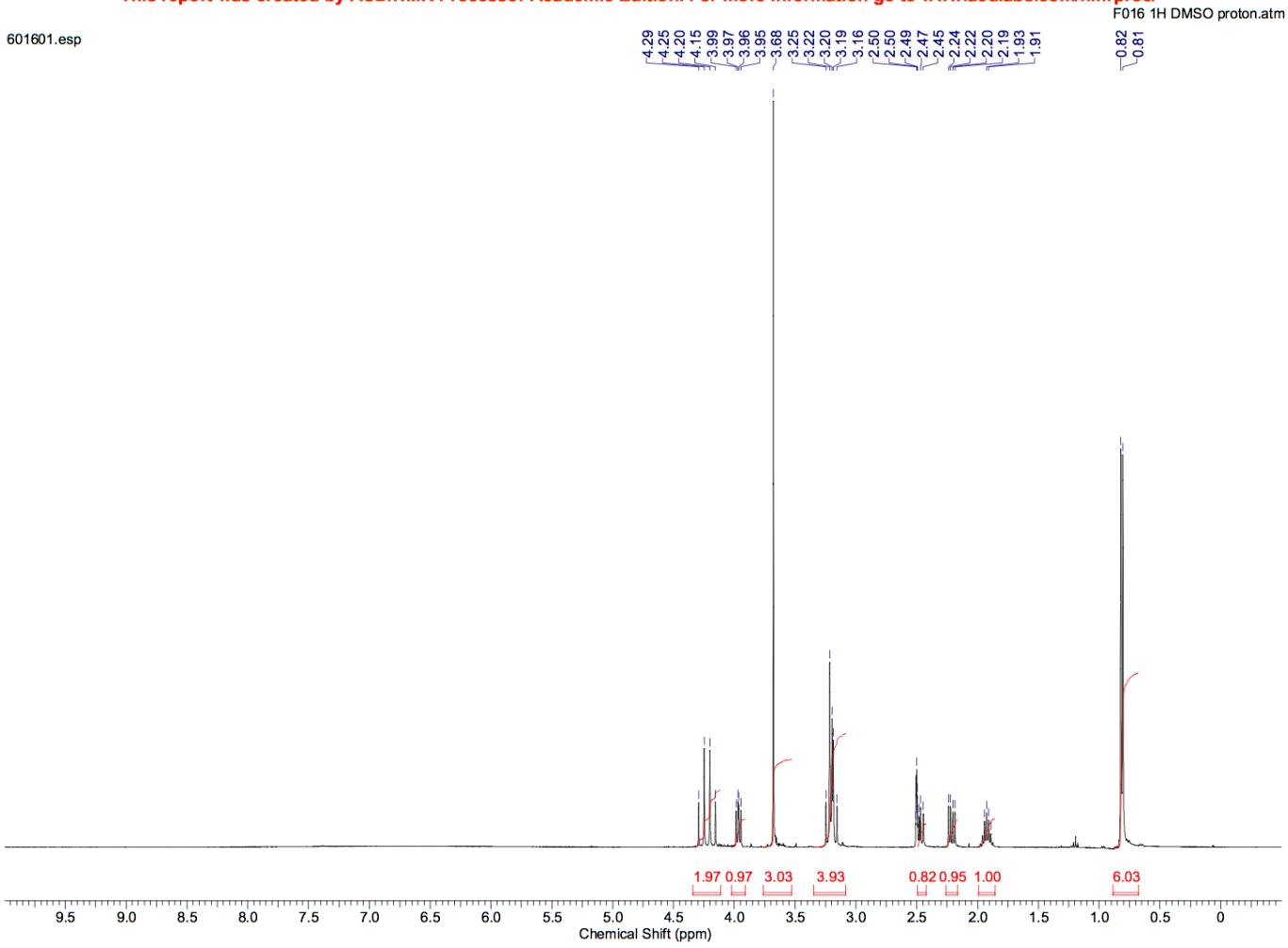

G_071_01.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
Chemical Shift (ppm)
5.27
2.27
1.07
3.23
1.12
1.41
1.35
2.01
2.18
1.00
9.33
6.22
7.36
7.35
7.35
7.34
7.33
7.33
7.32
7.27
5.30
5.18
5.16
5.15
5.12
5.09
4.43
4.39
4.37
4.10
4.09
4.07
4.02
3.99
3.98
3.86
3.72
3.66
3.37
3.36
3.35
3.34
2.66
2.59
2.58
2.56
2.55
2.06
1.68
1.50
1.46
1.42
1.42
1.38
1.38
1.34
0.90
0.90
0.89
0.88
0.04
0.01
0.00
-0.01
-0.04

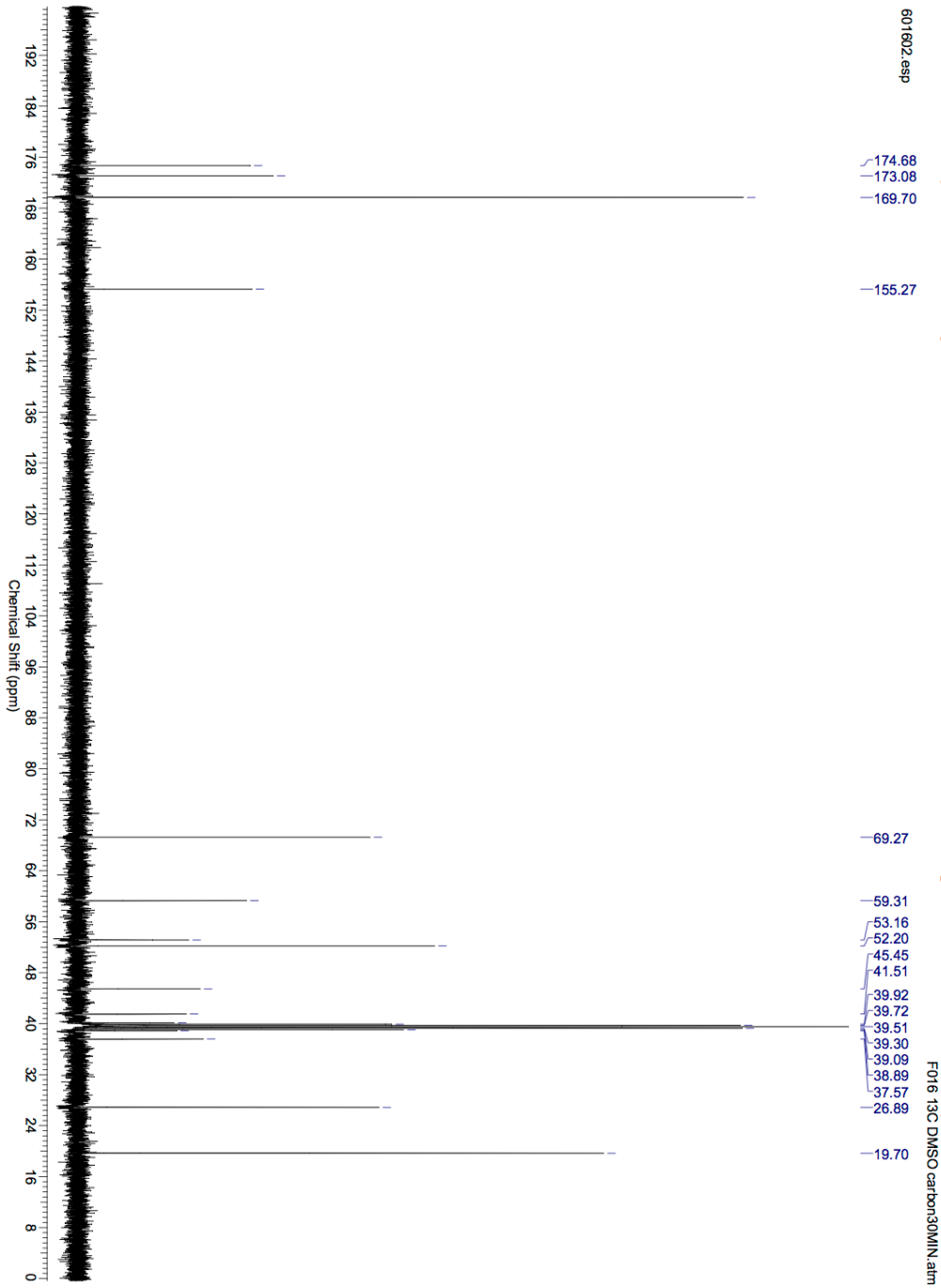

G_071_02.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
171.92
171.75
169.51
168.93
168.86
155.60
154.17
153.75
135.98
128.57
128.50
128.29
128.25
128.09
128.02
82.58
77.30
77.05
76.79
68.91
67.97
67.66
59.09
58.55
54.24
54.11
52.79
52.63
46.59
41.81
41.77
39.02
38.03
27.91
27.81
27.24
19.84
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0

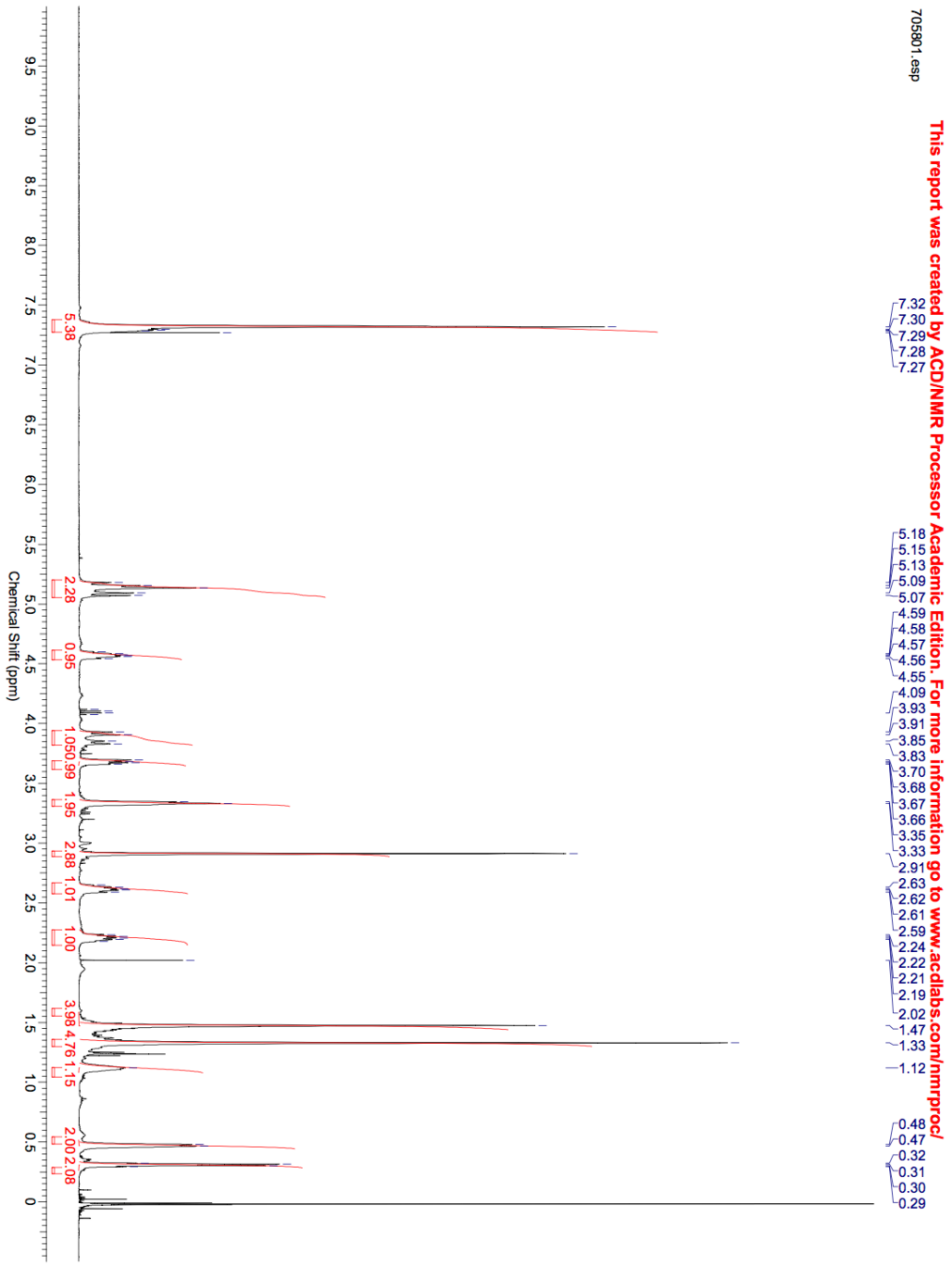
**4 – HPLC @ 220 nm**

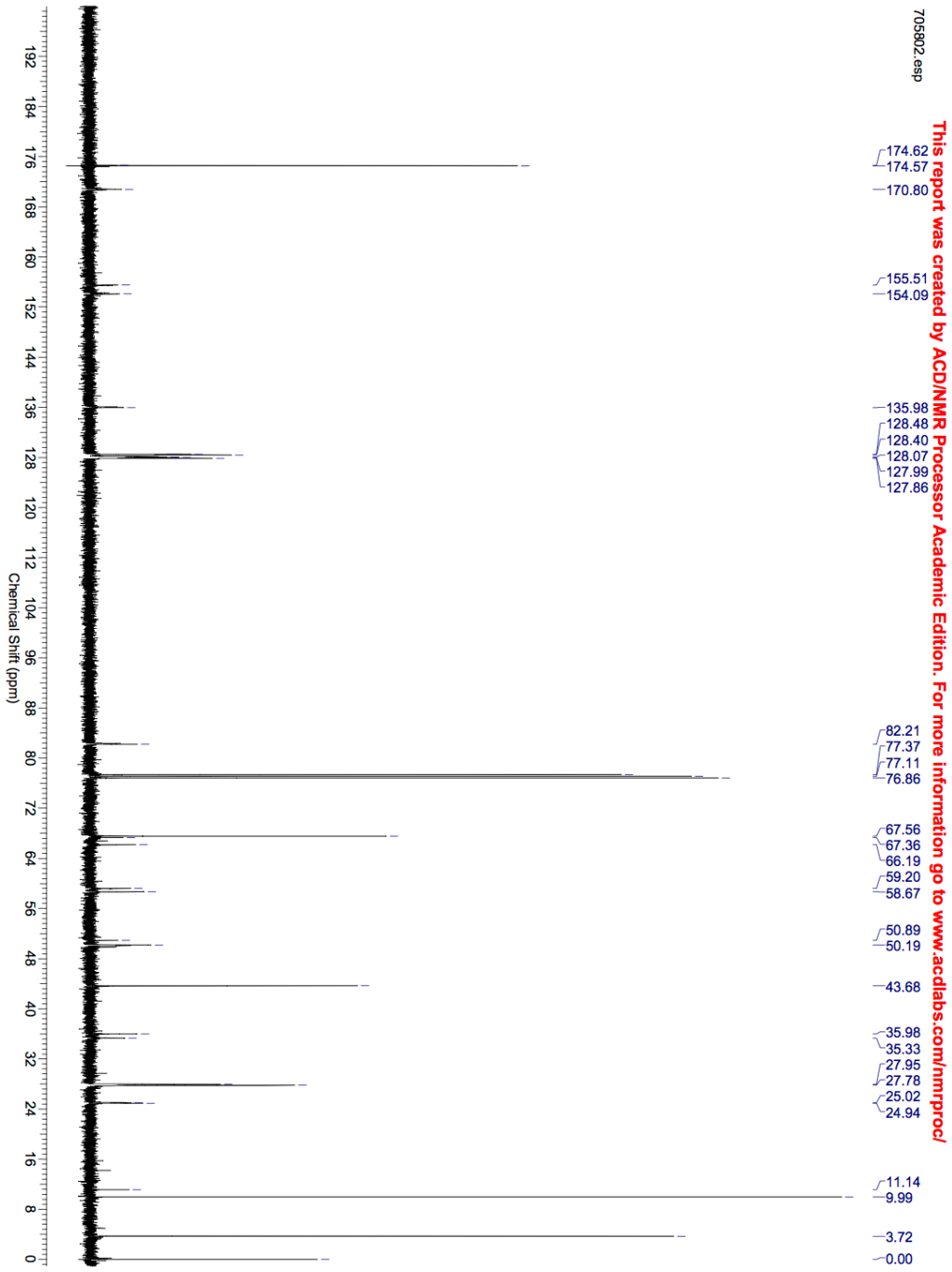

O
O
O
N
(S)
O
OH
(R)
NH
N
O


DAD1 A, Sig=220,4 Ref=360,100 (2015-05-12 AT 12-22-09 HOURS\JDN_F_016_CF10.D)
mAU
250
200
150
100
50
0
-50
0.5
1
1.5
2
2.5
3
3.5
4
4.5
min

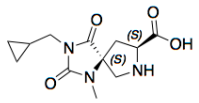


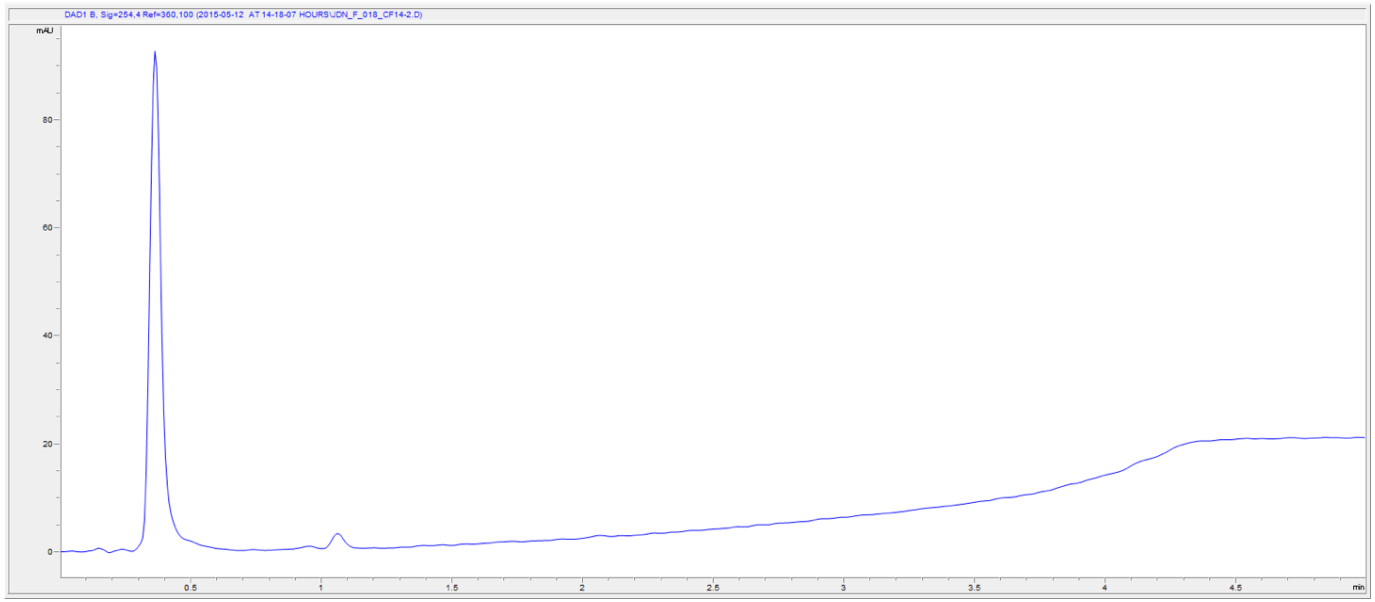

601602.esp
174.68
173.08
169.70
155.27
69.27
59.31
53.16
52.20
45.45
41.51
39.92
39.72
39.51
39.30
39.09
38.89
37.57
26.89
19.70
Chemical Shift (ppm)
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
F016 13C DMSO carbon30MIN.atm


705801.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
7.32
7.30
7.29
7.28
7.27
5.18
5.15
5.13
5.09
5.07
4.59
4.58
4.57
4.56
4.55
4.09
3.93
3.91
3.85
3.83
3.70
3.68
3.67
3.66
3.35
3.33
2.91
2.63
2.62
2.61
2.59
2.24
2.22
2.21
2.19
2.02
1.47
1.33
1.12
0.48
0.47
0.32
0.31
0.30
0.29
5.38
2.28
0.95
1.05
0.99
1.95
2.88
1.01
1.00
3.98
4.76
1.15
2.00
2.08
Chemical Shift (ppm)

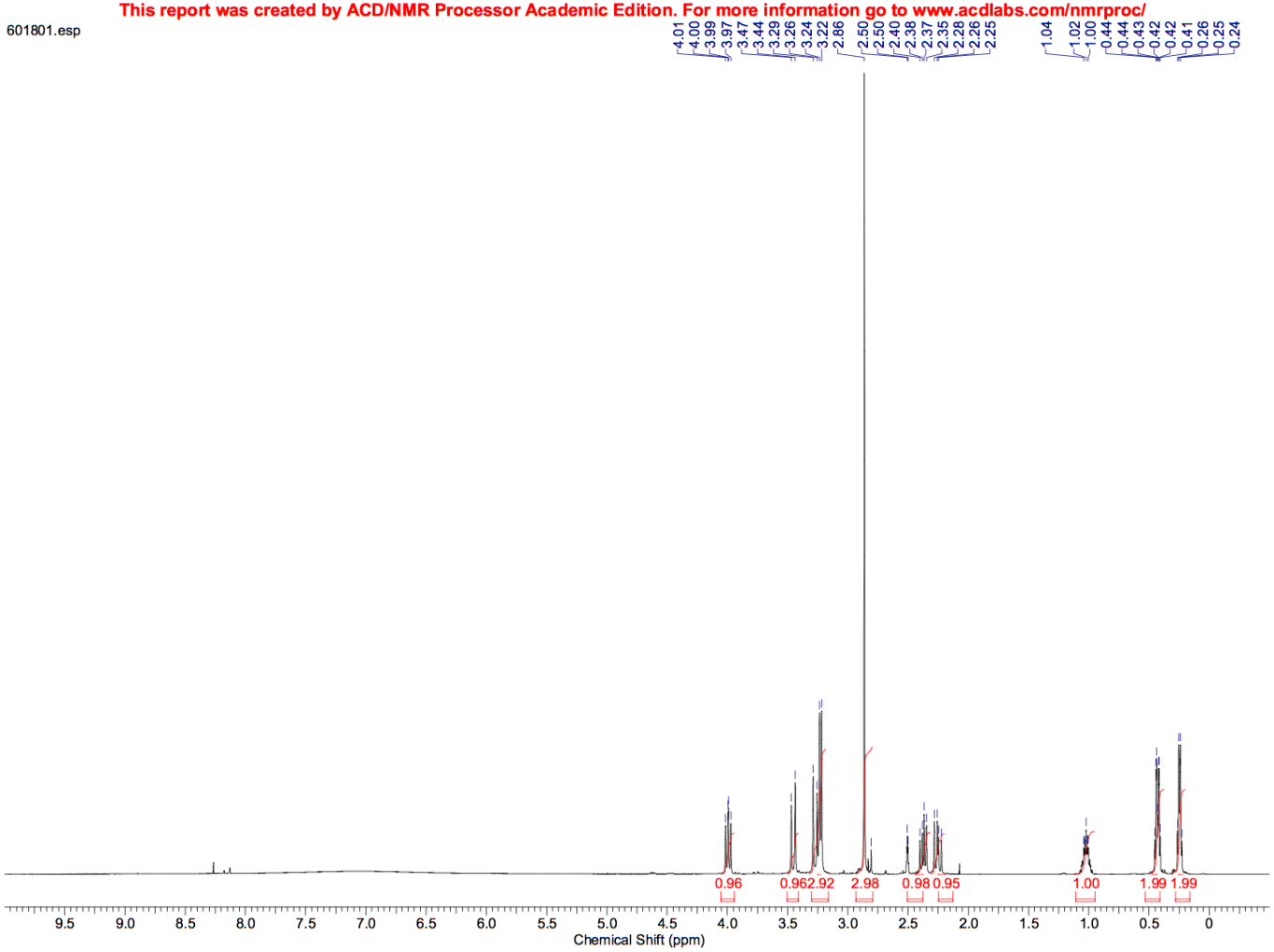

705802.esp
174.62
174.57
170.80
155.51
154.09
135.98
128.48
128.40
128.07
127.99
127.86
82.21
77.37
77.11
76.86
67.56
67.36
66.19
59.20
58.67
50.89
50.19
43.68
35.98
35.33
27.95
27.78
25.02
24.94
11.14
9.99
3.72
0.00
Chemical Shift (ppm)
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/

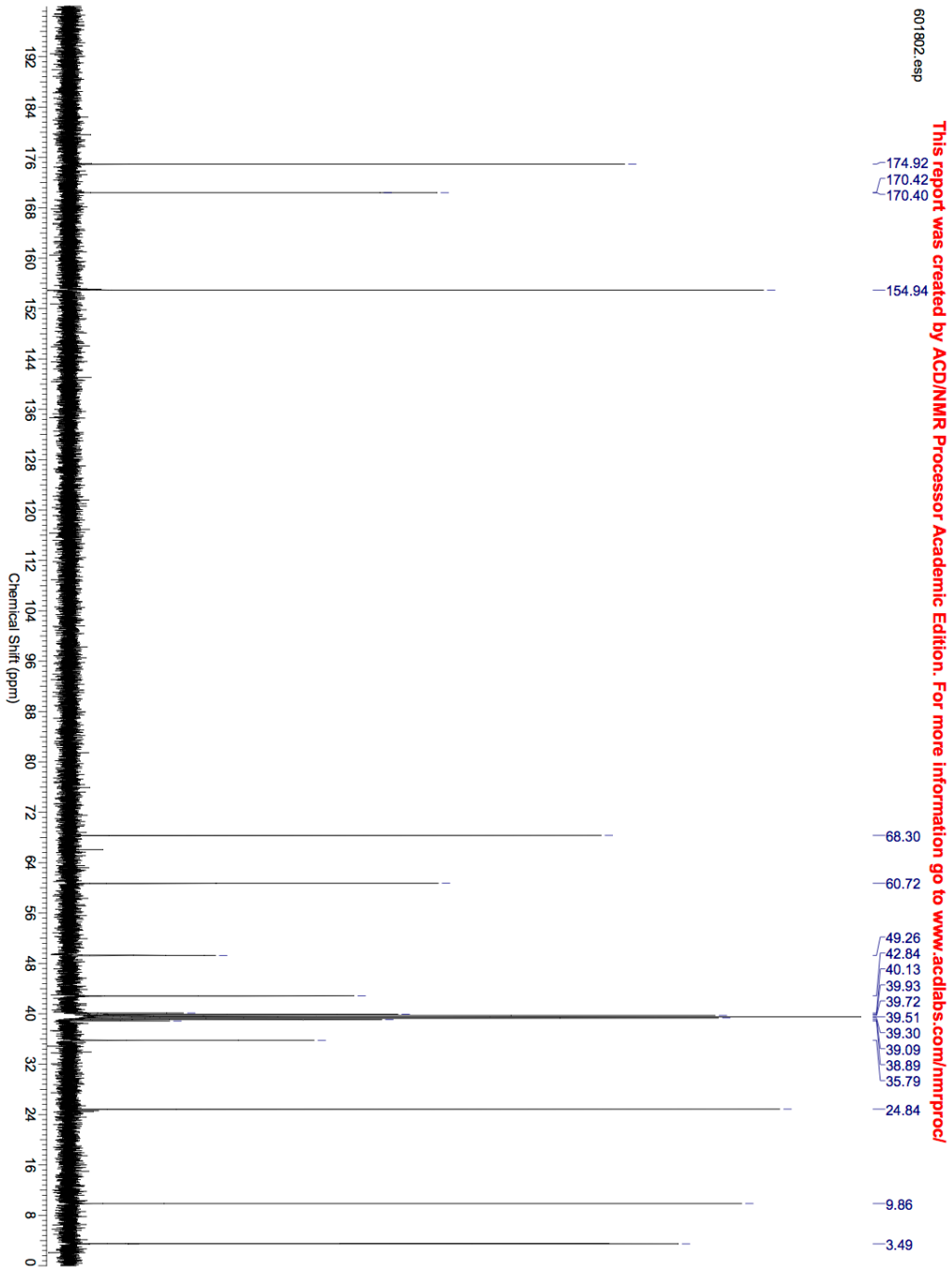
**5 – HPLC @ 254 nm**

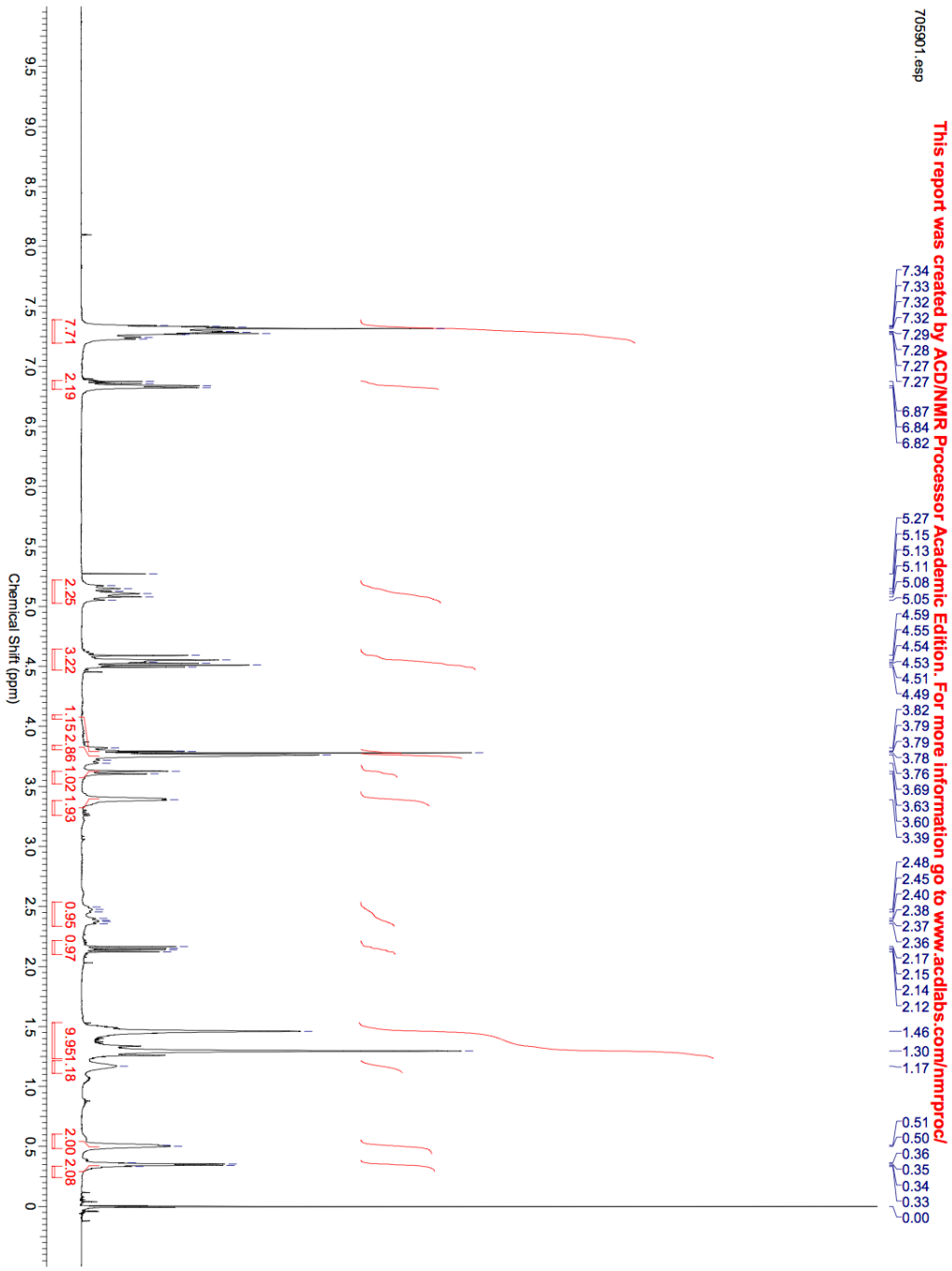

O
O
(S)
OH
N
(S)
NH
O
N

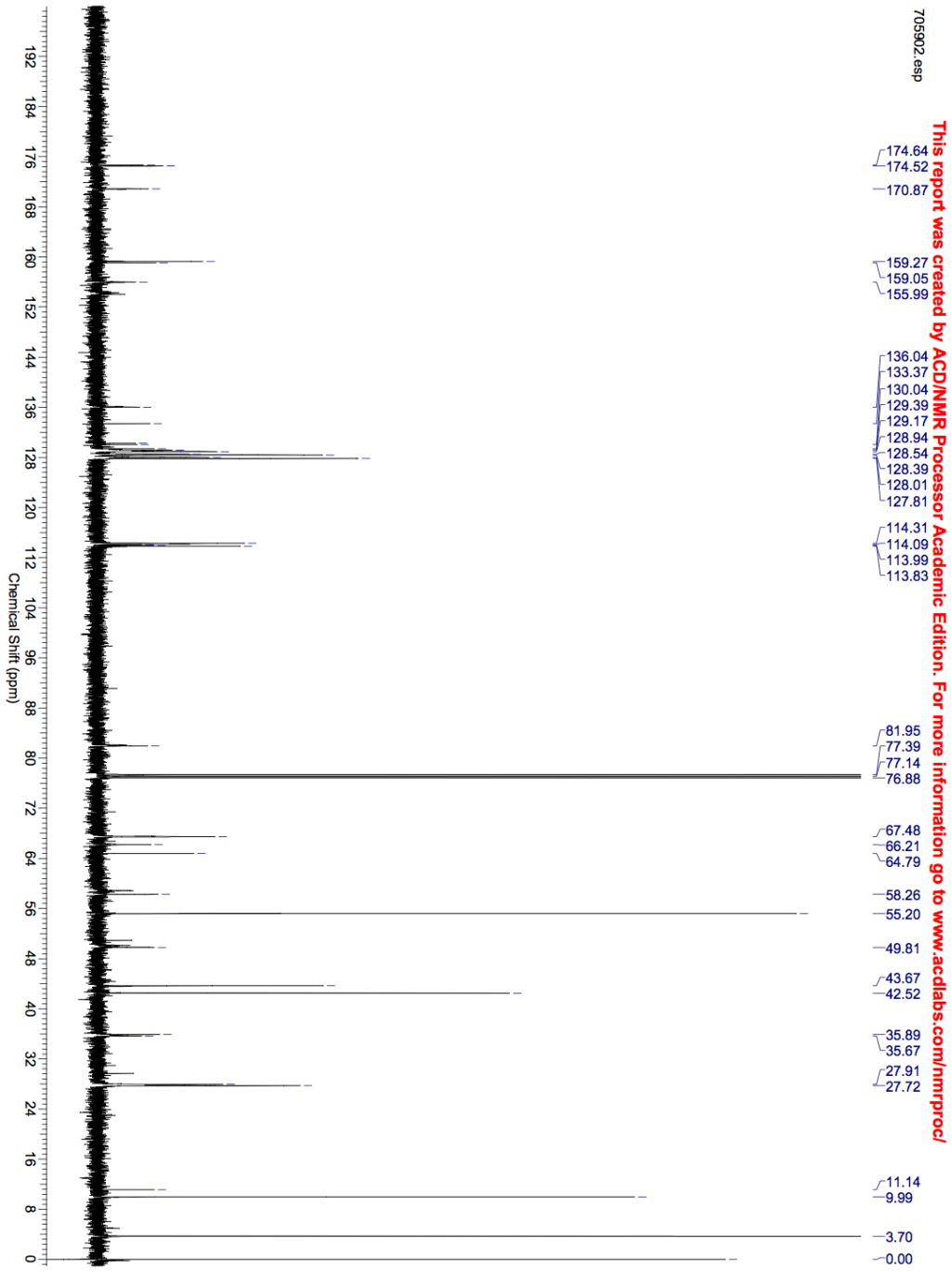

DAD1 B, Sig=254,4 Ref=360,100 (2015-05-12 AT 14-18-07 HOURS\JDN_F_018_CF14-2.D)
mAU
80
60
40
20
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
min

601801.esp

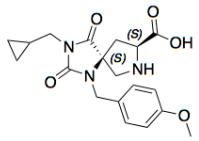


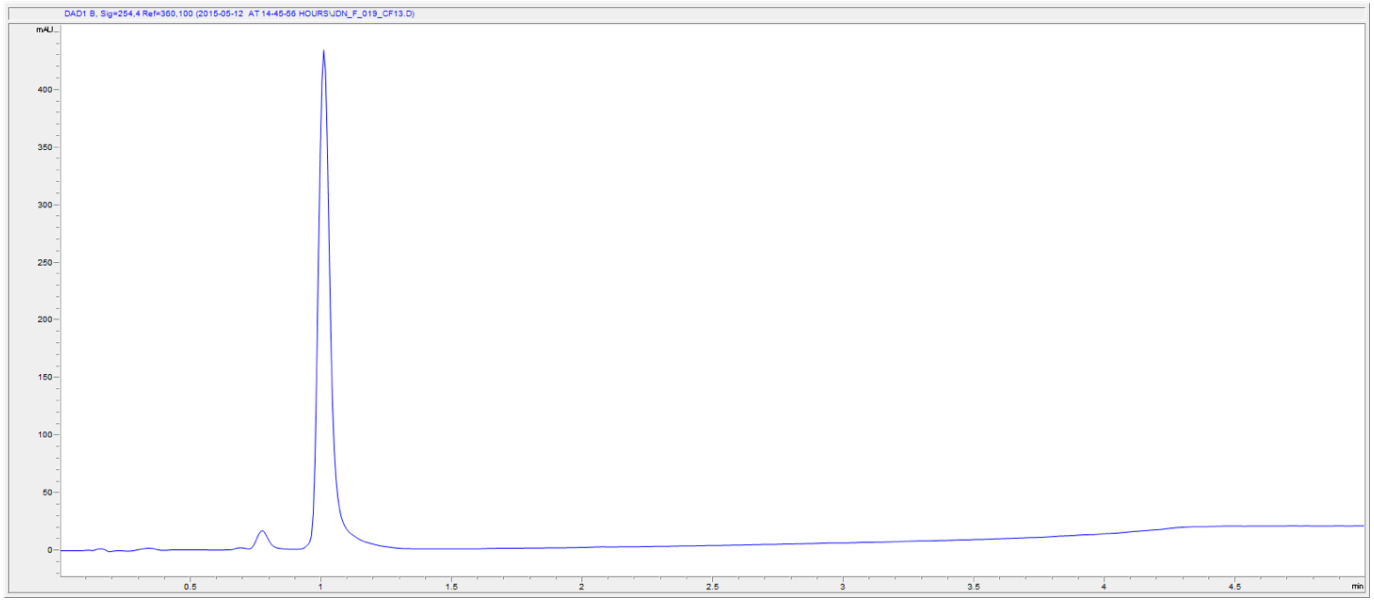

601802.esp
174.92
170.42
170.40
154.94
68.30
60.72
49.26
42.84
40.13
39.93
39.72
39.51
39.30
39.09
38.89
35.79
24.84
9.86
3.49
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/


705901.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
Chemical Shift (ppm)
7.71
2.19
2.25
3.22
1.15 2.86 1.02 1.93
0.95 0.97
9.95 1.18
2.00 2.08


705902.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
174.64
174.52
170.87
159.27
159.05
155.99
136.04
133.37
130.04
129.39
129.17
128.94
128.54
128.39
128.01
127.81
114.31
114.09
113.99
113.83
81.95
77.39
77.14
76.88
67.48
66.21
64.79
58.26
55.20
49.81
43.67
42.52
35.89
35.67
27.91
27.72
11.14
9.99
3.70
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0

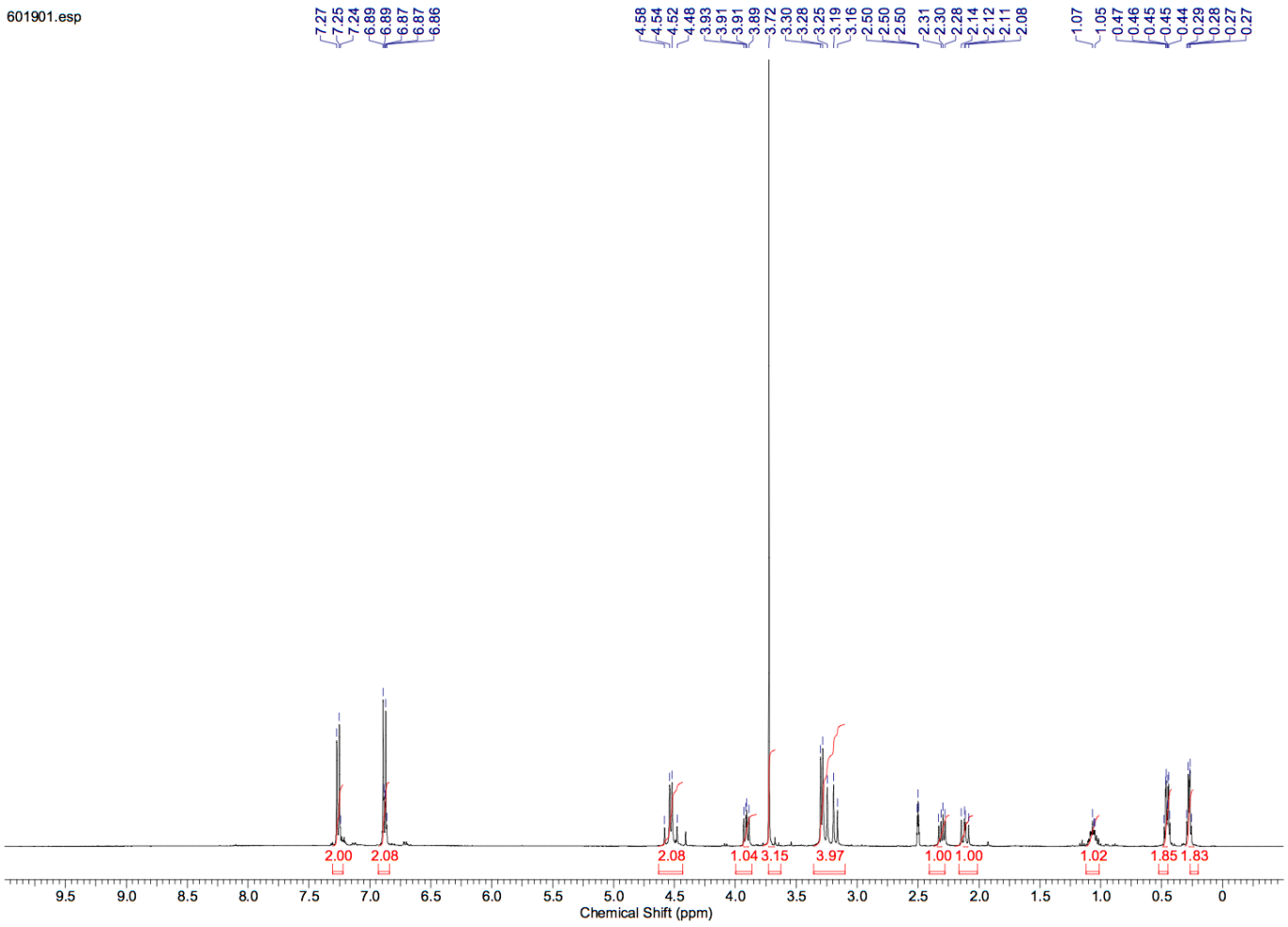
**6 – HPLC @ 254 nm**

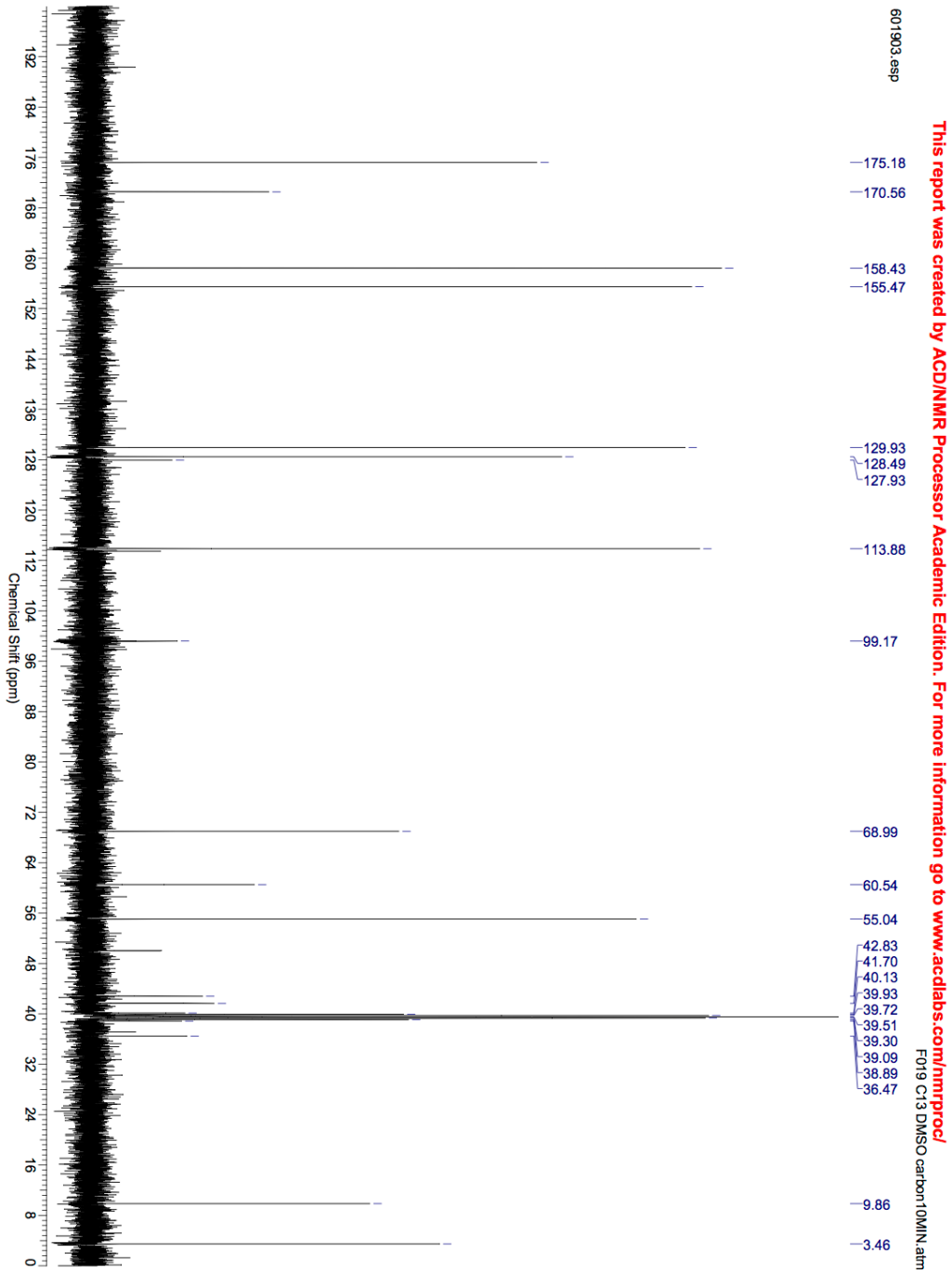

O
O
OH
(S)
(S)
NH
N
N
O
O

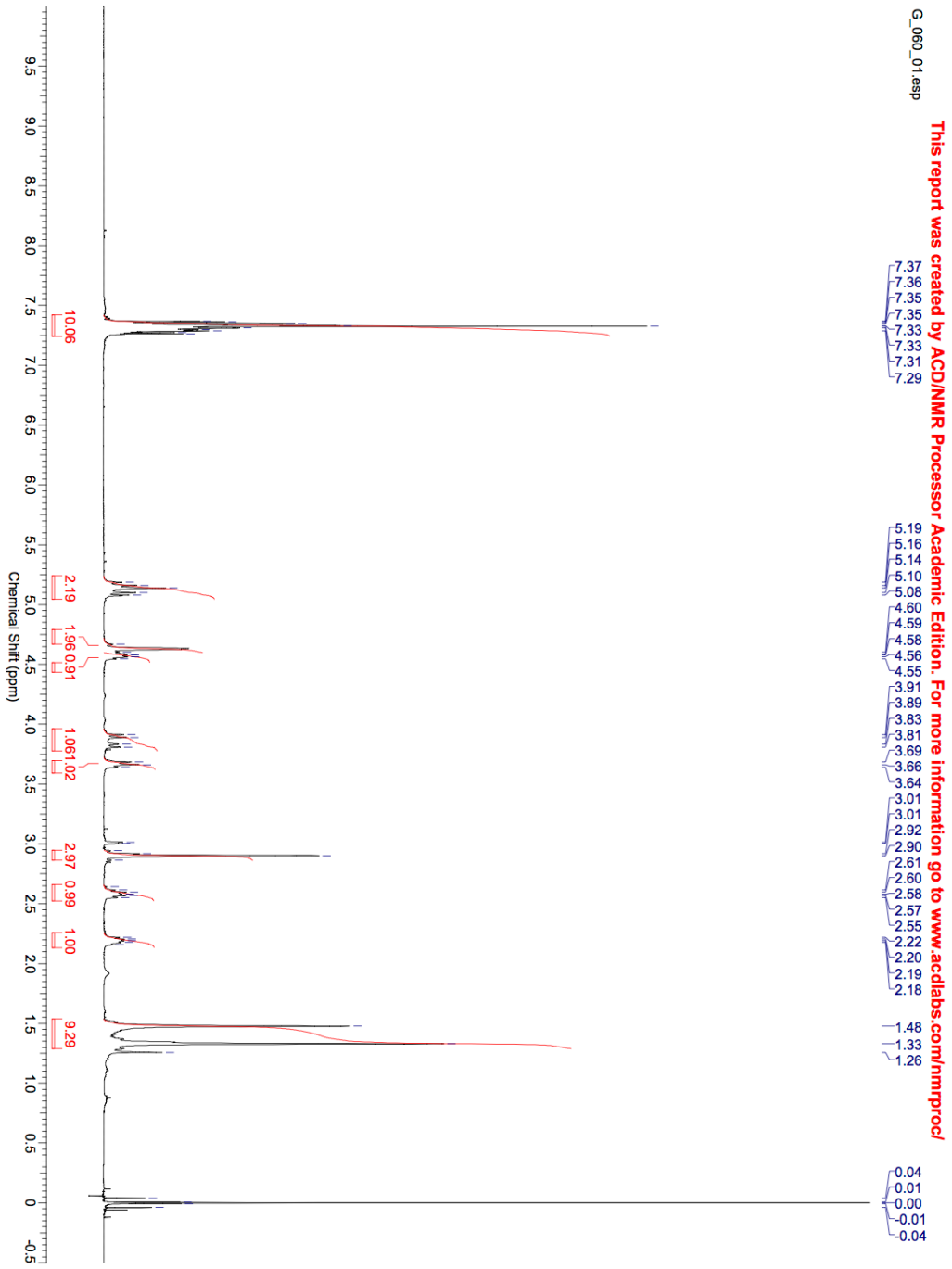

DAD1 B, Sig=254,4 Ref=360,100 (2015-05-12 AT 14-45-56 HOURS\JDN_F_019_CF13.D)
mAU
400
350
300
250
200
150
100
50
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
min

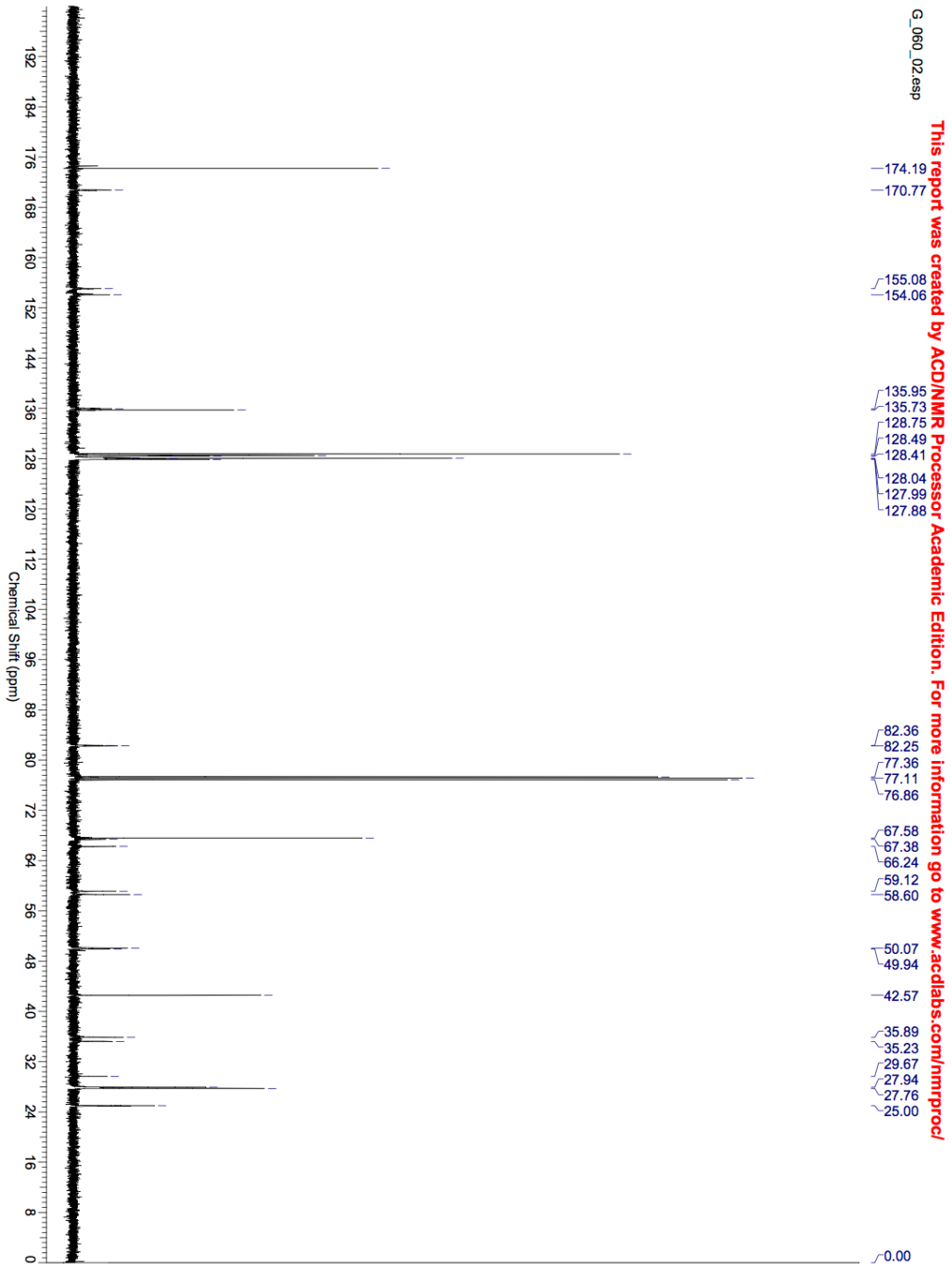
601901.esp

F019 Proton DMSO proton.atm

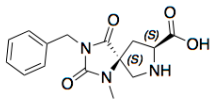


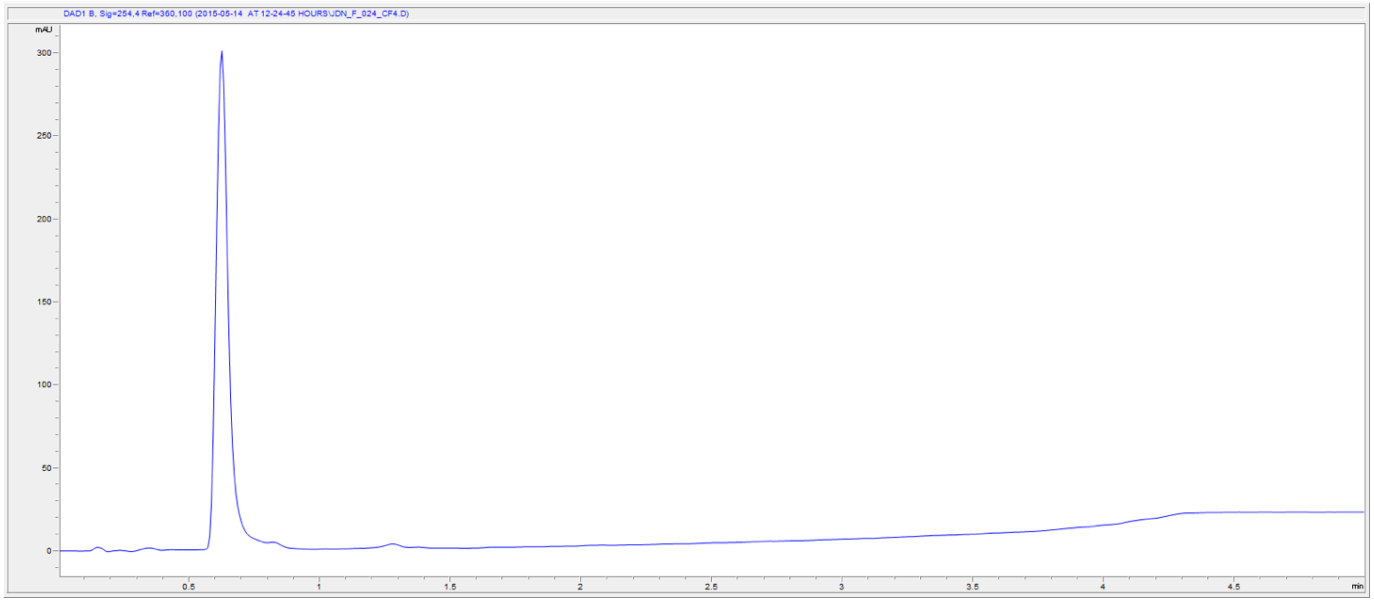

601903.esp
175.18
170.56
158.43
155.47
129.93
128.49
127.93
113.88
99.17
68.99
60.54
55.04
42.83
41.70
40.13
39.93
39.72
39.51
39.30
39.09
38.89
36.47
9.86
3.46
Chemical Shift (ppm)
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
F019 C13 DMSO carbon10MIN.atm


G_060_01.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
7.37
7.36
7.35
7.35
7.33
7.33
7.31
7.29
5.19
5.16
5.14
5.10
5.08
4.60
4.59
4.58
4.56
4.55
3.91
3.89
3.83
3.81
3.69
3.66
3.64
3.01
3.01
2.92
2.90
2.61
2.60
2.58
2.57
2.55
2.22
2.20
2.19
2.18
1.48
1.33
1.26
0.04
0.01
0.00
-0.01
-0.04
10.06
2.19
1.96
0.91
1.06
1.02
2.97
0.99
1.00
9.29
Chemical Shift (ppm)


G_060_02.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
174.19
170.77
155.08
154.06
135.95
135.73
128.75
128.49
128.41
128.04
127.99
127.88
82.36
82.25
77.36
77.11
76.86
67.58
67.38
66.24
59.12
58.60
50.07
49.94
42.57
35.89
35.23
29.67
27.94
27.76
25.00
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0

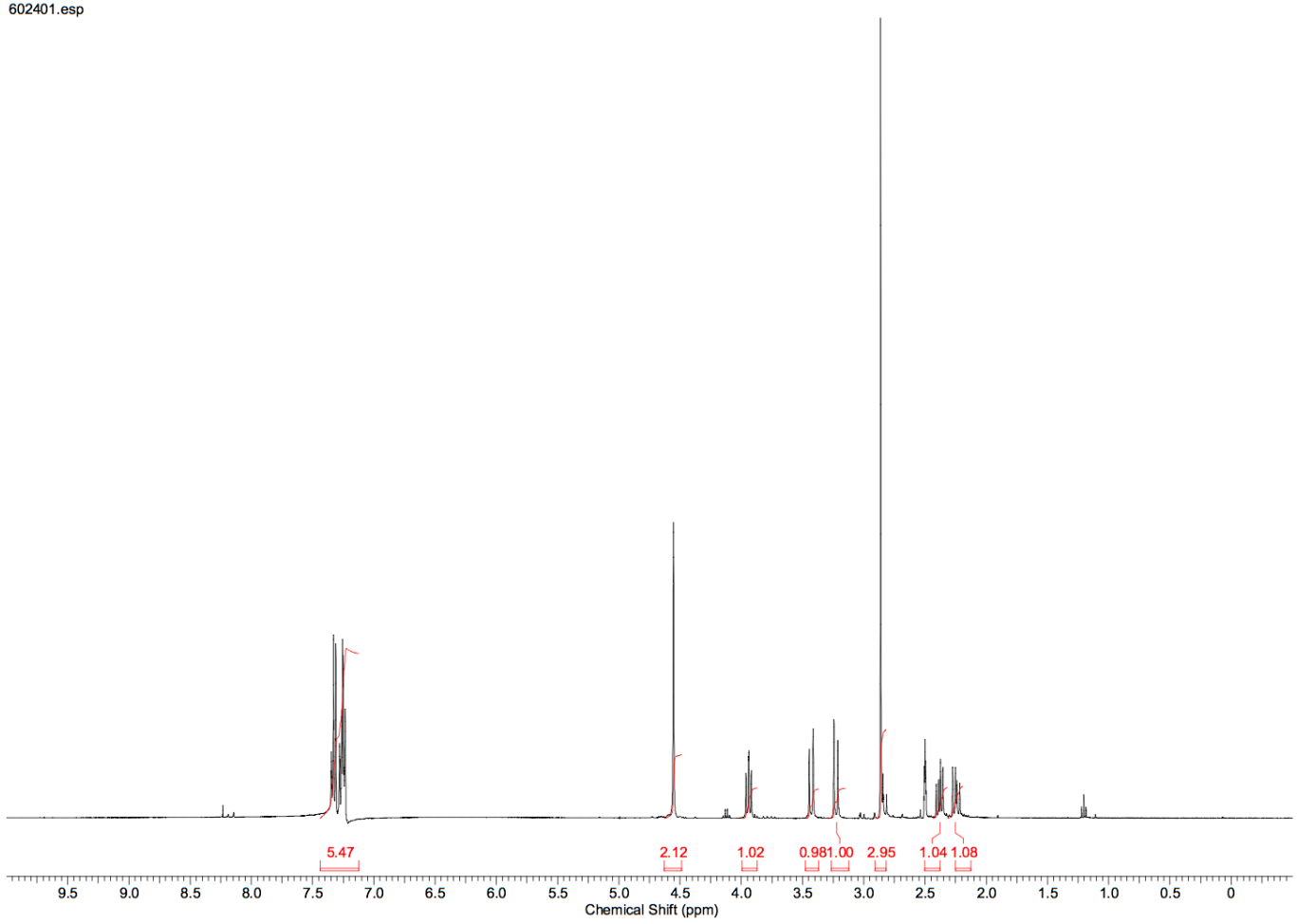
**7 – HPLC @ 254 nm**

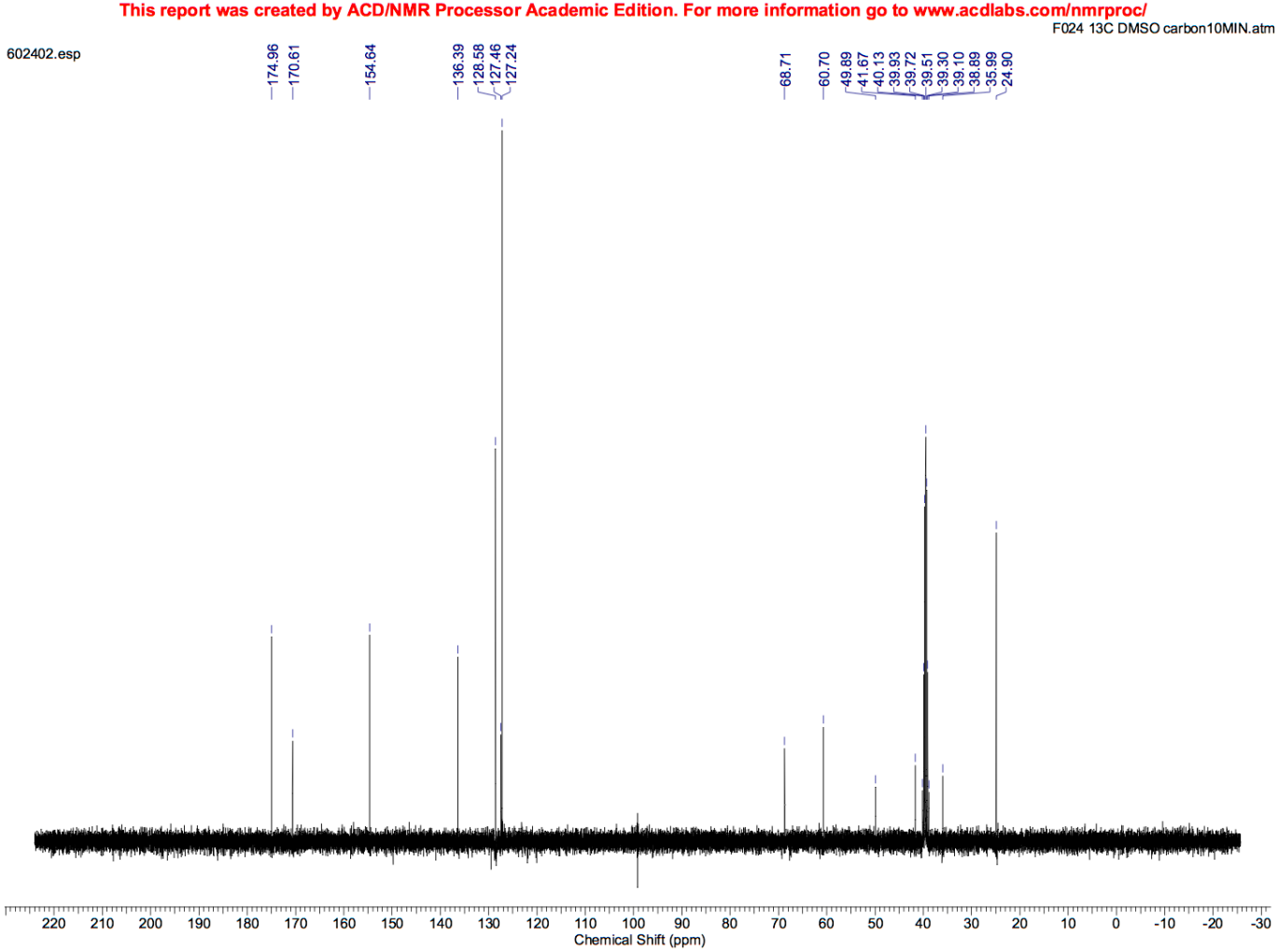

O
O
(S)
OH
N
(S)
NH
O
N

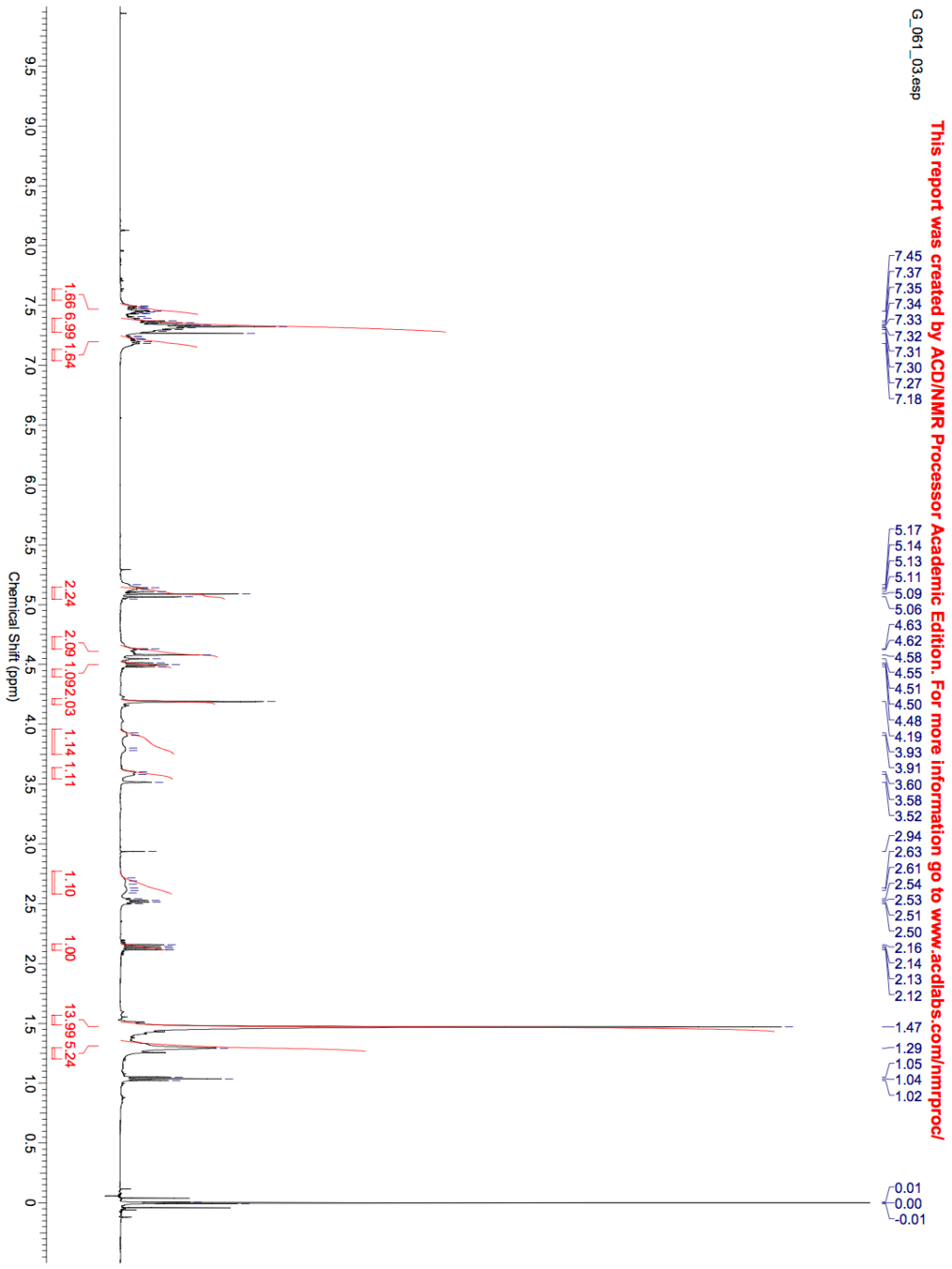

DAD1 B, Sig=254,4 Ref=360,100 (2015-05-14 AT 12-24-45 HOURS\JDN_F_024_CF4.D)
mAU
300
250
200
150
100
50
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
min

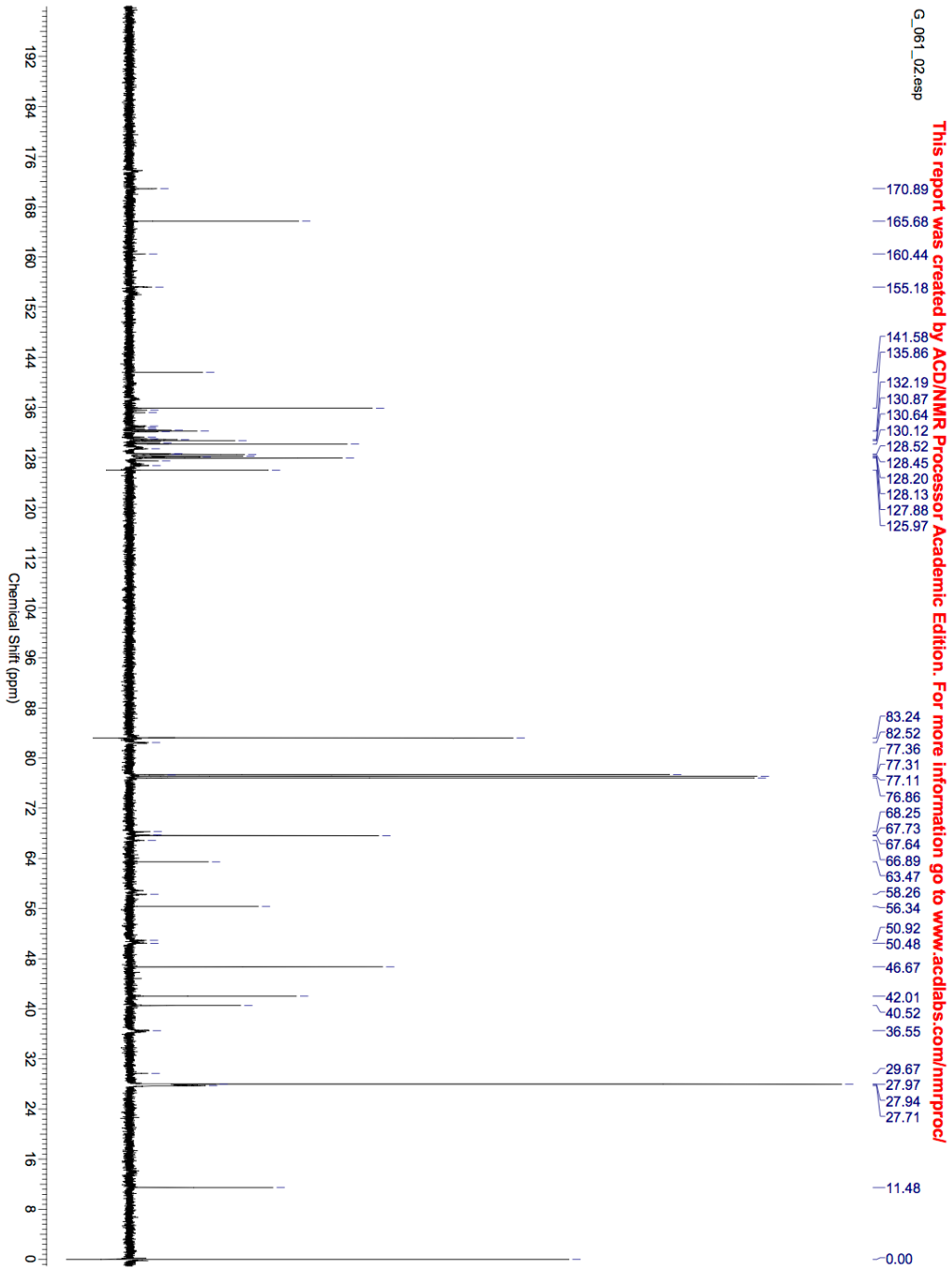
F024 1H DMSO proton.atm

602401.esp

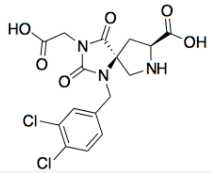


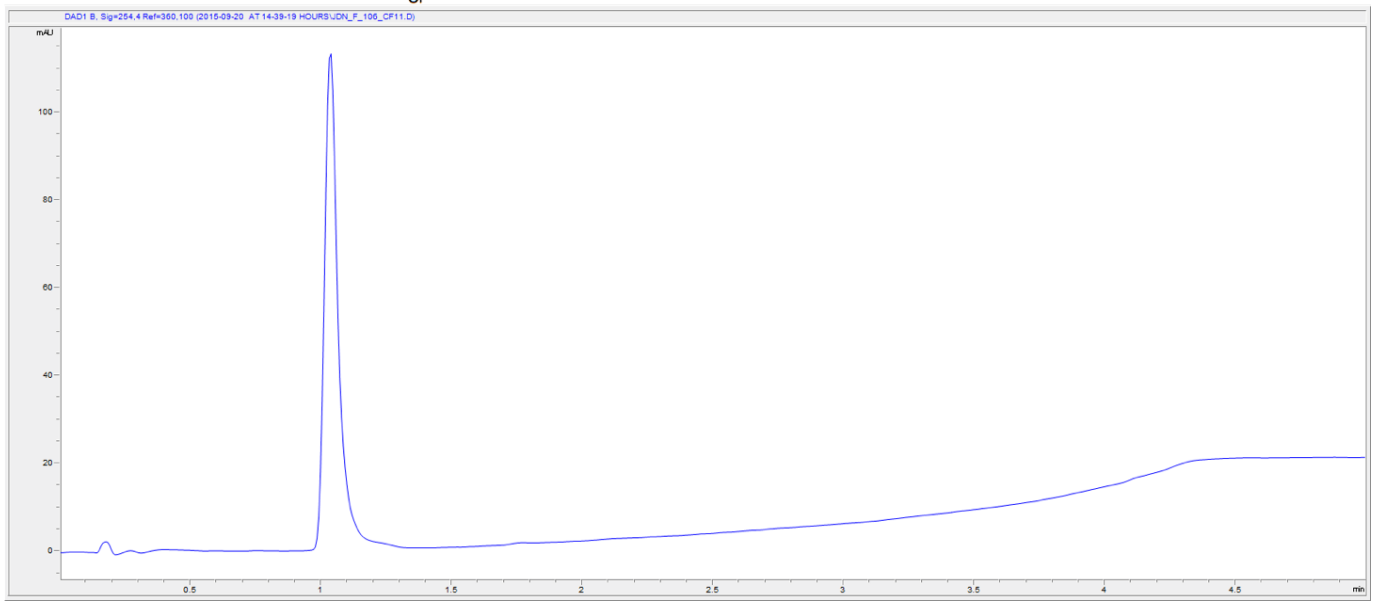


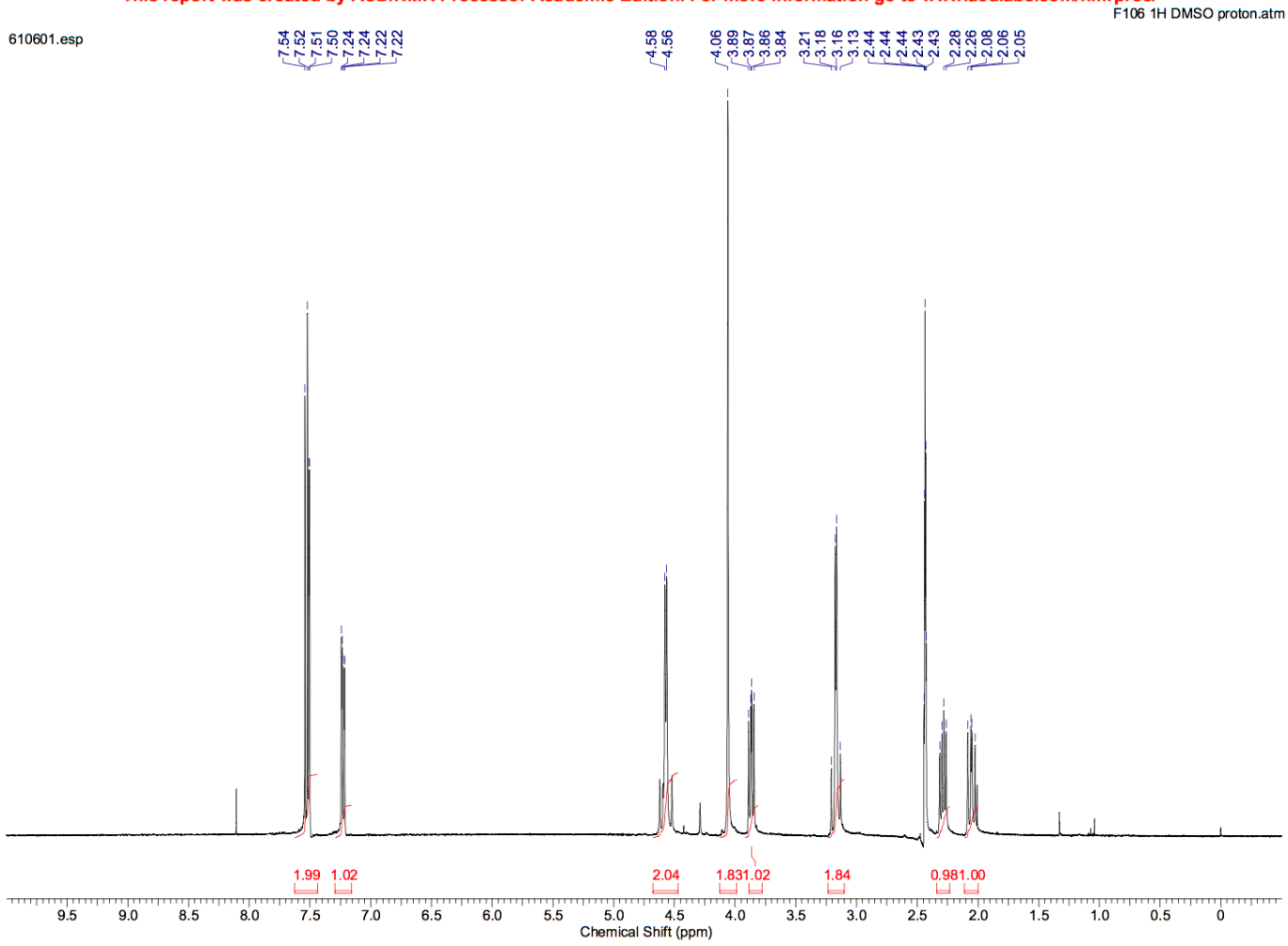

G_061_03.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
7.45
7.37
7.35
7.34
7.33
7.32
7.31
7.30
7.27
7.18
5.17
5.14
5.13
5.11
5.09
5.06
4.63
4.62
4.58
4.55
4.51
4.50
4.48
4.19
3.93
3.91
3.60
3.58
3.52
2.94
2.63
2.61
2.54
2.53
2.51
2.50
2.16
2.14
2.13
2.12
1.47
1.29
1.05
1.04
1.02
0.01
0.00
-0.01
1.66
6.99
1.64
2.24
2.09
1.09
2.03
1.14
1.11
1.10
1.00
13.99
5.24
Chemical Shift (ppm)

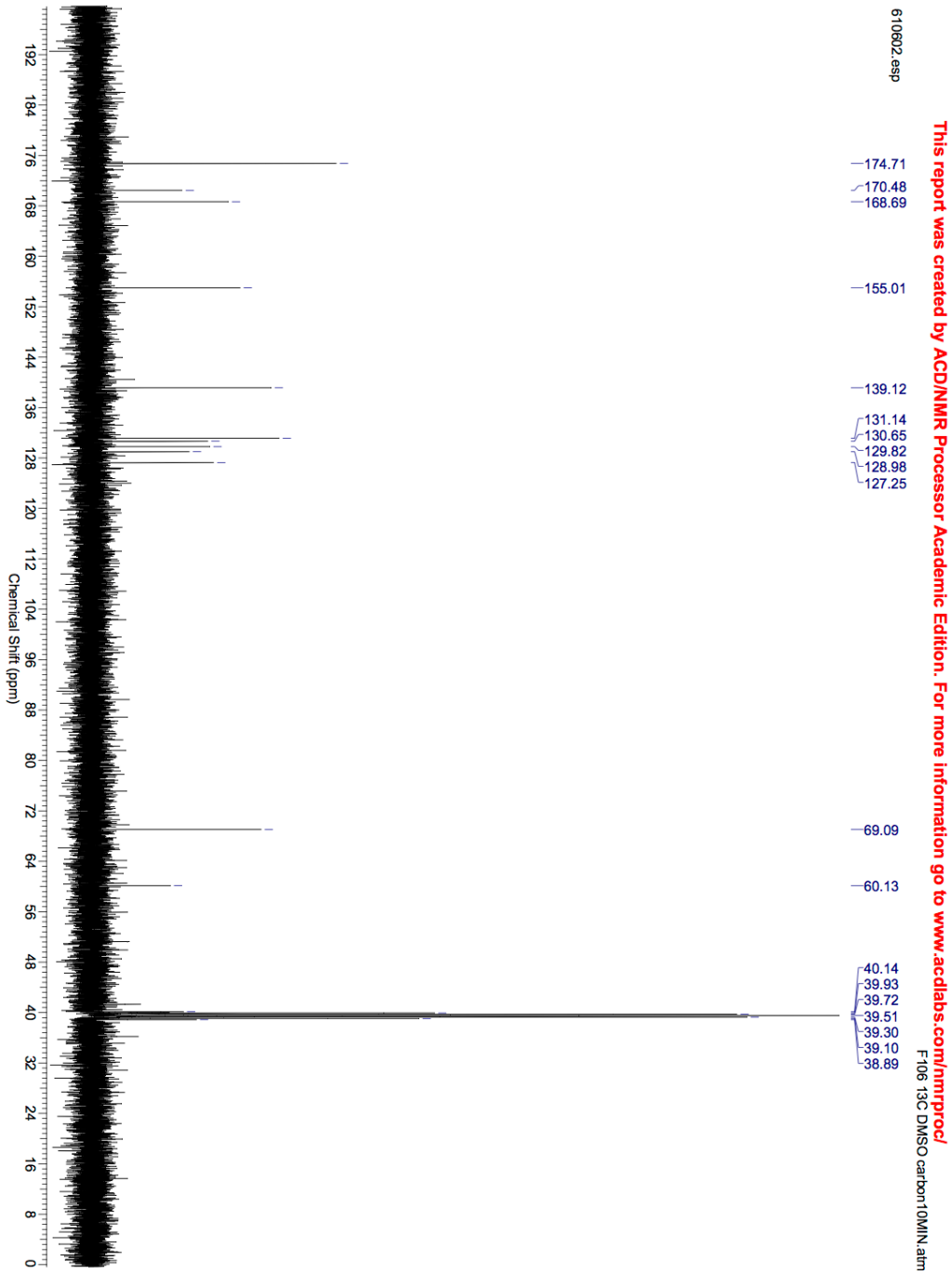

G_061_02.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
170.89
165.68
160.44
155.18
141.58
135.86
132.19
130.87
130.64
130.12
128.52
128.45
128.20
128.13
127.88
125.97
83.24
82.52
77.36
77.31
77.11
76.86
68.25
67.73
67.64
66.89
63.47
58.26
56.34
50.92
50.48
46.67
42.01
40.52
36.55
29.67
27.97
27.94
27.71
11.48
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0

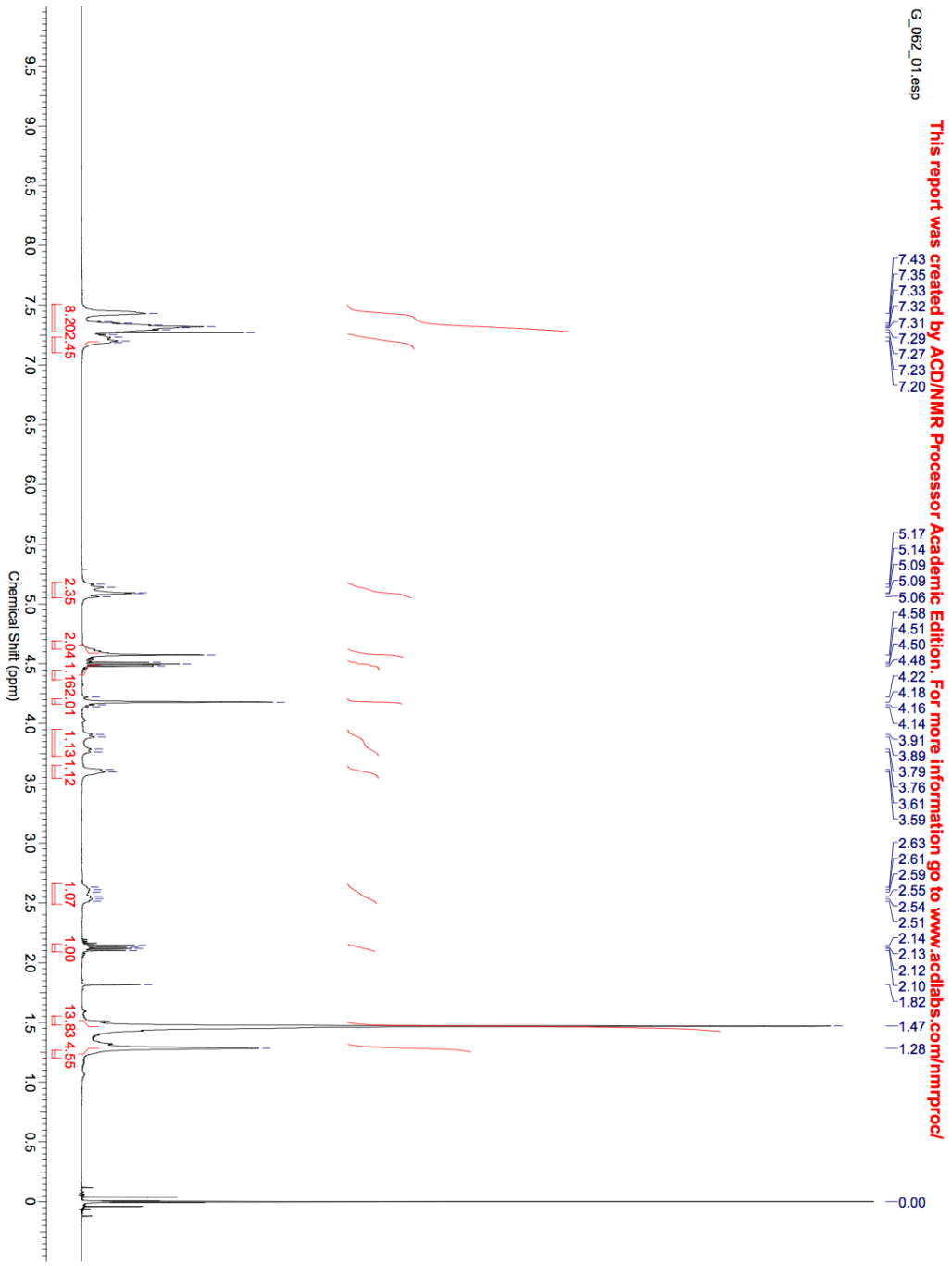
**8 – HPLC @ 254 nm**

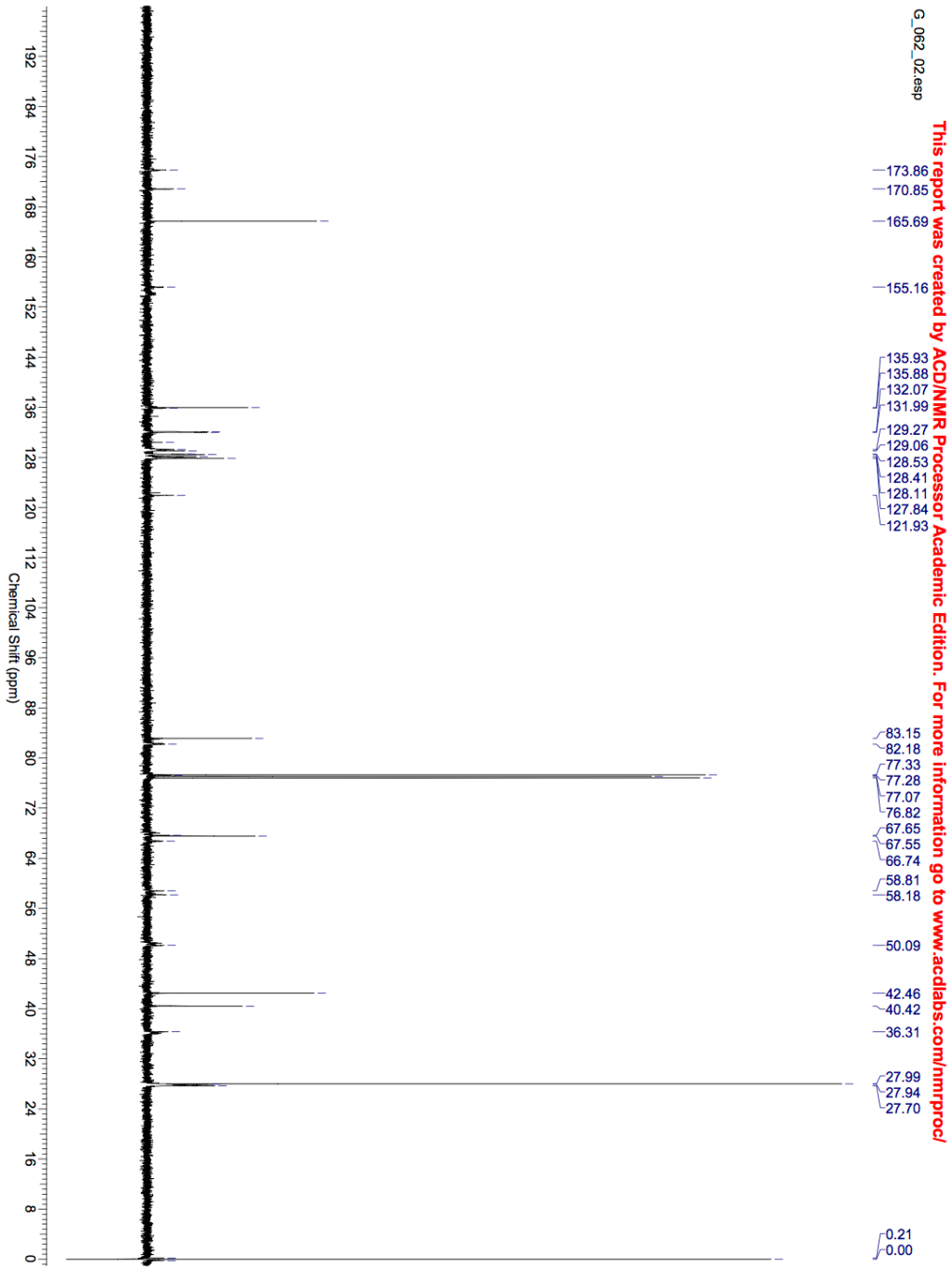

HO
O
N
O
O
N
O
OH
NH
Cl
Cl


DAD1 B, Sig=254,4 Ref=360,100 (2015-09-20 AT 14-39-19 HOURS\JDN_F_106_CF11.D)
mAU
100
80
60
40
20
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
min

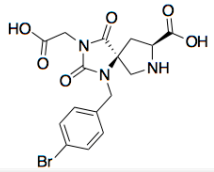


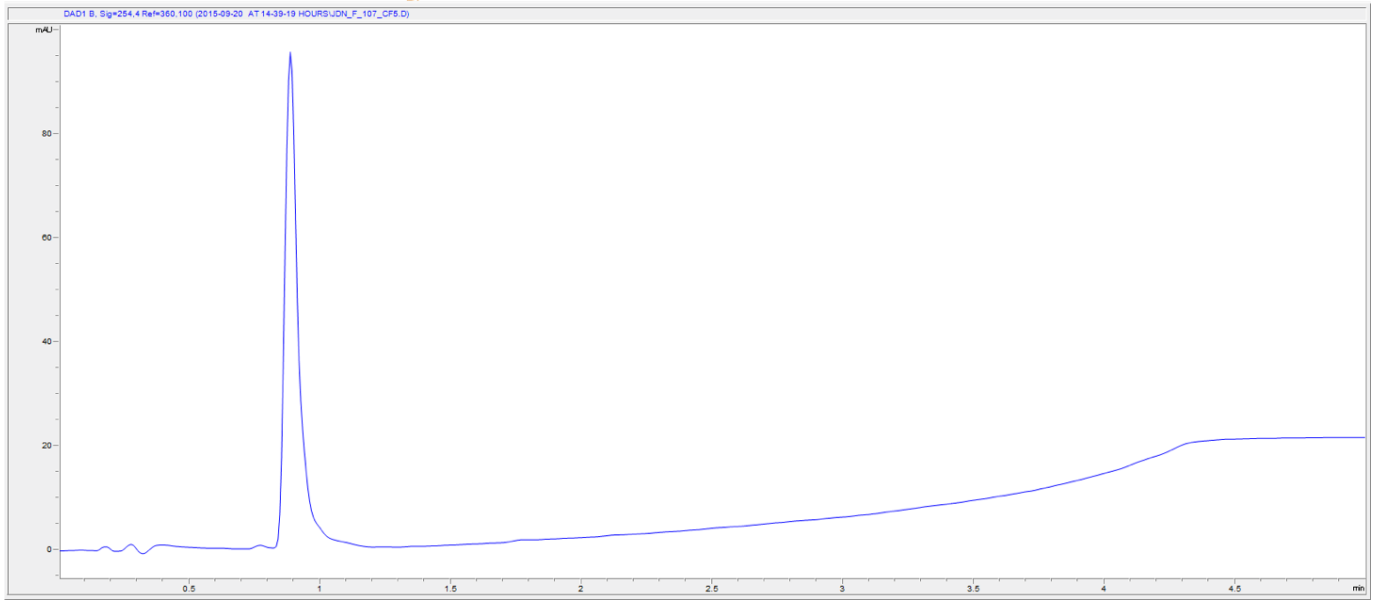

610602.esp
174.71
170.48
168.69
155.01
139.12
131.14
130.65
129.82
128.98
127.25
69.09
60.13
40.14
39.93
39.72
39.51
39.30
39.10
38.89
F106 13C DMSO carbon10MIN.atm
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0

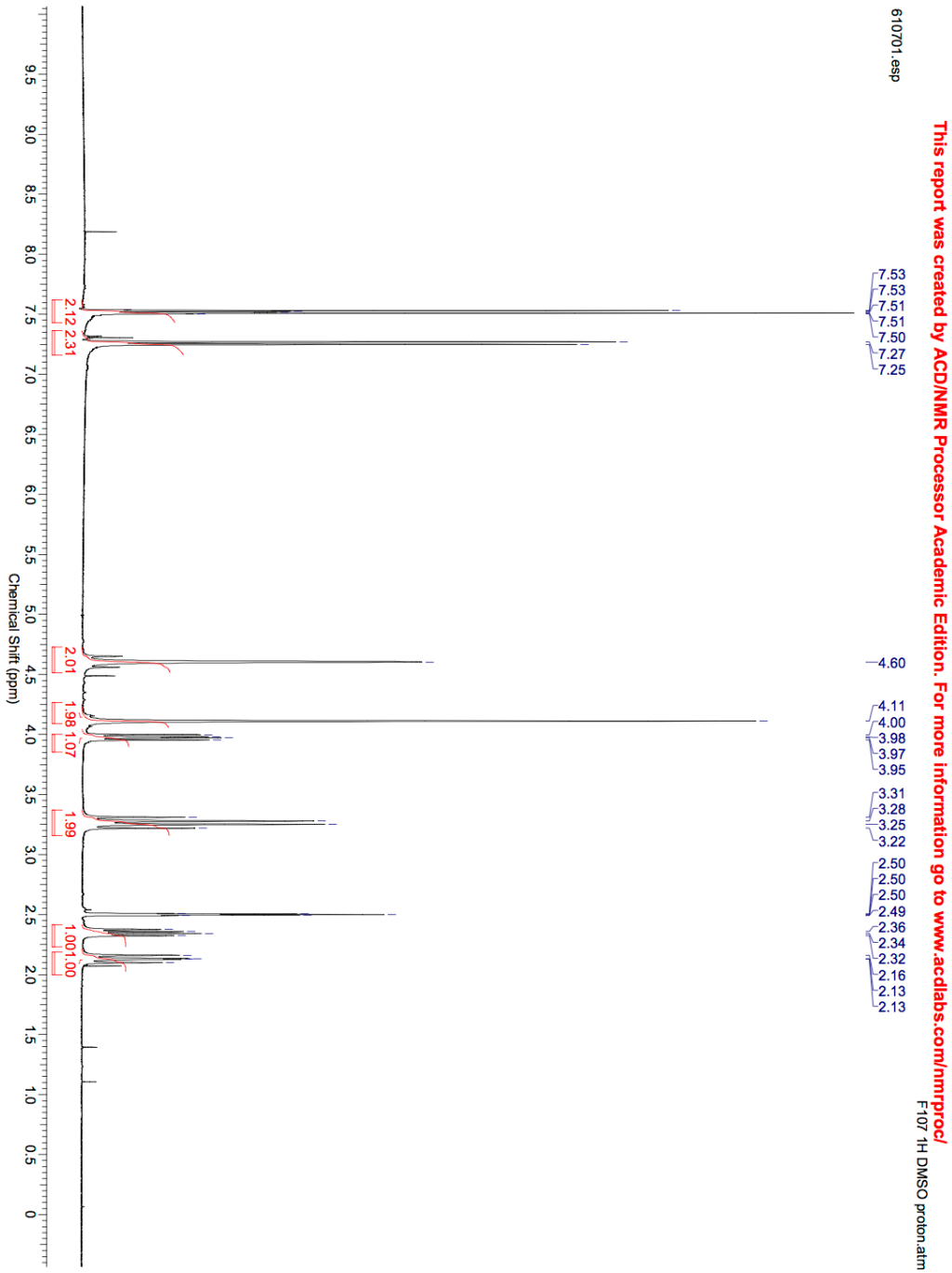

G_062_01.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
7.43
7.35
7.33
7.32
7.31
7.29
7.27
7.23
7.20
5.17
5.14
5.09
5.09
5.06
4.58
4.51
4.50
4.48
4.22
4.18
4.16
4.14
3.91
3.89
3.79
3.76
3.61
3.59
2.63
2.61
2.59
2.55
2.54
2.51
2.14
2.13
2.12
2.10
1.82
1.47
1.28
0.00
8.20
2.45
2.35
2.04
1.16
2.01
1.13
1.12
1.07
1.00
13.83
4.55
Chemical Shift (ppm)

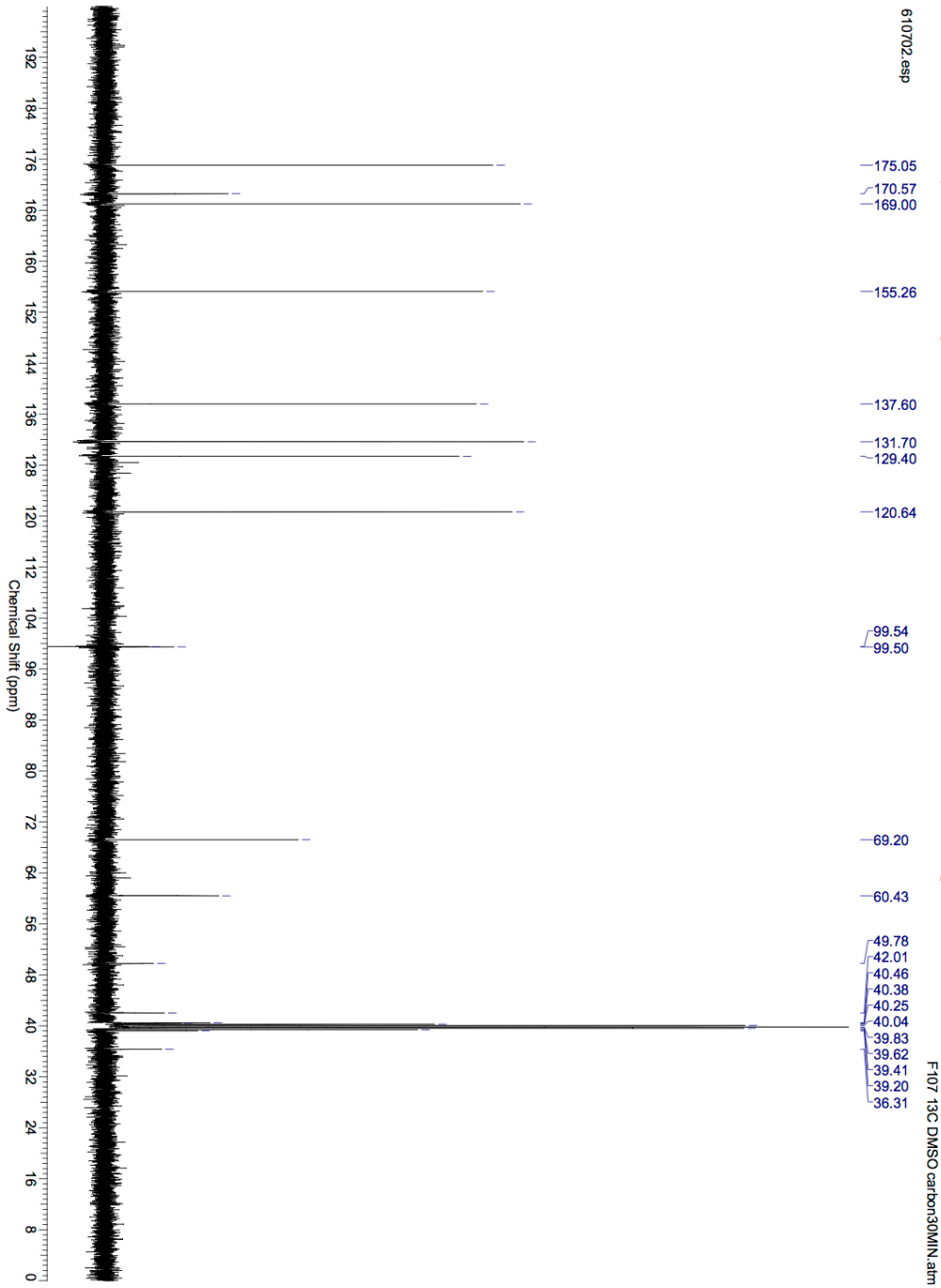

G_062_02.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
173.86
170.85
165.69
155.16
135.93
135.88
132.07
131.99
129.27
129.06
128.53
128.41
128.11
127.84
121.93
83.15
82.18
77.33
77.28
77.07
76.82
67.65
67.55
66.74
58.81
58.18
50.09
42.46
40.42
36.31
27.99
27.94
27.70
0.21
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0

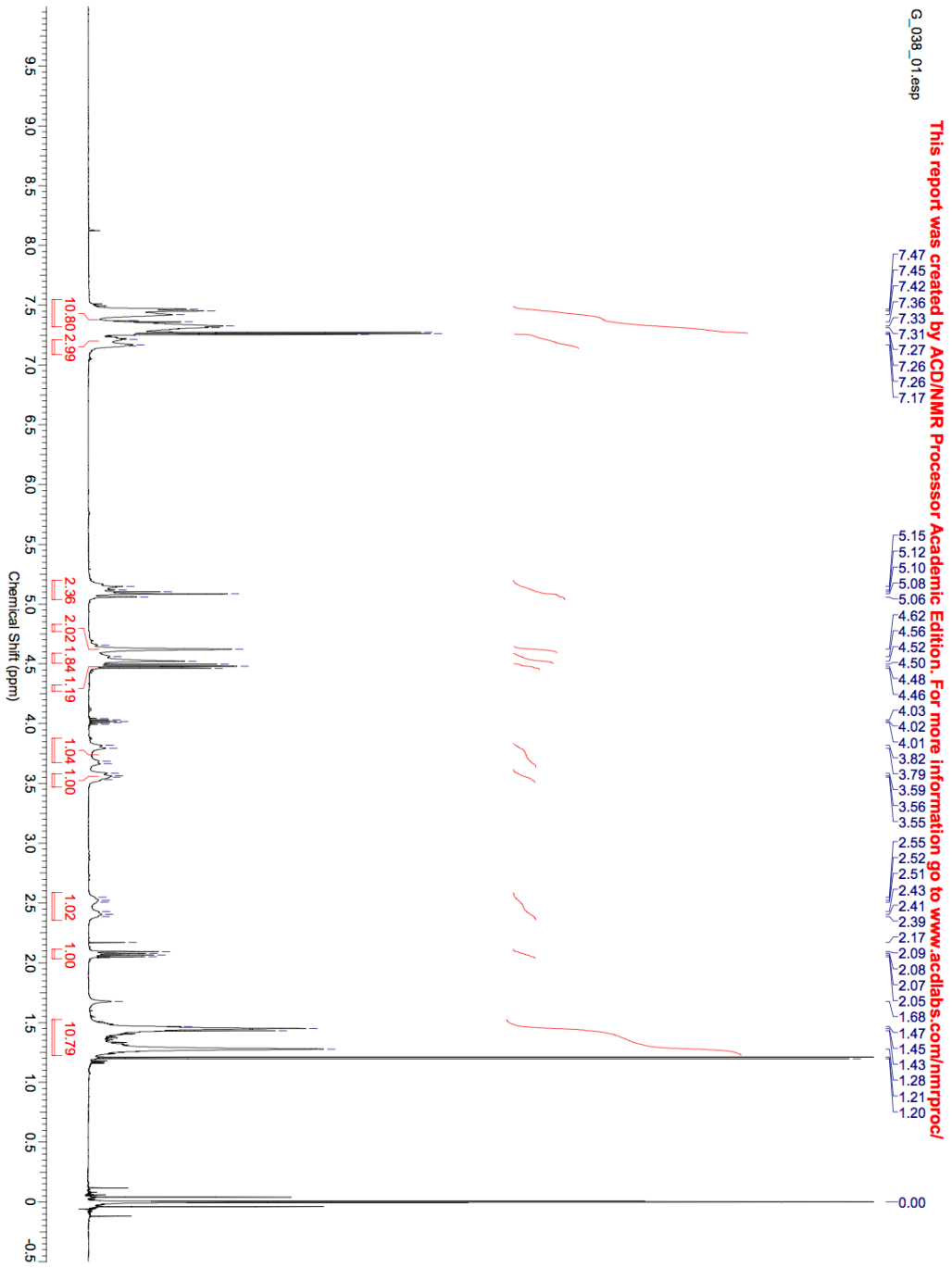
**9 – HPLC @ 254 nm**

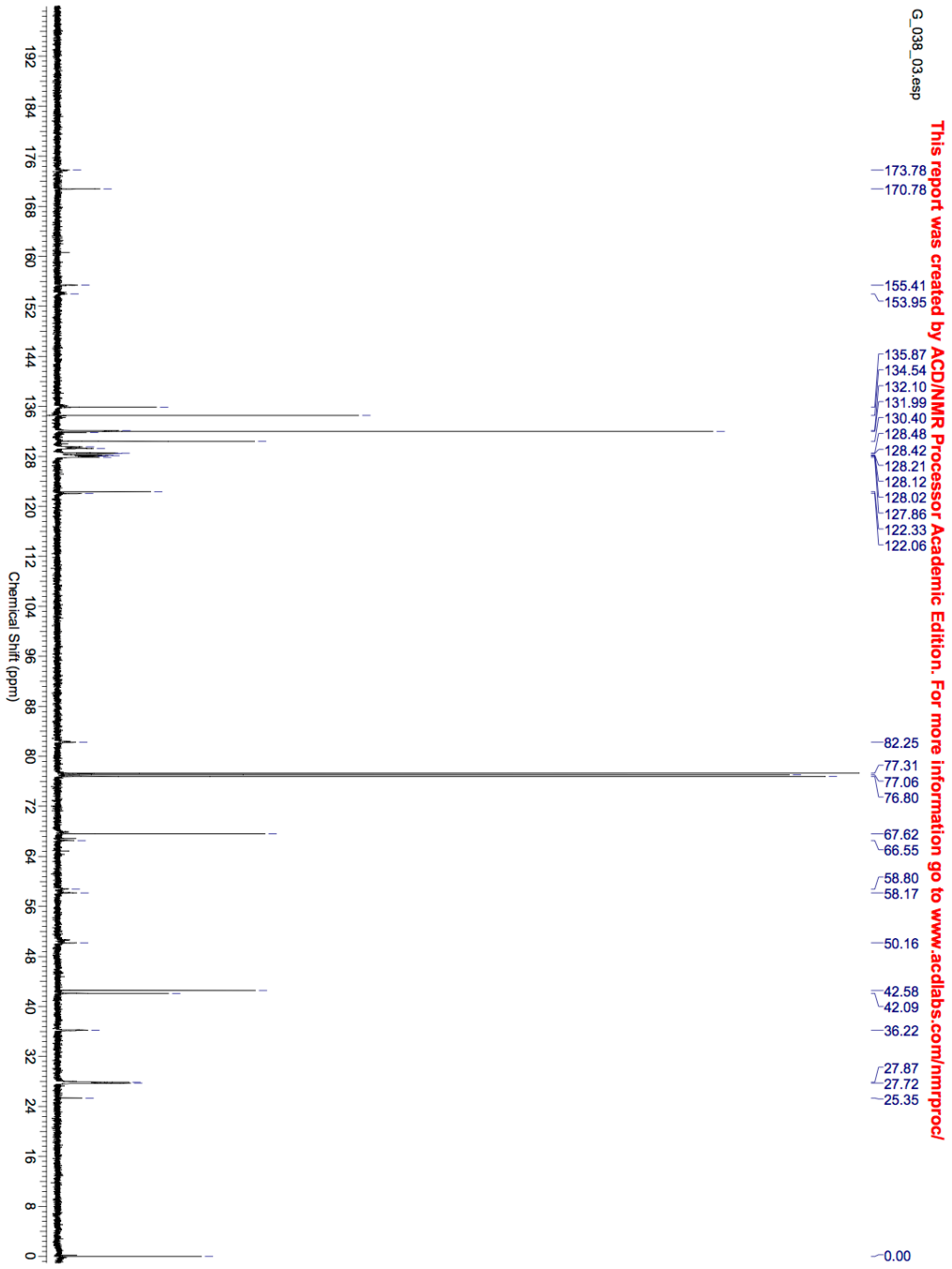

HO
O
N
O
O
N
O
OH
NH
Br


DAD1 B, Sig=254,4 Ref=360,100 (2015-09-20 AT 14-39-19 HOURS\JDN_F_107_CF5.D)
mAU
80
60
40
20
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
min

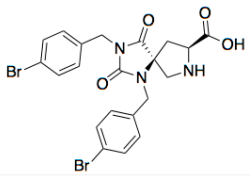

610701.esp
7.53
7.53
7.51
7.51
7.50
7.27
7.25
4.60
4.11
4.00
3.98
3.97
3.95
3.31
3.28
3.25
3.22
2.50
2.50
2.50
2.49
2.36
2.34
2.32
2.16
2.13
2.13
2.12 2.31
2.01
1.98 1.07
1.99
1.00 1.00
Chemical Shift (ppm)
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
F107 1H DMSO proton.atm

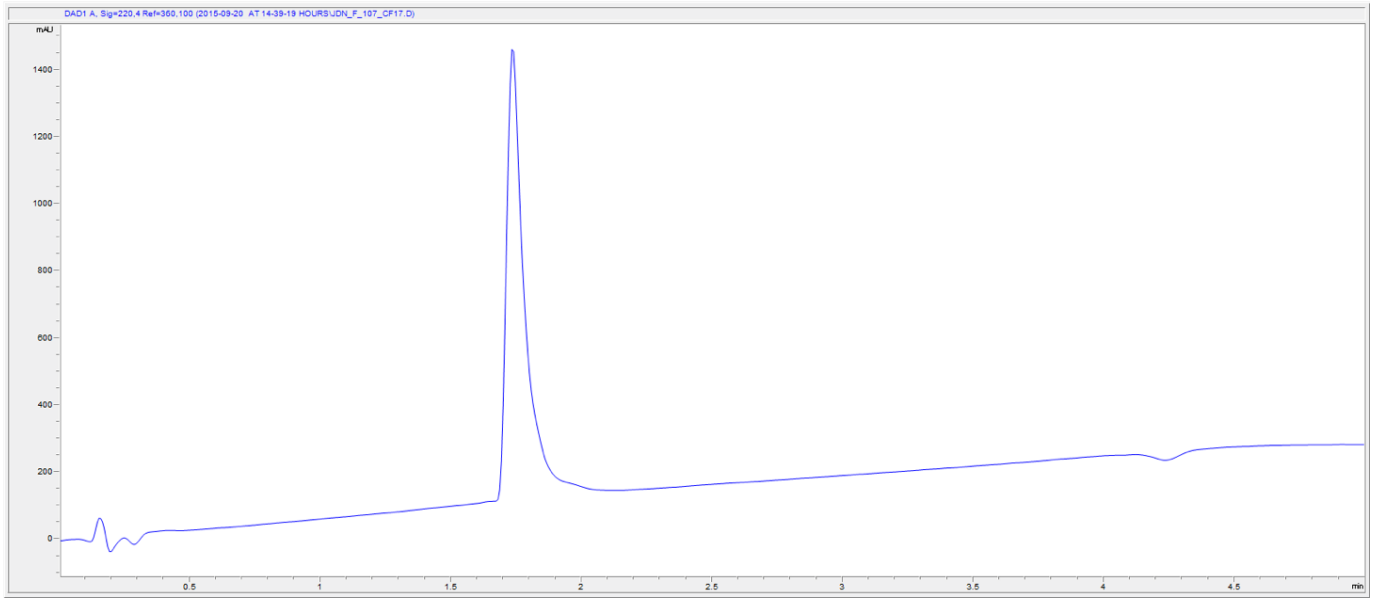

610702.esp
175.05
170.57
169.00
155.26
137.60
131.70
129.40
120.64
99.54
99.50
69.20
60.43
49.78
42.01
40.46
40.38
40.25
40.04
39.83
39.62
39.41
39.20
36.31
Chemical Shift (ppm)
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
F107 13C DMSO carbon30MIN.atm

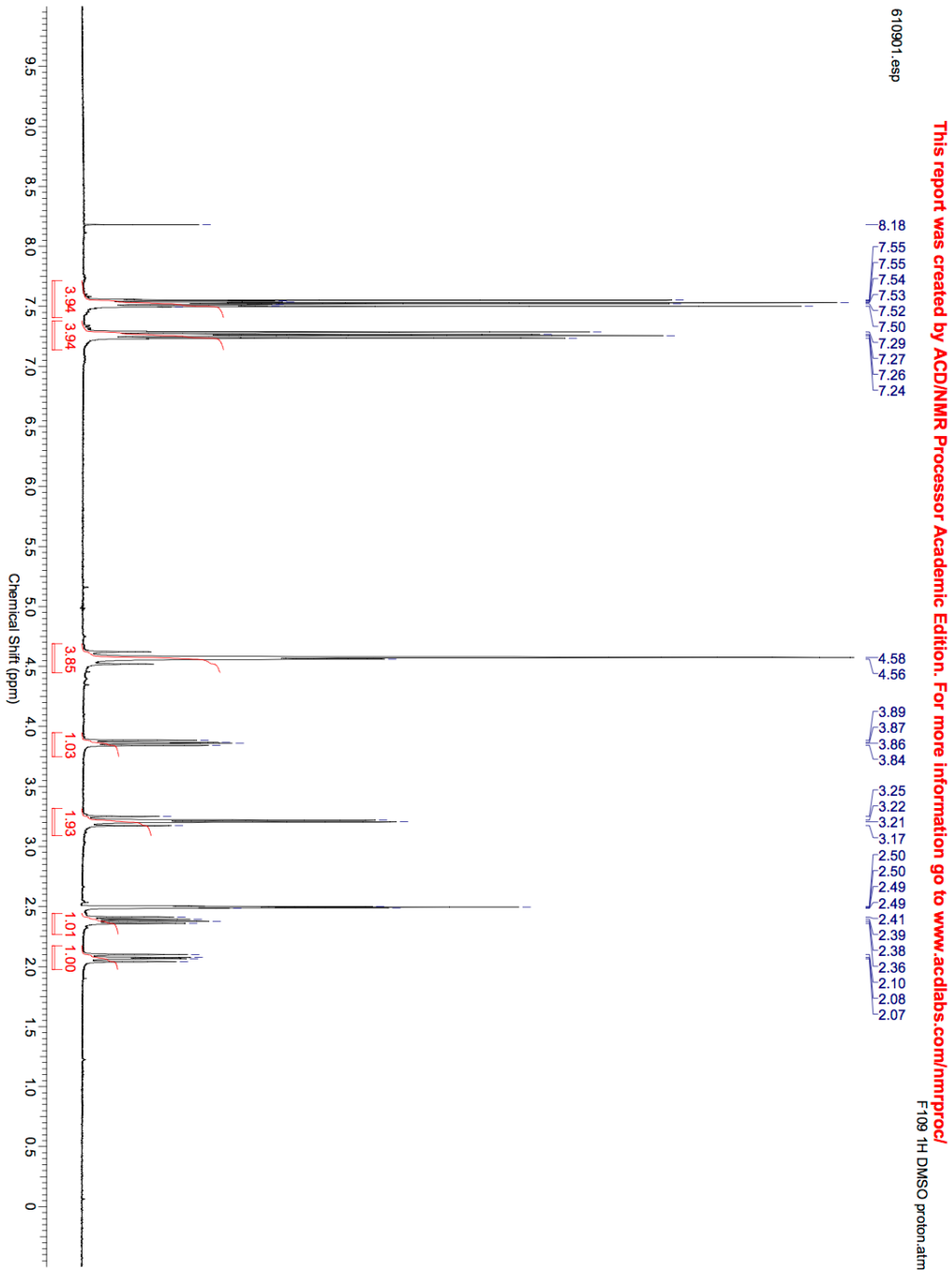

G_038_01.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
7.47
7.45
7.42
7.36
7.33
7.31
7.27
7.26
7.26
7.17
5.15
5.12
5.10
5.08
5.06
4.62
4.56
4.52
4.50
4.48
4.46
4.03
4.02
4.01
3.82
3.79
3.59
3.56
3.55
2.55
2.52
2.51
2.43
2.41
2.39
2.17
2.09
2.08
2.07
2.05
1.68
1.47
1.45
1.43
1.28
1.21
1.20
0.00
10.80
2.99
2.36
2.02
1.84
1.19
1.04
1.00
1.02
1.00
10.79
Chemical Shift (ppm)

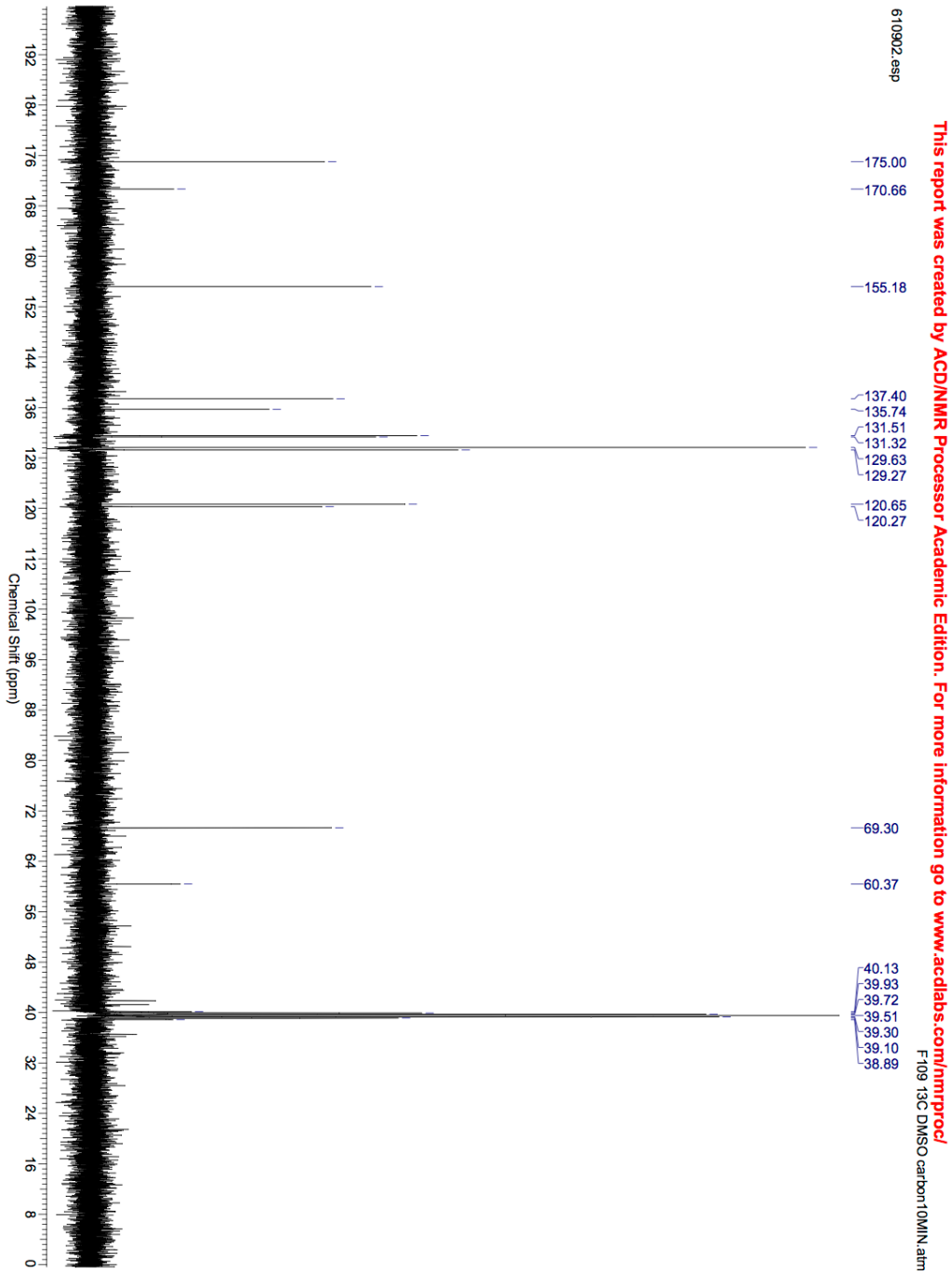

G_038_03.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
173.78
170.78
155.41
153.95
135.87
134.54
132.10
131.99
130.40
128.48
128.42
128.21
128.12
128.02
127.86
122.33
122.06
82.25
77.31
77.06
76.80
67.62
66.55
58.80
58.17
50.16
42.58
42.09
36.22
27.87
27.72
25.35
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0

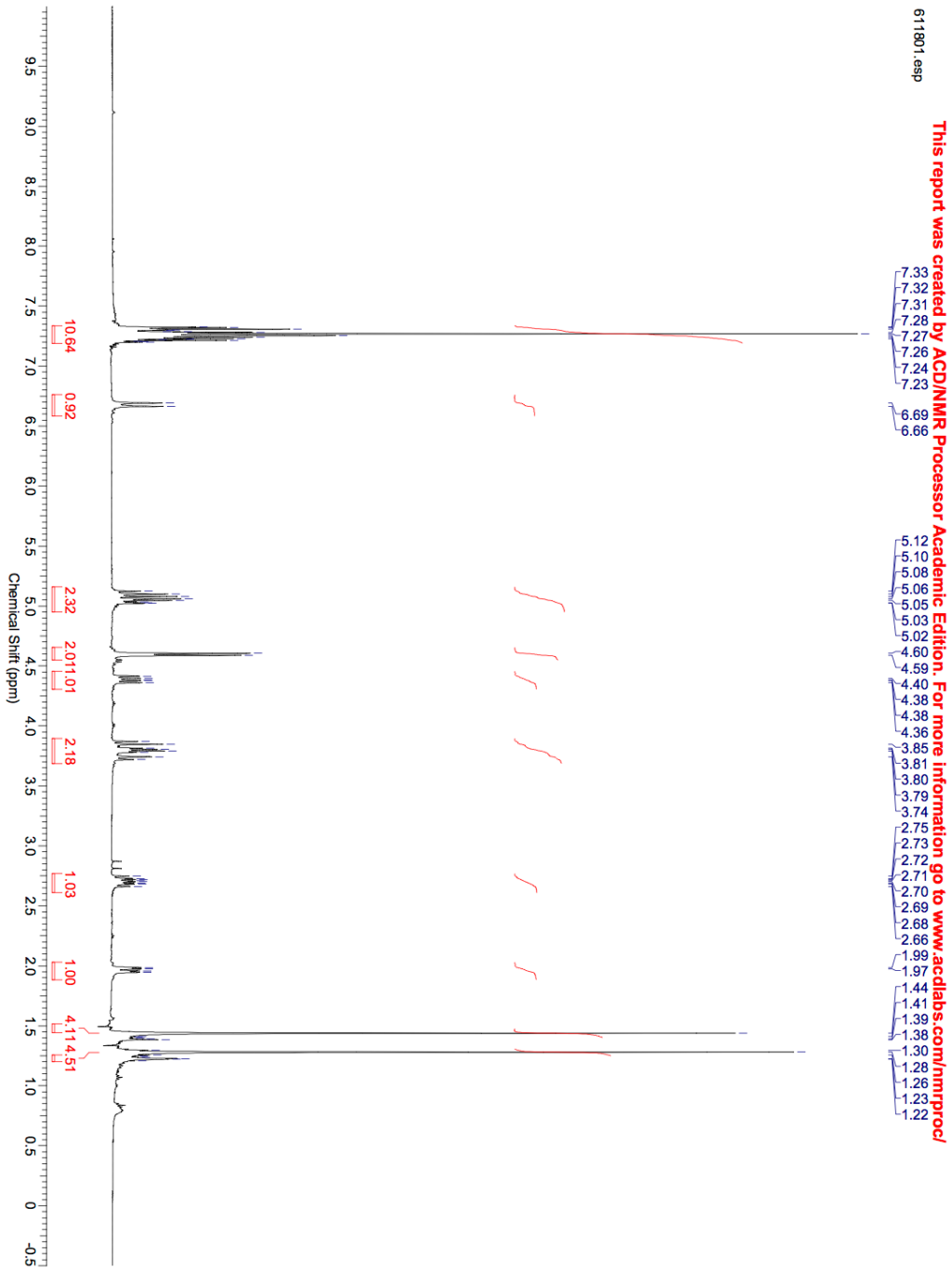
**10 – HPLC @ 220 nm**

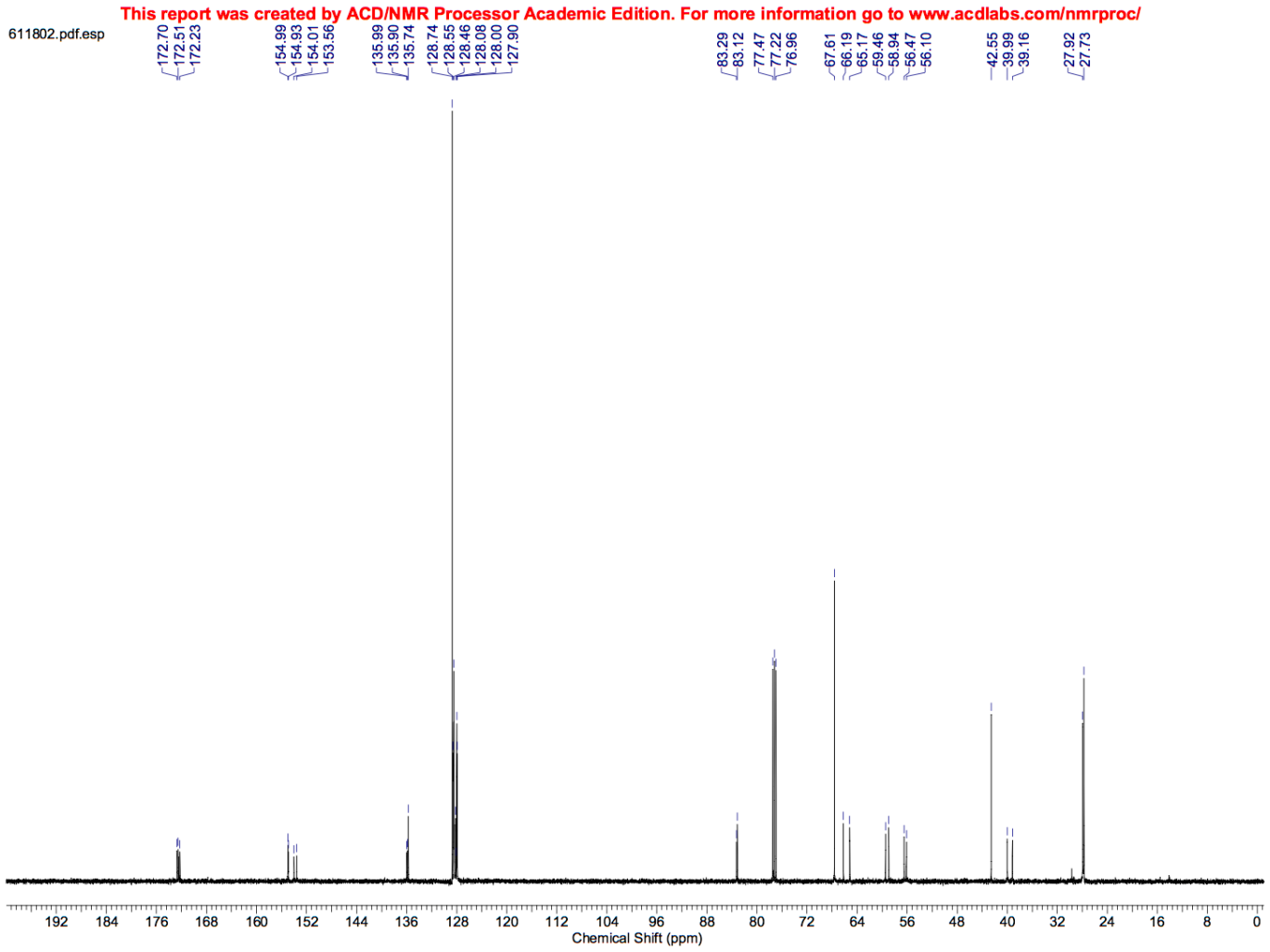


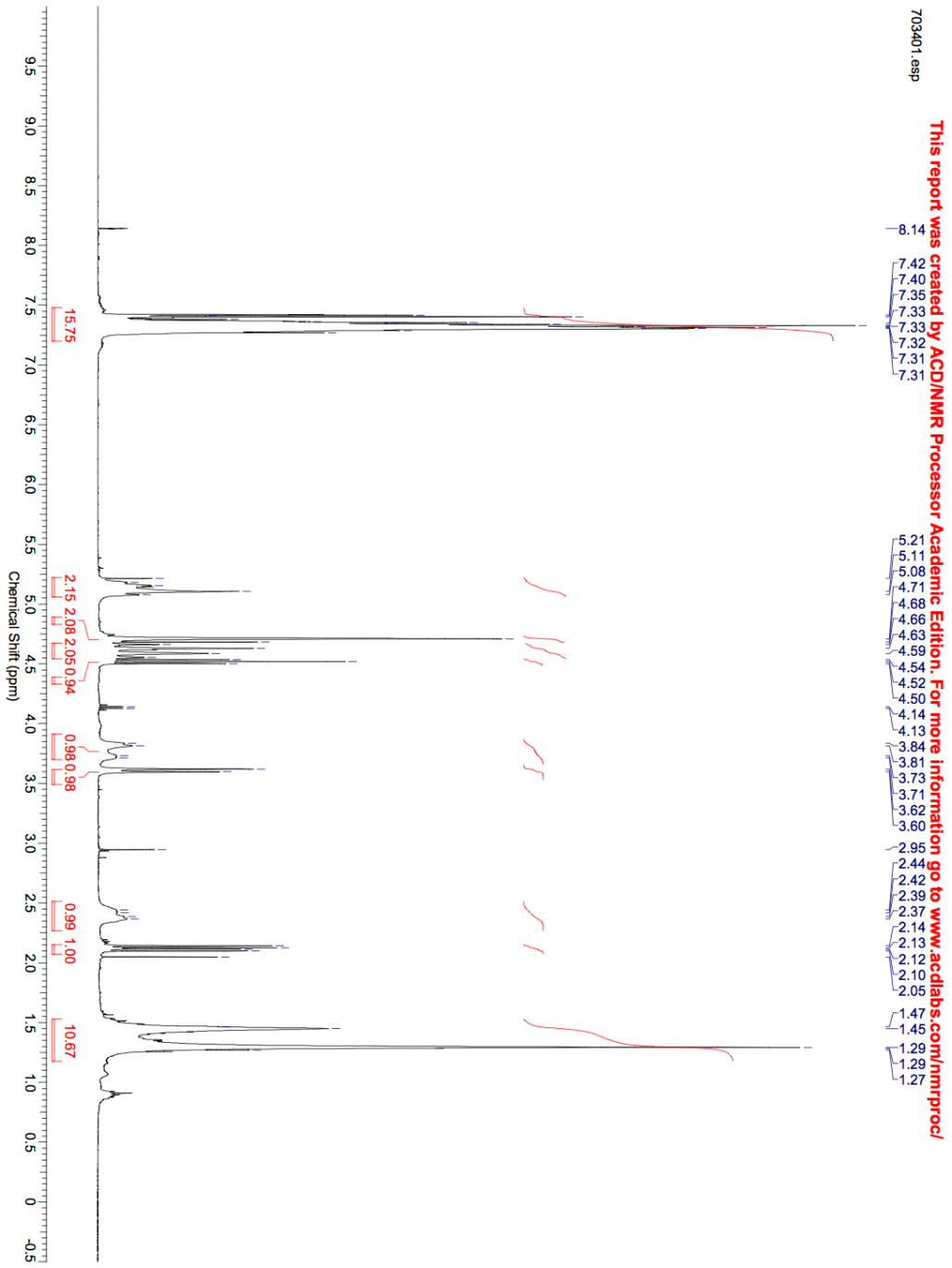



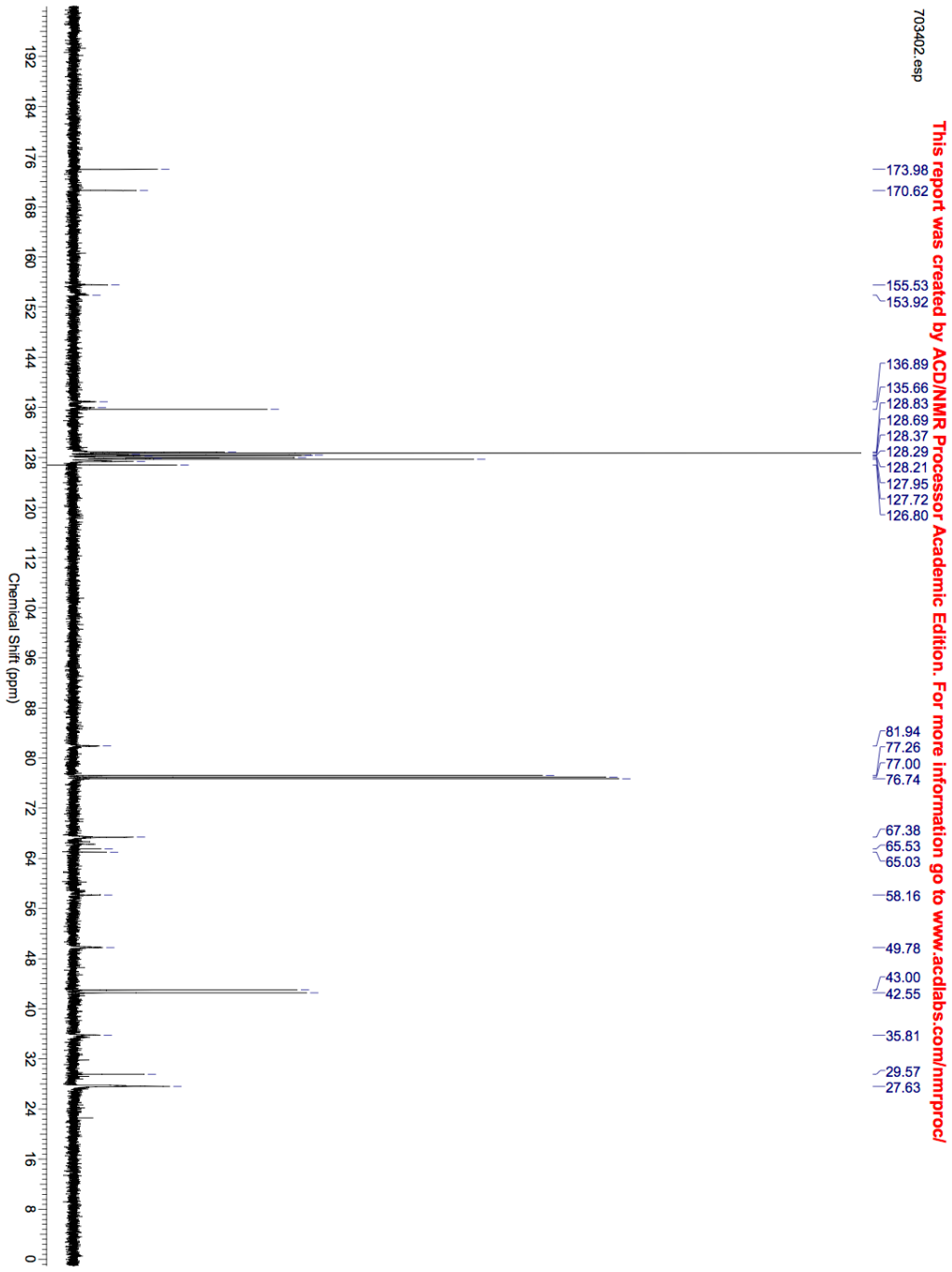

610901.esp
8.18
7.55
7.55
7.54
7.53
7.52
7.50
7.29
7.27
7.26
7.24
4.58
4.56
3.89
3.87
3.86
3.84
3.25
3.22
3.21
3.17
2.50
2.50
2.49
2.49
2.41
2.39
2.38
2.36
2.10
2.08
2.07
3.94
3.94
3.85
1.03
1.93
1.01
1.00
Chemical Shift (ppm)
F109 1H DMSO proton.atm
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/

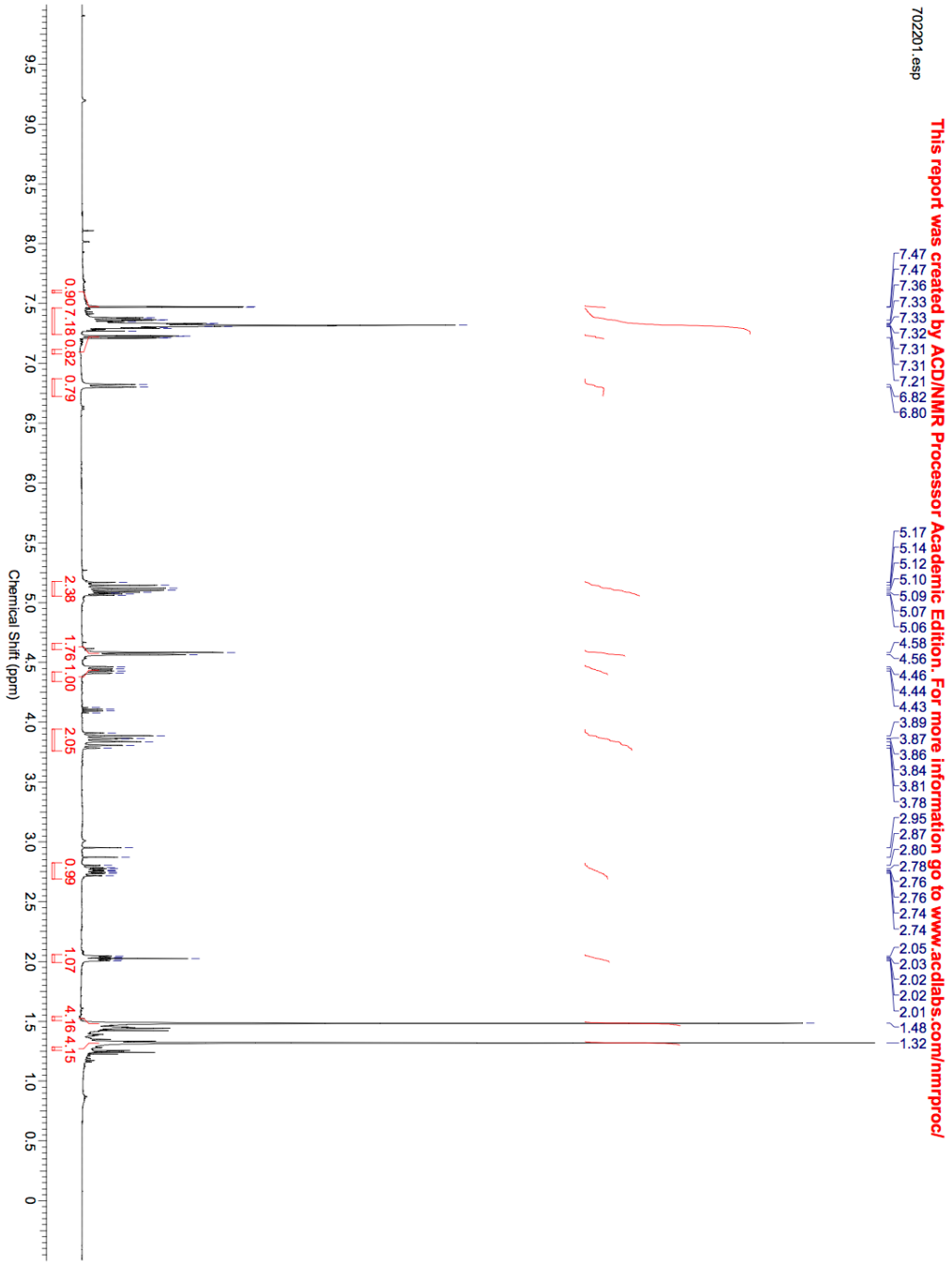

610902.esp
175.00
170.66
155.18
137.40
135.74
131.51
131.32
129.63
129.27
120.65
120.27
69.30
60.37
40.13
39.93
39.72
39.51
39.30
39.10
38.89
F109 13C DMSO carbon10MIN.atm
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0

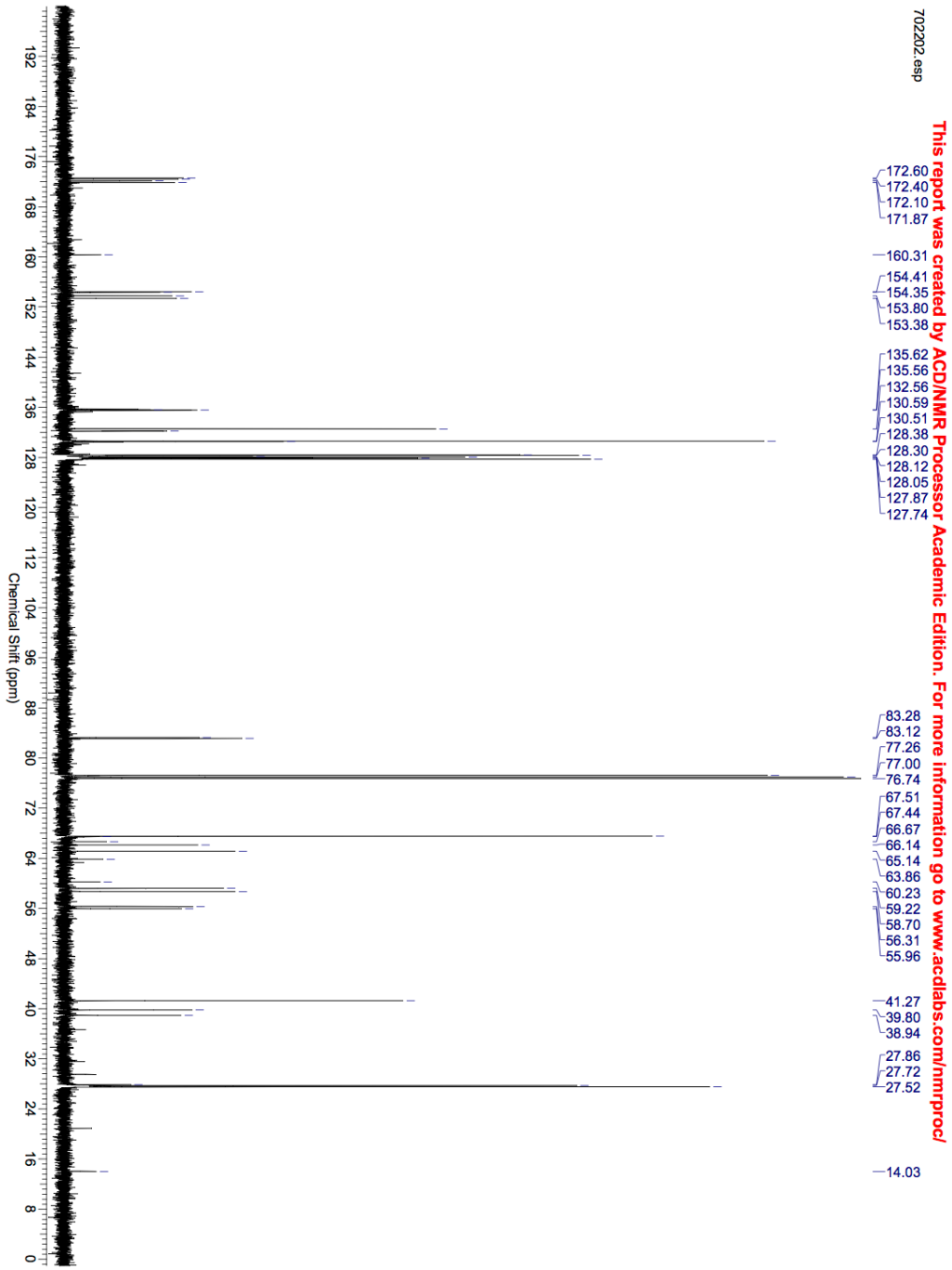

611801.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
7.33
7.32
7.31
7.28
7.27
7.26
7.24
7.23
6.69
6.66
5.12
5.10
5.08
5.06
5.05
5.03
5.02
4.60
4.59
4.40
4.38
4.38
4.36
3.85
3.81
3.80
3.79
3.74
2.75
2.73
2.72
2.71
2.70
2.69
2.68
2.66
1.99
1.97
1.44
1.41
1.39
1.38
1.30
1.28
1.26
1.23
1.22
10.64
0.92
2.32
2.01
1.01
2.18
1.03
1.00
4.11
4.51
Chemical Shift (ppm)
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
-0.5


This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
611802.pdf.esp
172.70
172.51
172.23
154.99
154.93
154.01
153.56
135.99
135.90
135.74
128.74
128.55
128.46
128.08
128.00
127.90
83.29
83.12
77.47
77.22
76.96
67.61
66.19
65.17
59.46
58.94
56.47
56.10
42.55
39.99
39.16
27.92
27.73
192 184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 16 8 0
Chemical Shift (ppm)


703401.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
8.14
7.42
7.40
7.35
7.33
7.33
7.32
7.31
7.31
5.21
5.11
5.08
4.71
4.68
4.66
4.63
4.59
4.54
4.52
4.50
4.14
4.13
3.84
3.81
3.73
3.71
3.62
3.60
2.95
2.44
2.42
2.39
2.37
2.14
2.13
2.12
2.10
2.05
1.47
1.45
1.29
1.29
1.27
15.75
2.15
2.08
2.05
0.94
0.98
0.98
0.99
1.00
10.67
Chemical Shift (ppm)


703402.esp
173.98
170.62
155.53
153.92
136.89
135.66
128.83
128.69
128.37
128.29
128.21
127.95
127.72
126.80
81.94
77.26
77.00
76.74
67.38
65.53
65.03
58.16
49.78
43.00
42.55
35.81
29.57
27.63
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0


This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
702201.esp
7.47
7.47
7.36
7.33
7.33
7.32
7.31
7.31
7.21
6.82
6.80
5.17
5.14
5.12
5.10
5.09
5.07
5.06
4.58
4.56
4.46
4.44
4.43
3.89
3.87
3.86
3.84
3.81
3.78
2.95
2.87
2.80
2.78
2.76
2.76
2.74
2.74
2.05
2.03
2.02
2.02
2.01
1.48
1.32
0.90
7.18
0.82
0.79
2.38
1.76
1.00
2.05
0.99
1.07
4.16
4.15
Chemical Shift (ppm)


702202.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
172.60
172.40
172.10
171.87
160.31
154.41
154.35
153.80
153.38
135.62
135.56
132.56
130.59
130.51
128.38
128.30
128.12
128.05
127.87
127.74
83.28
83.12
77.26
77.00
76.74
67.51
67.44
66.67
66.14
65.14
63.86
60.23
59.22
58.70
56.31
55.96
41.27
39.80
38.94
27.86
27.72
27.52
14.03
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
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88
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56
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16
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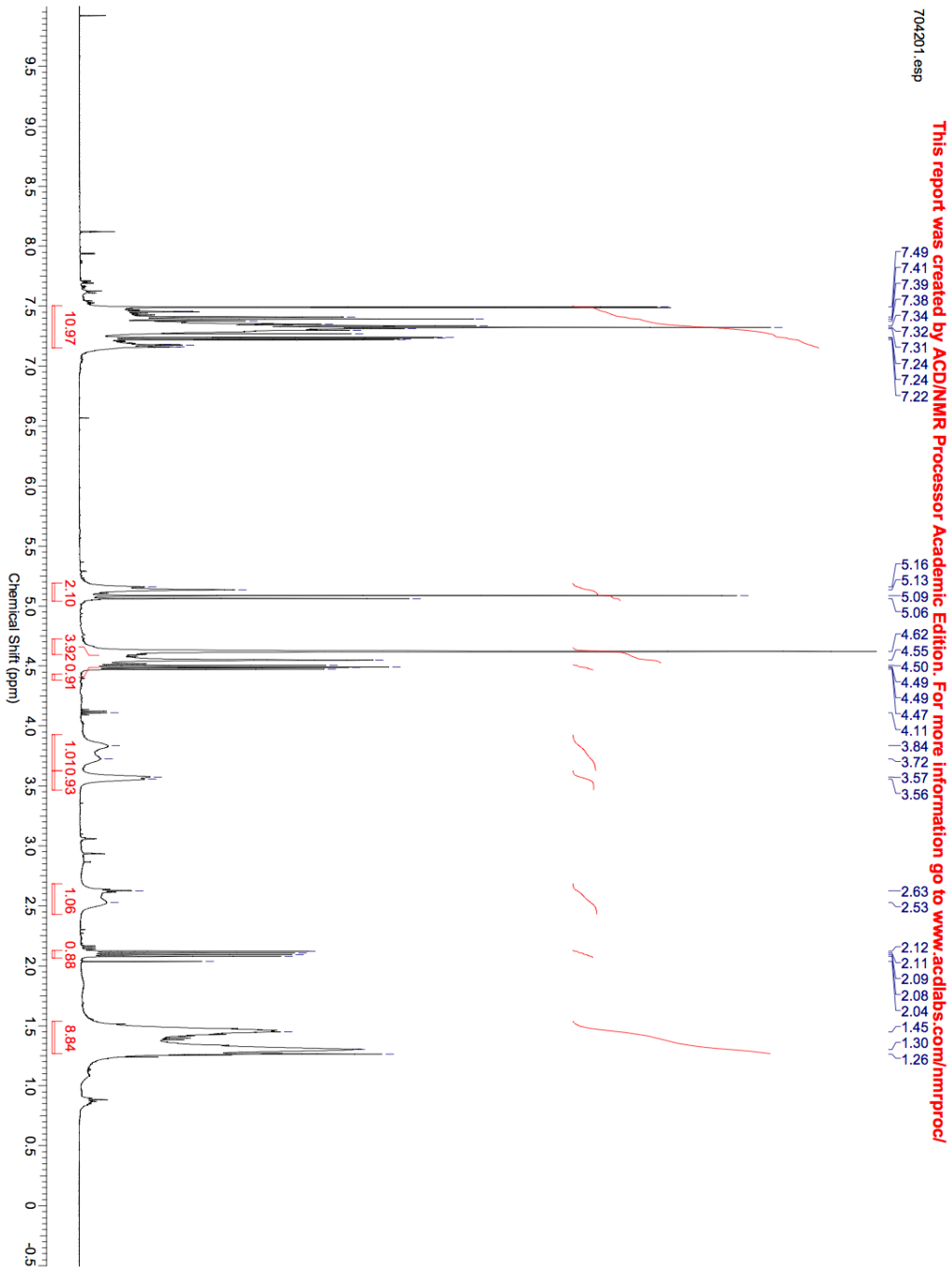
704201.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
7.49
7.41
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7.38
7.34
7.32
7.31
7.24
7.24
7.22
5.16
5.13
5.09
5.06
4.62
4.55
4.50
4.49
4.49
4.47
4.11
3.84
3.72
3.57
3.56
2.63
2.53
2.12
2.11
2.09
2.08
2.04
1.45
1.30
1.26
10.97
2.10
3.92
0.91
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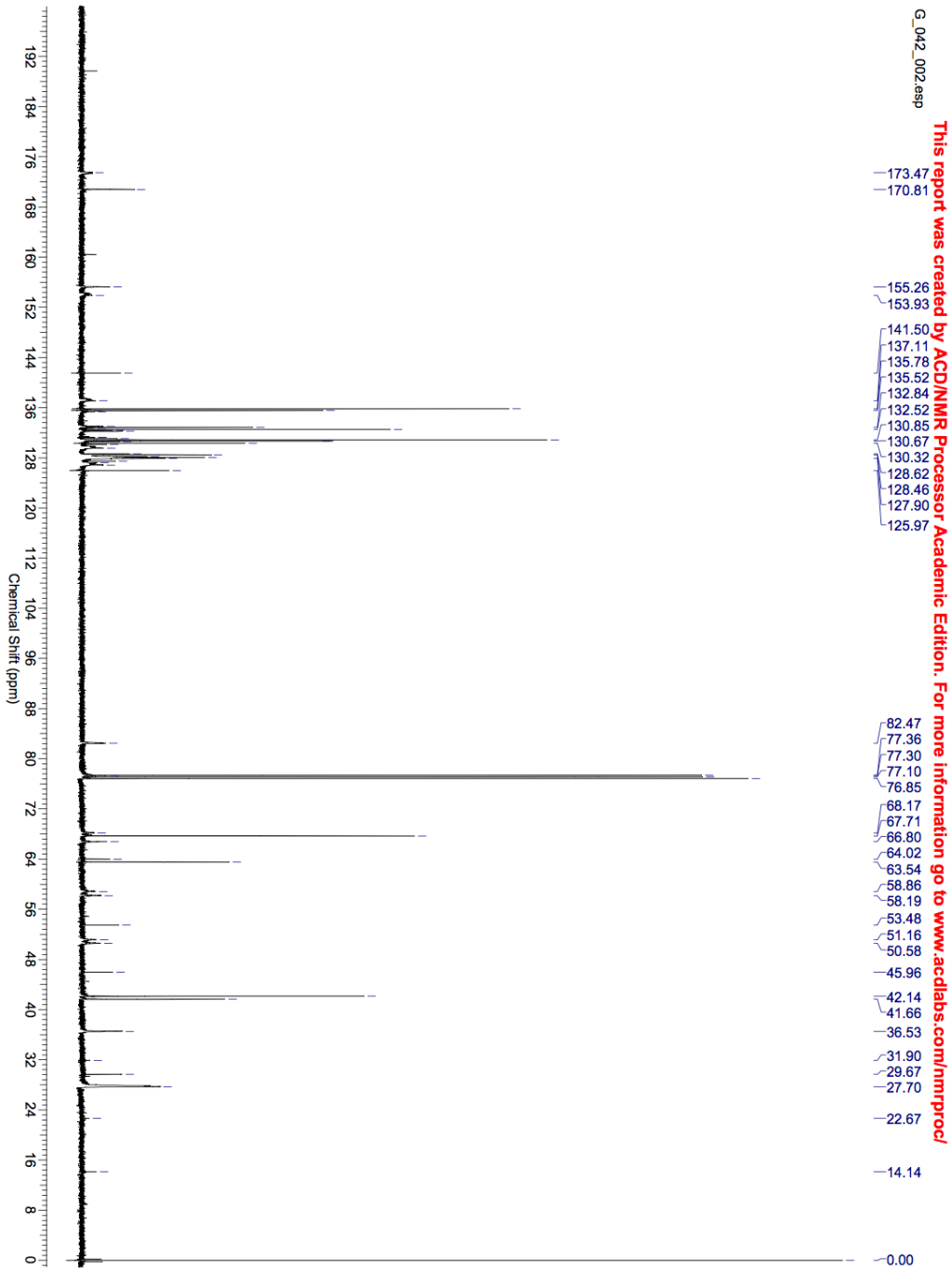
0.93
1.06
0.88
8.84
Chemical Shift (ppm)


G_042_002.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
173.47
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155.26
153.93
141.50
137.11
135.78
135.52
132.84
132.52
130.85
130.67
130.32
128.62
128.46
127.90
125.97
82.47
77.36
77.30
77.10
76.85
68.17
67.71
66.80
64.02
63.54
58.86
58.19
53.48
51.16
50.58
45.96
42.14
41.66
36.53
31.90
29.67
27.70
22.67
14.14
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
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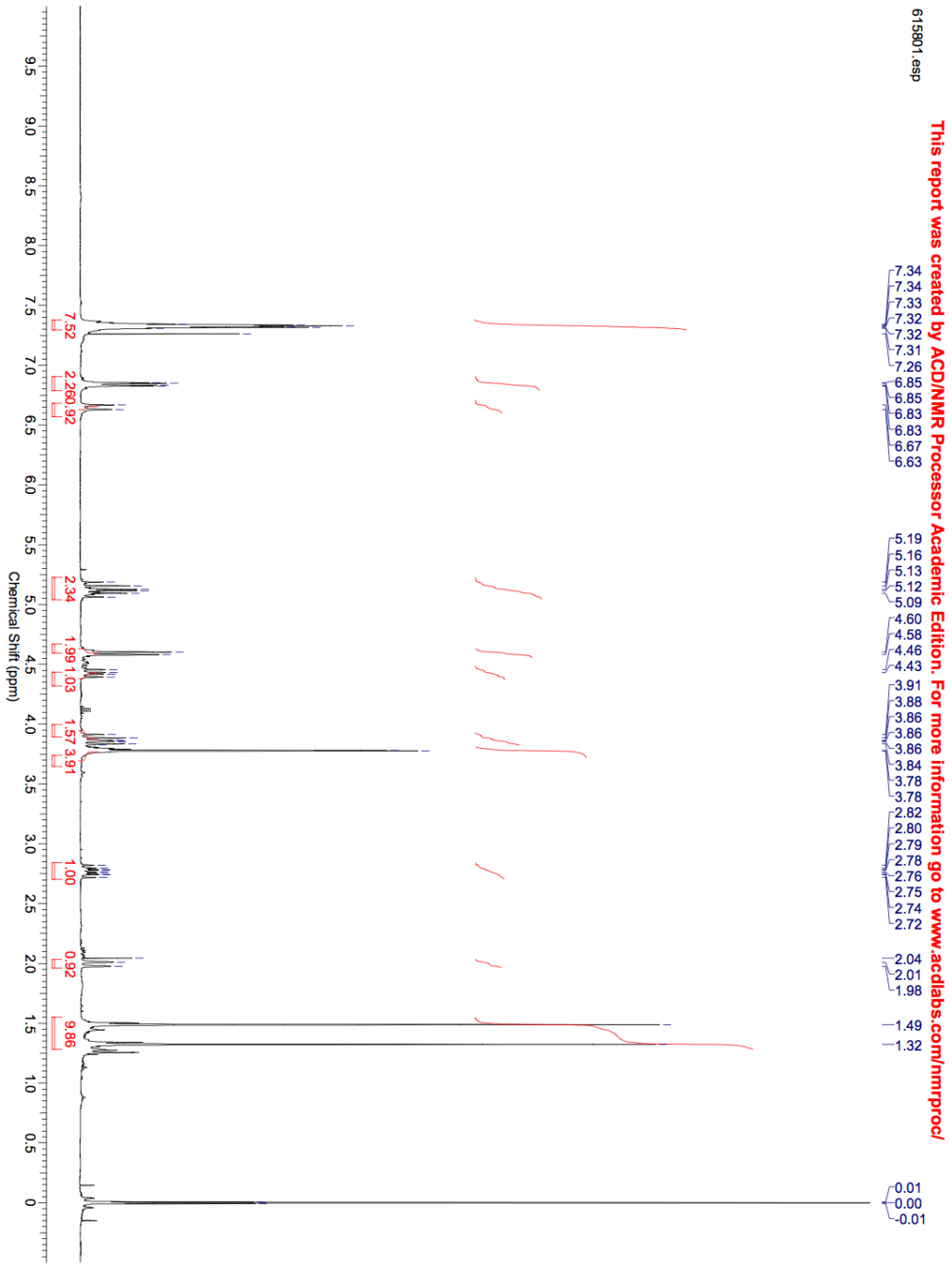
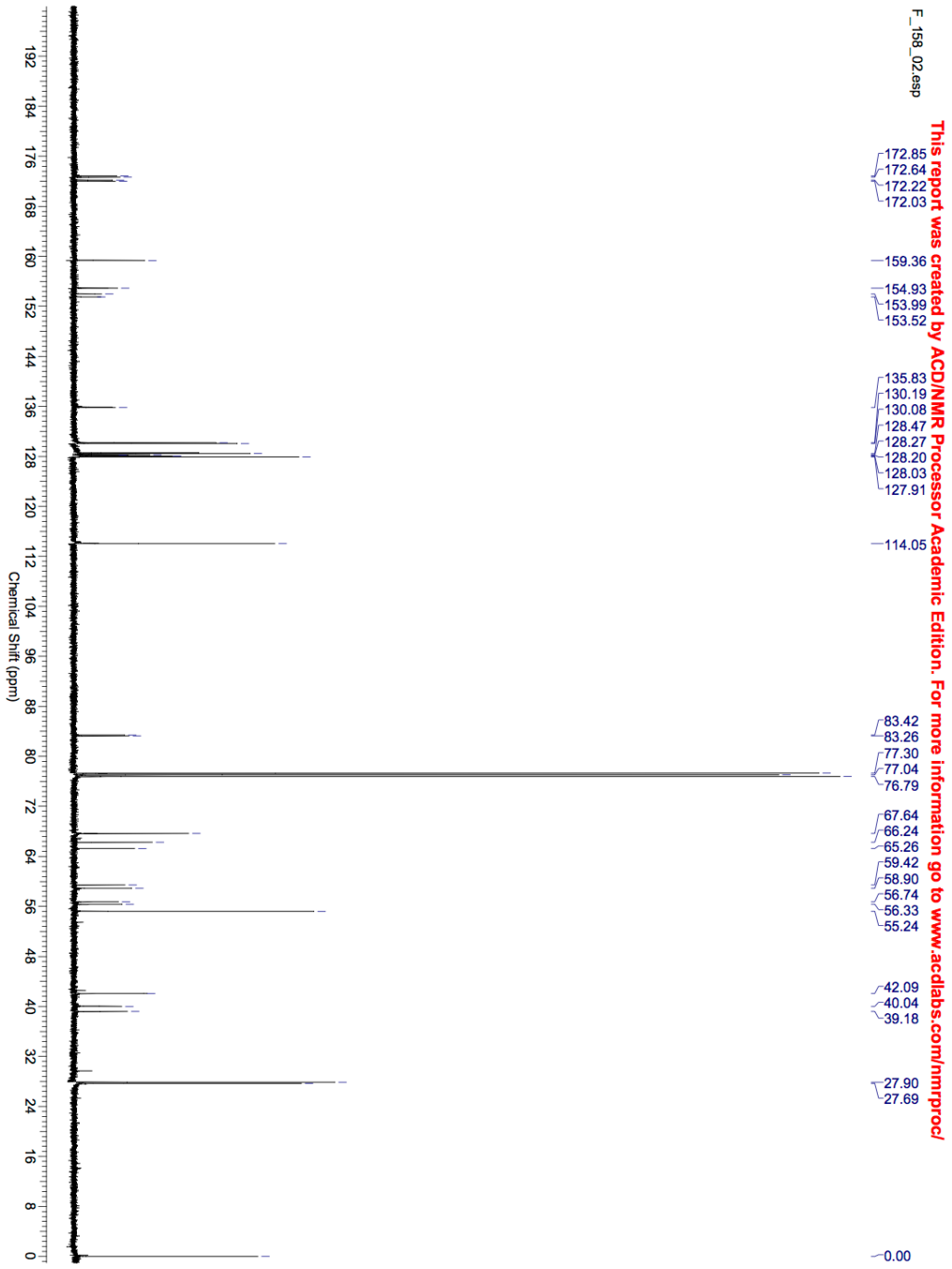

615801.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
Chemical Shift (ppm)
7.52
2.26
0.92
2.34
1.99
1.03
1.57
3.91
1.00
0.92
9.86
7.34
7.34
7.33
7.32
7.32
7.31
7.26
6.85
6.85
6.83
6.83
6.67
6.63
5.19
5.16
5.13
5.12
5.09
4.60
4.58
4.46
4.43
3.91
3.88
3.86
3.86
3.86
3.84
3.78
3.78
2.82
2.80
2.79
2.78
2.76
2.75
2.74
2.72
2.04
2.01
1.98
1.49
1.32
0.01
0.00
-0.01


F_158_02.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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172.64
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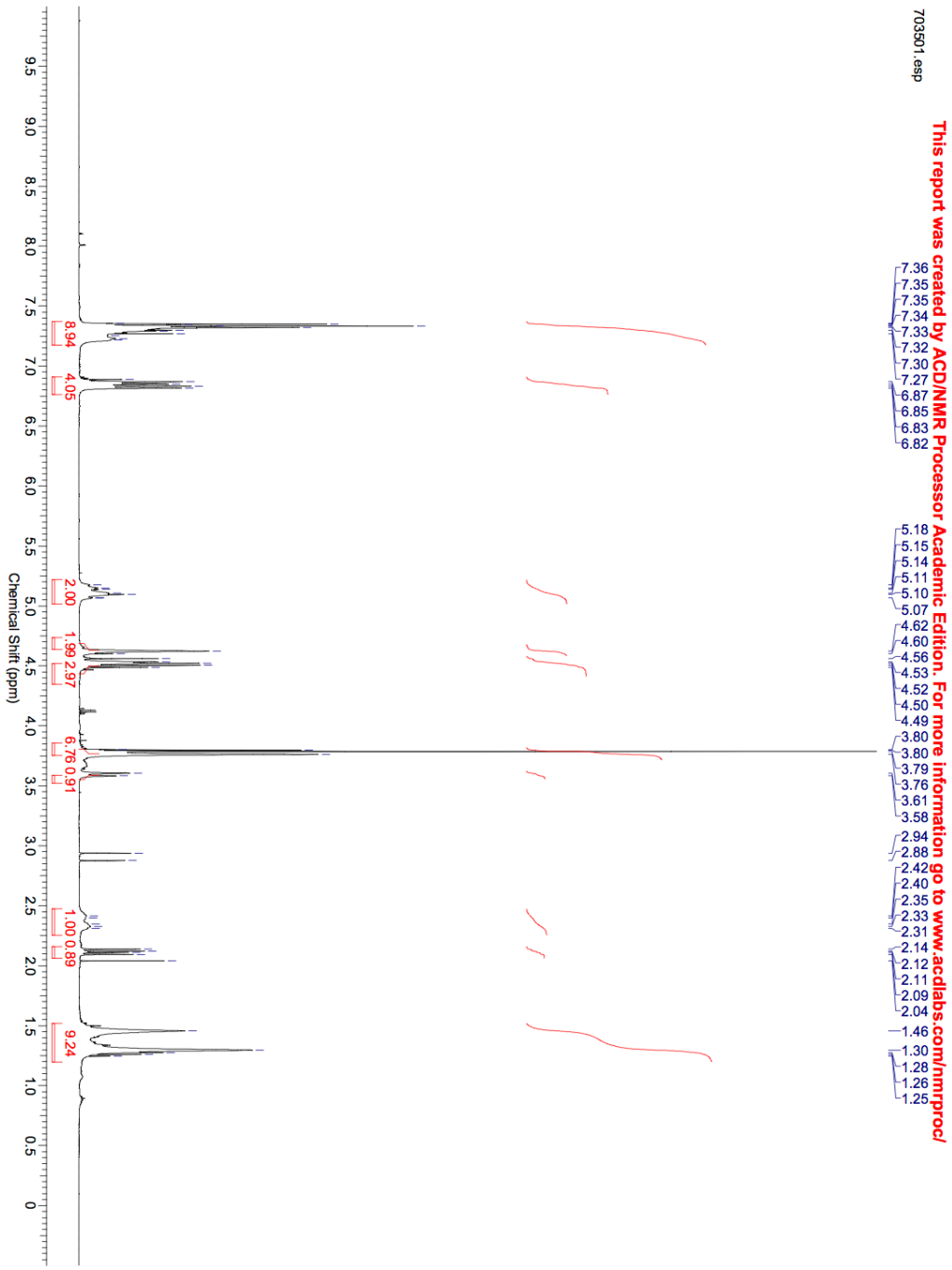
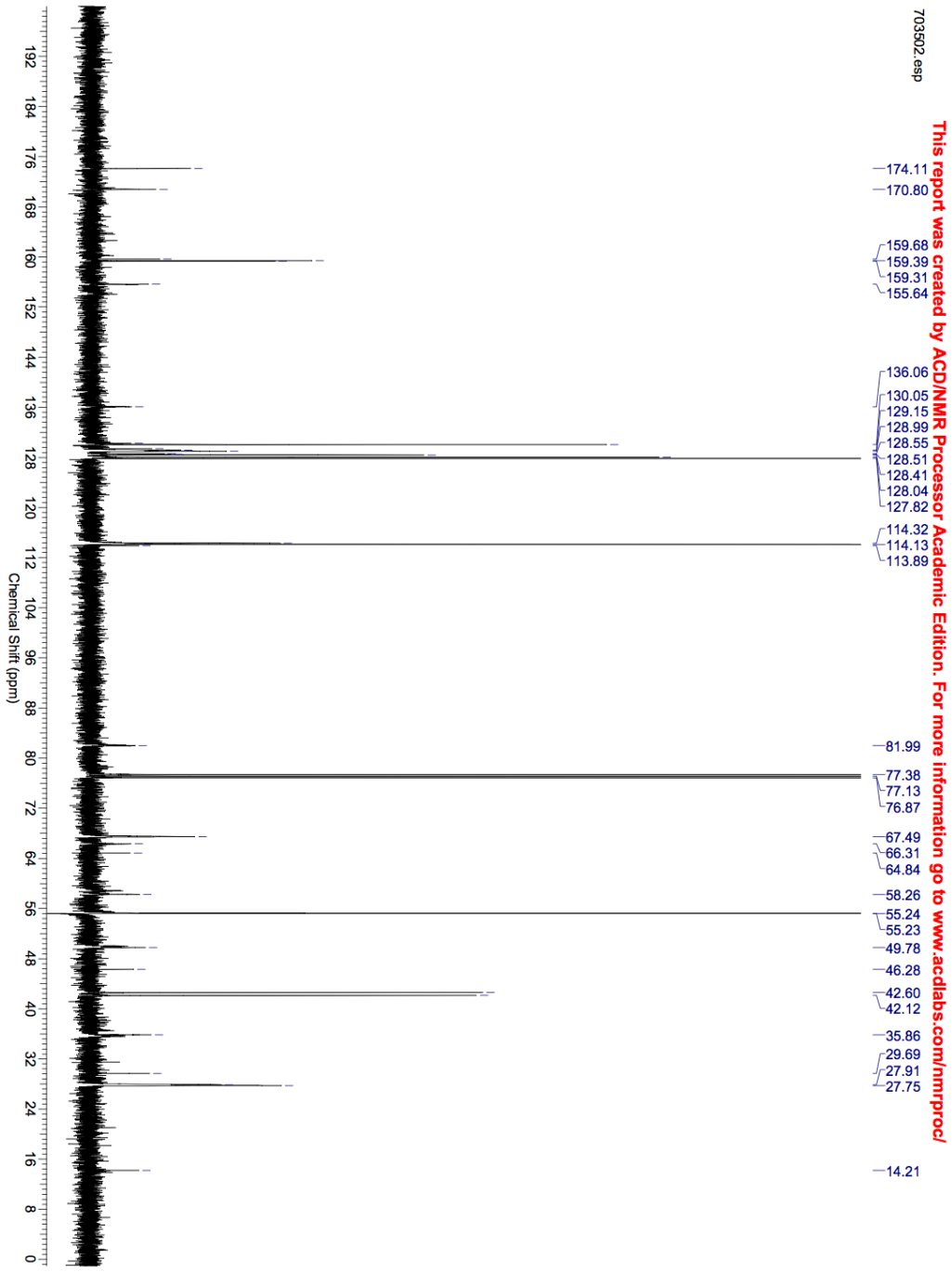
159.36
154.93
153.99
153.52
135.83
130.19
130.08
128.47
128.27
128.20
128.03
127.91
114.05
83.42
83.26
77.30
77.04
76.79
67.64
66.24
65.26
59.42
58.90
56.74
56.33
55.24
42.09
40.04
39.18
27.90
27.69
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0


703501.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
7.36
7.35
7.35
7.34
7.33
7.32
7.30
7.27
6.87
6.85
6.83
6.82
5.18
5.15
5.14
5.11
5.10
5.07
4.62
4.60
4.56
4.53
4.52
4.50
4.49
3.80
3.80
3.79
3.76
3.61
3.58
2.94
2.88
2.42
2.40
2.35
2.33
2.31
2.14
2.12
2.11
2.09
2.04
1.46
1.30
1.28
1.26
1.25
8.94
4.05
2.00
1.99
2.97
6.76
0.91
1.00
0.89
9.24
Chemical Shift (ppm)


703502.esp
174.11
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159.68
159.39
159.31
155.64
136.06
130.05
129.15
128.99
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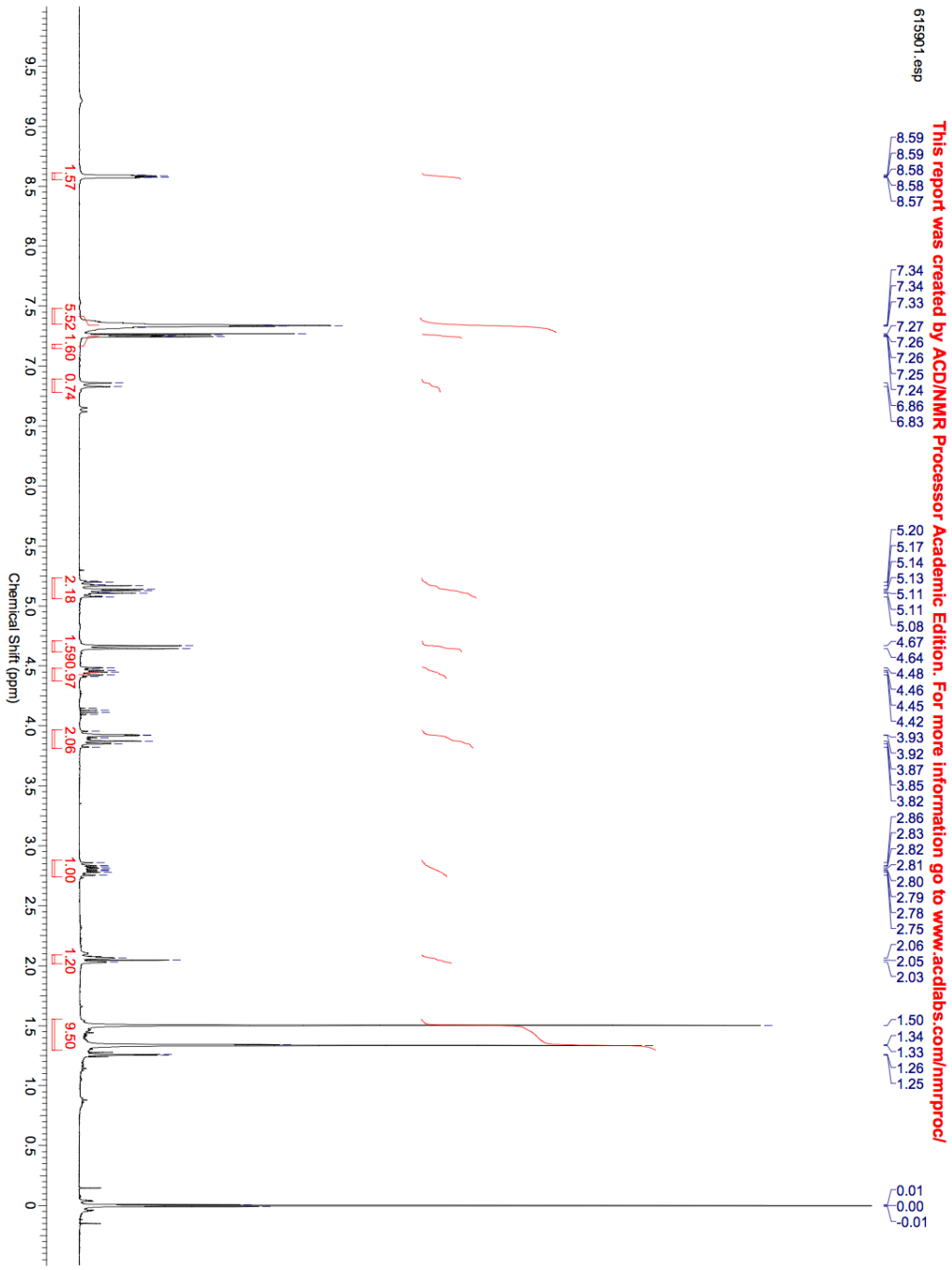
128.51
128.41
128.04
127.82
114.32
114.13
113.89
81.99
77.38
77.13
76.87
67.49
66.31
64.84
58.26
55.24
55.23
49.78
46.28
42.60
42.12
35.86
29.69
27.91
27.75
14.21
Chemical Shift (ppm)
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/


615901.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
Chemical Shift (ppm)
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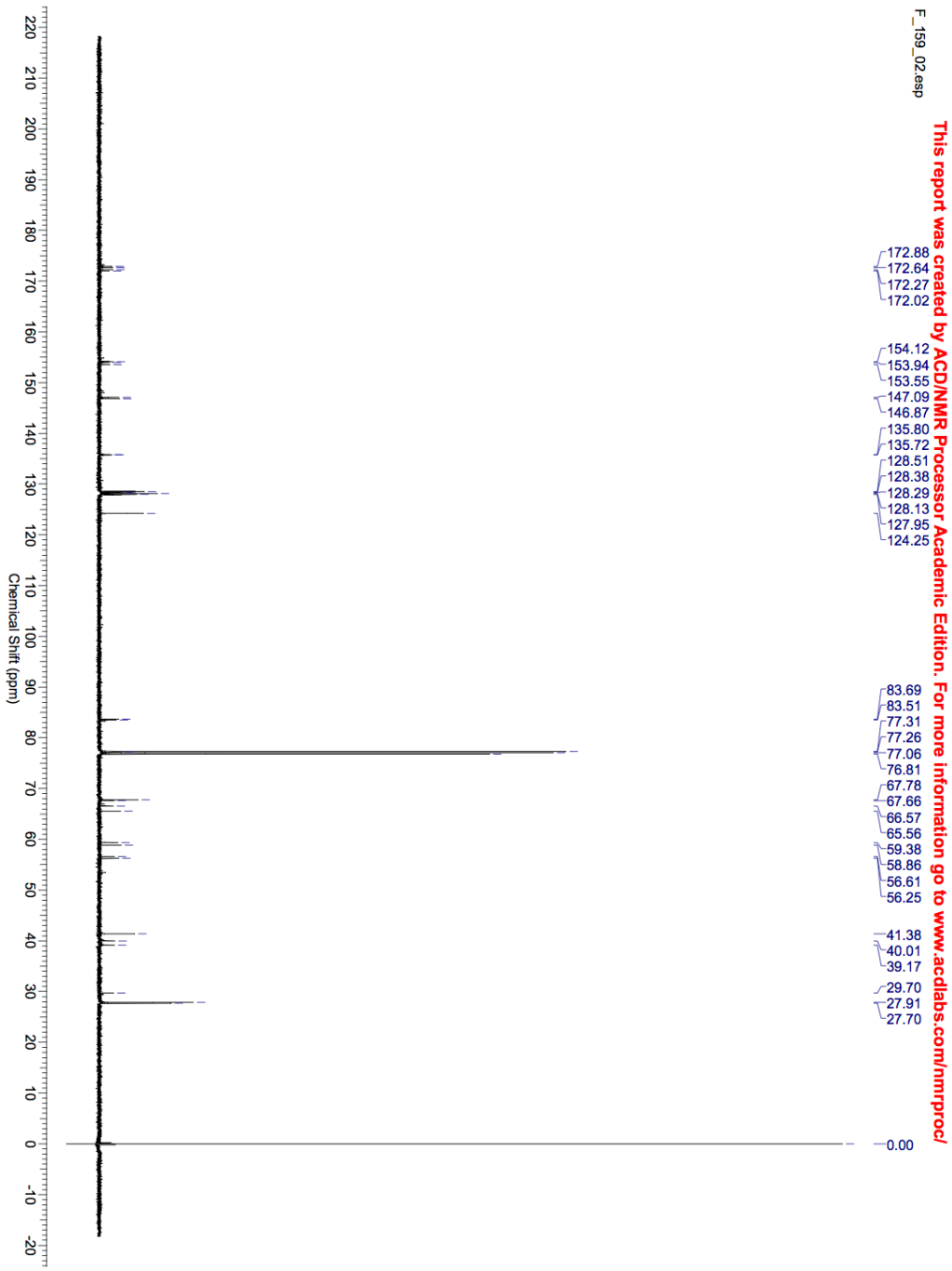
5.52
1.60
0.74
2.18
1.59
0.97
2.06
1.00
1.20
9.50


F_159_02.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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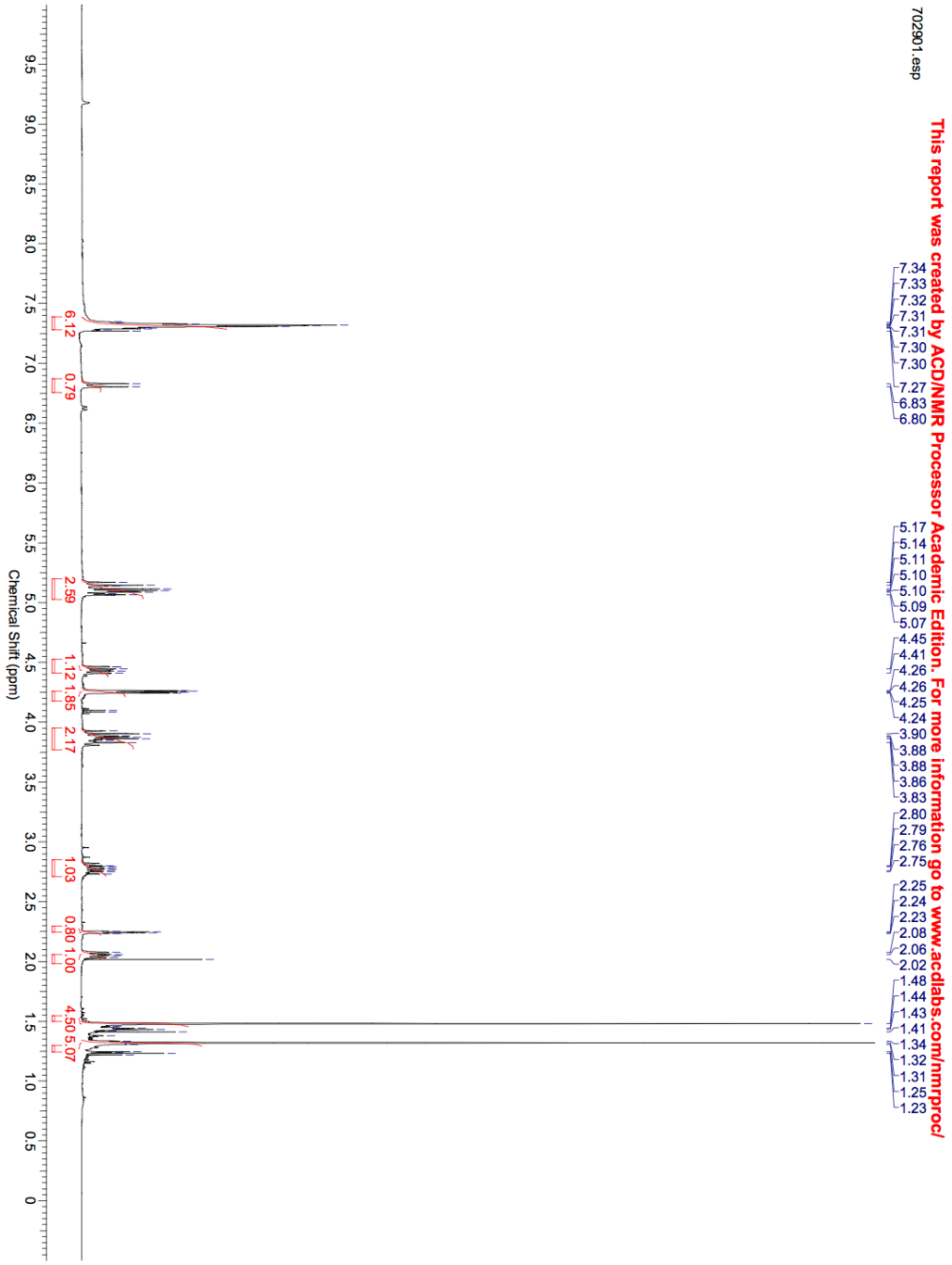
172.64
172.27
172.02
154.12
153.94
153.55
147.09
146.87
135.80
135.72
128.51
128.38
128.29
128.13
127.95
124.25
83.69
83.51
77.31
77.26
77.06
76.81
67.78
67.66
66.57
65.56
59.38
58.86
56.61
56.25
41.38
40.01
39.17
29.70
27.91
27.70
0.00
Chemical Shift (ppm)
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20


This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
702901.esp
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7.33
7.32
7.31
7.31
7.30
7.30
7.27
6.83
6.80
5.17
5.14
5.11
5.10
5.10
5.09
5.07
4.45
4.41
4.26
4.26
4.25
4.24
3.90
3.88
3.88
3.86
3.83
2.80
2.79
2.76
2.75
2.25
2.24
2.23
2.08
2.06
2.02
1.48
1.44
1.43
1.41
1.34
1.32
1.31
1.25
1.23
6.12
0.79
2.59
1.12
1.85
2.17
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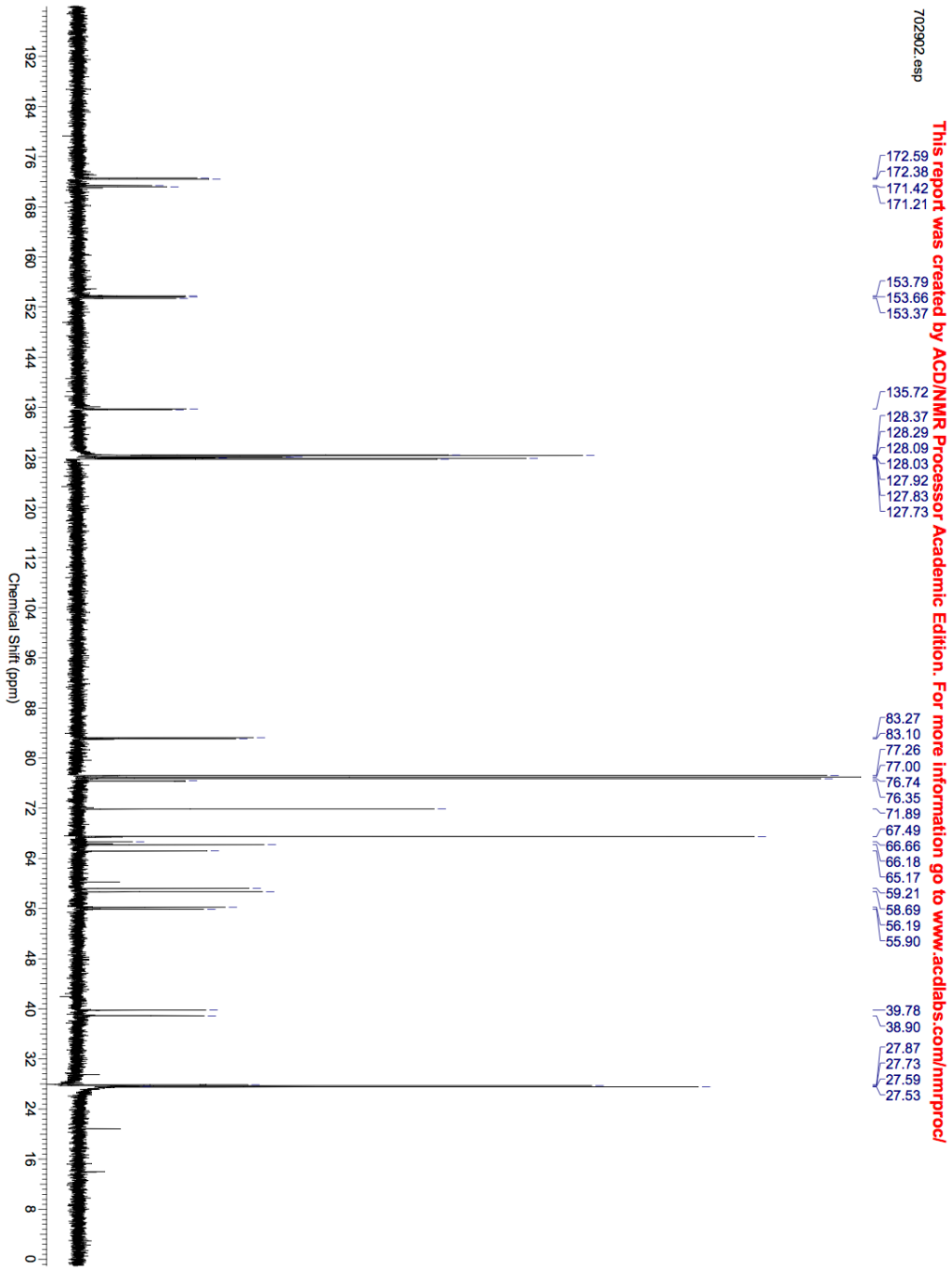
1.00
4.50
5.07
Chemical Shift (ppm)


702902.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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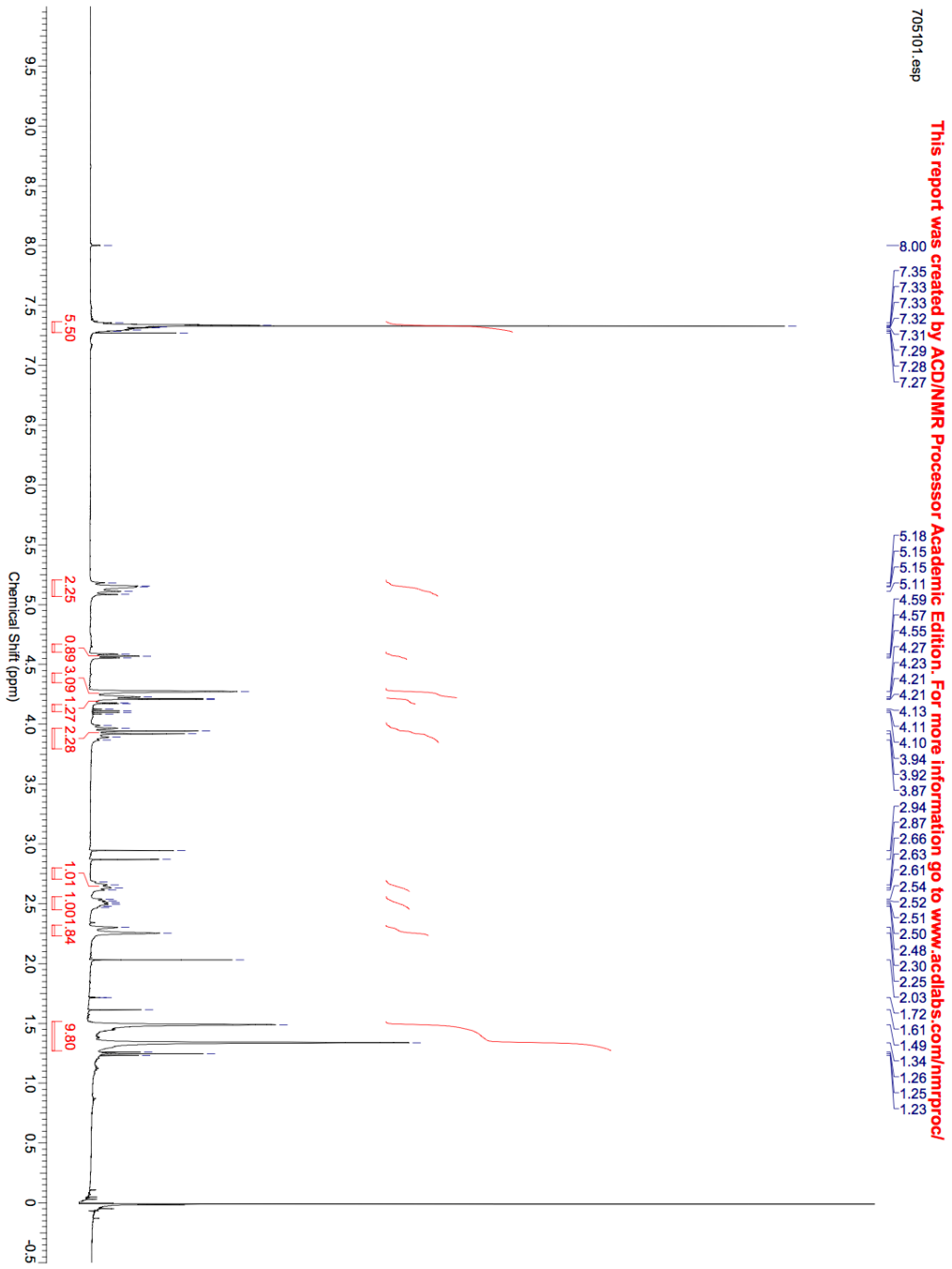
172.38
171.42
171.21
153.79
153.66
153.37
135.72
128.37
128.29
128.09
128.03
127.92
127.83
127.73
83.27
83.10
77.26
77.00
76.74
76.35
71.89
67.49
66.66
66.18
65.17
59.21
58.69
56.19
55.90
39.78
38.90
27.87
27.73
27.59
27.53
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0


705101.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
8.00
7.35
7.33
7.33
7.32
7.31
7.29
7.28
7.27
5.18
5.15
5.15
5.11
4.59
4.57
4.55
4.27
4.23
4.21
4.21
4.13
4.11
4.10
3.94
3.92
3.87
2.94
2.87
2.66
2.63
2.61
2.54
2.52
2.51
2.50
2.48
2.30
2.25
2.03
1.72
1.61
1.49
1.34
1.26
1.25
1.23
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3.09
1.27
2.28
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1.00
1.84
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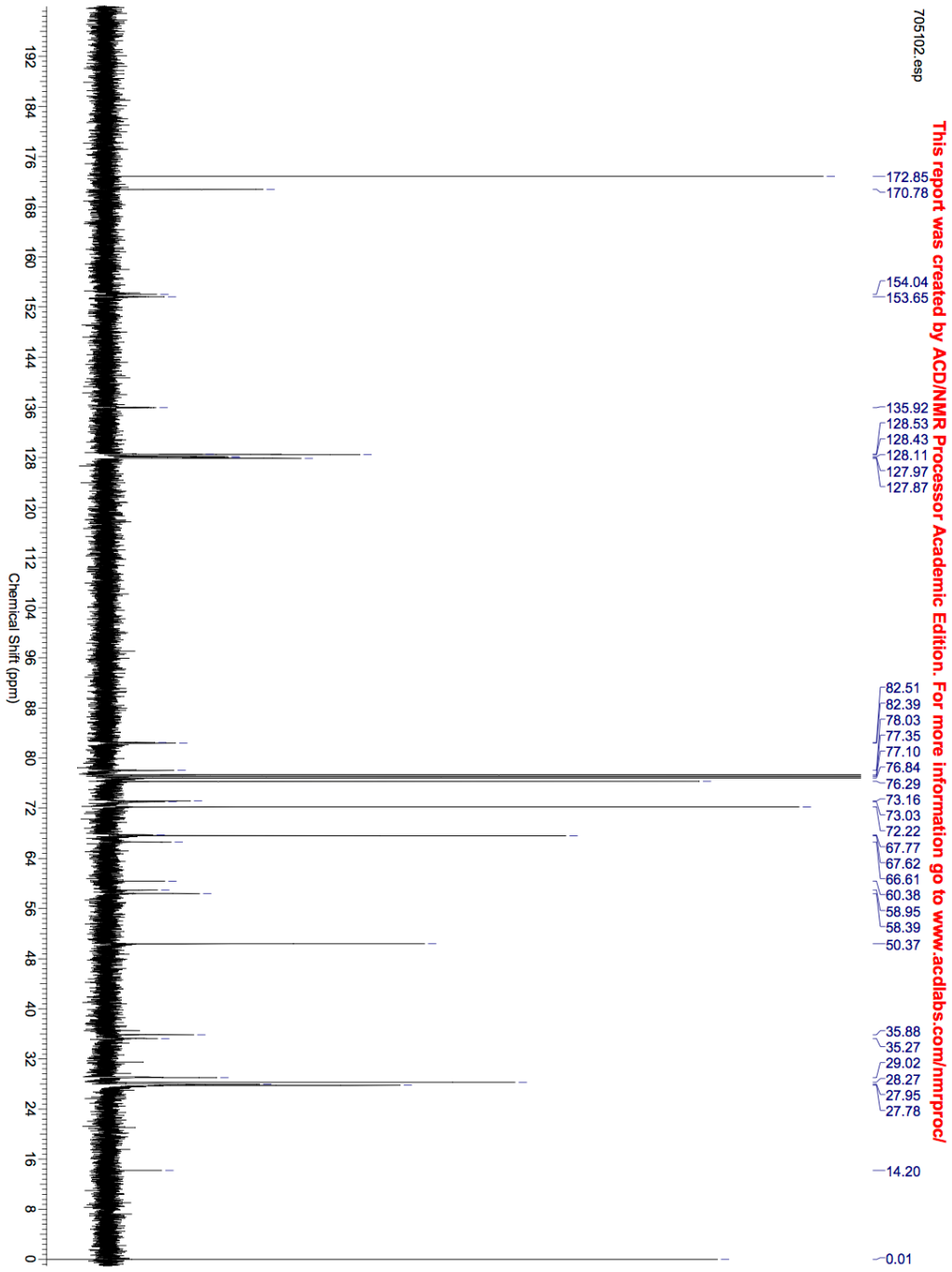
Chemical Shift (ppm)


705102.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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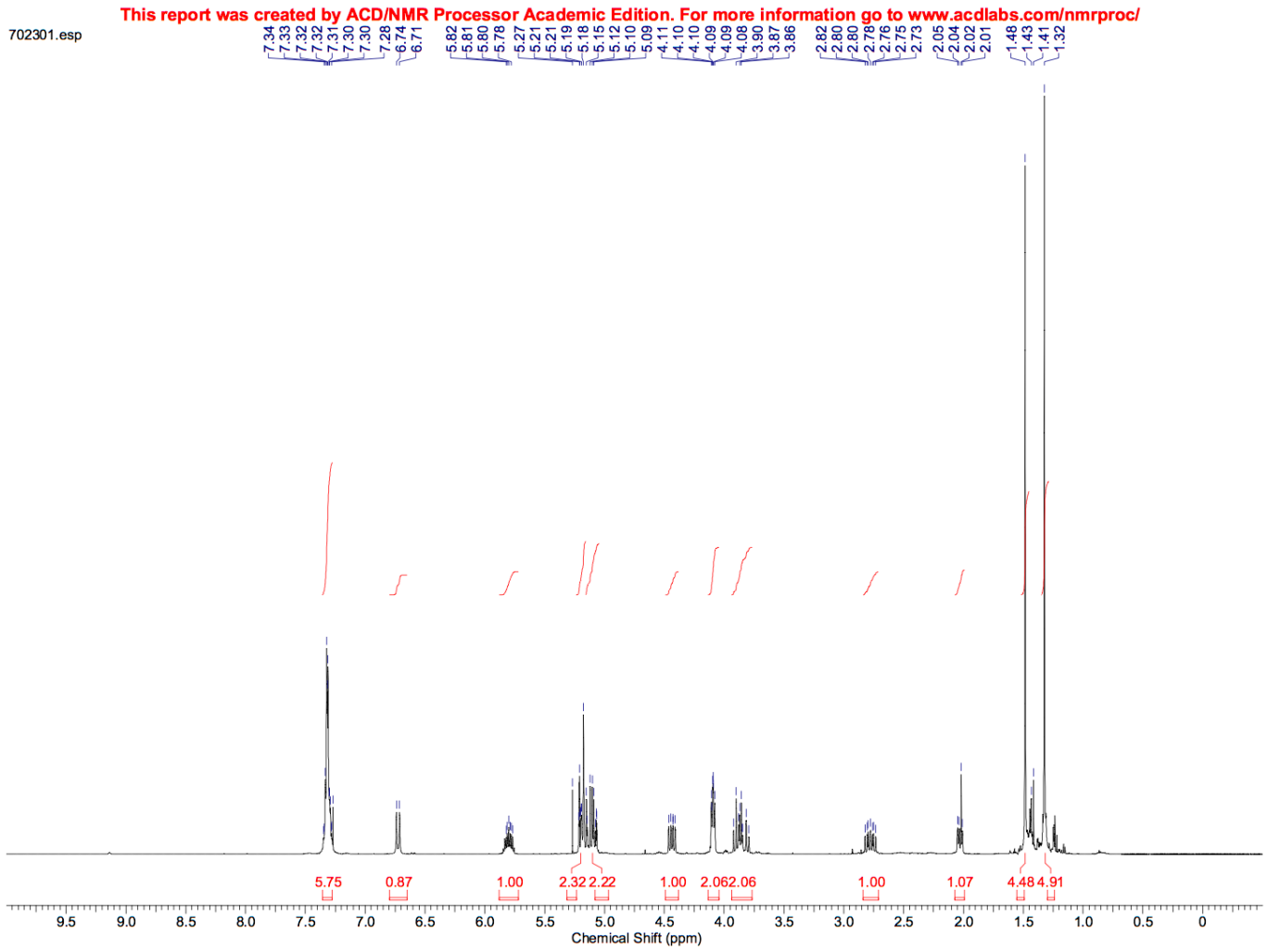
135.92
128.53
128.43
128.11
127.97
127.87
82.51
82.39
78.03
77.35
77.10
76.84
76.29
73.16
73.03
72.22
67.77
67.62
66.61
60.38
58.95
58.39
50.37
35.88
35.27
29.02
28.27
27.95
27.78
14.20
0.01
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0


This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
702301.esp
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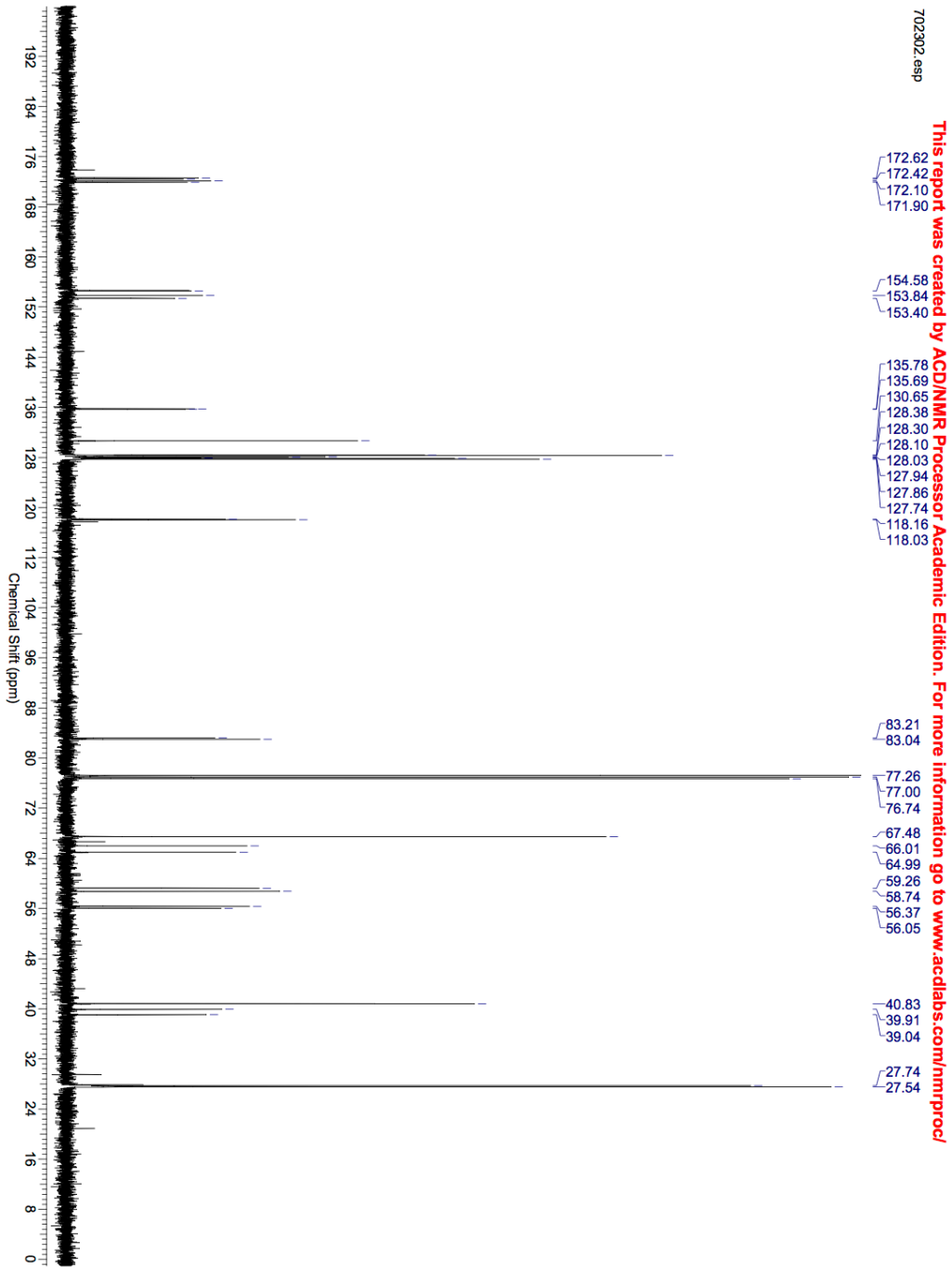
5.75 0.87 1.00 2.32 2.22 1.00 2.06 2.06 1.00 1.07 4.48 4.91
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
Chemical Shift (ppm)


702302.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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172.42
172.10
171.90
154.58
153.84
153.40
135.78
135.69
130.65
128.38
128.30
128.10
128.03
127.94
127.86
127.74
118.16
118.03
83.21
83.04
77.26
77.00
76.74
67.48
66.01
64.99
59.26
58.74
56.37
56.05
40.83
39.91
39.04
27.74
27.54
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
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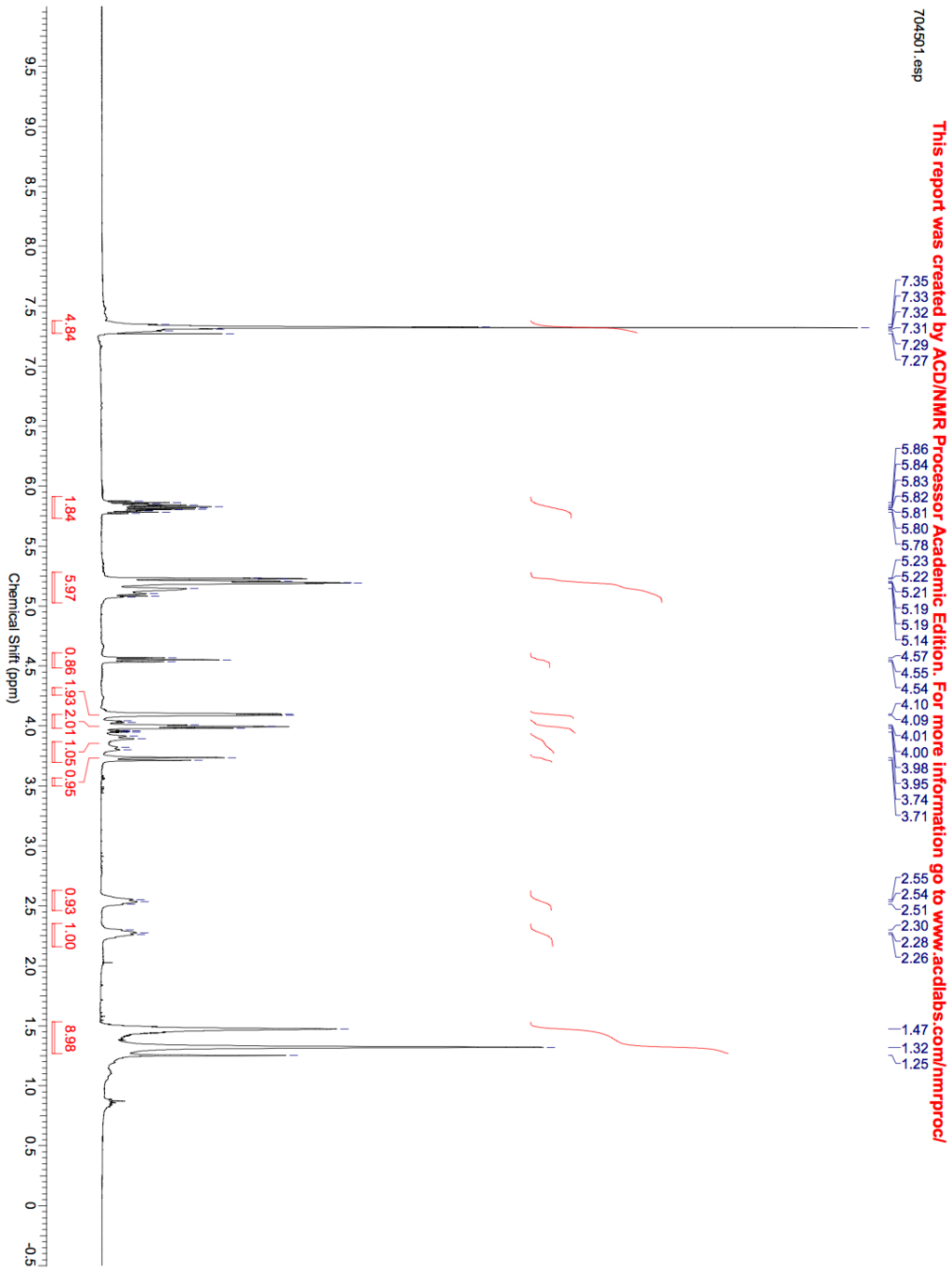
704501.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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5.84
5.83
5.82
5.81
5.80
5.78
5.23
5.22
5.21
5.19
5.19
5.14
4.57
4.55
4.54
4.10
4.09
4.01
4.00
3.98
3.95
3.74
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2.28
2.26
1.47
1.32
1.25
4.84
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1.93
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0.93
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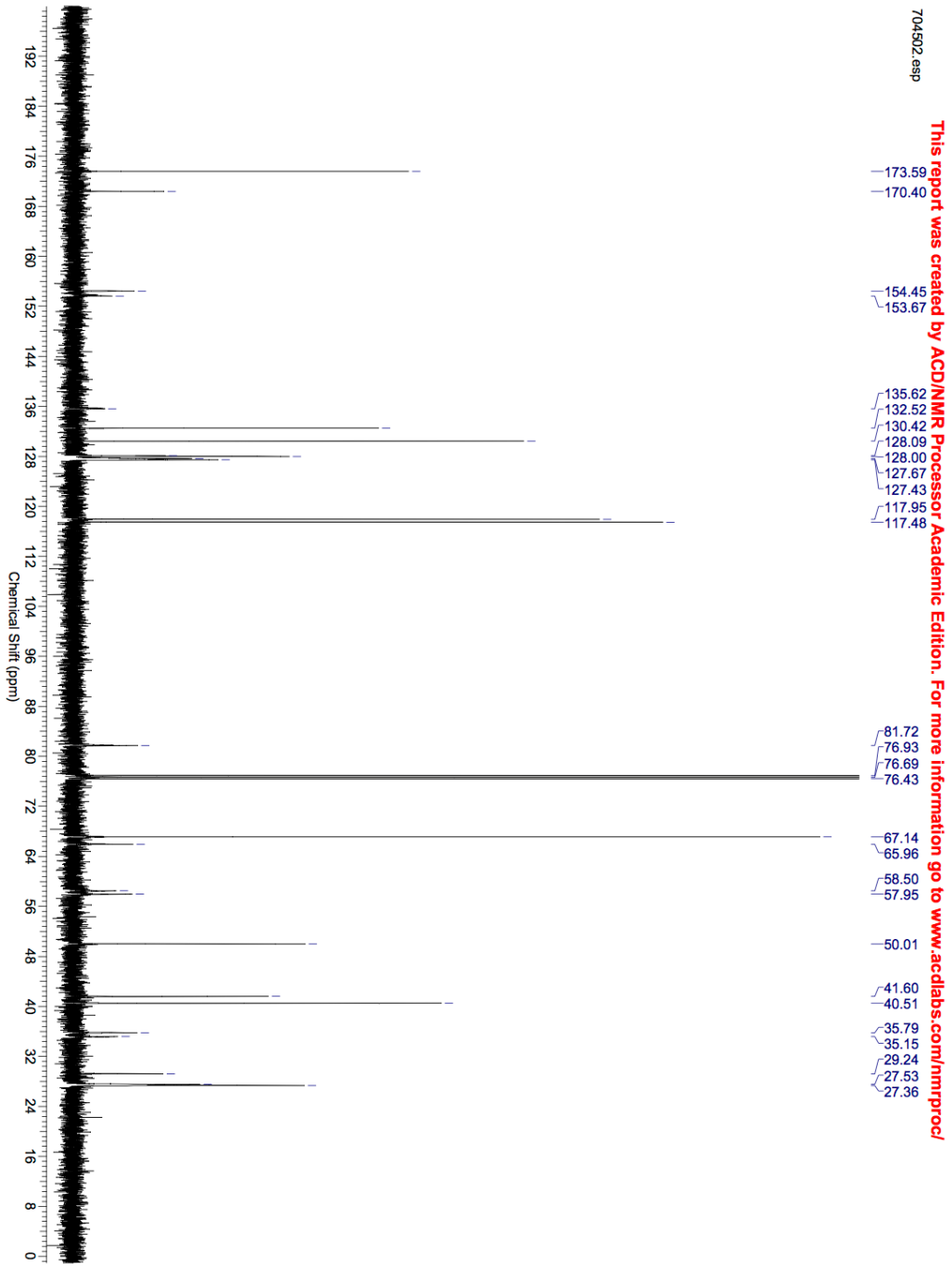
8.98
Chemical Shift (ppm)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117.48
81.72
76.93
76.69
76.43
67.14
65.96
58.50
57.95
50.01
41.60
40.51
35.79
35.15
29.24
27.53
27.36
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
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32
24
16
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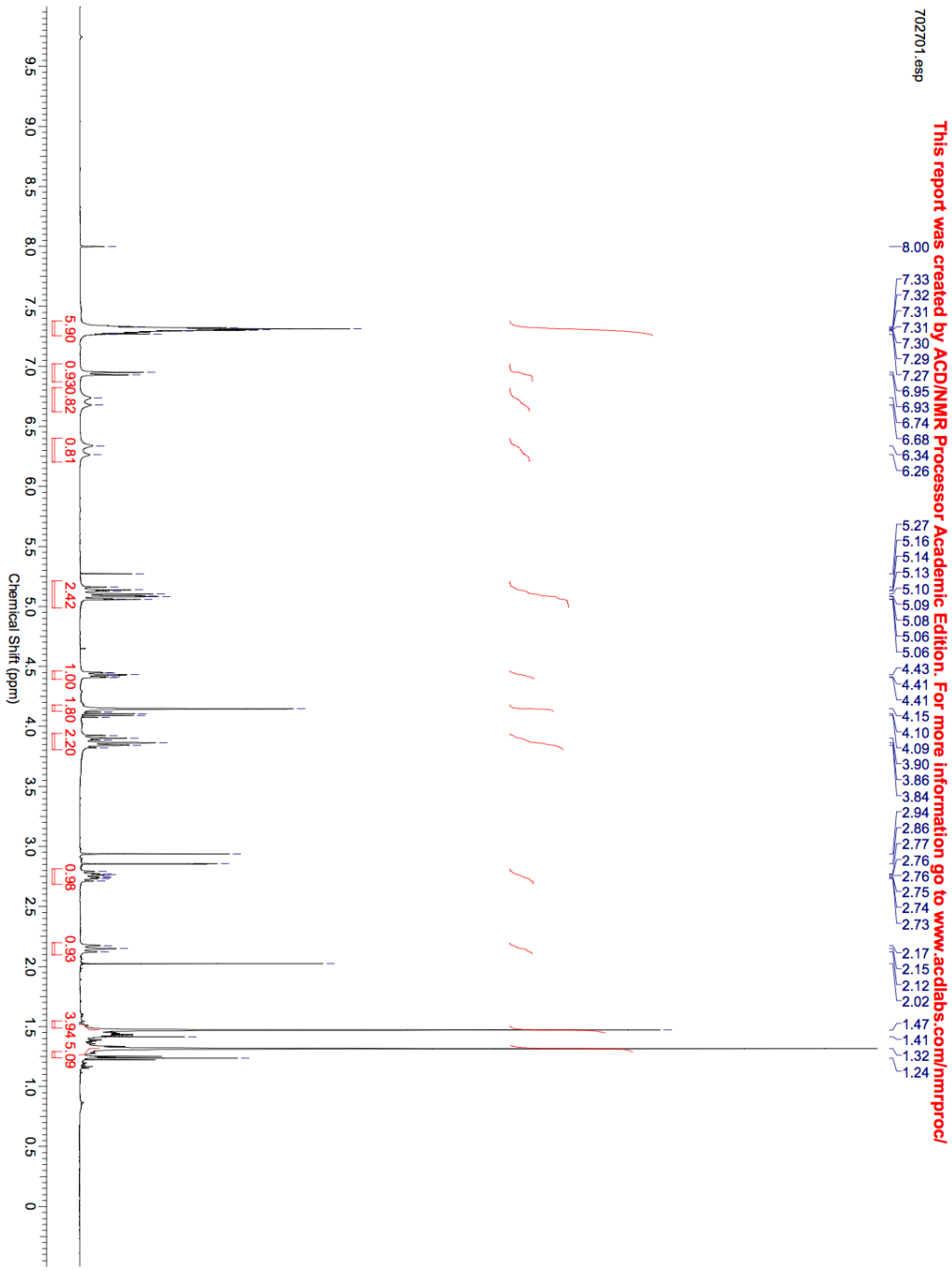
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/


This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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6.95
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5.16
5.14
5.13
5.10
5.09
5.08
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4.43
4.41
4.41
4.15
4.10
4.09
3.90
3.86
3.84
2.94
2.86
2.77
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2.76
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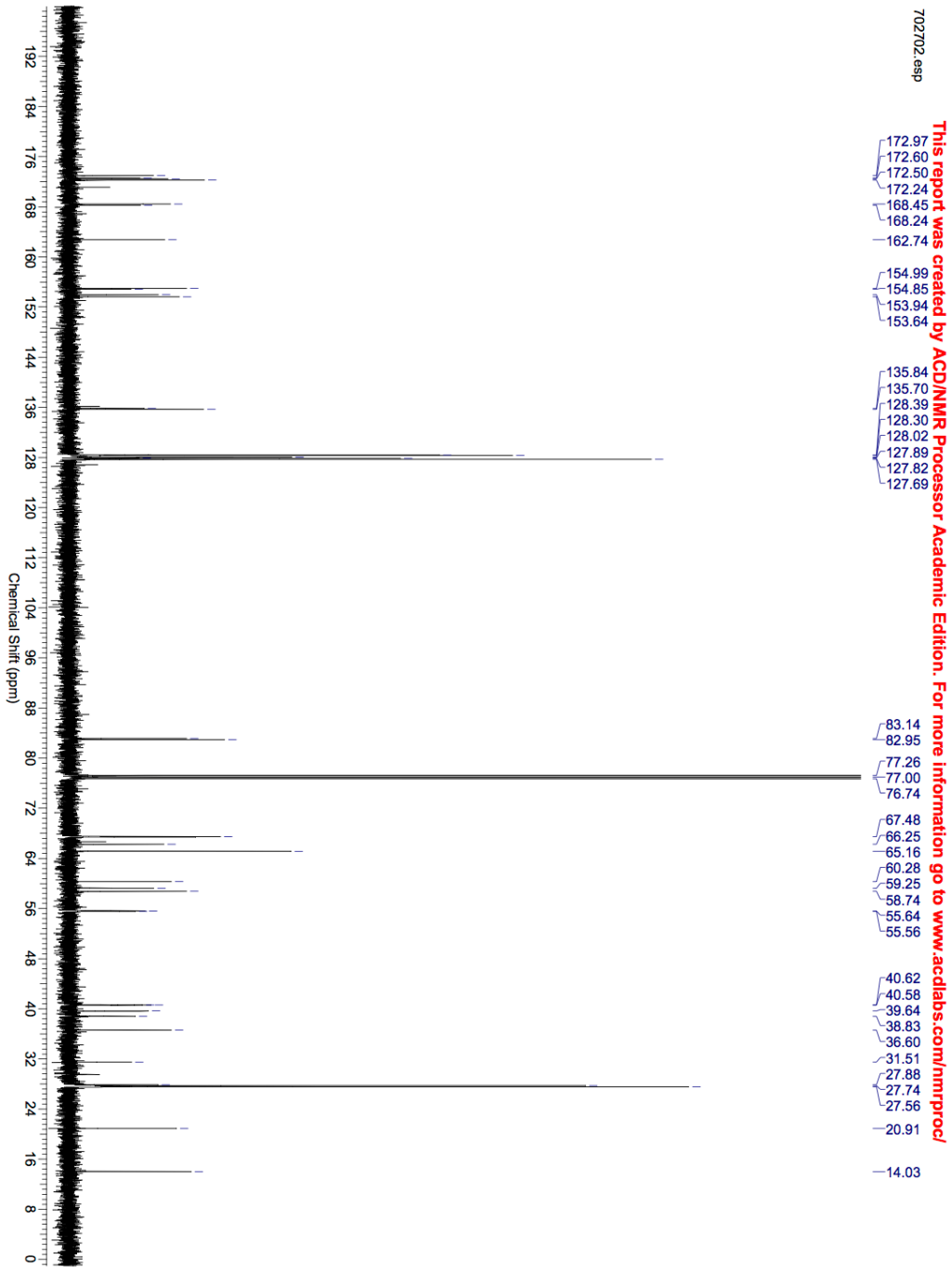
2.74
2.73
2.17
2.15
2.12
2.02
1.47
1.41
1.32
1.24
5.90
0.93
0.82
0.81
2.42
1.00
1.80
2.20
0.98
0.93
3.94
5.09
Chemical Shift (ppm)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153.64
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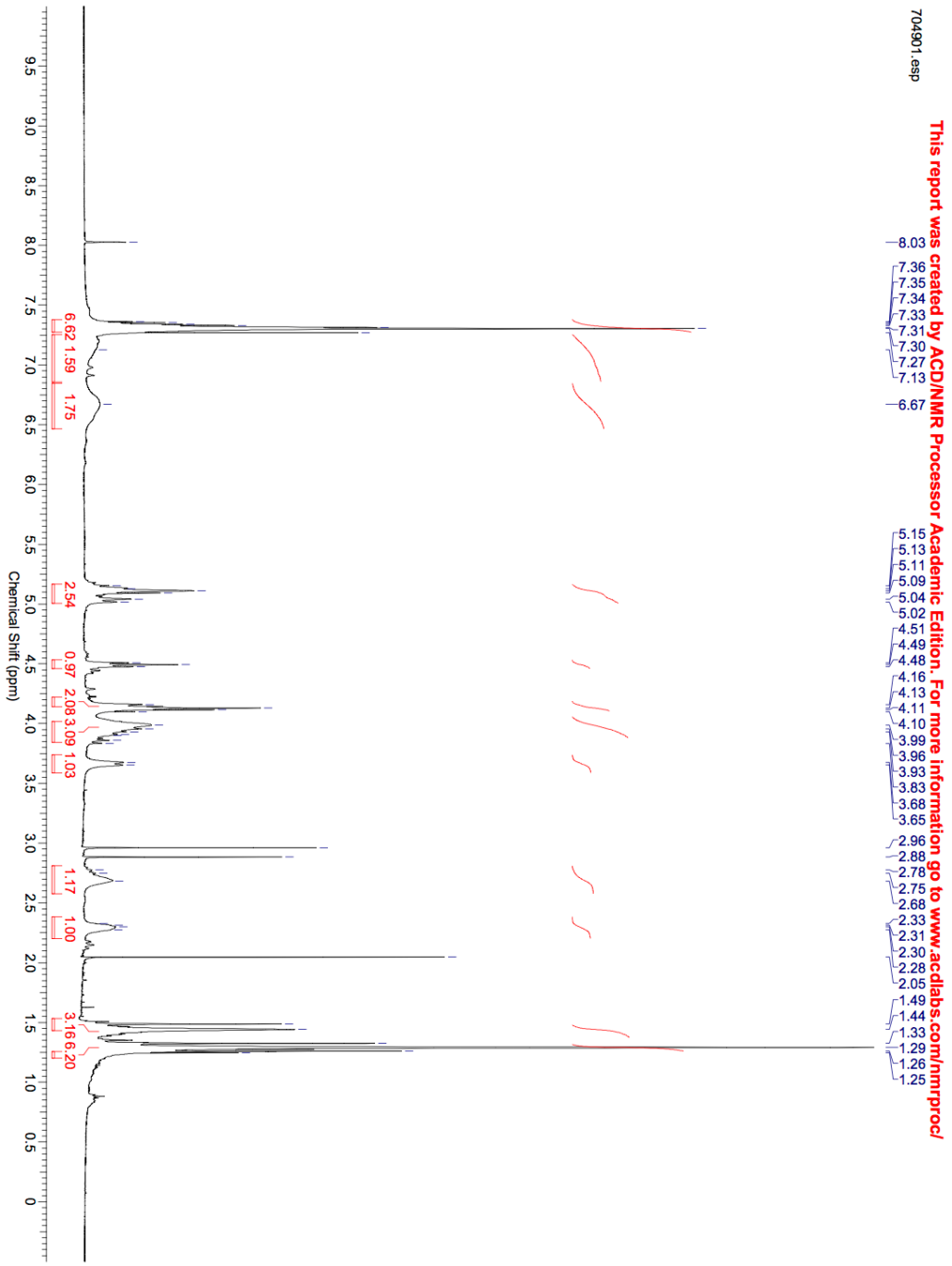
135.70
128.39
128.30
128.02
127.89
127.82
127.69
83.14
82.95
77.26
77.00
76.74
67.48
66.25
65.16
60.28
59.25
58.74
55.64
55.56
40.62
40.58
39.64
38.83
36.60
31.51
27.88
27.74
27.56
20.91
14.03
Chemical Shift (ppm)
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/


704901.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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7.33
7.31
7.30
7.27
7.13
6.67
5.15
5.13
5.11
5.09
5.04
5.02
4.51
4.49
4.48
4.16
4.13
4.11
4.10
3.99
3.96
3.93
3.83
3.68
3.65
2.96
2.88
2.78
2.75
2.68
2.33
2.31
2.30
2.28
2.05
1.49
1.44
1.33
1.29
1.26
1.25
6.62
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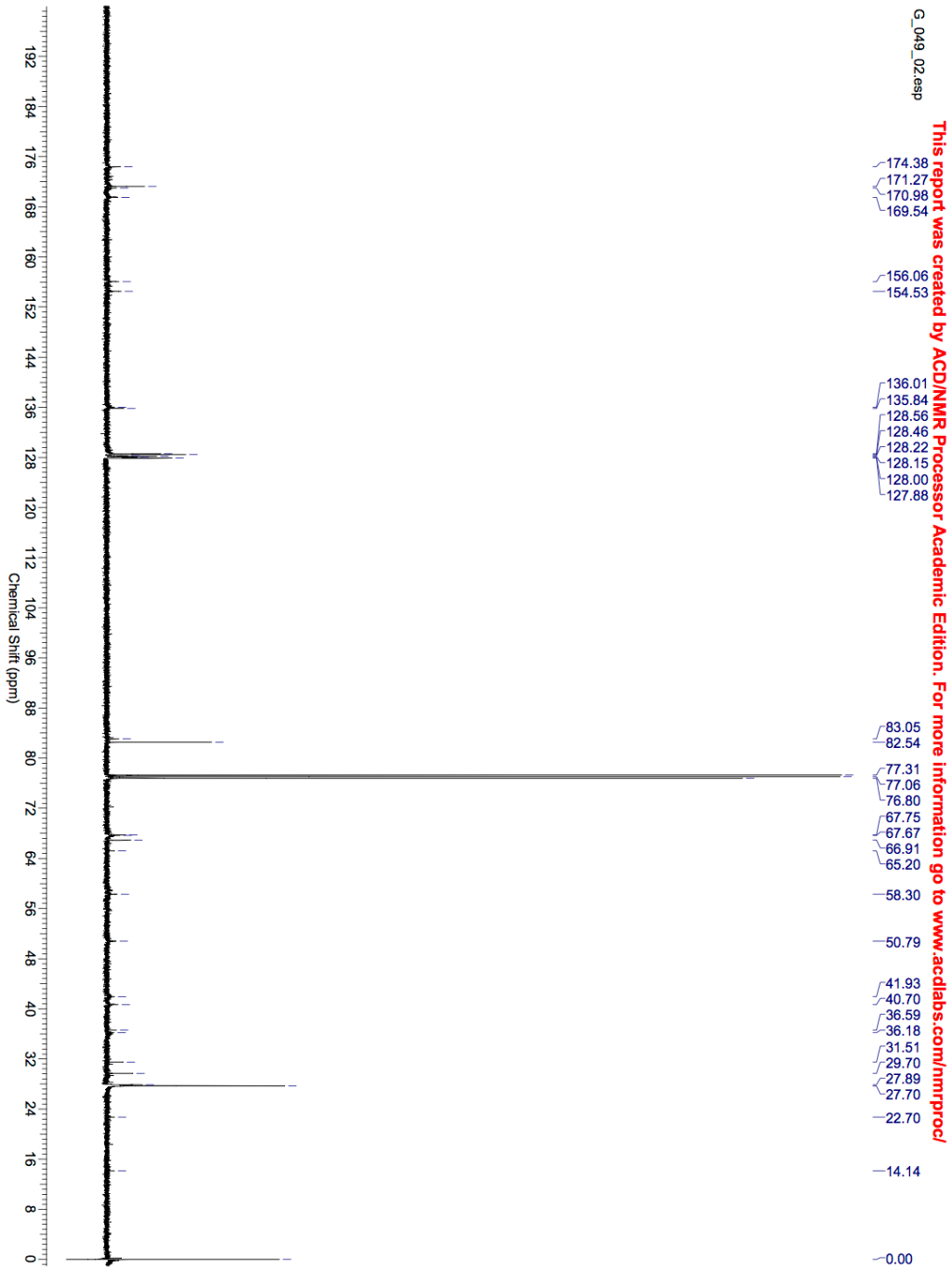
Chemical Shift (ppm)


G_049_02.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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136.01
135.84
128.56
128.46
128.22
128.15
128.00
127.88
83.05
82.54
77.31
77.06
76.80
67.75
67.67
66.91
65.20
58.30
50.79
41.93
40.70
36.59
36.18
31.51
29.70
27.89
27.70
22.70
14.14
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
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56
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32
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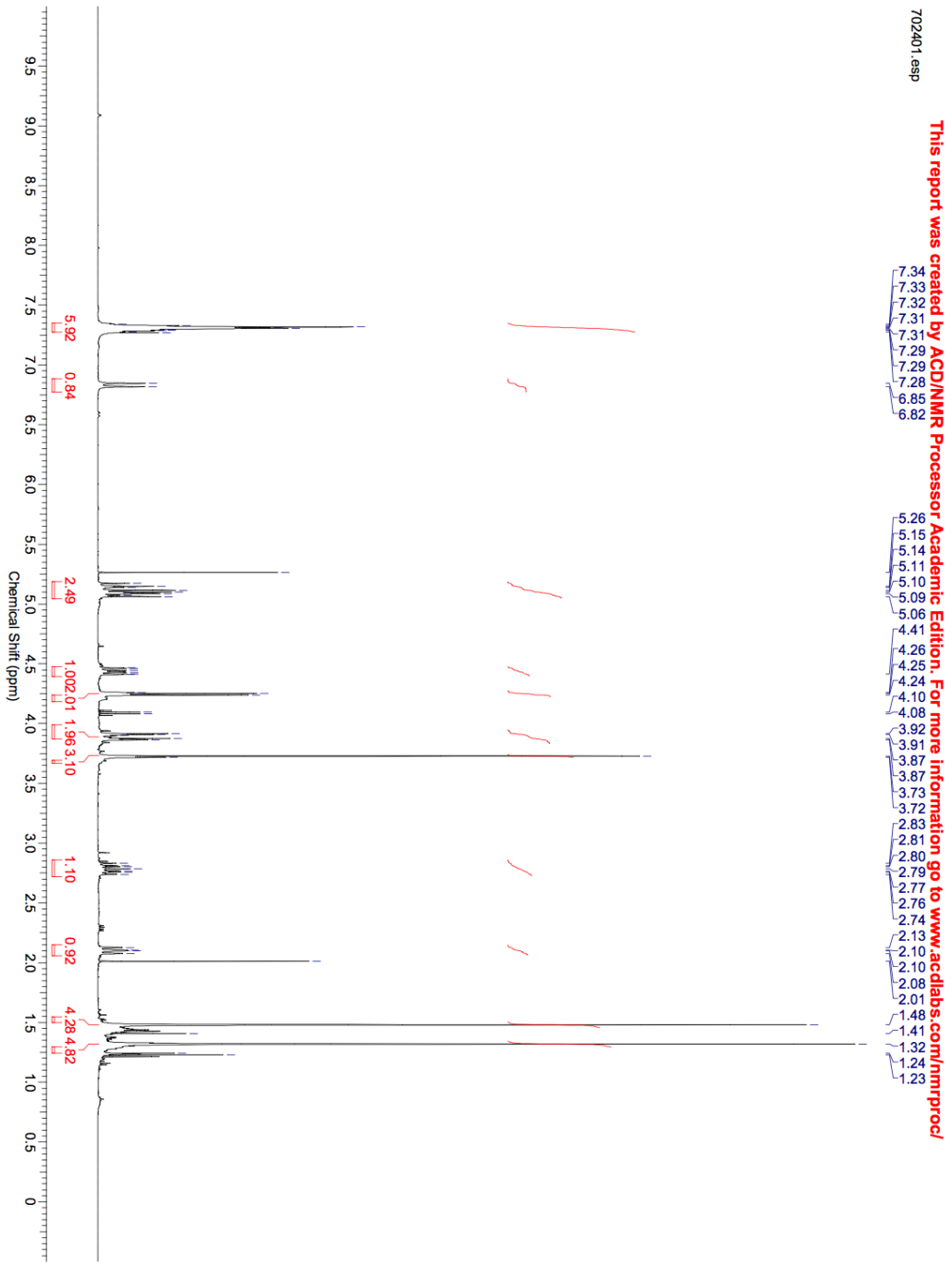
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702401.esp
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7.28
6.85
6.82
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5.14
5.11
5.10
5.09
5.06
4.41
4.26
4.25
4.24
4.10
4.08
3.92
3.91
3.87
3.87
3.73
3.72
2.83
2.81
2.80
2.79
2.77
2.76
2.74
2.13
2.10
2.10
2.08
2.01
1.48
1.41
1.32
1.24
1.23
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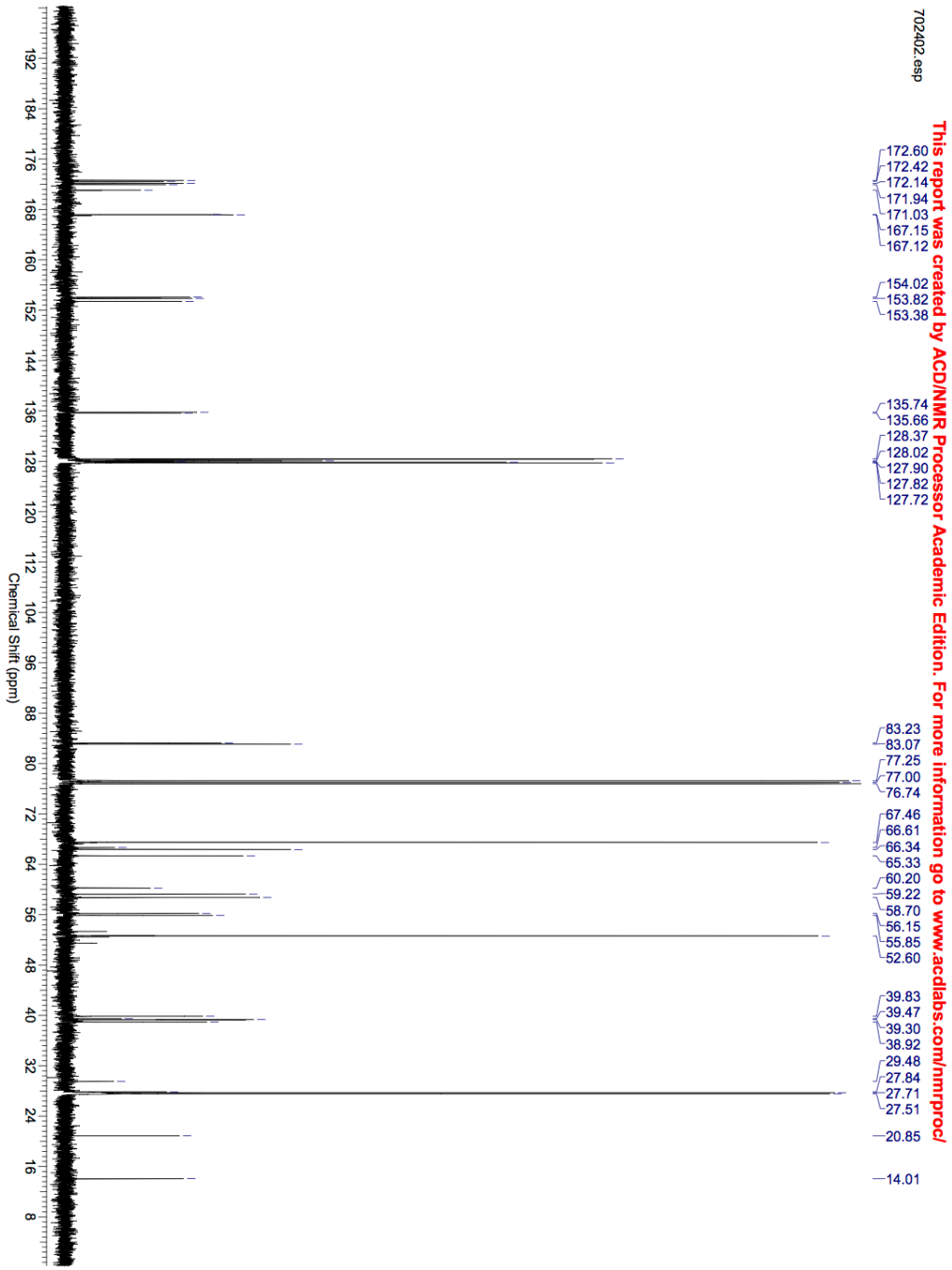
1.10
0.92
4.28
4.82
Chemical Shift (ppm)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128.37
128.02
127.90
127.82
127.72
83.23
83.07
77.25
77.00
76.74
67.46
66.61
66.34
65.33
60.20
59.22
58.70
56.15
55.85
52.60
39.83
39.47
39.30
38.92
29.48
27.84
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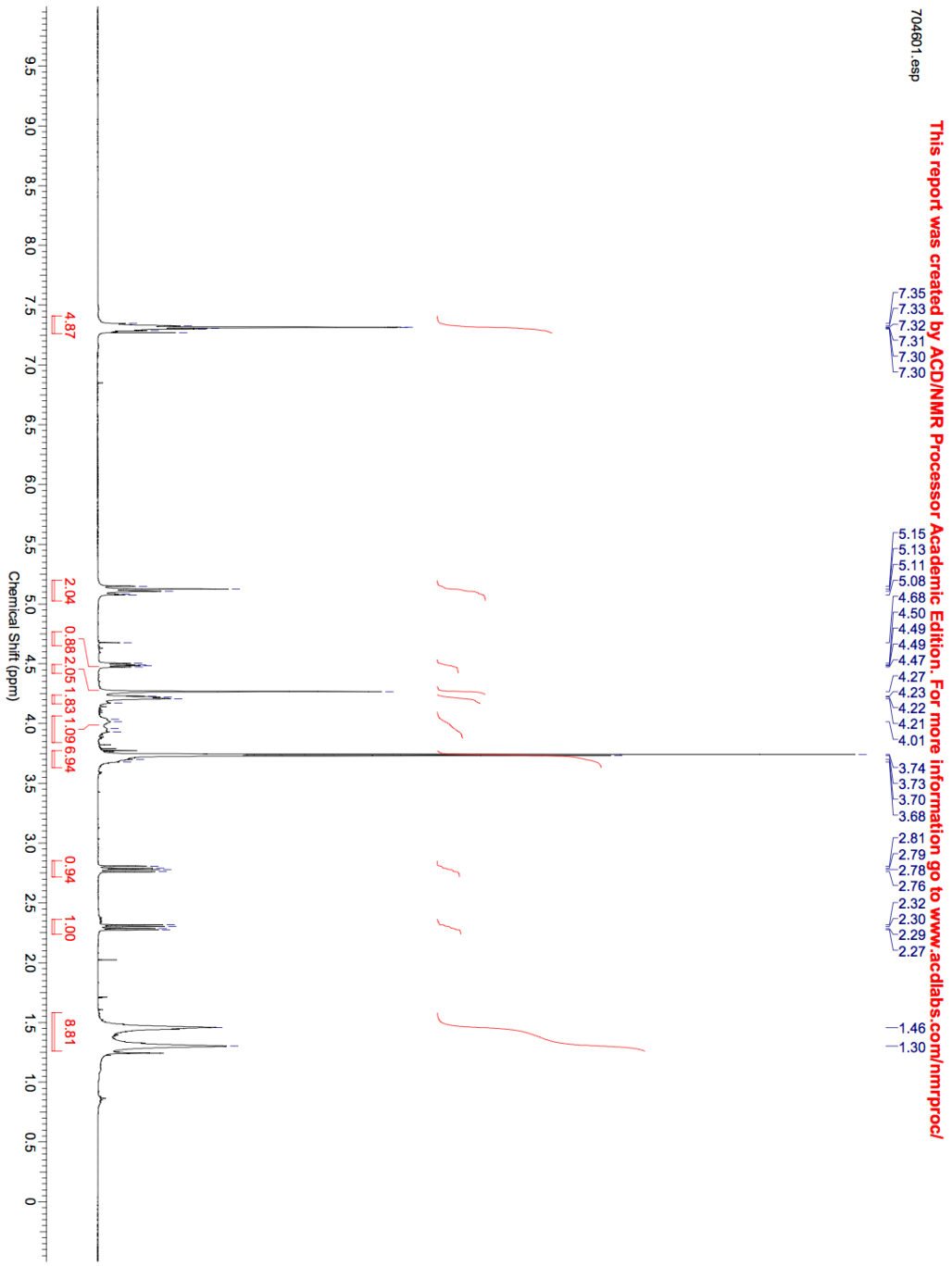
14.01
Chemical Shift (ppm)
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/


704601.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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7.30
5.15
5.13
5.11
5.08
4.68
4.50
4.49
4.49
4.47
4.27
4.23
4.22
4.21
4.01
3.74
3.73
3.70
3.68
2.81
2.79
2.78
2.76
2.32
2.30
2.29
2.27
1.46
1.30
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2.05
1.83
1.09
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0.94
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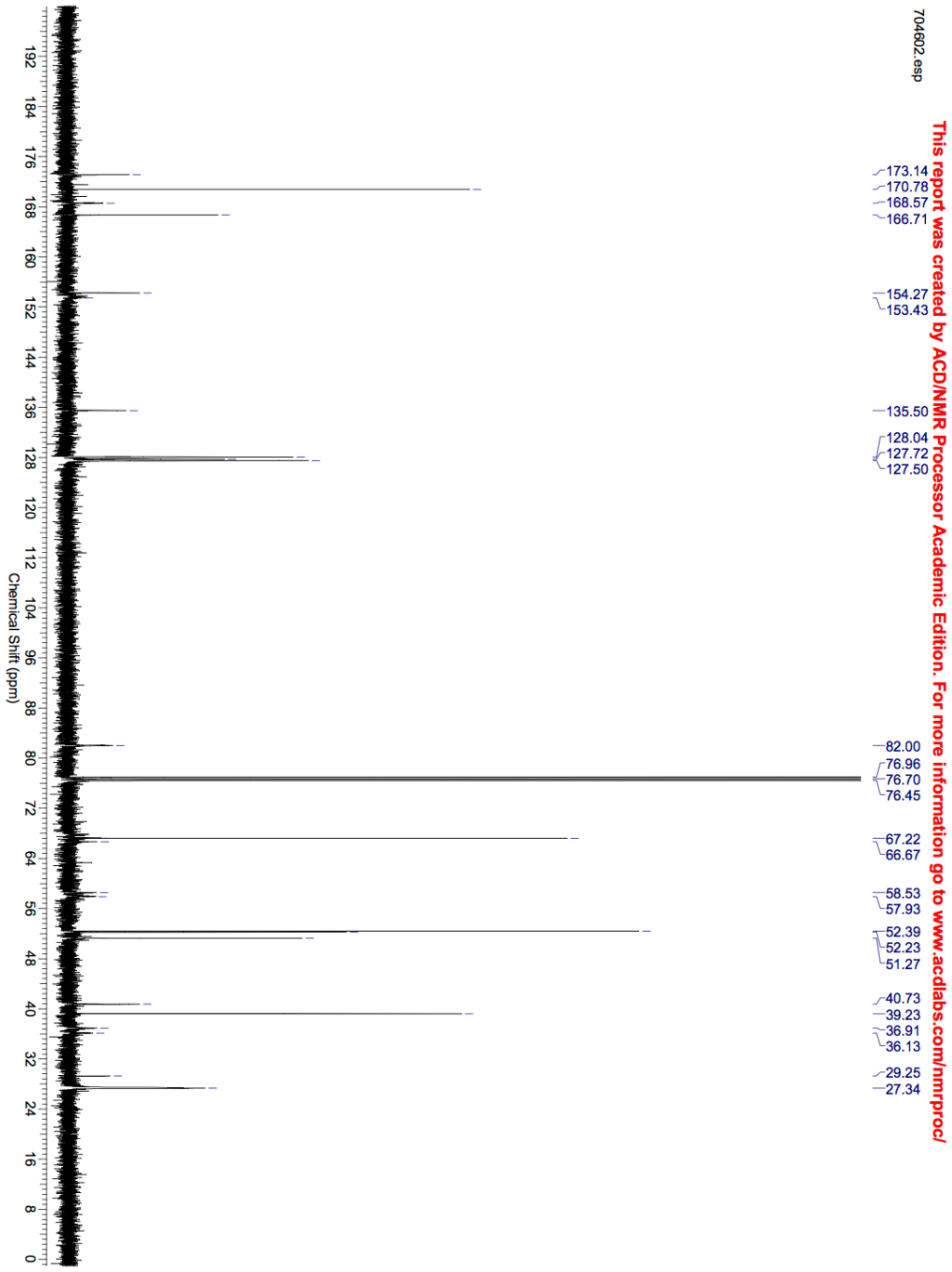
8.81
Chemical Shift (ppm)


704602.esp
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153.43
135.50
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127.72
127.50
82.00
76.96
76.70
76.45
67.22
66.67
58.53
57.93
52.39
52.23
51.27
40.73
39.23
36.91
36.13
29.25
27.34
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
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32
24
16
8
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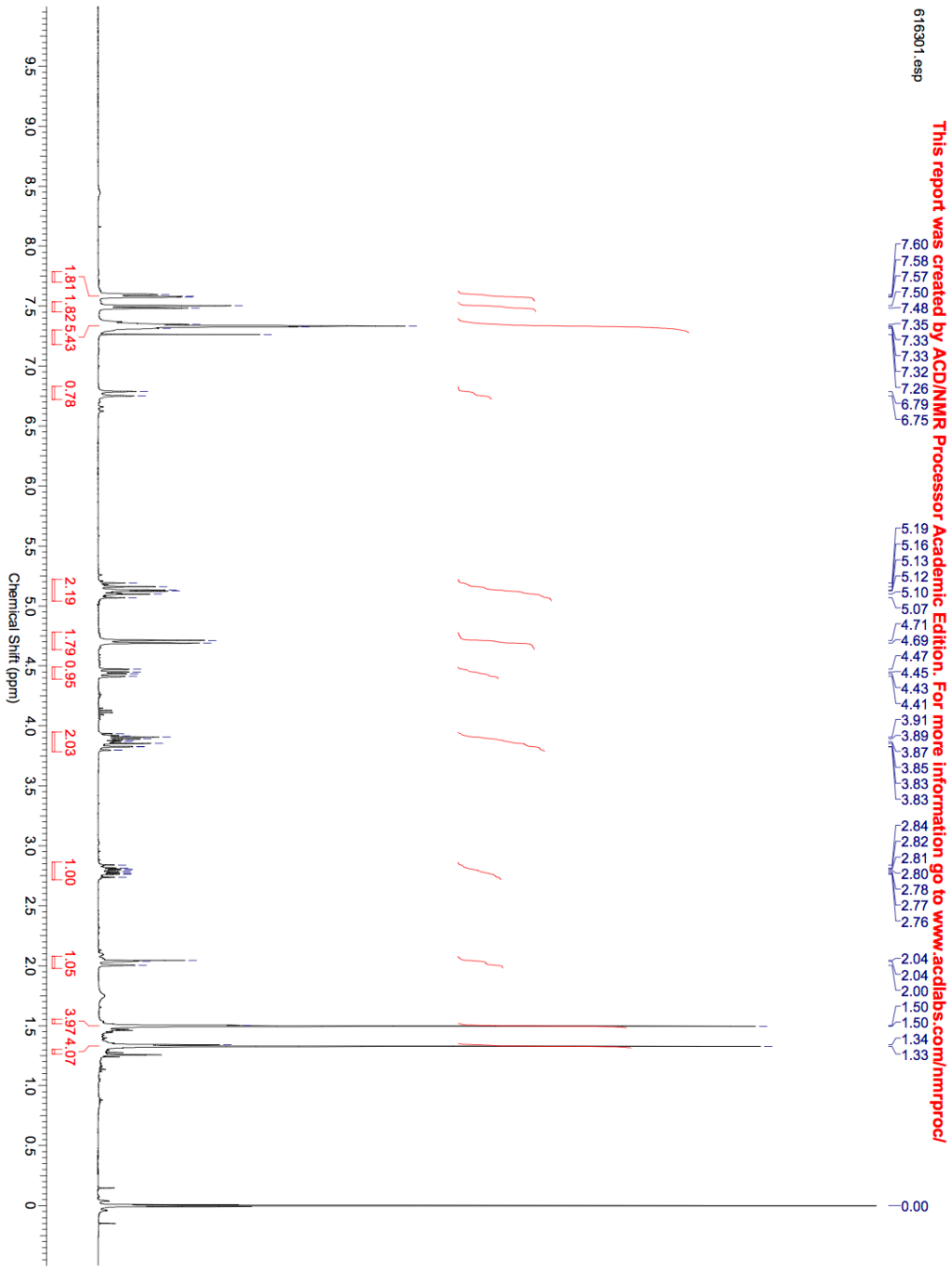
616301.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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7.33
7.33
7.32
7.26
6.79
6.75
5.19
5.16
5.13
5.12
5.10
5.07
4.71
4.69
4.47
4.45
4.43
4.41
3.91
3.89
3.87
3.85
3.83
3.83
2.84
2.82
2.81
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2.78
2.77
2.76
2.04
2.04
2.00
1.50
1.50
1.34
1.33
0.00
1.81
1.82
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2.19
1.79
0.95
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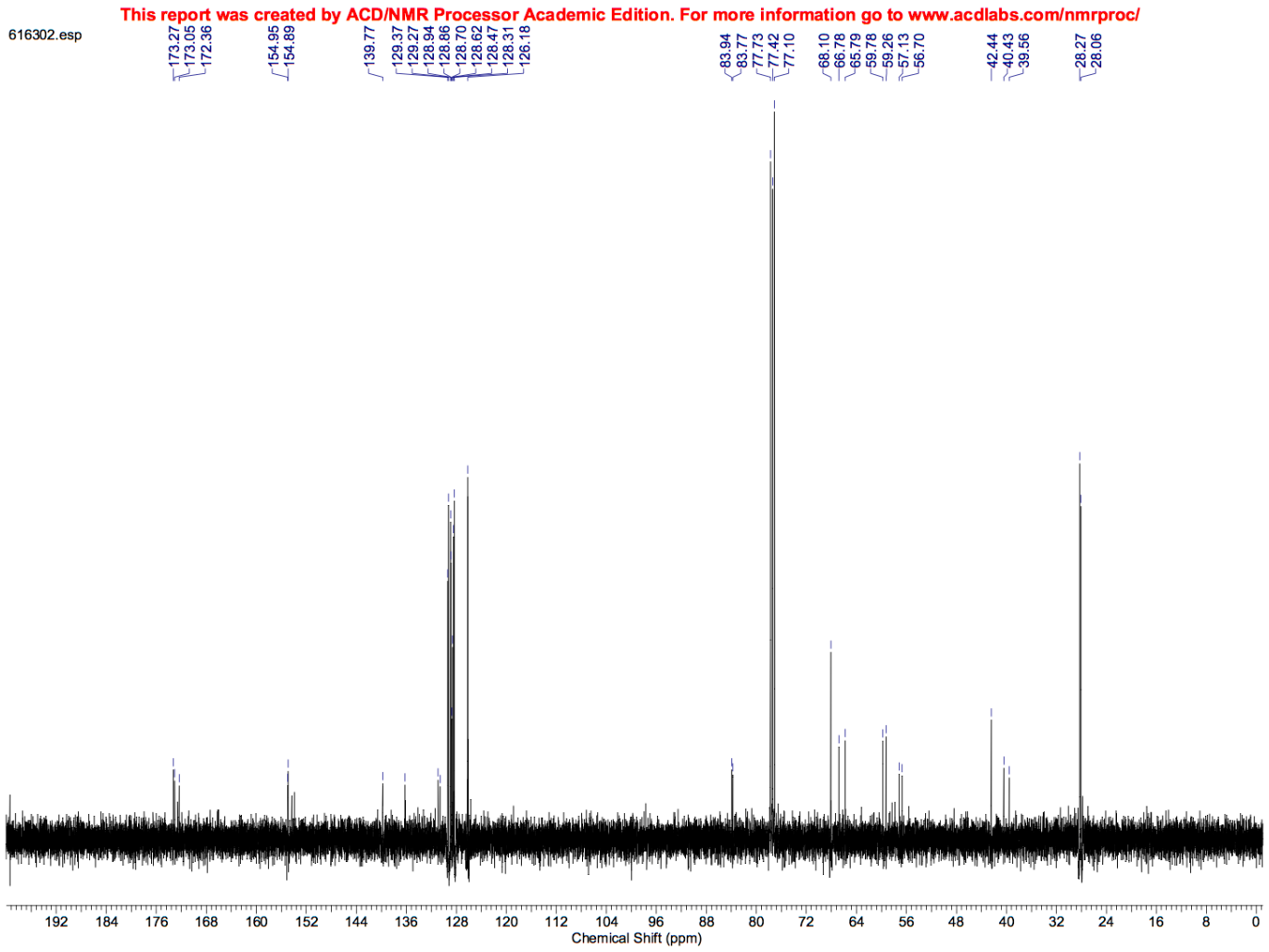
1.00
1.05
3.97
4.07
Chemical Shift (ppm)


This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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128.94
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128.70
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128.47
128.31
126.18
83.94
83.77
77.73
77.42
77.10
68.10
66.78
65.79
59.78
59.26
57.13
56.70
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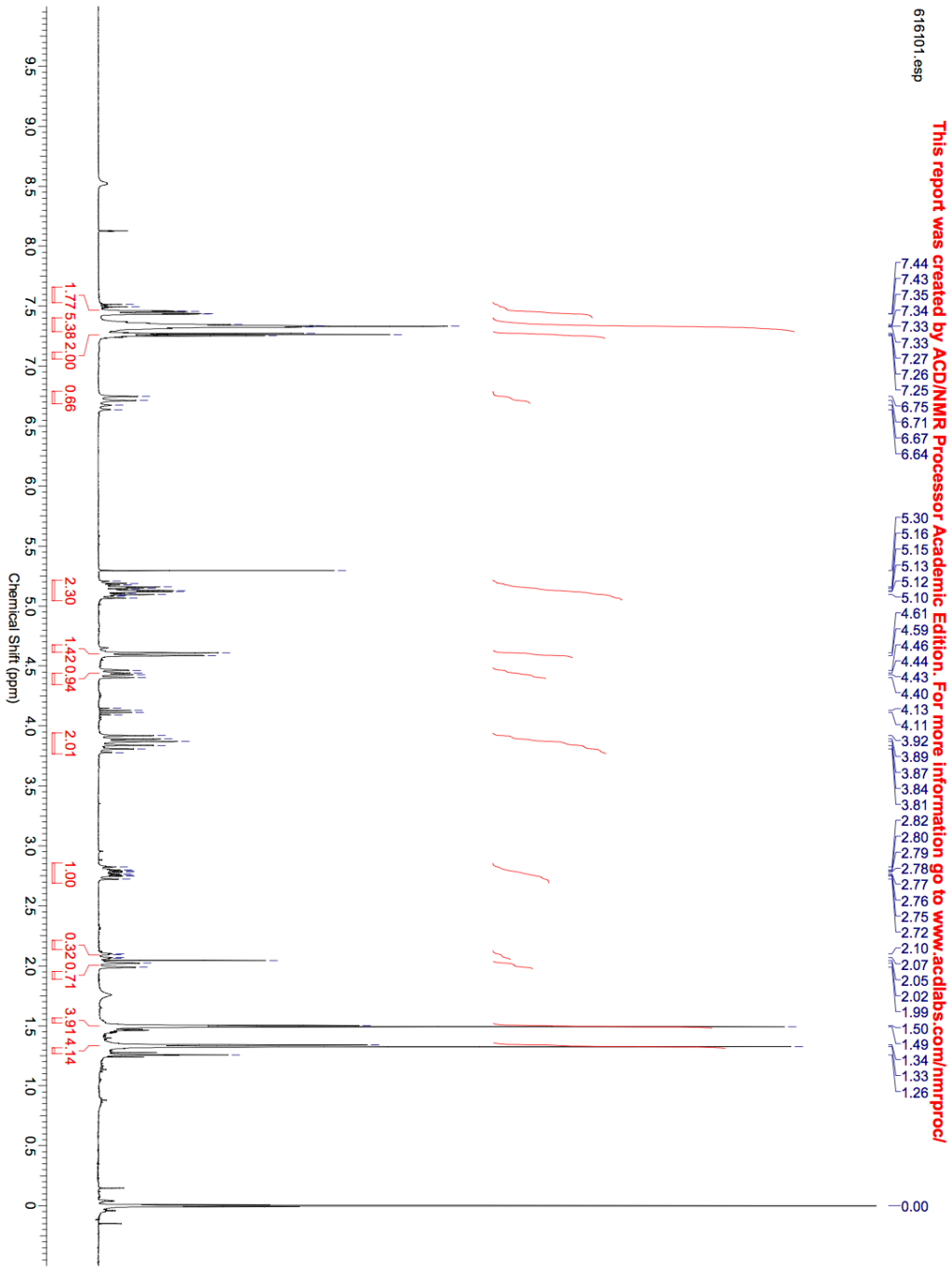
40.43
39.56
28.27
28.06
192 184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 16 8 0
Chemical Shift (ppm)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This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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7.25
6.75
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6.67
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5.30
5.16
5.15
5.13
5.12
5.10
4.61
4.59
4.46
4.44
4.43
4.40
4.13
4.11
3.92
3.89
3.87
3.84
3.81
2.82
2.80
2.79
2.78
2.77
2.76
2.75
2.72
2.10
2.07
2.05
2.02
1.99
1.50
1.49
1.34
1.33
1.26
0.00
1.77
5.38
2.00
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0.94
2.01
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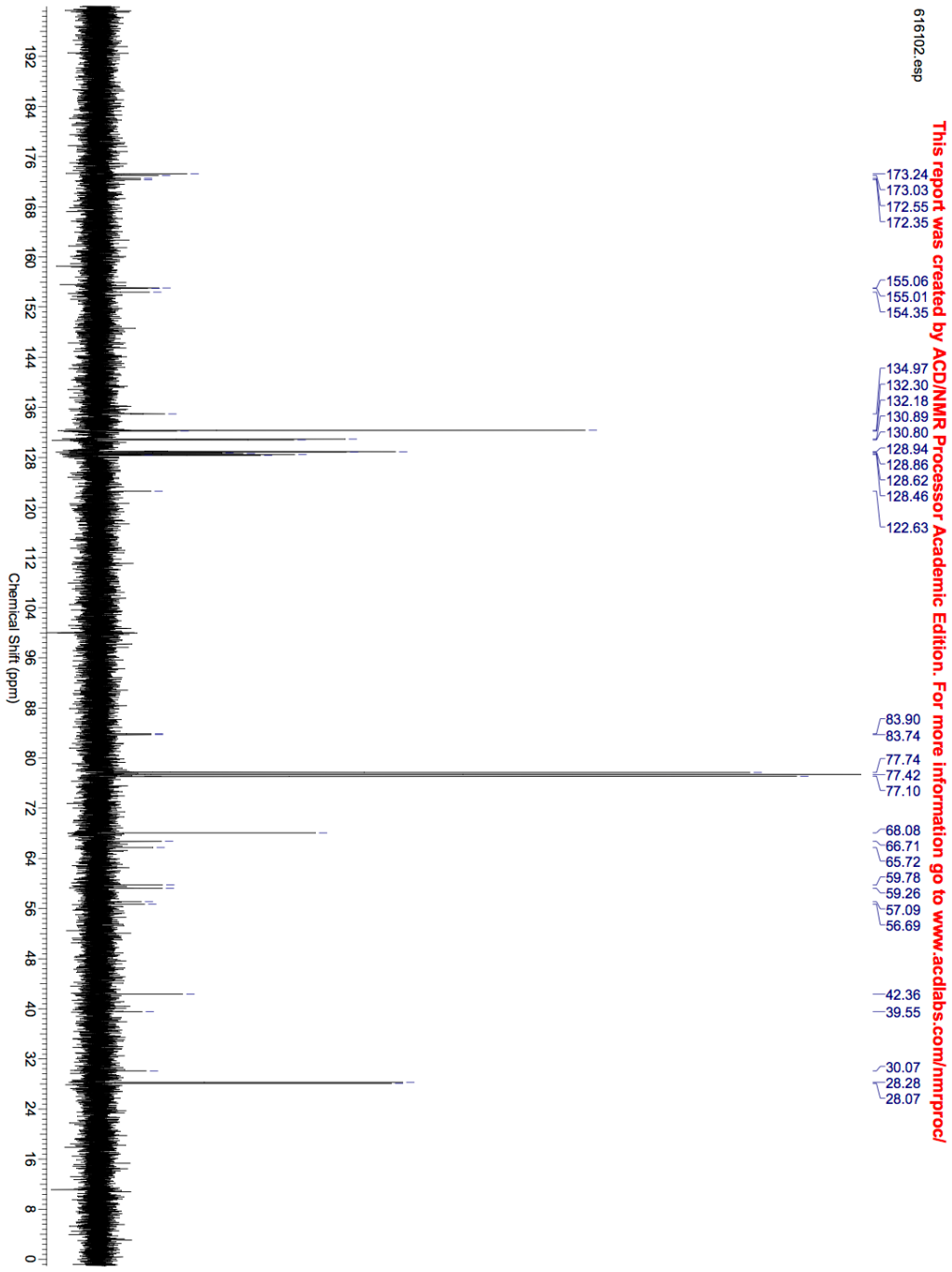
0.71
3.91
4.14
Chemical Shift (ppm)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128.46
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83.74
77.74
77.42
77.10
68.08
66.71
65.72
59.78
59.26
57.09
56.69
42.36
39.55
30.07
28.28
28.07
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
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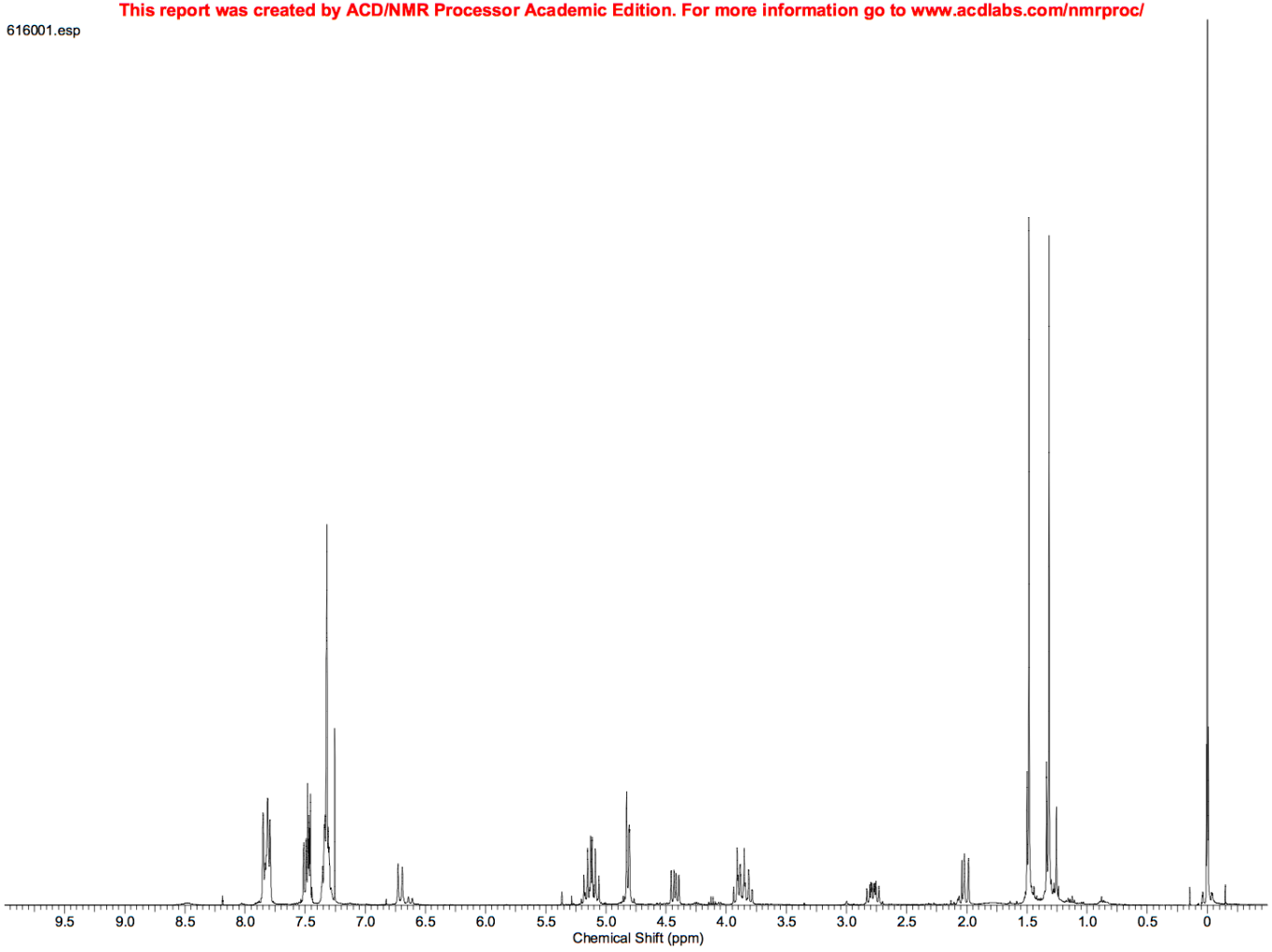
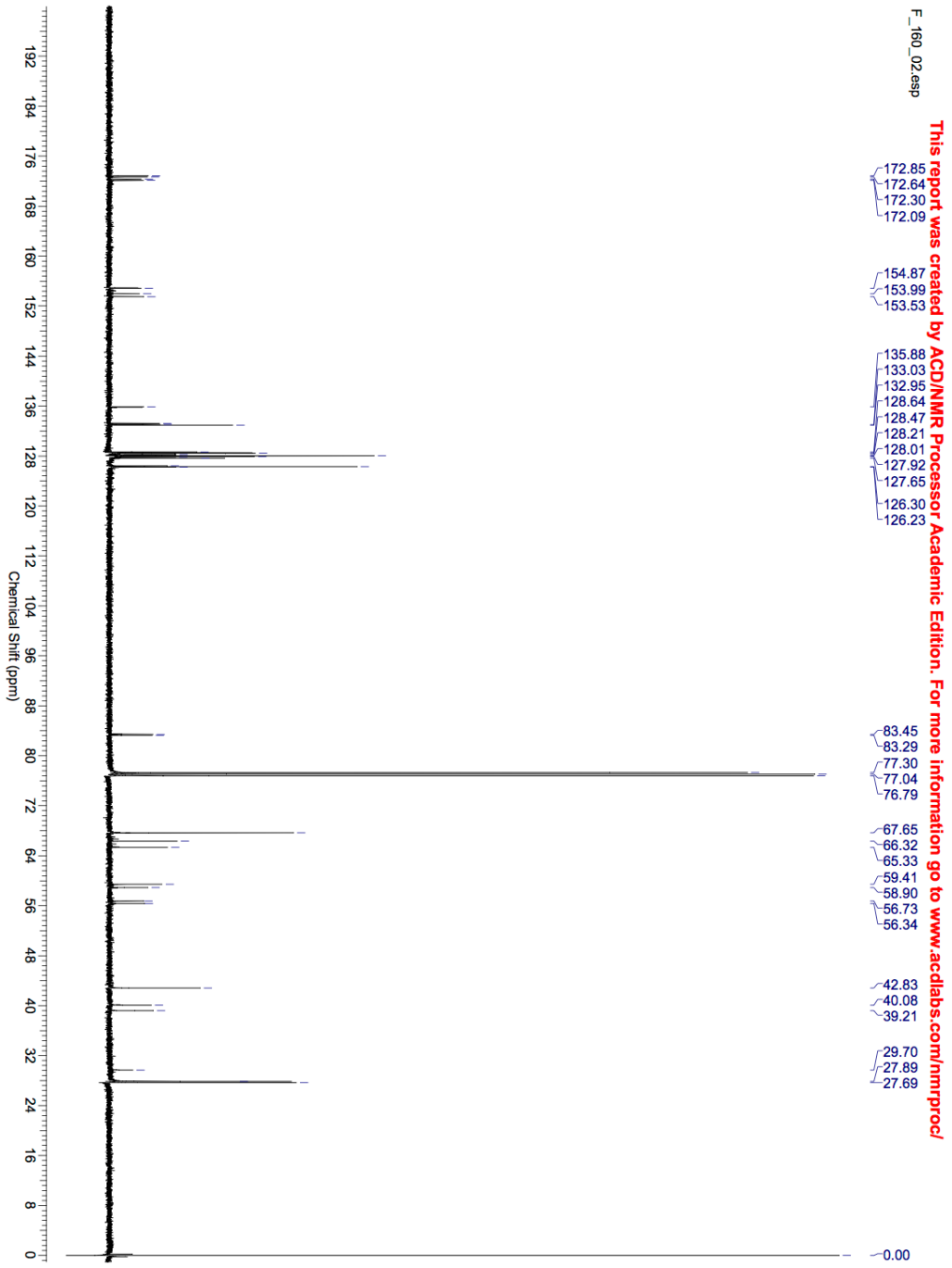
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/

616001.esp




F_160_02.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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132.95
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128.21
128.01
127.92
127.65
126.30
126.23
83.45
83.29
77.30
77.04
76.79
67.65
66.32
65.33
59.41
58.90
56.73
56.34
42.83
40.08
39.21
29.70
27.89
27.69
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
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56
48
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16
8
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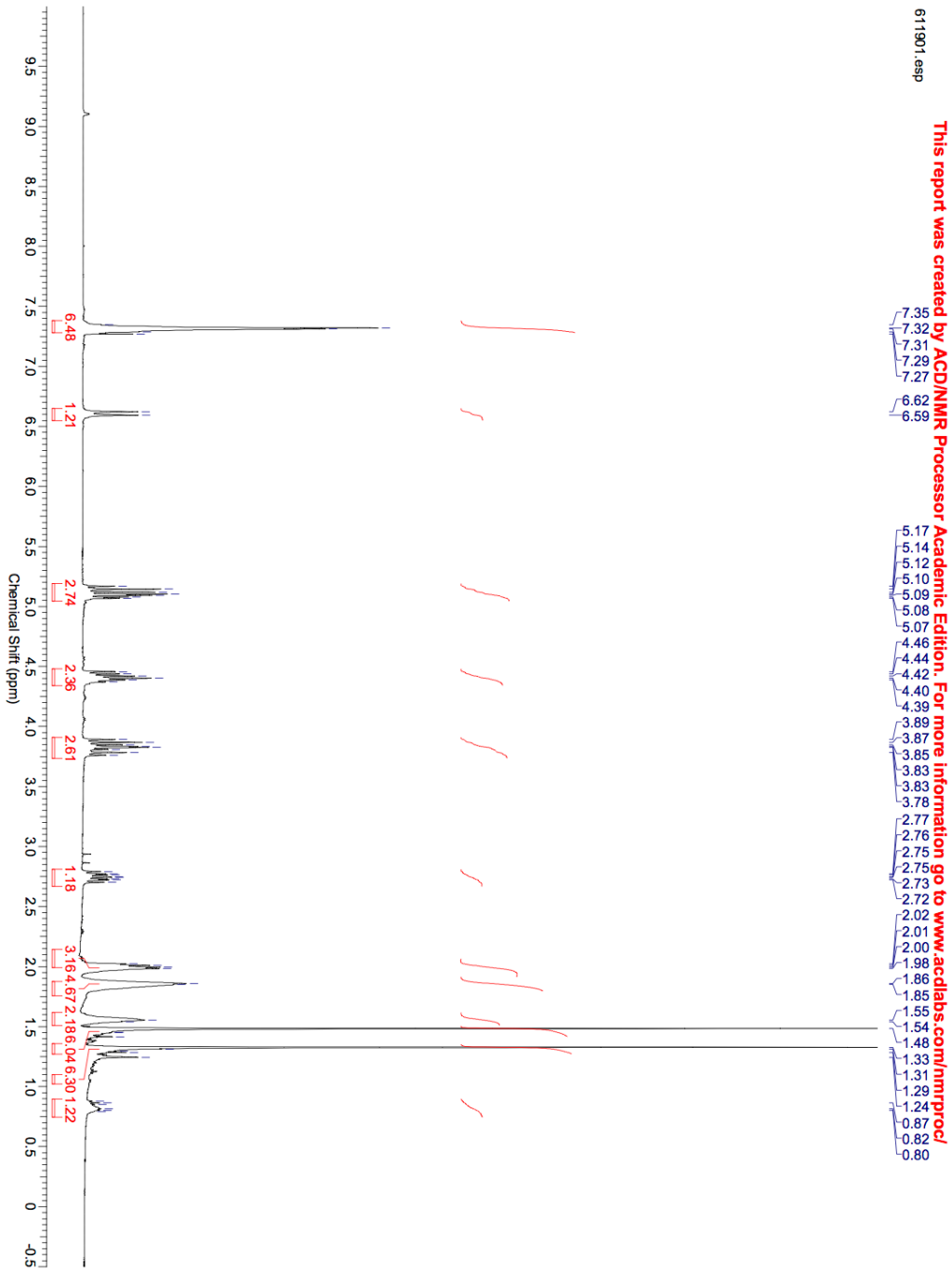
611901.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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5.12
5.10
5.09
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5.07
4.46
4.44
4.42
4.40
4.39
3.89
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2.01
2.00
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1.86
1.85
1.55
1.54
1.48
1.33
1.31
1.29
1.24
0.87
0.82
0.80
6.48
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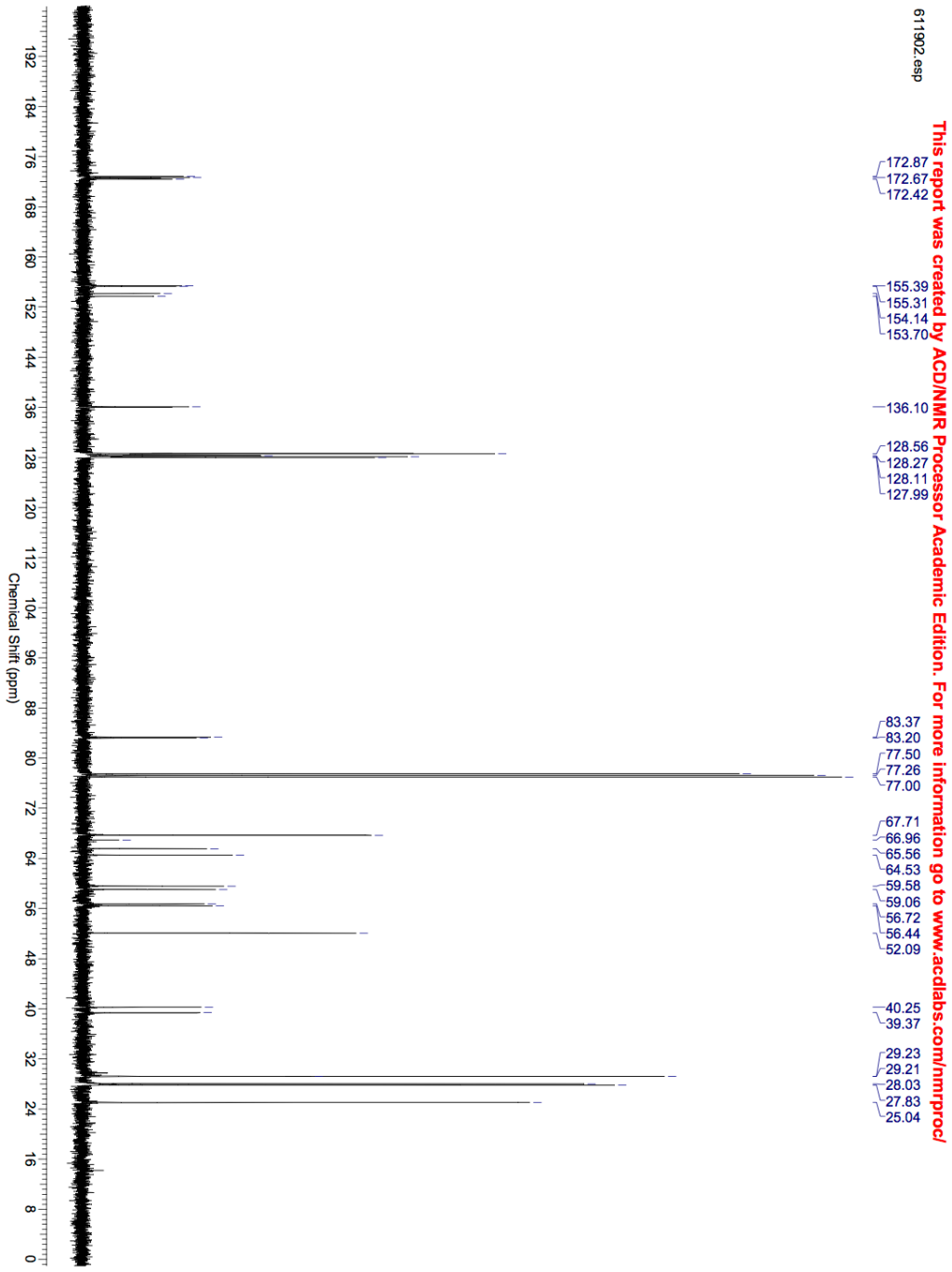
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127.99
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83.20
77.50
77.26
77.00
67.71
66.96
65.56
64.53
59.58
59.06
56.72
56.44
52.09
40.25
39.37
29.23
29.21
28.03
27.83
25.04
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
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16
8
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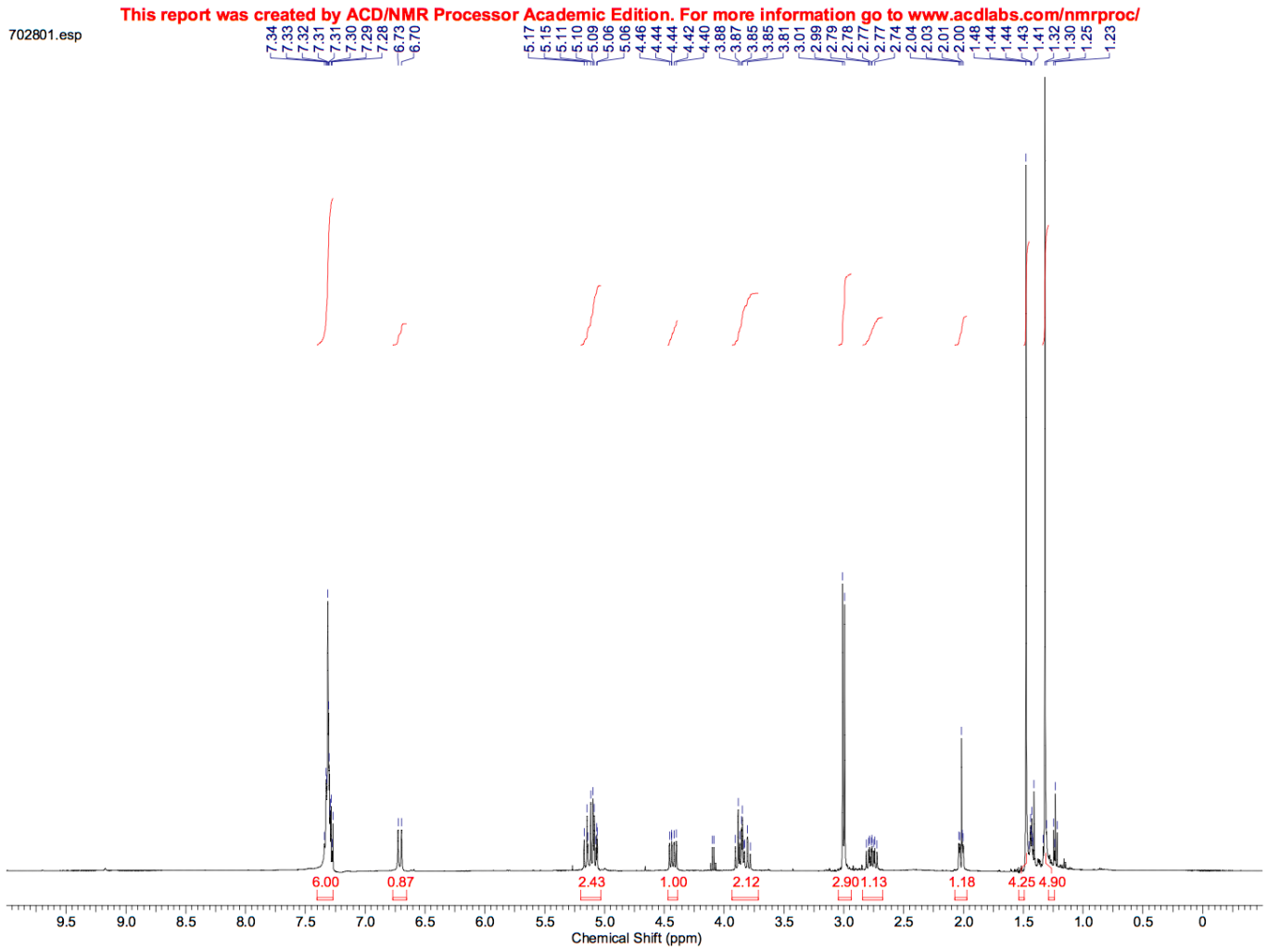
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
702801.esp
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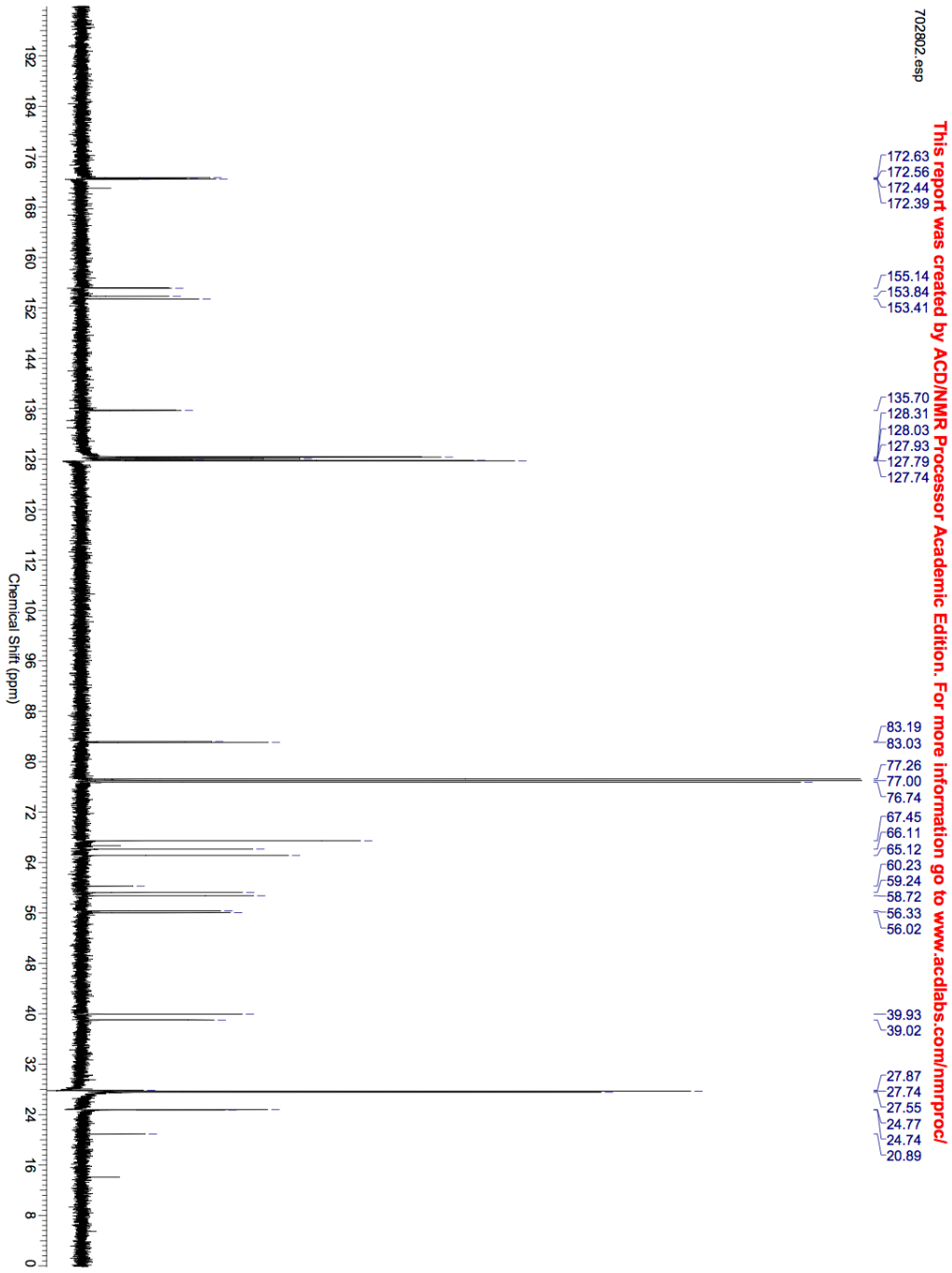
5.17 5.15 5.11 5.10 5.09 5.06 5.06 4.46 4.44 4.44 4.42 4.40 3.88 3.87 3.85 3.85 3.81 3.01 2.99 2.79 2.78 2.77 2.77 2.74 2.04 2.03 2.01 2.00 1.48 1.44 1.44 1.43 1.41 1.32 1.30 1.25 1.23
6.00 0.87 2.43 1.00 2.12 2.90 1.13 1.18 4.25 4.90
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
Chemical Shift (ppm)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127.79
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83.19
83.03
77.26
77.00
76.74
67.45
66.11
65.12
60.23
59.24
58.72
56.33
56.02
39.93
39.02
27.87
27.74
27.55
24.77
24.74
20.89
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
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32
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16
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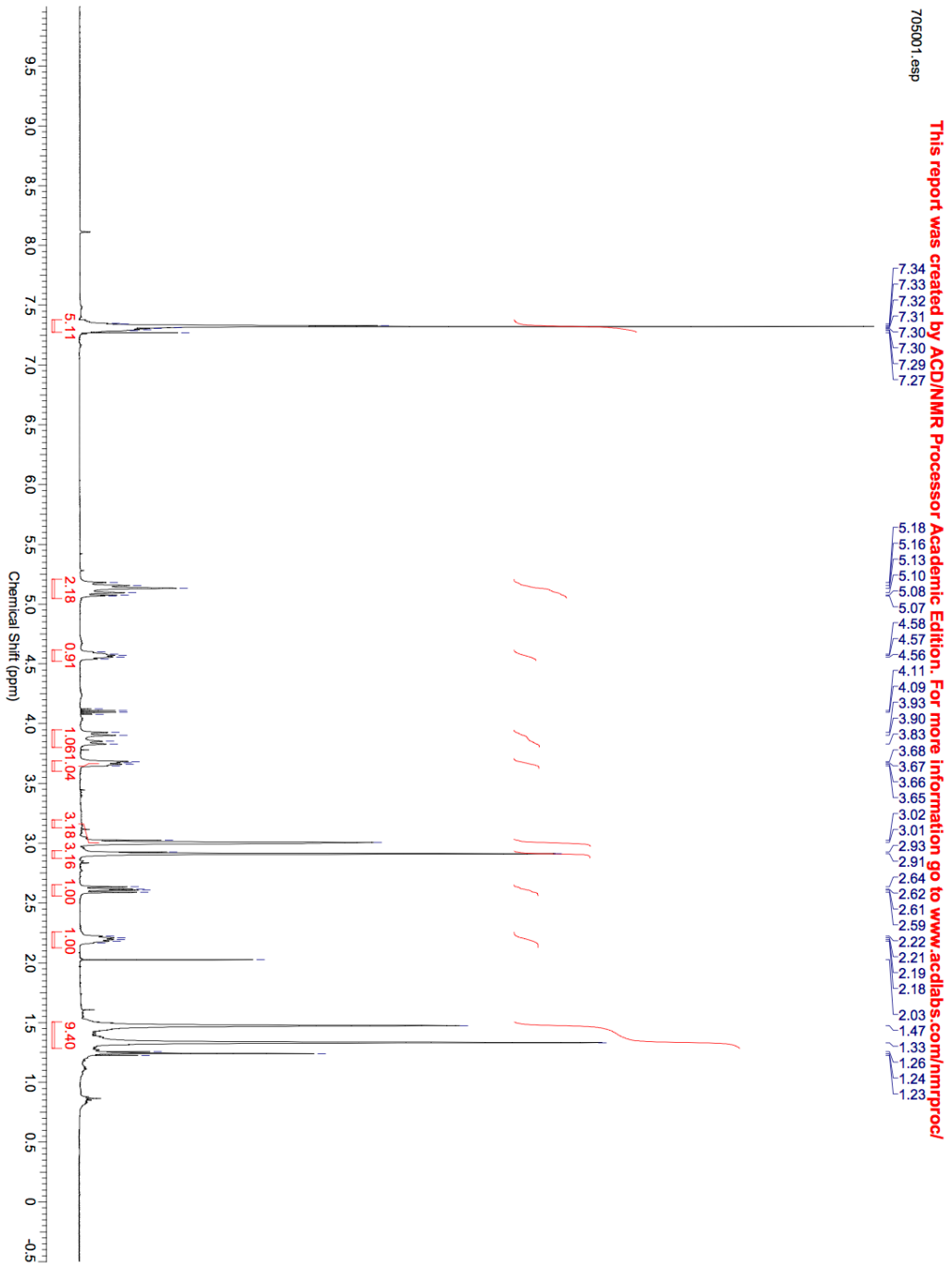
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/


705001.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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7.32
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7.30
7.30
7.29
7.27
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5.16
5.13
5.10
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5.07
4.58
4.57
4.56
4.11
4.09
3.93
3.90
3.83
3.68
3.67
3.66
3.65
3.02
3.01
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2.91
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2.62
2.61
2.59
2.22
2.21
2.19
2.18
2.03
1.47
1.33
1.26
1.24
1.23
5.11
2.18
0.91
1.06
1.04
3.18
3.16
1.00
1.00
9.40
Chemical Shift (ppm)
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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4.0
3.5
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1.5
1.0
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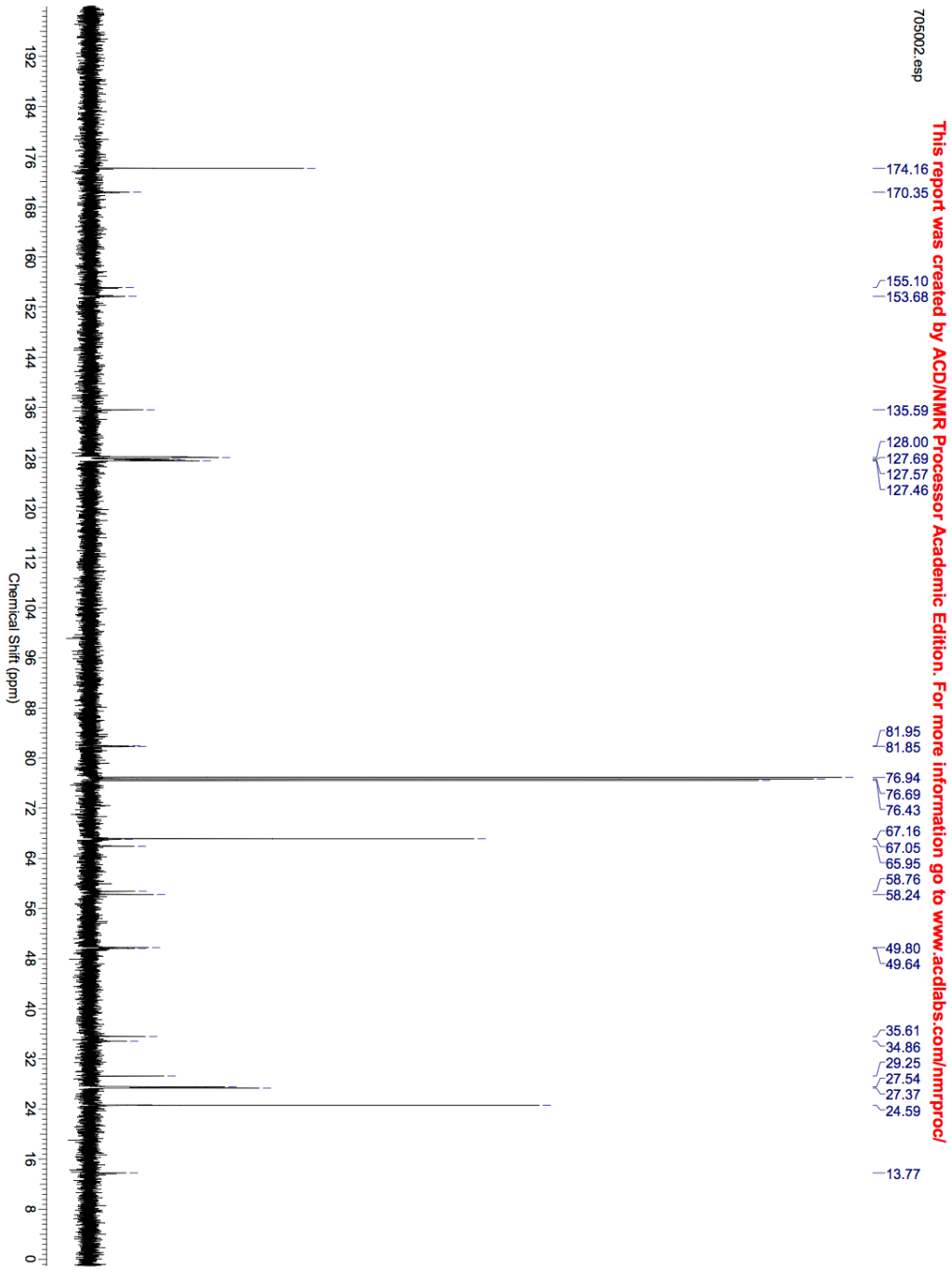
-0.5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76.69
76.43
67.16
67.05
65.95
58.76
58.24
49.80
49.64
35.61
34.86
29.25
27.54
27.37
24.59
13.77
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
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24
16
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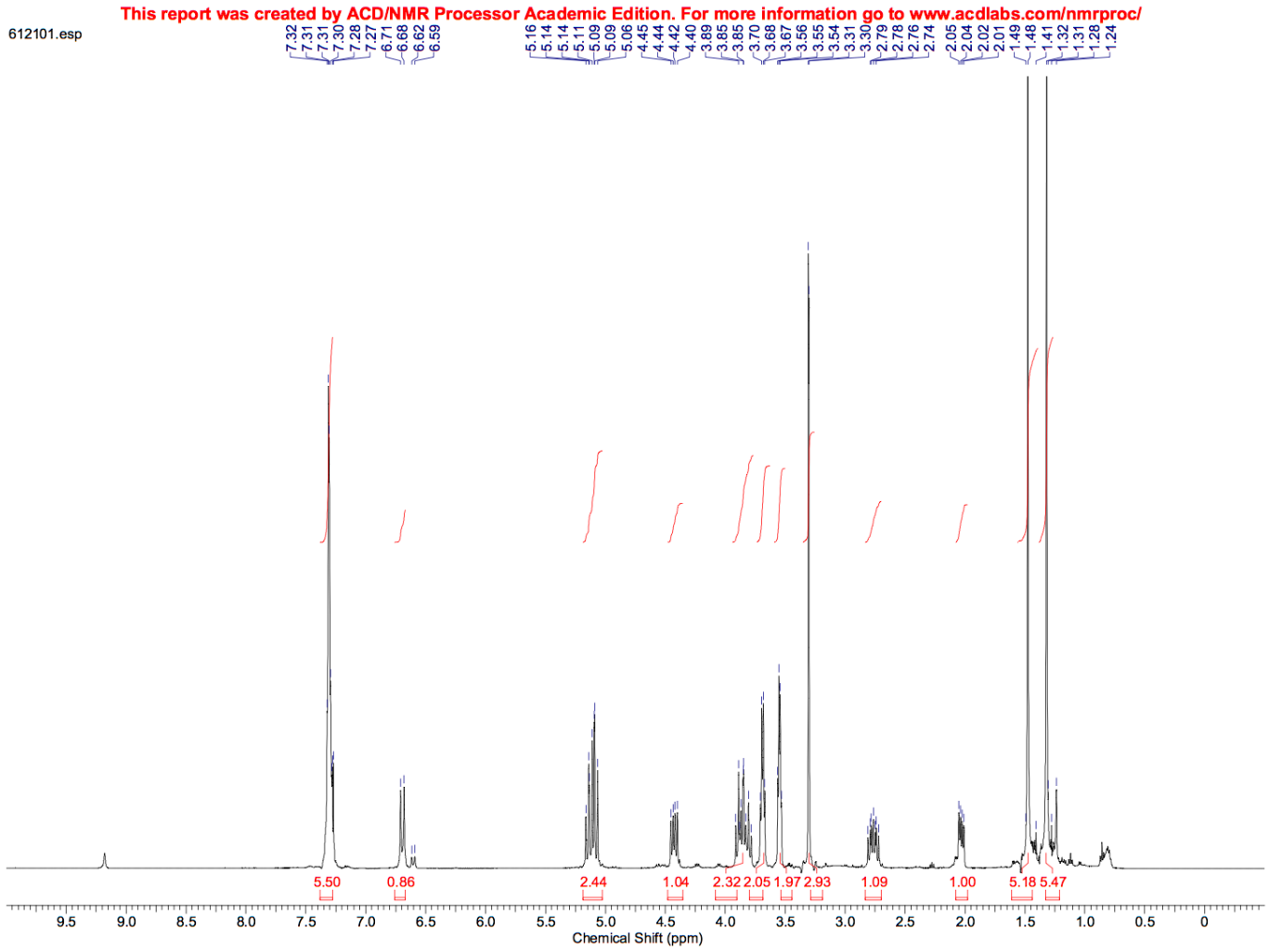
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
612101.esp
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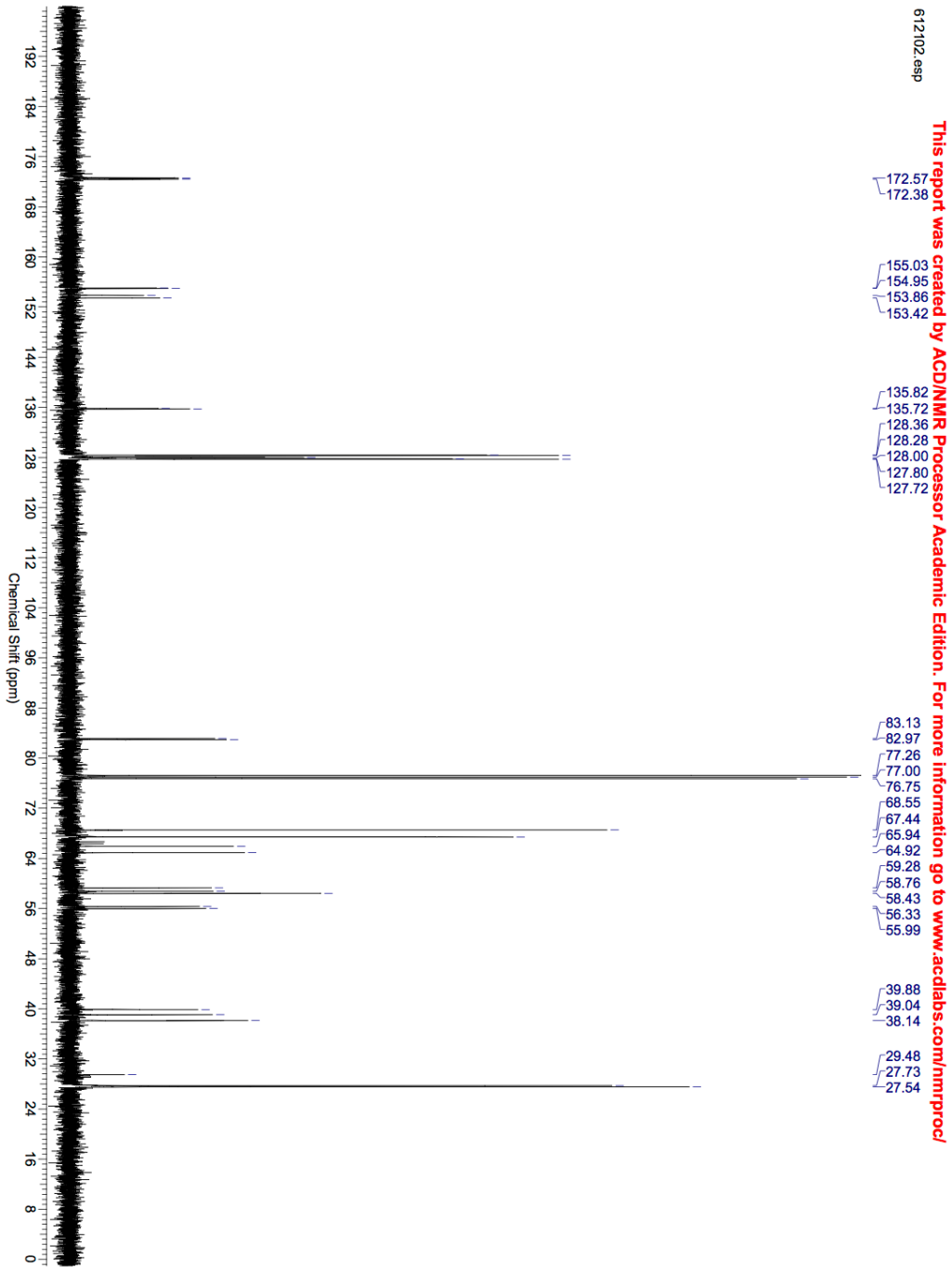
5.16 5.14 5.14 5.11 5.09 5.09 5.06 4.45 4.44 4.42 4.40 3.89 3.85 3.85 3.70 3.68 3.67 3.56 3.55 3.54 3.31 3.30 2.79 2.78 2.76 2.74 2.05 2.04 2.02 2.01 1.49 1.48 1.41 1.32 1.31 1.28 1.24
5.50 0.86 2.44 1.04 2.32 2.05 1.97 2.93 1.09 1.00 5.18 5.47
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
Chemical Shift (ppm)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This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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135.72
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128.00
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127.72
83.13
82.97
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77.00
76.75
68.55
67.44
65.94
64.92
59.28
58.76
58.43
56.33
55.99
39.88
39.04
38.14
29.48
27.73
27.54
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
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64
56
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32
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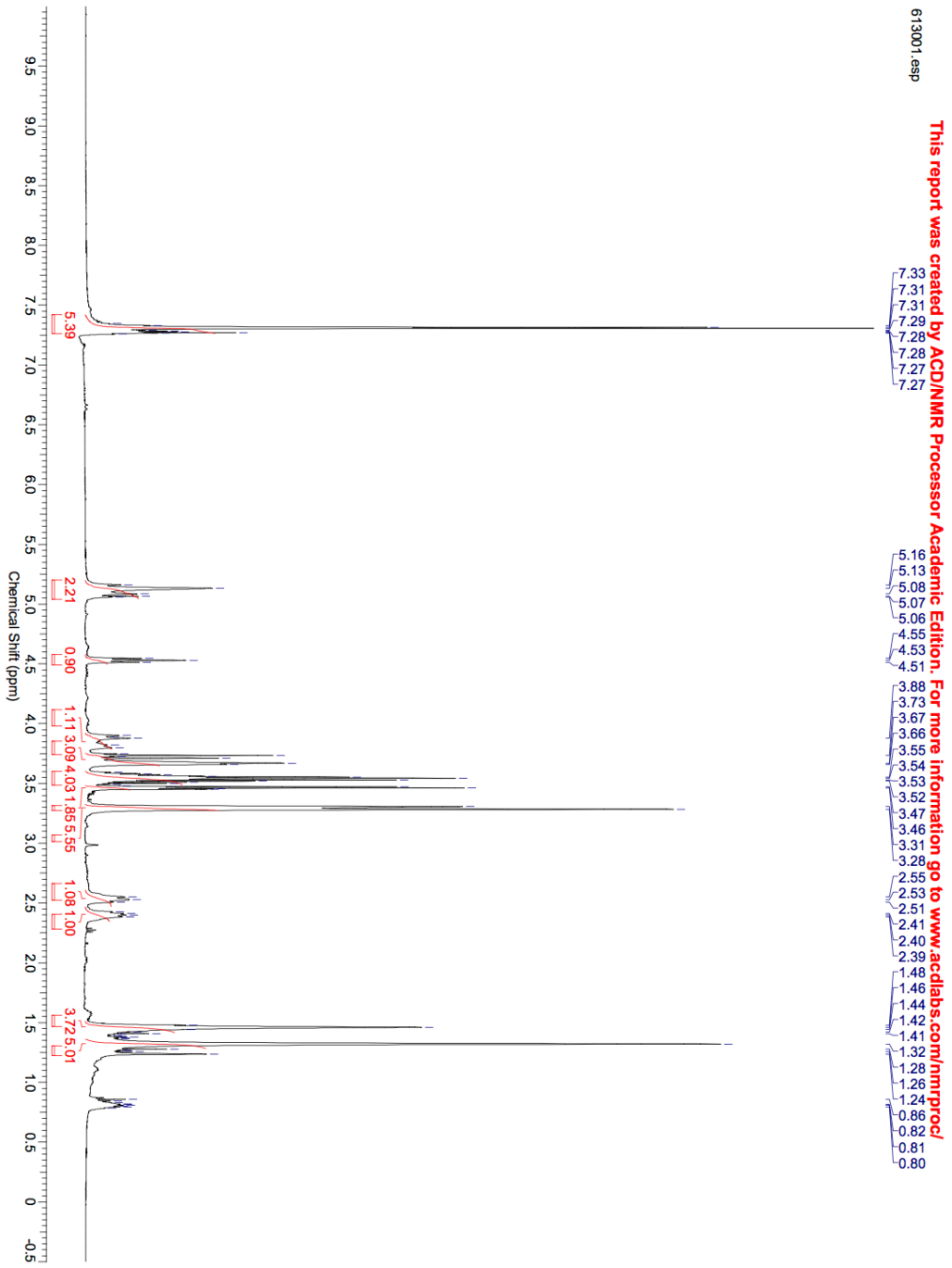
613001.esp
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7.27
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5.07
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4.53
4.51
3.88
3.73
3.67
3.66
3.55
3.54
3.53
3.52
3.47
3.46
3.31
3.28
2.55
2.53
2.51
2.41
2.40
2.39
1.48
1.46
1.44
1.42
1.41
1.32
1.28
1.26
1.24
0.86
0.82
0.81
0.80
5.39
2.21
0.90
1.11
3.09
4.03
1.85
5.55
1.08
1.00
3.72
5.01
Chemical Shift (ppm)
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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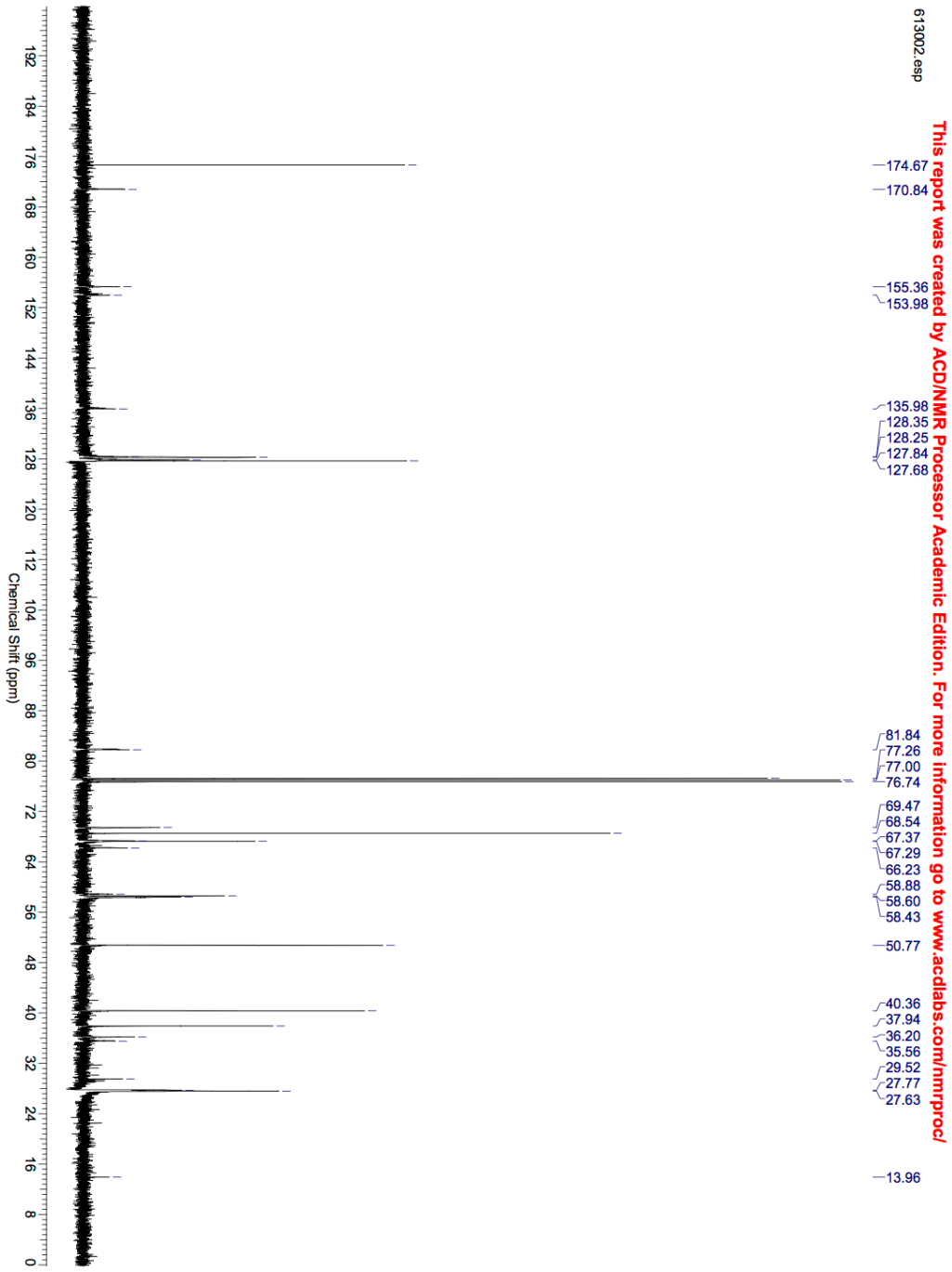
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68.54
67.37
67.29
66.23
58.88
58.60
58.43
50.77
40.36
37.94
36.20
35.56
29.52
27.77
27.63
13.96
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
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32
24
16
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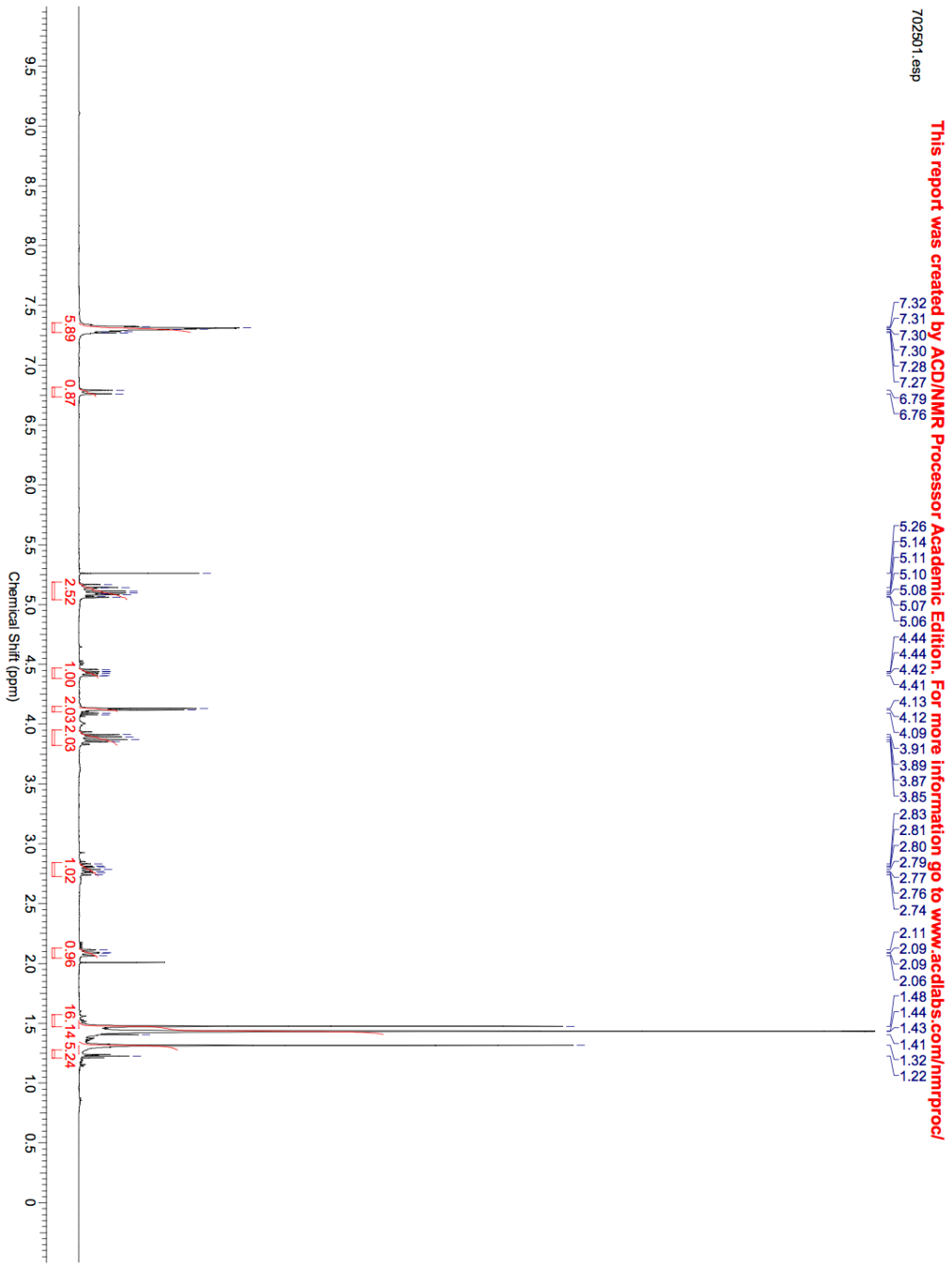
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This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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6.79
6.76
5.26
5.14
5.11
5.10
5.08
5.07
5.06
4.44
4.44
4.42
4.41
4.13
4.12
4.09
3.91
3.89
3.87
3.85
2.83
2.81
2.80
2.79
2.77
2.76
2.74
2.11
2.09
2.09
2.06
1.48
1.44
1.43
1.41
1.32
1.22
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2.52
1.00
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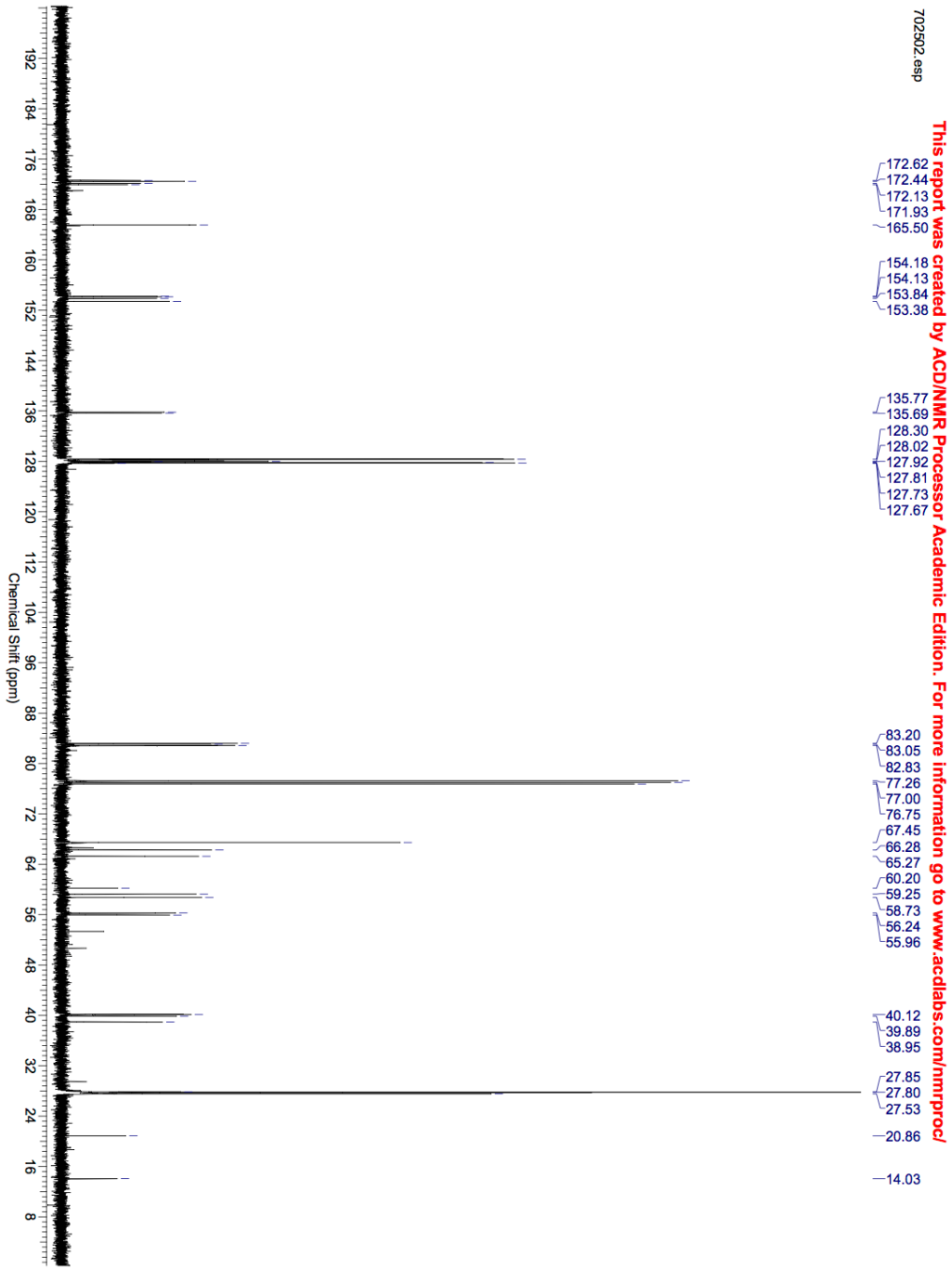
2.03
1.02
0.96
16.14
5.24
Chemical Shift (ppm)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172.44
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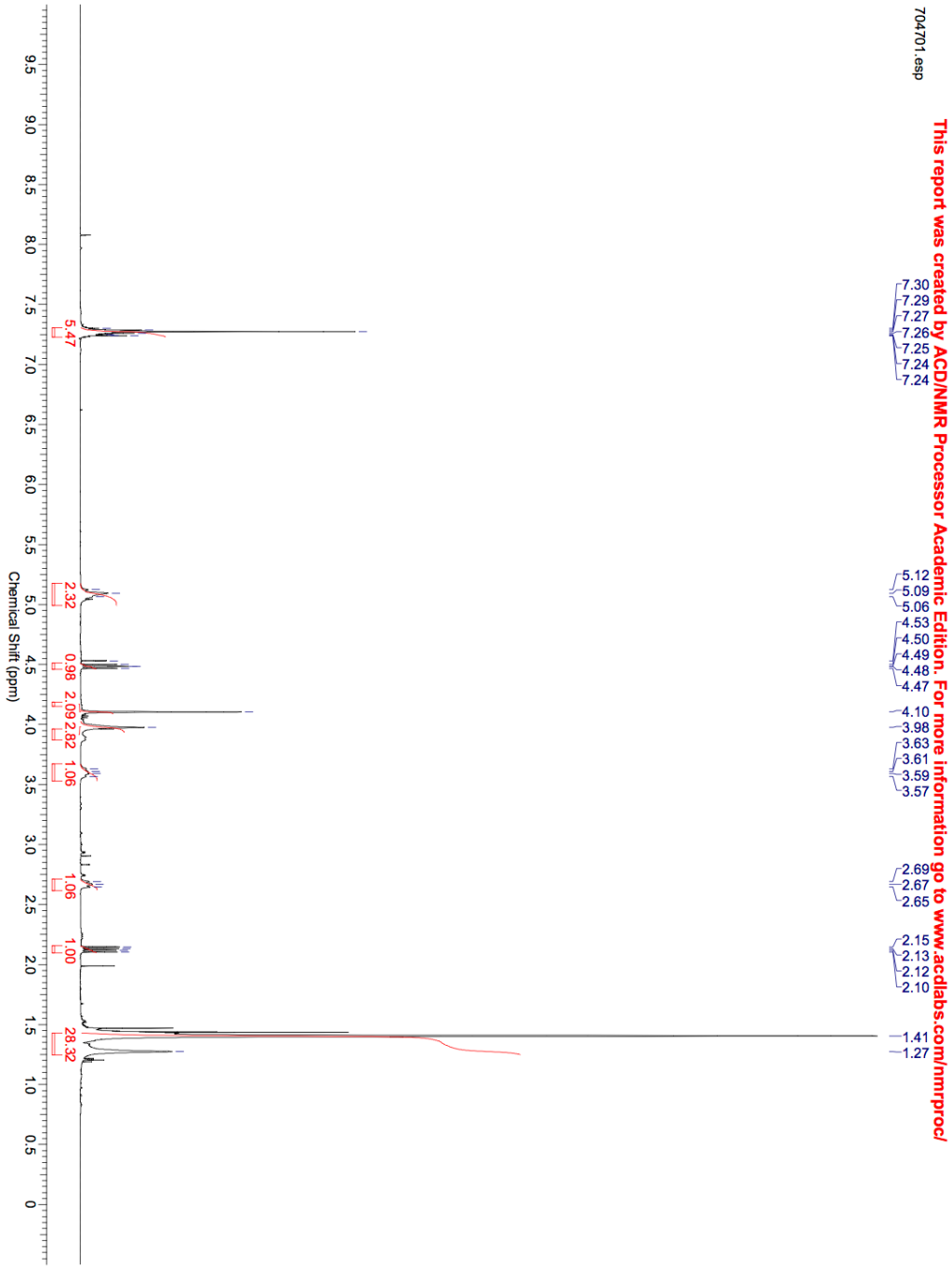
153.38
135.77
135.69
128.30
128.02
127.92
127.81
127.73
127.67
83.20
83.05
82.83
77.26
77.00
76.75
67.45
66.28
65.27
60.20
59.25
58.73
56.24
55.96
40.12
39.89
38.95
27.85
27.80
27.53
20.86
14.03
Chemical Shift (ppm)
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/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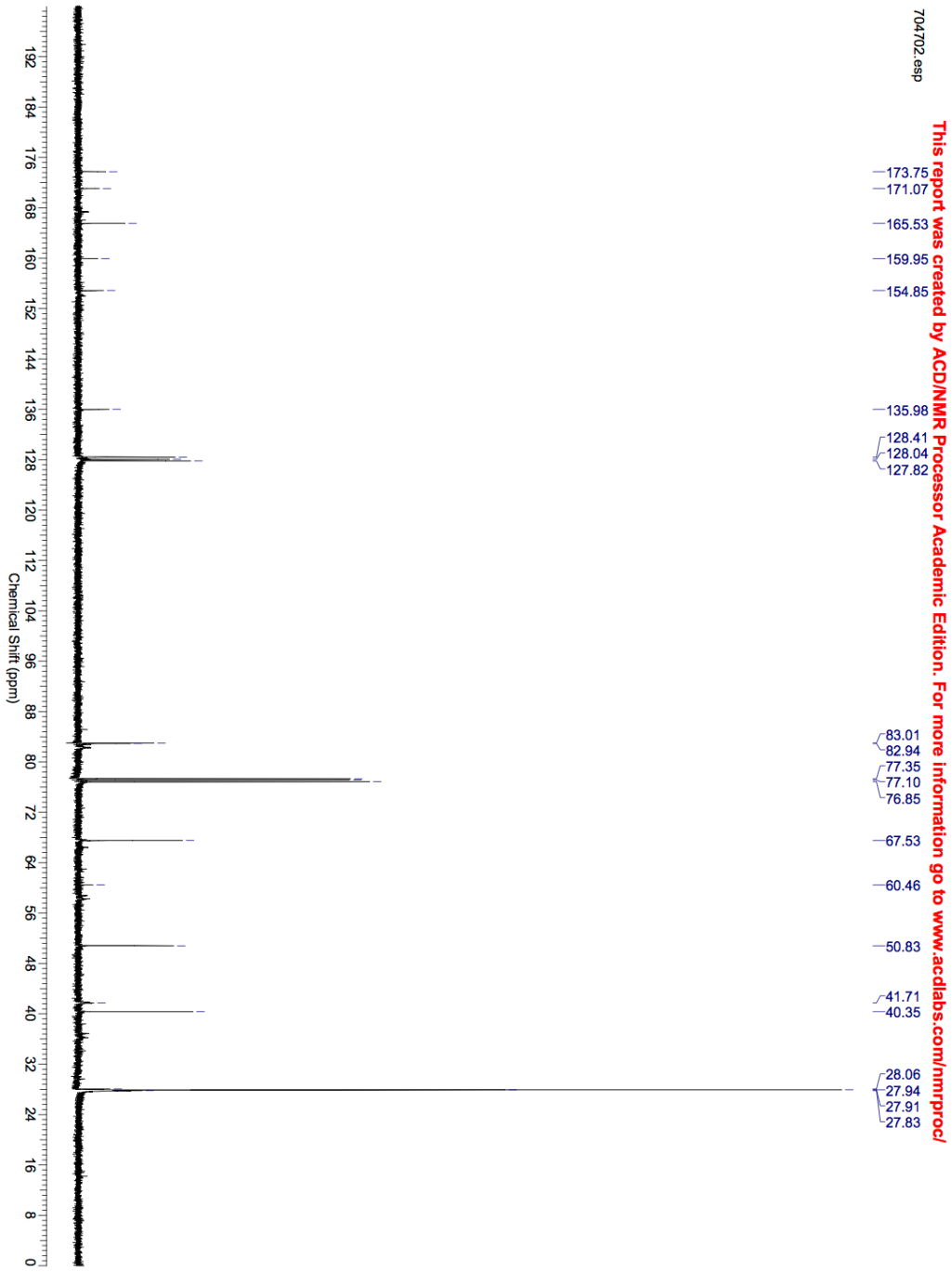
7.25
7.24
7.24
5.12
5.09
5.06
4.53
4.50
4.49
4.48
4.47
4.10
3.98
3.63
3.61
3.59
3.57
2.69
2.67
2.65
2.15
2.13
2.12
2.10
1.41
1.27
5.47
2.32
0.98
2.09
2.82
1.06
1.06
1.00
28.32
Chemical Shift (ppm)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135.98
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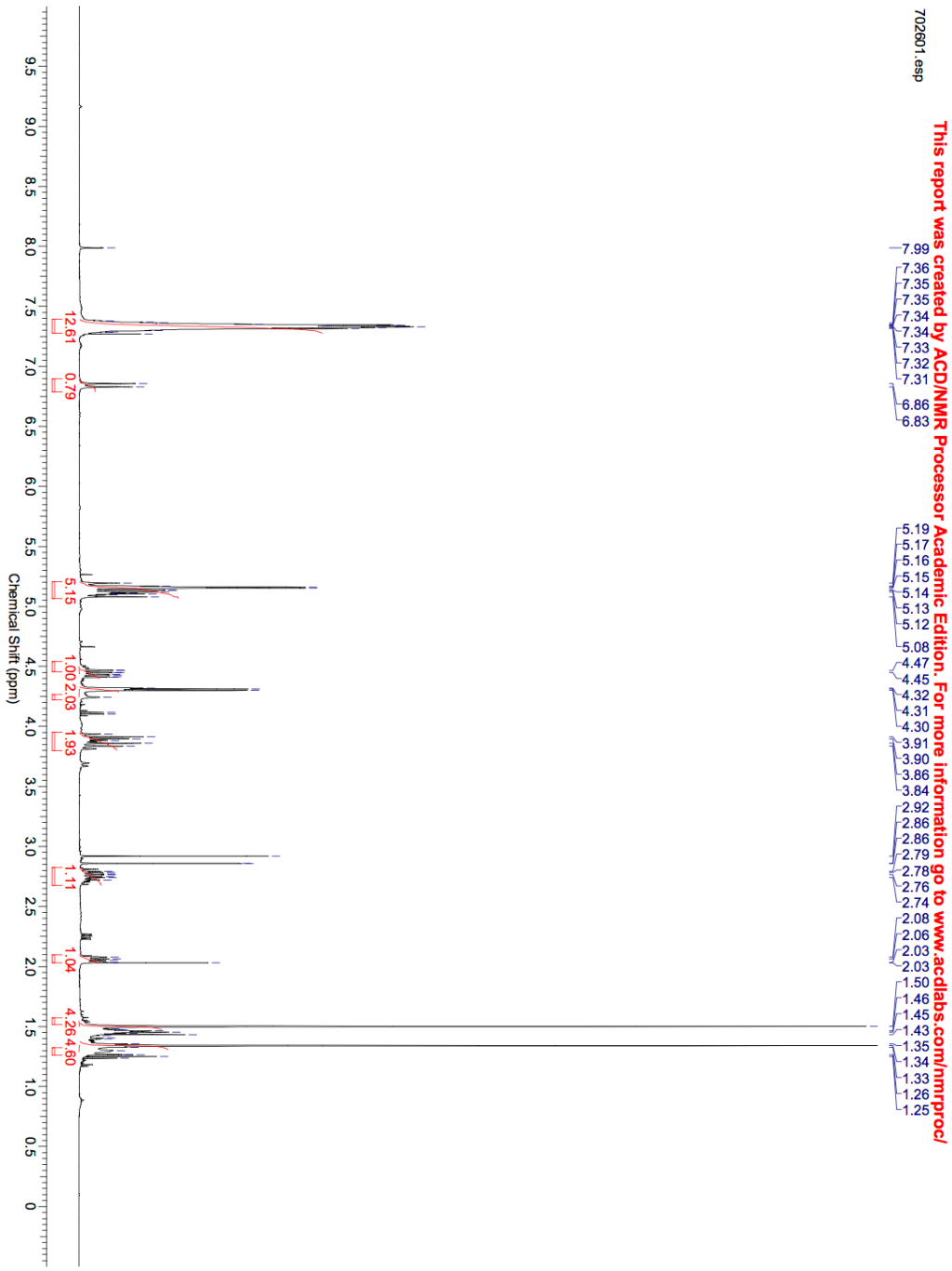
77.35
77.10
76.85
67.53
60.46
50.83
41.71
40.35
28.06
27.94
27.91
27.83
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0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7.34
7.33
7.32
7.31
6.86
6.83
5.19
5.17
5.16
5.15
5.14
5.13
5.12
5.08
4.47
4.45
4.32
4.31
4.30
3.91
3.90
3.86
3.84
2.92
2.86
2.86
2.79
2.78
2.76
2.74
2.08
2.06
2.03
2.03
1.50
1.46
1.45
1.43
1.35
1.34
1.33
1.26
1.25
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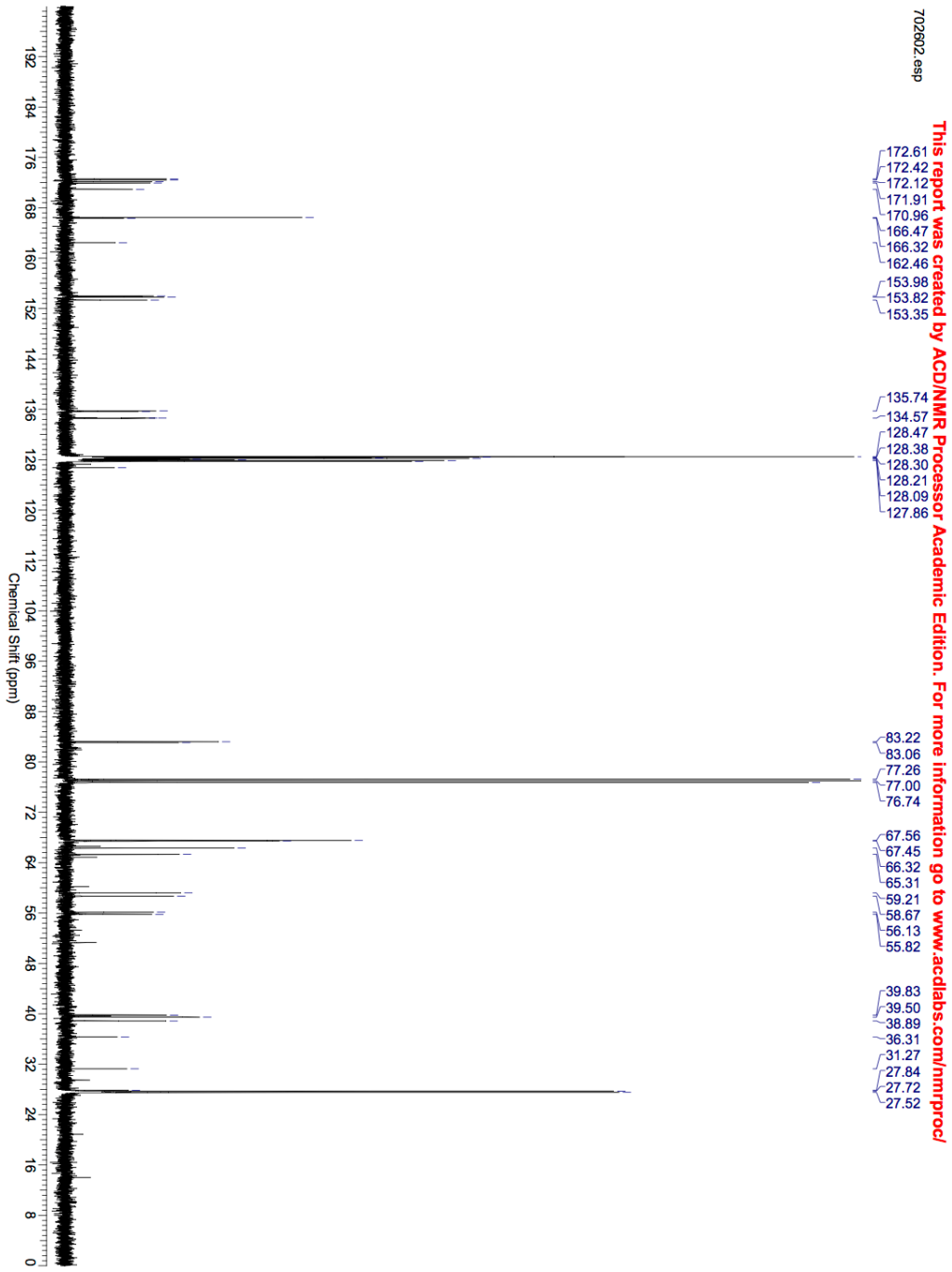
1.11
1.04
4.26
4.60
Chemical Shift (ppm)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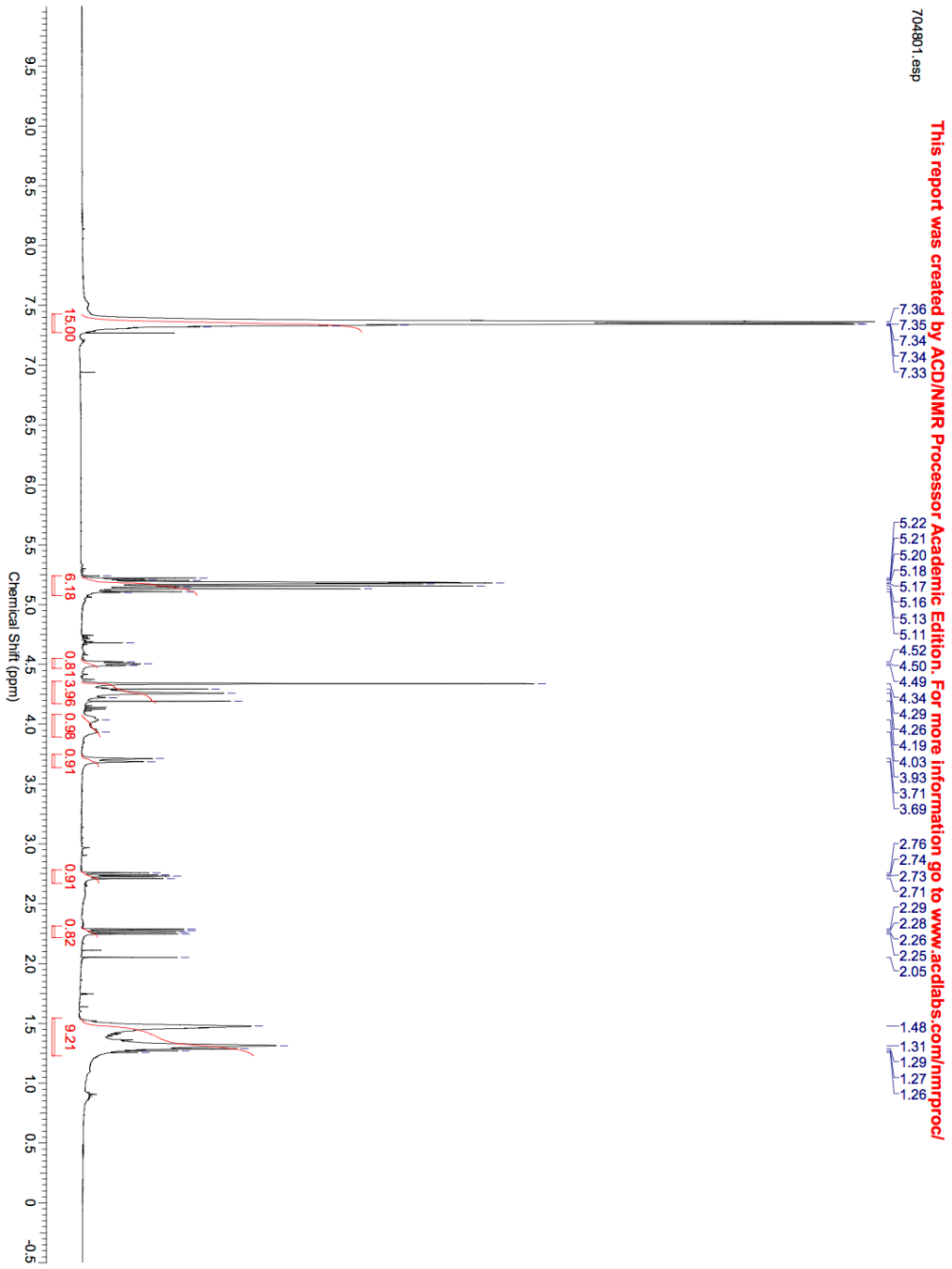
153.98
153.82
153.35
135.74
134.57
128.47
128.38
128.30
128.21
128.09
127.86
83.22
83.06
77.26
77.00
76.74
67.56
67.45
66.32
65.31
59.21
58.67
56.13
55.82
39.83
39.50
38.89
36.31
31.27
27.84
27.72
27.52
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0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5.20
5.18
5.17
5.16
5.13
5.11
4.52
4.50
4.49
4.34
4.29
4.26
4.19
4.03
3.93
3.71
3.69
2.76
2.74
2.73
2.71
2.29
2.28
2.26
2.25
2.05
1.48
1.31
1.29
1.27
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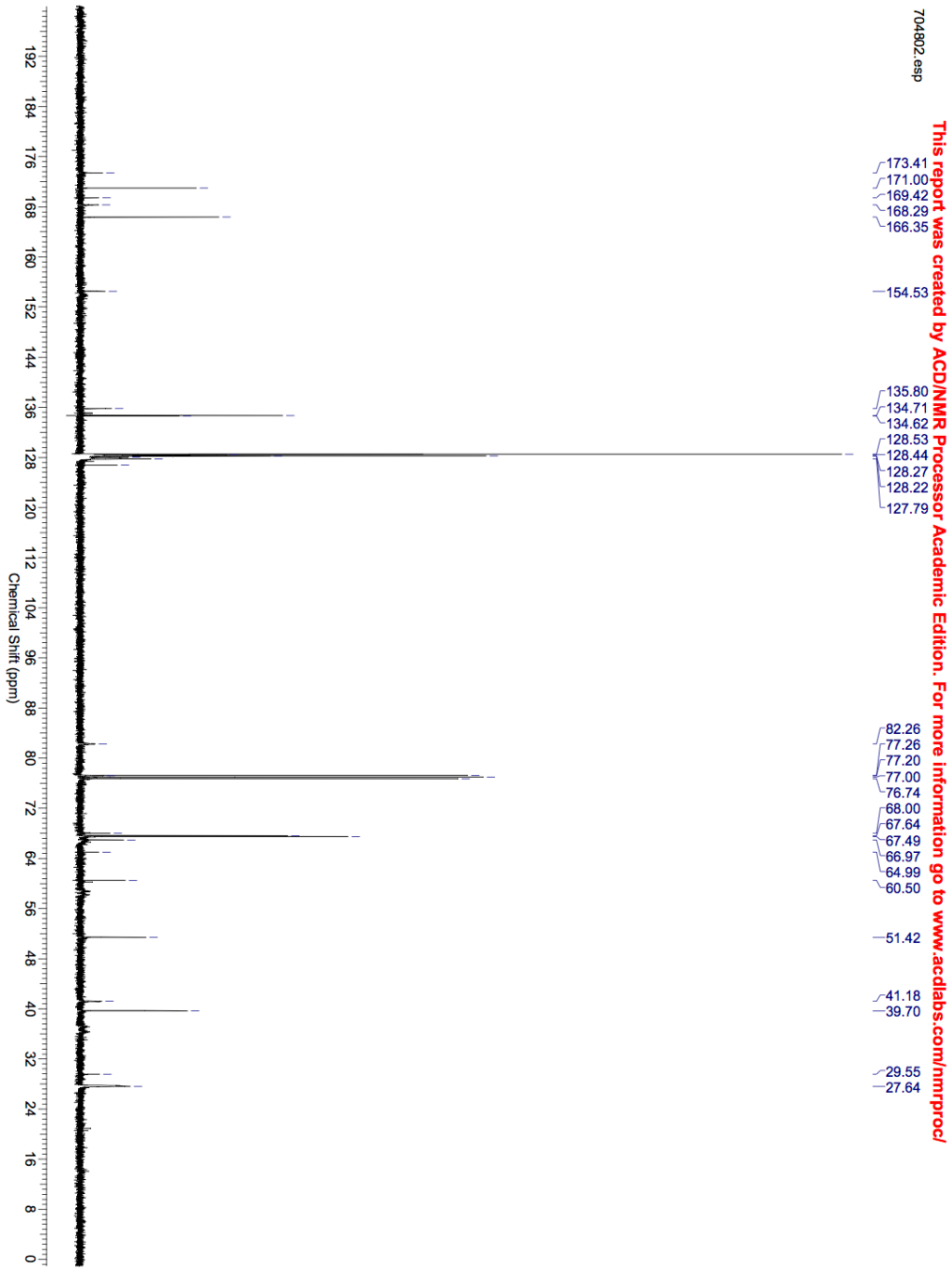
0.91
0.82
9.21
Chemical Shift (ppm)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128.22
127.79
82.26
77.26
77.20
77.00
76.74
68.00
67.64
67.49
66.97
64.99
60.50
51.42
41.18
39.70
29.55
27.64
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
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120
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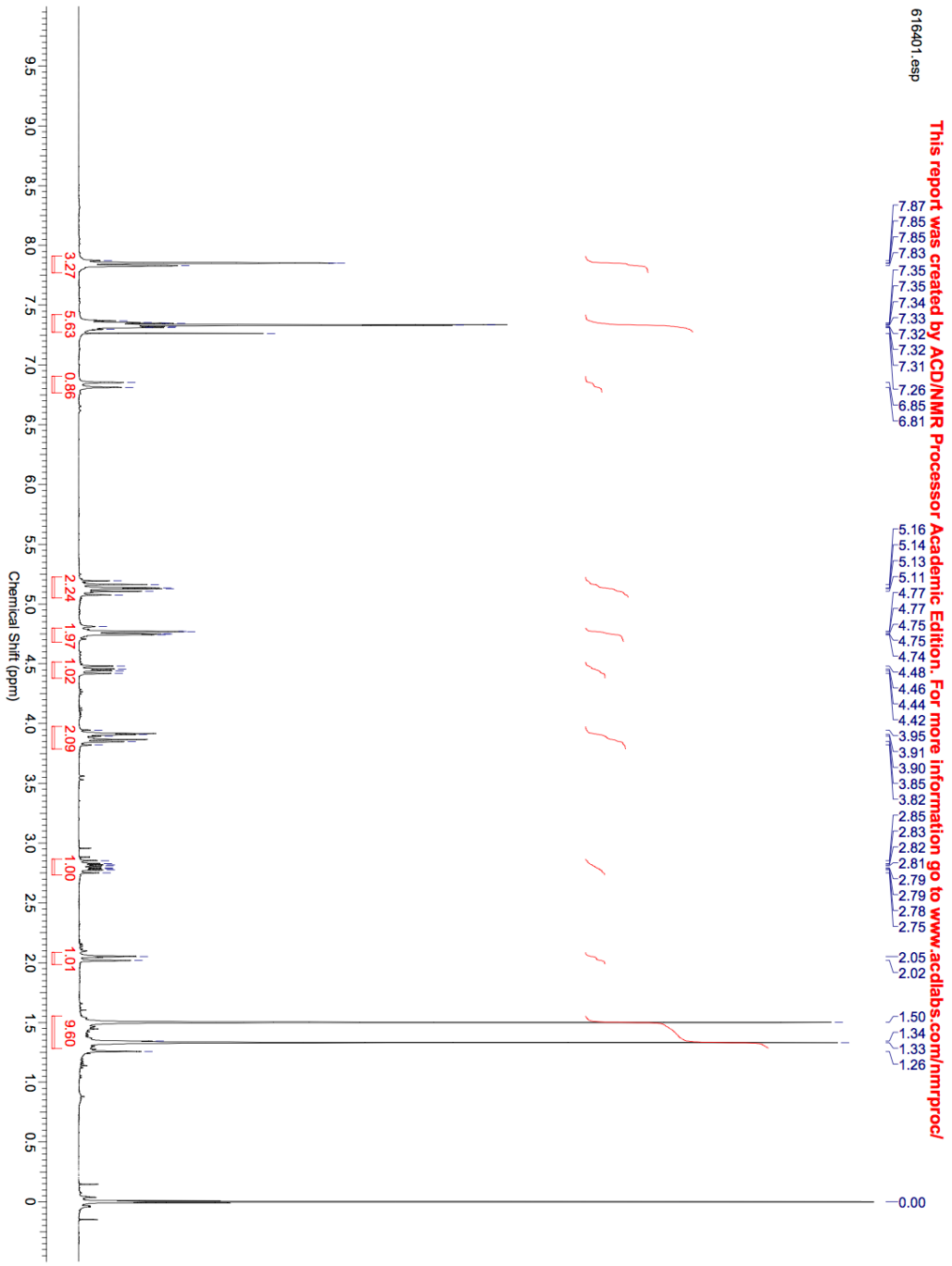
104
96
88
80
72
64
56
48
40
32
24
16
8
0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This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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1.02
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7.35
7.35
7.34
7.33
7.32
7.32
7.31
7.26
6.85
6.81
5.16
5.14
5.13
5.11
4.77
4.77
4.75
4.75
4.74
4.48
4.46
4.44
4.42
3.95
3.91
3.90
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2.85
2.83
2.82
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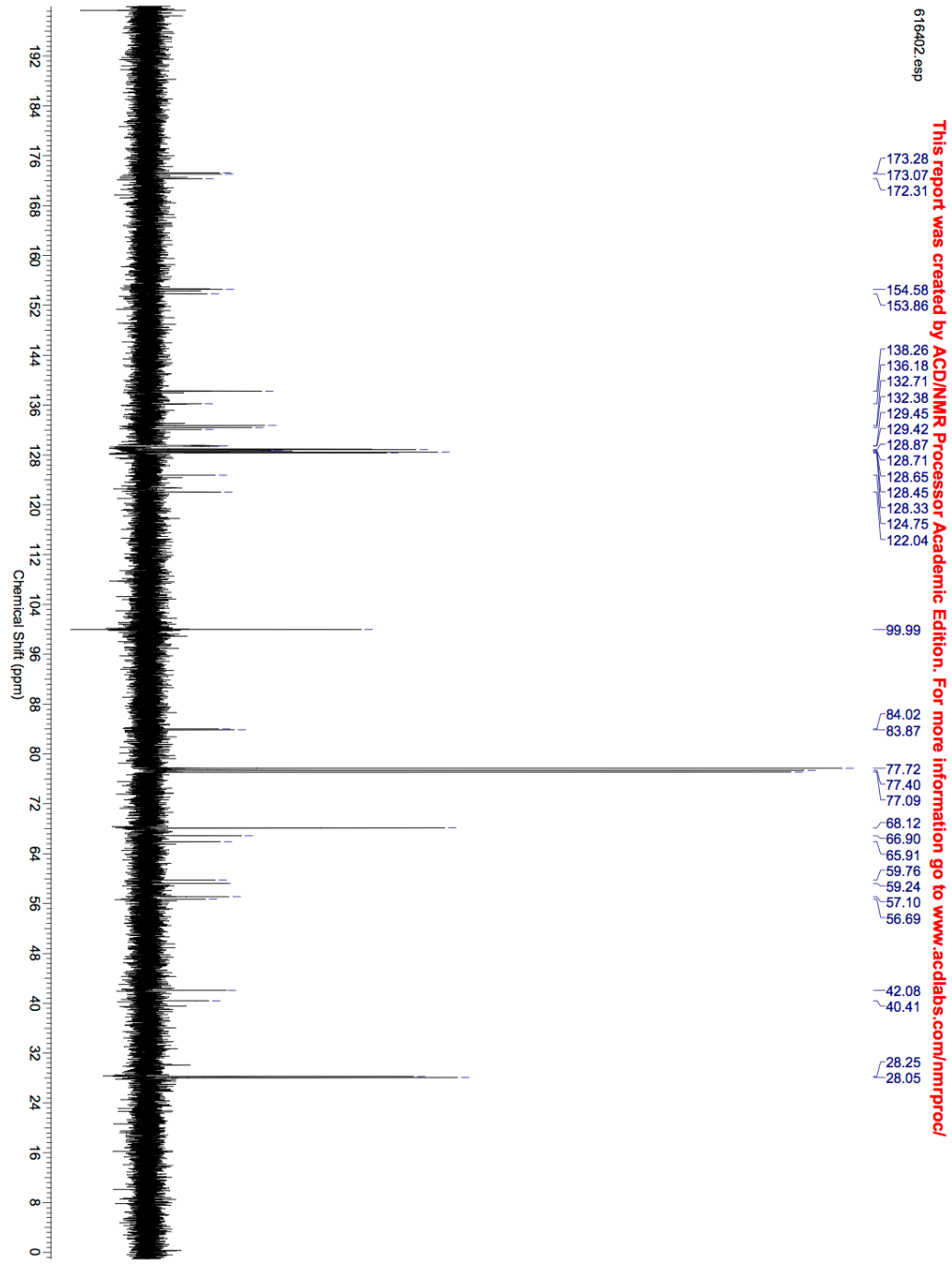
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128.45
128.33
124.75
122.04
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84.02
83.87
77.72
77.40
77.09
68.12
66.90
65.91
59.76
59.24
57.10
56.69
42.08
40.41
28.25
28.05
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
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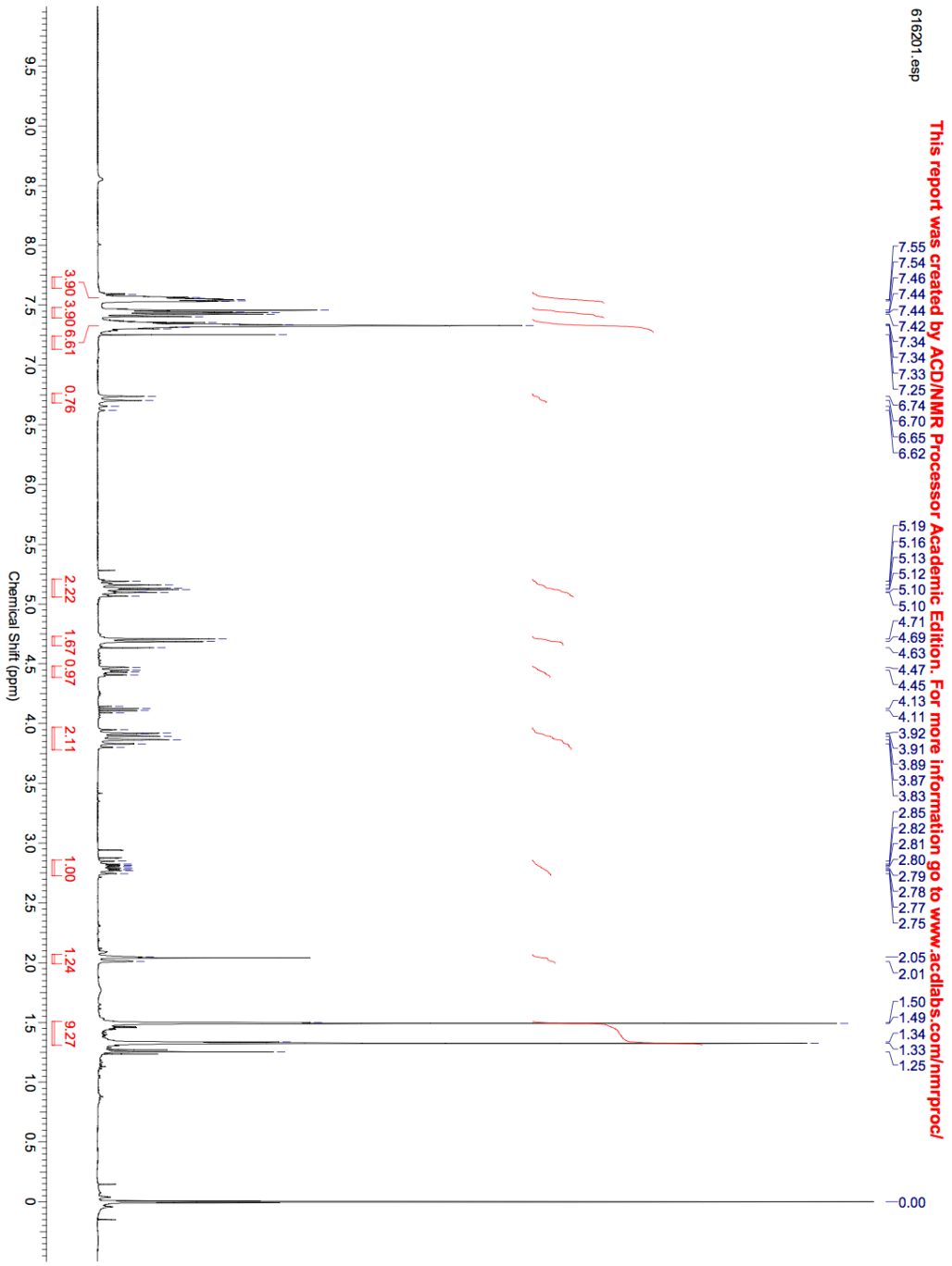
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7.33
7.25
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5.19
5.16
5.13
5.12
5.10
5.10
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4.69
4.63
4.47
4.45
4.13
4.11
3.92
3.91
3.89
3.87
3.83
2.85
2.82
2.81
2.80
2.79
2.78
2.77
2.75
2.05
2.01
1.50
1.49
1.34
1.33
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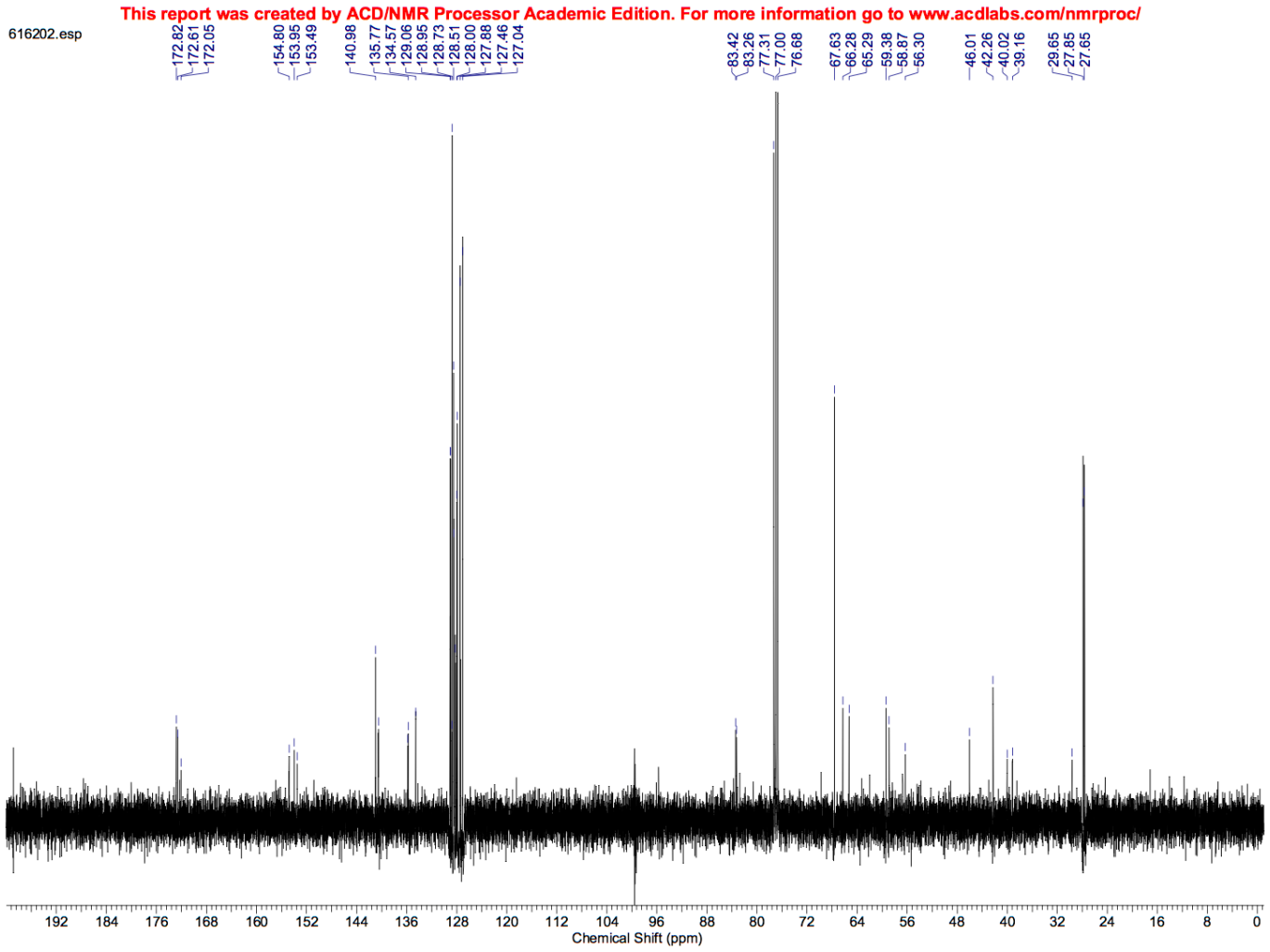
Chemical Shift (ppm)


This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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128.00
127.88
127.46
127.04
83.42
83.26
77.31
77.00
76.68
67.63
66.28
65.29
59.38
58.87
56.30
46.01
42.26
40.02
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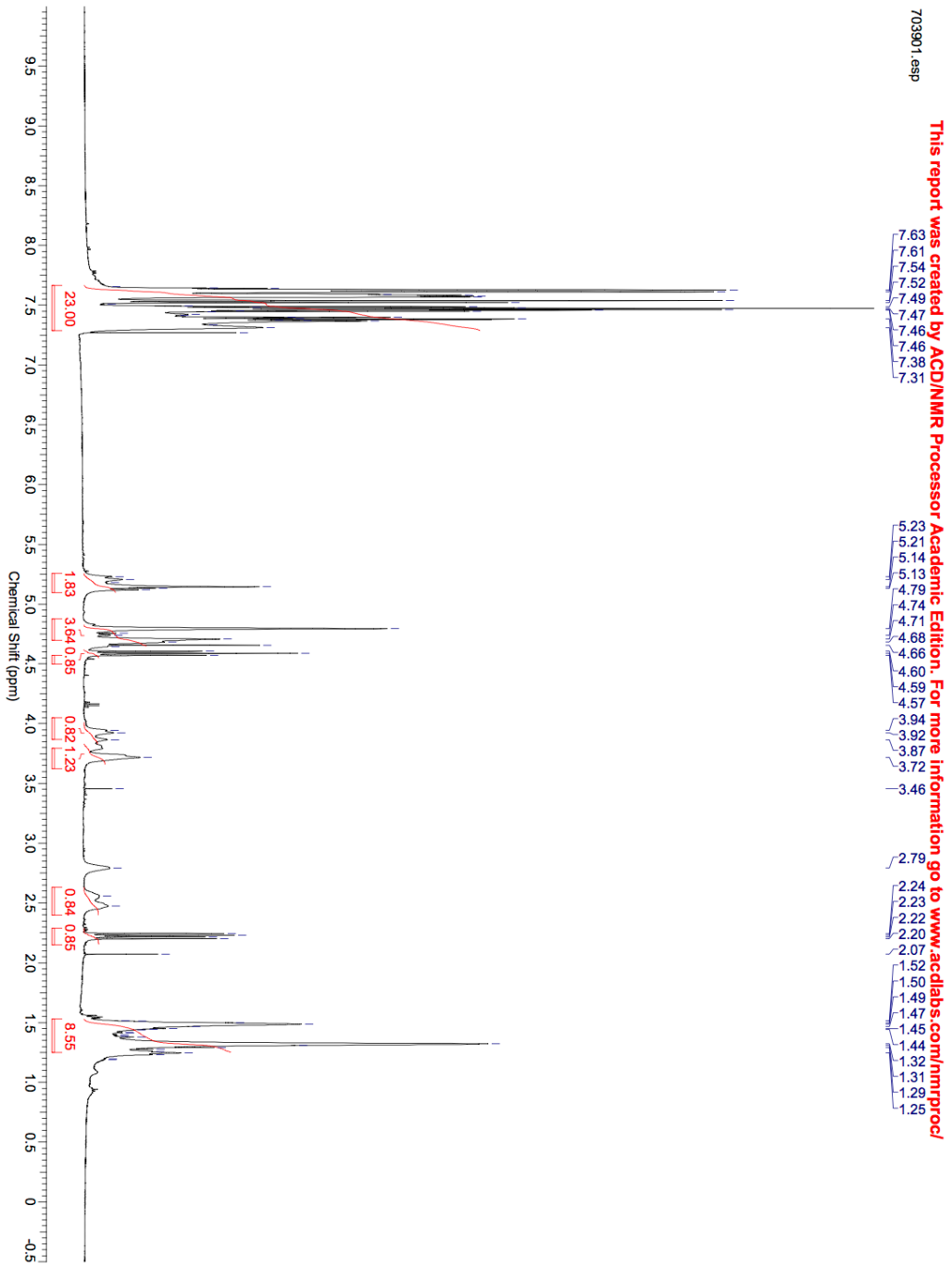
29.65
27.85
27.65
192 184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 16 8 0
Chemical Shift (ppm)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This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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7.46
7.38
7.31
5.23
5.21
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1.50
1.49
1.47
1.45
1.44
1.32
1.31
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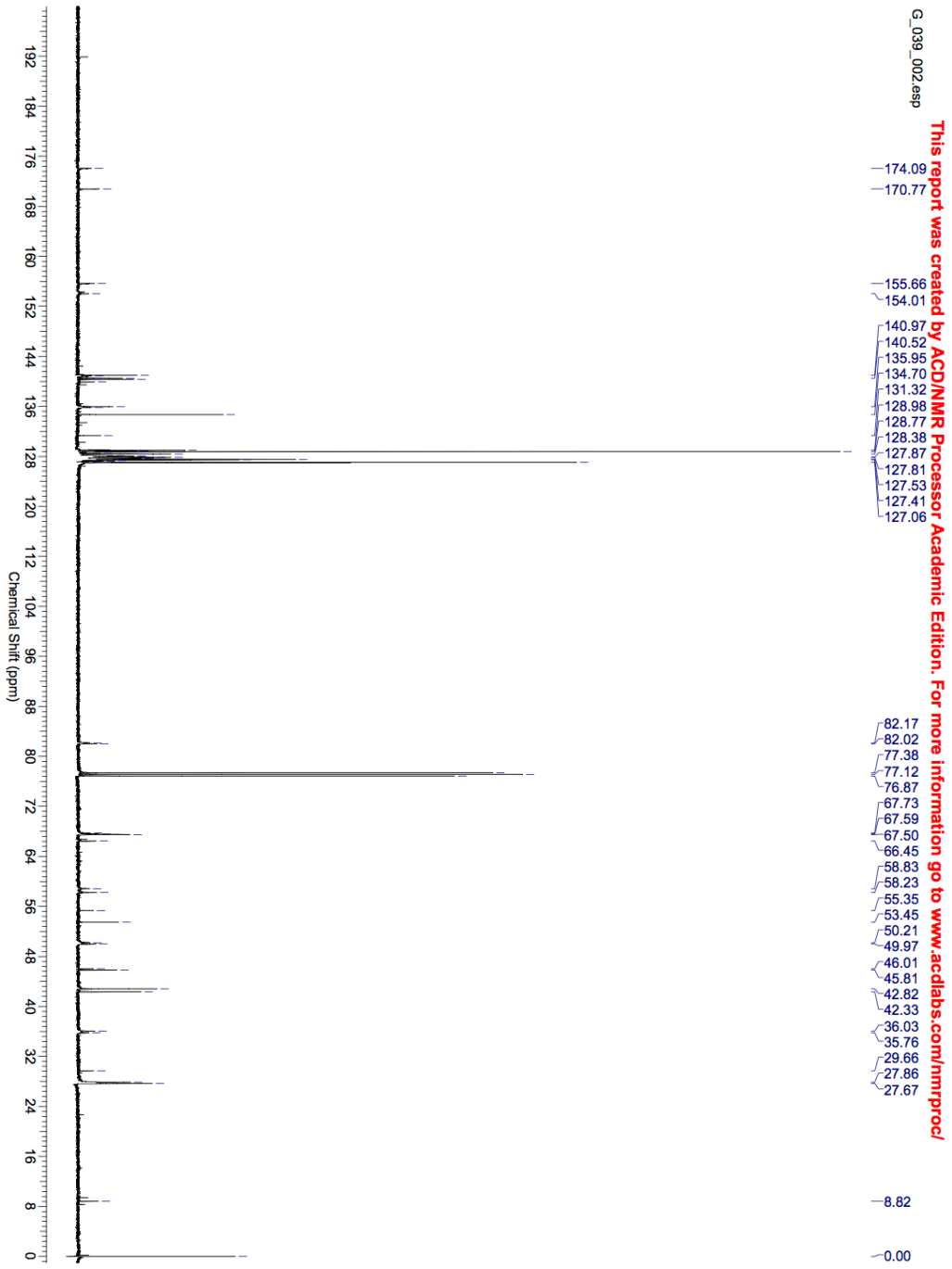
0.85
8.55
Chemical Shift (ppm)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127.53
127.41
127.06
82.17
82.02
77.38
77.12
76.87
67.73
67.59
67.50
66.45
58.83
58.23
55.35
53.45
50.21
49.97
46.01
45.81
42.82
42.33
36.03
35.76
29.66
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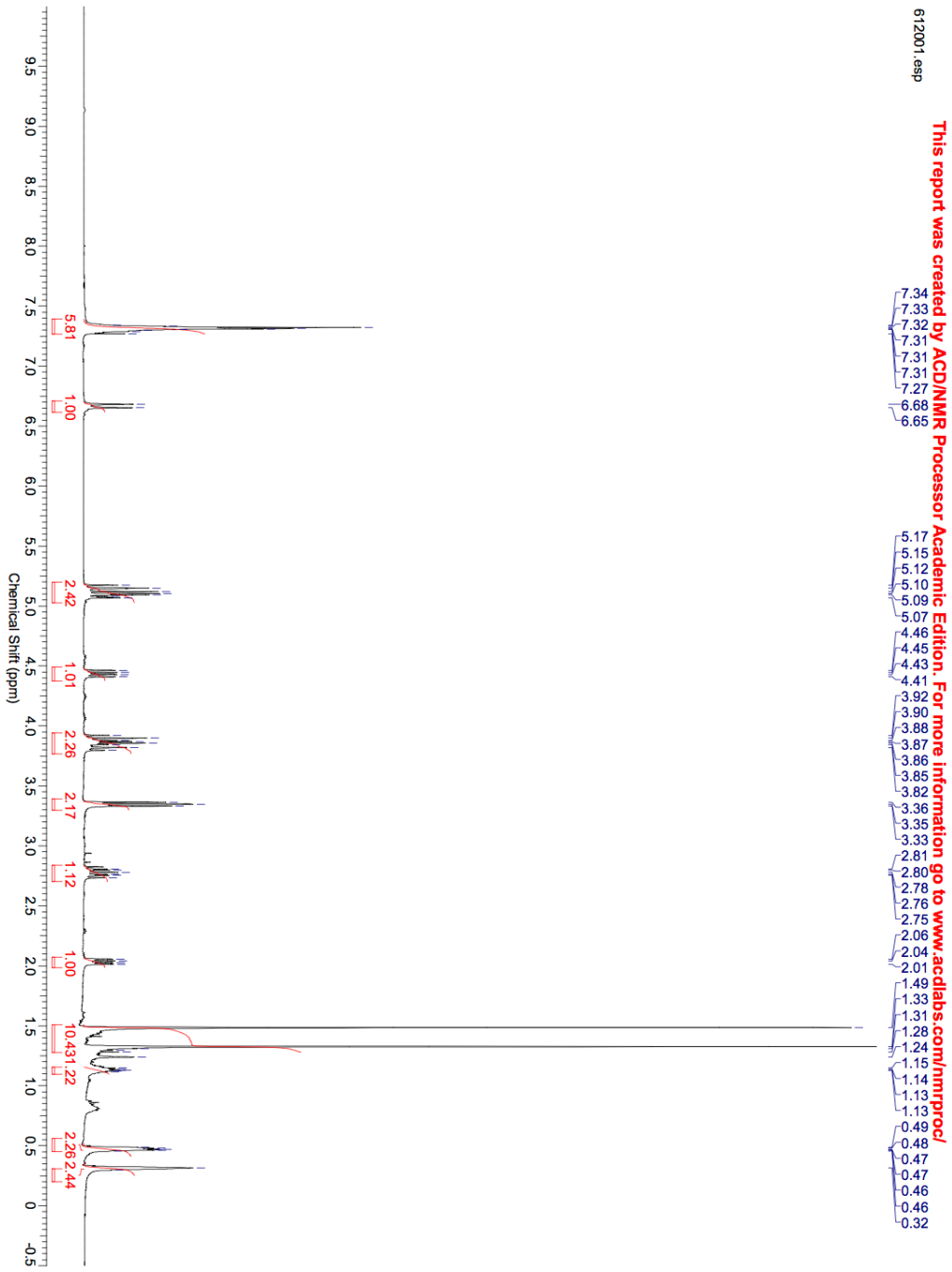
0.00
Chemical Shift (ppm)
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/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This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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7.27
6.68
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5.17
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5.10
5.09
5.07
4.46
4.45
4.43
4.41
3.92
3.90
3.88
3.87
3.86
3.85
3.82
3.36
3.35
3.33
2.81
2.80
2.78
2.76
2.75
2.06
2.04
2.01
1.49
1.33
1.31
1.28
1.24
1.15
1.14
1.13
1.13
0.49
0.48
0.47
0.47
0.46
0.46
0.32
5.81
1.00
2.42
1.01
2.26
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1.12
1.00
10.43
1.22
2.26
2.44
Chemical Shift (ppm)
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9.0
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8.0
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7.0
6.5
6.0
5.5
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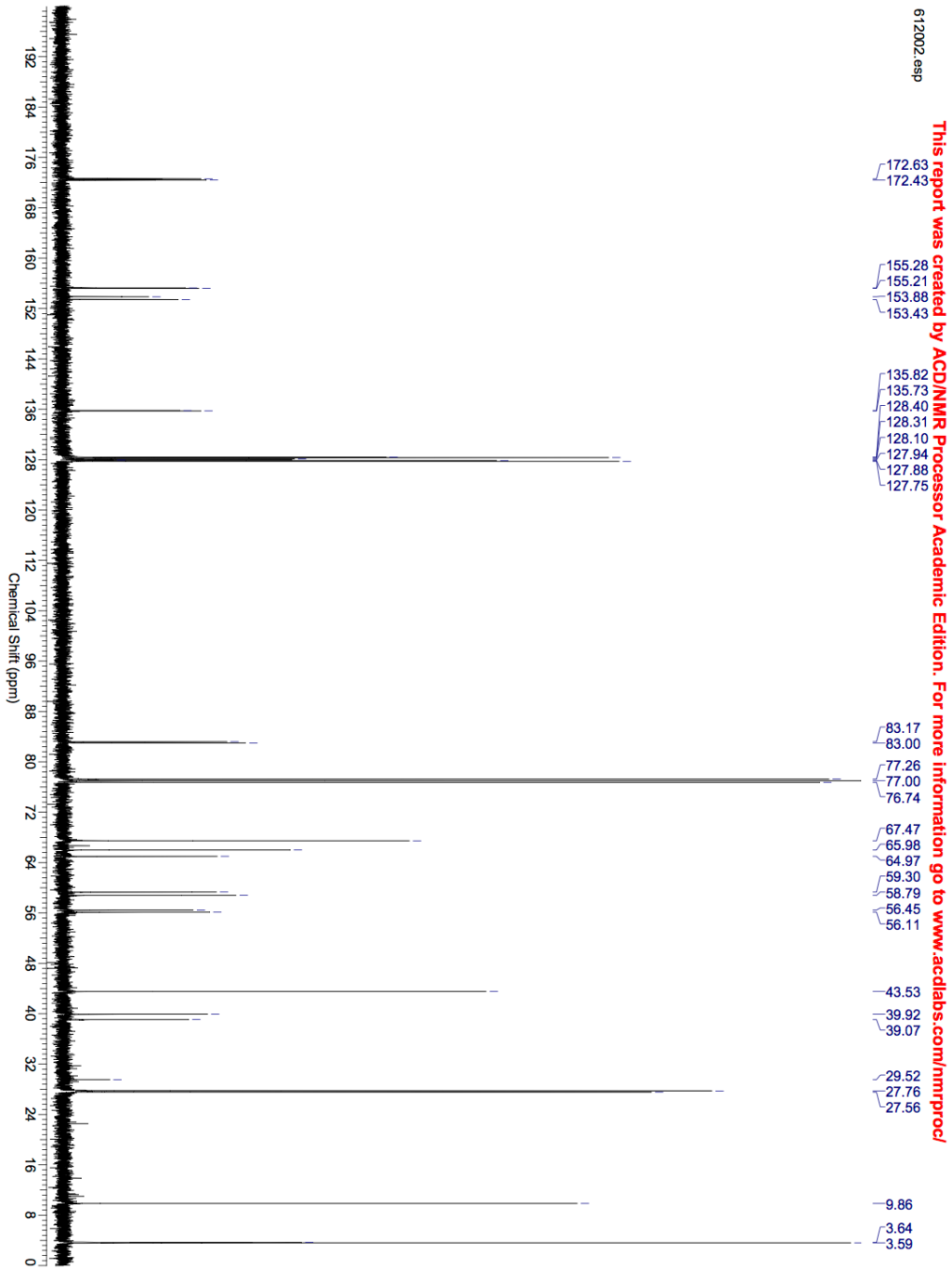
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153.88
153.43
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128.40
128.31
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127.88
127.75
83.17
83.00
77.26
77.00
76.74
67.47
65.98
64.97
59.30
58.79
56.45
56.11
43.53
39.92
39.07
29.52
27.76
27.56
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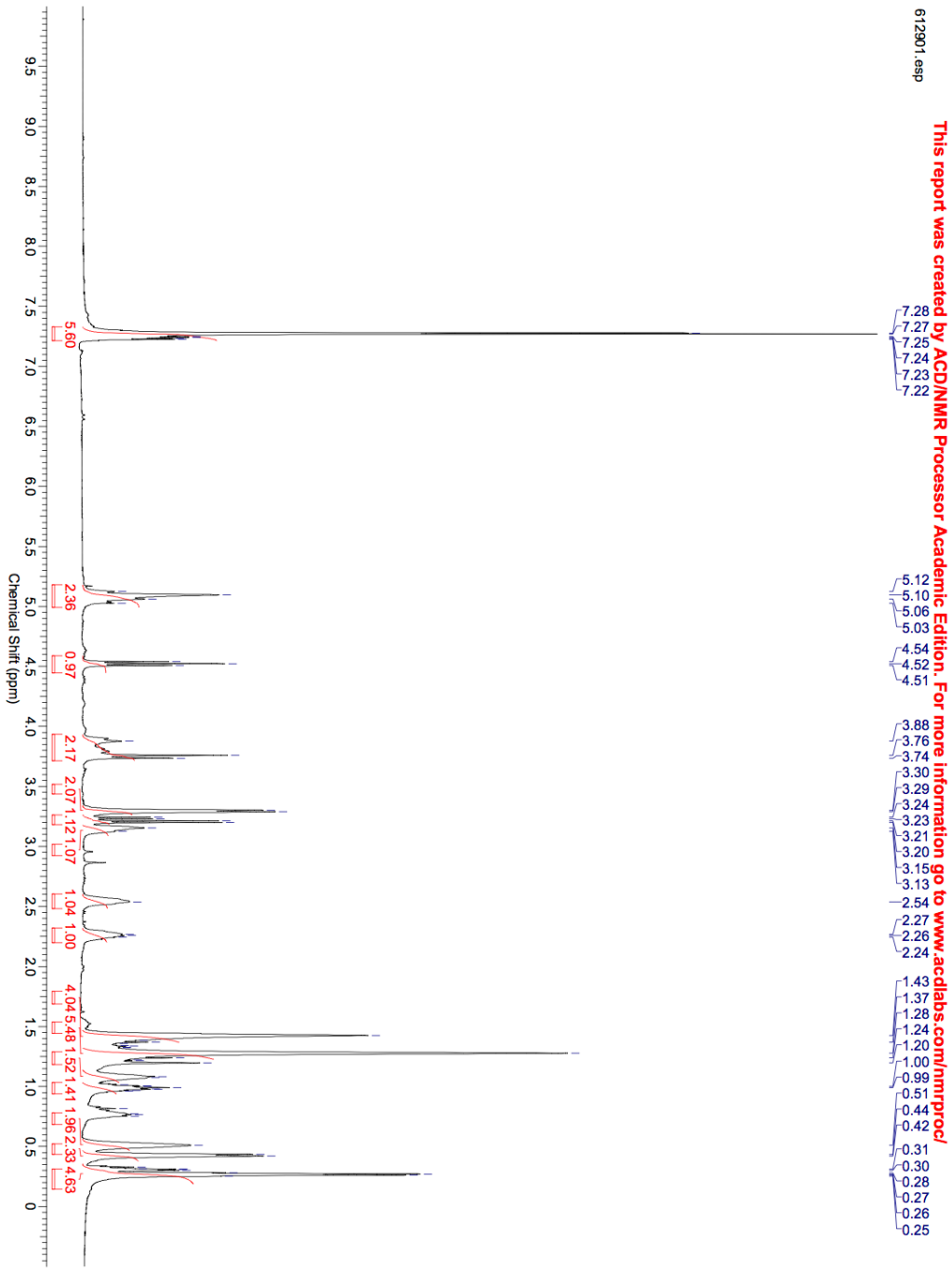
3.64
3.59
Chemical Shift (ppm)
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/


612901.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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7.23
7.22
5.12
5.10
5.06
5.03
4.54
4.52
4.51
3.88
3.76
3.74
3.30
3.29
3.24
3.23
3.21
3.20
3.15
3.13
2.54
2.27
2.26
2.24
1.43
1.37
1.28
1.24
1.20
1.00
0.99
0.51
0.44
0.42
0.31
0.30
0.28
0.27
0.26
0.25
5.60
2.36
0.97
2.17
2.07
1.12
1.21
1.07
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1.00
4.04
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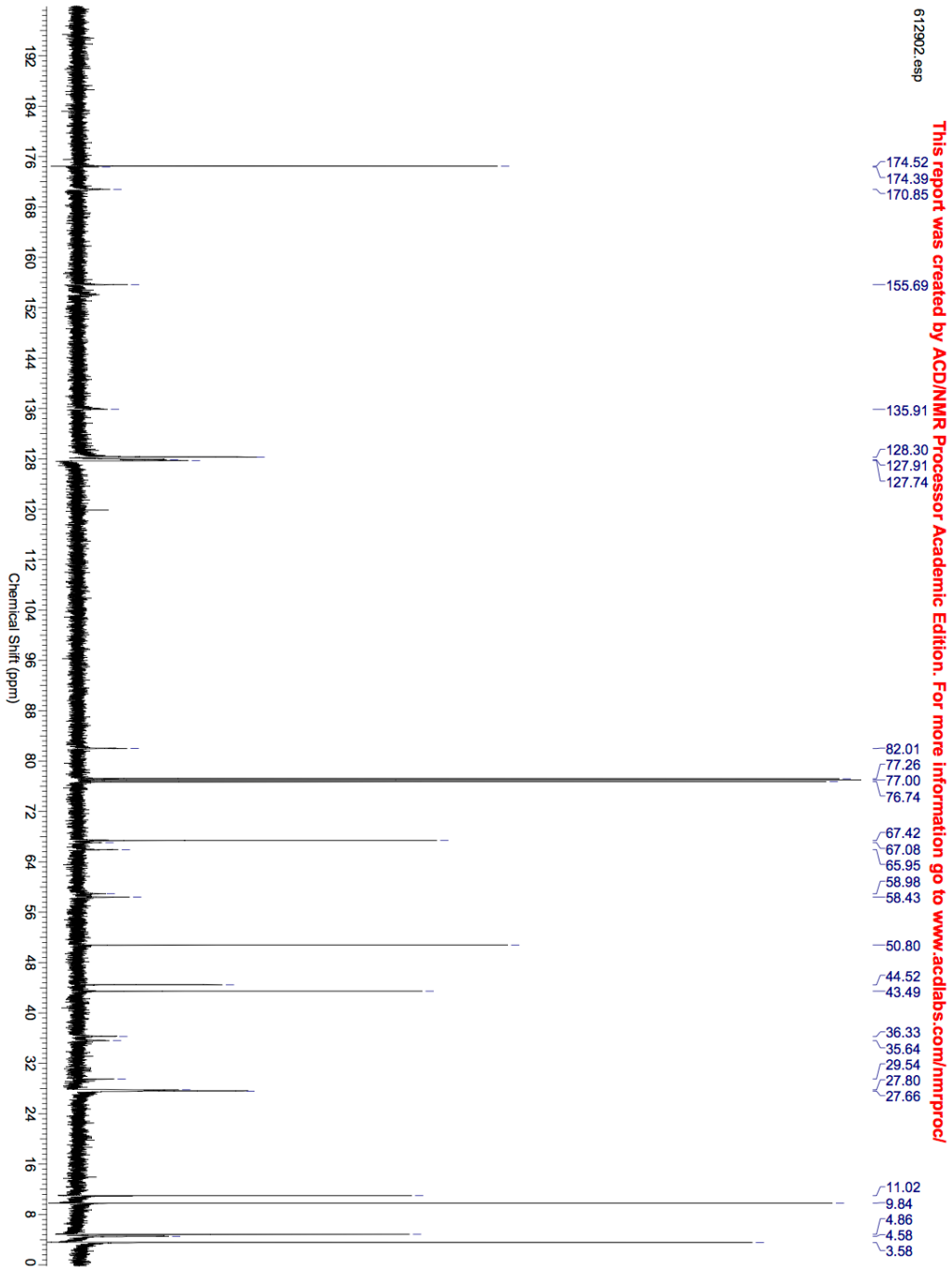
1.96
2.33
4.63
Chemical Shift (ppm)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174.39
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67.08
65.95
58.98
58.43
50.80
44.52
43.49
36.33
35.64
29.54
27.80
27.66
11.02
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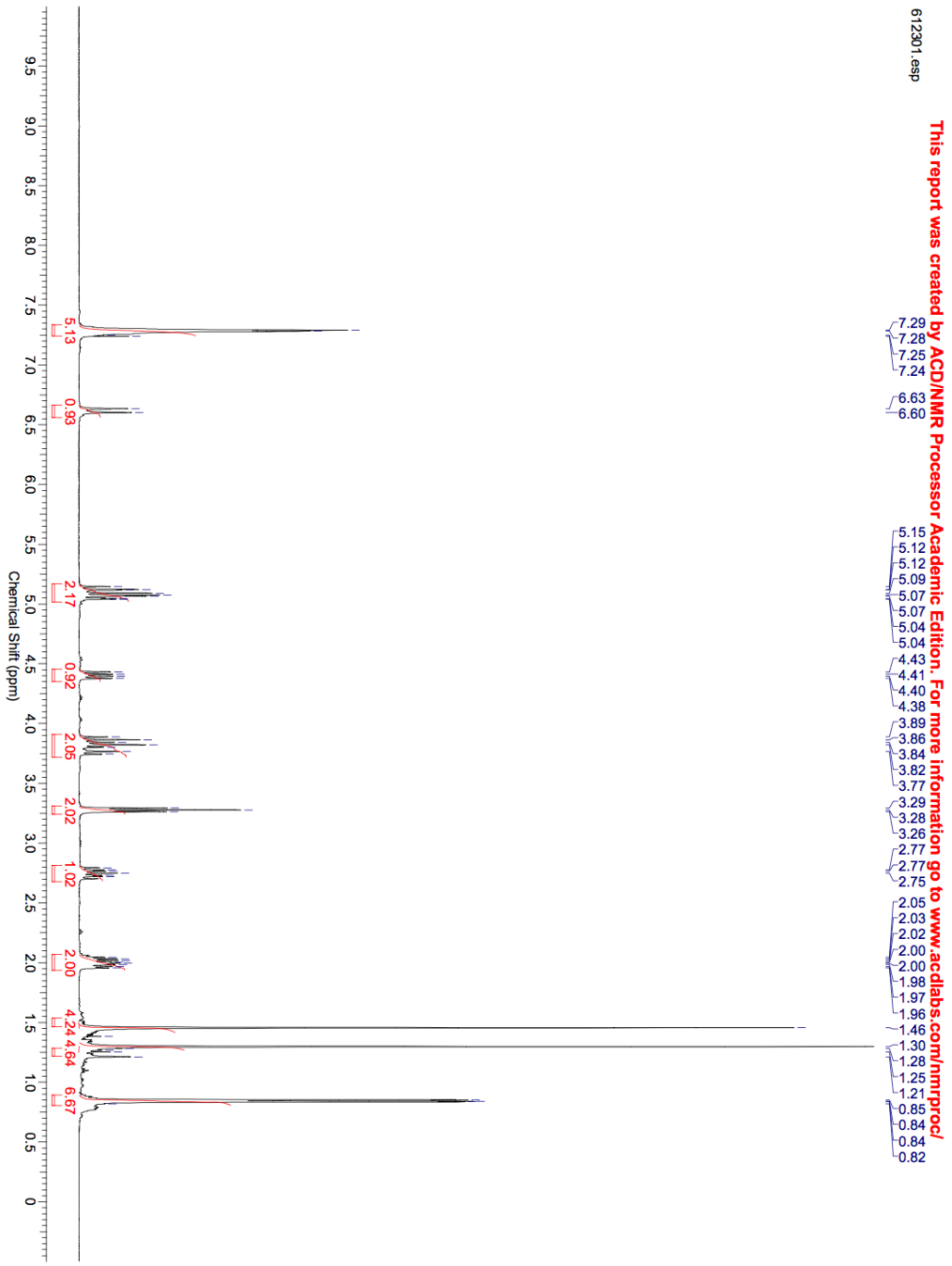
3.58
Chemical Shift (ppm)
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/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This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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5.12
5.12
5.09
5.07
5.07
5.04
5.04
4.43
4.41
4.40
4.38
3.89
3.86
3.84
3.82
3.77
3.29
3.28
3.26
2.77
2.77
2.75
2.05
2.03
2.02
2.00
2.00
1.98
1.97
1.96
1.46
1.30
1.28
1.25
1.21
0.85
0.84
0.84
0.82
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1.02
2.00
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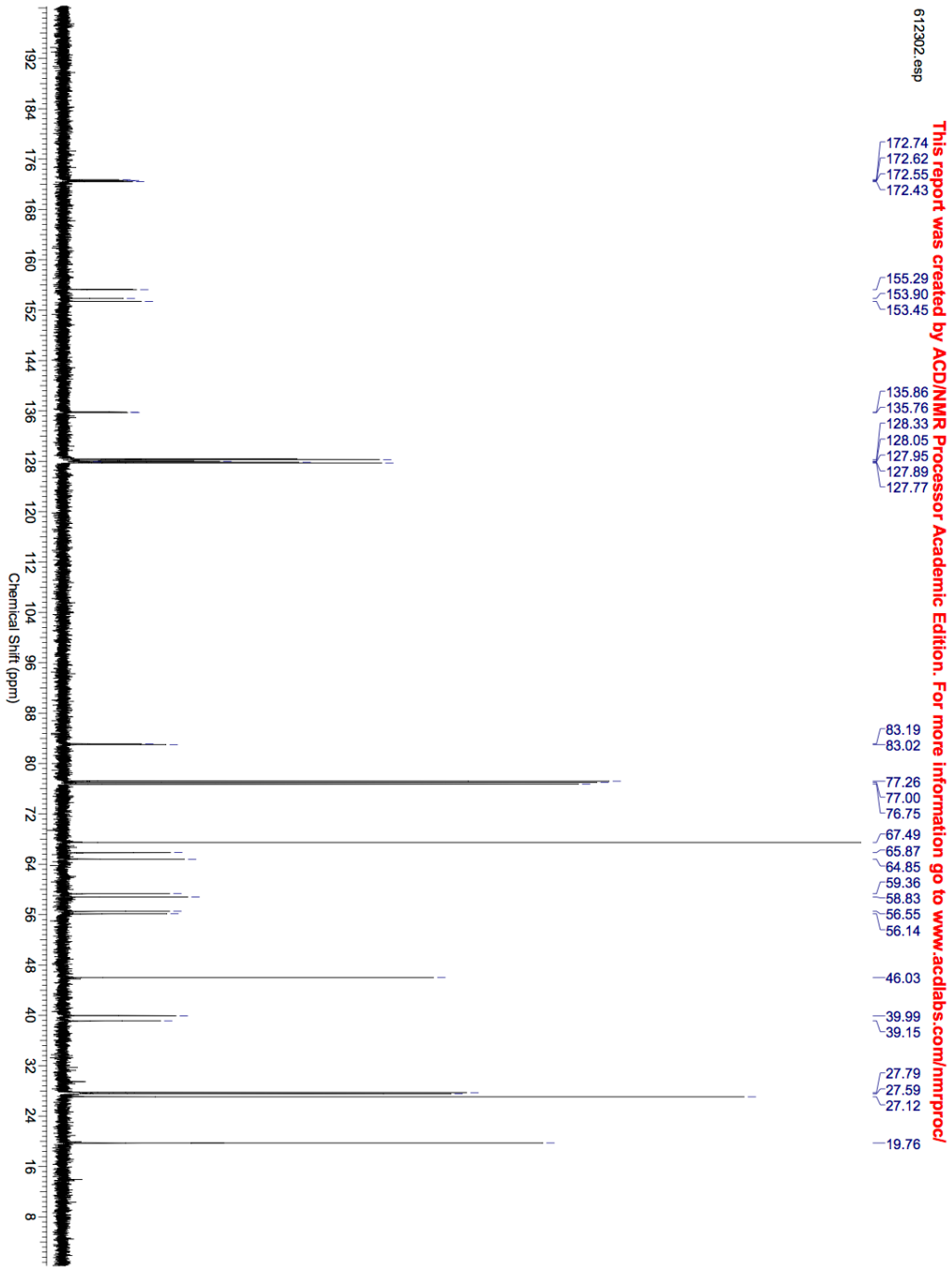
Chemical Shift (ppm)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135.76
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127.77
83.19
83.02
77.26
77.00
76.75
67.49
65.87
64.85
59.36
58.83
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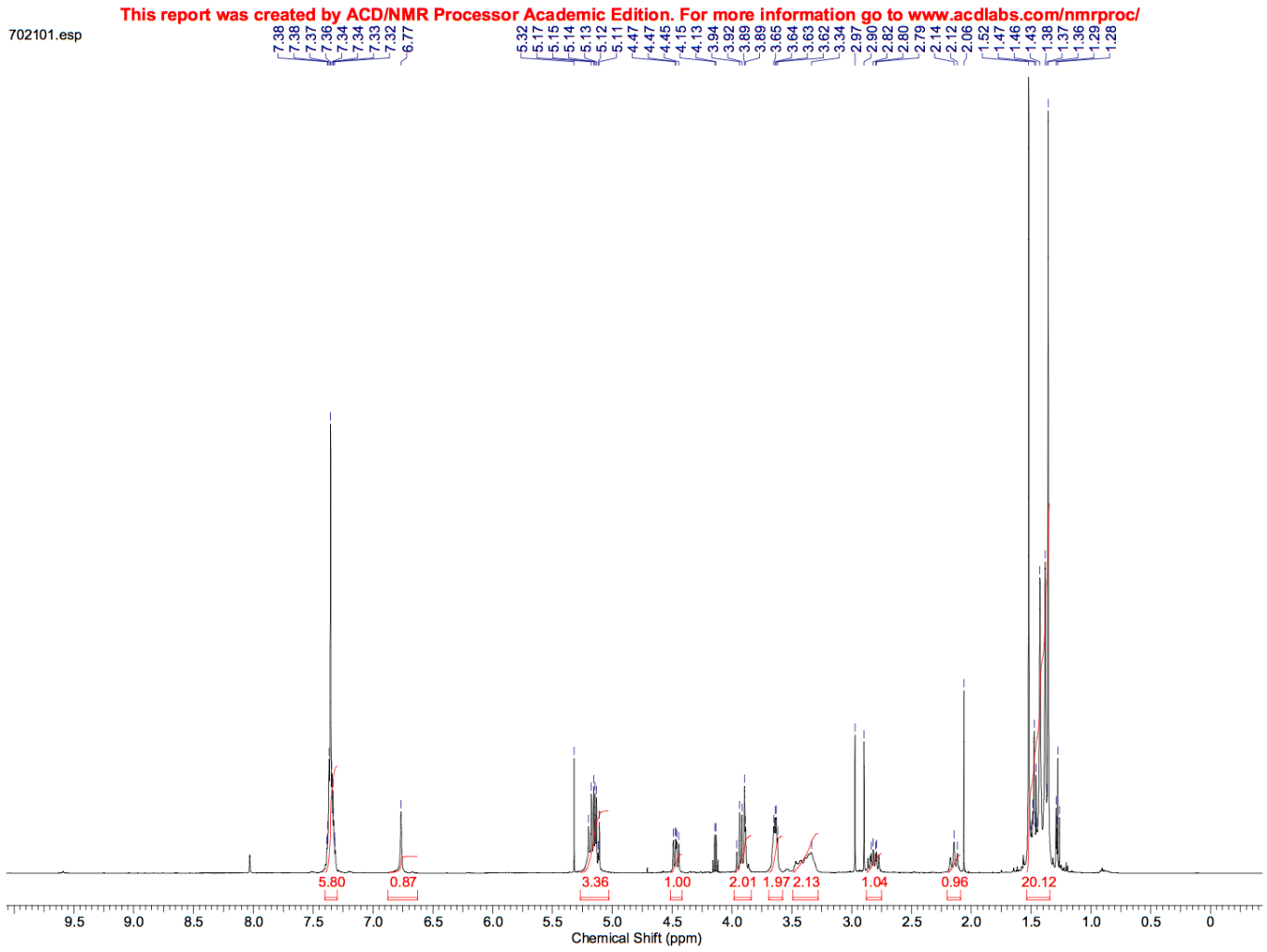
56.14
46.03
39.99
39.15
27.79
27.59
27.12
19.76
Chemical Shift (ppm)
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/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7.34
7.34
7.33
7.32
6.77
5.32
5.17
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5.14
5.13
5.12
5.11
4.47
4.47
4.45
4.15
4.13
3.94
3.92
3.89
3.89
3.65
3.64
3.63
3.62
3.34
2.97
2.90
2.82
2.80
2.79
2.14
2.12
2.06
1.52
1.47
1.46
1.43
1.38
1.37
1.36
1.29
1.28
5.80
0.87
3.36
1.00
2.01
1.97
2.13
1.04
0.96
20.12
9.5
9.0
8.5
8.0
7.5
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6.0
5.5
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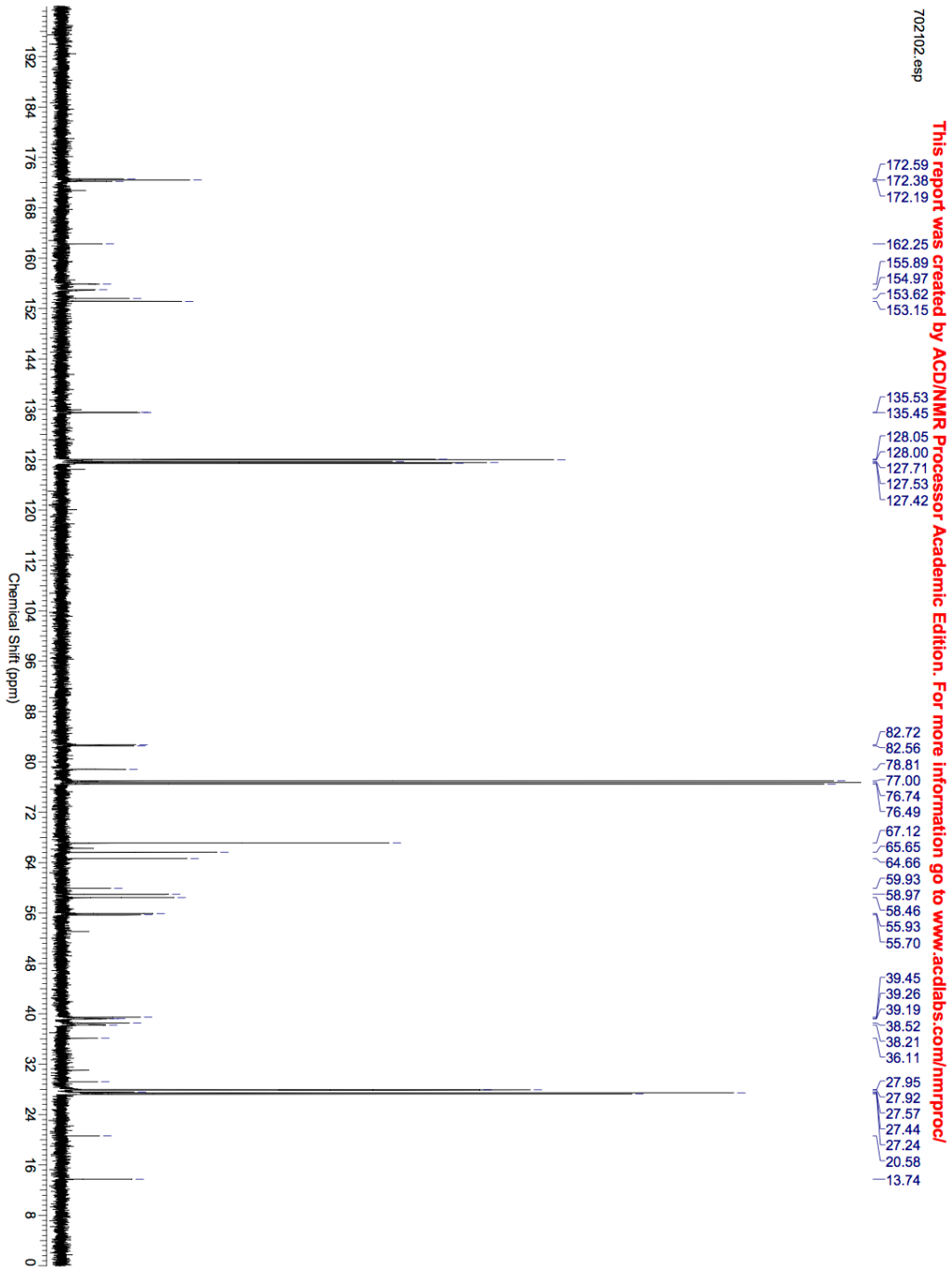
Chemical Shift (ppm)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127.71
127.53
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82.72
82.56
78.81
77.00
76.74
76.49
67.12
65.65
64.66
59.93
58.97
58.46
55.93
55.70
39.45
39.26
39.19
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27.92
27.57
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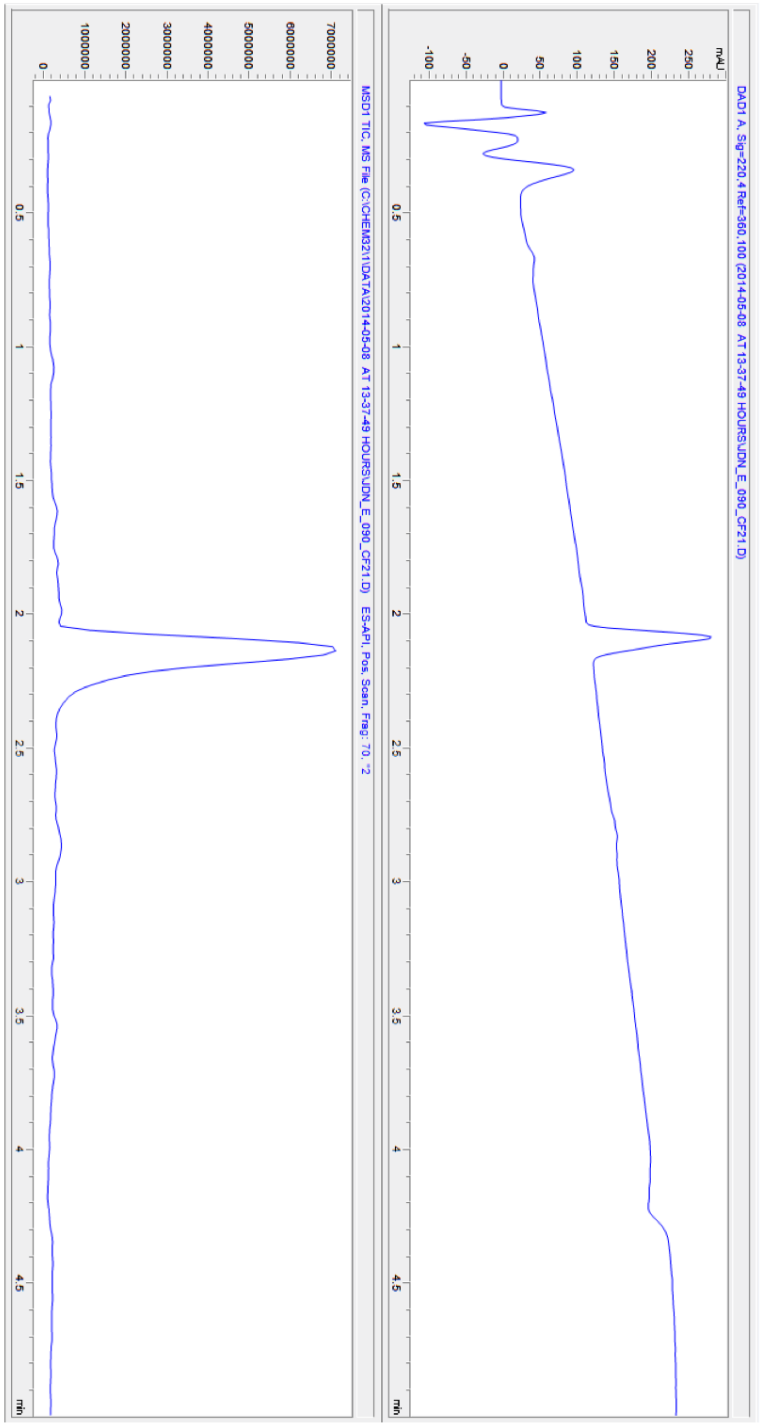
13.74
Chemical Shift (ppm)
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/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mAU
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200
150
100
50
0
-50
-100
0.5
1
1.5
2
2.5
3
3.5
4
4.5
min
MSD1 TIC, MS File (C:\CHEM32\1\DATA\2014-05-08 AT 13-37-49 HOURS\JDN_E_090_CF21.D) ES-API, Pos, Scan, Frag: 70, "2
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6000000
5000000
4000000
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1000000
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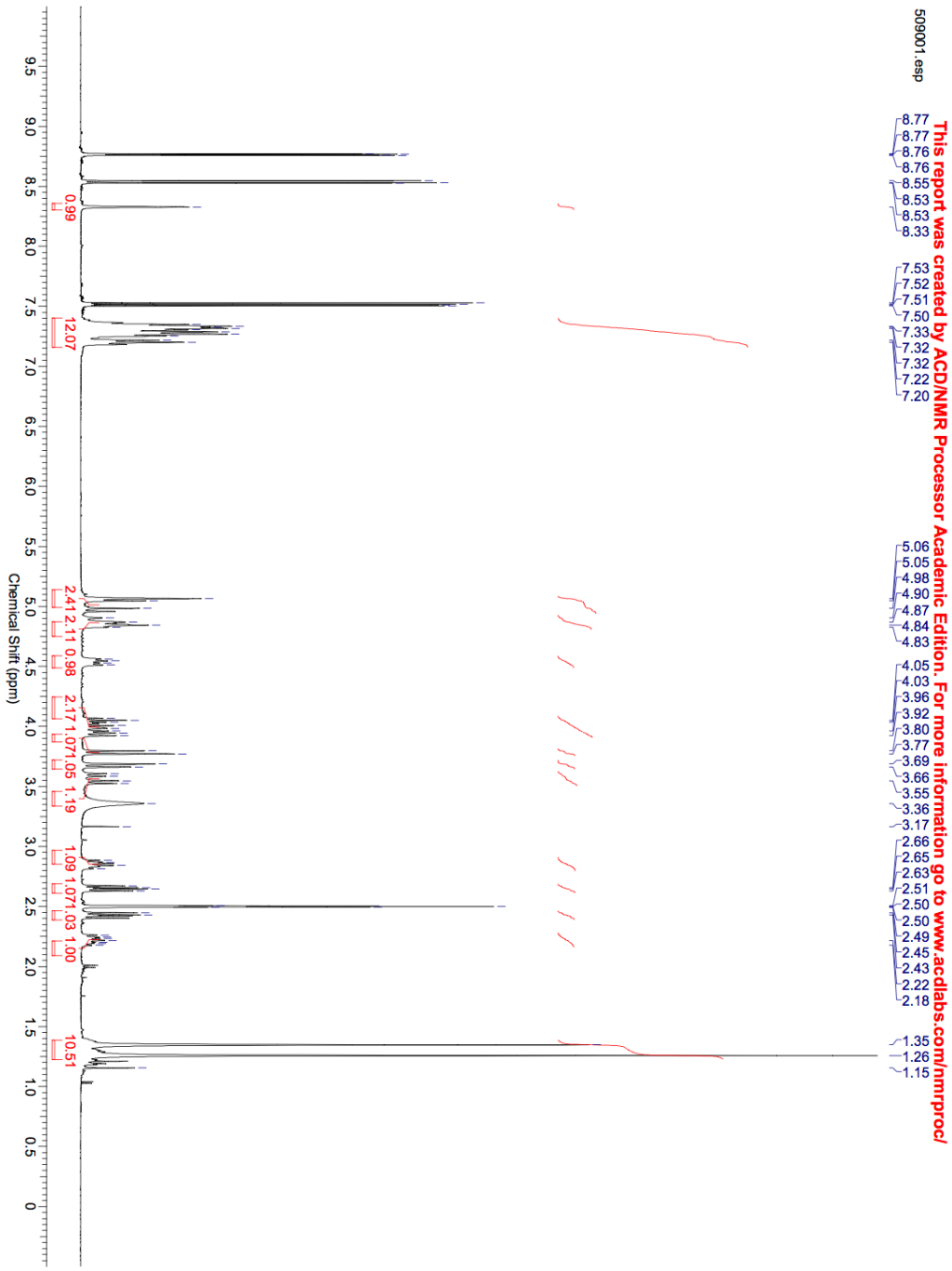
1
1.5
2
2.5
3
3.5
4
4.5
min


509001.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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8.76
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8.53
8.33
7.53
7.52
7.51
7.50
7.33
7.32
7.32
7.22
7.20
5.06
5.05
4.98
4.90
4.87
4.84
4.83
4.05
4.03
3.96
3.92
3.80
3.77
3.69
3.66
3.55
3.36
3.17
2.66
2.65
2.63
2.51
2.50
2.50
2.49
2.45
2.43
2.22
2.18
1.35
1.26
1.15
0.99
12.07
2.41
2.11
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1.07
1.05
1.19
1.09
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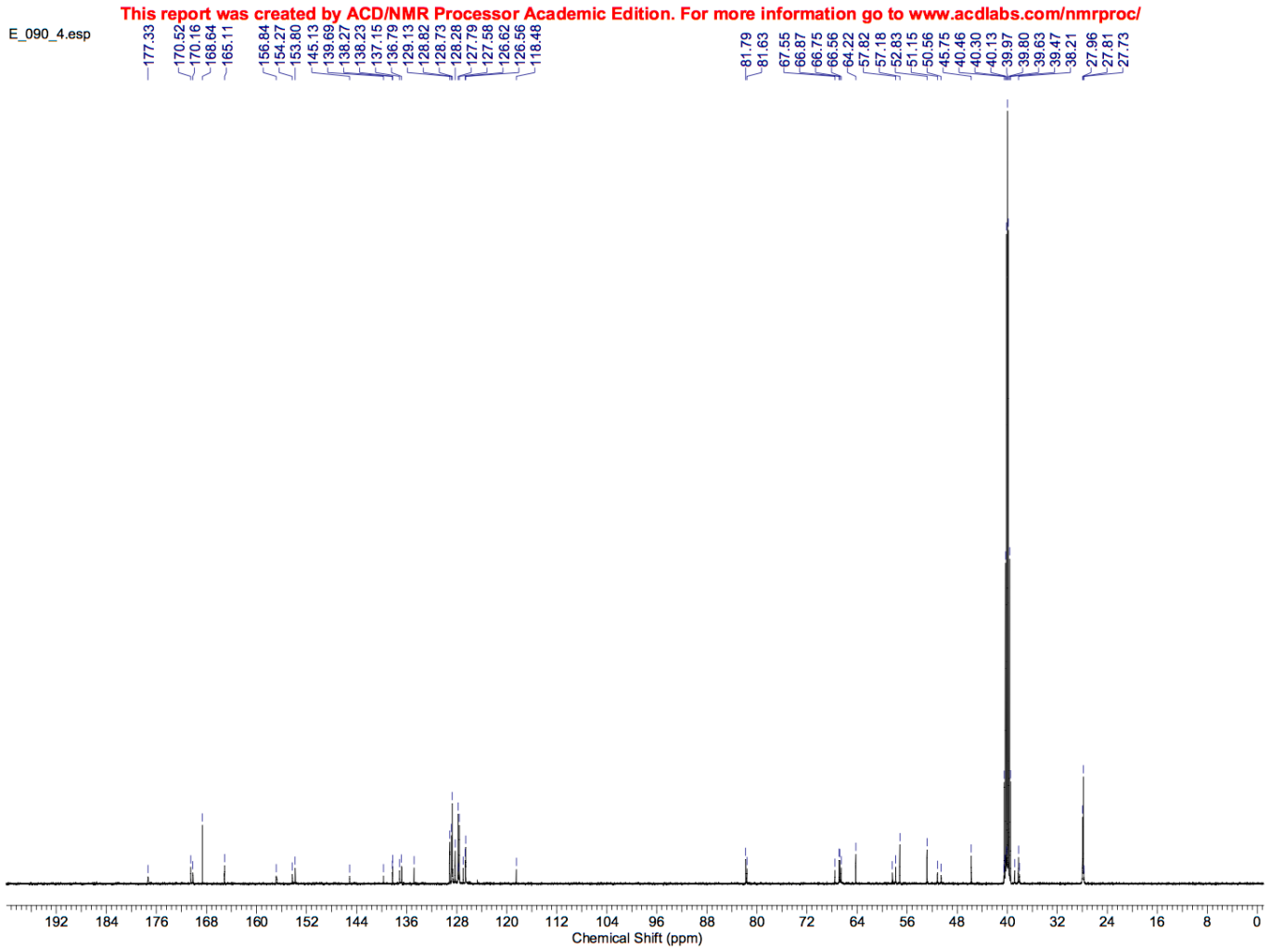
1.03
1.00
10.51
Chemical Shift (ppm)


This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
E_090_4.esp
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170.52
170.16
168.64
165.11
156.84
154.27
153.80
145.13
139.69
138.27
138.23
137.15
136.79
129.13
128.82
128.73
128.28
127.79
127.58
126.62
126.56
118.48
81.79
81.63
67.55
66.87
66.75
66.56
64.22
57.82
57.18
52.83
51.15
50.56
45.75
40.46
40.30
40.13
39.97
39.80
39.63
39.47
38.21
27.96
27.81
27.73
192 184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 16 8 0
Chemical Shift (ppm)


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400
300
200
100
0
-100
0.5
1
1.5
2
2.5
3
3.5
4
4.5
min
MSD1 691: EIC=690.692 (C:\CHEM32\1\DATA\2014-05-15 AT 12-35-33 HOURSUDN_E_091_P.D) ES-API, Pos, Scan, Frag: 70, "
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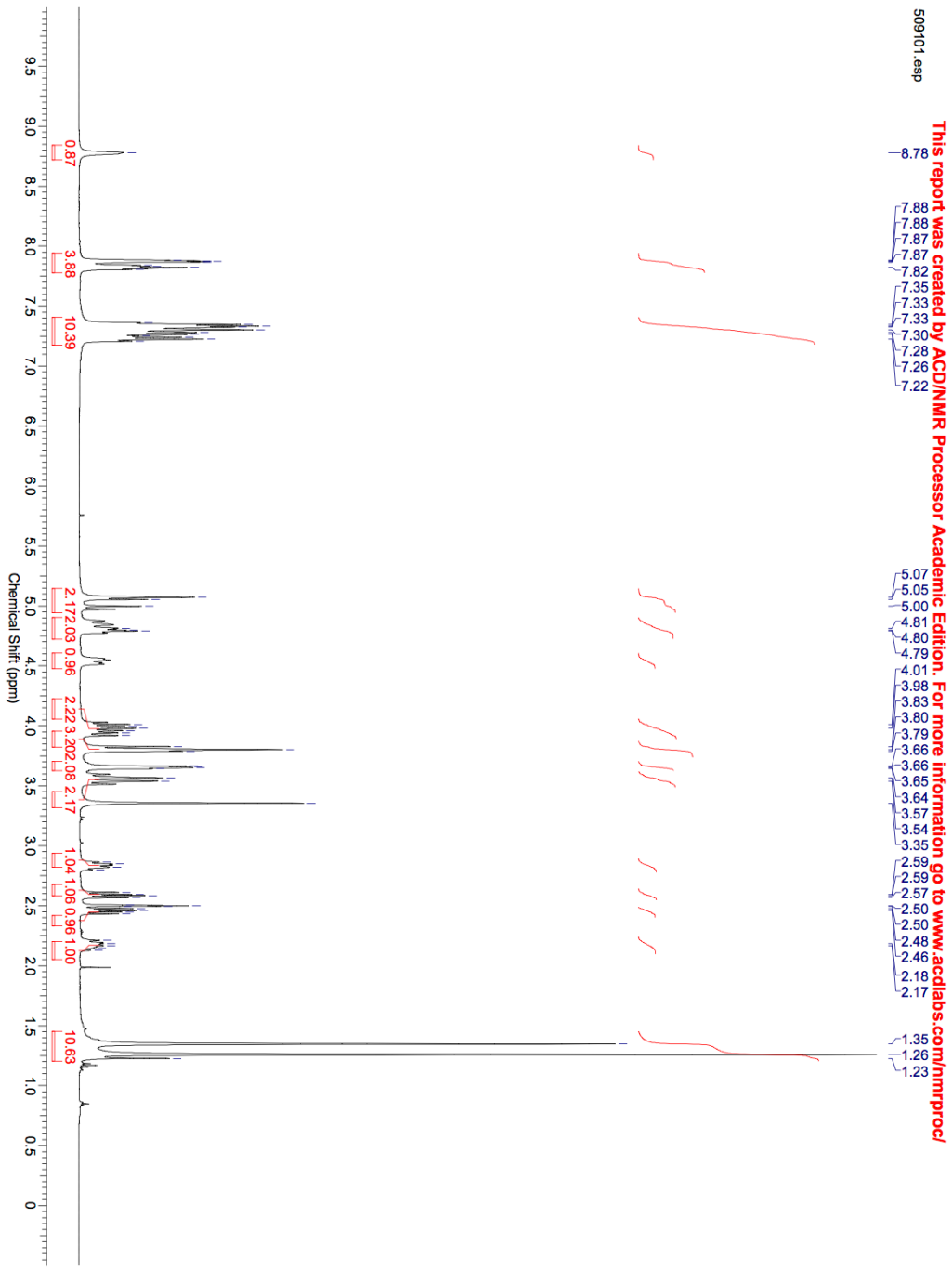
1
1.5
2
2.5
3
3.5
4
4.5
min


509101.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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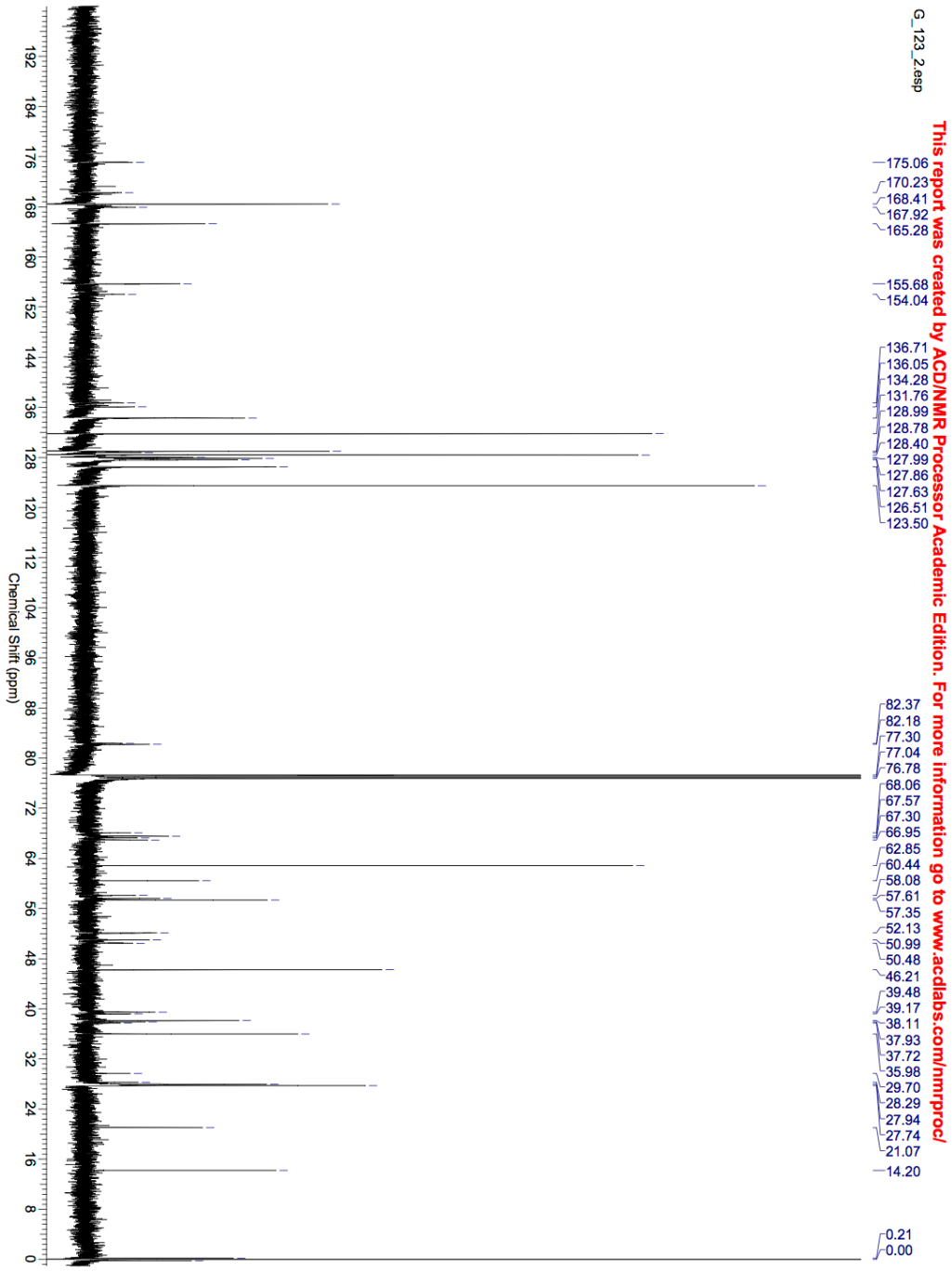
7.88
7.88
7.87
7.87
7.82
7.35
7.33
7.33
7.30
7.28
7.26
7.22
5.07
5.05
5.00
4.81
4.80
4.79
4.01
3.98
3.83
3.80
3.79
3.66
3.66
3.65
3.64
3.57
3.54
3.35
2.59
2.59
2.57
2.50
2.50
2.48
2.46
2.18
2.17
1.35
1.26
1.23
0.87
3.88
10.39
2.17
2.03
0.96
2.22
3.20
2.08
2.17
1.04
1.06
0.96
1.00
10.63
Chemical Shift (ppm)


G_123_2.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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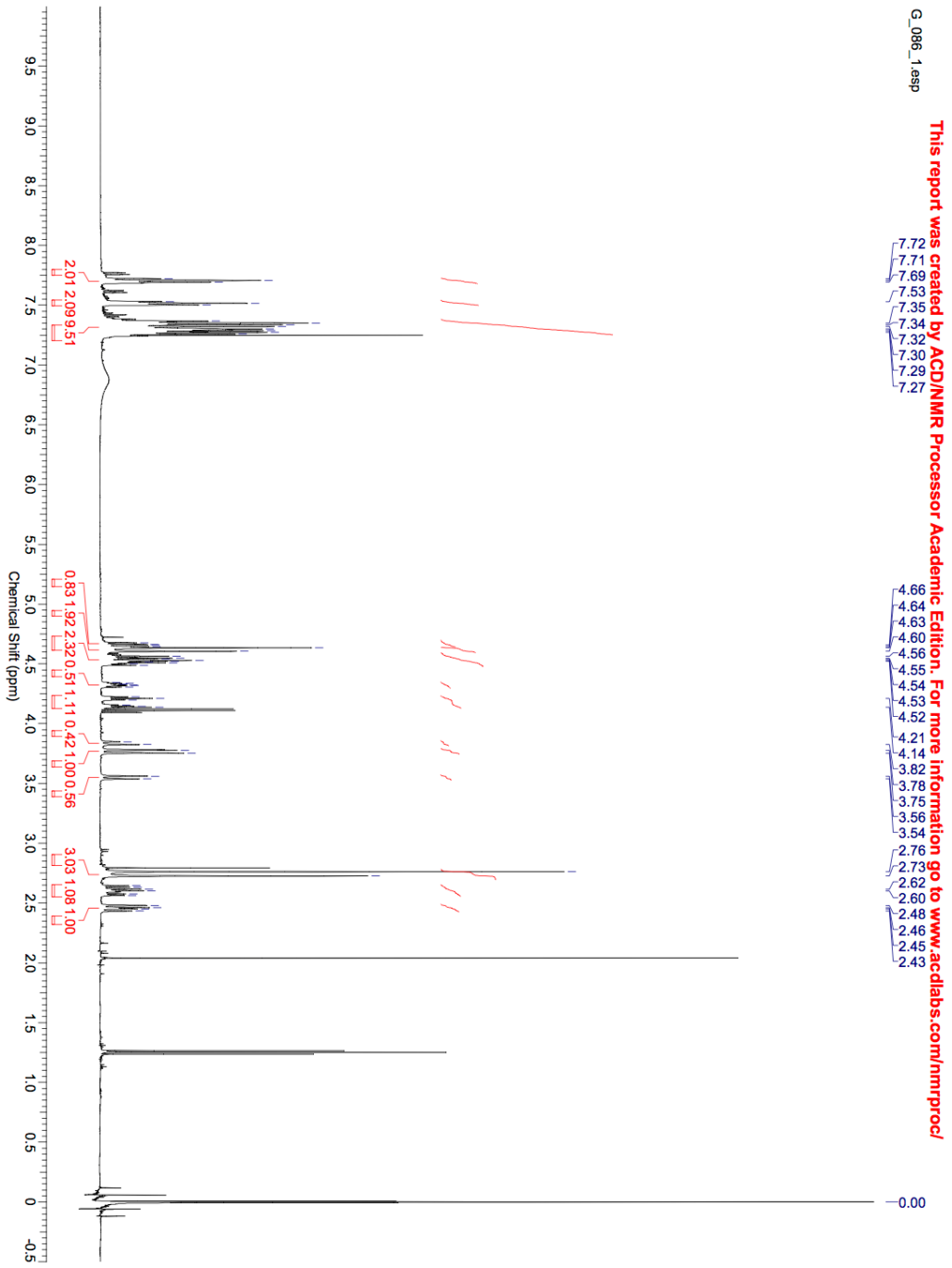
170.23
168.41
167.92
165.28
155.68
154.04
136.71
136.05
134.28
131.76
128.99
128.78
128.40
127.99
127.86
127.63
126.51
123.50
82.37
82.18
77.30
77.04
76.78
68.06
67.57
67.30
66.95
62.85
60.44
58.08
57.61
57.35
52.13
50.99
50.48
46.21
39.48
39.17
38.11
37.93
37.72
35.98
29.70
28.29
27.94
27.74
21.07
14.20
0.21
0.00
Chemical Shift (ppm)

## APPENDIX B

## CHAPTER 3 NMR SPECTRA


G_086_1.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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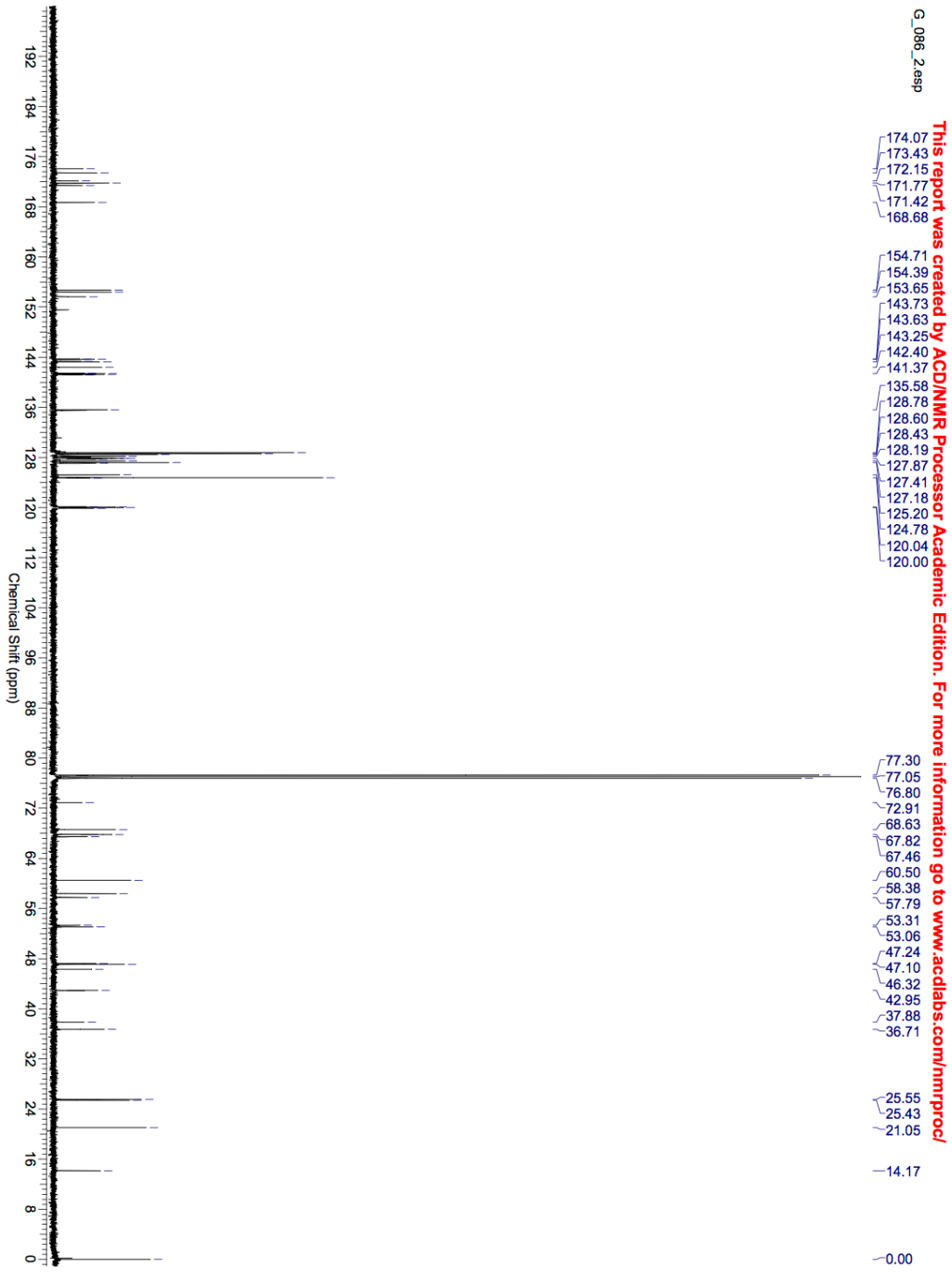
7.53
7.35
7.34
7.32
7.30
7.29
7.27
4.66
4.64
4.63
4.60
4.56
4.55
4.54
4.53
4.52
4.21
4.14
3.82
3.78
3.75
3.56
3.54
2.76
2.73
2.62
2.60
2.48
2.46
2.45
2.43
0.00
2.01 2.09 9.51
0.83 1.92 2.32 0.51 1.11 0.42 1.00 0.56
3.03 1.08 1.00
Chemical Shift (ppm)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This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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171.77
171.42
168.68
154.71
154.39
153.65
143.73
143.63
143.25
142.40
141.37
135.58
128.78
128.60
128.43
128.19
127.87
127.41
127.18
125.20
124.78
120.04
120.00
77.30
77.05
76.80
72.91
68.63
67.82
67.46
60.50
58.38
57.79
53.31
53.06
47.24
47.10
46.32
42.95
37.88
36.71
25.55
25.43
21.05
14.17
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
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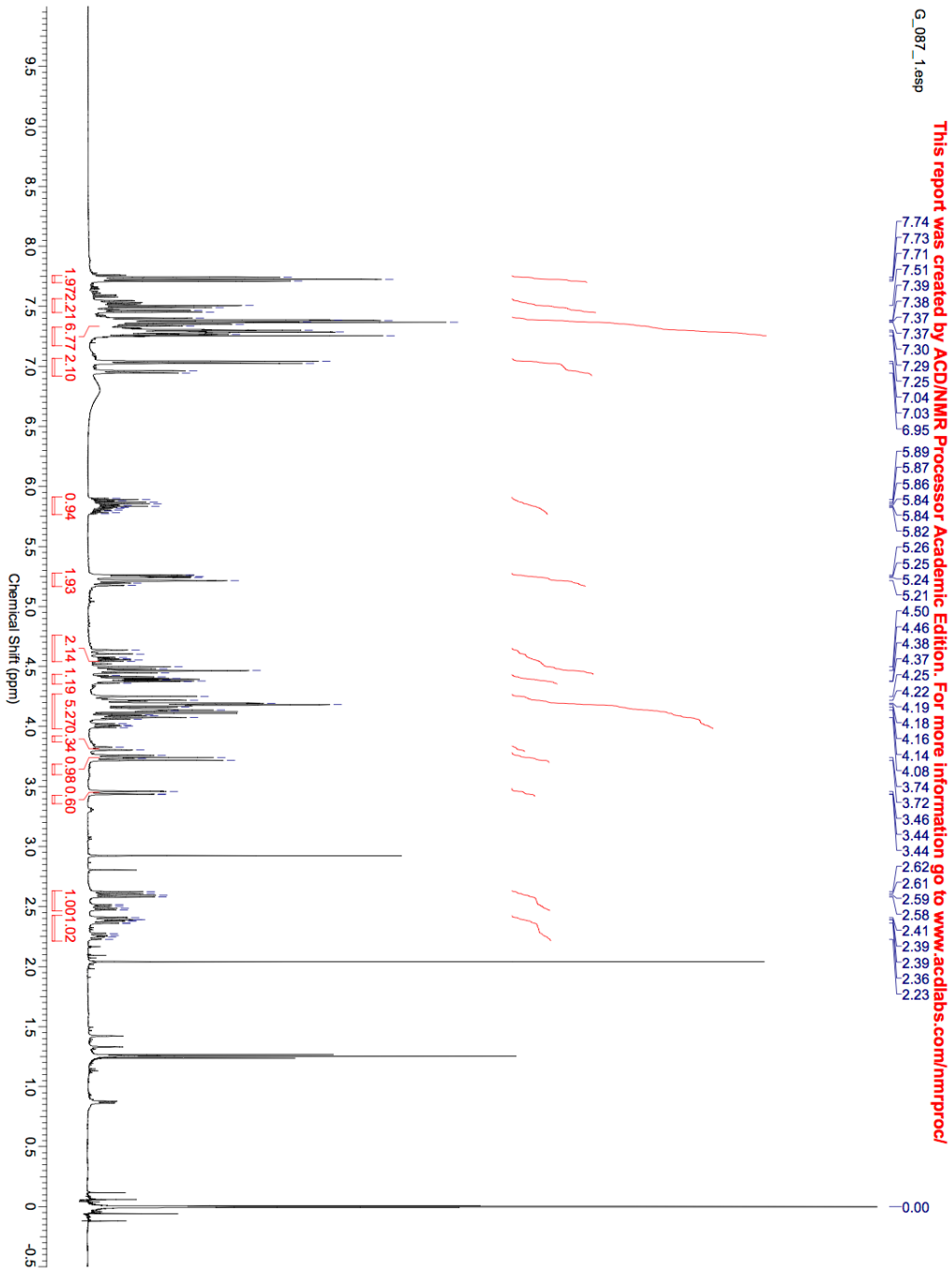
G_087_1.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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7.38
7.37
7.37
7.30
7.29
7.25
7.04
7.03
6.95
5.89
5.87
5.86
5.84
5.84
5.82
5.26
5.25
5.24
5.21
4.50
4.46
4.38
4.37
4.25
4.22
4.19
4.18
4.16
4.14
4.08
3.74
3.72
3.46
3.44
3.44
2.62
2.61
2.59
2.58
2.41
2.39
2.39
2.36
2.23
0.00
1.97 2.21 6.77 2.10
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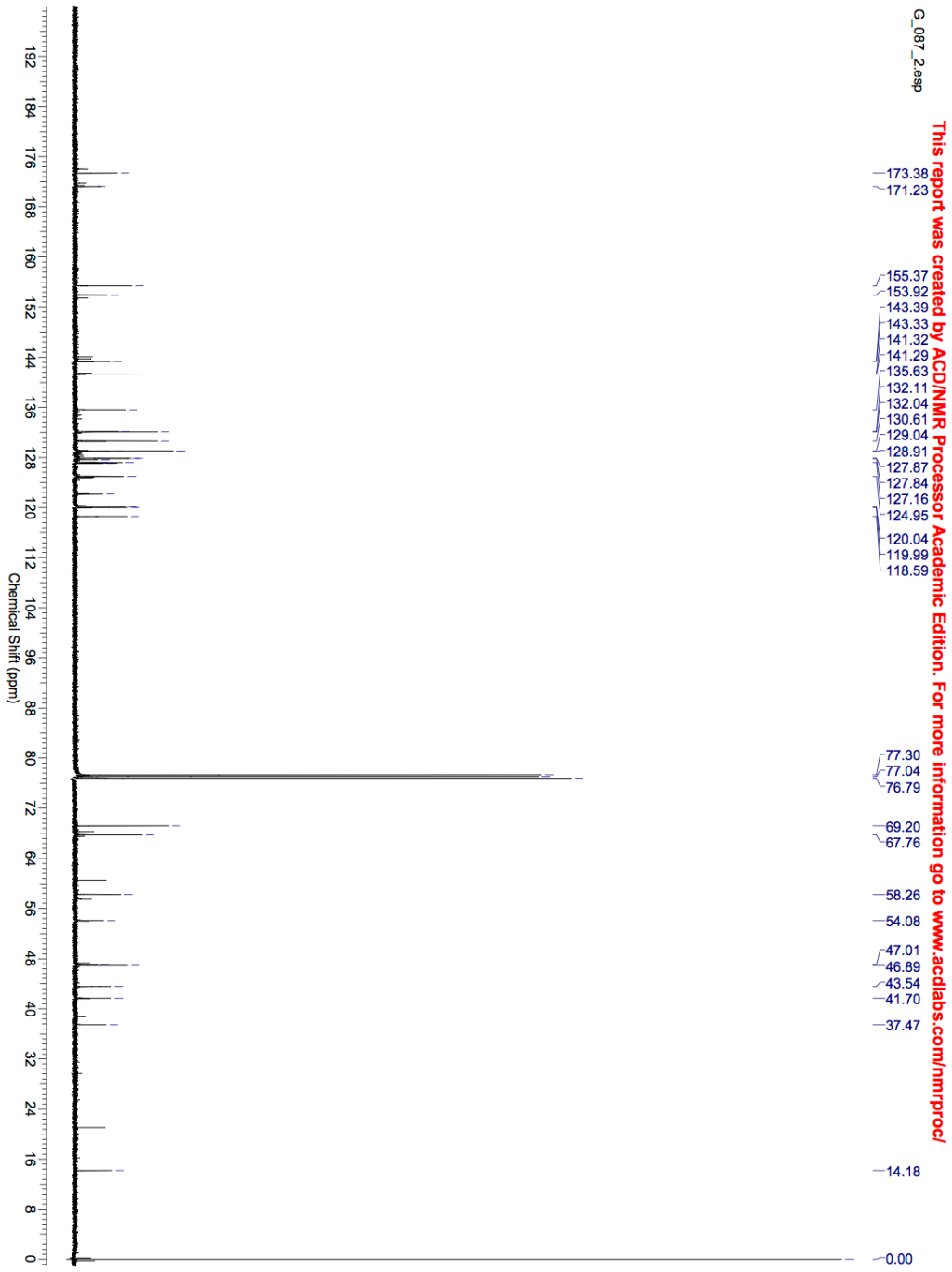
1.93
2.14 1.19 5.27 0.34 0.98 0.60
1.00 1.02
Chemical Shift (ppm)


G_087_2.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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143.33
141.32
141.29
135.63
132.11
132.04
130.61
129.04
128.91
127.87
127.84
127.16
124.95
120.04
119.99
118.59
77.30
77.04
76.79
69.20
67.76
58.26
54.08
47.01
46.89
43.54
41.70
37.47
14.18
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
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128
120
112
104
96
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56
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40
32
24
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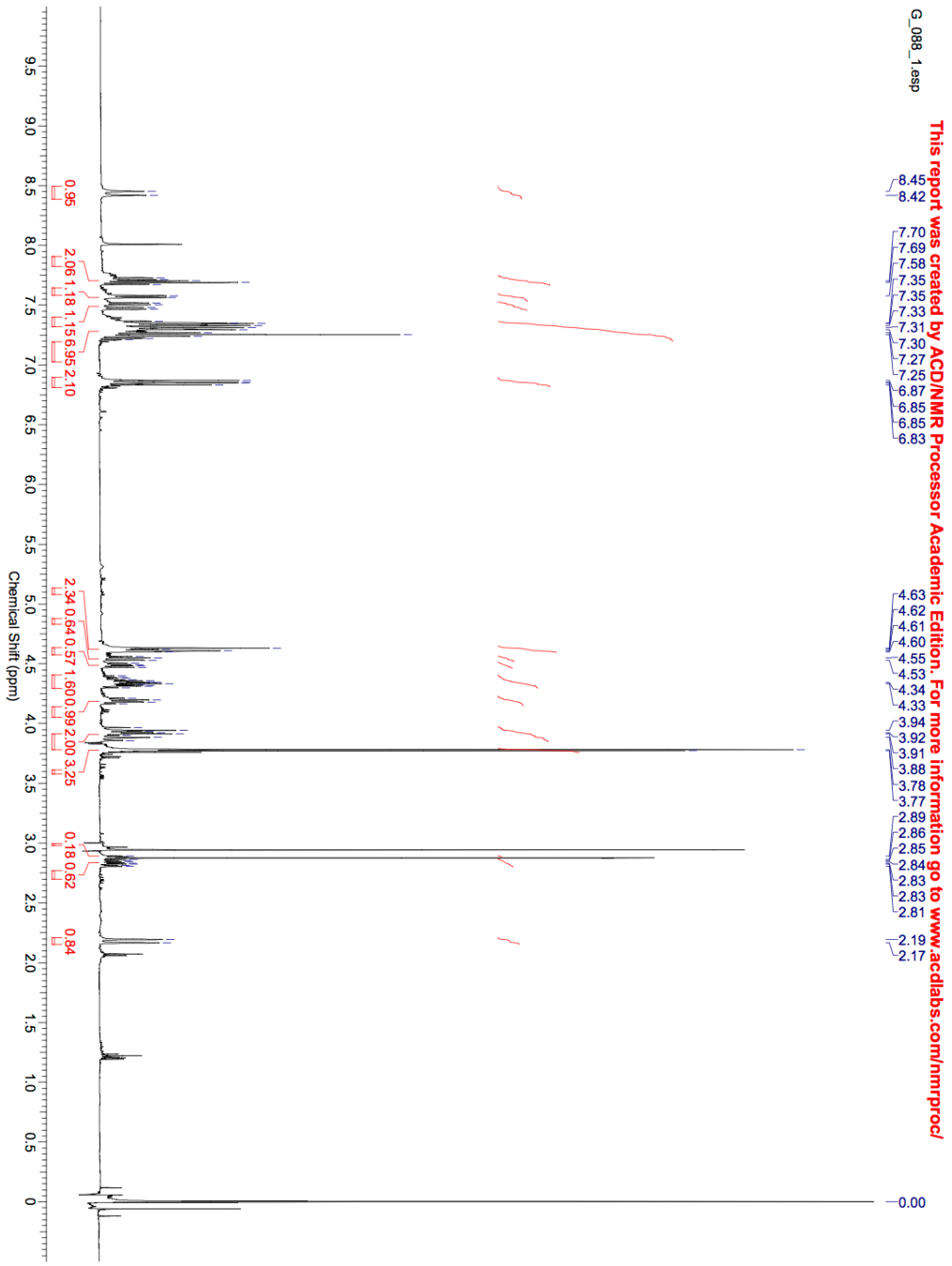
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This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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8.42
7.70
7.69
7.58
7.35
7.35
7.33
7.31
7.30
7.27
7.25
6.87
6.85
6.85
6.83
4.63
4.62
4.61
4.60
4.55
4.53
4.34
4.33
3.94
3.92
3.91
3.88
3.78
3.77
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2.86
2.85
2.84
2.83
2.83
2.81
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2.17
0.00
0.95
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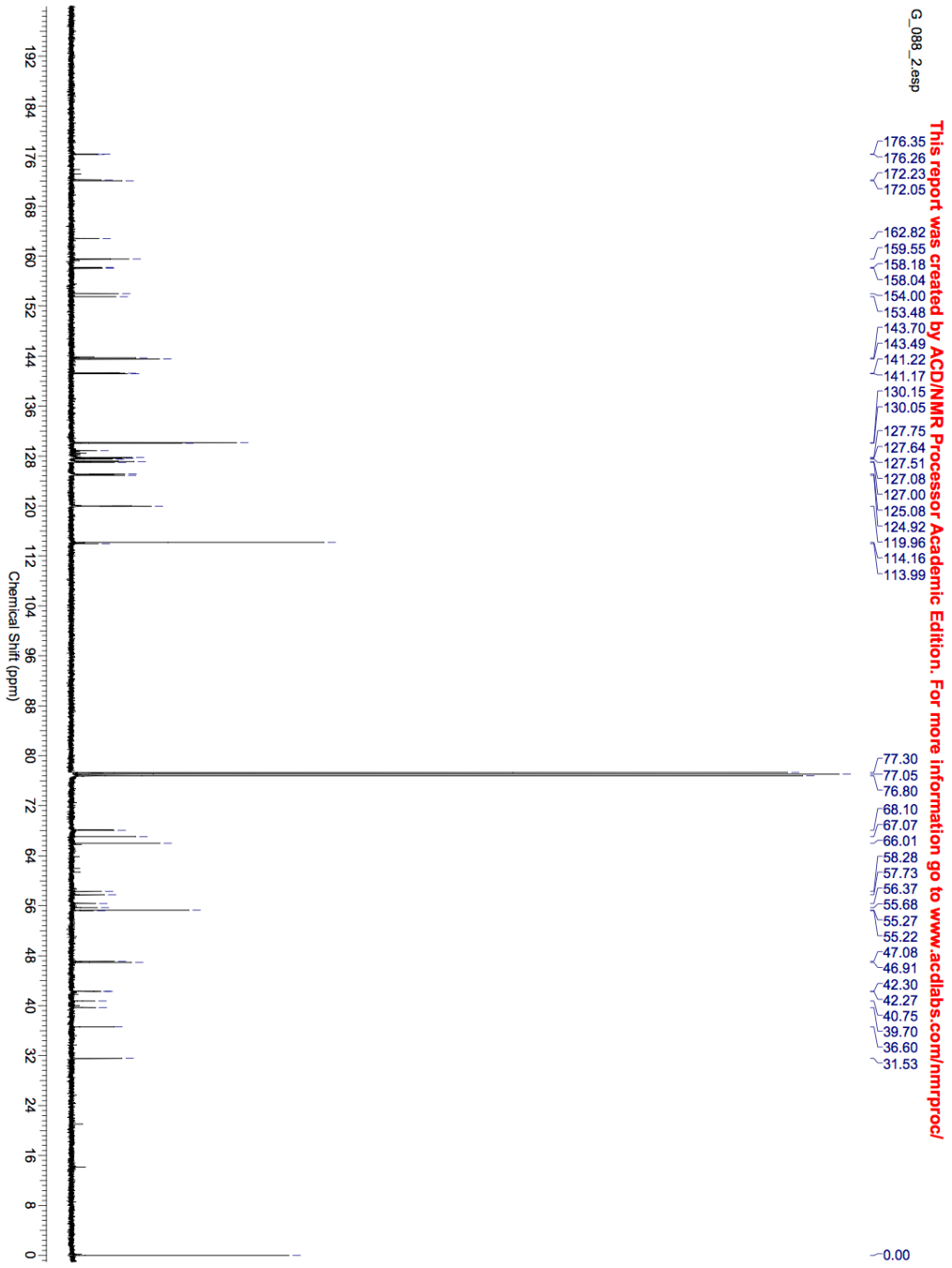
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This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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176.26
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172.05
162.82
159.55
158.18
158.04
154.00
153.48
143.70
143.49
141.22
141.17
130.15
130.05
127.75
127.64
127.51
127.08
127.00
125.08
124.92
119.96
114.16
113.99
77.30
77.05
76.80
68.10
67.07
66.01
58.28
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56.37
55.68
55.27
55.22
47.08
46.91
42.30
42.27
40.75
39.70
36.60
31.53
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
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88
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56
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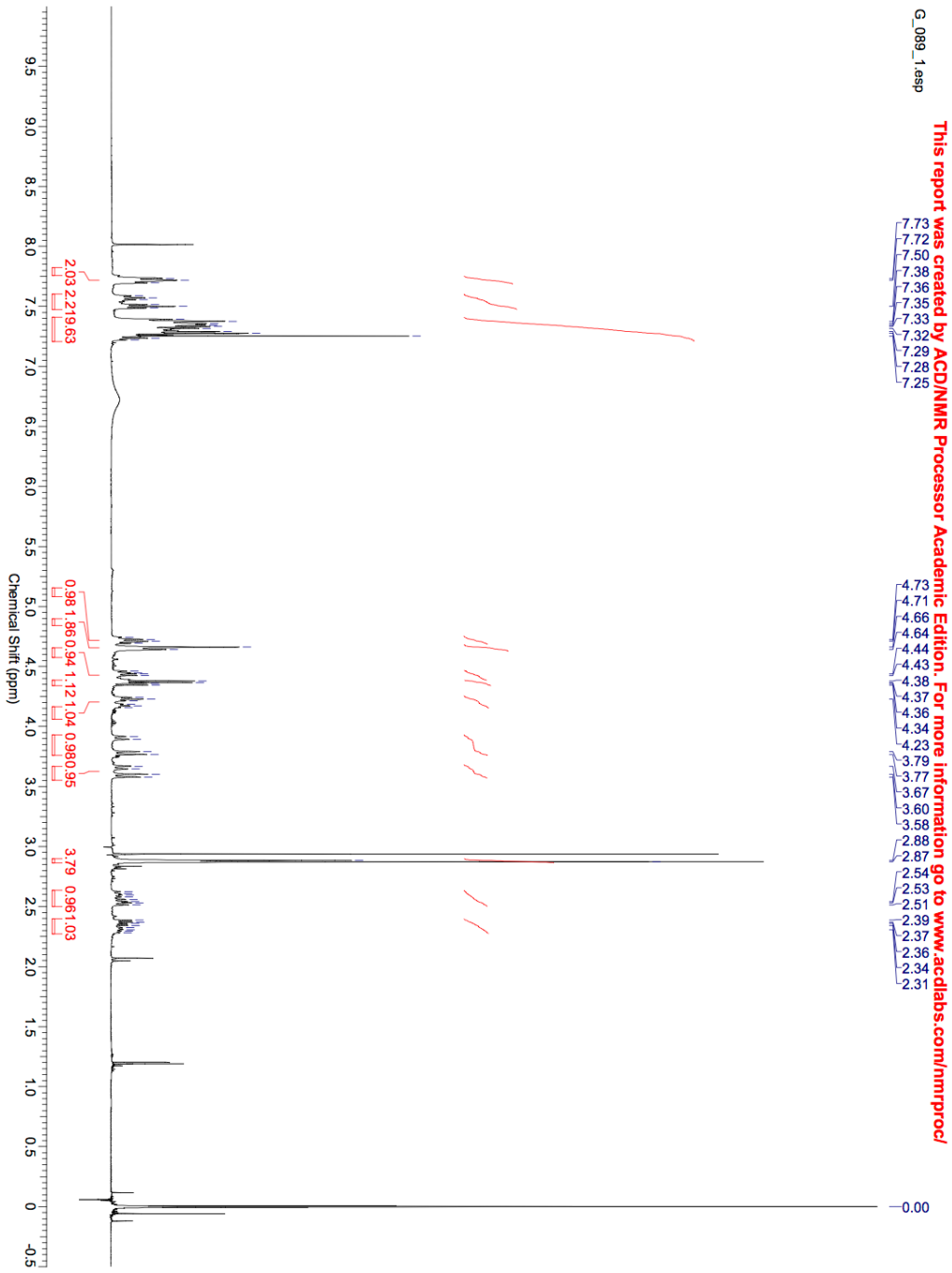
G_089_1.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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7.72
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7.38
7.36
7.35
7.33
7.32
7.29
7.28
7.25
4.73
4.71
4.66
4.64
4.44
4.43
4.38
4.37
4.36
4.34
4.23
3.79
3.77
3.67
3.60
3.58
2.88
2.87
2.54
2.53
2.51
2.39
2.37
2.36
2.34
2.31
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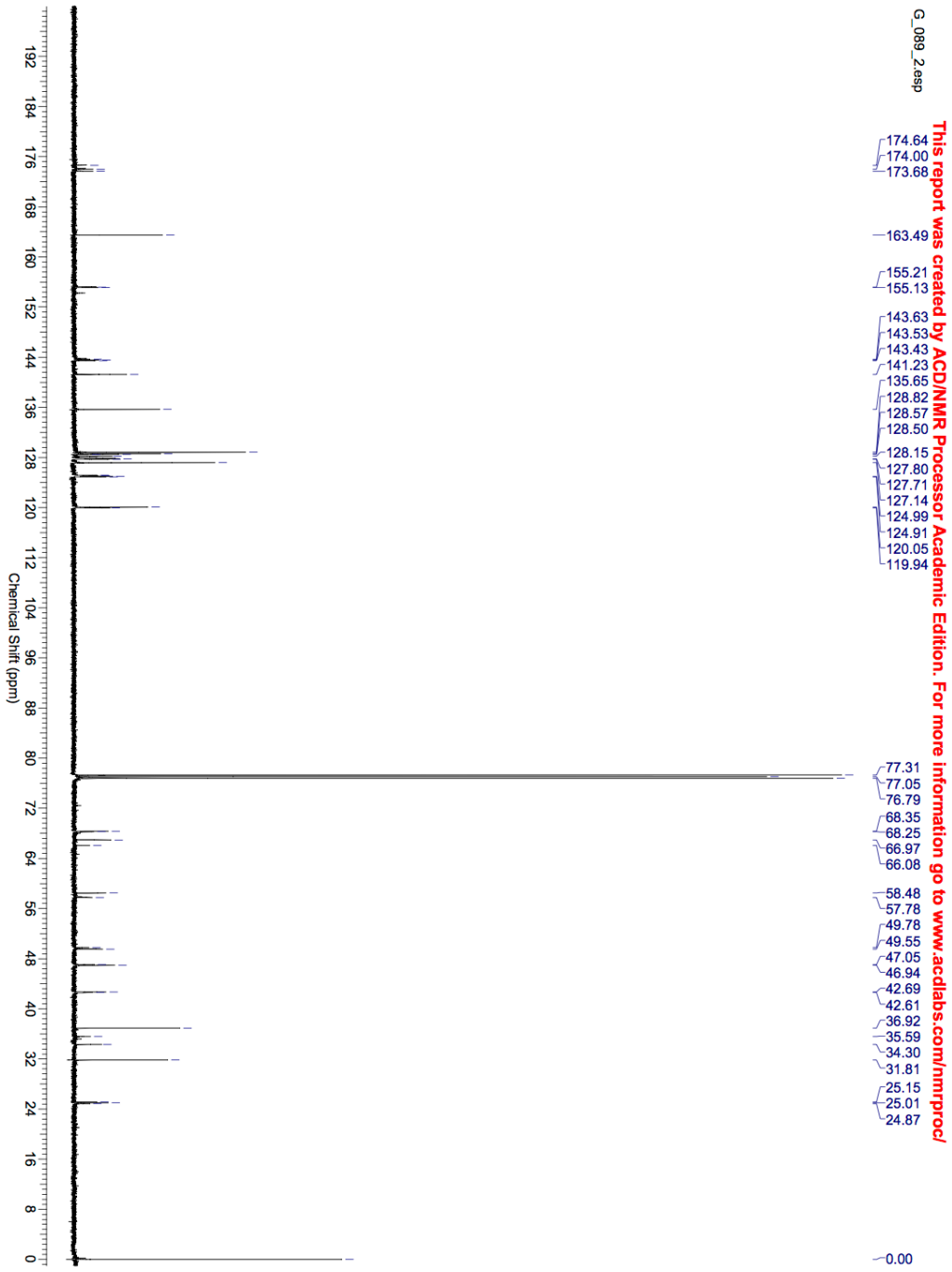
0.98 1.86 0.94 1.12 1.04 0.98 0.95
3.79 0.96 1.03
Chemical Shift (ppm)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This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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143.53
143.43
141.23
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127.71
127.14
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124.91
120.05
119.94
77.31
77.05
76.79
68.35
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66.08
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57.78
49.78
49.55
47.05
46.94
42.69
42.61
36.92
35.59
34.30
31.81
25.15
25.01
24.87
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
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120
112
104
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88
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56
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24
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8
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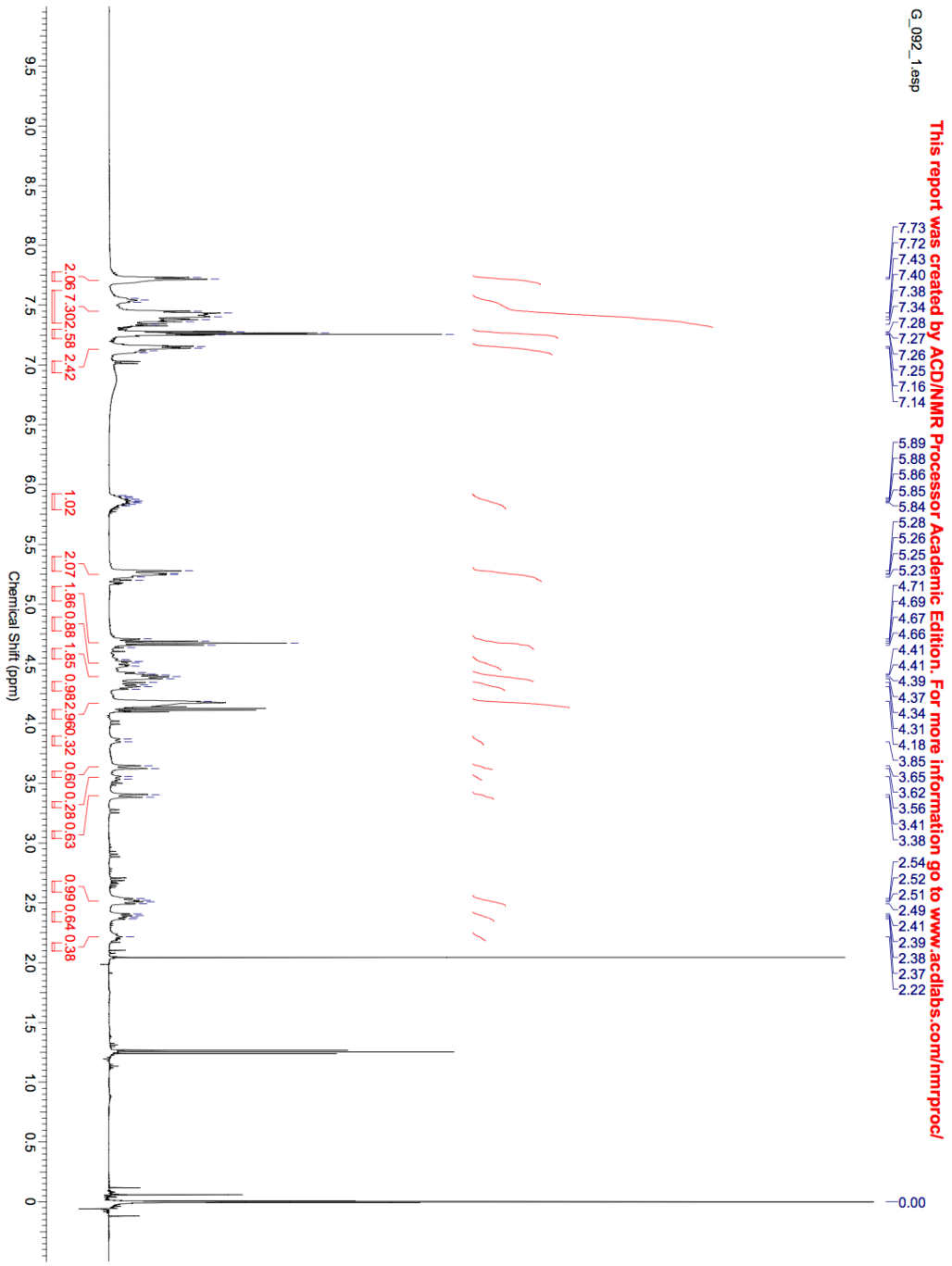
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This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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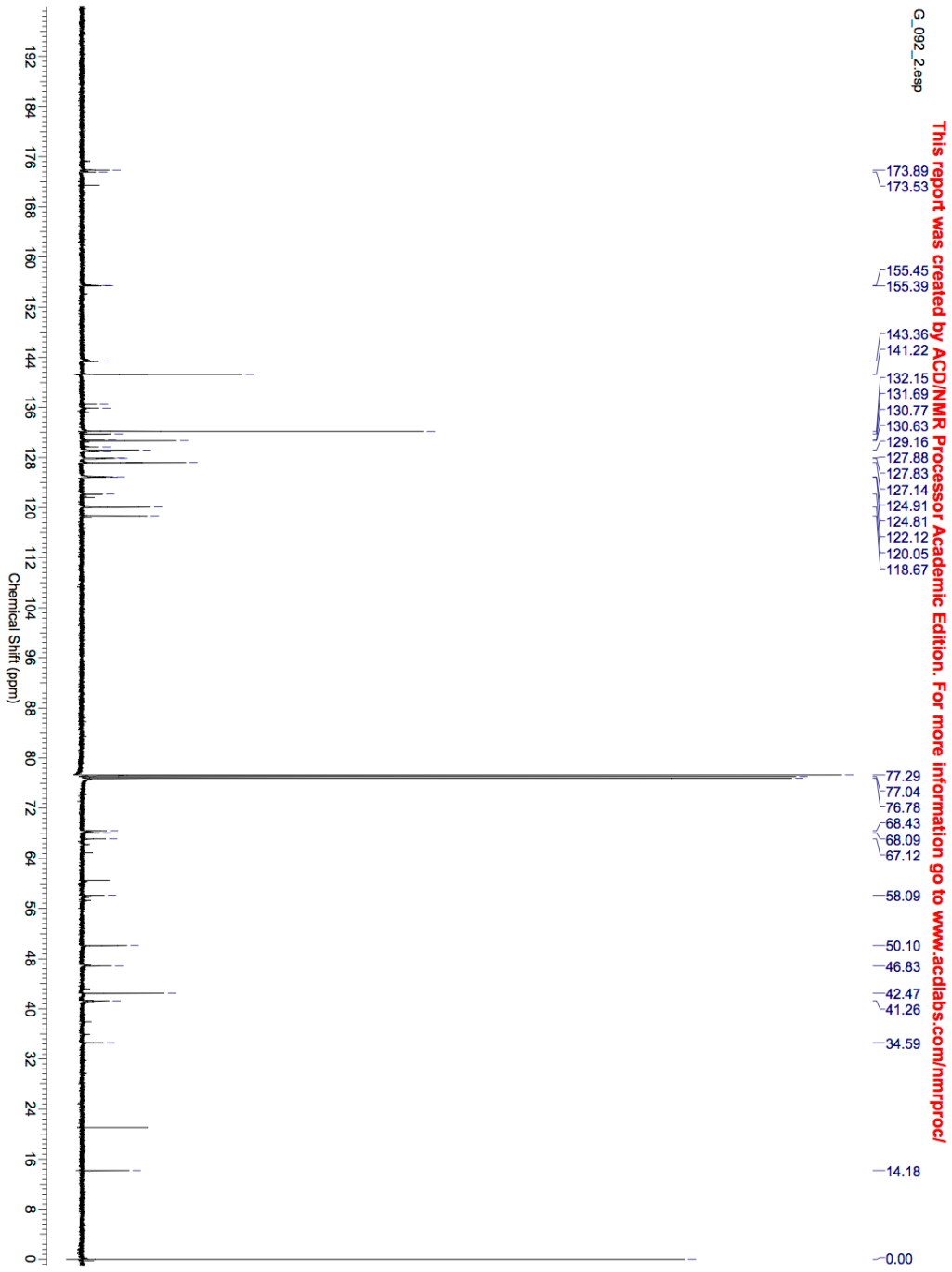
1.02
2.07 1.86 0.88 1.85 0.98 2.96 0.32 0.60 0.28 0.63
0.99 0.64 0.38
7.73 7.72 7.43 7.40 7.38 7.34 7.28 7.27 7.26 7.25 7.16 7.14
5.89 5.88 5.86 5.85 5.84
5.28 5.26 5.25 5.23
4.71 4.69 4.67 4.66 4.41 4.41 4.39 4.37 4.34 4.31 4.18
3.85 3.65 3.62 3.56 3.41 3.38
2.54 2.52 2.51 2.49 2.41 2.39 2.38 2.37 2.22
0.00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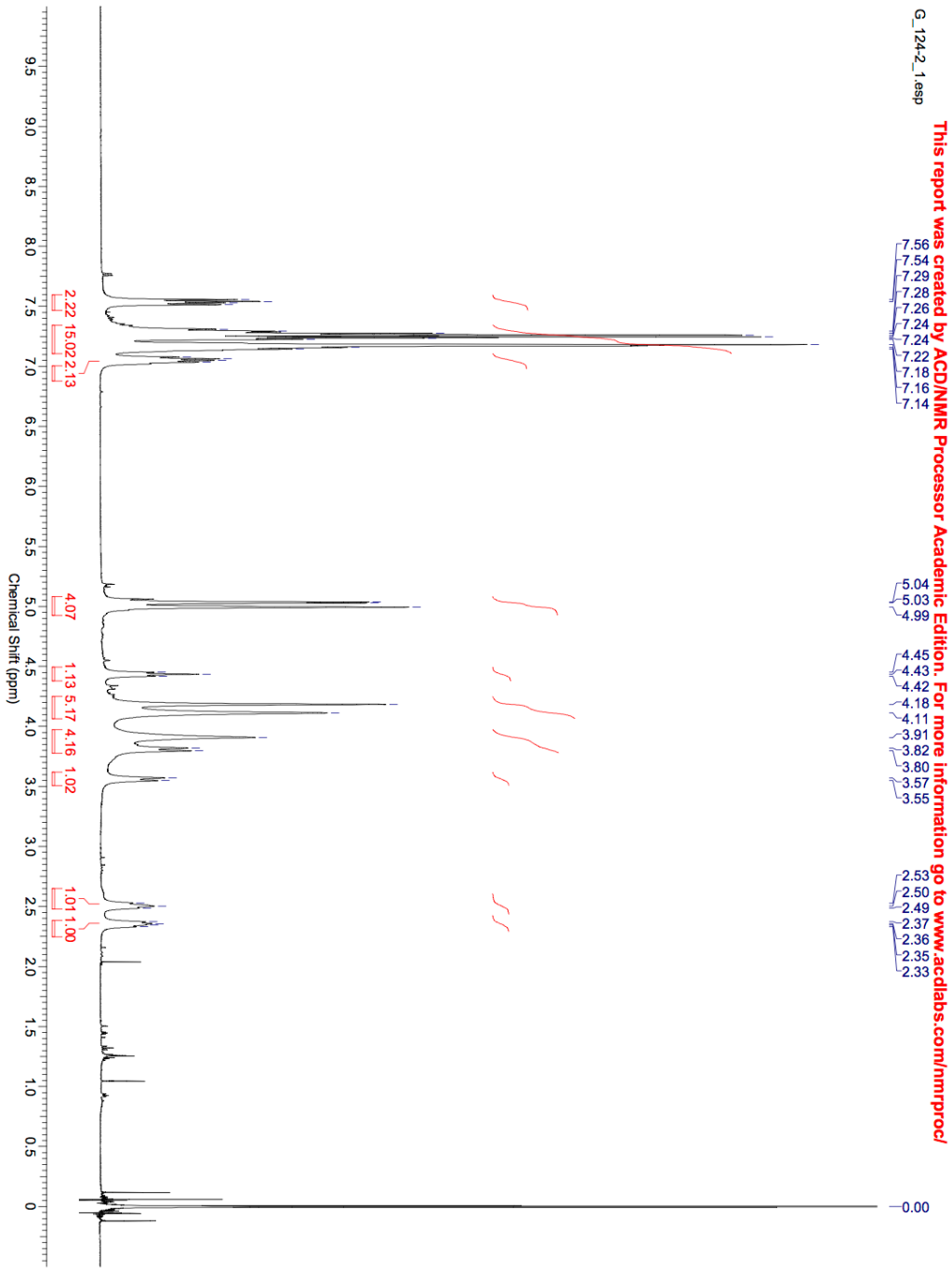
131.69
130.77
130.63
129.16
127.88
127.83
127.14
124.91
124.81
122.12
120.05
118.67
77.29
77.04
76.78
68.43
68.09
67.12
58.09
50.10
46.83
42.47
41.26
34.59
14.18
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0


G_124-2_1.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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7.28
7.26
7.24
7.24
7.22
7.18
7.16
7.14
5.04
5.03
4.99
4.45
4.43
4.42
4.18
4.11
3.91
3.82
3.80
3.57
3.55
2.53
2.50
2.49
2.37
2.36
2.35
2.33
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2.13
4.07
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5.17
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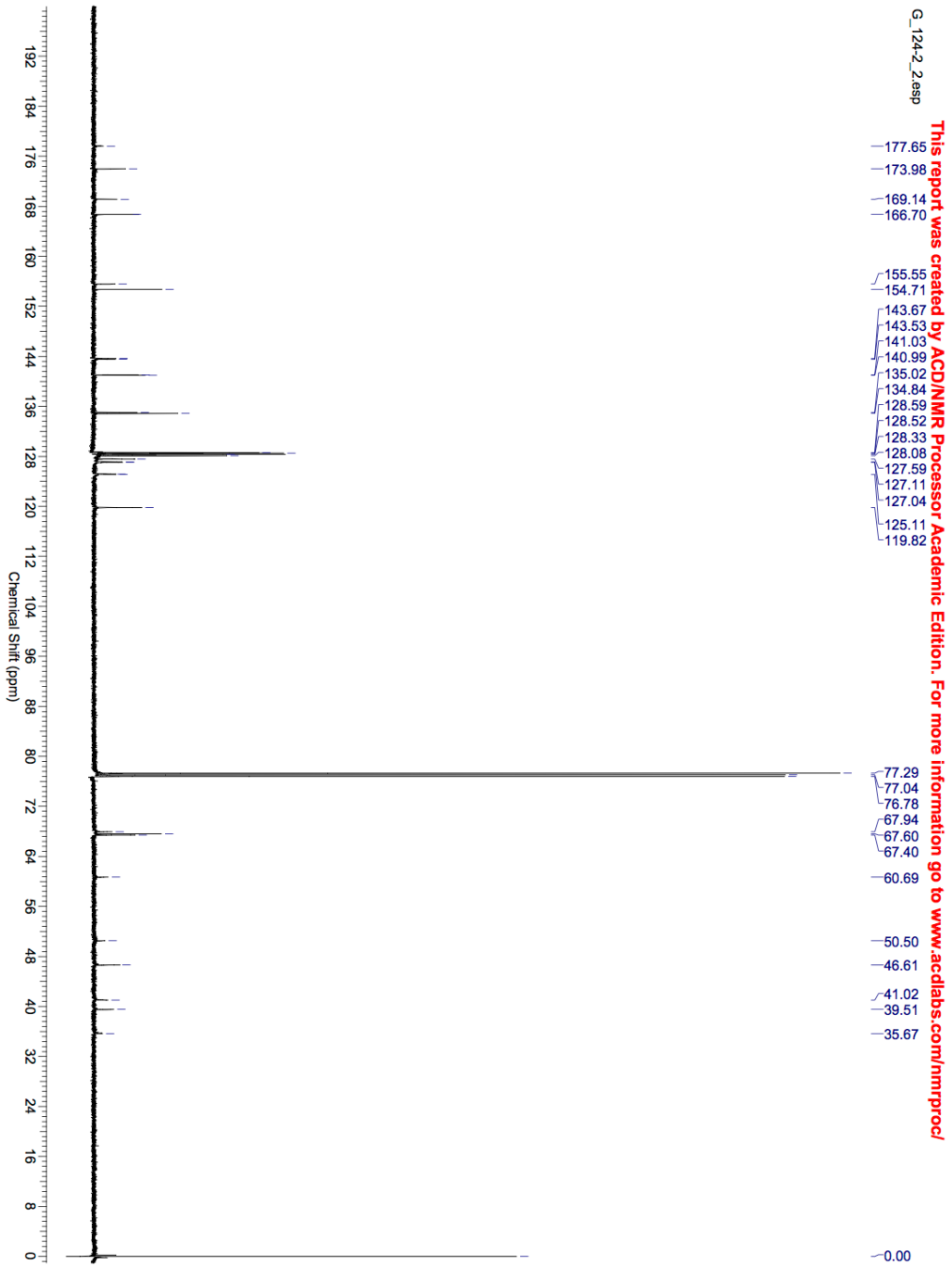
1.02
1.01
1.00
Chemical Shift (ppm)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166.70
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154.71
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140.99
135.02
134.84
128.59
128.52
128.33
128.08
127.59
127.11
127.04
125.11
119.82
77.29
77.04
76.78
67.94
67.60
67.40
60.69
50.50
46.61
41.02
39.51
35.67
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
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32
24
16
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0
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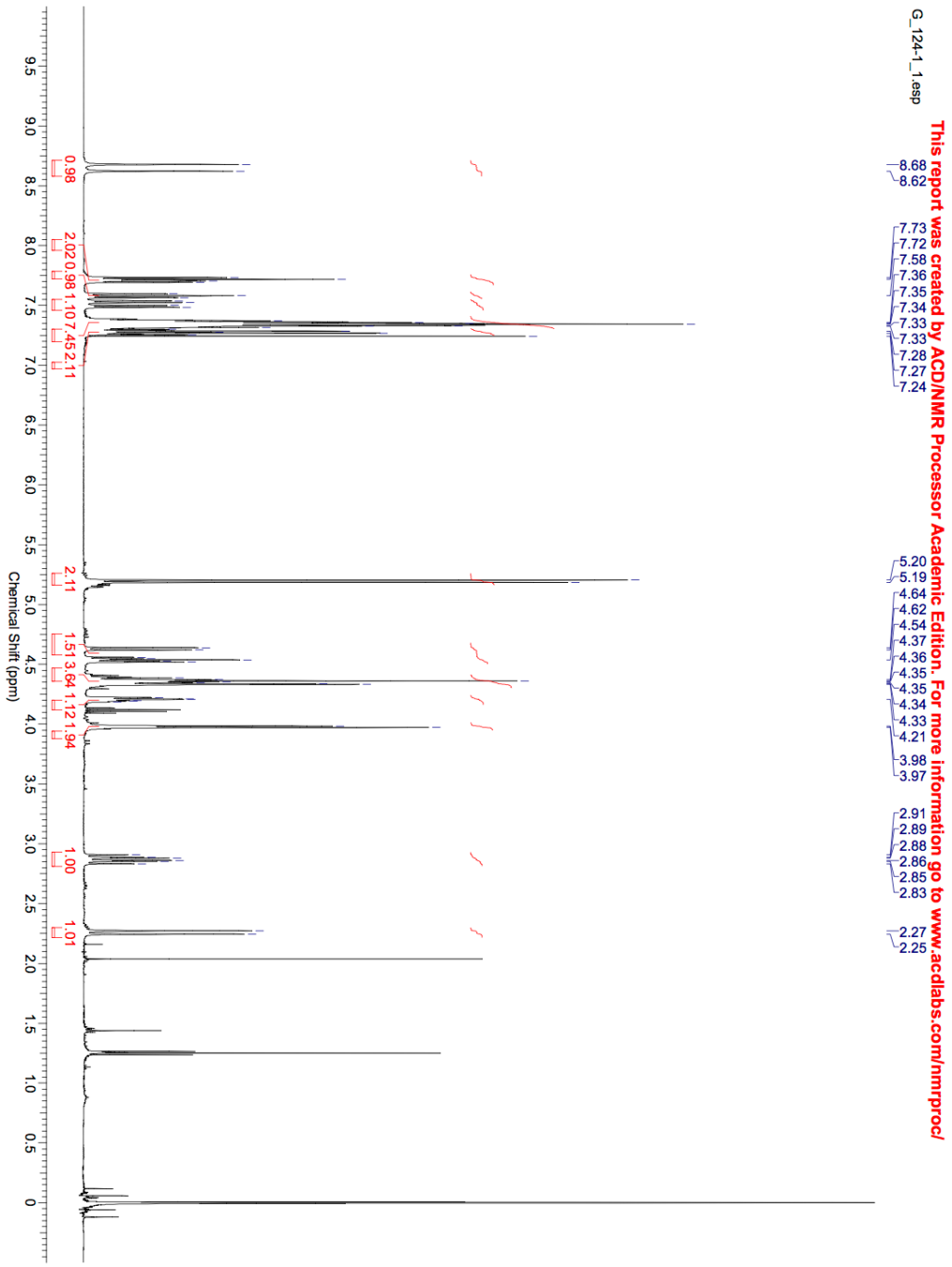
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This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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7.72
7.58
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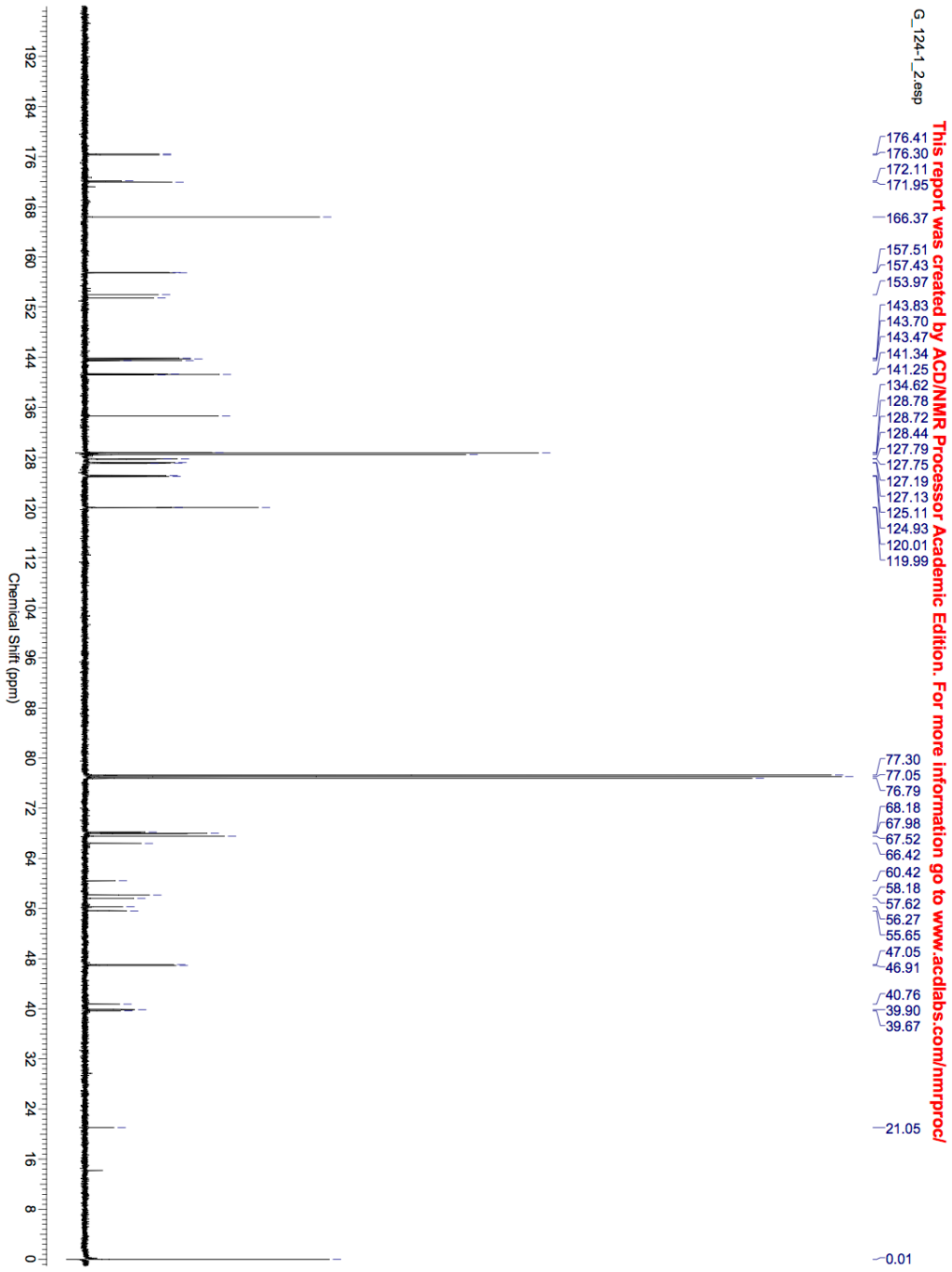
7.35
7.34
7.33
7.33
7.28
7.27
7.24
5.20
5.19
4.64
4.62
4.54
4.37
4.36
4.35
4.35
4.34
4.33
4.21
3.98
3.97
2.91
2.89
2.88
2.86
2.85
2.83
2.27
2.25
0.98
2.02
0.98
1.10
7.45
2.11
2.11
1.51
3.64
1.12
1.94
1.00
1.01
Chemical Shift (ppm)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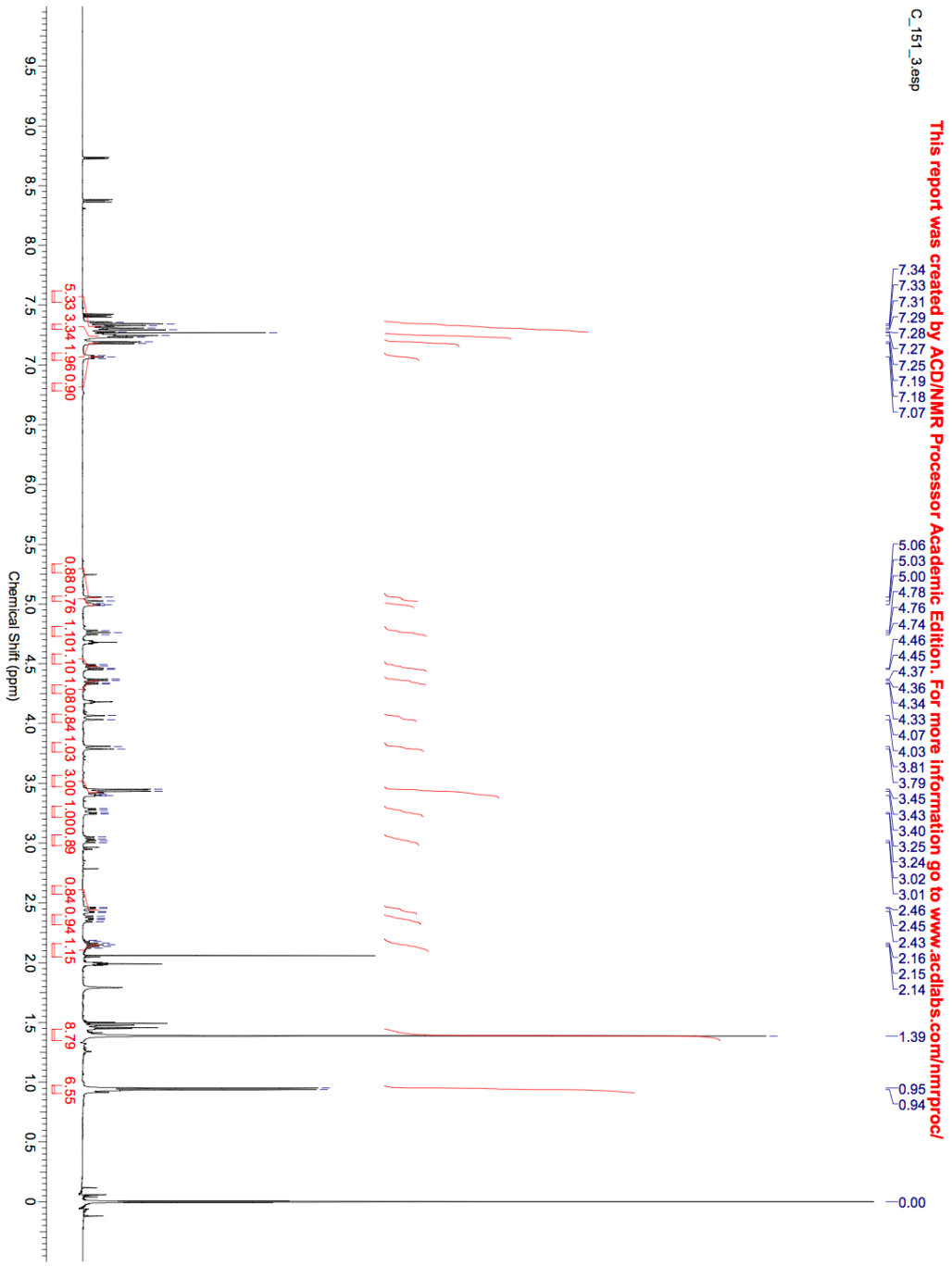
157.51
157.43
153.97
143.83
143.70
143.47
141.34
141.25
134.62
128.78
128.72
128.44
127.79
127.75
127.19
127.13
125.11
124.93
120.01
119.99
77.30
77.05
76.79
68.18
67.98
67.52
66.42
60.42
58.18
57.62
56.27
55.65
47.05
46.91
40.76
39.90
39.67
21.05
0.01
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0

# APPENDIX C

## CHAPTER 4 NMR SPECTRA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This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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7.27
7.25
7.19
7.18
7.07
5.06
5.03
5.00
4.78
4.76
4.74
4.46
4.45
4.37
4.36
4.34
4.33
4.07
4.03
3.81
3.79
3.45
3.43
3.40
3.25
3.24
3.02
3.01
2.46
2.45
2.43
2.16
2.15
2.14
1.39
0.95
0.94
0.00
5.33
3.34
1.96
0.90
0.88
0.76
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1.08
1.03
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1.00
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0.84
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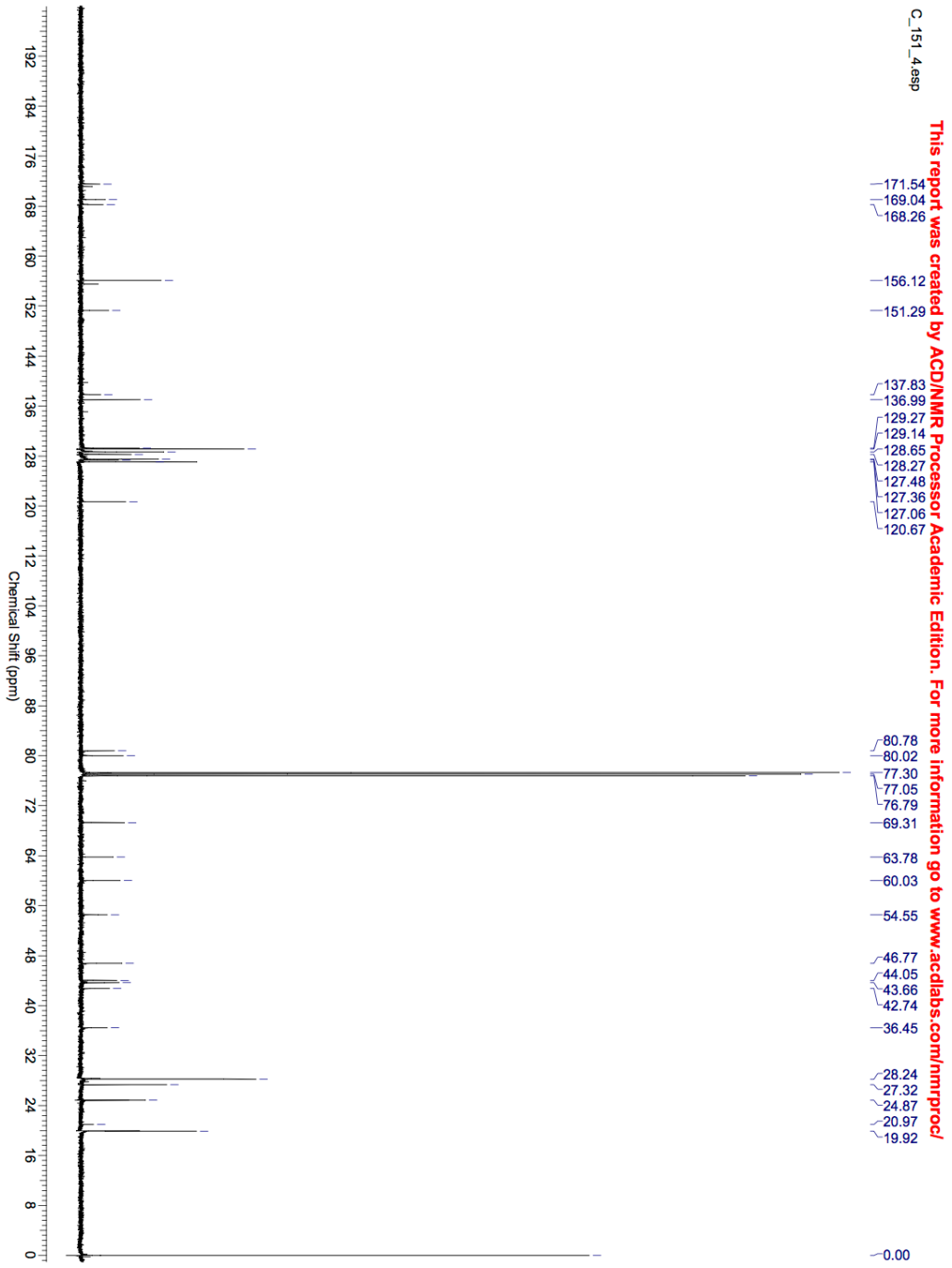
1.15
8.79
6.55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151.29
137.83
136.99
129.27
129.14
128.65
128.27
127.48
127.36
127.06
120.67
80.78
80.02
77.30
77.05
76.79
69.31
63.78
60.03
54.55
46.77
44.05
43.66
42.74
36.45
28.24
27.32
24.87
20.97
19.92
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
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56
48
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24
16
8
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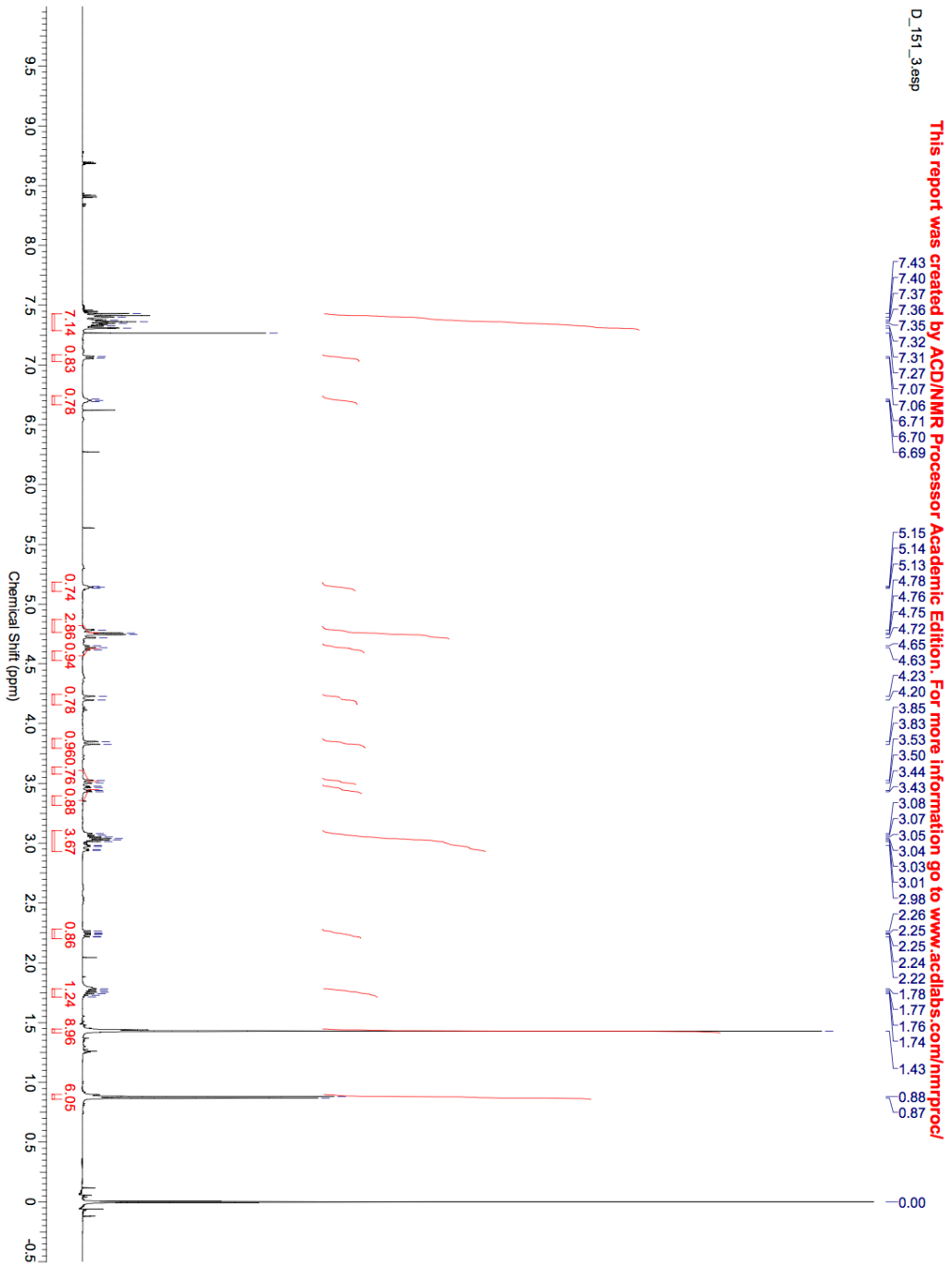
D_151_3.esp
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7.31
7.27
7.07
7.06
6.71
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6.69
5.15
5.14
5.13
4.78
4.76
4.75
4.72
4.65
4.63
4.23
4.20
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3.83
3.53
3.50
3.44
3.43
3.08
3.07
3.05
3.04
3.03
3.01
2.98
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2.25
2.25
2.24
2.22
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1.76
1.74
1.43
0.88
0.87
0.00
7.14
0.83
0.78
0.74
2.86
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3.67
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1.24
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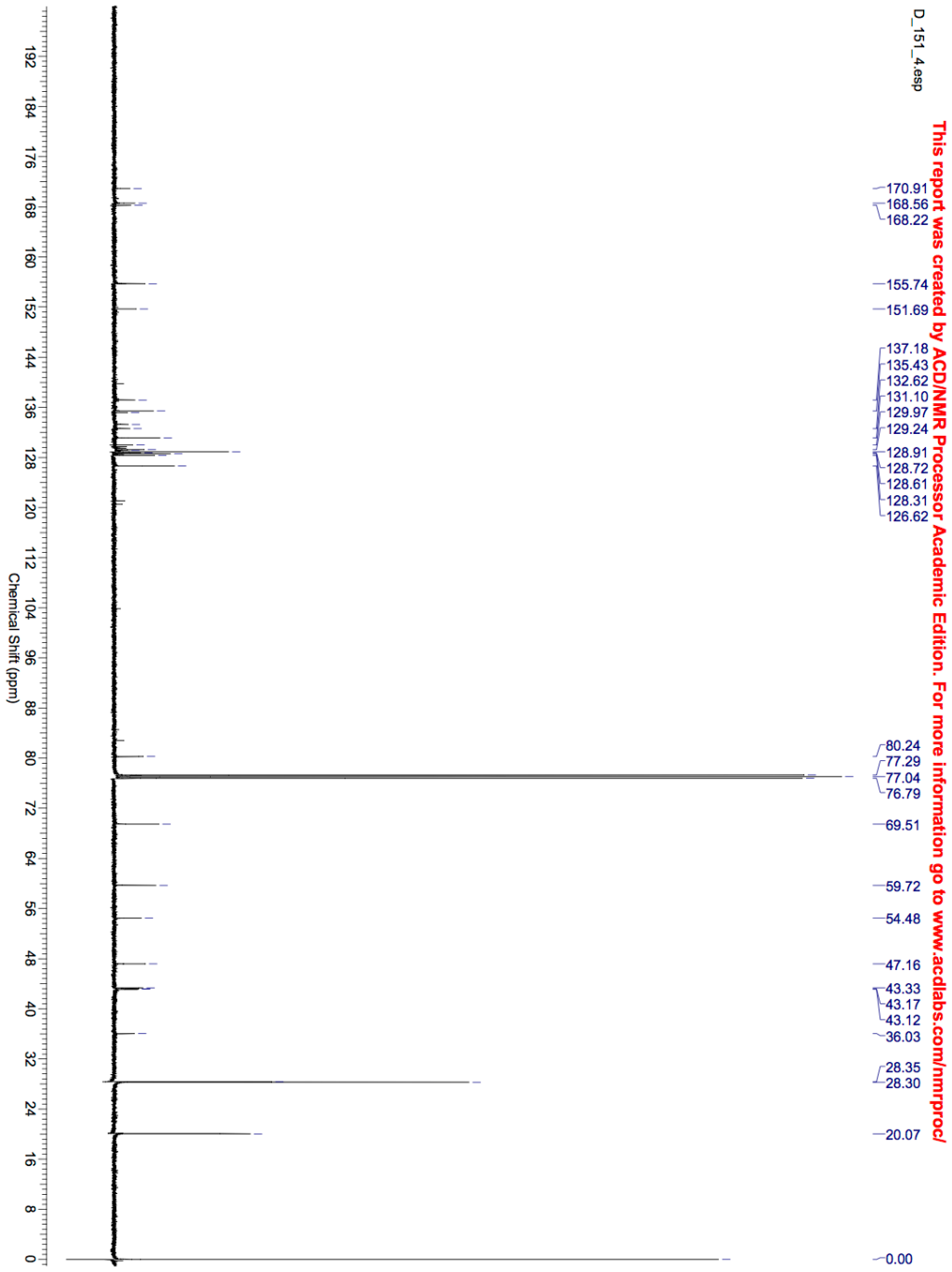
6.05
Chemical Shift (ppm)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This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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129.97
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128.72
128.61
128.31
126.62
80.24
77.29
77.04
76.79
69.51
59.72
54.48
47.16
43.33
43.17
43.12
36.03
28.35
28.30
20.07
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
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136
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120
112
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88
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56
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8
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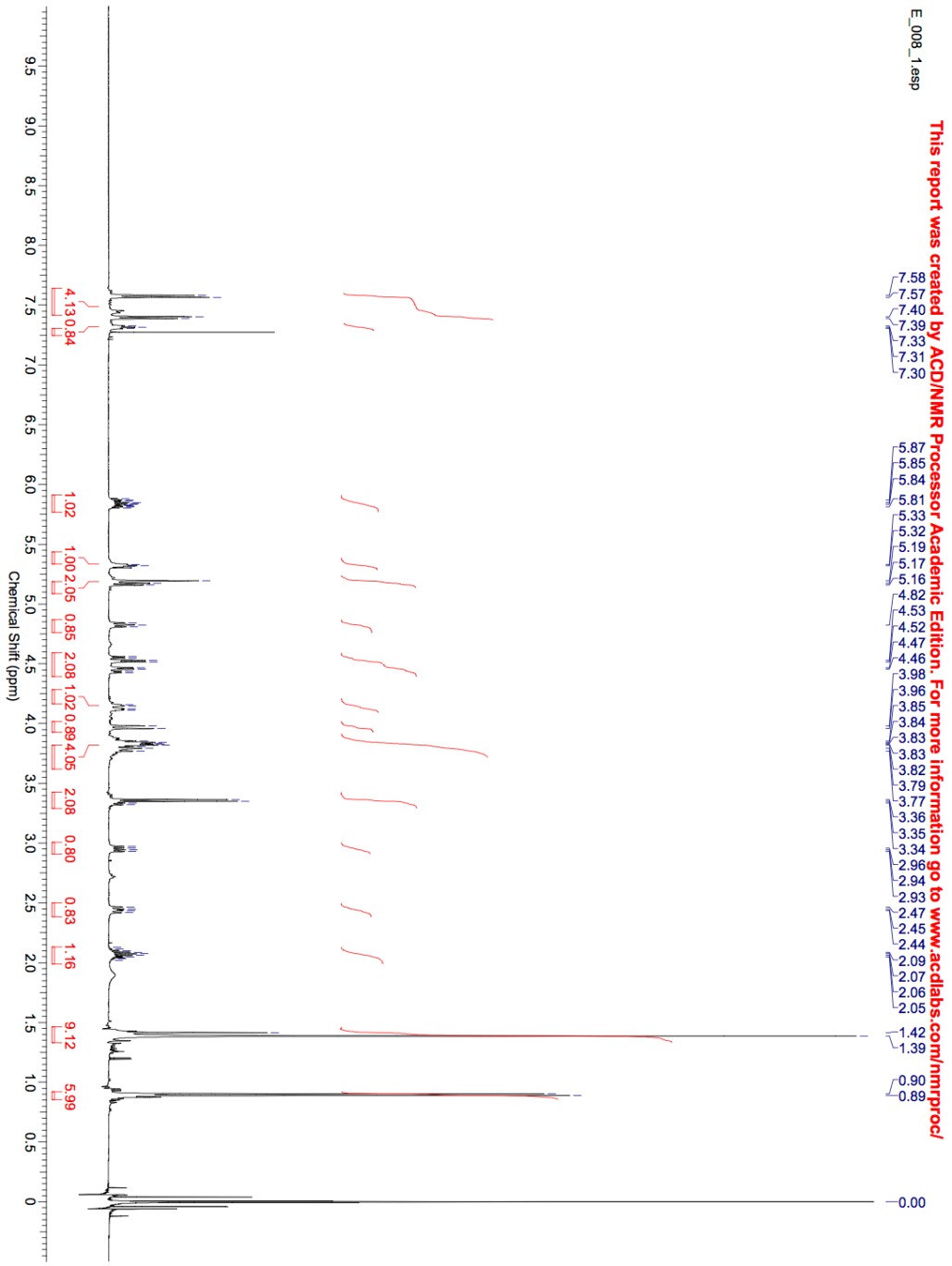
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5.84
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5.19
5.17
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4.53
4.52
4.47
4.46
3.98
3.96
3.85
3.84
3.83
3.83
3.82
3.79
3.77
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3.35
3.34
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2.94
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2.47
2.45
2.44
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2.07
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1.00
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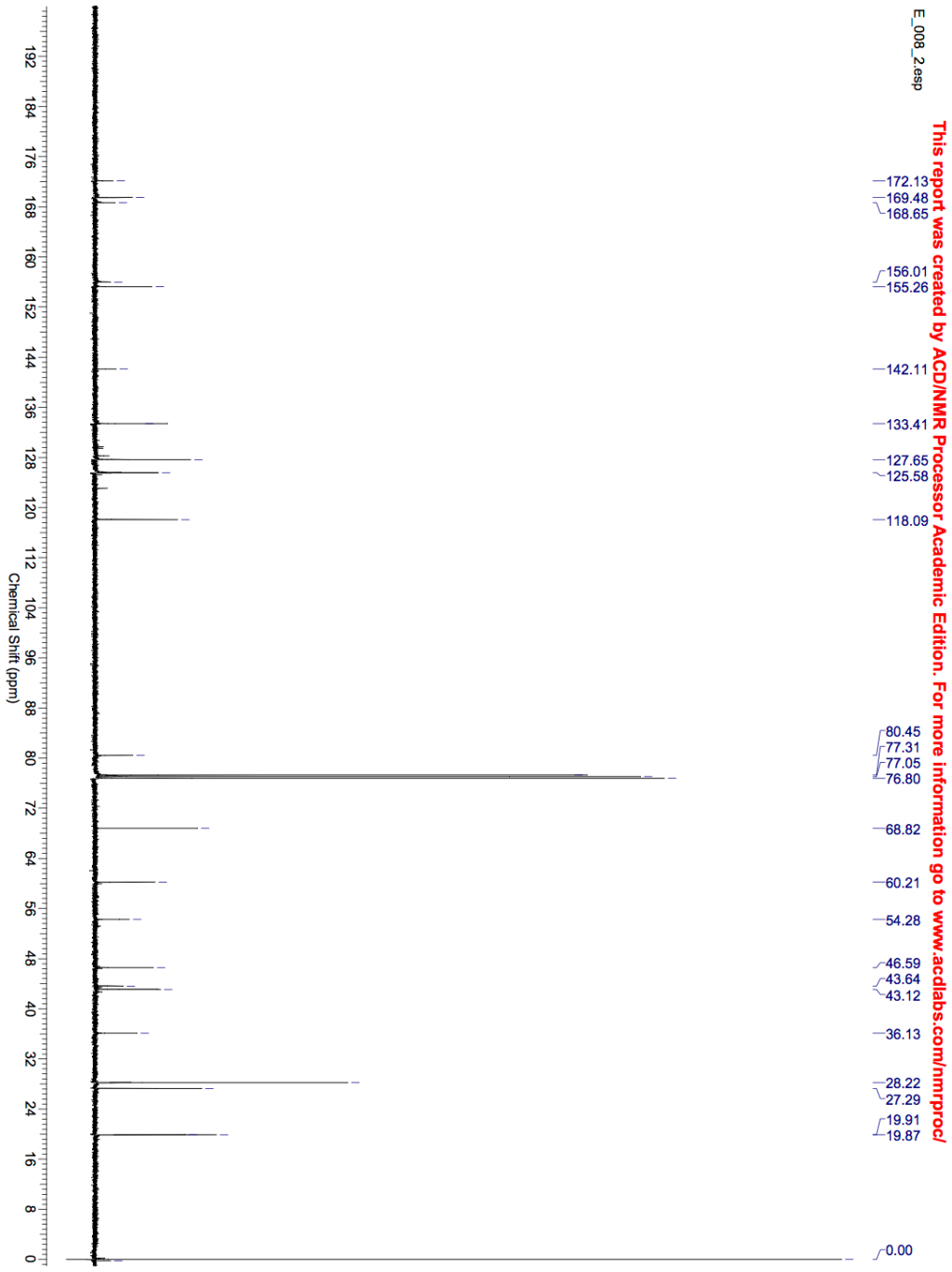
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125.58
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80.45
77.31
77.05
76.80
68.82
60.21
54.28
46.59
43.64
43.12
36.13
28.22
27.29
19.91
19.87
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
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120
112
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56
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8
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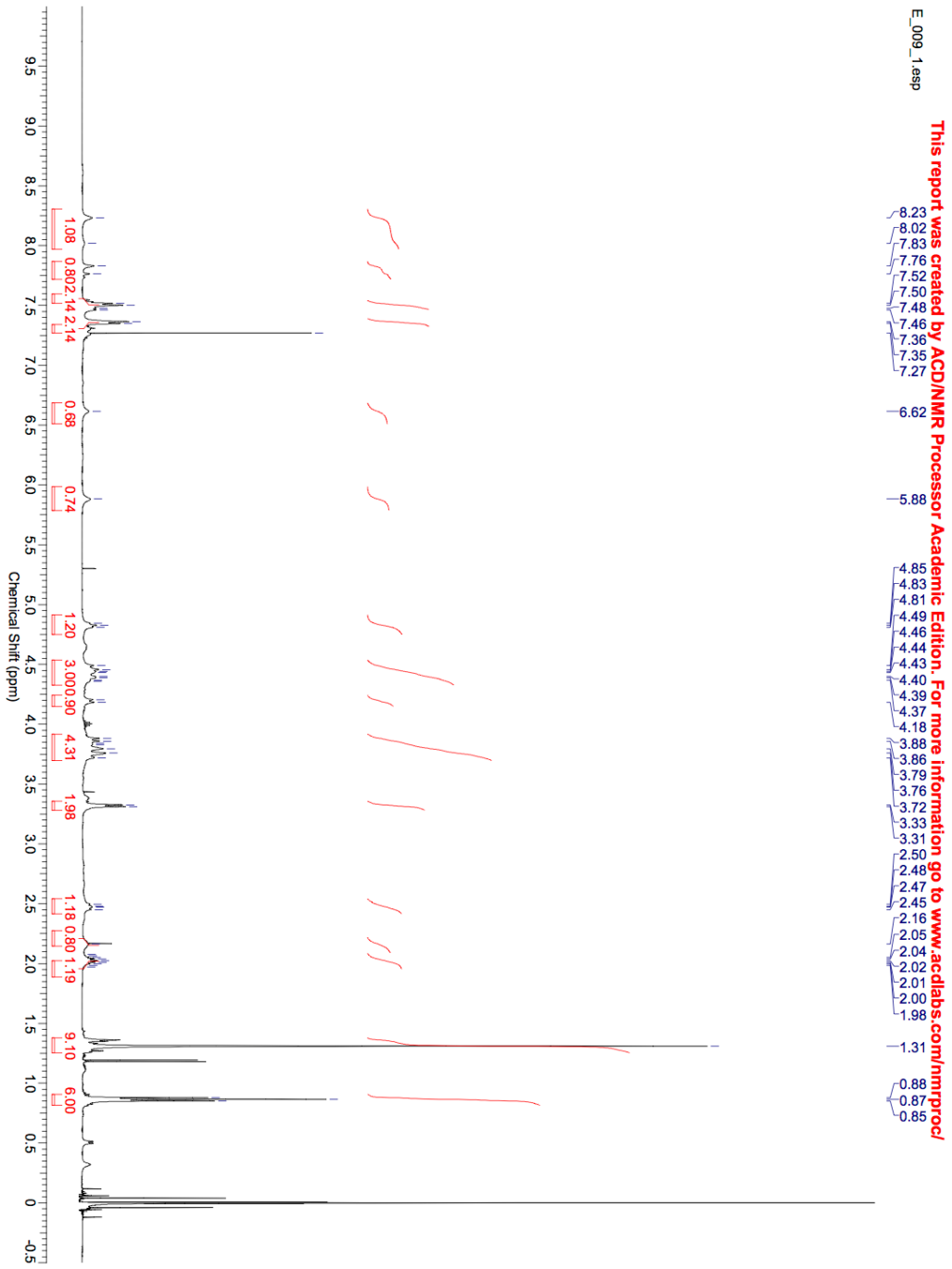
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This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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7.46
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7.35
7.27
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4.85
4.83
4.81
4.49
4.46
4.44
4.43
4.40
4.39
4.37
4.18
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3.76
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3.33
3.31
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2.47
2.45
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2.04
2.02
2.01
2.00
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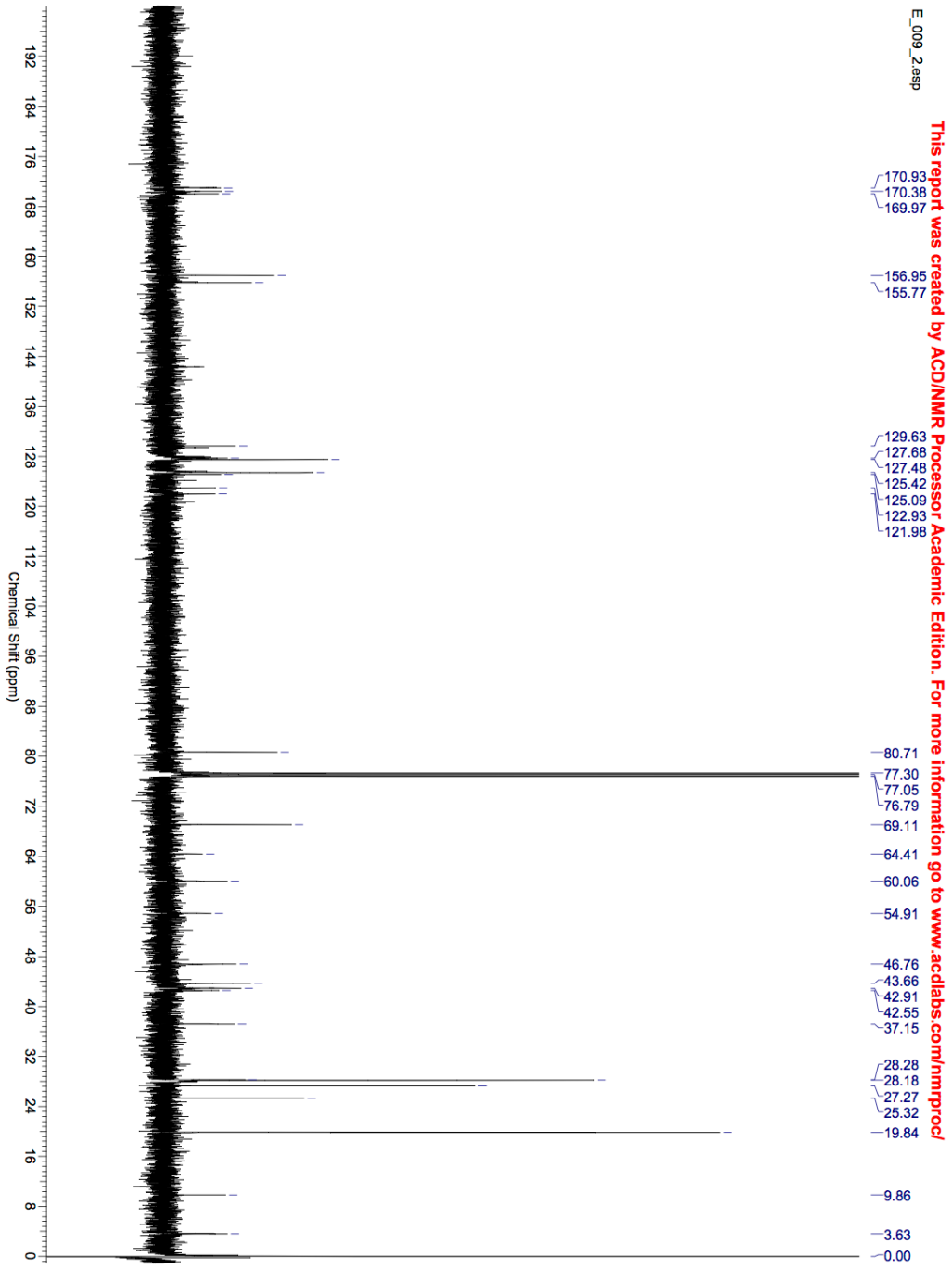
1.31
0.88
0.87
0.85
1.08
0.80
2.14
2.14
0.88
0.74
1.20
3.00
0.90
4.31
1.98
1.18
0.80
1.19
9.10
6.00
Chemical Shift (ppm)


E_009_2.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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170.38
169.97
156.95
155.77
129.63
127.68
127.48
125.42
125.09
122.93
121.98
80.71
77.30
77.05
76.79
69.11
64.41
60.06
54.91
46.76
43.66
42.91
42.55
37.15
28.28
28.18
27.27
25.32
19.84
9.86
3.63
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
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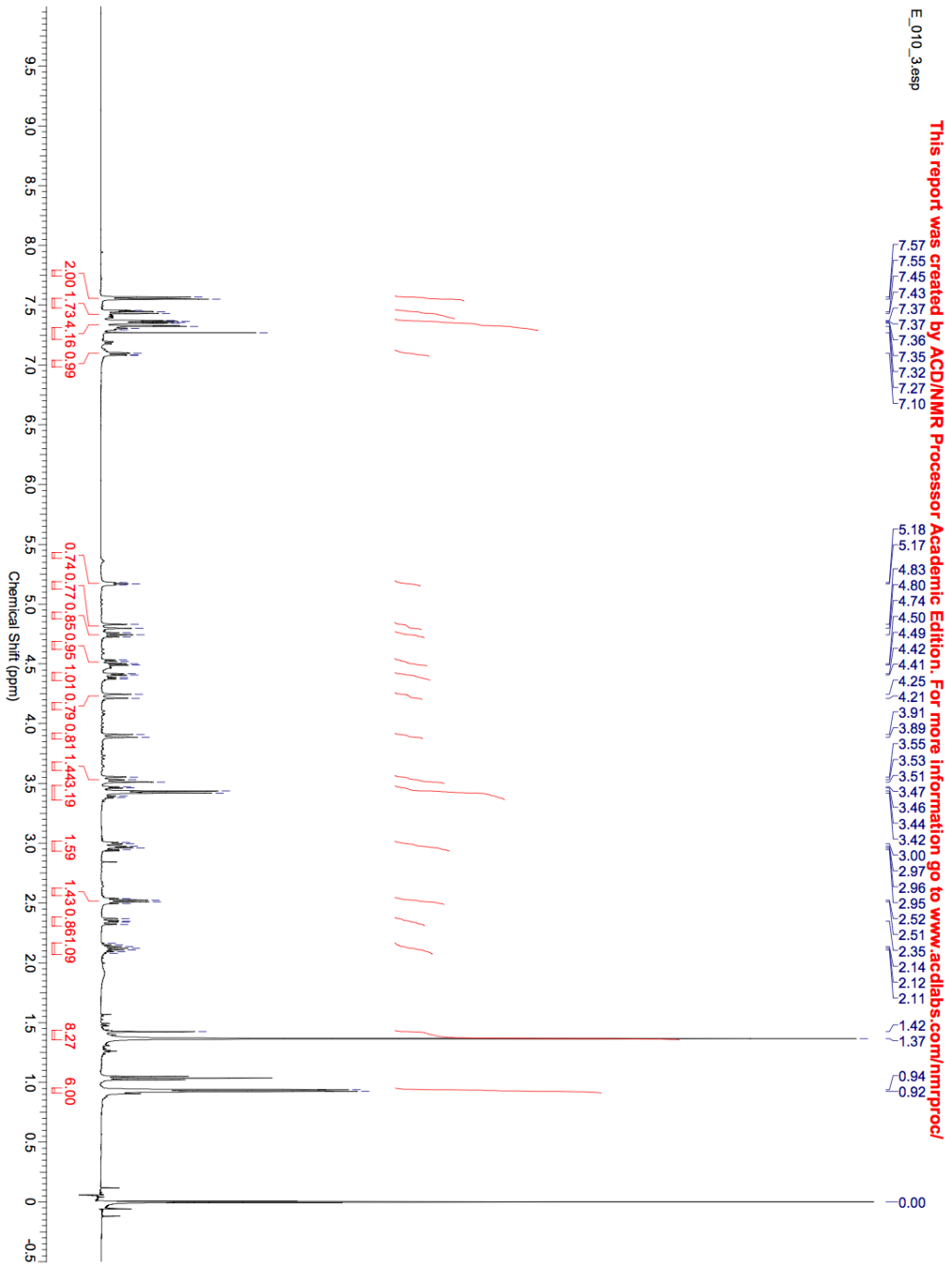
E_010_3.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
Chemical Shift (ppm)
7.57
7.55
7.45
7.43
7.37
7.37
7.36
7.35
7.32
7.27
7.10
5.18
5.17
4.83
4.80
4.74
4.50
4.49
4.42
4.41
4.25
4.21
3.91
3.89
3.55
3.53
3.51
3.47
3.46
3.44
3.42
3.00
2.97
2.96
2.95
2.52
2.51
2.35
2.14
2.12
2.11
1.42
1.37
0.94
0.92
0.00
2.00
1.73
4.16
0.99
0.74
0.77
0.85
0.95
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0.79
0.81
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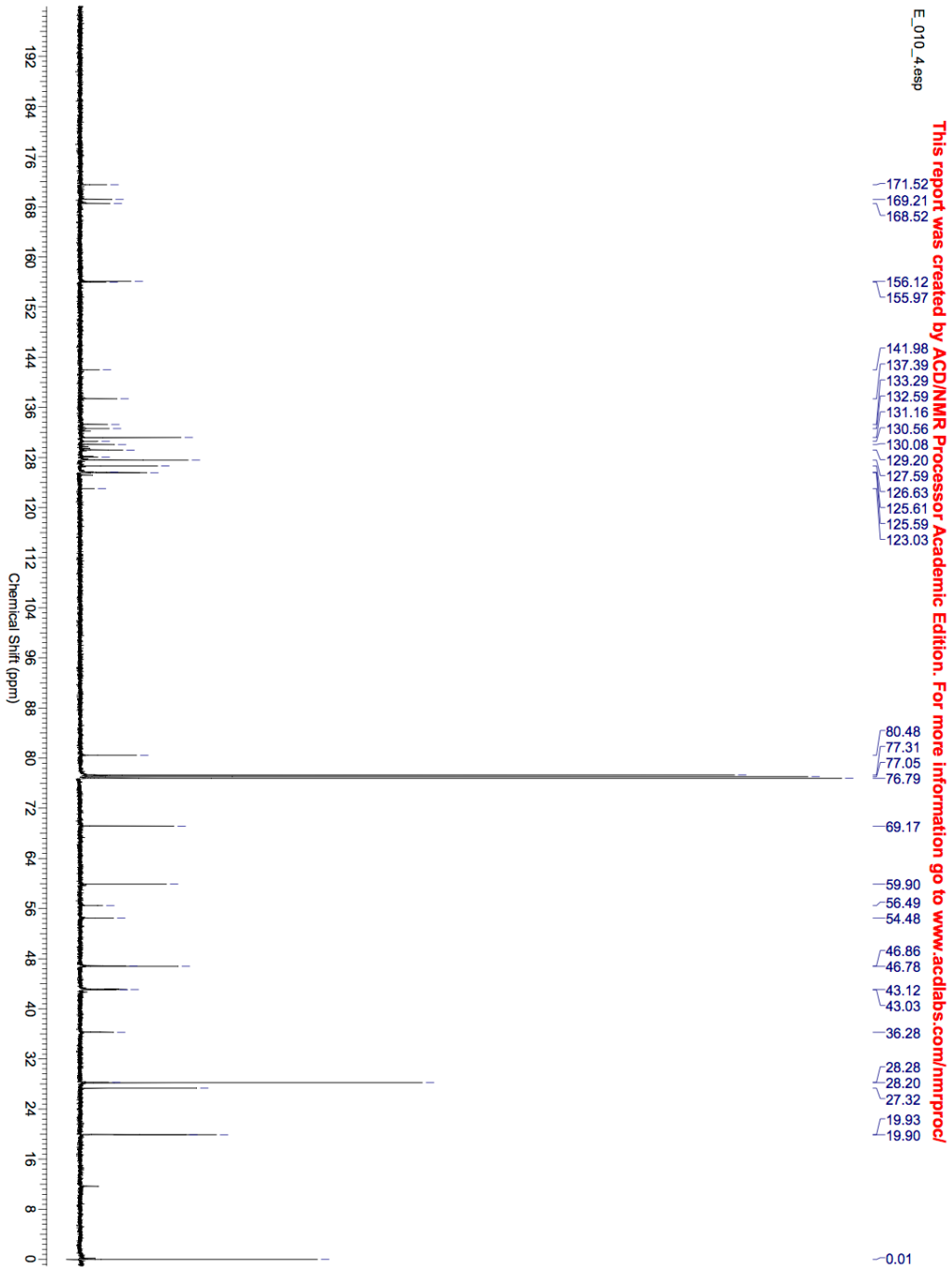
3.19
1.59
1.43
0.86
1.09
8.27
6.00


E_010_4.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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168.52
156.12
155.97
141.98
137.39
133.29
132.59
131.16
130.56
130.08
129.20
127.59
126.63
125.61
125.59
123.03
80.48
77.31
77.05
76.79
69.17
59.90
56.49
54.48
46.86
46.78
43.12
43.03
36.28
28.28
28.20
27.32
19.93
19.90
0.01
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
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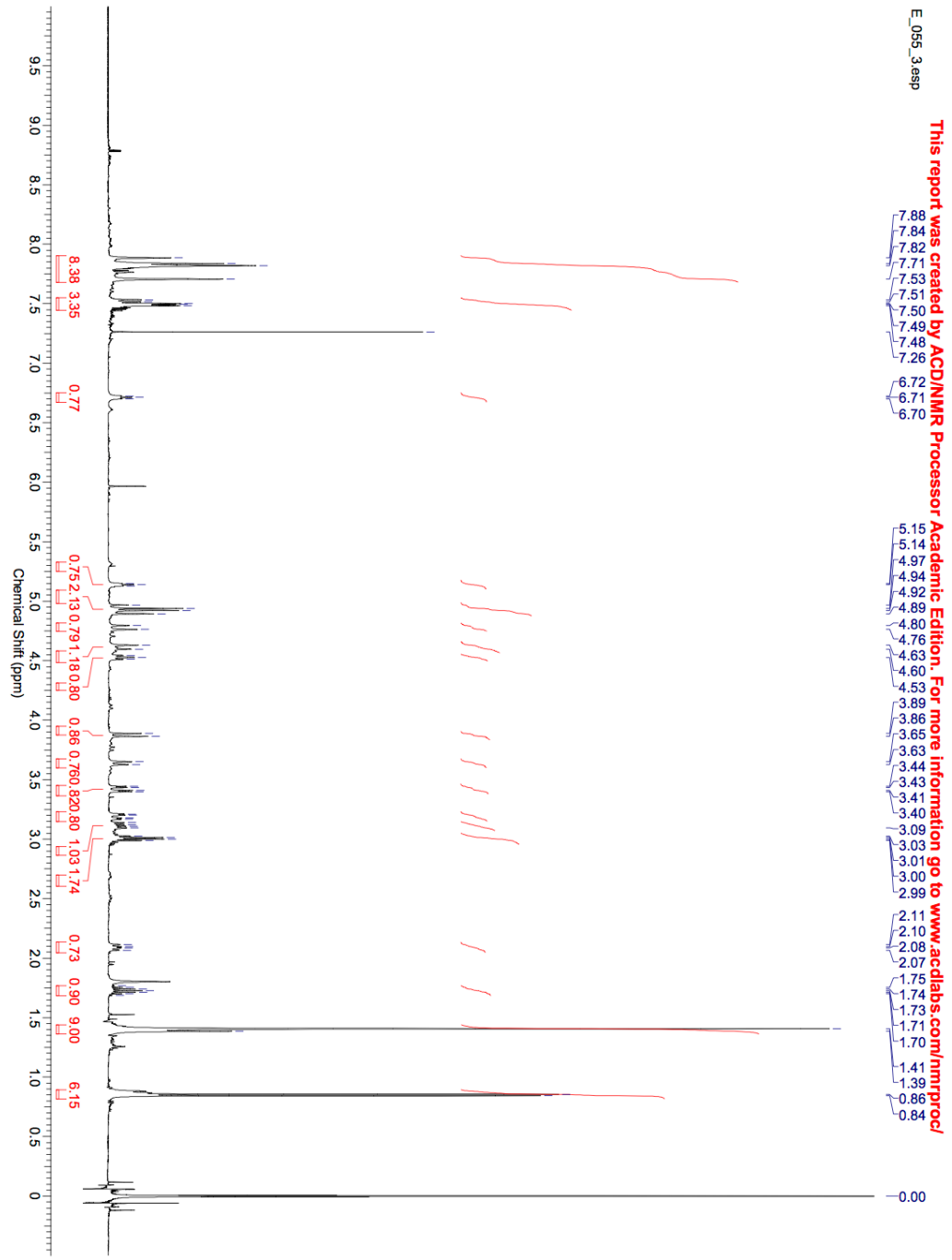
E_055_3.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
Chemical Shift (ppm)
7.88
7.84
7.82
7.71
7.53
7.51
7.50
7.49
7.48
7.26
6.72
6.71
6.70
5.15
5.14
4.97
4.94
4.92
4.89
4.80
4.76
4.63
4.60
4.53
3.89
3.86
3.65
3.63
3.44
3.43
3.41
3.40
3.09
3.03
3.01
3.00
2.99
2.11
2.10
2.08
2.07
1.75
1.74
1.73
1.71
1.70
1.41
1.39
0.86
0.84
0.00
8.38
3.35
0.77
0.75
2.13
0.79
1.18
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0.86
0.76
0.82
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1.74
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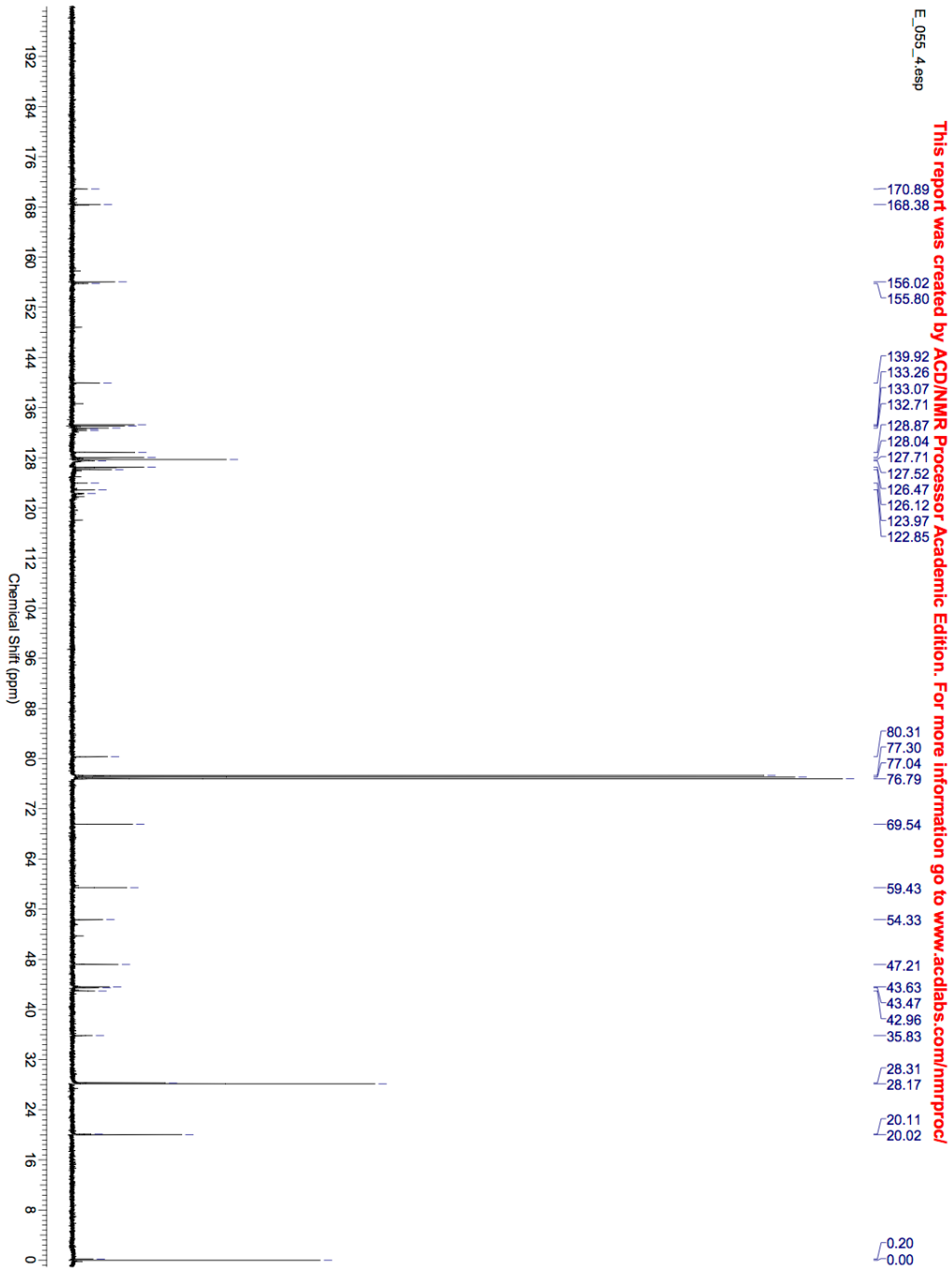
9.00
6.15


E_055_4.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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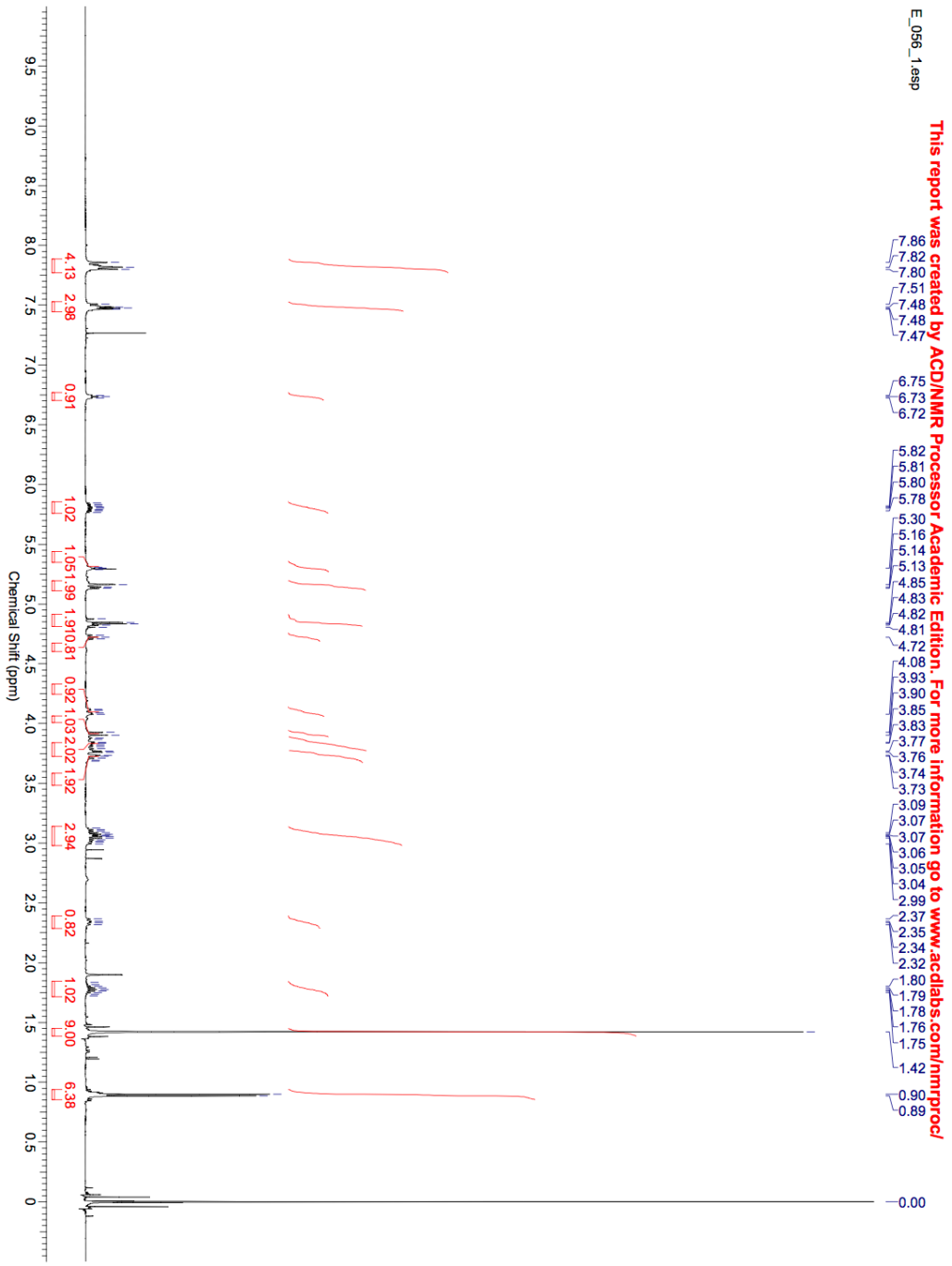
139.92
133.26
133.07
132.71
128.87
128.04
127.71
127.52
126.47
126.12
123.97
122.85
80.31
77.30
77.04
76.79
69.54
59.43
54.33
47.21
43.63
43.47
42.96
35.83
28.31
28.17
20.11
20.02
0.20
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0


E_056_1.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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7.51
7.48
7.48
7.47
6.75
6.73
6.72
5.82
5.81
5.80
5.78
5.30
5.16
5.14
5.13
4.85
4.83
4.82
4.81
4.72
4.08
3.93
3.90
3.85
3.83
3.77
3.76
3.74
3.73
3.09
3.07
3.07
3.06
3.05
3.04
2.99
2.37
2.35
2.34
2.32
1.80
1.79
1.78
1.76
1.75
1.42
0.90
0.89
0.00
4.13
2.98
0.91
1.02
1.05
1.99
1.99
1.91
0.81
0.92
1.03
2.02
1.92
2.94
0.82
1.02
9.00
6.38
Chemical Shift (ppm)
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
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4.5
4.0
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2.0
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1.0
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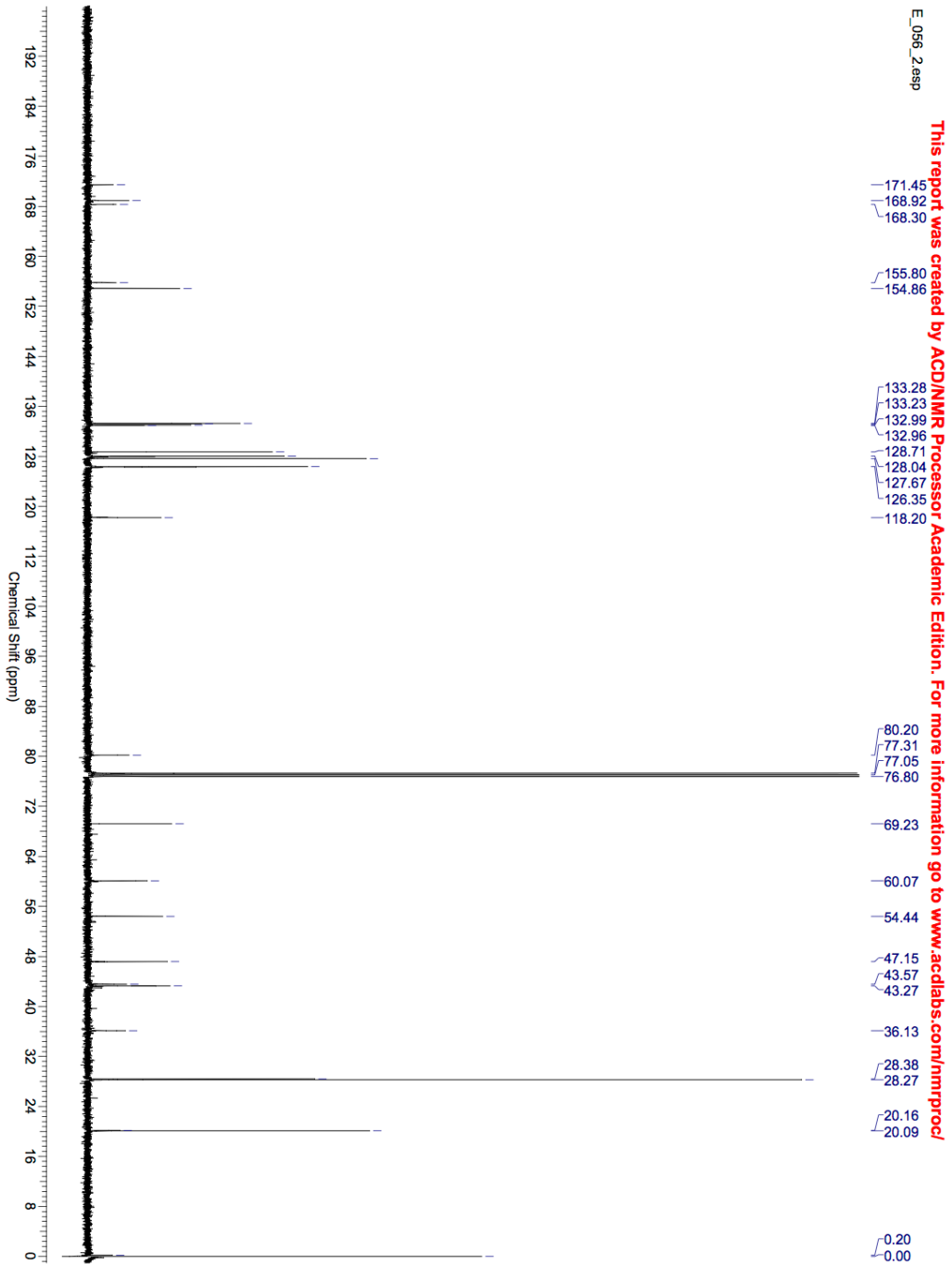
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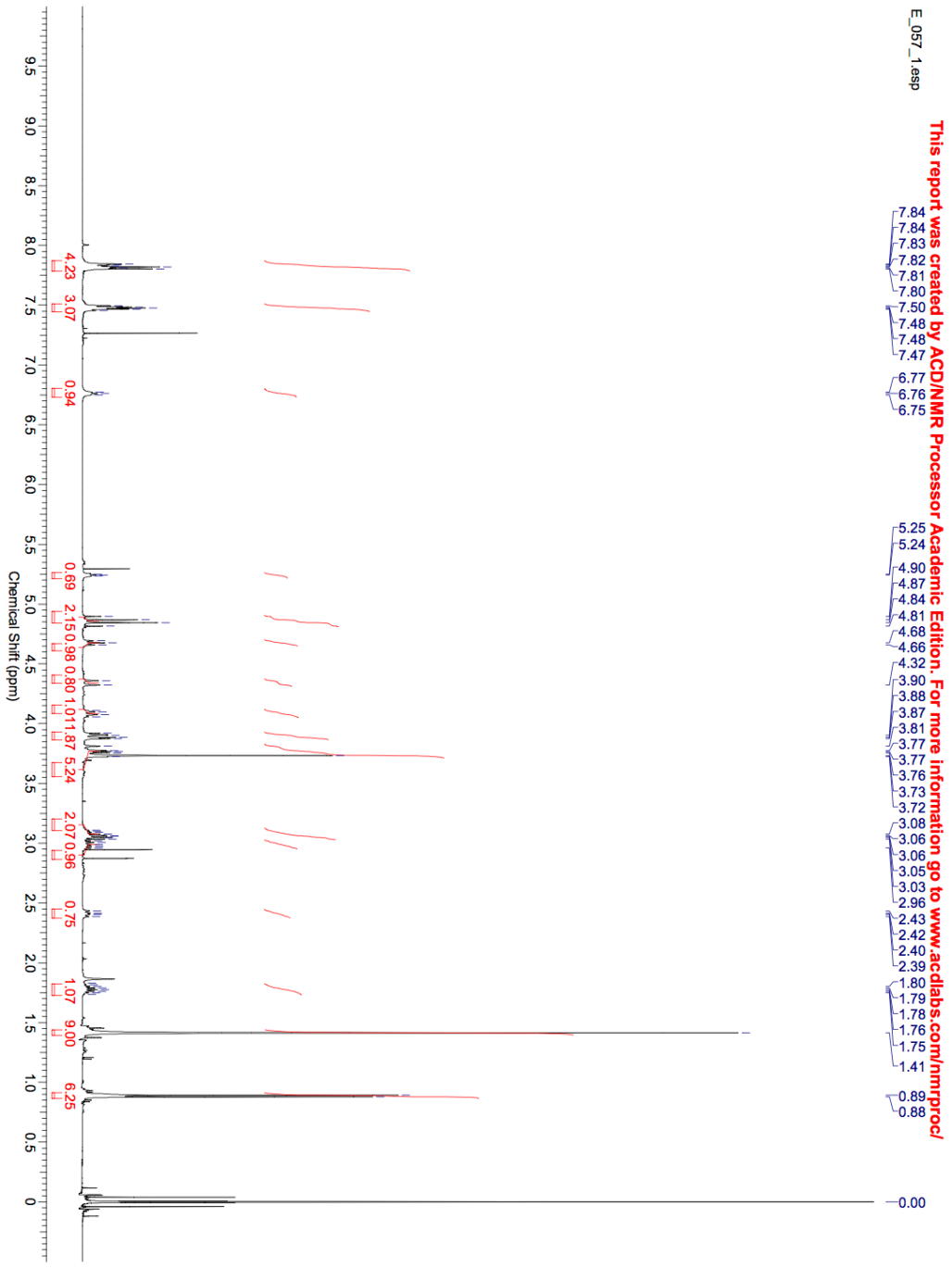
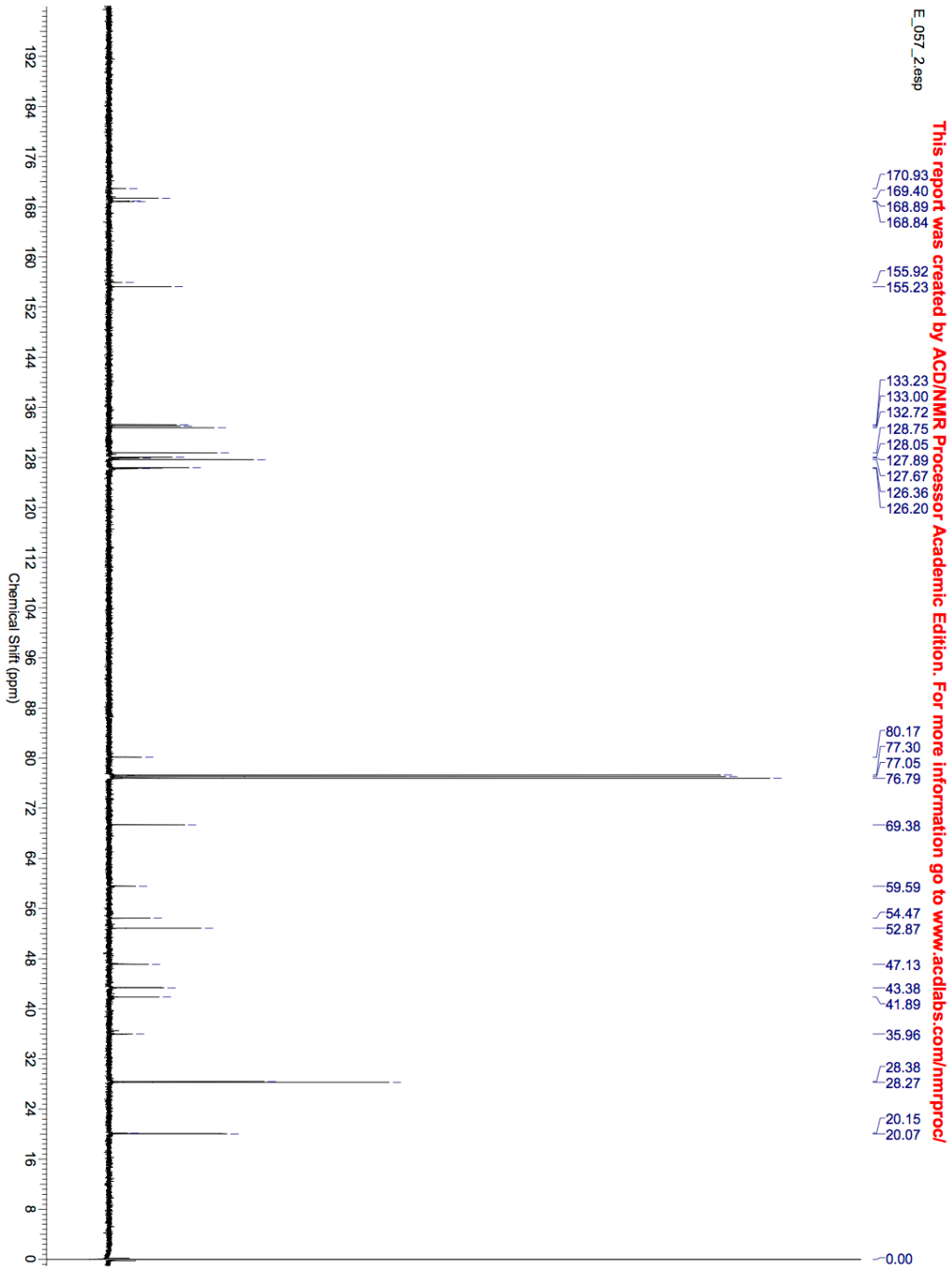
168.30
155.80
154.86
133.28
133.23
132.99
132.96
128.71
128.04
127.67
126.35
118.20
80.20
77.31
77.05
76.80
69.23
60.07
54.44
47.15
43.57
43.27
36.13
28.38
28.27
20.16
20.09
0.20
0.00
Chemical Shift (ppm)
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/


E_057_1.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
Chemical Shift (ppm)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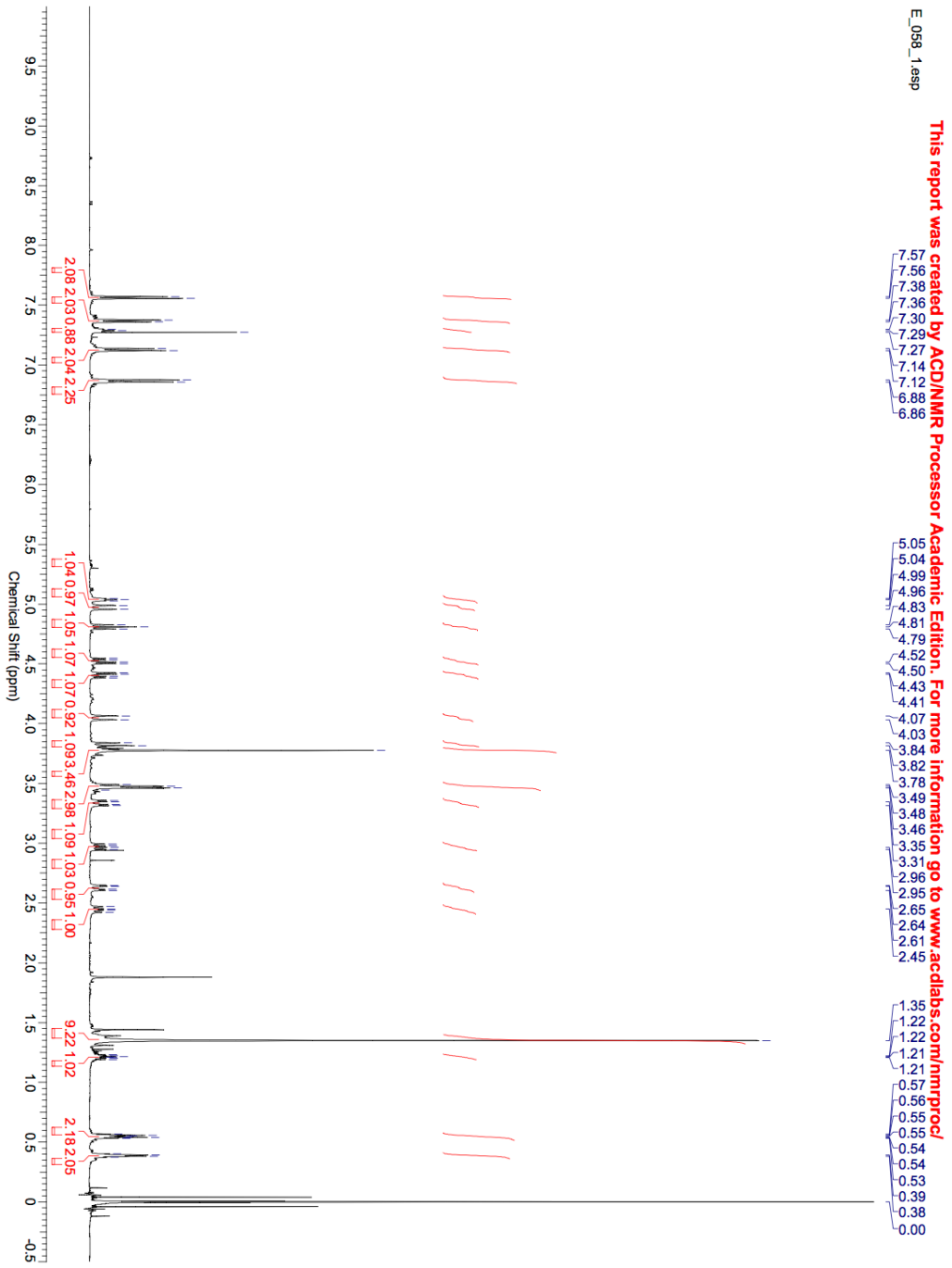
155.23
133.23
133.00
132.72
128.75
128.05
127.89
127.67
126.36
126.20
80.17
77.30
77.05
76.79
69.38
59.59
54.47
52.87
47.13
43.38
41.89
35.96
28.38
28.27
20.15
20.07
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0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This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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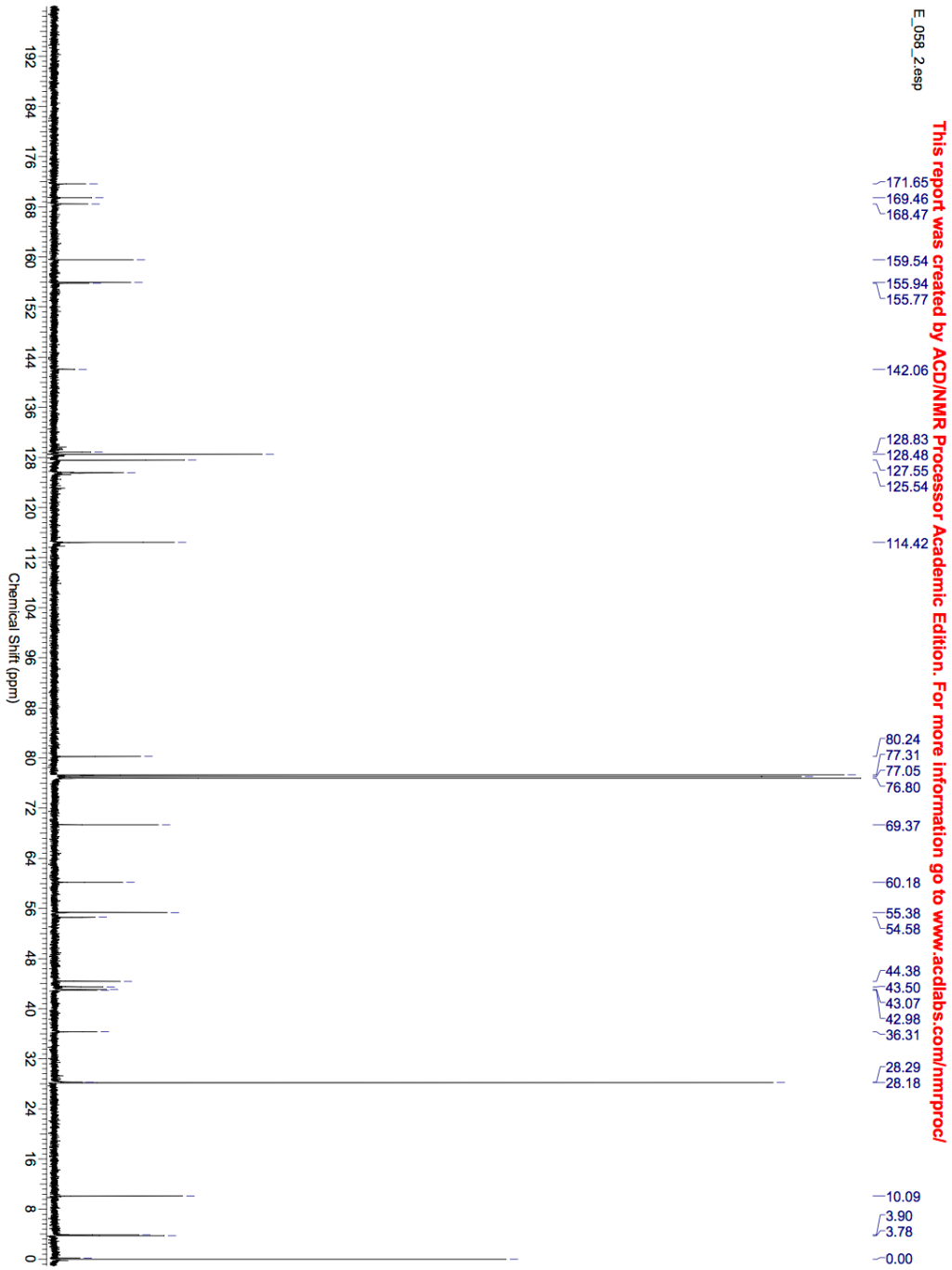
7.38
7.36
7.30
7.29
7.27
7.14
7.12
6.88
6.86
5.05
5.04
4.99
4.96
4.83
4.81
4.79
4.52
4.50
4.43
4.41
4.07
4.03
3.84
3.82
3.78
3.49
3.48
3.46
3.35
3.31
2.96
2.95
2.65
2.64
2.61
2.45
1.35
1.22
1.22
1.21
1.21
0.57
0.56
0.55
0.55
0.54
0.54
0.53
0.39
0.38
0.00
2.08 2.03 0.88 2.04 2.25
1.04 0.97 1.05 1.07 1.07 0.92 1.09 3.46 2.98 1.09 1.03 0.95 1.00
9.22 1.02
2.18 2.05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128.48
127.55
125.54
114.42
80.24
77.31
77.05
76.80
69.37
60.18
55.38
54.58
44.38
43.50
43.07
42.98
36.31
28.29
28.18
10.09
3.90
3.78
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
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32
24
16
8
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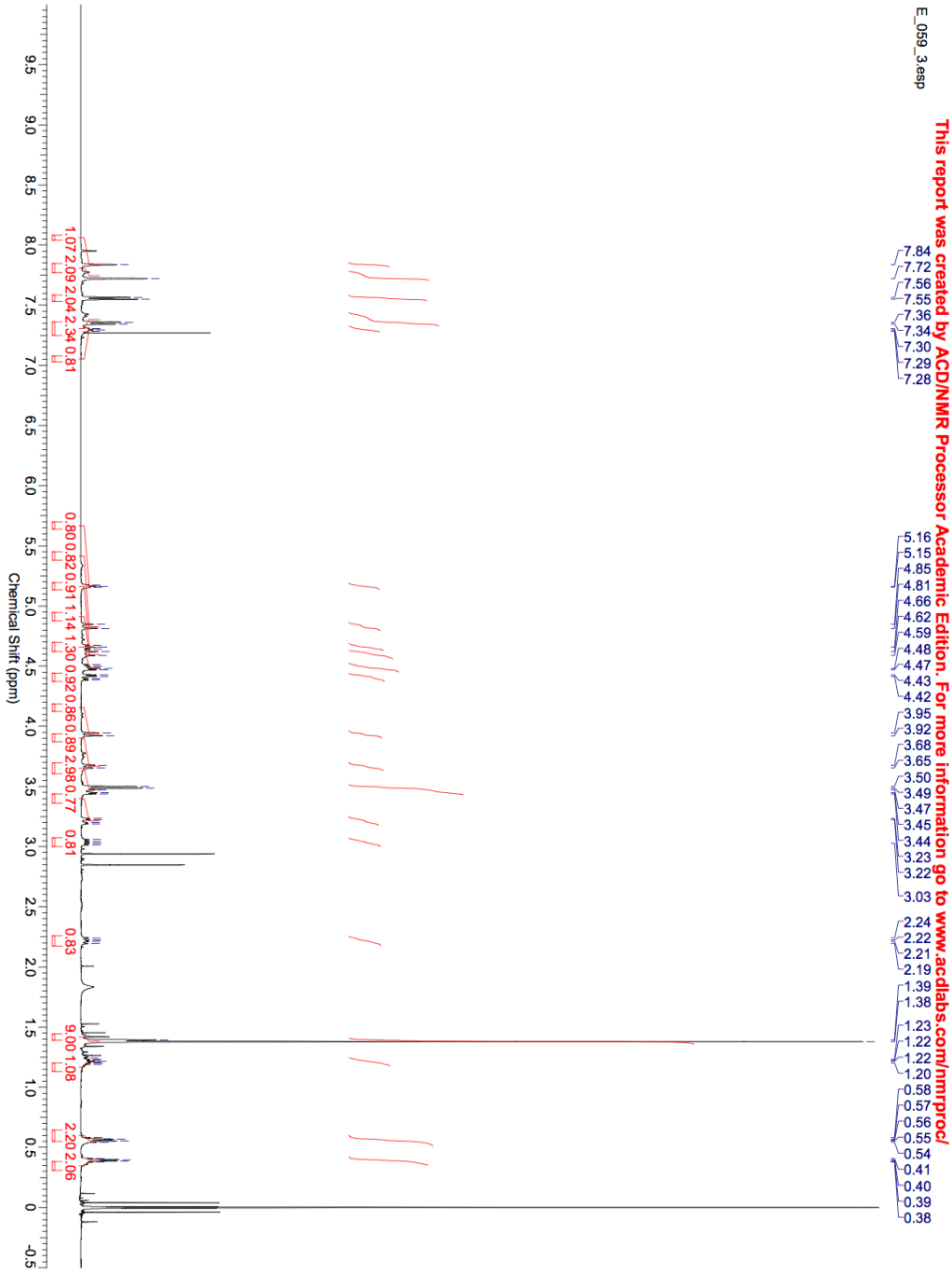
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/


E_059_3.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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7.56
7.55
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7.34
7.30
7.29
7.28
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5.15
4.85
4.81
4.66
4.62
4.59
4.48
4.47
4.43
4.42
3.95
3.92
3.68
3.65
3.50
3.49
3.47
3.45
3.44
3.23
3.22
3.03
2.24
2.22
2.21
2.19
1.39
1.38
1.23
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0.54
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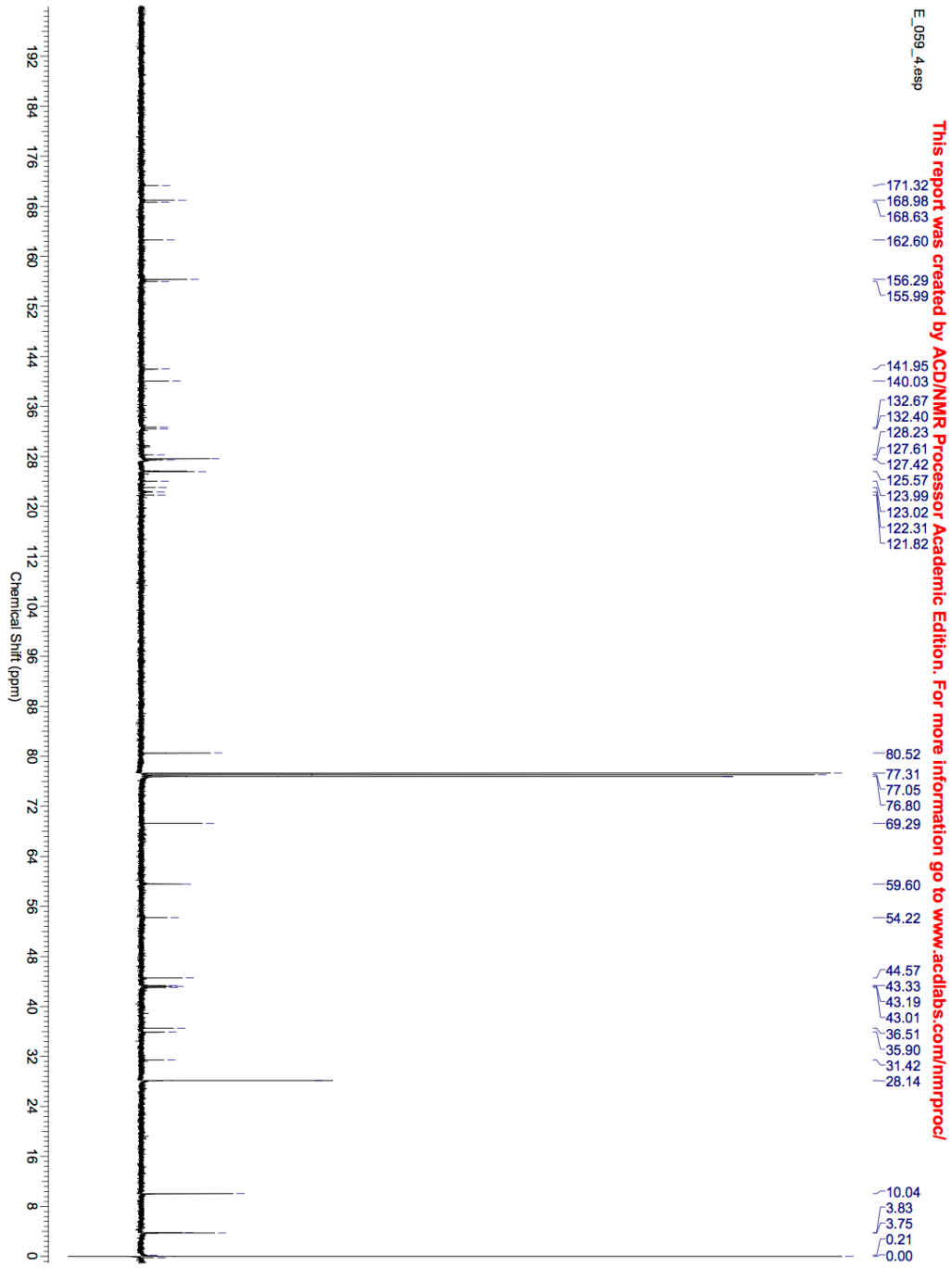
0.39
0.38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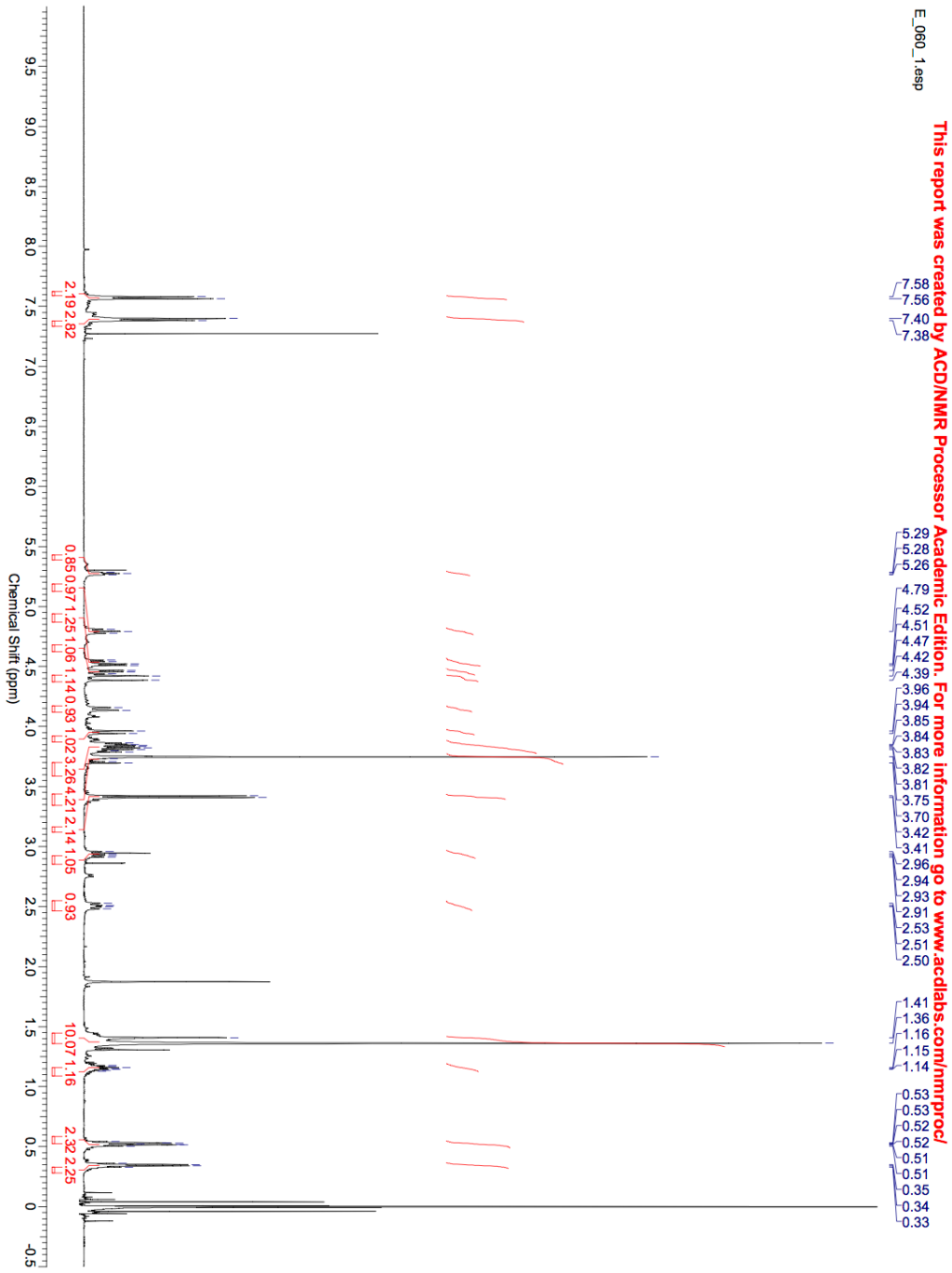
127.61
127.42
125.57
123.99
123.02
122.31
121.82
80.52
77.31
77.05
76.80
69.29
59.60
54.22
44.57
43.33
43.19
43.01
36.51
35.90
31.42
28.14
10.04
3.83
3.75
0.21
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/


E_060_1.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
Chemical Shift (ppm)
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7.40
7.38
5.29
5.28
5.26
4.79
4.52
4.51
4.47
4.42
4.39
3.96
3.94
3.85
3.84
3.83
3.82
3.81
3.75
3.70
3.42
3.41
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2.94
2.93
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2.53
2.51
2.50
1.41
1.36
1.16
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0.52
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0.51
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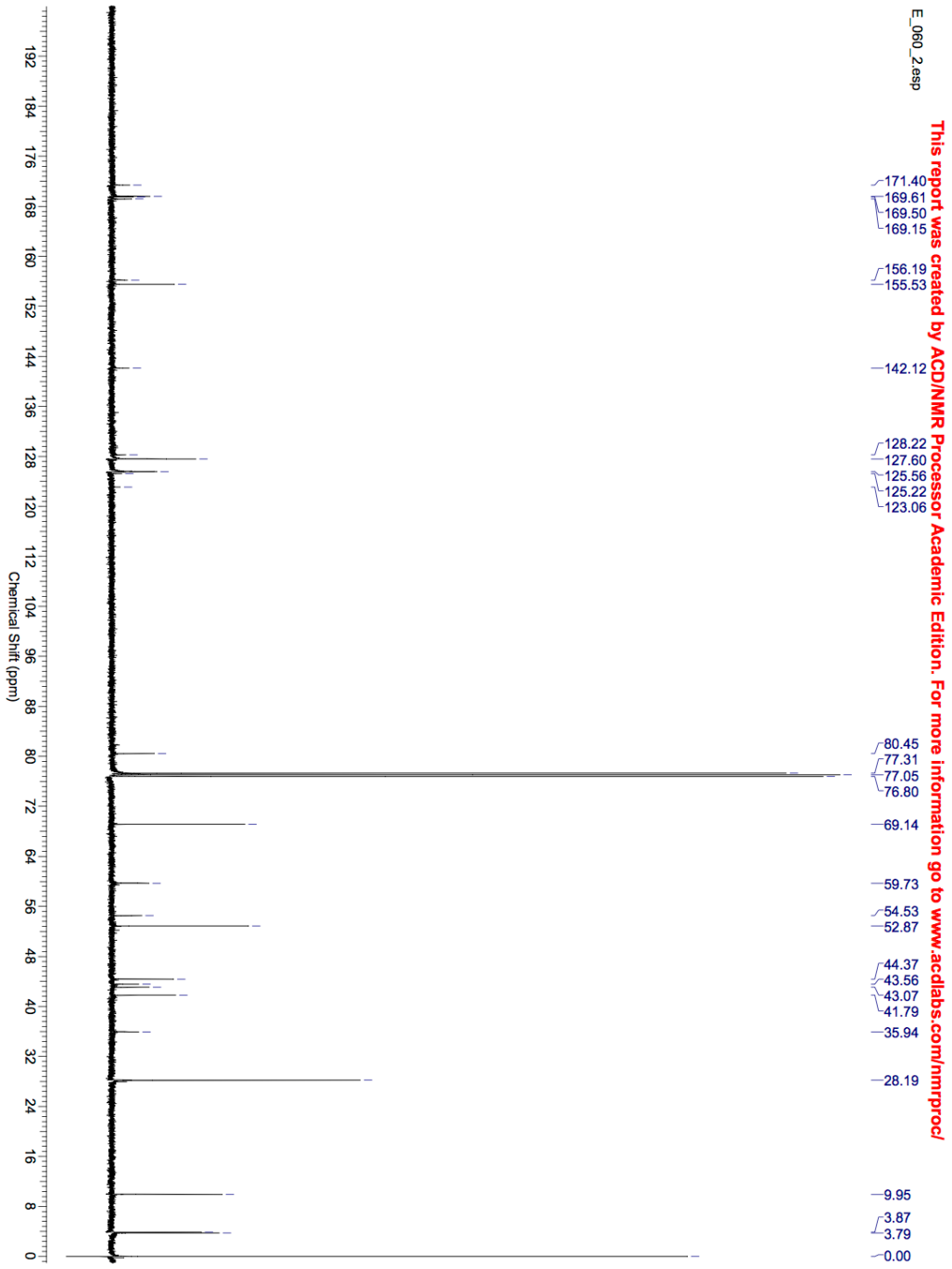
0.33


E_060_2.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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169.50
169.15
156.19
155.53
142.12
128.22
127.60
125.56
125.22
123.06
80.45
77.31
77.05
76.80
69.14
59.73
54.53
52.87
44.37
43.56
43.07
41.79
35.94
28.19
9.95
3.87
3.79
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
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24
16
8
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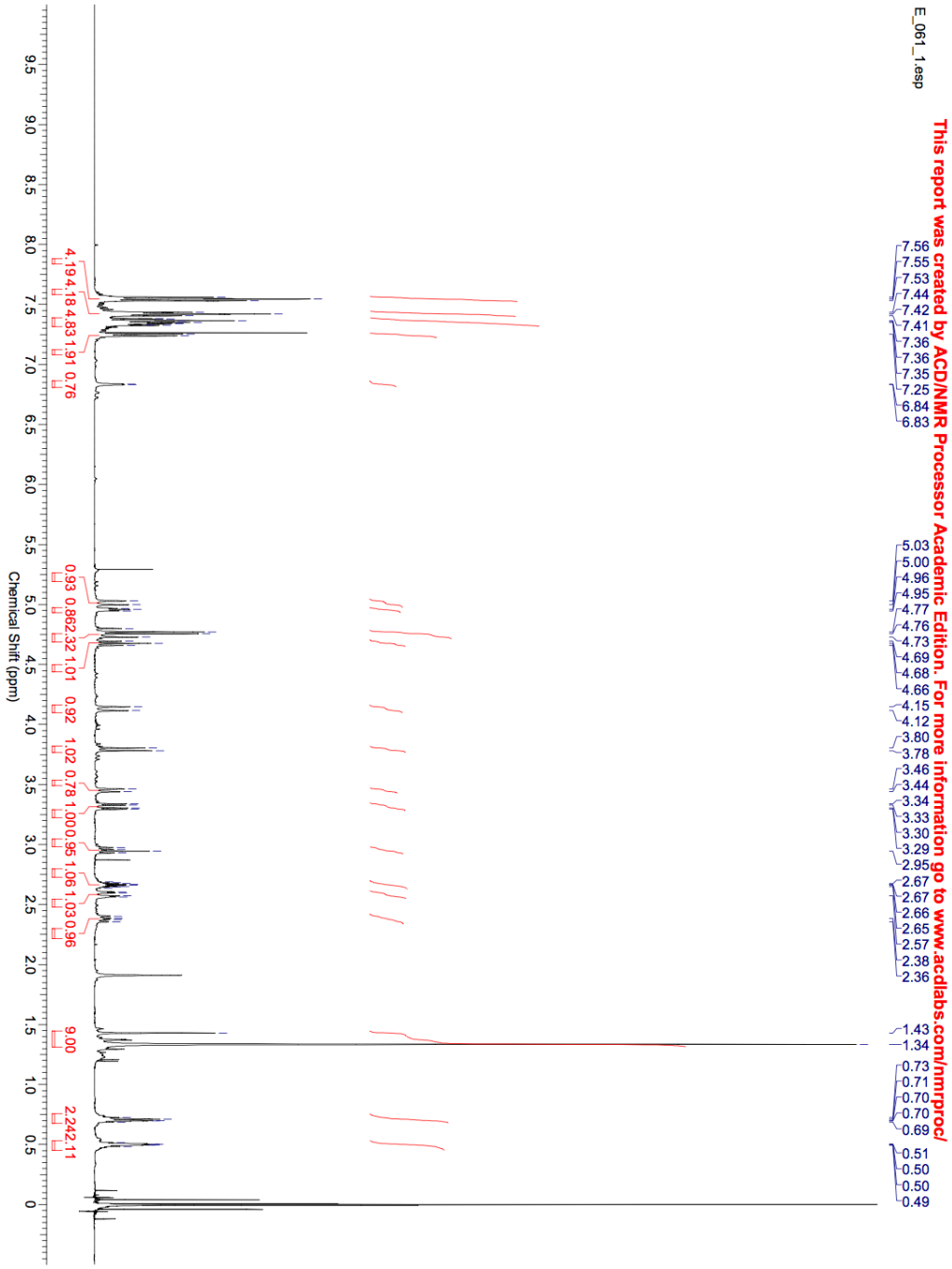
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This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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7.42
7.41
7.36
7.36
7.35
7.25
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6.83
5.03
5.00
4.96
4.95
4.77
4.76
4.73
4.69
4.68
4.66
4.15
4.12
3.80
3.78
3.46
3.44
3.34
3.33
3.30
3.29
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2.67
2.67
2.66
2.65
2.57
2.38
2.36
1.43
1.34
0.73
0.71
0.70
0.70
0.69
0.51
0.50
0.50
0.49
4.19
4.18
4.83
1.91
0.76
0.93
0.86
2.32
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0.78
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1.03
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9.00
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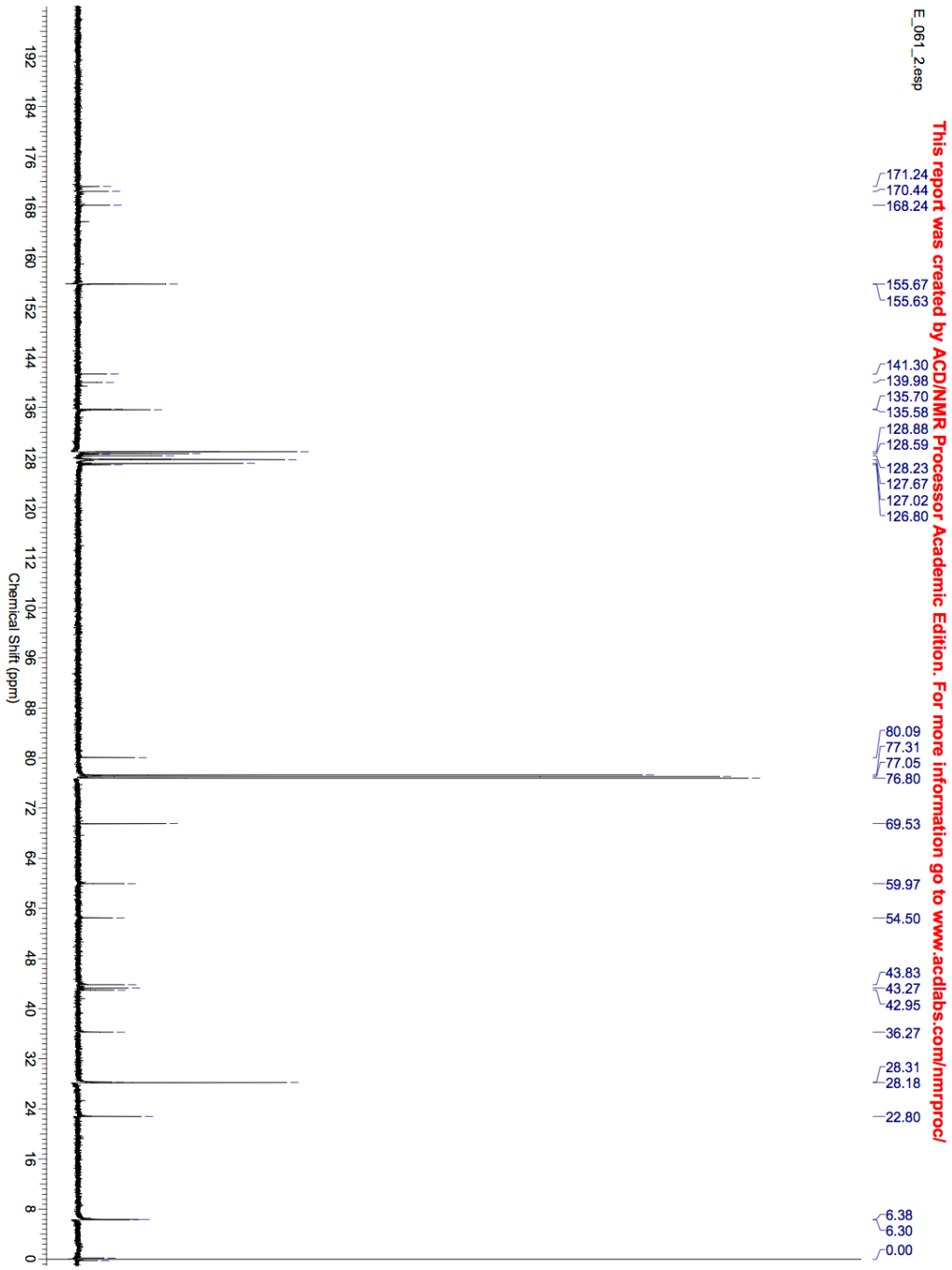
2.11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135.58
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128.59
128.23
127.67
127.02
126.80
80.09
77.31
77.05
76.80
69.53
59.97
54.50
43.83
43.27
42.95
36.27
28.31
28.18
22.80
6.38
6.30
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
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48
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32
24
16
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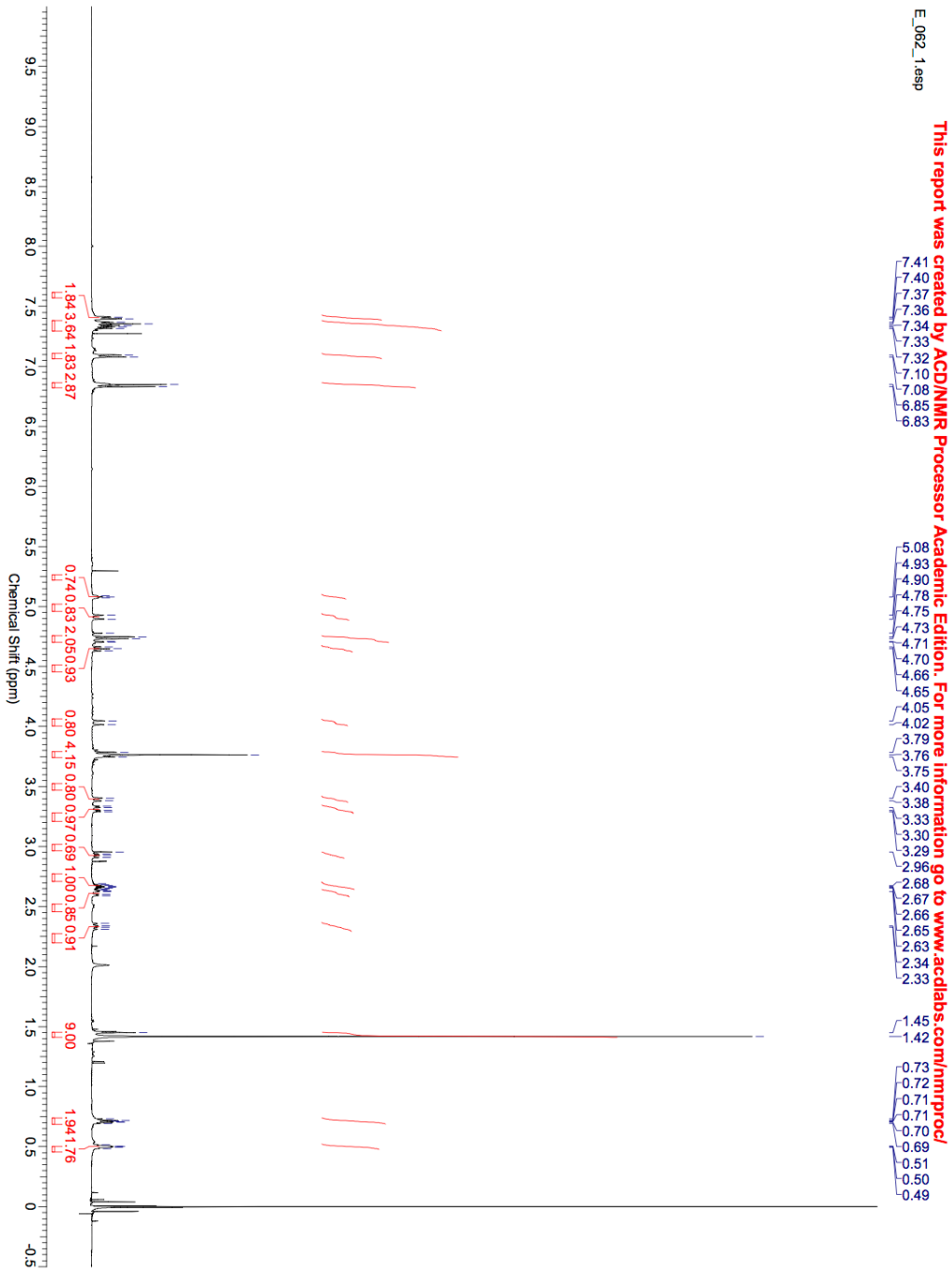
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/


E_062_1.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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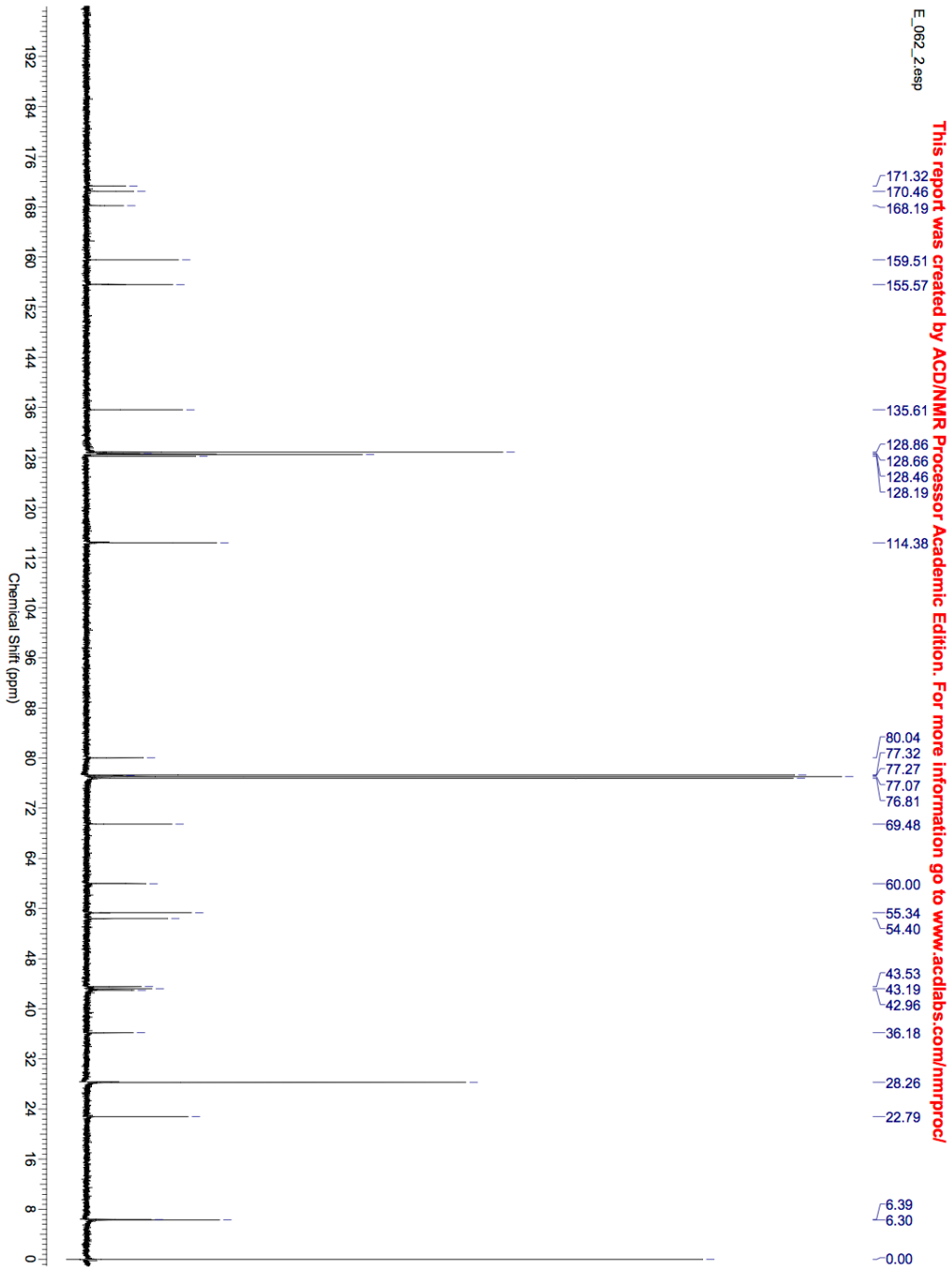
7.32
7.10
7.08
6.85
6.83
5.08
4.93
4.90
4.78
4.75
4.73
4.71
4.70
4.66
4.65
4.05
4.02
3.79
3.76
3.75
3.40
3.38
3.33
3.30
3.29
2.96
2.68
2.67
2.66
2.65
2.63
2.34
2.33
1.45
1.42
0.73
0.72
0.71
0.71
0.70
0.69
0.51
0.50
0.49
1.84 3.64 1.83 2.87
0.74 0.83 2.05 0.93
0.80 4.15 0.80 0.97 0.69 1.00 0.85 0.91
9.00
1.94 1.76
Chemical Shift (ppm)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128.46
128.19
114.38
80.04
77.32
77.27
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76.81
69.48
60.00
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54.40
43.53
43.19
42.96
36.18
28.26
22.79
6.39
6.30
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
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32
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16
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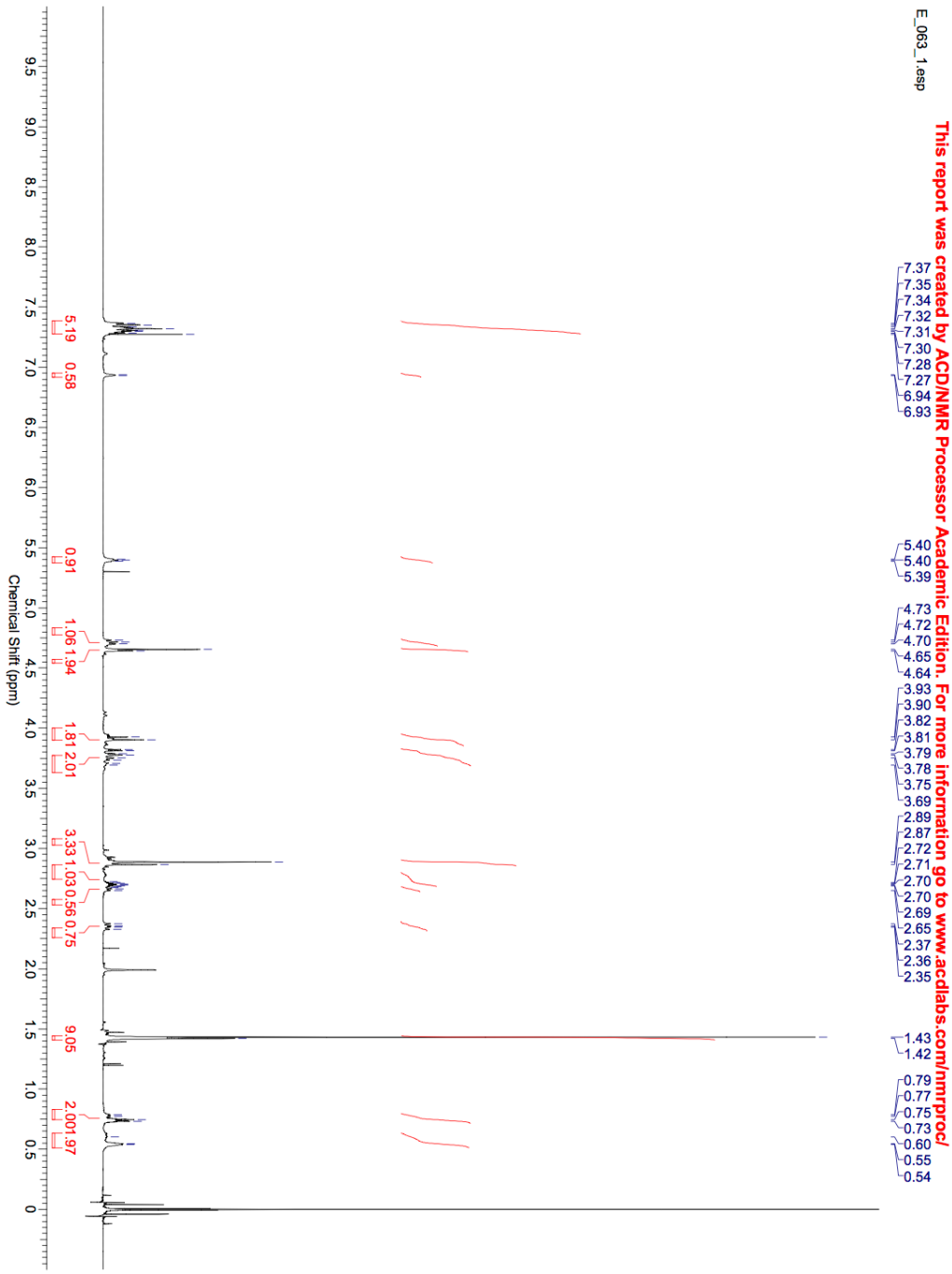
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/


E_063_1.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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7.28
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6.93
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5.40
5.39
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4.72
4.70
4.65
4.64
3.93
3.90
3.82
3.81
3.79
3.78
3.75
3.69
2.89
2.87
2.72
2.71
2.70
2.70
2.69
2.65
2.37
2.36
2.35
1.43
1.42
0.79
0.77
0.75
0.73
0.60
0.55
0.54
5.19
0.58
0.91
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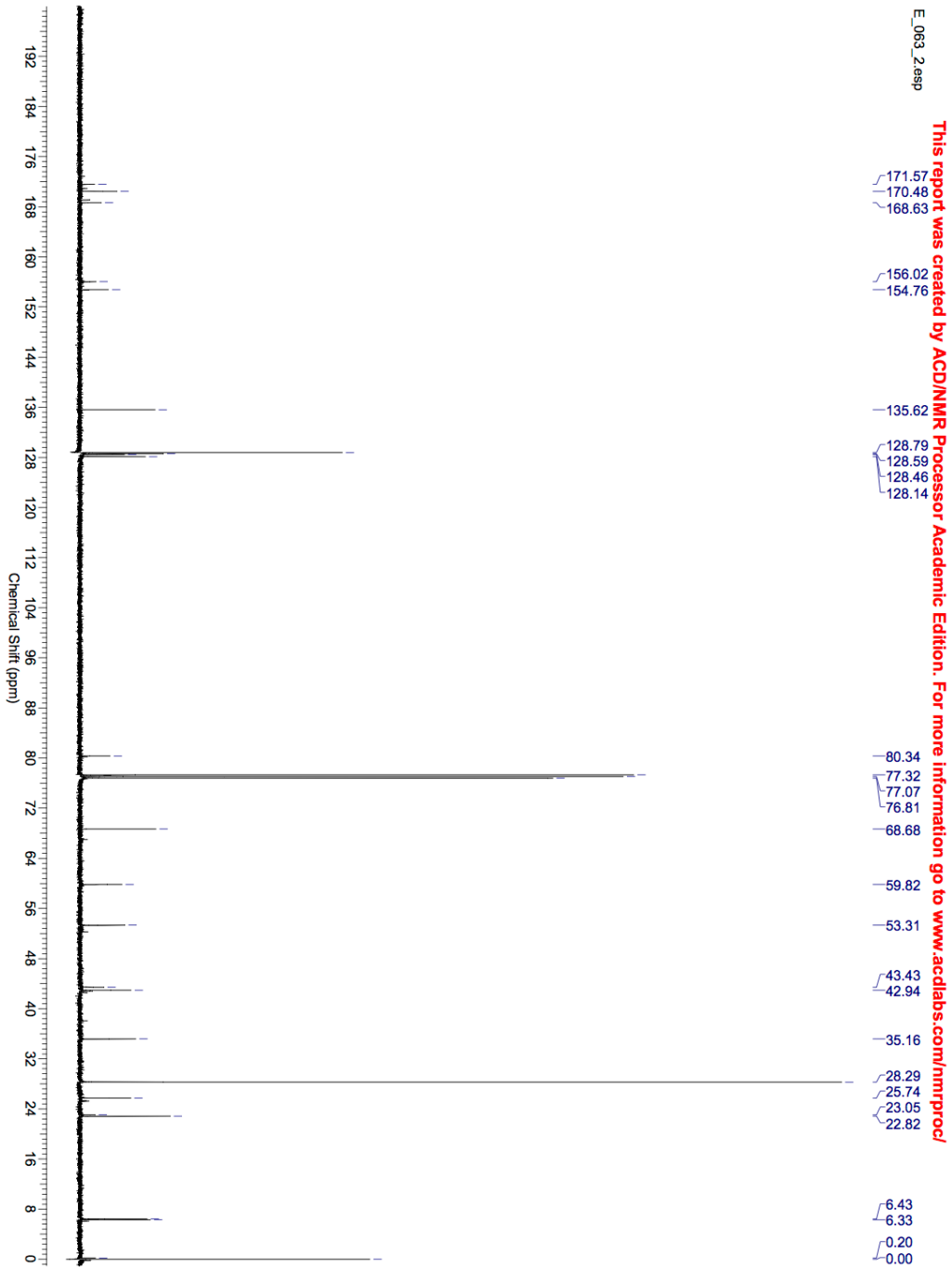
Chemical Shift (ppm)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77.07
76.81
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53.31
43.43
42.94
35.16
28.29
25.74
23.05
22.82
6.43
6.33
0.20
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
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32
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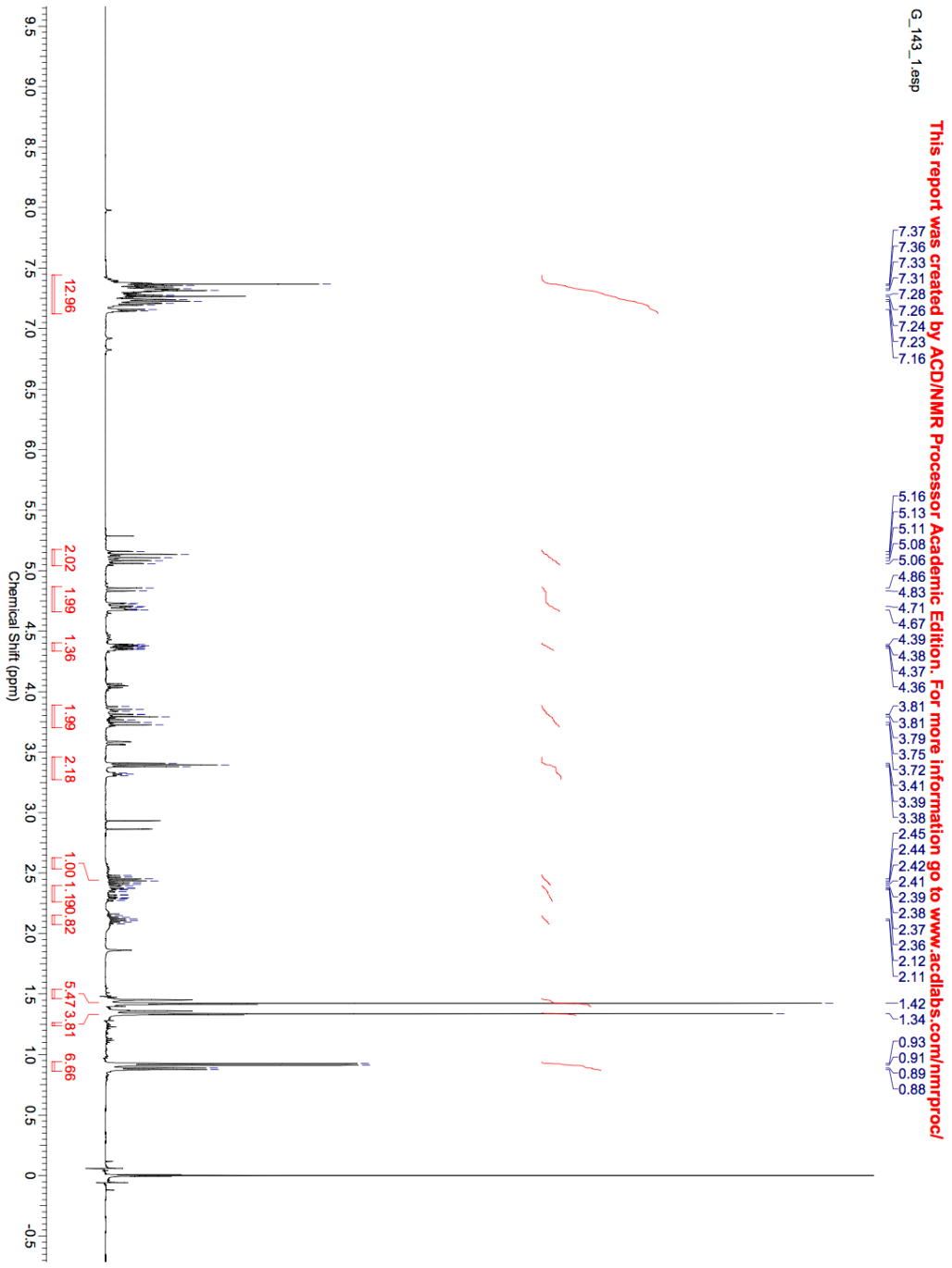
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/


This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
G_143_1.esp
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7.28
7.26
7.24
7.23
7.16
5.16
5.13
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5.08
5.06
4.86
4.83
4.71
4.67
4.39
4.38
4.37
4.36
3.81
3.81
3.79
3.75
3.72
3.41
3.39
3.38
2.45
2.44
2.42
2.41
2.39
2.38
2.37
2.36
2.12
2.11
1.42
1.34
0.93
0.91
0.89
0.88
12.96
2.02
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1.99
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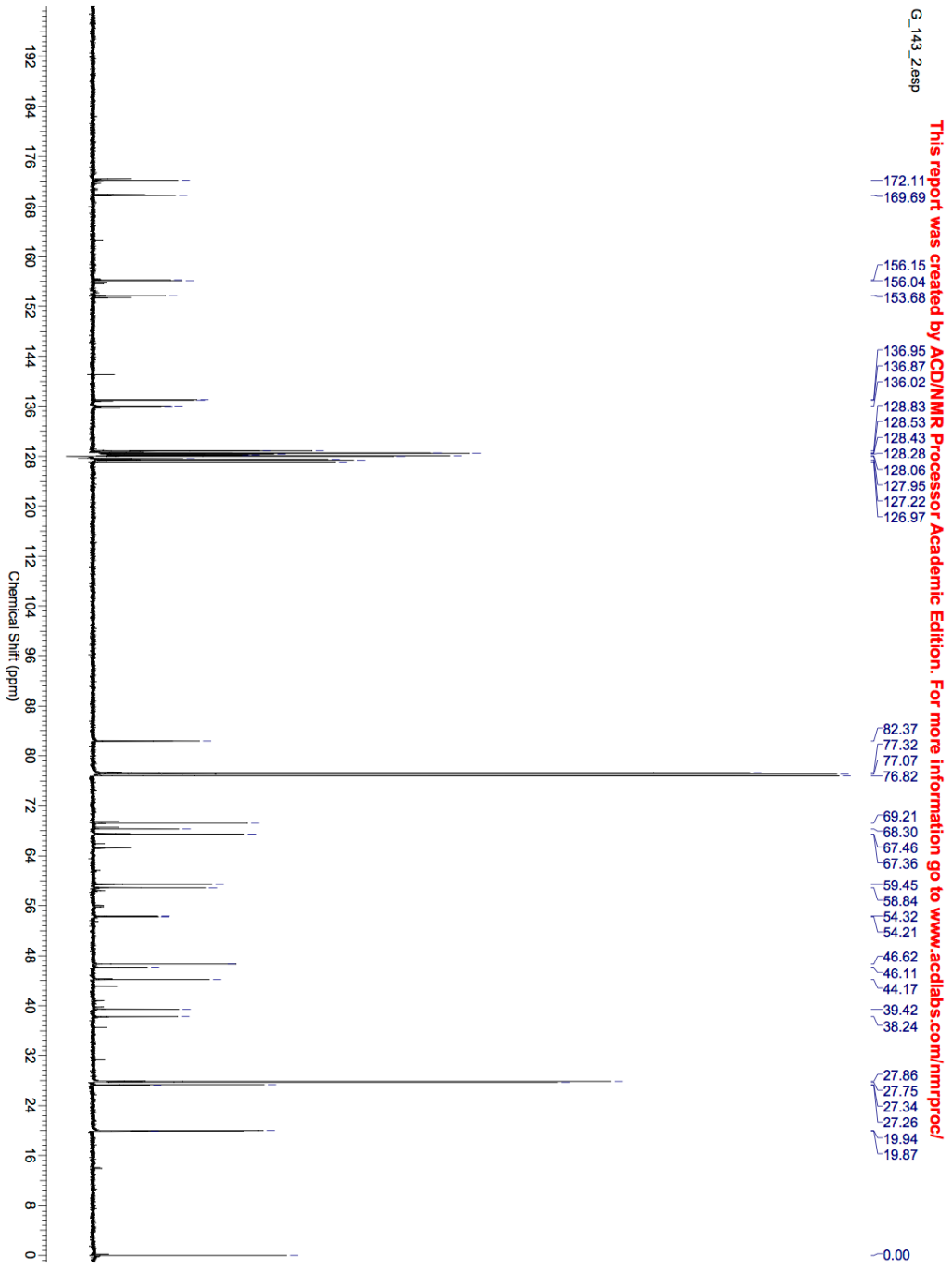
0.82
5.47
3.81
6.66
Chemical Shift (ppm)


G_143_2.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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156.15
156.04
153.68
136.95
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136.02
128.83
128.53
128.43
128.28
128.06
127.95
127.22
126.97
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77.32
77.07
76.82
69.21
68.30
67.46
67.36
59.45
58.84
54.32
54.21
46.62
46.11
44.17
39.42
38.24
27.86
27.75
27.34
27.26
19.94
19.87
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
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88
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72
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56
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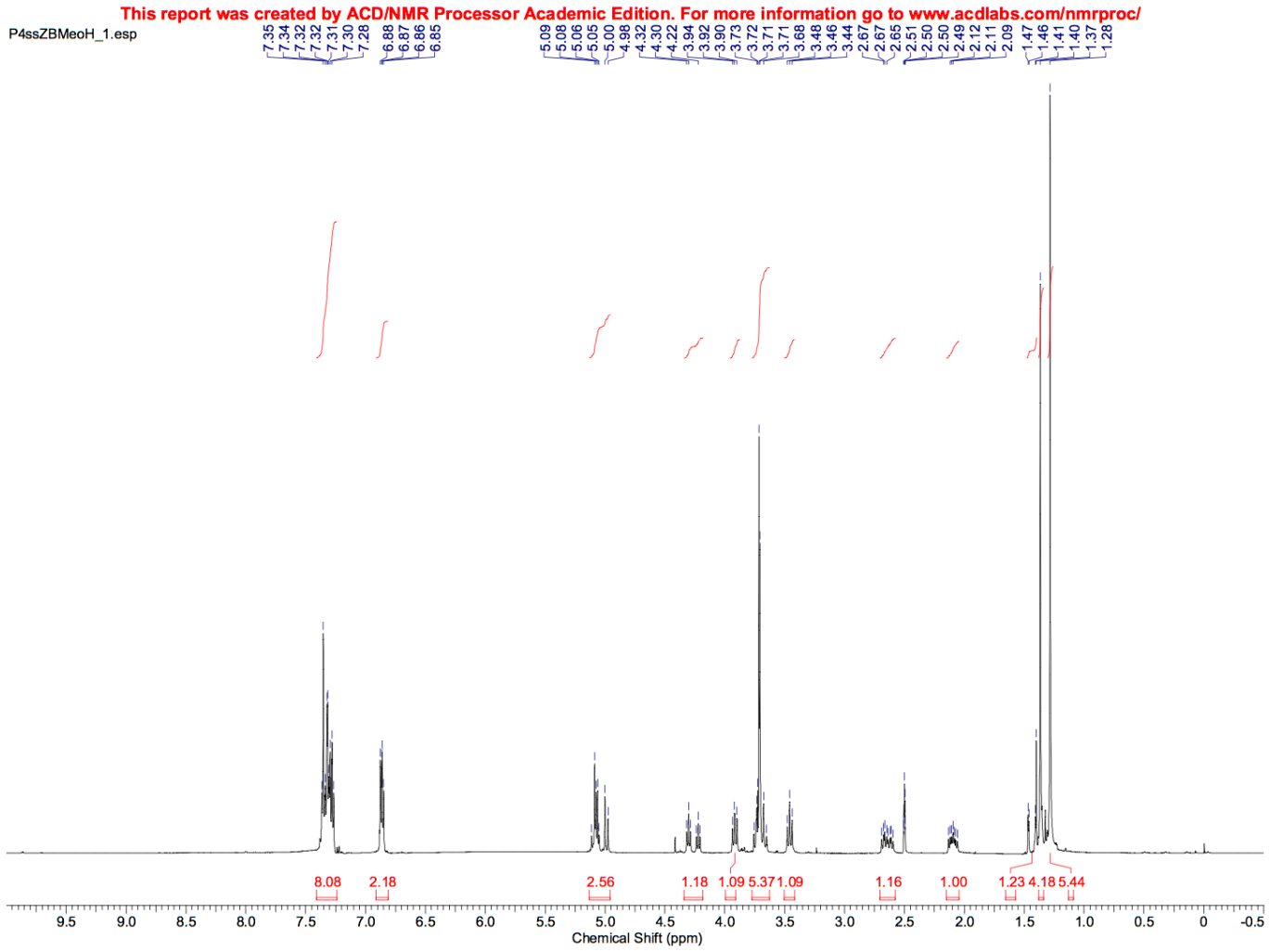
40
32
24
16
8
0


This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
P4ssZBMeoH_1.esp
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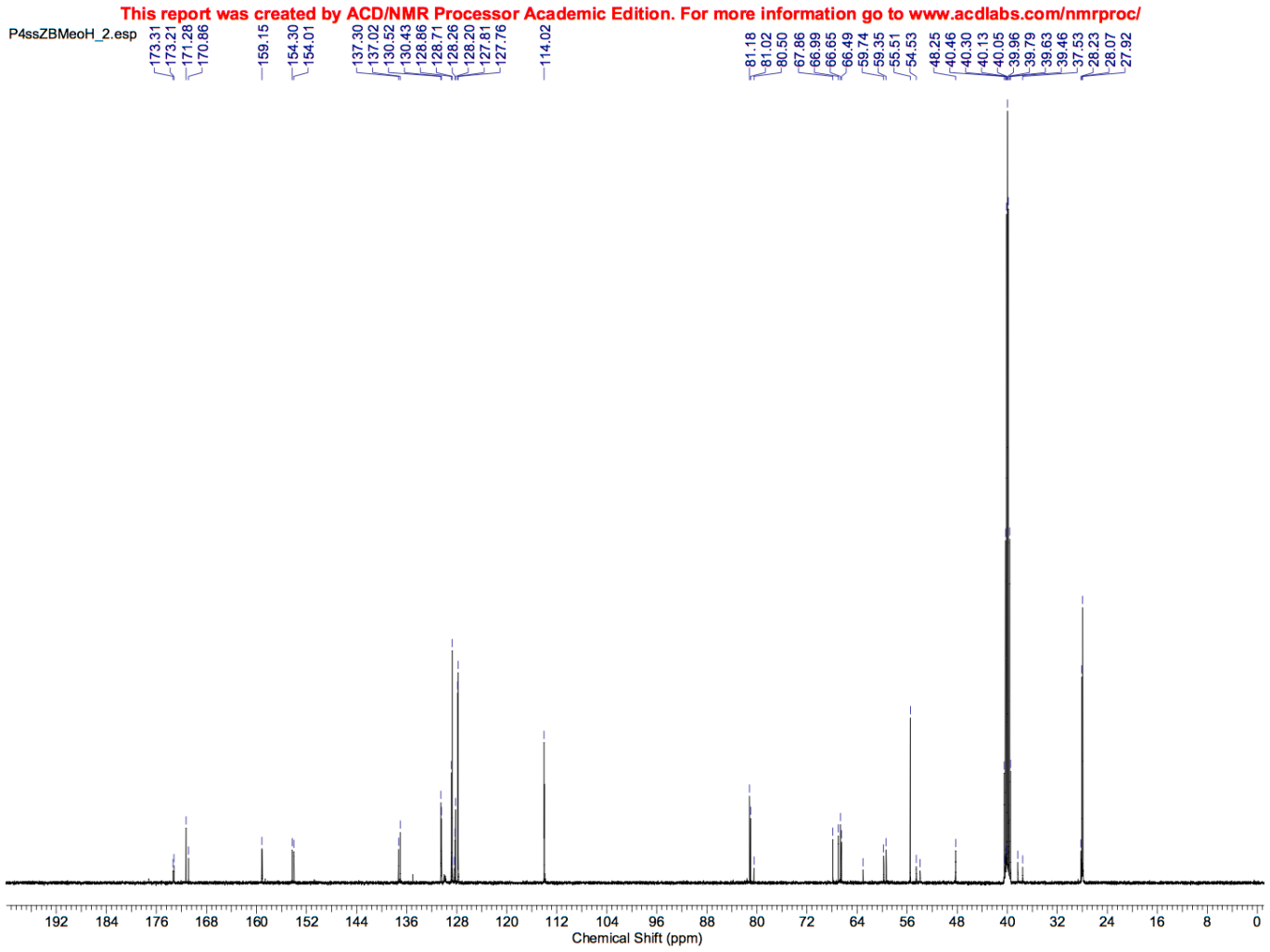
6.88 6.87 6.86 6.85
5.09 5.08 5.06 5.05 5.00 4.98
4.32 4.30 4.22 3.94 3.92 3.90 3.73 3.72 3.71 3.71 3.68 3.48 3.46 3.44
2.67 2.67 2.65 2.51 2.50 2.50 2.49 2.12 2.11 2.09
1.47 1.46 1.41 1.40 1.37 1.28
8.08 2.18 2.56 1.18 1.09 5.37 1.09 1.16 1.00 1.23 4.18 5.44
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0 -0.5
Chemical Shift (ppm)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P4ssZBMeoH_2.esp
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170.86
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154.30
154.01
137.30
137.02
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130.43
128.86
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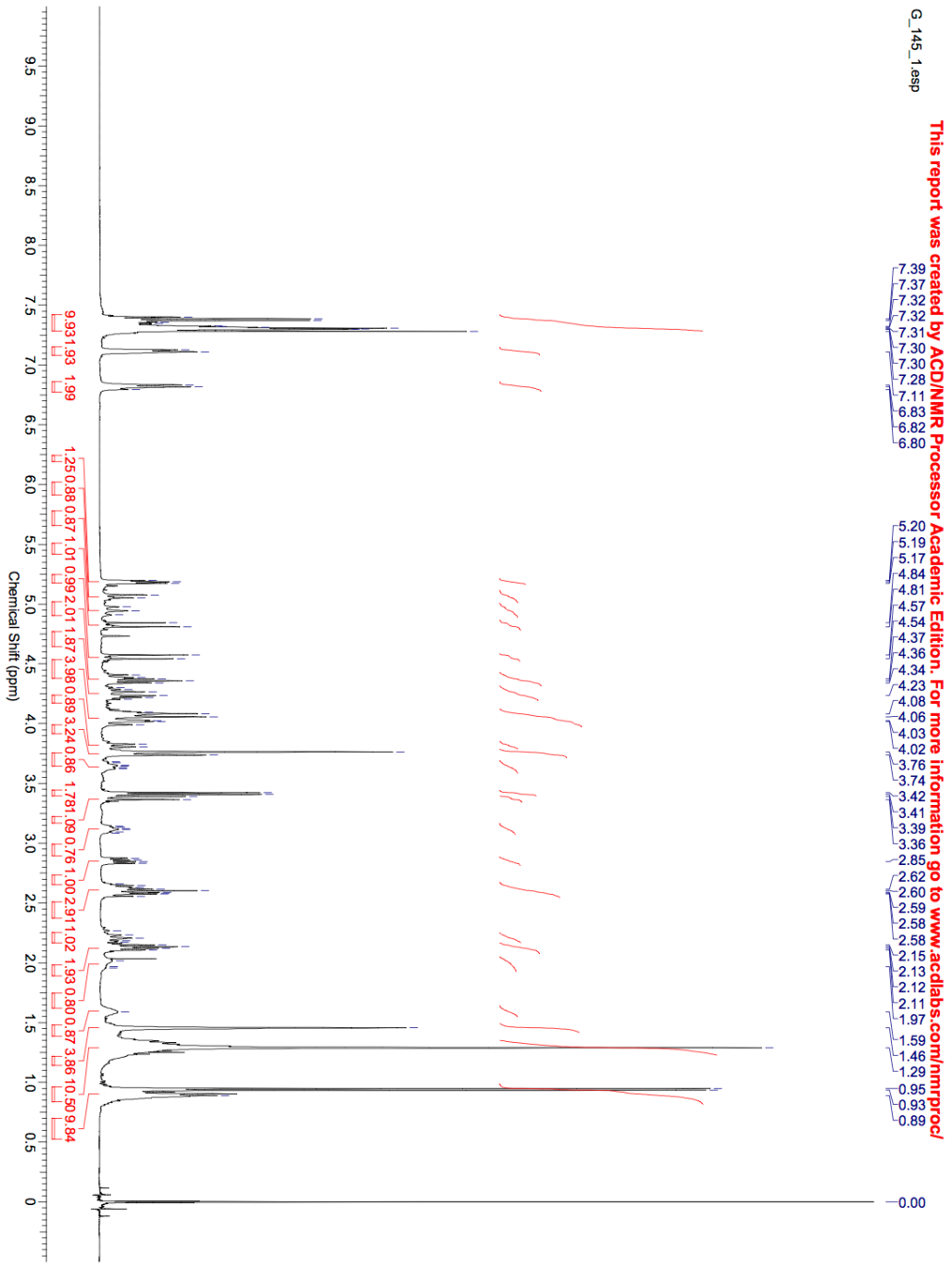
128.26
128.20
127.81
127.76
114.02
81.18
81.02
80.50
67.86
66.99
66.65
66.49
59.74
59.35
55.51
54.53
48.25
40.46
40.30
40.13
40.05
39.96
39.79
39.63
39.46
37.53
28.23
28.07
27.92
192 184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 16 8 0
Chemical Shift (ppm)


G_145_1.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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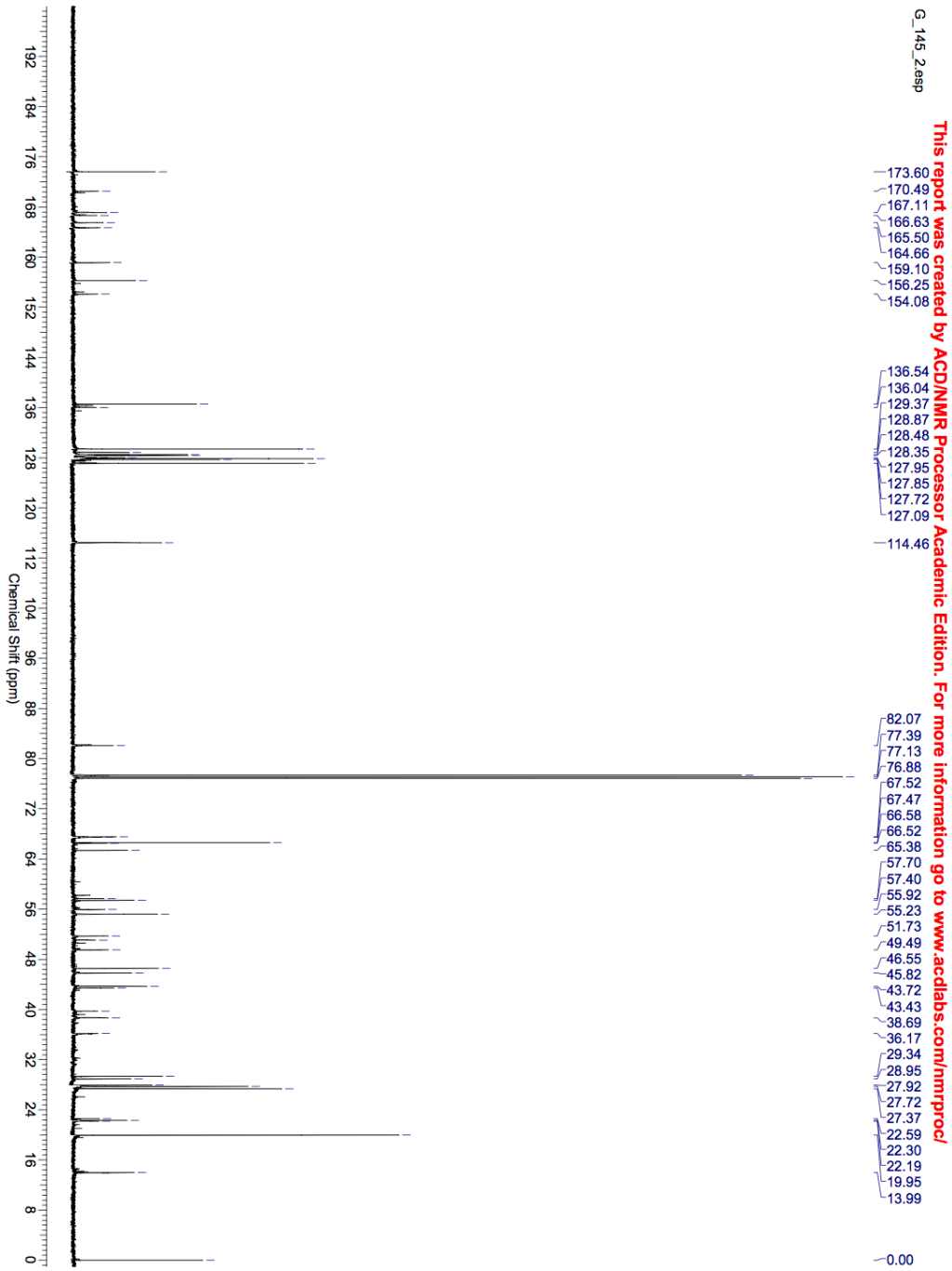
G_145_2.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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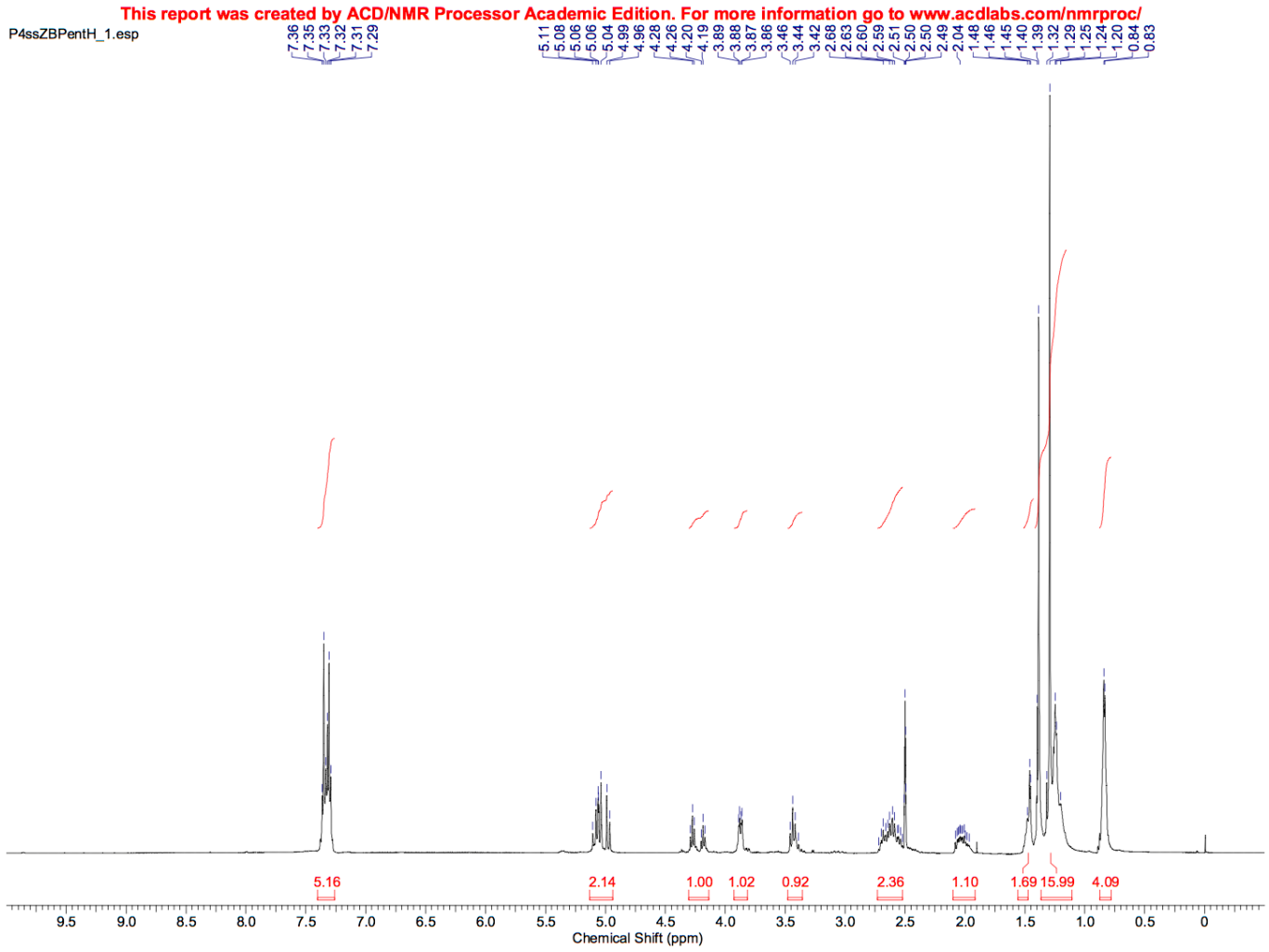
159.10
156.25
154.08
136.54
136.04
129.37
128.87
128.48
128.35
127.95
127.85
127.72
127.09
114.46
82.07
77.39
77.13
76.88
67.52
67.47
66.58
66.52
65.38
57.70
57.40
55.92
55.23
51.73
49.49
46.55
45.82
43.72
43.43
38.69
36.17
29.34
28.95
27.92
27.72
27.37
22.59
22.30
22.19
19.95
13.99
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0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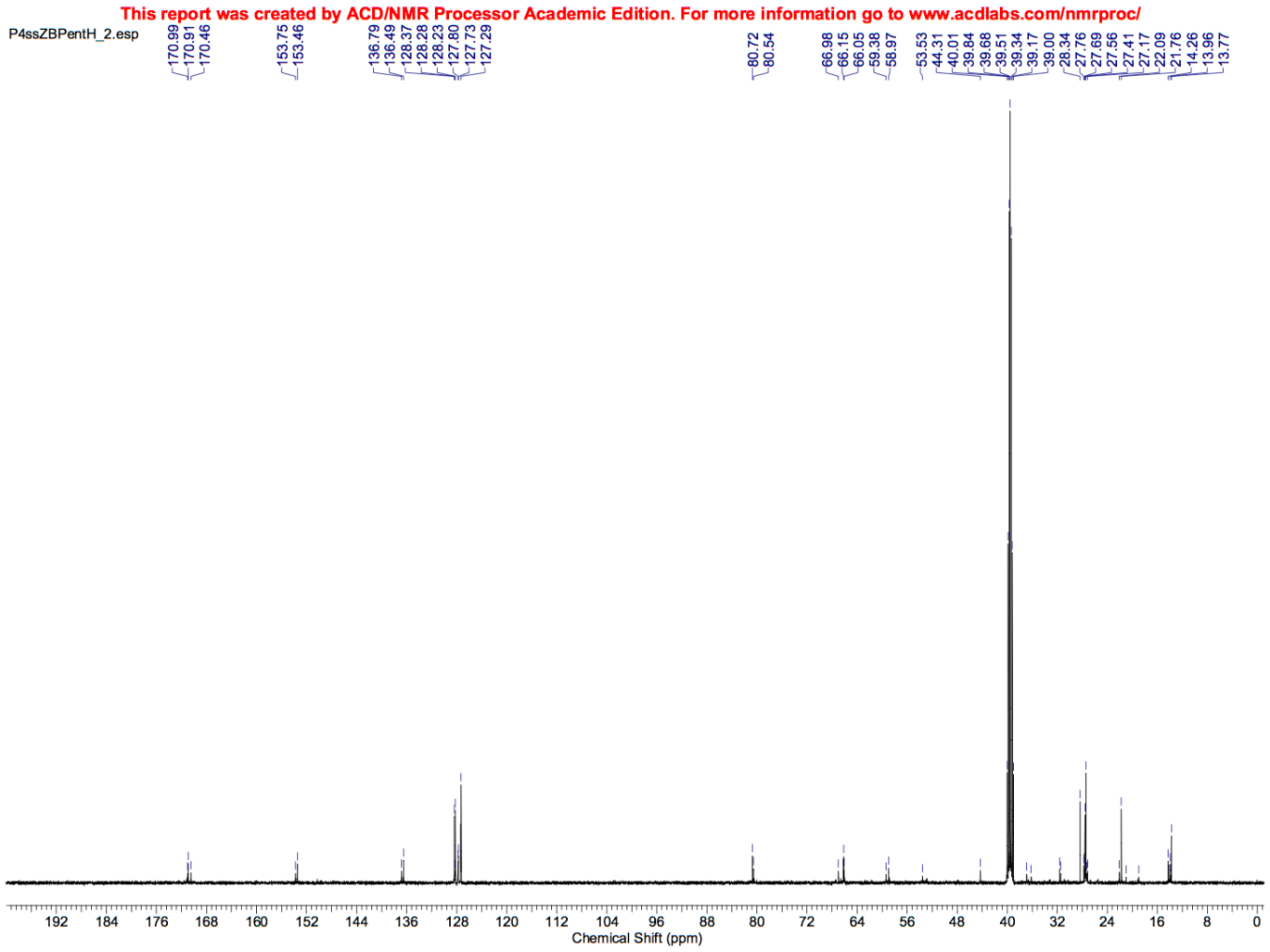
5.11 5.08 5.06 5.06 5.04 4.99 4.96 4.28 4.26 4.20 4.19 3.89 3.88 3.87 3.86 3.46 3.44 3.42 2.68 2.63 2.60 2.59 2.51 2.50 2.50 2.49 2.04 1.48 1.46 1.45 1.40 1.39 1.32 1.29 1.25 1.24 1.20 0.84 0.83
5.16 2.14 1.00 1.02 0.92 2.36 1.10 1.69 15.99 4.09
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
Chemical Shift (ppm)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128.28
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127.80
127.73
127.29
80.72
80.54
66.98
66.15
66.05
59.38
58.97
53.53
44.31
40.01
39.84
39.68
39.51
39.34
39.17
39.00
28.34
27.76
27.69
27.56
27.41
27.17
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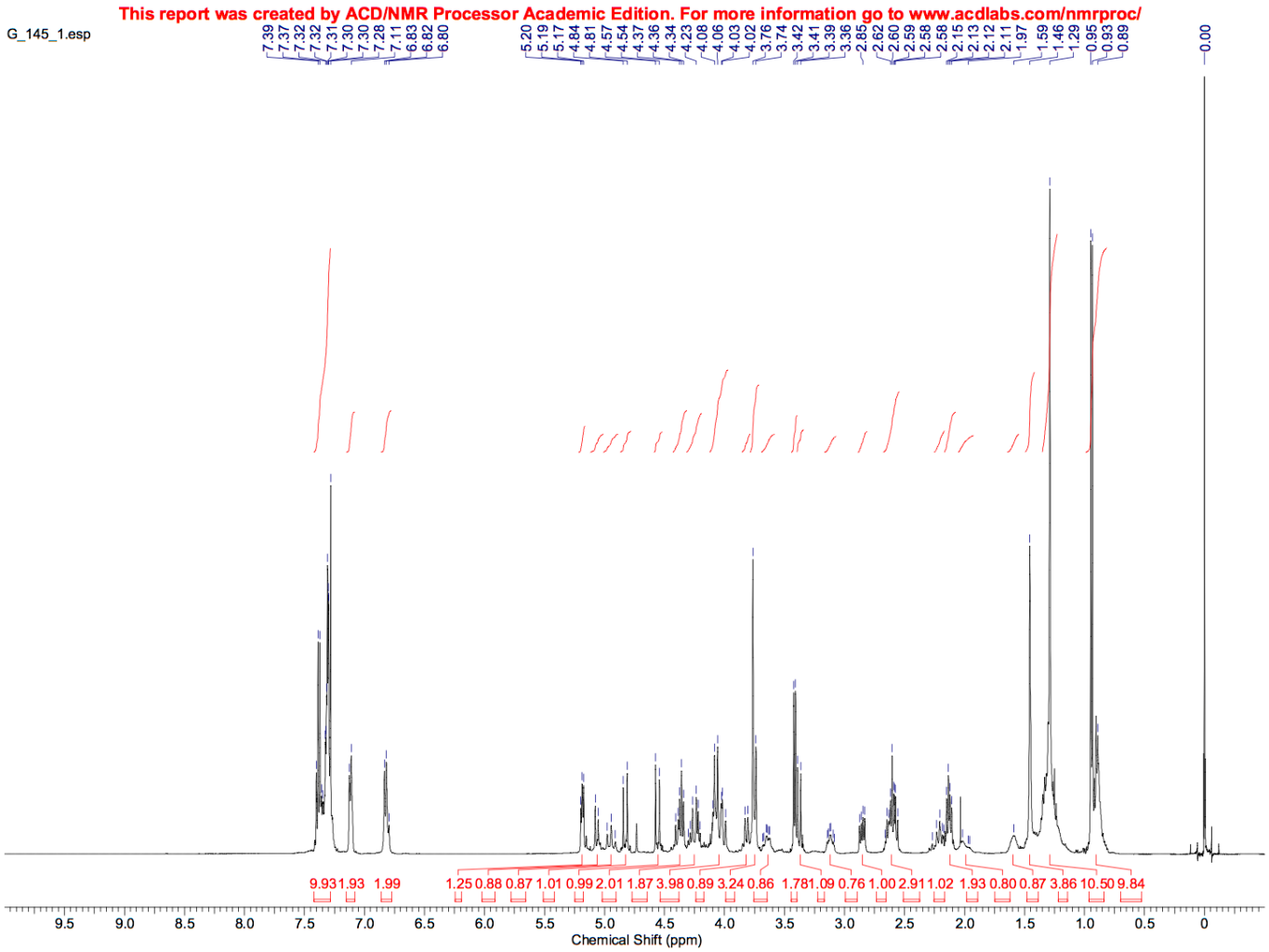
21.76
14.26
13.96
13.77
192 184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 16 8 0
Chemical Shift (ppm)


This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
G_145_1.esp
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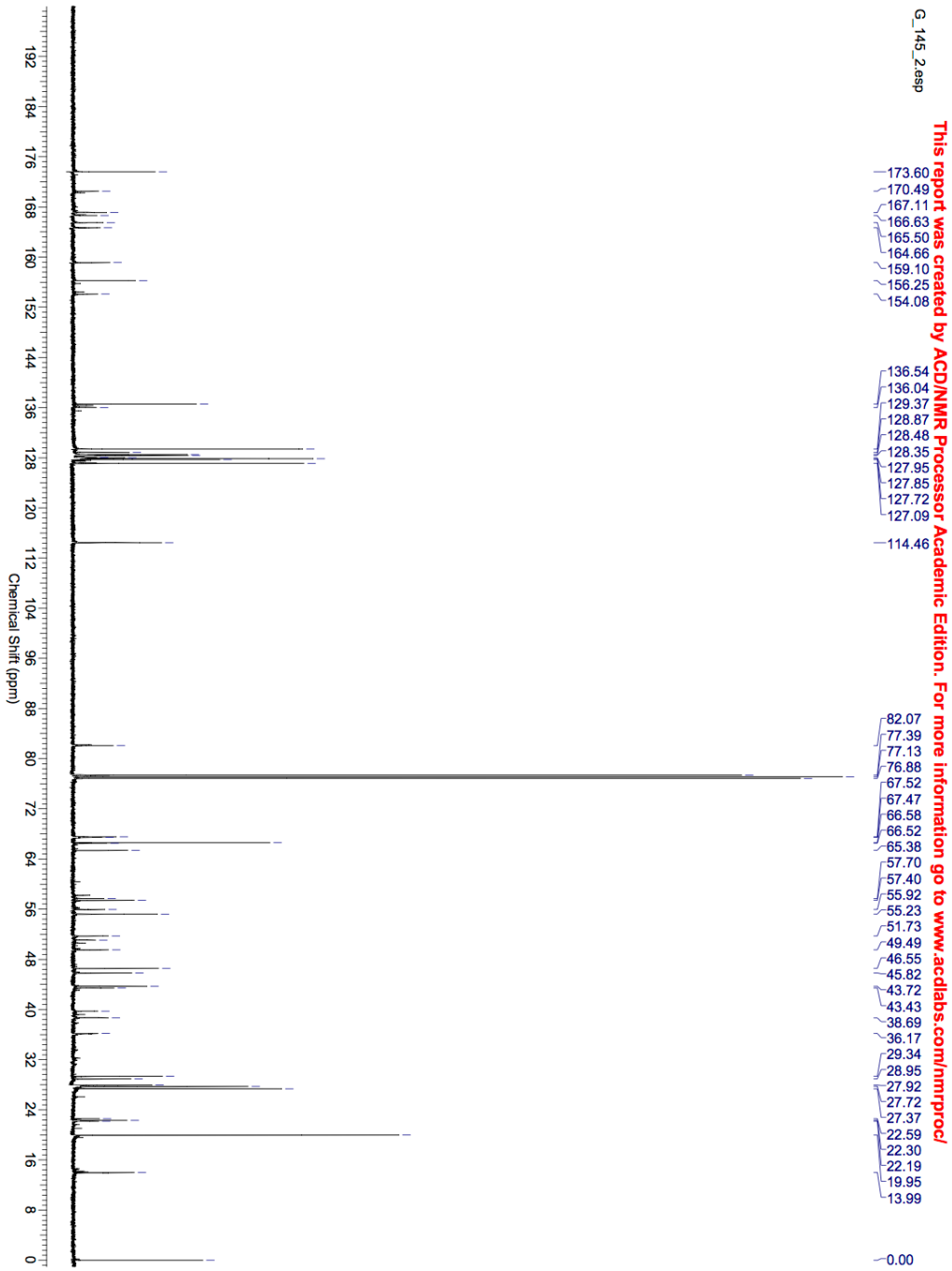
5.20 5.19 5.17 4.84 4.81 4.57 4.54 4.37 4.36 4.34 4.23 4.08 4.06 4.03 4.02 3.76 3.74 3.42 3.41 3.39 3.36 2.85 2.62 2.60 2.59 2.58 2.58 2.15 2.13 2.12 2.11 1.97 1.59 1.46 1.29 0.95 0.93 0.89
0.00
9.93 1.93 1.99 1.25 0.88 0.87 1.01 0.99 2.01 1.87 3.98 0.89 3.24 0.86 1.78 1.09 0.76 1.00 2.91 1.02 1.93 0.80 0.87 3.86 10.50 9.84
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
Chemical Shift (ppm)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This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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165.50
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136.04
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128.35
127.95
127.85
127.72
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77.39
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67.52
67.47
66.58
66.52
65.38
57.70
57.40
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45.82
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43.43
38.69
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28.95
27.92
27.72
27.37
22.59
22.30
22.19
19.95
13.99
0.00
Chemical Shift (ppm)
192
184
176
168
160
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128
120
112
104
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88
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72
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56
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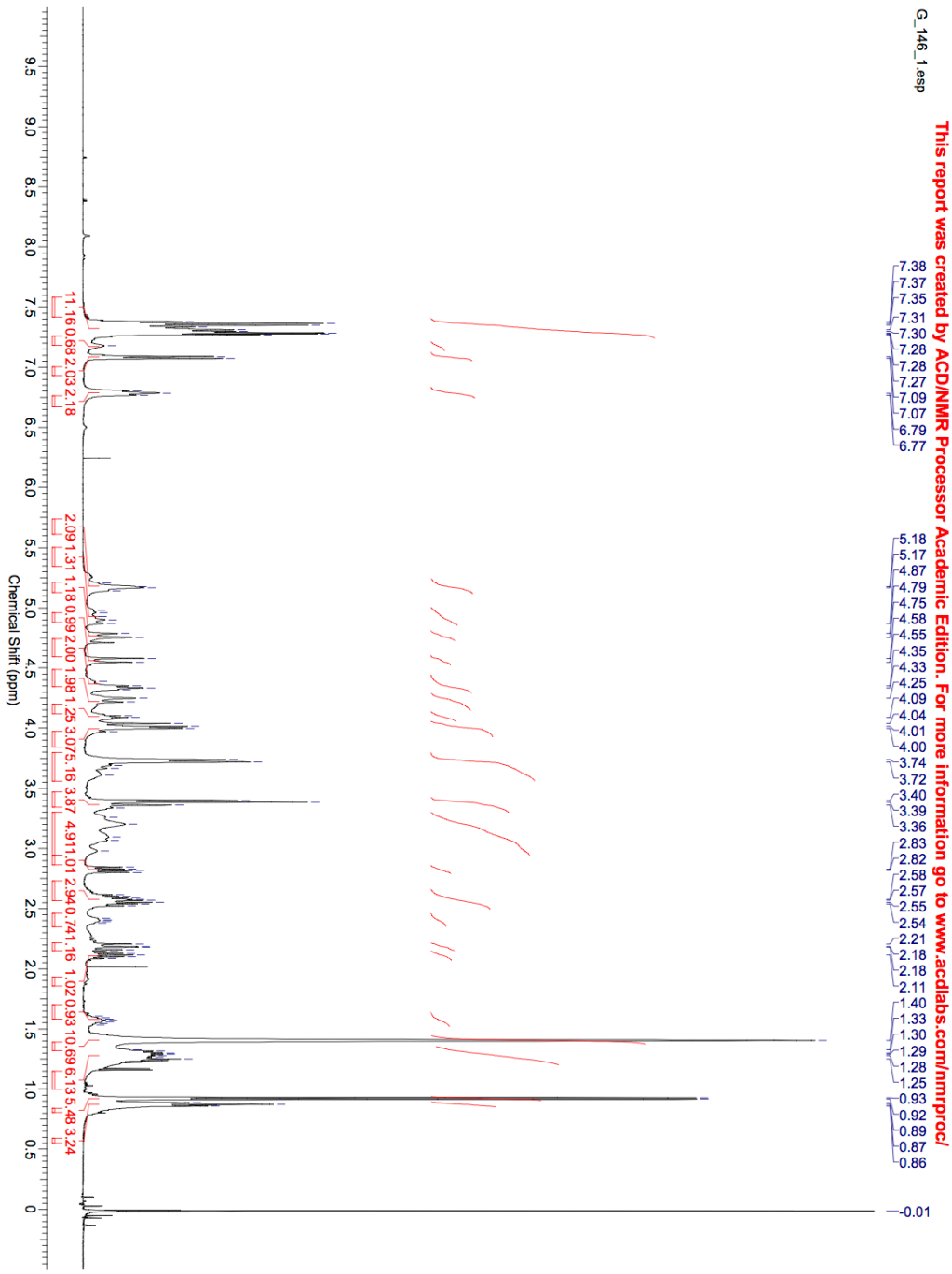
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This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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4.75
4.58
4.55
4.35
4.33
4.25
4.09
4.04
4.01
4.00
3.74
3.72
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3.39
3.36
2.83
2.82
2.58
2.57
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2.54
2.21
2.18
2.18
2.11
1.40
1.33
1.30
1.29
1.28
1.25
0.93
0.92
0.89
0.87
0.86
-0.01
11.16 0.68 2.03 2.18
2.09 1.31 1.18 0.99 2.00 1.98 1.25 3.07 5.16 3.87 4.91 1.01 2.94 0.74 1.16 1.02 0.93 10.69 6.13 5.48 3.24
Chemical Shift (ppm)
9.5
9.0
8.5
8.0
7.5
7.0
6.5
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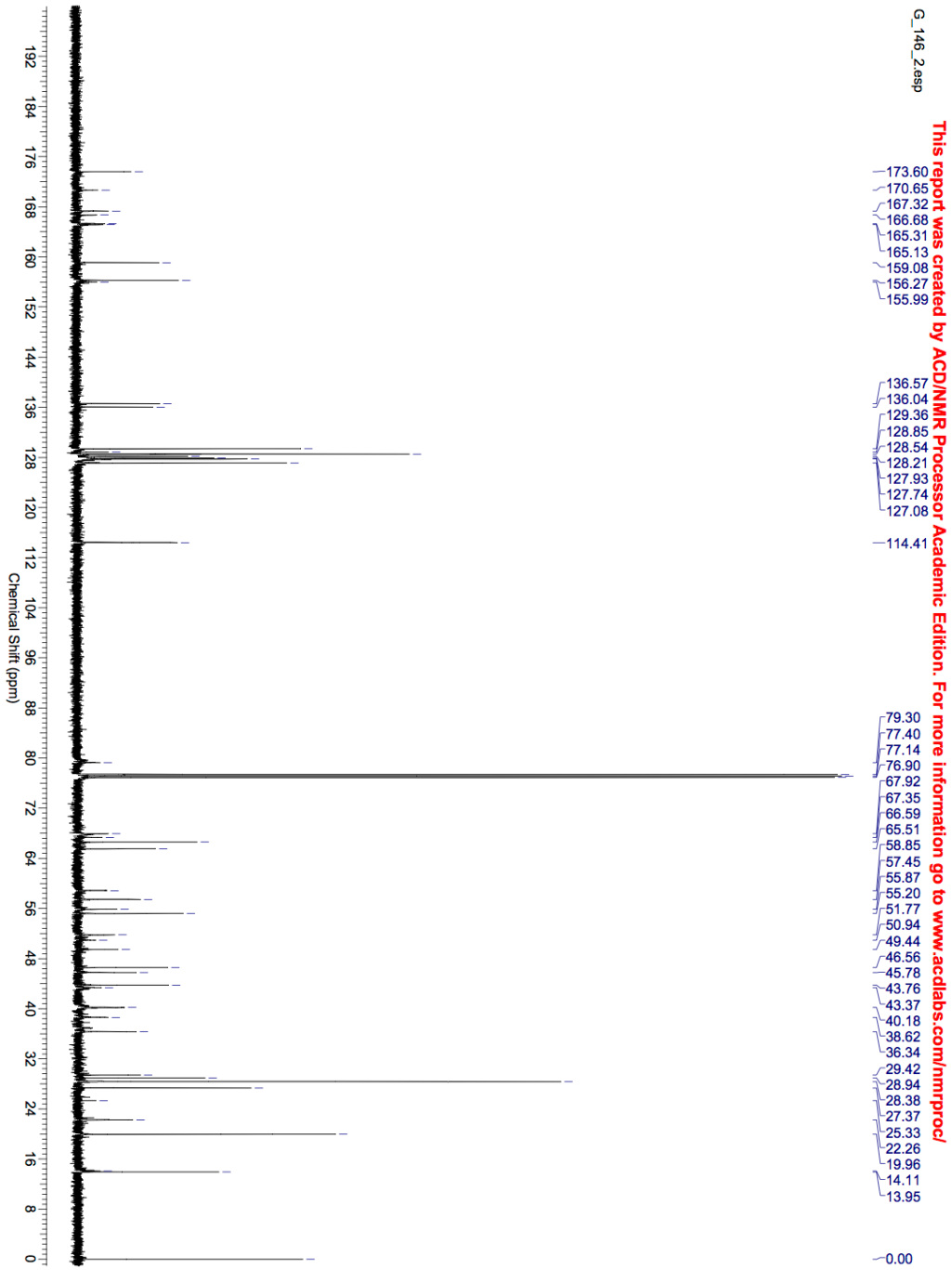
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136.04
129.36
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128.54
128.21
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127.74
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114.41
79.30
77.40
77.14
76.90
67.92
67.35
66.59
65.51
58.85
57.45
55.87
55.20
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50.94
49.44
46.56
45.78
43.76
43.37
40.18
38.62
36.34
29.42
28.94
28.38
27.37
25.33
22.26
19.96
14.11
13.95
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
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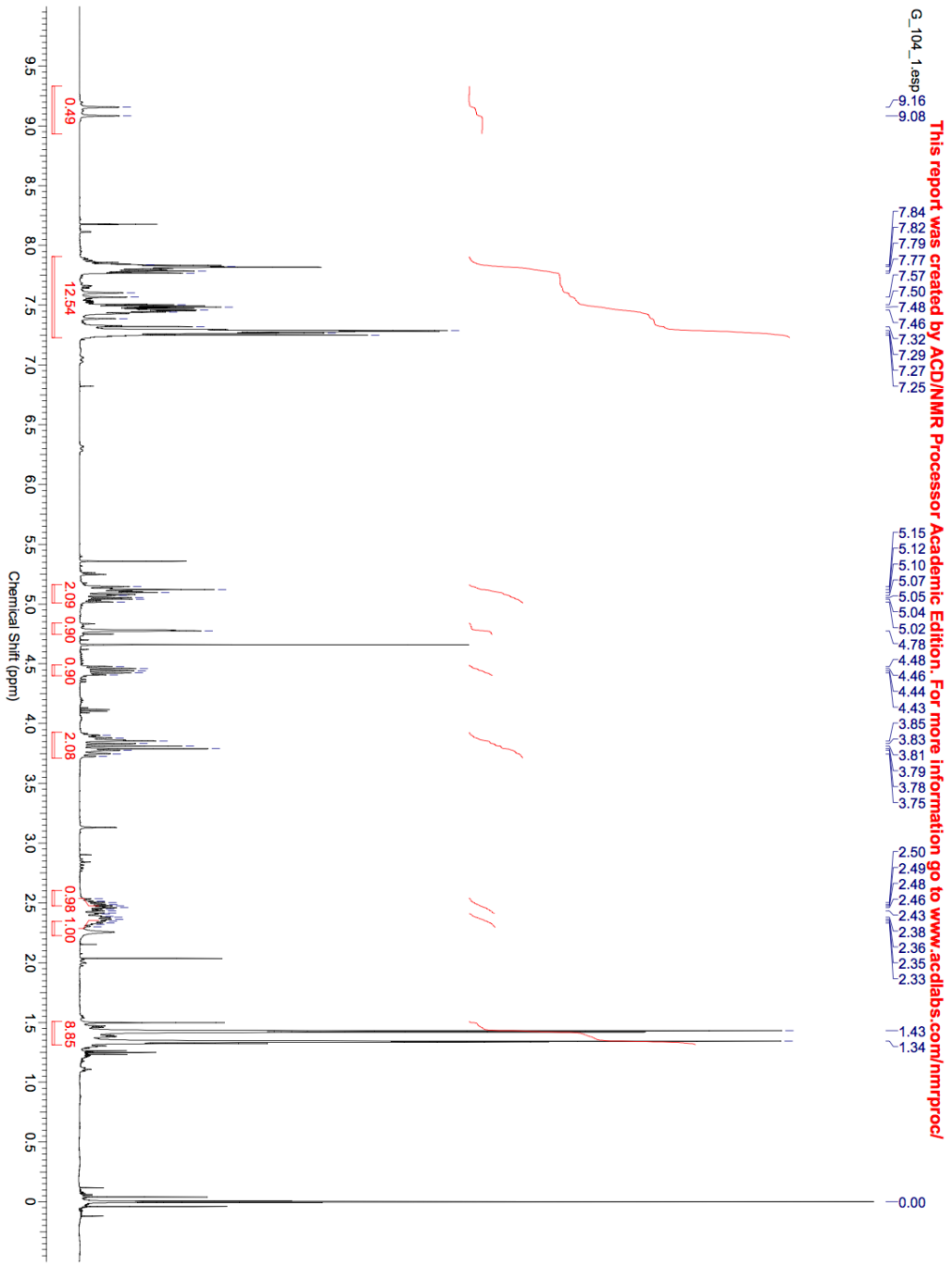
16
8
0


G_104_1.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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7.79
7.77
7.57
7.50
7.48
7.46
7.32
7.29
7.27
7.25
5.15
5.12
5.10
5.07
5.05
5.04
5.02
4.78
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4.46
4.44
4.43
3.85
3.83
3.81
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3.78
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2.46
2.43
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2.36
2.35
2.33
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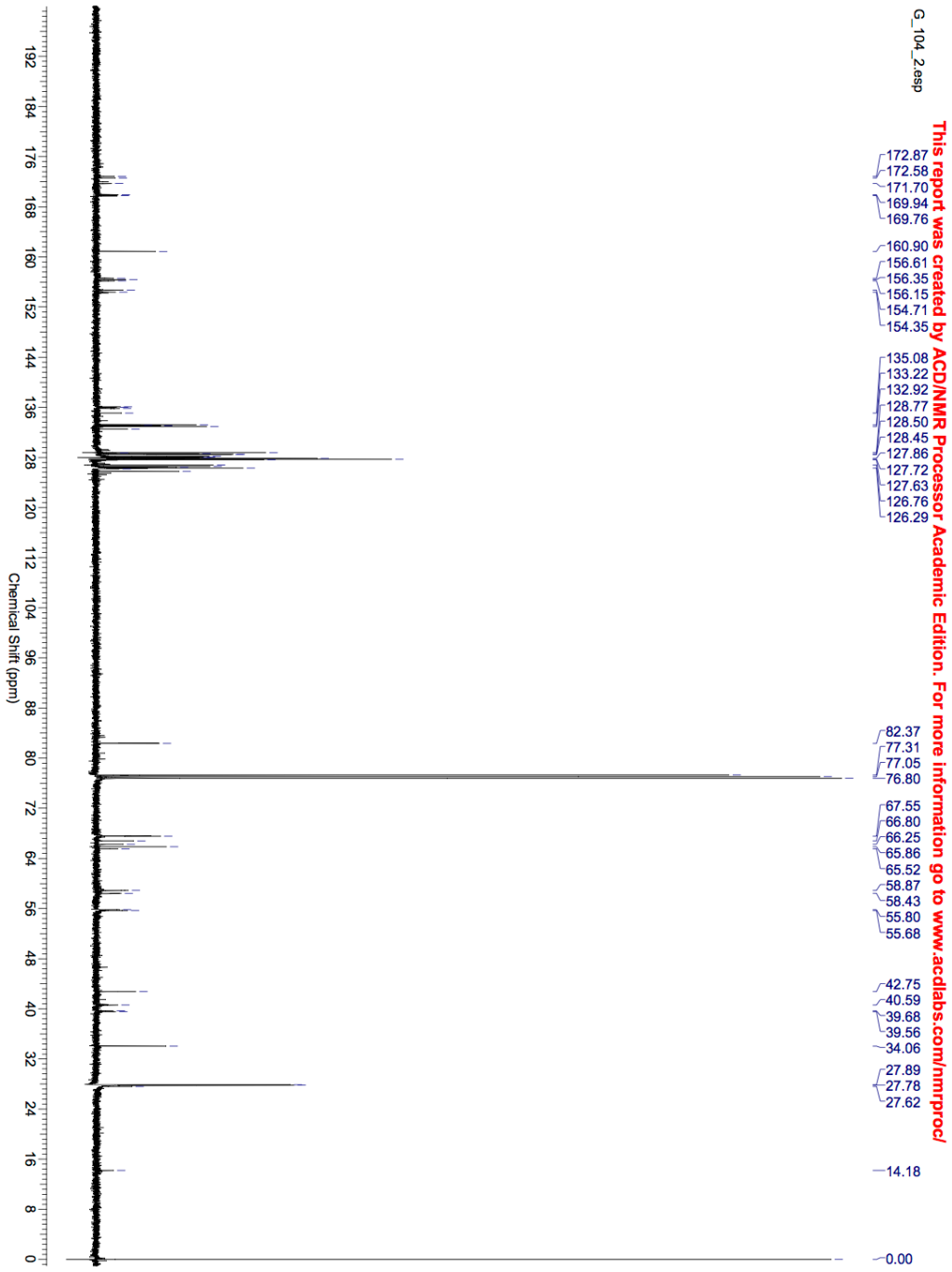
8.85


G_104_2.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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126.76
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55.80
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42.75
40.59
39.68
39.56
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27.89
27.78
27.62
14.18
0.00
Chemical Shift (ppm)
192
184
176
168
160
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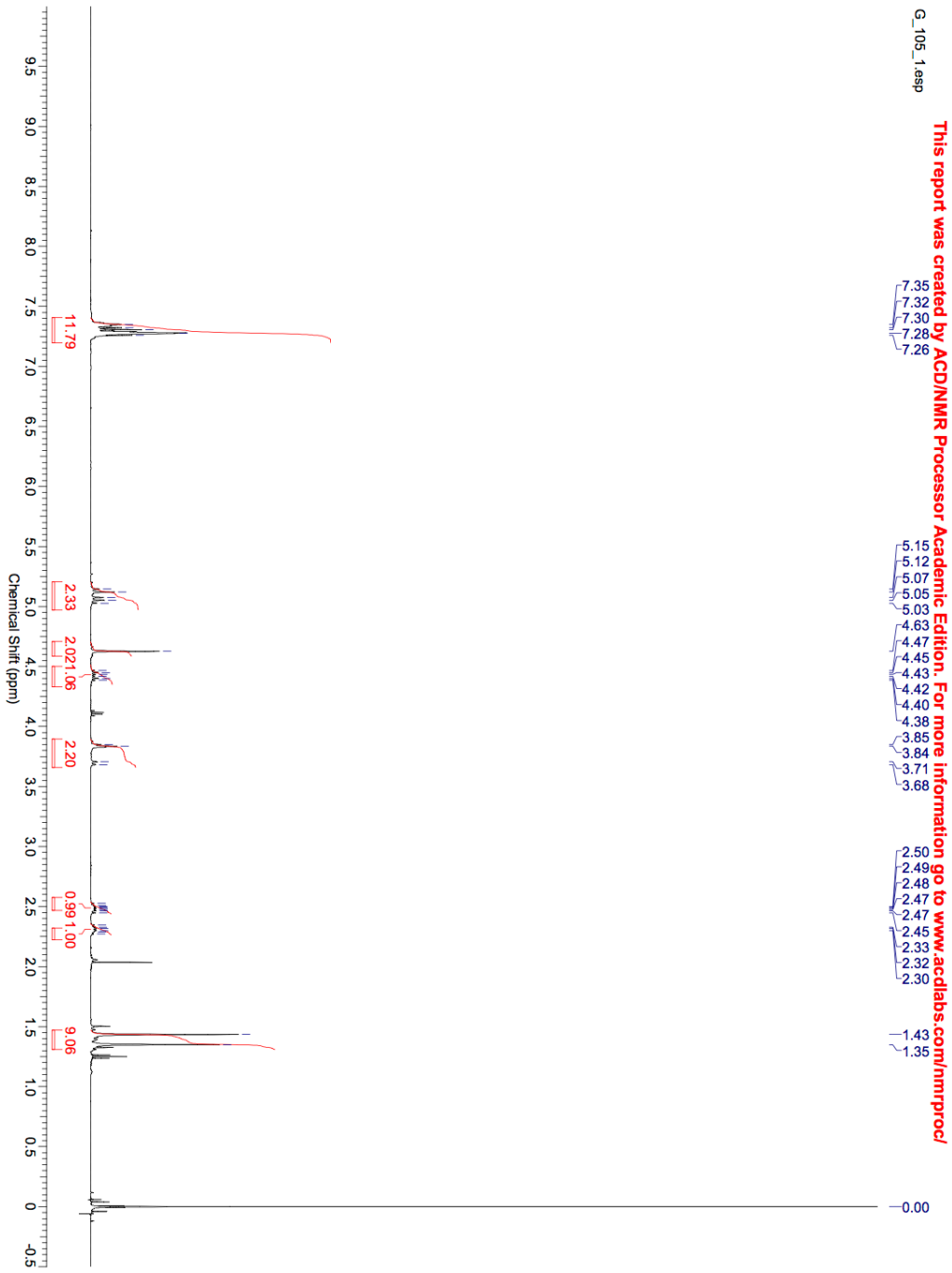
G_105_1.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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7.32
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7.28
7.26
5.15
5.12
5.07
5.05
5.03
4.63
4.47
4.45
4.43
4.42
4.40
4.38
3.85
3.84
3.71
3.68
2.50
2.49
2.48
2.47
2.47
2.45
2.33
2.32
2.30
1.43
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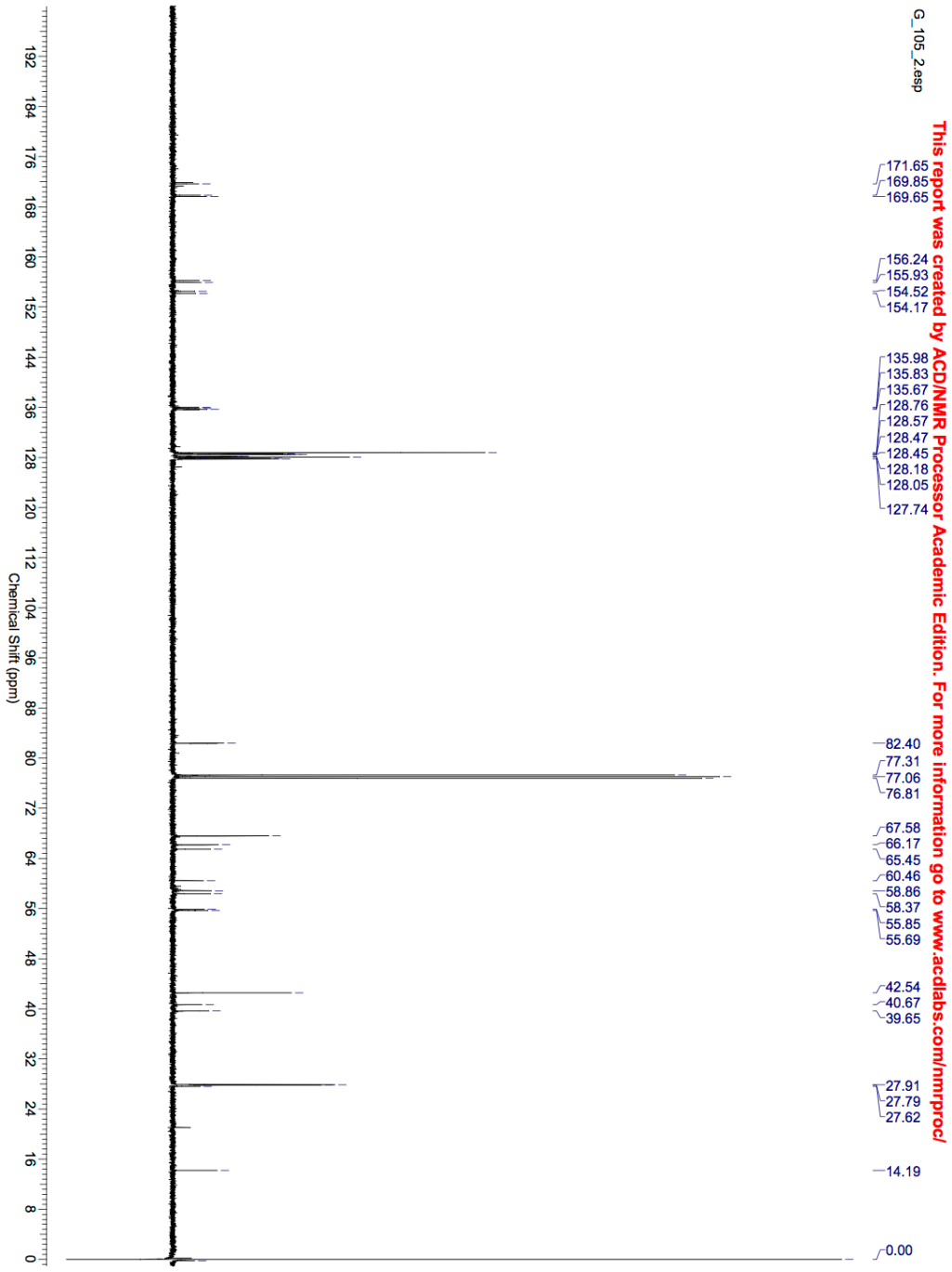
2.33
2.02
1.06
2.20
0.99
1.00
9.06
Chemical Shift (ppm)


G_105_2.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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169.85
169.65
156.24
155.93
154.52
154.17
135.98
135.83
135.67
128.76
128.57
128.47
128.45
128.18
128.05
127.74
82.40
77.31
77.06
76.81
67.58
66.17
65.45
60.46
58.86
58.37
55.85
55.69
42.54
40.67
39.65
27.91
27.79
27.62
14.19
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
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56
48
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32
24
16
8
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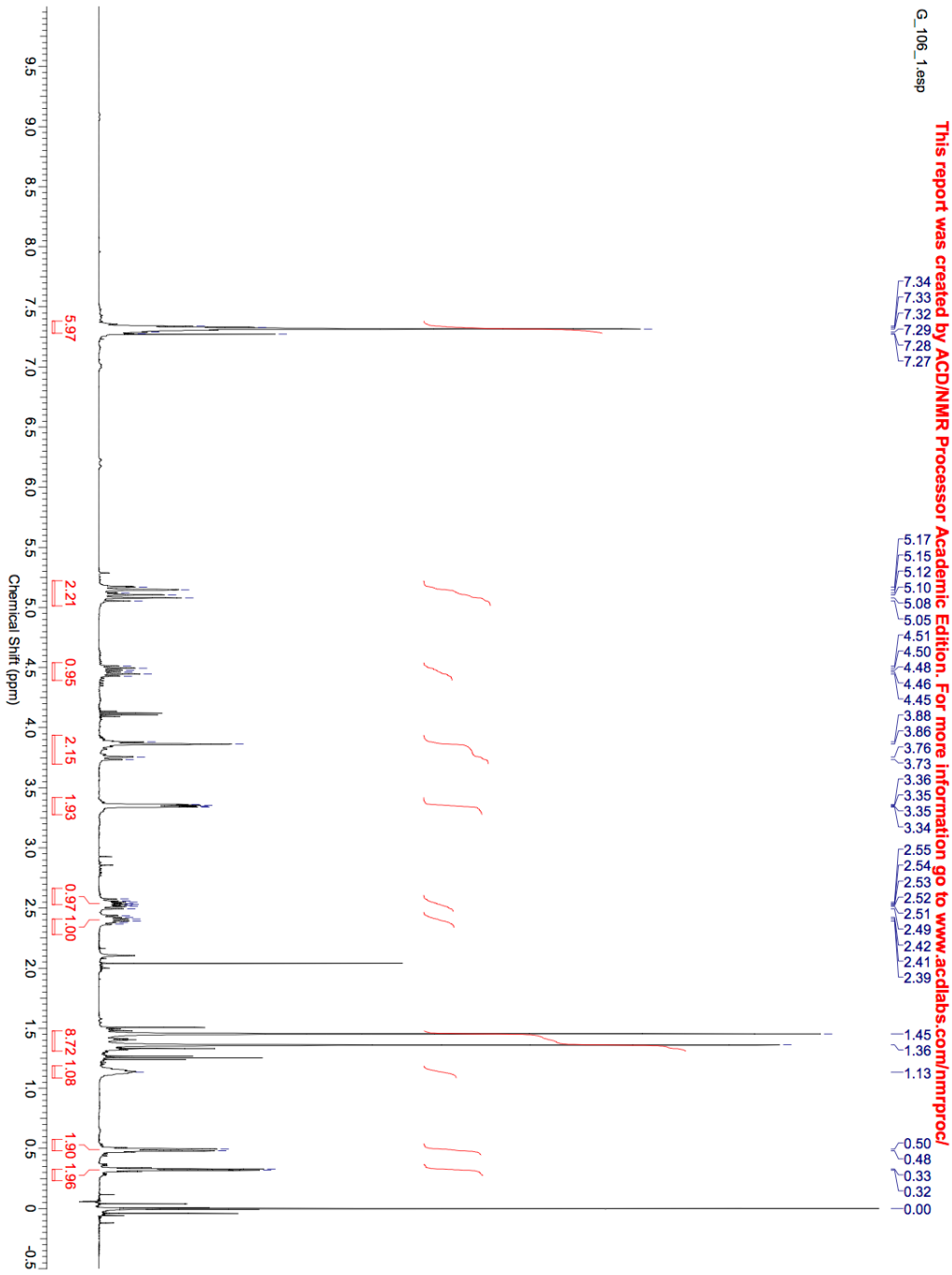
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This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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7.28
7.27
5.17
5.15
5.12
5.10
5.08
5.05
4.51
4.50
4.48
4.46
4.45
3.88
3.86
3.76
3.73
3.36
3.35
3.35
3.34
2.55
2.54
2.53
2.52
2.51
2.49
2.42
2.41
2.39
1.45
1.36
1.13
0.50
0.48
0.33
0.32
0.00
5.97
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2.15
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0.97
1.00
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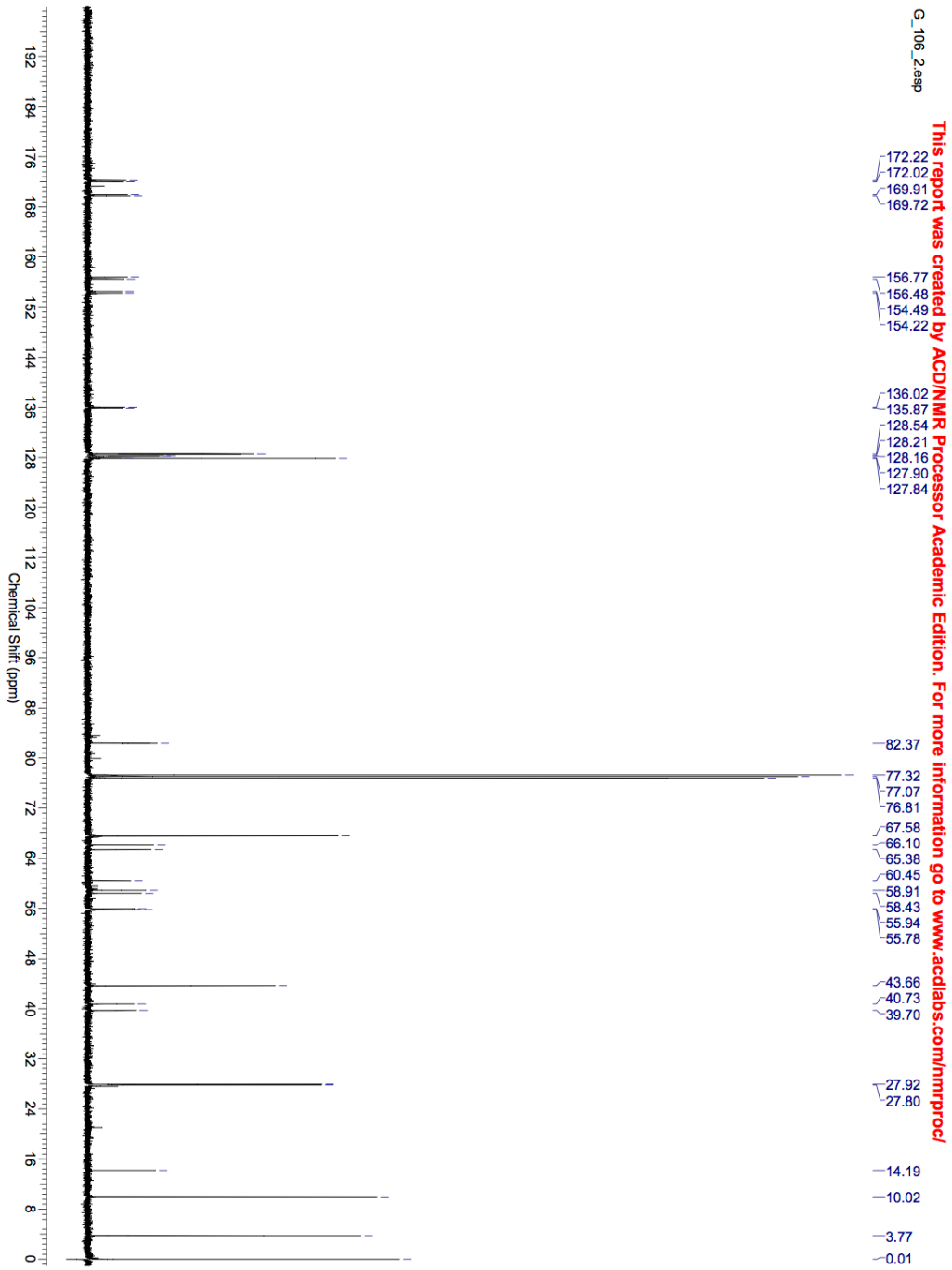
1.08
1.90
1.96
Chemical Shift (ppm)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This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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172.02
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156.77
156.48
154.49
154.22
136.02
135.87
128.54
128.21
128.16
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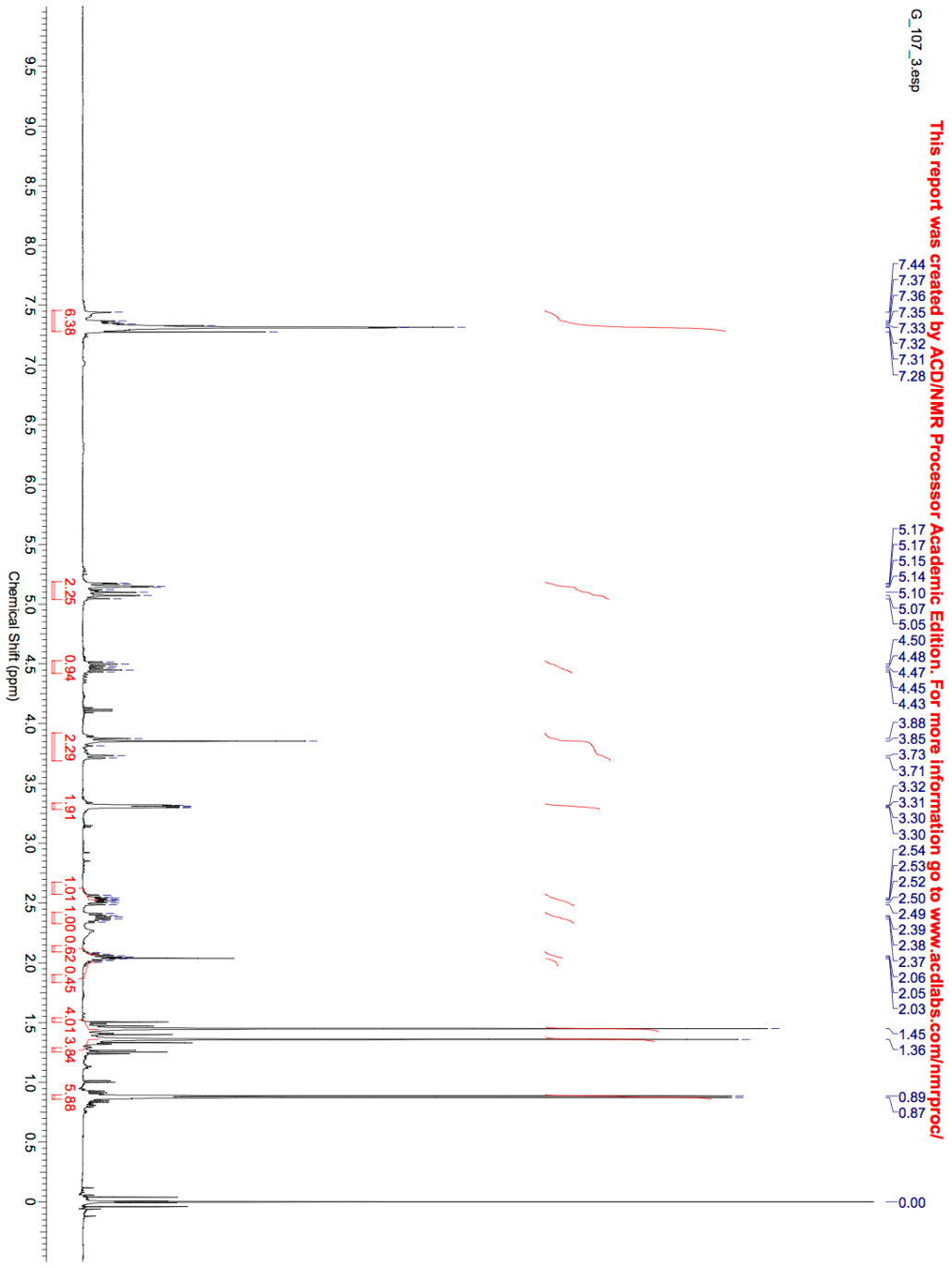
127.84
82.37
77.32
77.07
76.81
67.58
66.10
65.38
60.45
58.91
58.43
55.94
55.78
43.66
40.73
39.70
27.92
27.80
14.19
10.02
3.77
0.01
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0


G_107_3.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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7.37
7.36
7.35
7.33
7.32
7.31
7.28
5.17
5.17
5.15
5.14
5.10
5.07
5.05
4.50
4.48
4.47
4.45
4.43
3.88
3.85
3.73
3.71
3.32
3.31
3.30
3.30
2.54
2.53
2.52
2.50
2.49
2.39
2.38
2.37
2.06
2.05
2.03
1.45
1.36
0.89
0.87
0.00
6.38
2.25
0.94
2.29
1.91
1.01
1.00
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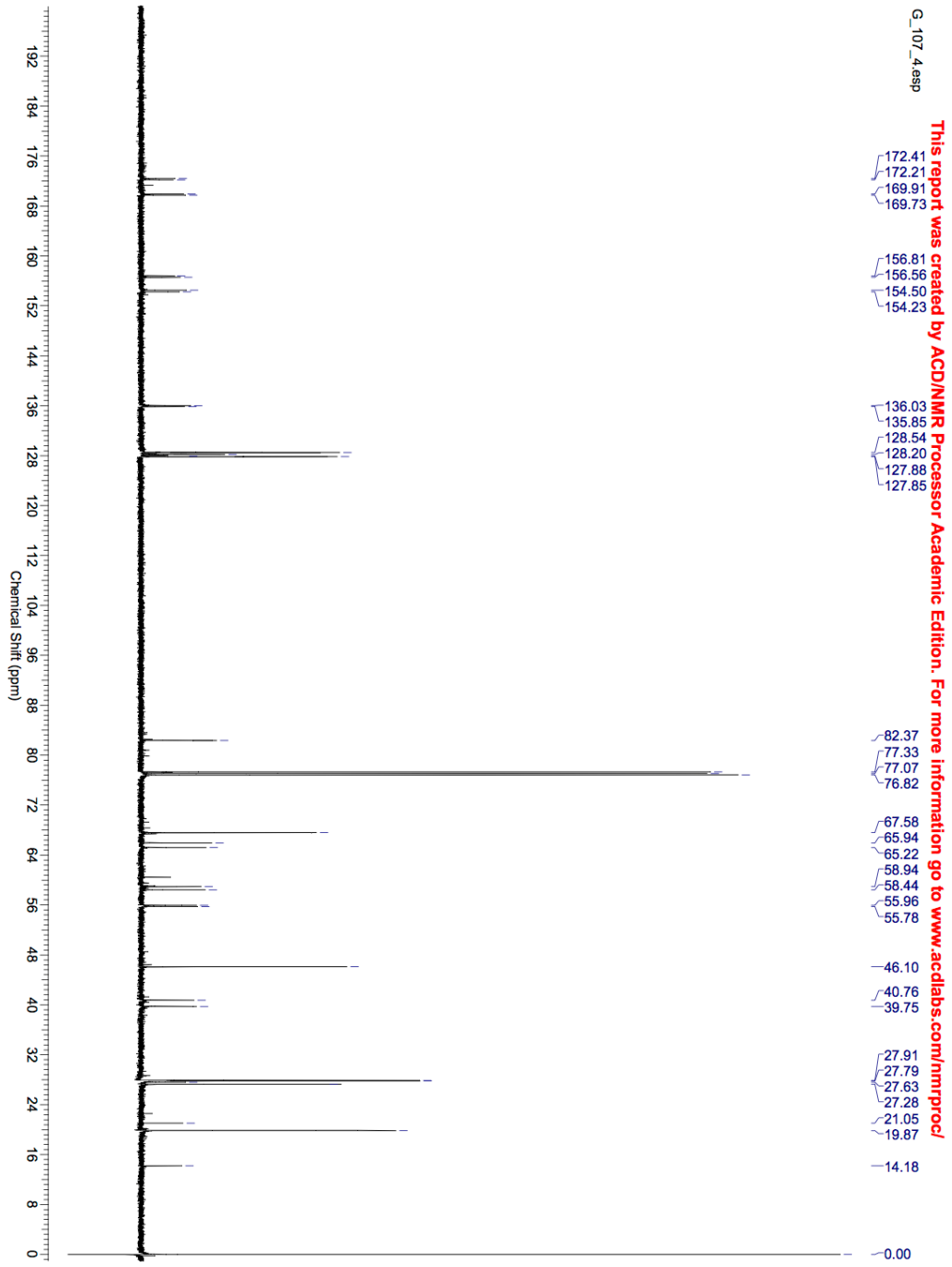
0.45
4.01
3.84
5.88
Chemical Shift (ppm)


G_107_4.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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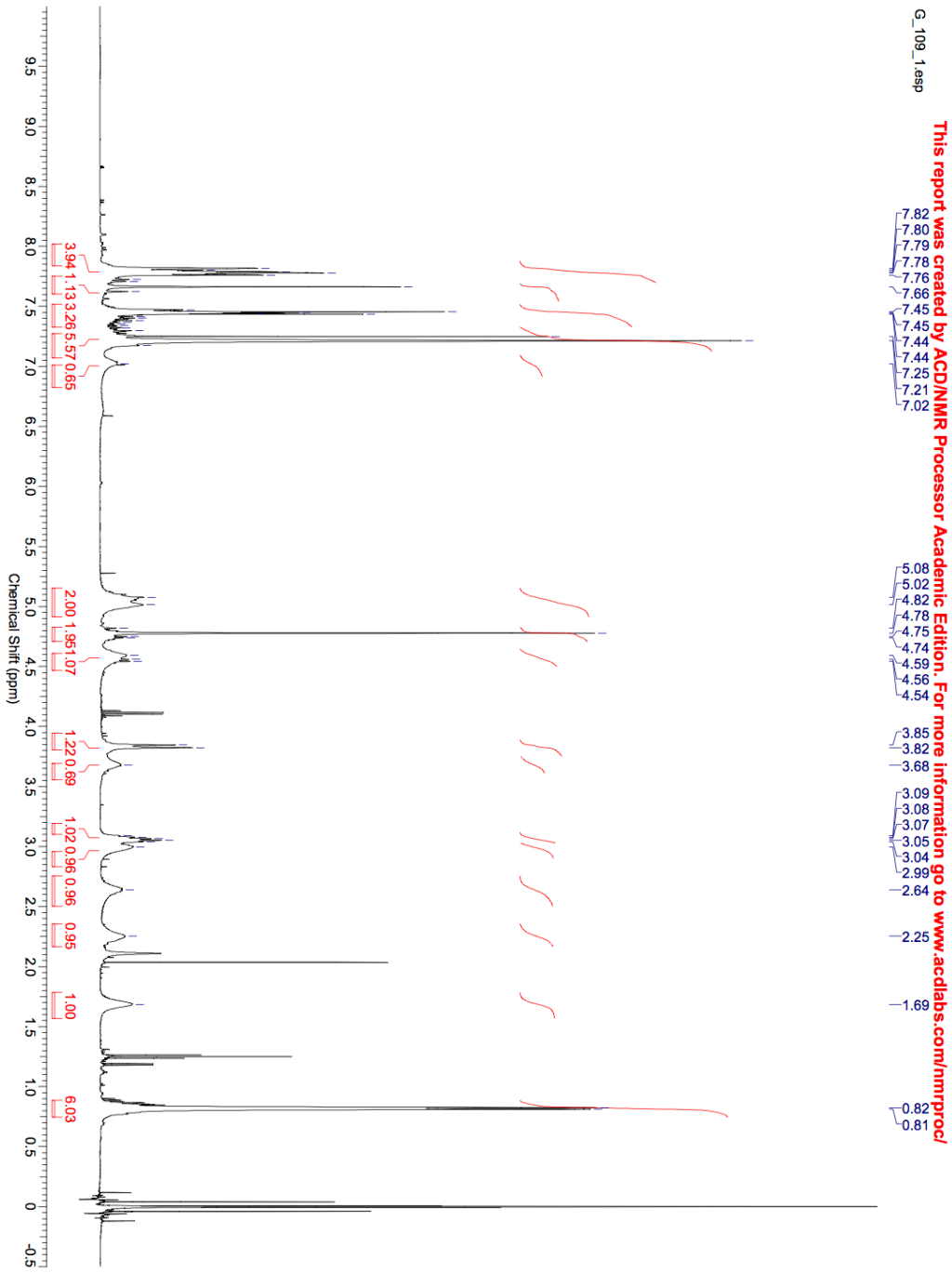
172.21
169.91
169.73
156.81
156.56
154.50
154.23
136.03
135.85
128.54
128.20
127.88
127.85
82.37
77.33
77.07
76.82
67.58
65.94
65.22
58.94
58.44
55.96
55.78
46.10
40.76
39.75
27.91
27.79
27.63
27.28
21.05
19.87
14.18
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0


G_109_1.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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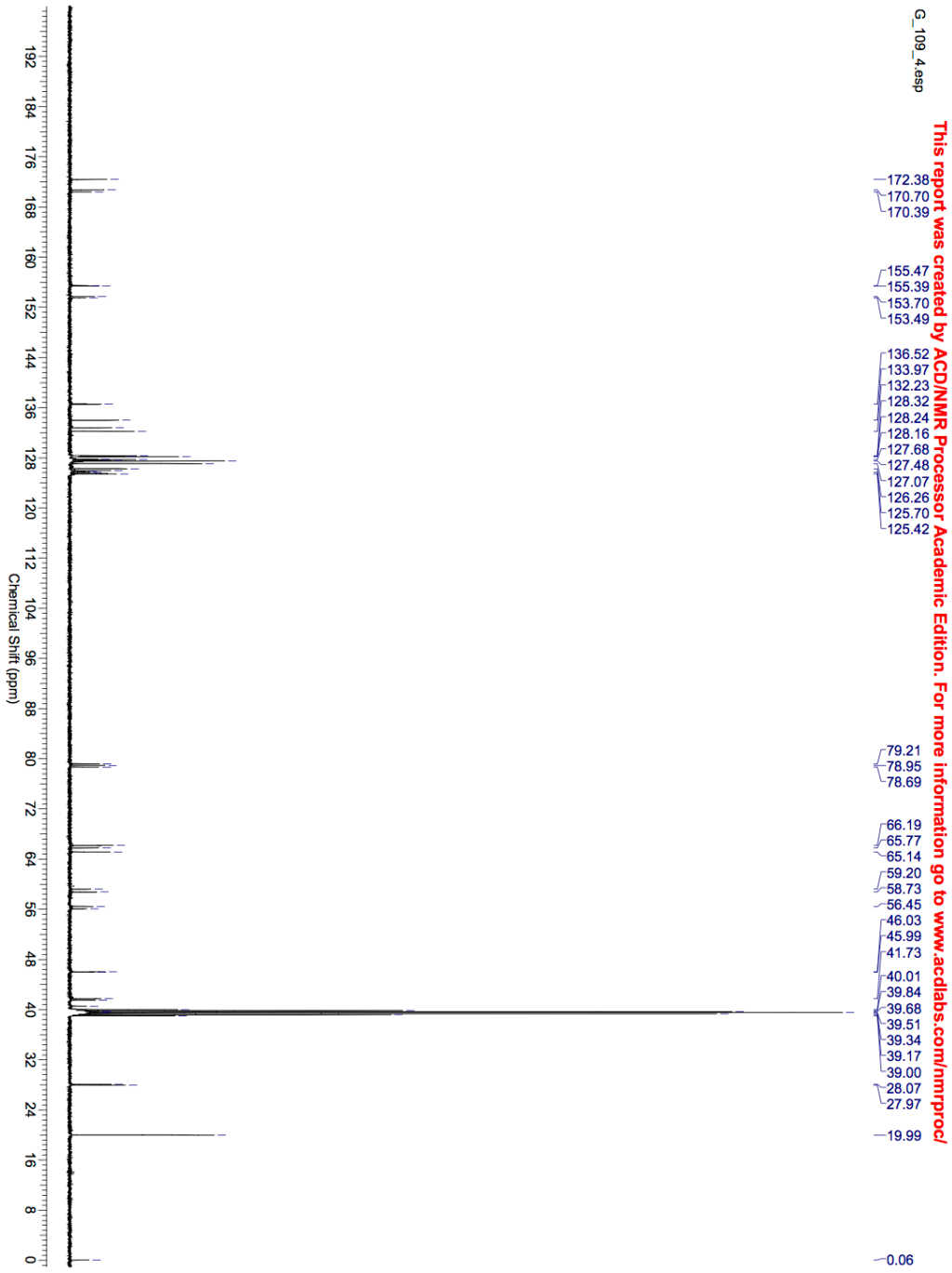
7.80
7.79
7.78
7.76
7.66
7.45
7.45
7.44
7.44
7.25
7.21
7.02
5.08
5.02
4.82
4.78
4.75
4.74
4.59
4.56
4.54
3.85
3.82
3.68
3.09
3.08
3.07
3.05
3.04
2.99
2.64
2.25
1.69
0.82
0.81
3.94
1.13
3.26
5.57
0.65
2.00
1.95
1.07
1.22
0.69
1.02
0.96
0.96
0.95
1.00
6.03
Chemical Shift (ppm)


G_109_4.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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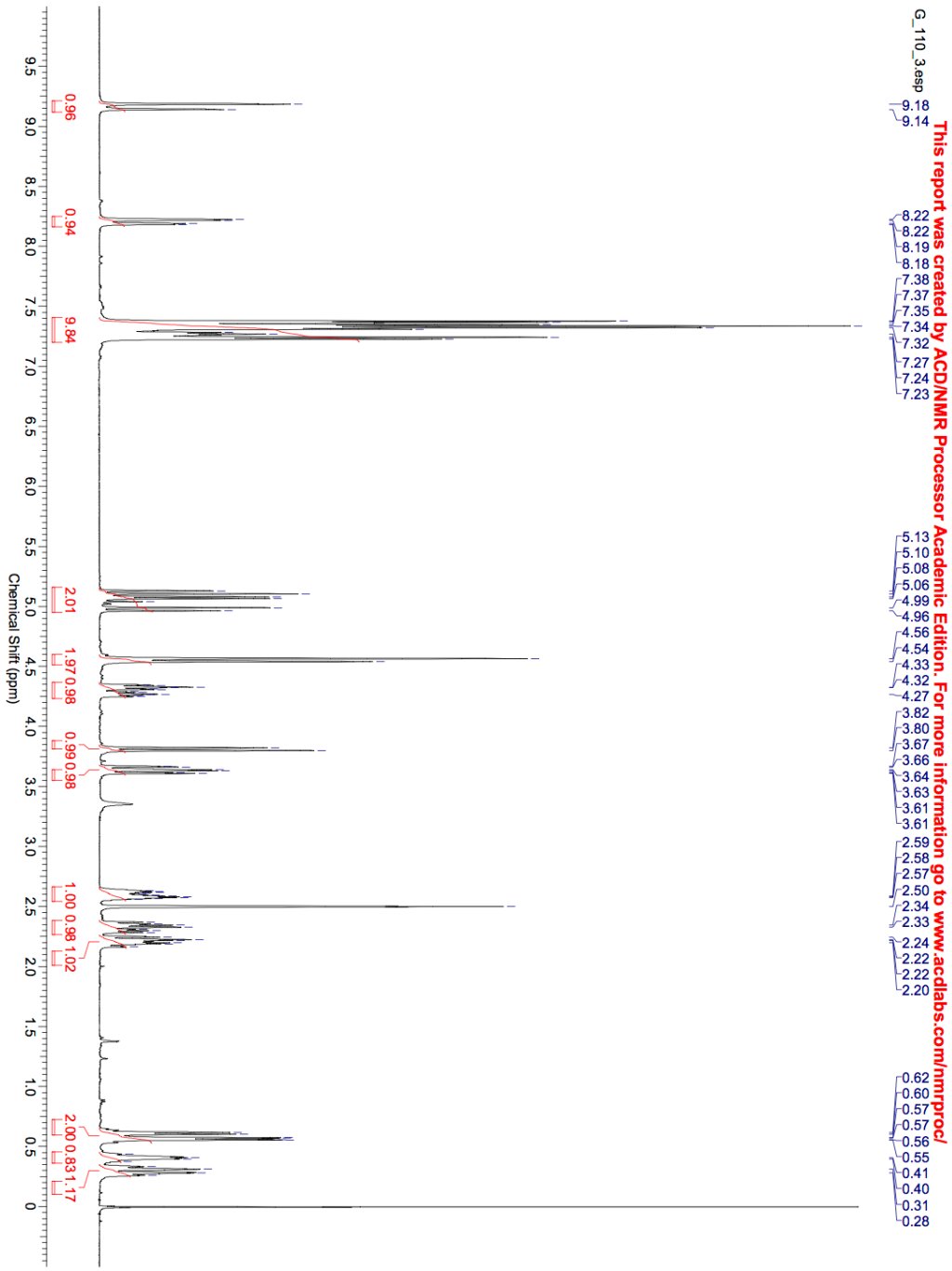
155.39
153.70
153.49
136.52
133.97
132.23
128.32
128.24
128.16
127.68
127.48
127.07
126.26
125.70
125.42
79.21
78.95
78.69
66.19
65.77
65.14
59.20
58.73
56.45
46.03
45.99
41.73
40.01
39.84
39.68
39.51
39.34
39.17
39.00
28.07
27.97
19.99
0.06
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0


G_110_3.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
Chemical Shift (ppm)
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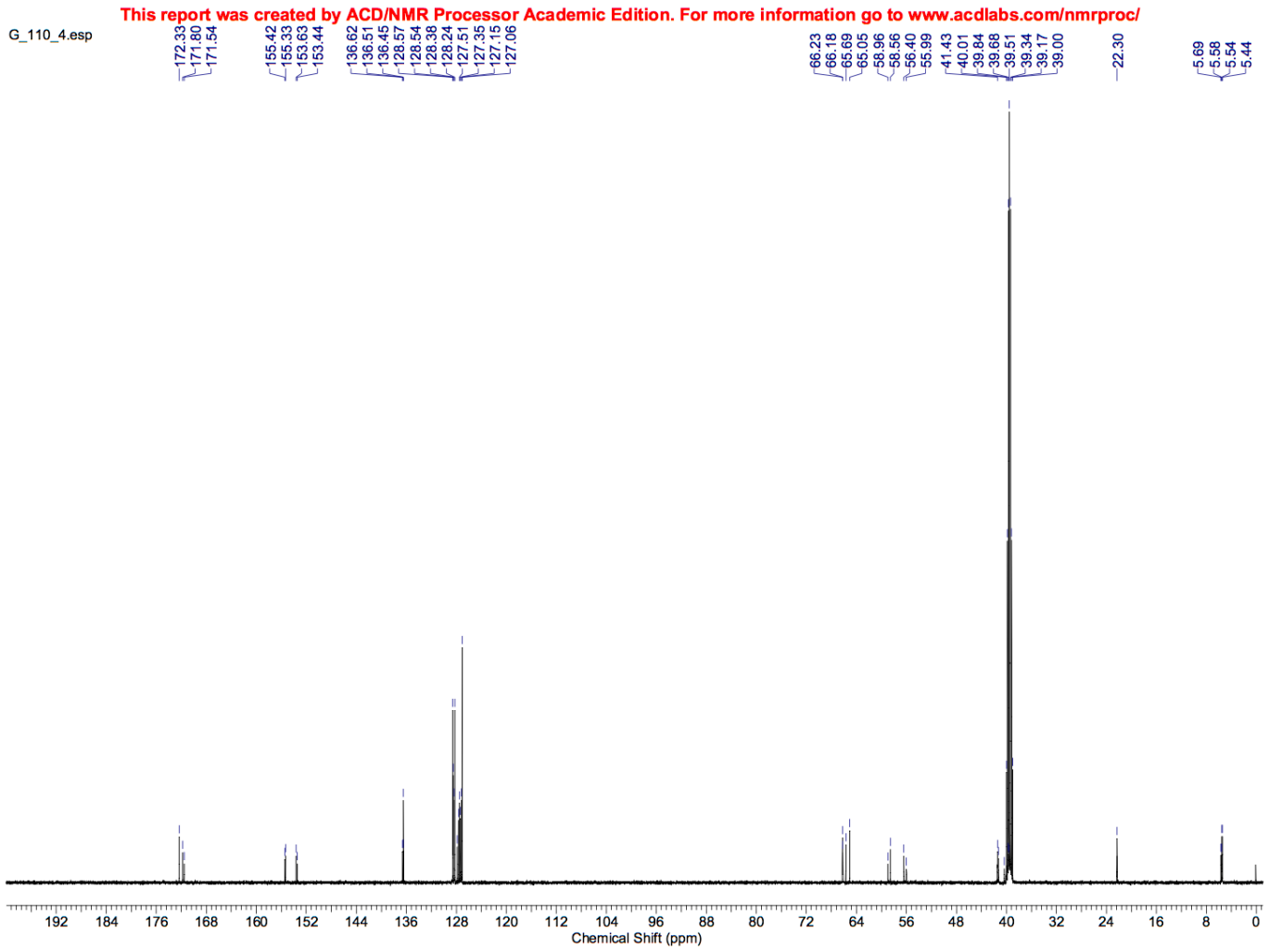
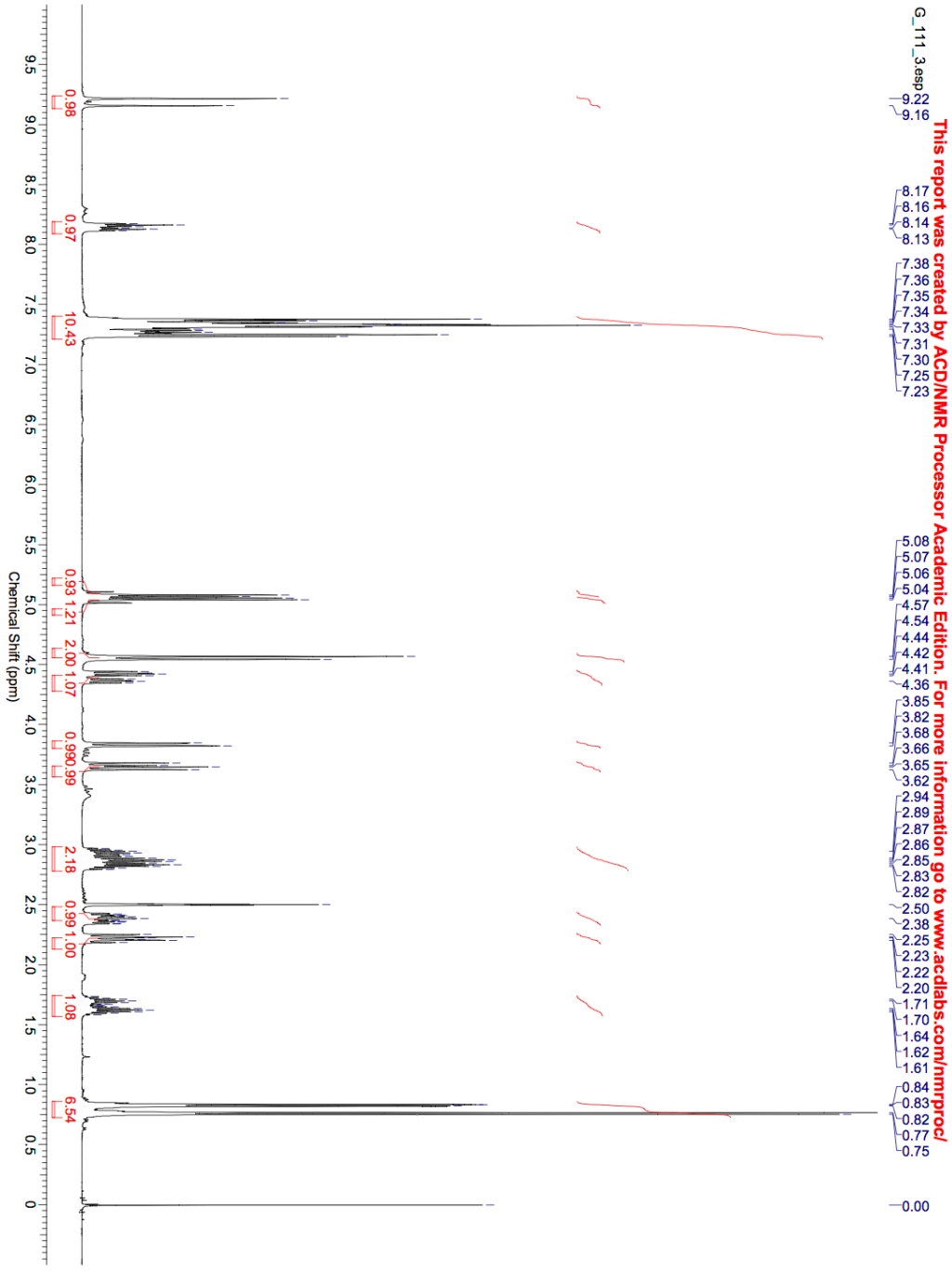
9.14
8.22
8.22
8.19
8.18
7.38
7.37
7.35
7.34
7.32
7.27
7.24
7.23
5.13
5.10
5.08
5.06
4.99
4.96
4.56
4.54
4.33
4.32
4.27
3.82
3.80
3.67
3.66
3.64
3.63
3.61
3.61
2.59
2.58
2.57
2.50
2.34
2.33
2.24
2.22
2.22
2.20
0.62
0.60
0.57
0.57
0.56
0.55
0.41
0.40
0.31
0.28
0.96
0.94
9.84
2.01
1.97
0.98
0.99
0.99
0.98
1.00
0.98
1.02
2.00
0.83
1.17




G_111_3.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
Chemical Shift (ppm)
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8.17
8.16
8.14
8.13
7.38
7.36
7.35
7.34
7.33
7.31
7.30
7.25
7.23
5.08
5.07
5.06
5.04
4.57
4.54
4.44
4.42
4.41
4.36
3.85
3.82
3.68
3.66
3.65
3.62
2.94
2.89
2.87
2.86
2.85
2.83
2.82
2.50
2.38
2.25
2.23
2.22
2.20
1.71
1.70
1.64
1.62
1.61
0.84
0.83
0.82
0.77
0.75
0.00
0.98
0.97
10.43
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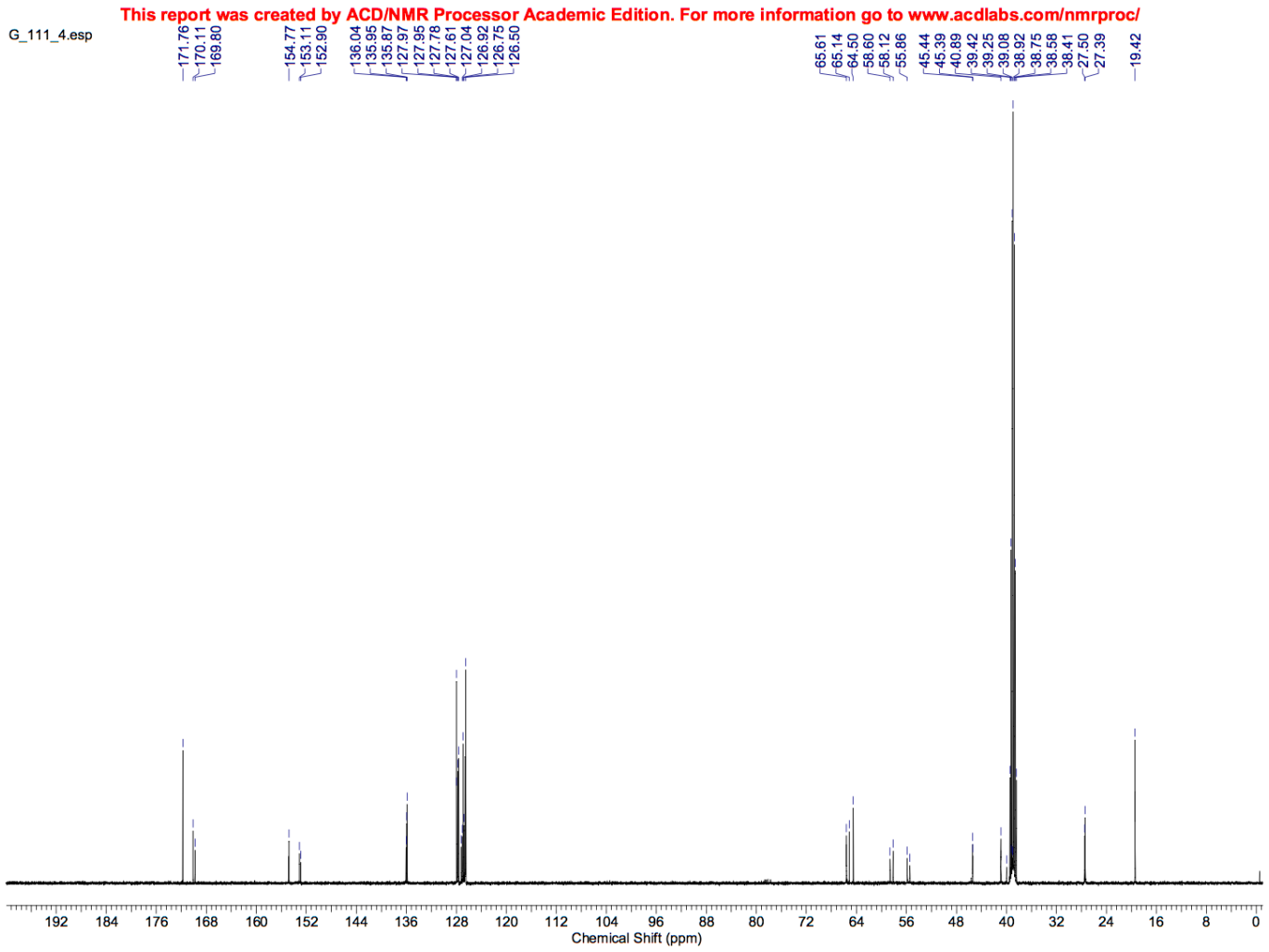
2.00
1.07
0.99
0.99
2.18
0.99
1.00
1.08
6.54


This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
G_111_4.esp
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169.80
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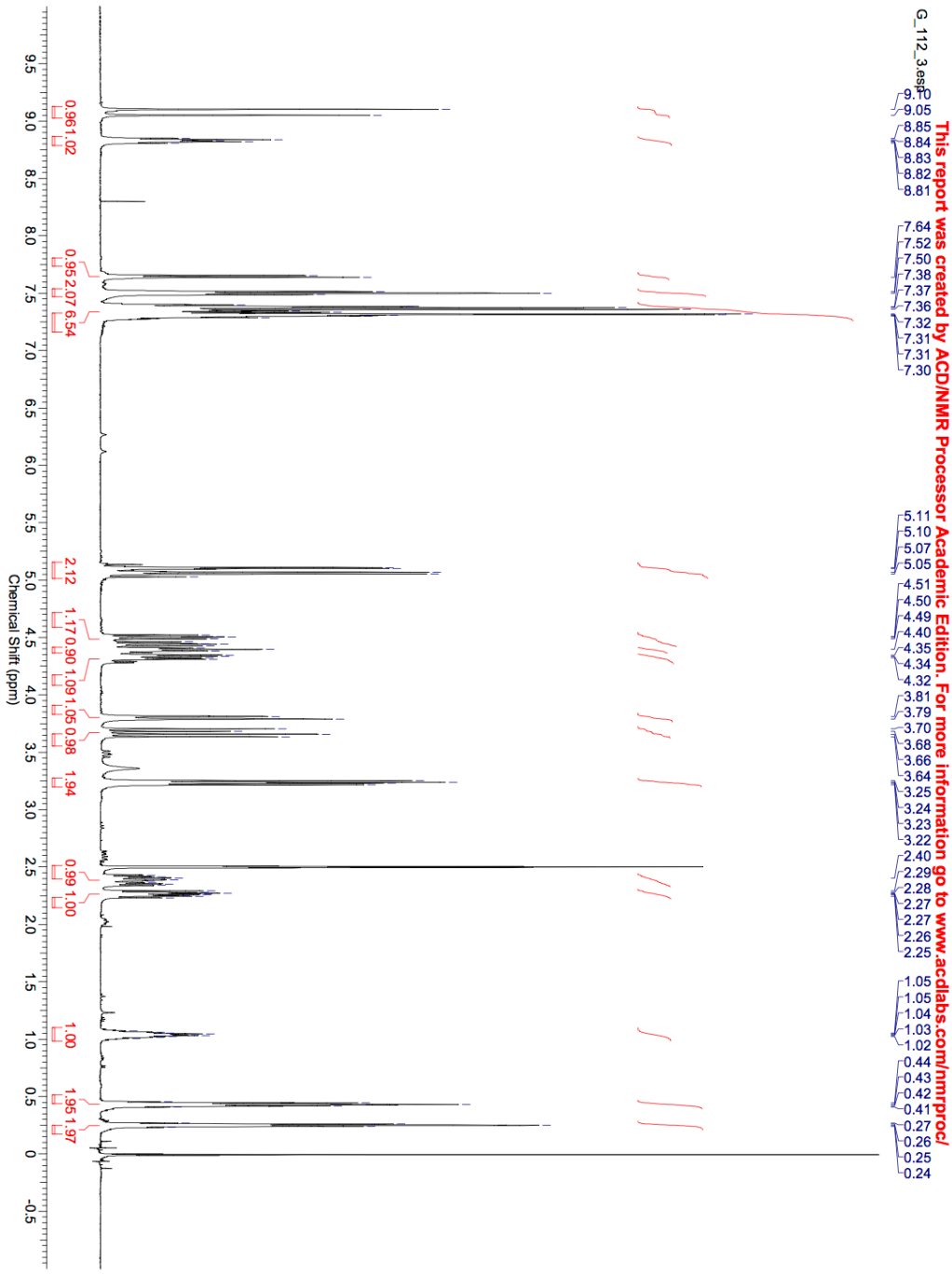
153.11
152.90
136.04
135.95
135.87
127.97
127.95
127.78
127.61
127.04
126.92
126.75
126.50
65.61
65.14
64.50
58.60
58.12
55.86
45.44
45.39
40.89
39.42
39.25
39.08
38.92
38.75
38.58
38.41
27.50
27.39
19.42
192 184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 16 8 0
Chemical Shift (ppm)


G_112_3.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
Chemical Shift (ppm)
0.96 1.02
0.95 2.07 6.54
2.12
1.17 0.90 1.09 1.05 0.98
1.94
0.99 1.00
1.00
1.95 1.97
9.10
9.05
8.85
8.84
8.83
8.82
8.81
7.64
7.52
7.50
7.38
7.37
7.36
7.32
7.31
7.31
7.30
5.11
5.10
5.07
5.05
4.51
4.50
4.49
4.40
4.35
4.34
4.32
3.81
3.79
3.70
3.68
3.66
3.64
3.25
3.24
3.23
3.22
2.40
2.29
2.28
2.27
2.27
2.26
2.25
1.05
1.05
1.04
1.03
1.02
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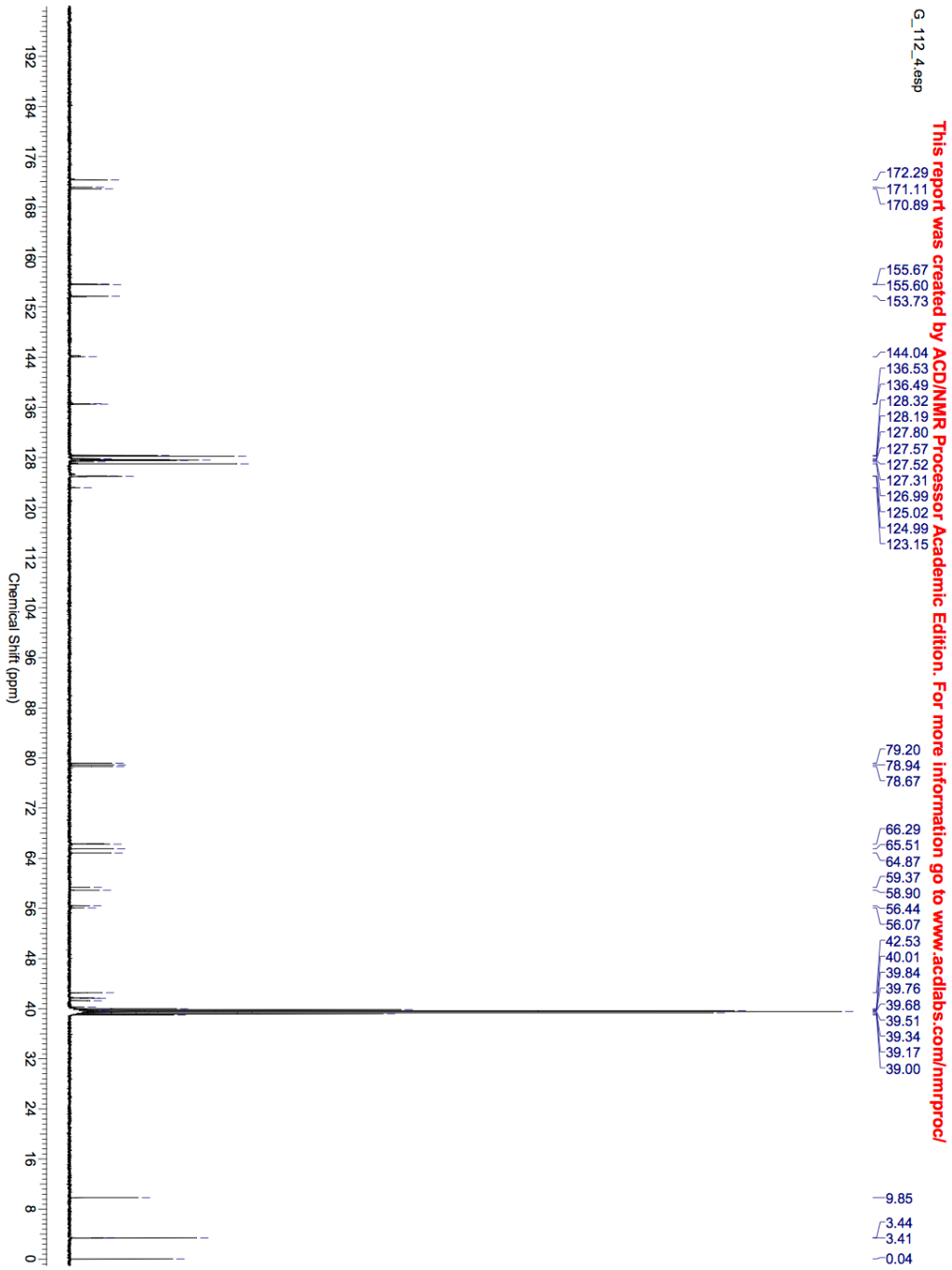
0.43
0.42
0.41
0.27
0.26
0.25
0.24


G_112_4.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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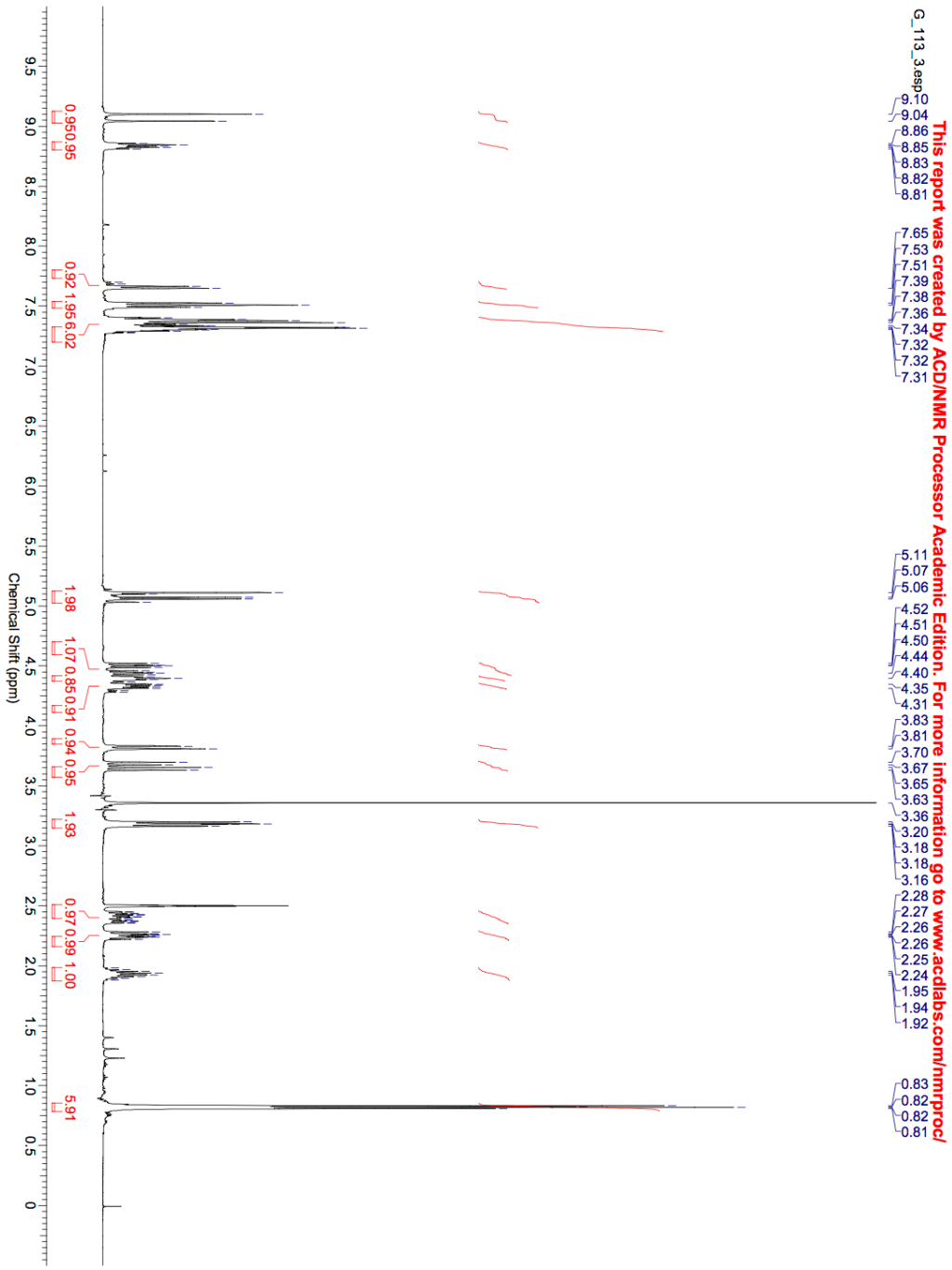
155.60
153.73
144.04
136.53
136.49
128.32
128.19
127.80
127.57
127.52
127.31
126.99
125.02
124.99
123.15
79.20
78.94
78.67
66.29
65.51
64.87
59.37
58.90
56.44
56.07
42.53
40.01
39.84
39.76
39.68
39.51
39.34
39.17
39.00
9.85
3.44
3.41
0.04
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0


G_113_3.esp
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8.85
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8.82
8.81
7.65
7.53
7.51
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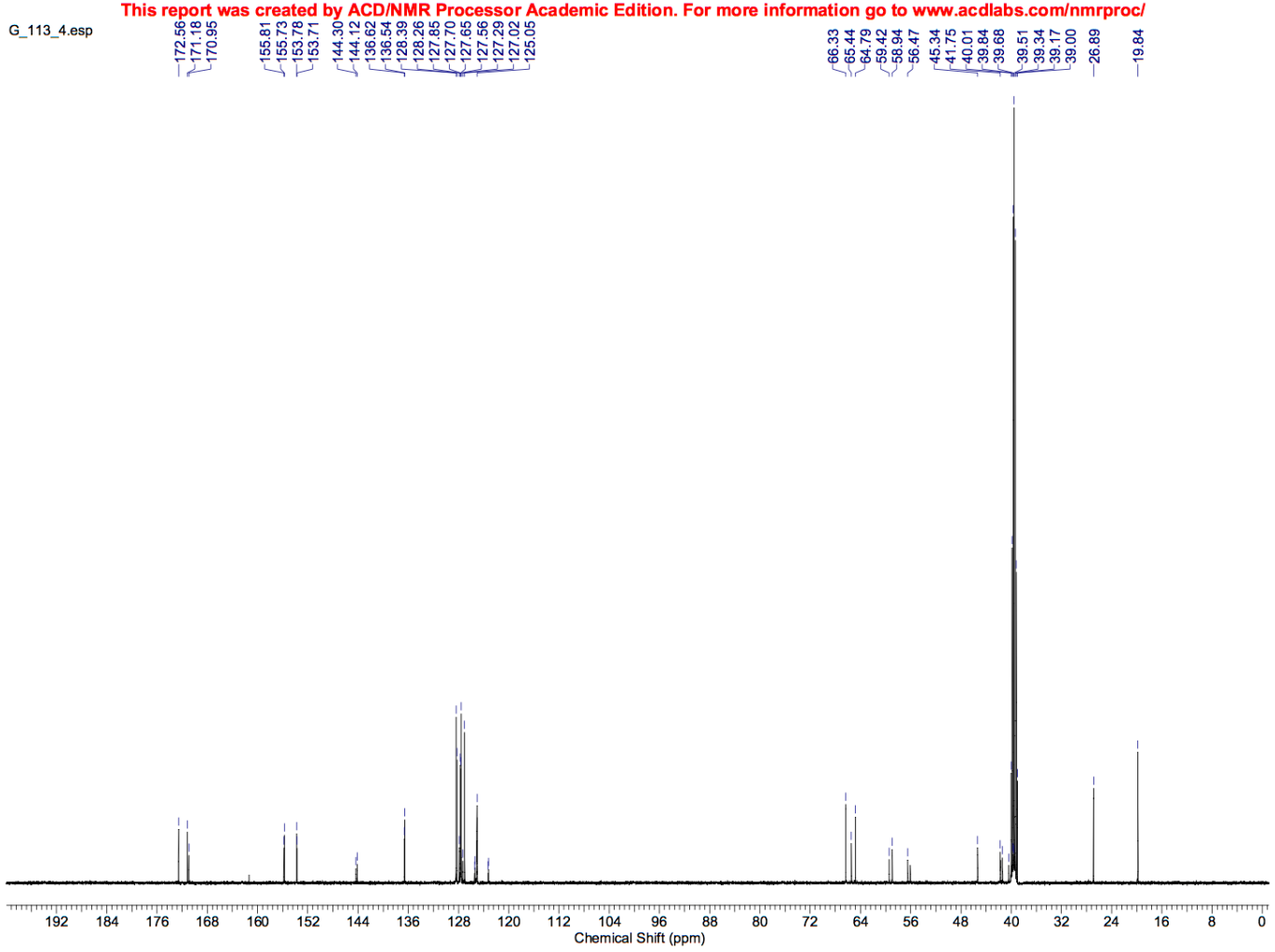
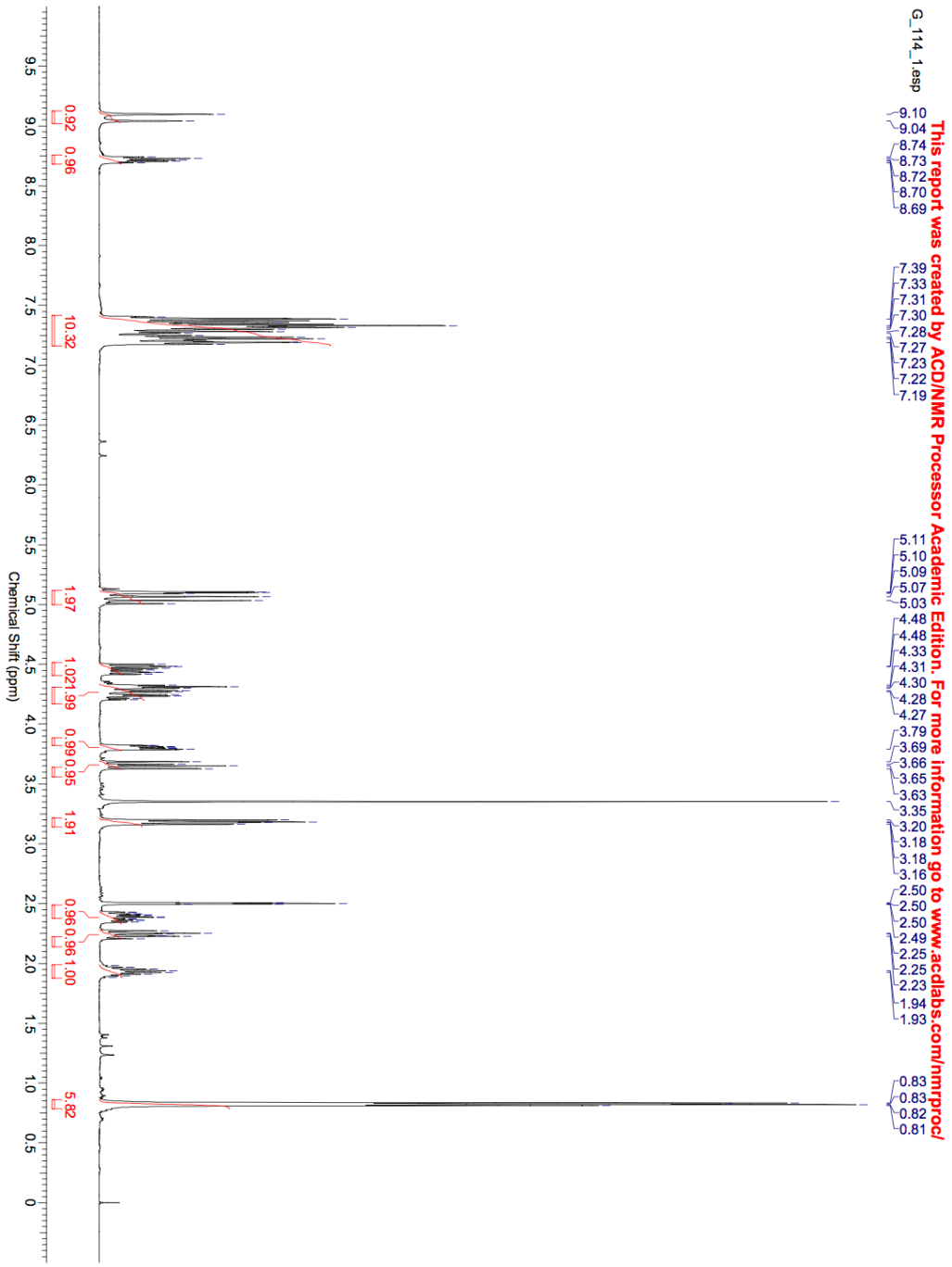
7.38
7.36
7.34
7.32
7.32
7.31
5.11
5.07
5.06
4.52
4.51
4.50
4.44
4.40
4.35
4.31
3.83
3.81
3.70
3.67
3.65
3.63
3.36
3.20
3.18
3.18
3.16
2.28
2.27
2.26
2.26
2.25
2.24
1.95
1.94
1.92
0.83
0.82
0.82
0.81
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
0.95 0.95
0.92 1.95 6.02
1.98
1.07 0.85 0.91 0.94 0.95
1.93
0.97 0.99 1.00
5.91
Chemical Shift (ppm)




G_114_1.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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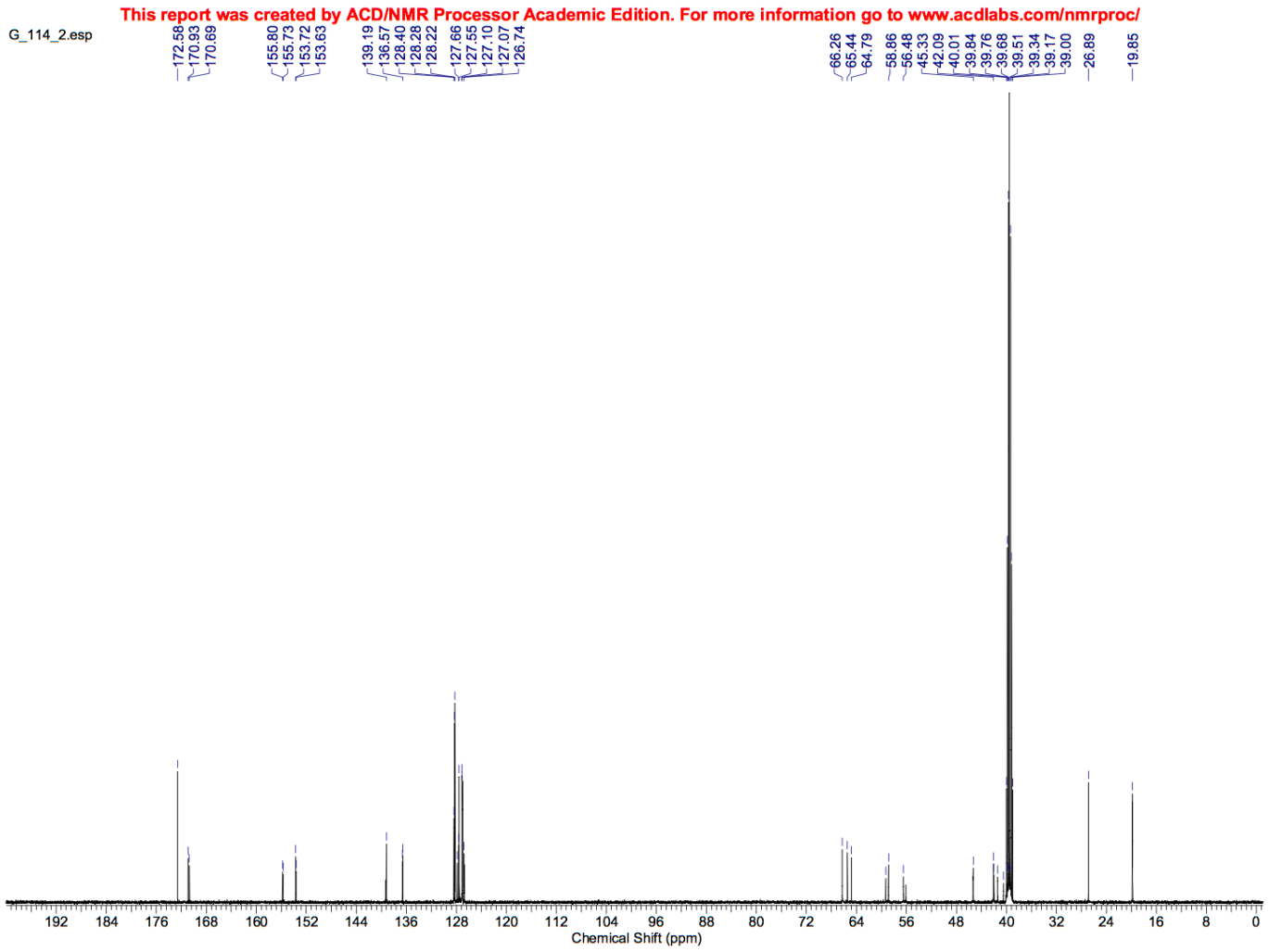
9.04
8.74
8.73
8.72
8.70
8.69
7.39
7.33
7.31
7.30
7.28
7.27
7.23
7.22
7.19
5.11
5.10
5.09
5.07
5.03
4.48
4.48
4.33
4.31
4.30
4.28
4.27
3.79
3.69
3.66
3.65
3.63
3.35
3.20
3.18
3.18
3.16
2.50
2.50
2.50
2.49
2.25
2.25
2.23
1.94
1.93
0.83
0.83
0.82
0.81
0.92
0.96
10.32
1.97
1.02
1.99
0.99
0.95
1.91
0.96
0.96
1.00
5.82
Chemical Shift (ppm)


This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
G_114_2.esp
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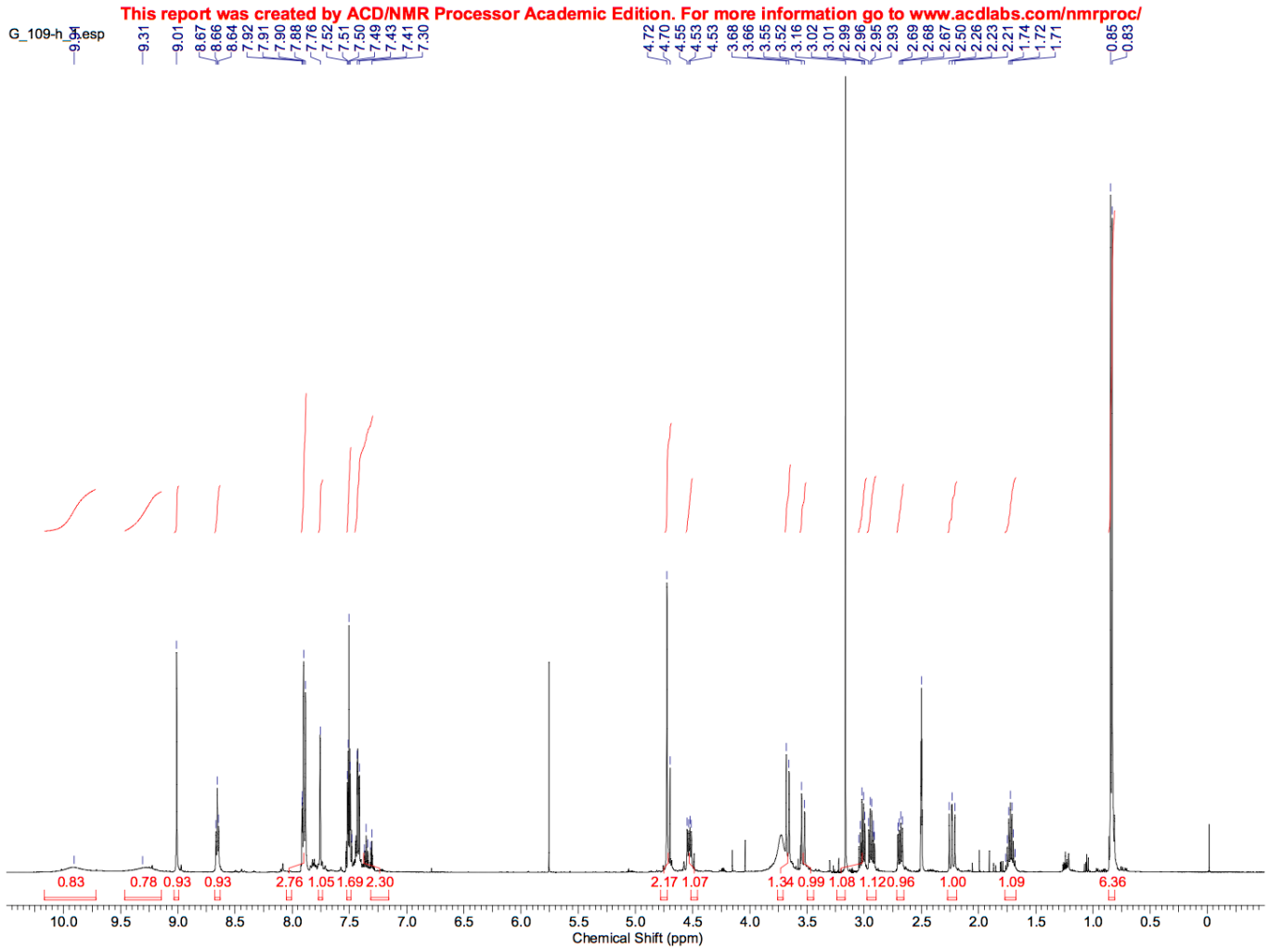
155.73
153.72
153.63
139.19
136.57
128.40
128.28
128.22
127.66
127.55
127.10
127.07
126.74
66.26
65.44
64.79
58.86
56.48
45.33
42.09
40.01
39.84
39.76
39.68
39.51
39.34
39.17
39.00
26.89
19.85
192 184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 16 8 0
Chemical Shift (ppm)


This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
G_109-h_1.esp
9.31
9.01
8.67
8.66
8.64
7.92
7.91
7.90
7.88
7.76
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7.51
7.50
7.49
7.43
7.41
7.30
4.72
4.70
4.55
4.53
3.68
3.66
3.55
3.52
3.16
3.02
3.01
2.99
2.96
2.95
2.93
2.69
2.68
2.67
2.50
2.26
2.23
2.21
1.74
1.72
1.71
0.85
0.83
0.83
0.78
0.93
0.93
2.76
1.05
1.69
2.30
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1.34
0.99
1.08
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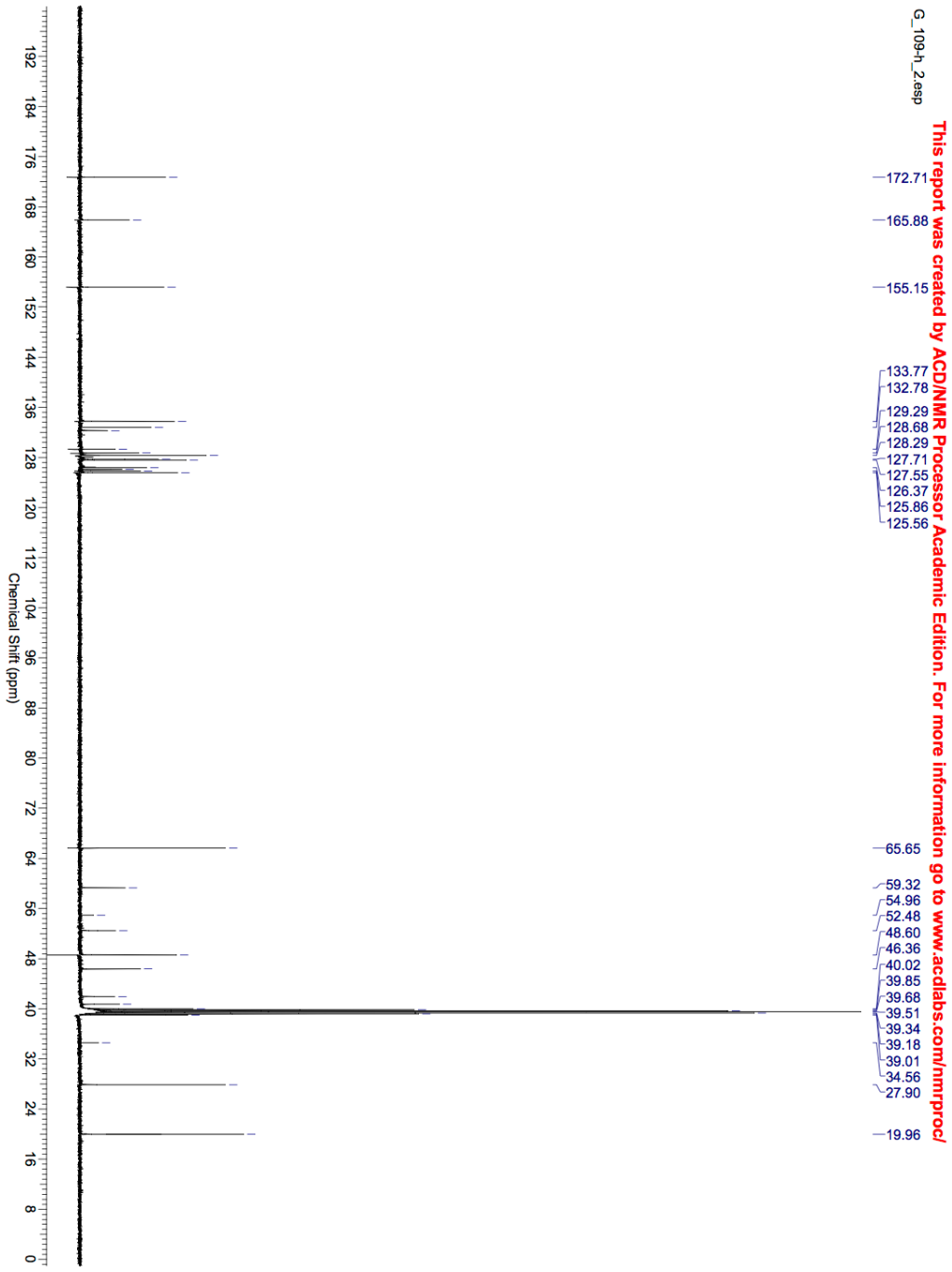
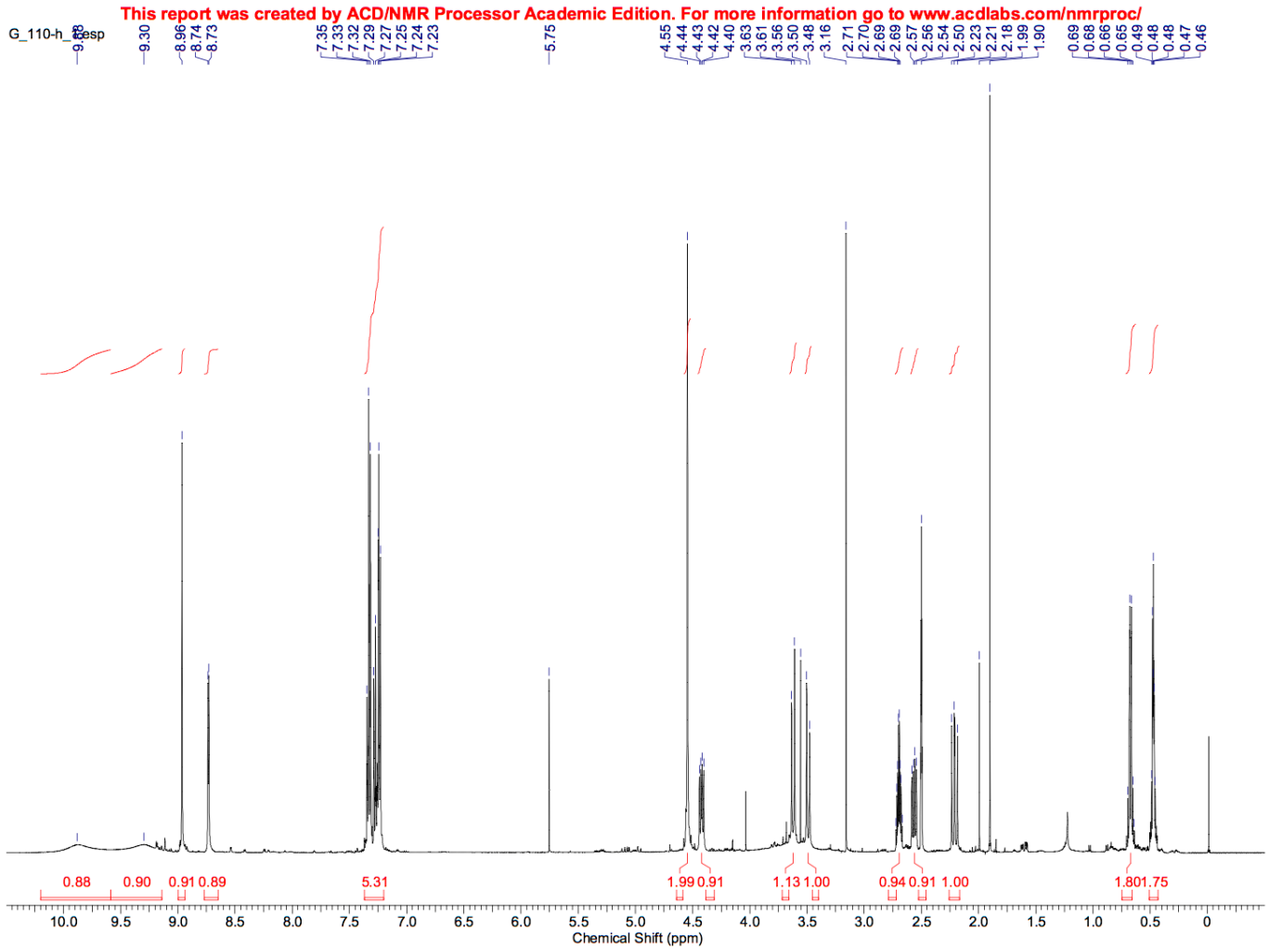
1.00
1.09
6.36
Chemical Shift (ppm)


G_109-h_2.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
172.71
165.88
155.15
133.77
132.78
129.29
128.68
128.29
127.71
127.55
126.37
125.86
125.56
65.65
59.32
54.96
52.48
48.60
46.36
40.02
39.85
39.68
39.51
39.34
39.18
39.01
34.56
27.90
19.96
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0


This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
G_110-h_cesp
9.30
8.96
8.74
8.73
7.35
7.33
7.32
7.29
7.27
7.25
7.24
7.23
5.75
4.55
4.44
4.43
4.42
4.40
3.63
3.61
3.56
3.50
3.48
3.16
2.71
2.70
2.69
2.69
2.57
2.56
2.54
2.50
2.23
2.21
2.18
1.99
1.90
0.69
0.68
0.66
0.65
0.49
0.48
0.48
0.47
0.46
0.88
0.90
0.91
0.89
5.31
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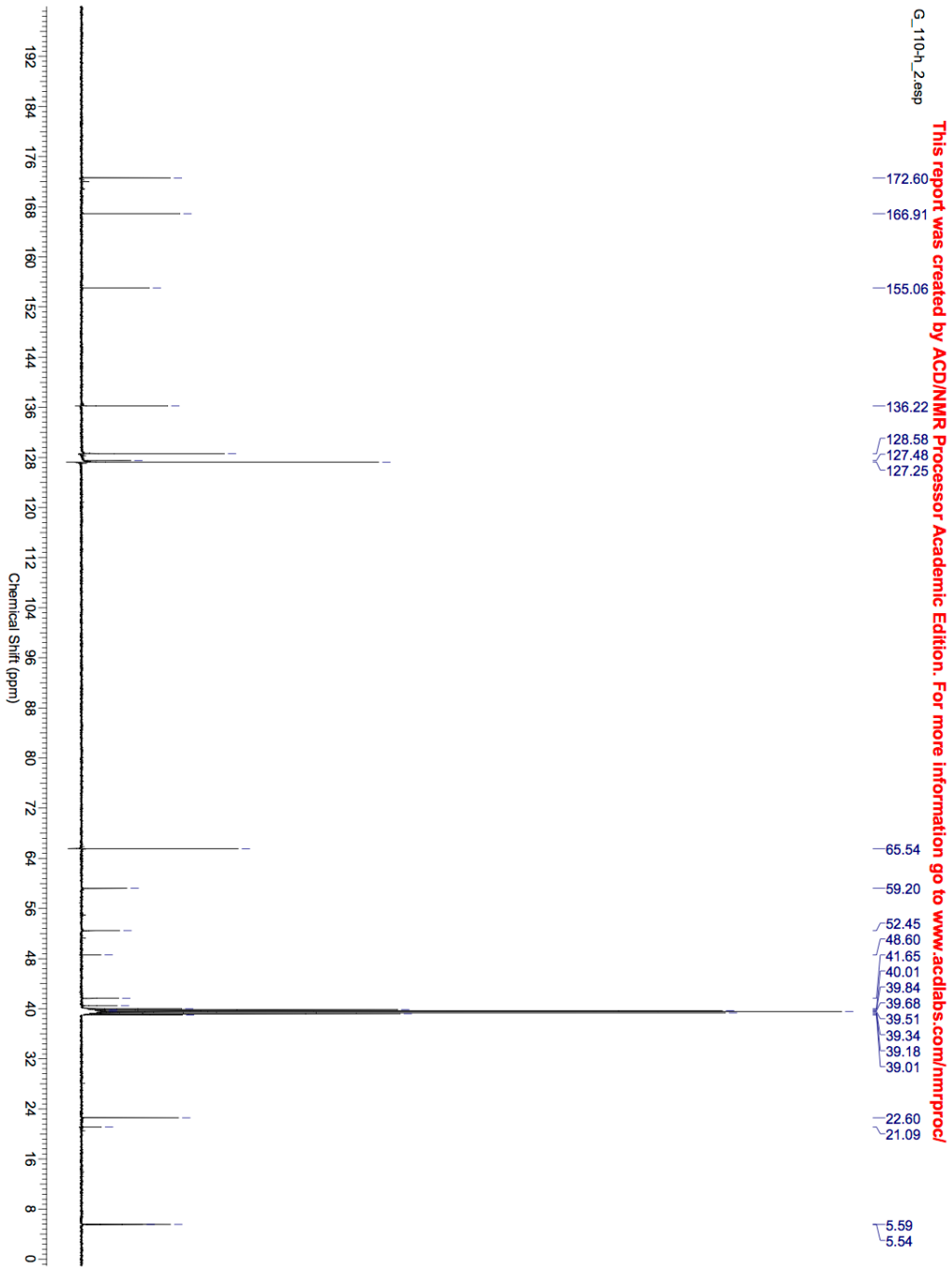
0.91
1.13
1.00
0.94
0.91
1.00
1.80
1.75
Chemical Shift (ppm)


G_110-h_2.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
172.60
166.91
155.06
136.22
128.58
127.48
127.25
65.54
59.20
52.45
48.60
41.65
40.01
39.84
39.68
39.51
39.34
39.18
39.01
22.60
21.09
5.59
5.54
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
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8
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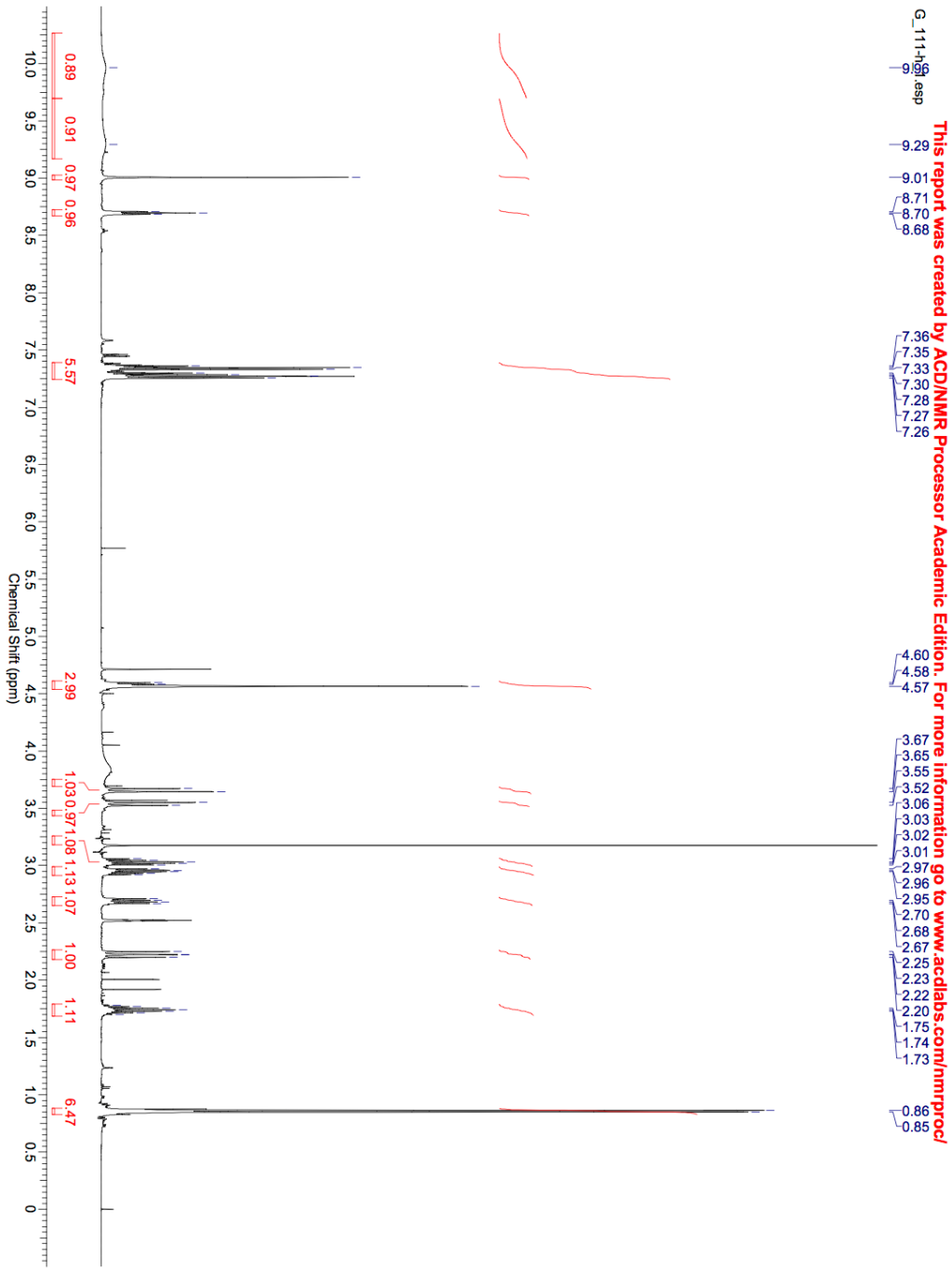
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8.68
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7.35
7.33
7.30
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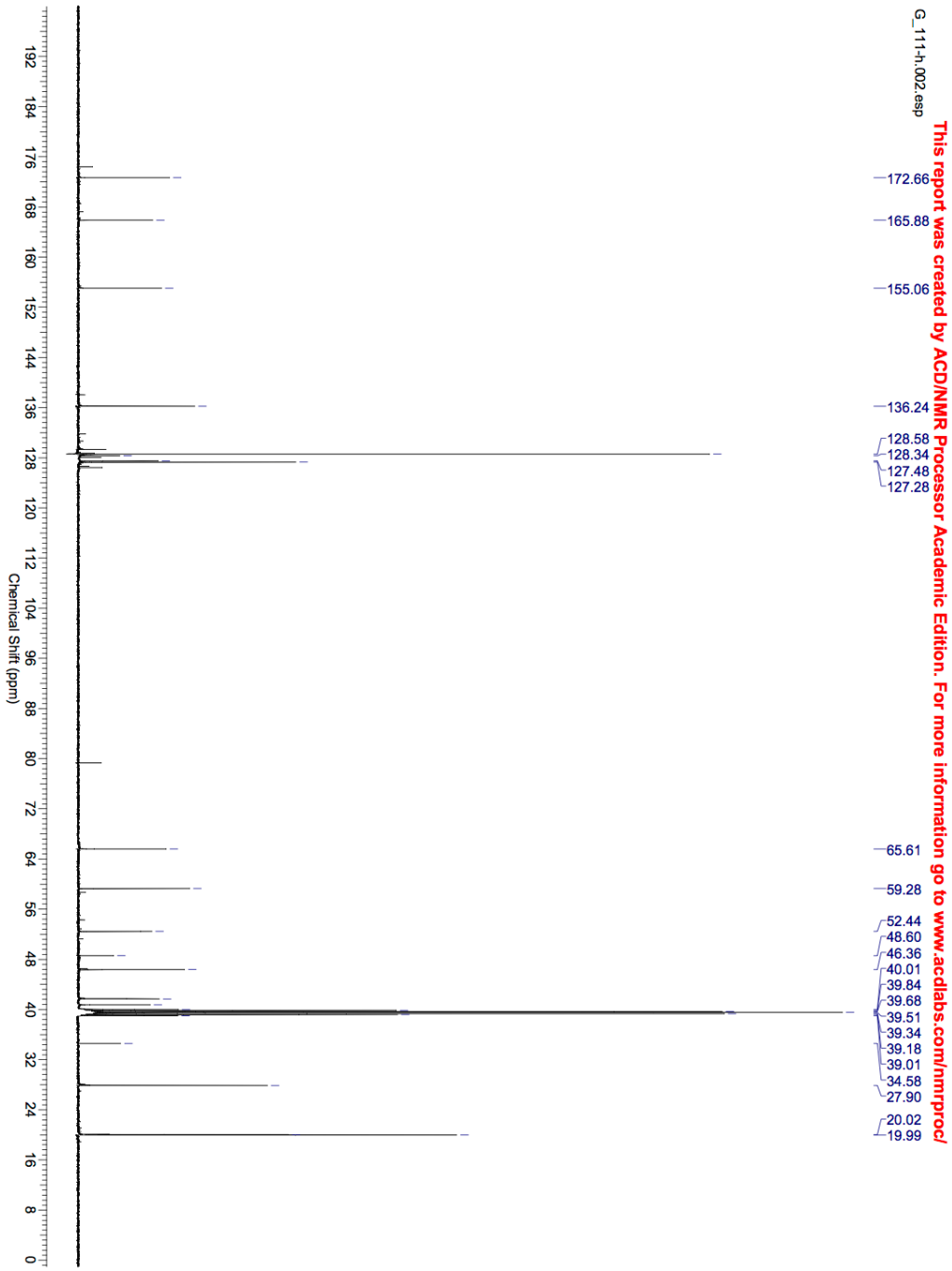
7.26
4.60
4.58
4.57
3.67
3.65
3.55
3.52
3.06
3.03
3.02
3.01
2.97
2.96
2.95
2.70
2.68
2.67
2.25
2.23
2.22
2.20
1.75
1.74
1.73
0.86
0.85
0.89
0.91
0.97
0.96
5.57
2.99
1.03
0.97
1.08
1.13
1.07
1.00
1.11
6.47
Chemical Shift (ppm)
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/


G_111-h.002.esp
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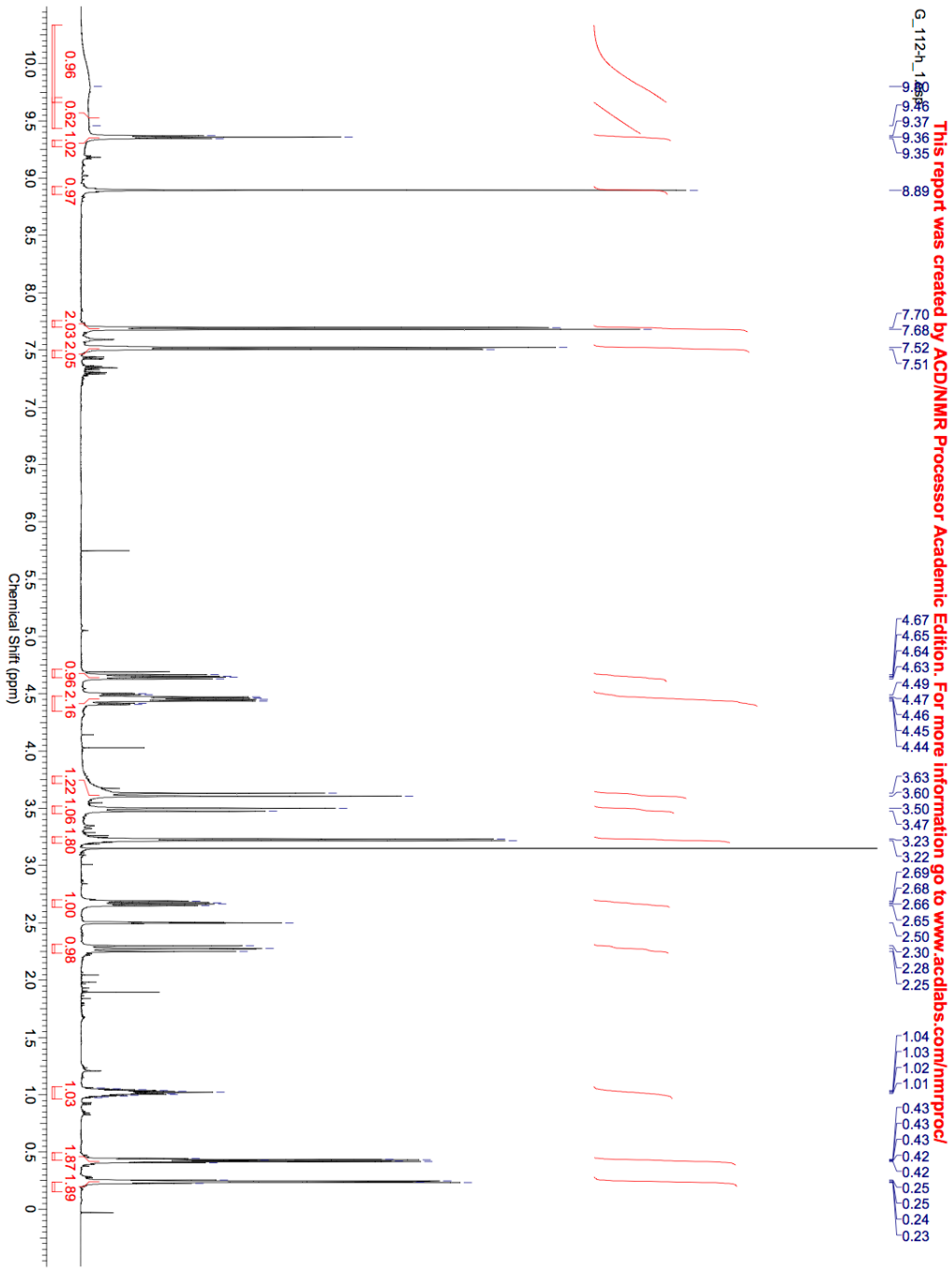
155.06
136.24
128.58
128.34
127.48
127.28
65.61
59.28
52.44
48.60
46.36
40.01
39.84
39.68
39.51
39.34
39.18
39.01
34.58
27.90
20.02
19.99
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/


G_112-h_1
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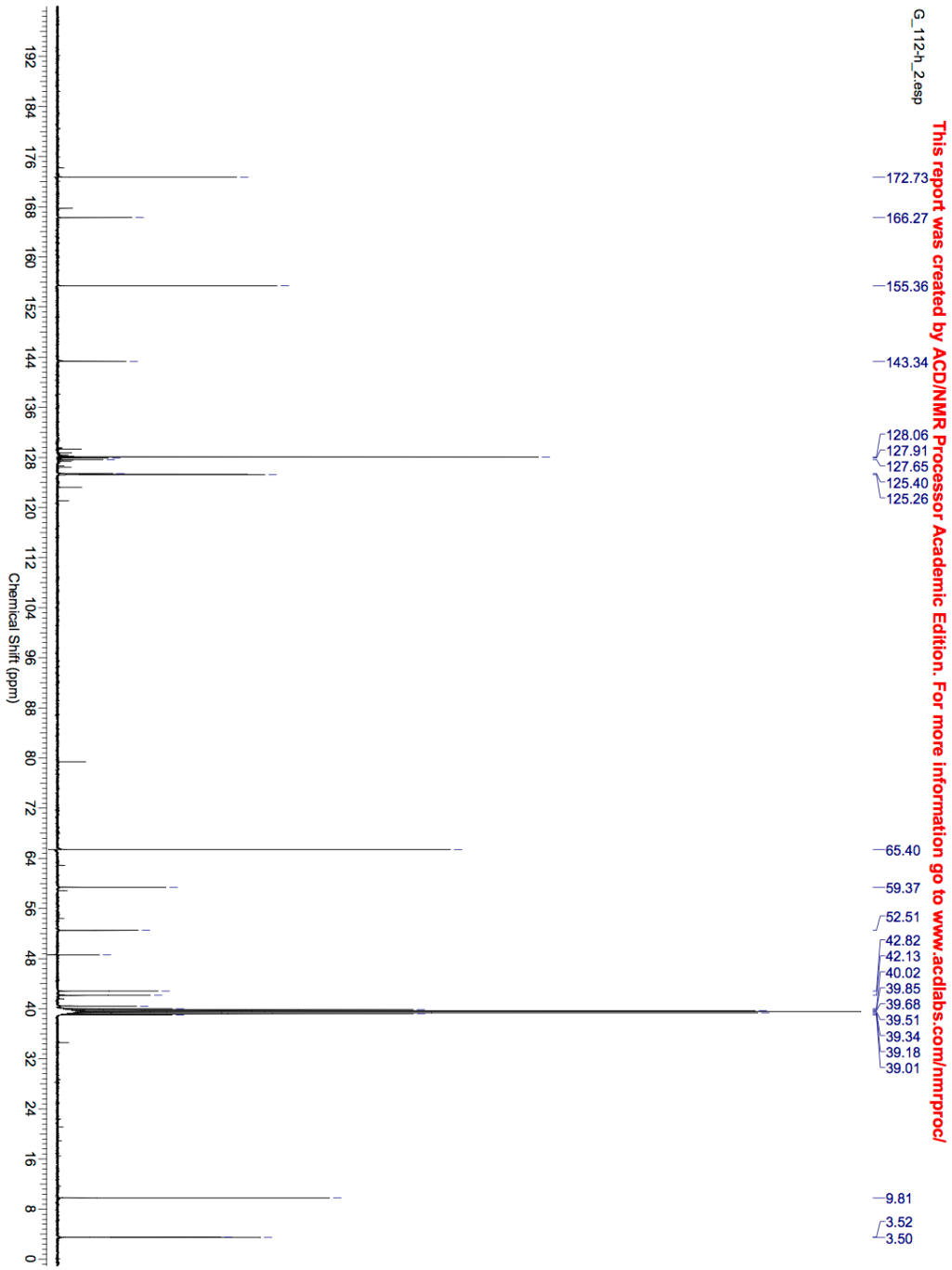
9.46
9.37
9.36
9.35
8.89
7.70
7.68
7.52
7.51
4.67
4.65
4.64
4.63
4.49
4.47
4.46
4.45
4.44
3.63
3.60
3.50
3.47
3.23
3.22
2.69
2.68
2.66
2.65
2.50
2.30
2.28
2.25
1.04
1.03
1.02
1.01
0.43
0.43
0.43
0.42
0.42
0.25
0.25
0.24
0.23
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
Chemical Shift (ppm)


G_112-h_2.esp
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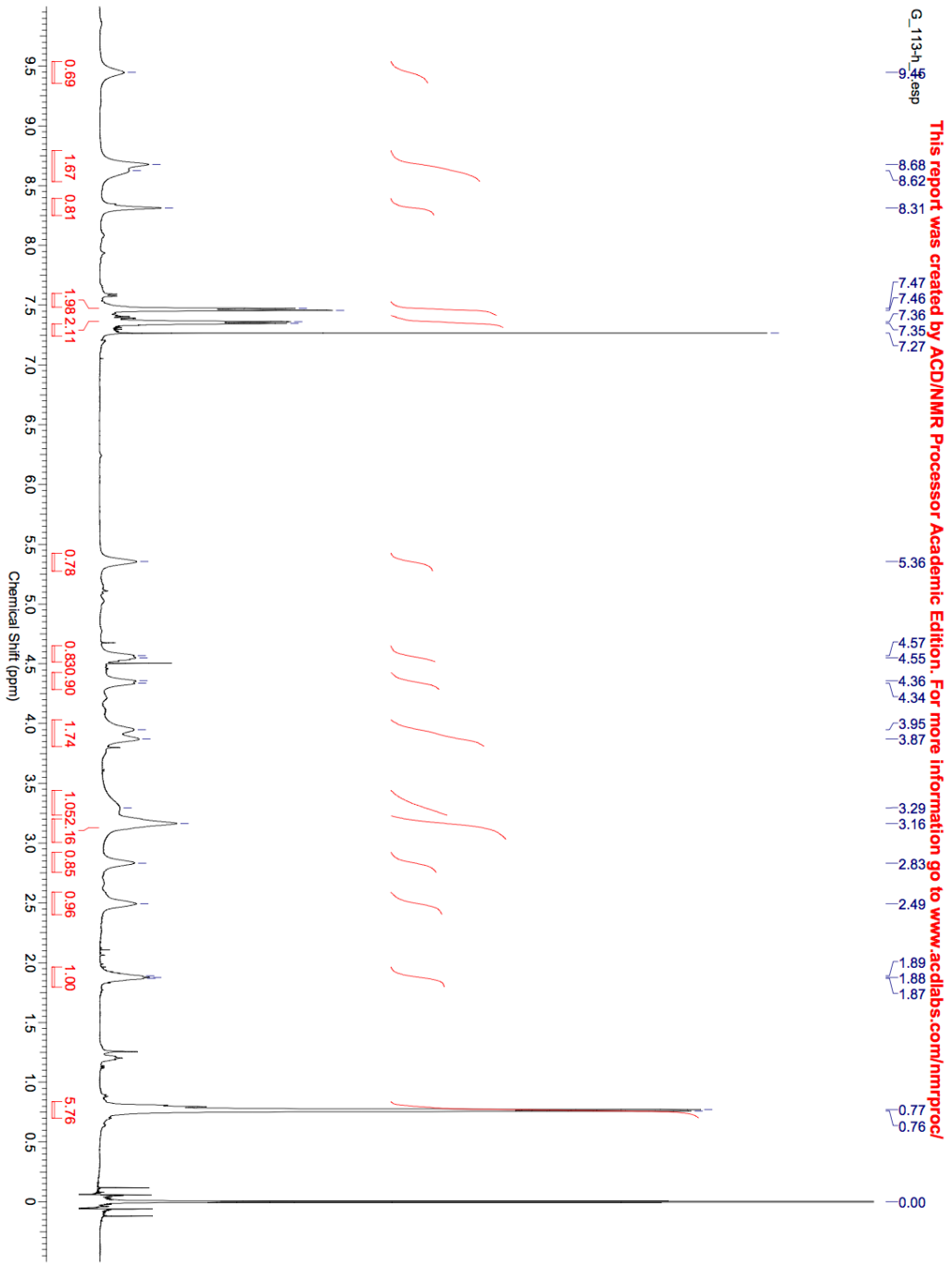
155.36
143.34
128.06
127.91
127.65
125.40
125.26
65.40
59.37
52.51
42.82
42.13
40.02
39.85
39.68
39.51
39.34
39.18
39.01
9.81
3.52
3.50
Chemical Shift (ppm)
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/


G_113-h.esp
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8.68
8.62
8.31
7.47
7.46
7.36
7.35
7.27
5.36
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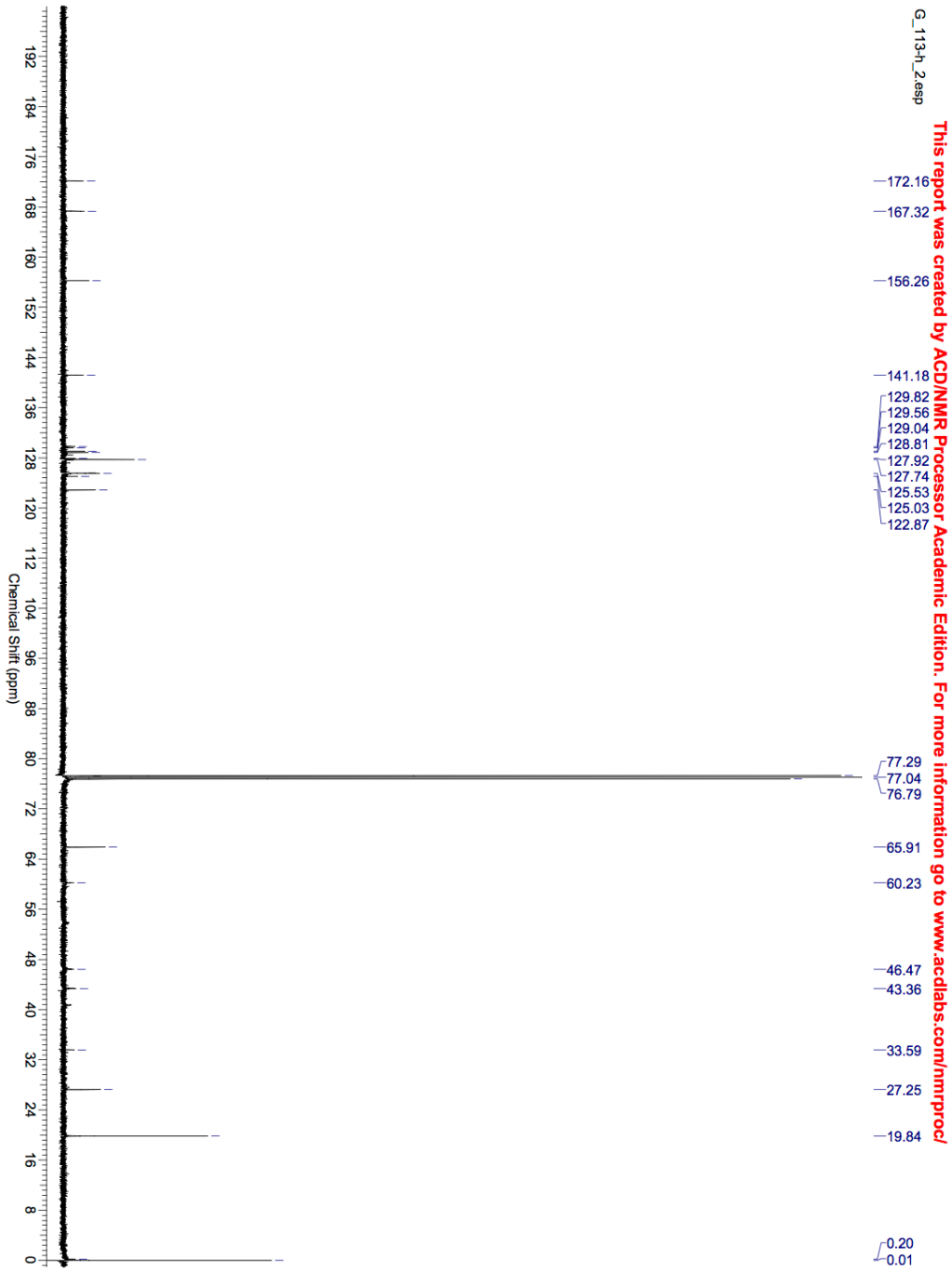
4.55
4.36
4.34
3.95
3.87
3.29
3.16
2.83
2.49
1.89
1.88
1.87
0.77
0.76
0.00
0.69
1.67
0.81
1.98
2.11
0.78
0.83
0.90
1.74
1.05
2.16
0.85
0.96
1.00
5.76
Chemical Shift (ppm)
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/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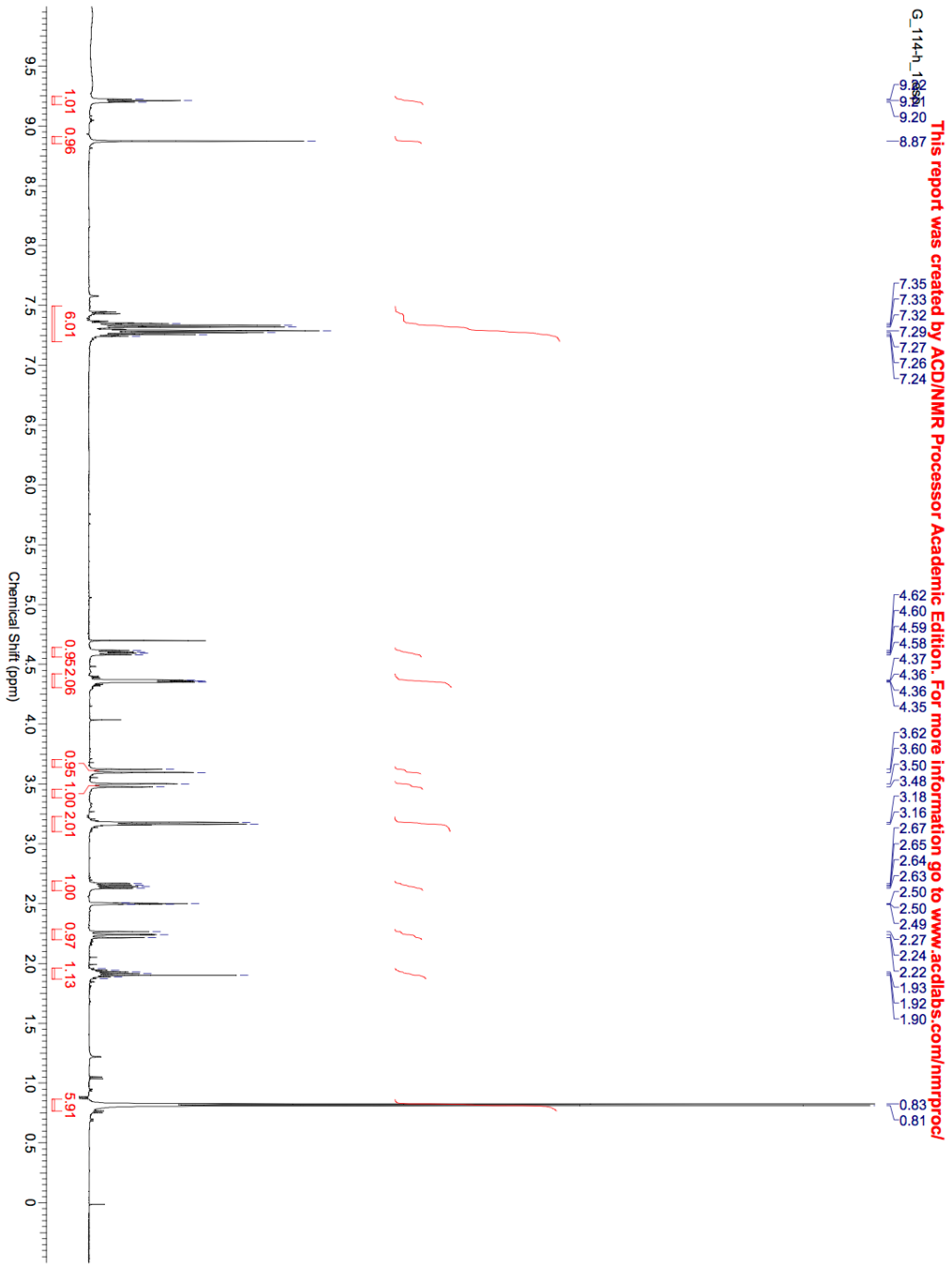
156.26
141.18
129.82
129.56
129.04
128.81
127.92
127.74
125.53
125.03
122.87
77.29
77.04
76.79
65.91
60.23
46.47
43.36
33.59
27.25
19.84
0.20
0.01
Chemical Shift (ppm)
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/


G_114-h_1
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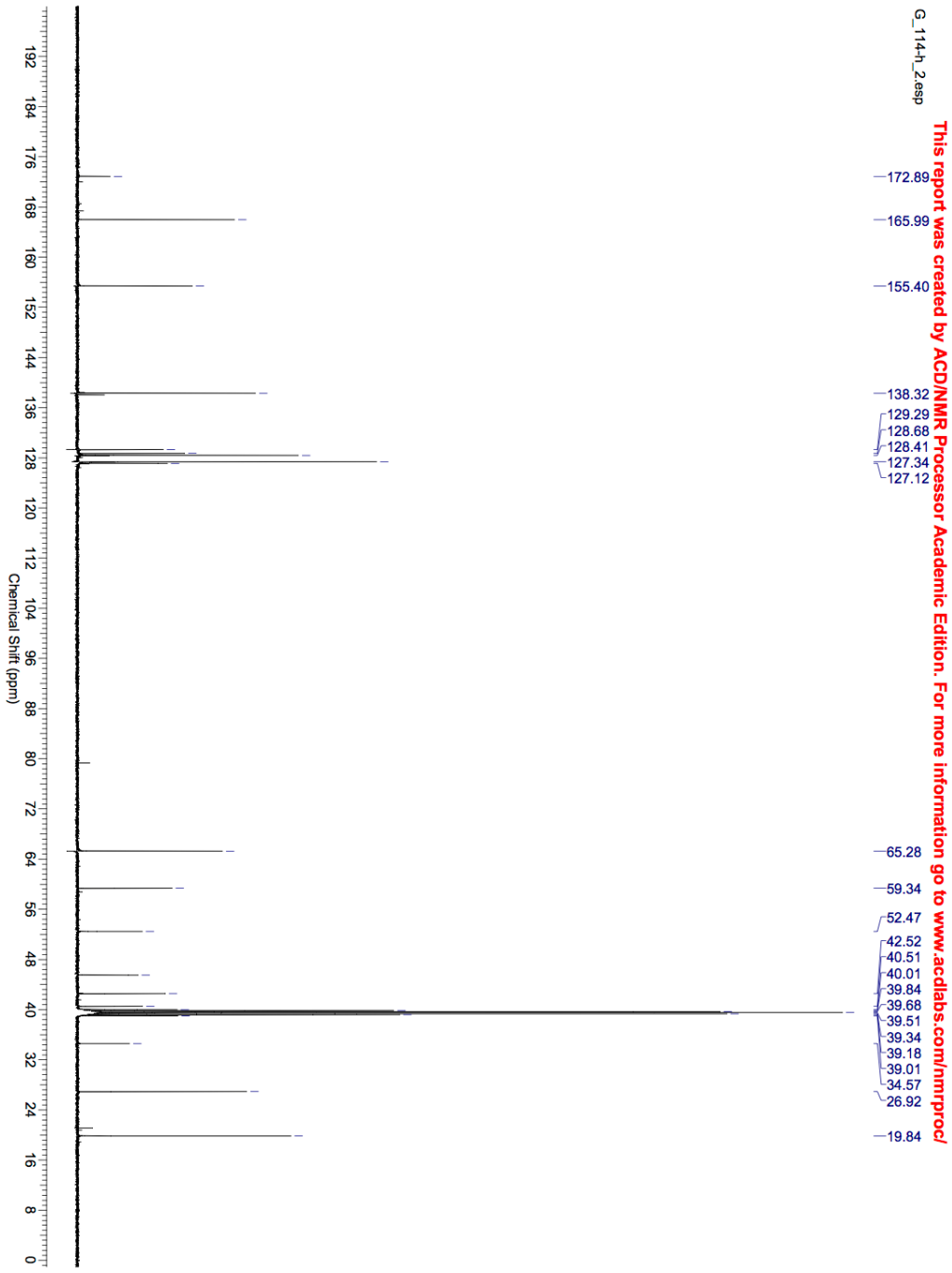
9.21
9.20
8.87
7.35
7.33
7.32
7.29
7.27
7.26
7.24
4.62
4.60
4.59
4.58
4.37
4.36
4.36
4.35
3.62
3.60
3.50
3.48
3.18
3.16
2.67
2.65
2.64
2.63
2.50
2.50
2.49
2.27
2.24
2.22
1.93
1.92
1.90
0.83
0.81
1.01
0.96
6.01
0.95
2.06
0.95
1.00
2.01
1.00
0.97
1.13
5.91
Chemical Shift (ppm)
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/


G_114-h_2.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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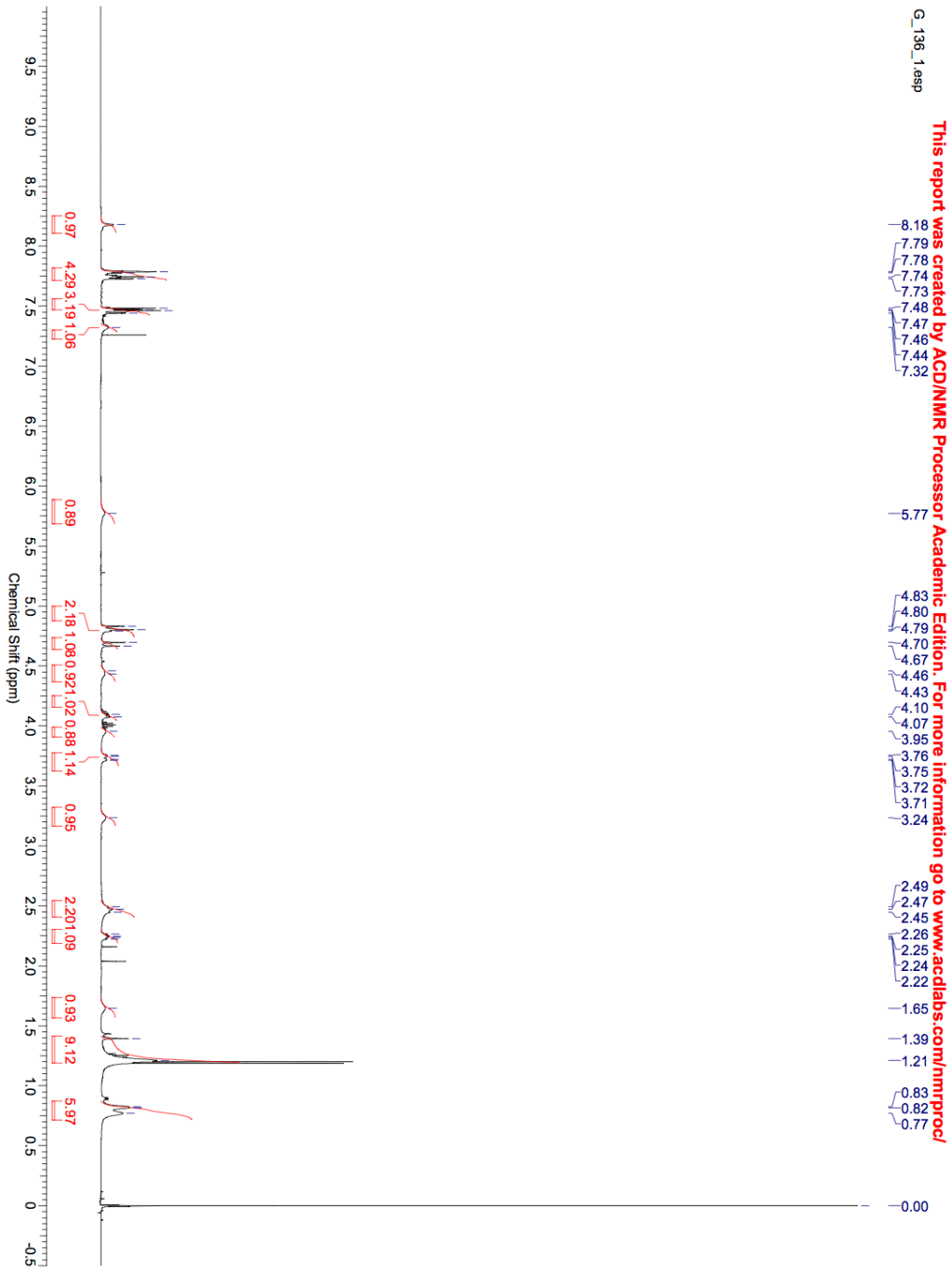
138.32
129.29
128.68
128.41
127.34
127.12
65.28
59.34
52.47
42.52
40.51
40.01
39.84
39.68
39.51
39.34
39.18
39.01
34.57
26.92
19.84
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0


G_136_1.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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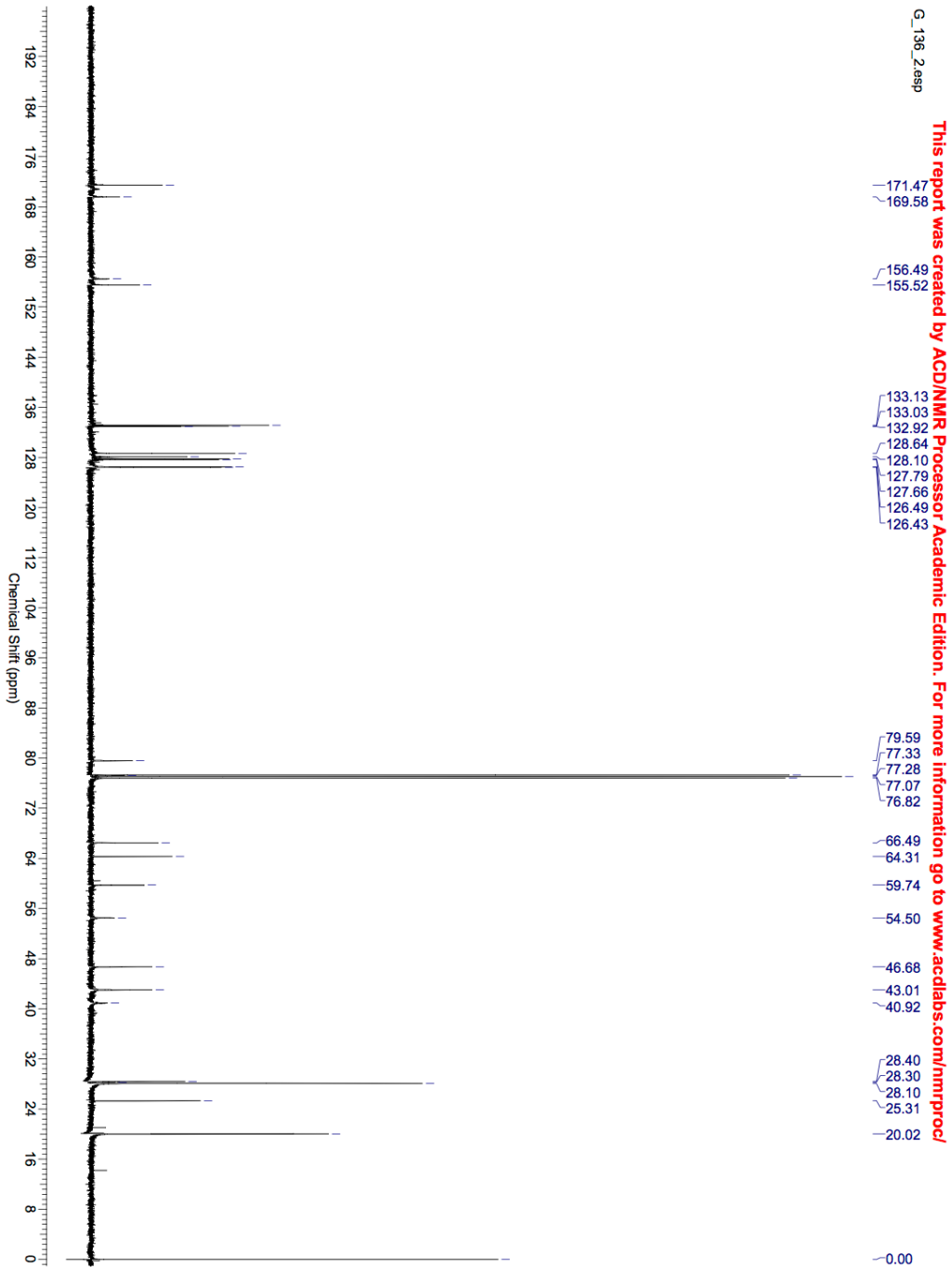
7.79
7.78
7.74
7.73
7.48
7.47
7.46
7.44
7.32
5.77
4.83
4.80
4.79
4.70
4.67
4.46
4.43
4.10
4.07
3.95
3.76
3.75
3.72
3.71
3.24
2.49
2.47
2.45
2.26
2.25
2.24
2.22
1.65
1.39
1.21
0.83
0.82
0.77
0.00
0.97
4.29
3.19
1.06
0.89
2.18
1.08
0.92
1.02
0.88
1.14
0.95
2.20
1.09
0.93
9.12
5.97
Chemical Shift (ppm)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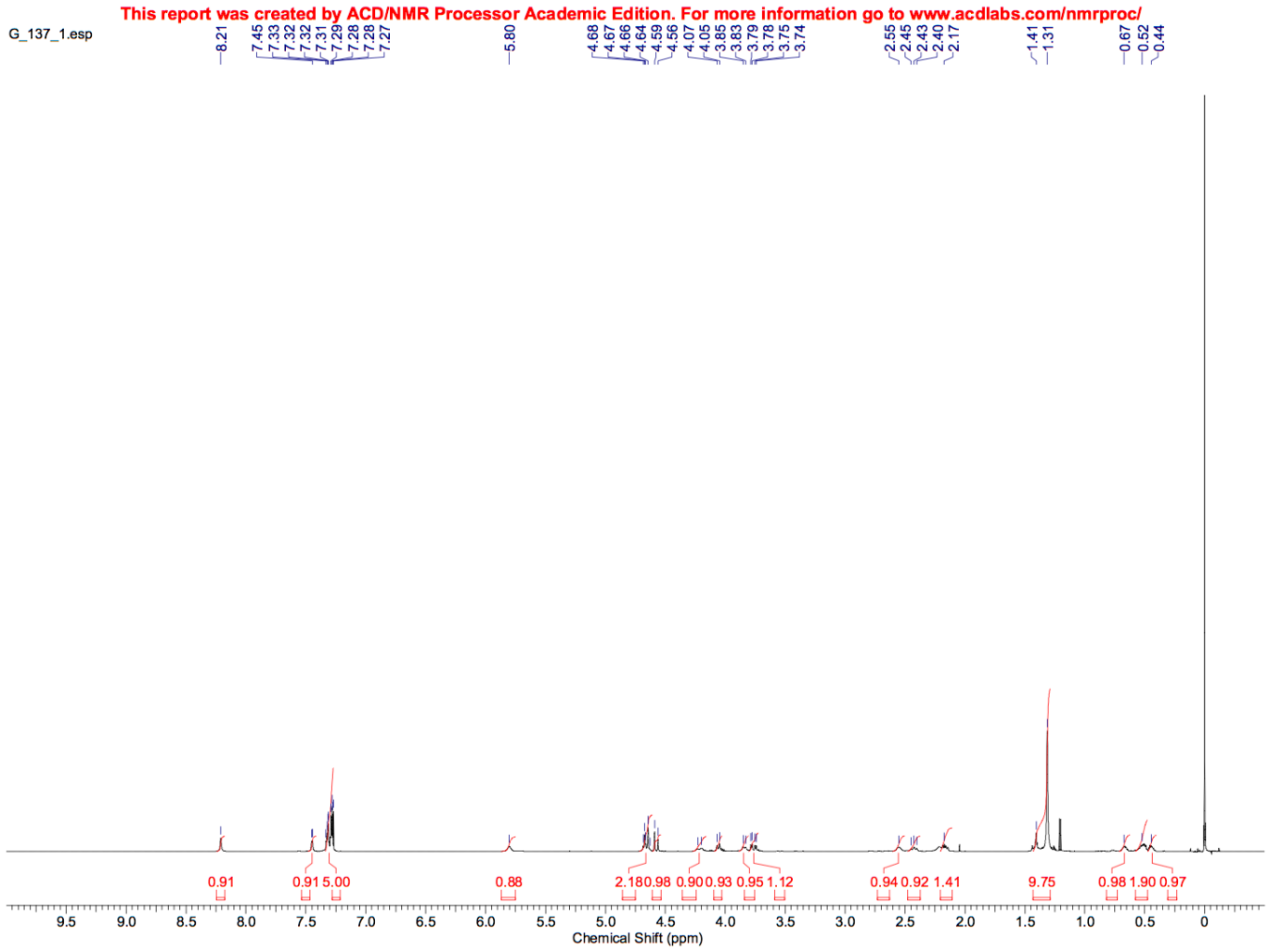
155.52
133.13
133.03
132.92
128.64
128.10
127.79
127.66
126.49
126.43
79.59
77.33
77.28
77.07
76.82
66.49
64.31
59.74
54.50
46.68
43.01
40.92
28.40
28.30
28.10
25.31
20.02
0.00
Chemical Shift (ppm)
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/


This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
G_137_1.esp
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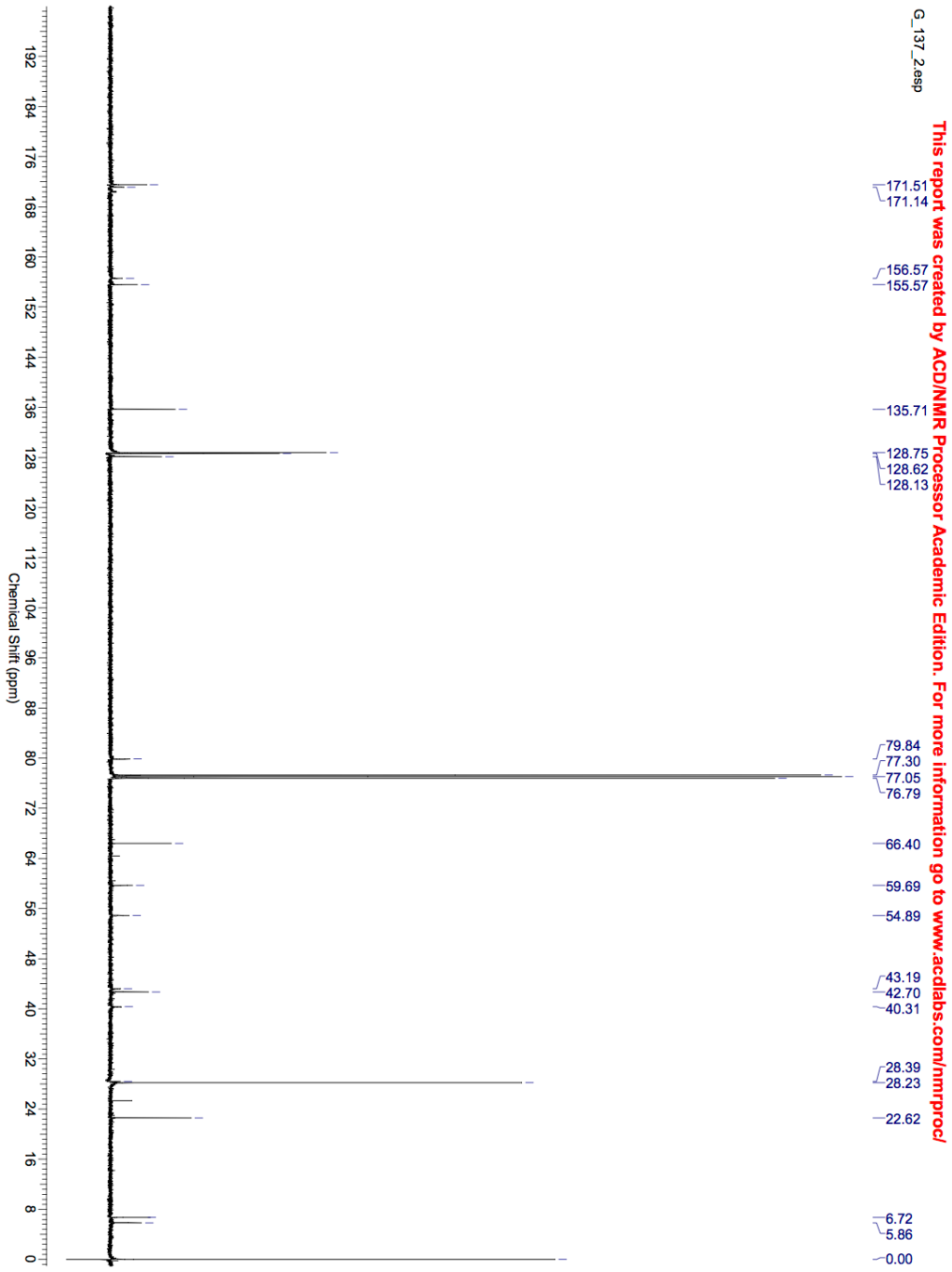
7.32
7.32
7.31
7.29
7.28
7.28
7.27
5.80
4.68
4.67
4.66
4.64
4.59
4.56
4.07
4.05
3.85
3.83
3.79
3.78
3.75
3.74
2.55
2.45
2.43
2.40
2.17
1.41
1.31
0.67
0.52
0.44
0.91
0.91
5.00
0.88
2.18
0.98
0.90
0.93
0.95
1.12
0.94
0.92
1.41
9.75
0.98
1.90
0.97
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Chemical Shift (ppm)


G_137_2.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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171.14
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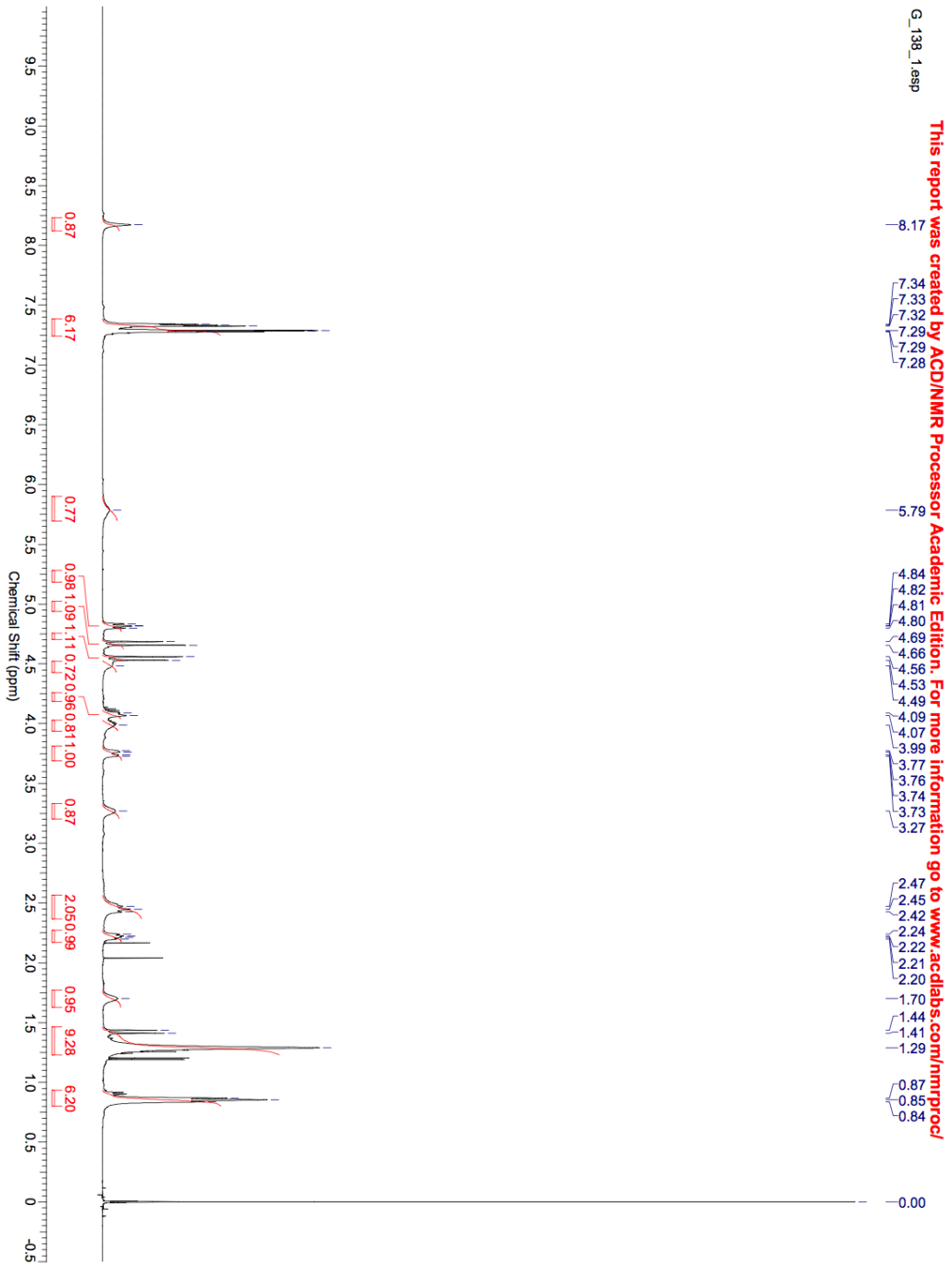
155.57
135.71
128.75
128.62
128.13
79.84
77.30
77.05
76.79
66.40
59.69
54.89
43.19
42.70
40.31
28.39
28.23
22.62
6.72
5.86
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0


G_138_1.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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7.34
7.33
7.32
7.29
7.29
7.28
5.79
4.84
4.82
4.81
4.80
4.69
4.66
4.56
4.53
4.49
4.09
4.07
3.99
3.77
3.76
3.74
3.73
3.27
2.47
2.45
2.42
2.24
2.22
2.21
2.20
1.70
1.44
1.41
1.29
0.87
0.85
0.84
0.00
0.87
6.17
0.77
0.98
1.09
1.11
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0.96
0.81
1.00
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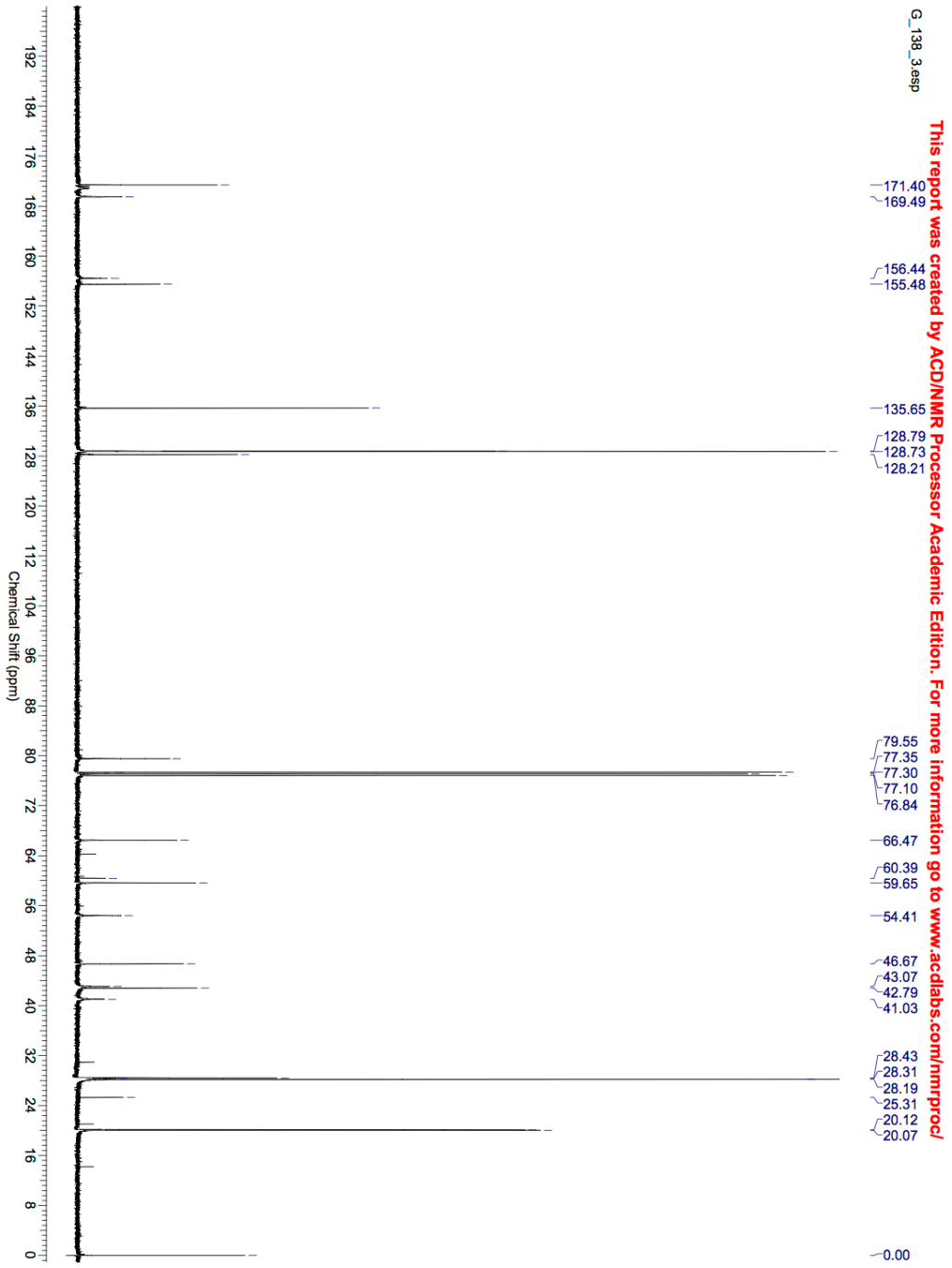
2.05
0.99
0.95
9.28
6.20
Chemical Shift (ppm)


G_138_3.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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169.49
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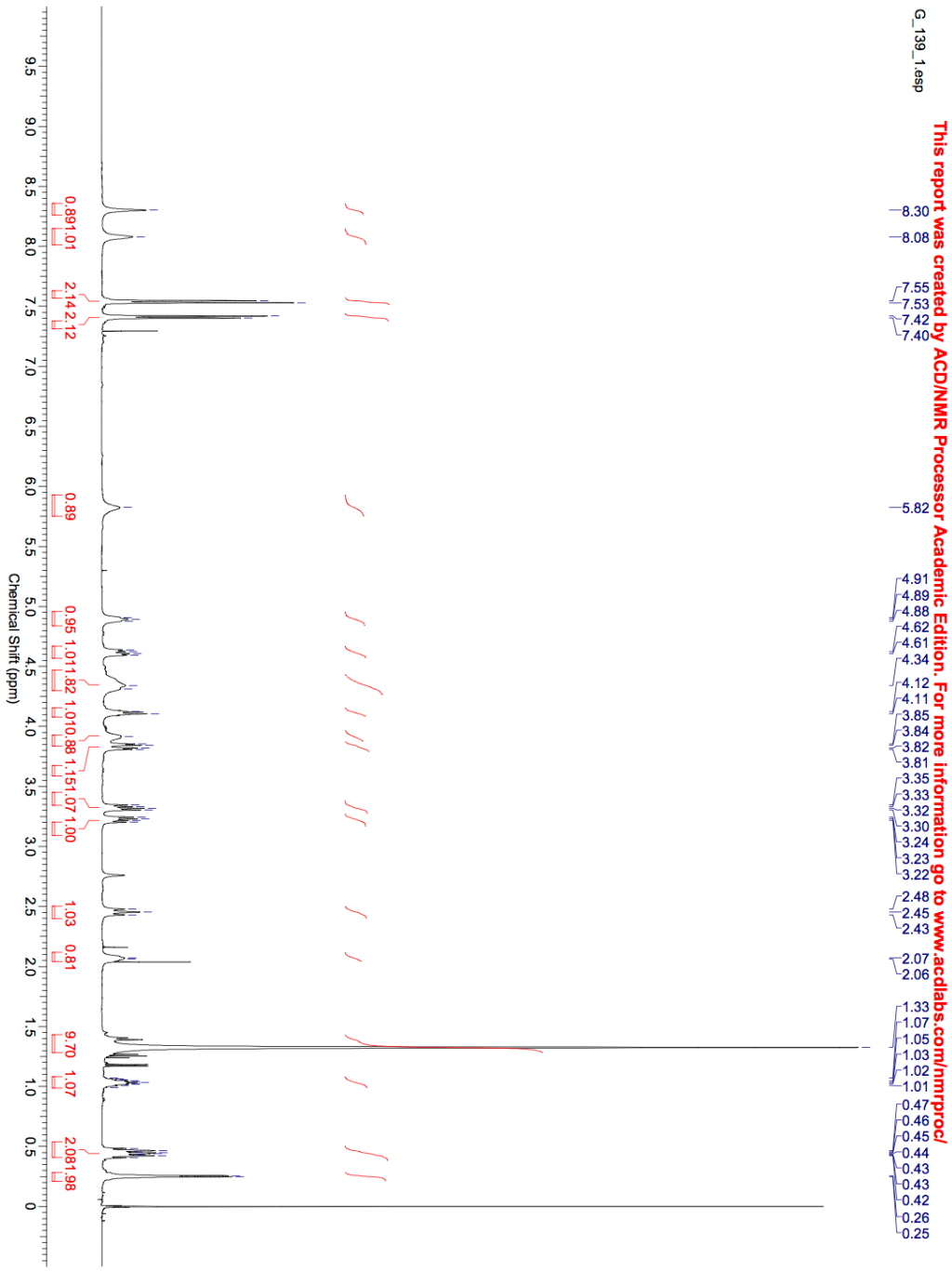
155.48
135.65
128.79
128.73
128.21
79.55
77.35
77.30
77.10
76.84
66.47
60.39
59.65
54.41
46.67
43.07
42.79
41.03
28.43
28.31
28.19
25.31
20.12
20.07
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0


G_139_1.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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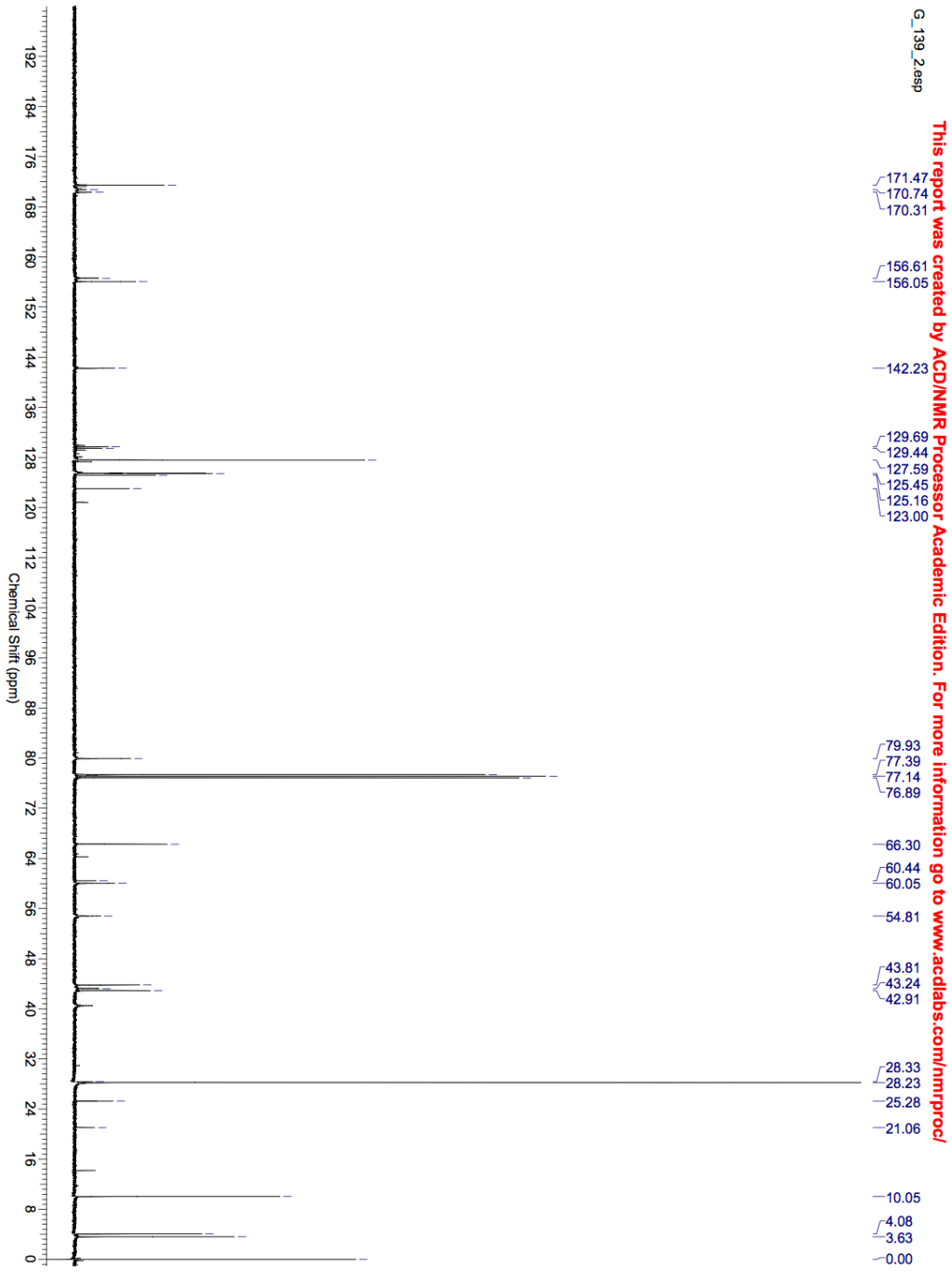
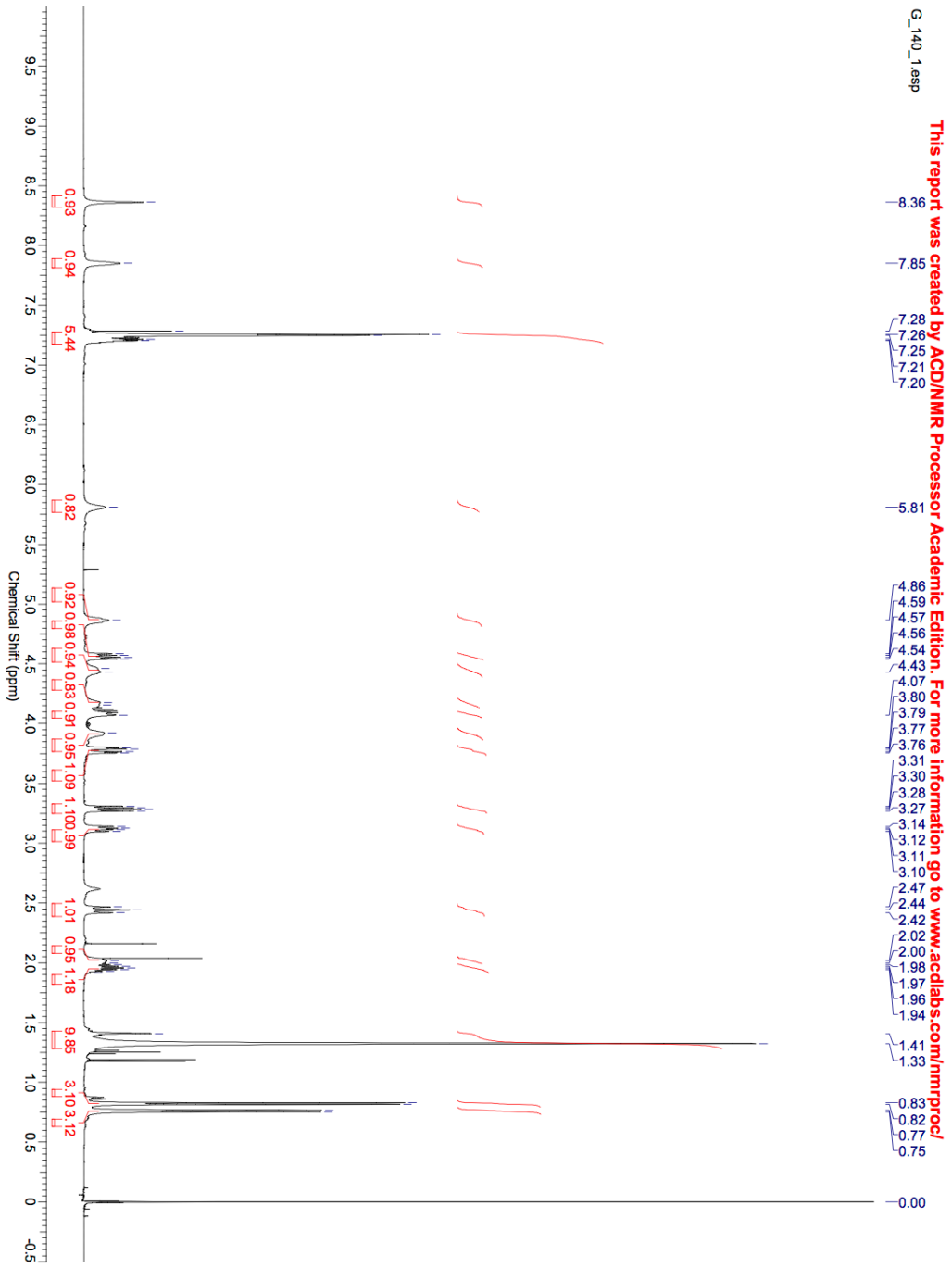
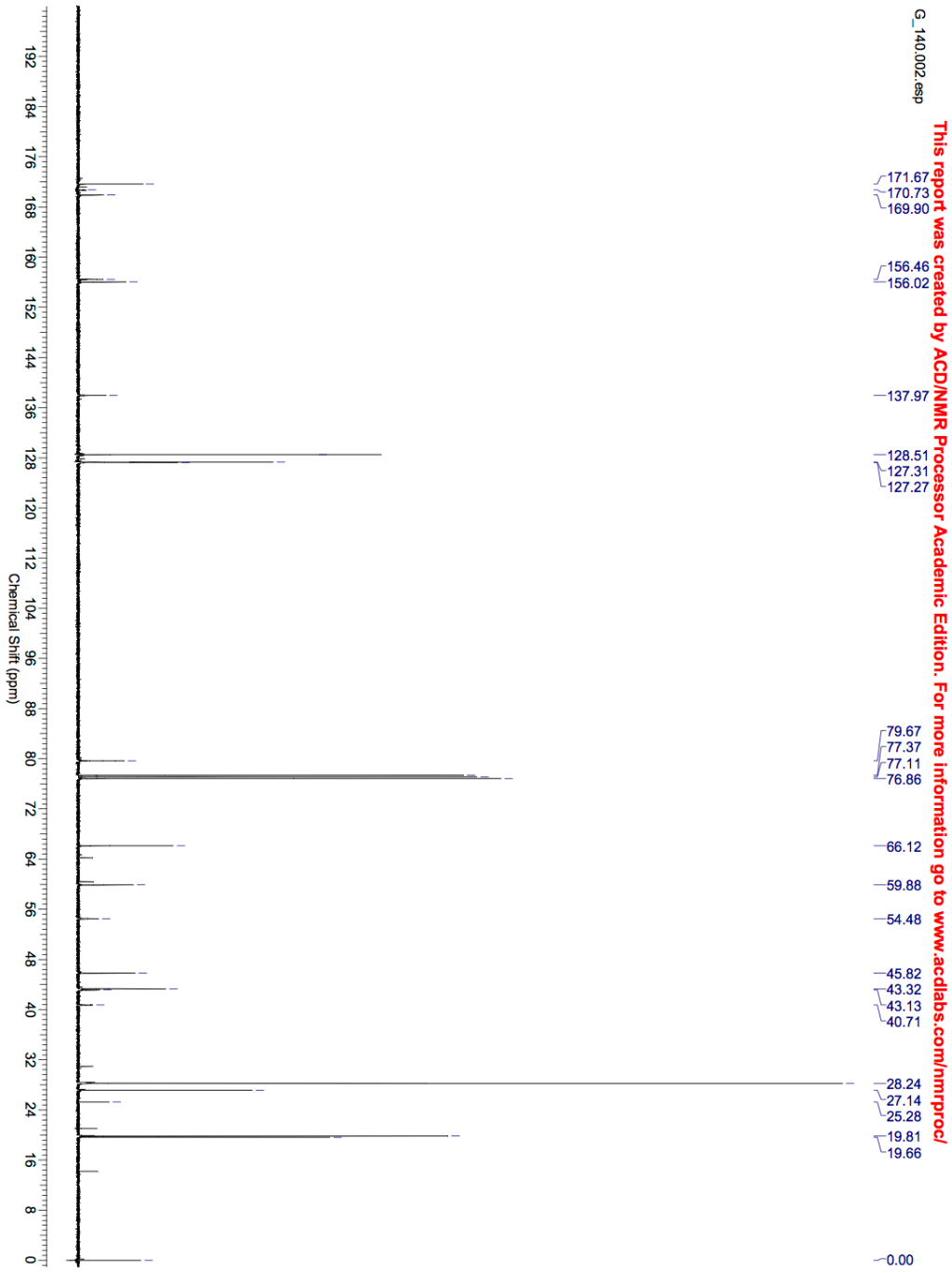
8.08
7.55
7.53
7.42
7.40
5.82
4.91
4.89
4.88
4.62
4.61
4.34
4.12
4.11
3.85
3.84
3.82
3.81
3.35
3.33
3.32
3.30
3.24
3.23
3.22
2.48
2.45
2.43
2.07
2.06
1.33
1.07
1.05
1.03
1.02
1.01
0.47
0.46
0.45
0.44
0.43
0.43
0.42
0.26
0.25
0.89
1.01
2.14
2.12
0.89
0.95
1.01
1.82
1.01
0.88
1.15
1.07
1.00
1.03
0.81
9.70
1.07
2.08
1.98
Chemical Shift (ppm)


G_139_2.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
171.47
170.74
170.31
156.61
156.05
142.23
129.69
129.44
127.59
125.45
125.16
123.00
79.93
77.39
77.14
76.89
66.30
60.44
60.05
54.81
43.81
43.24
42.91
28.33
28.23
25.28
21.06
10.05
4.08
3.63
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0


G_140_1.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
Chemical Shift (ppm)
8.36
7.85
7.28
7.26
7.25
7.21
7.20
5.81
4.86
4.59
4.57
4.56
4.54
4.43
4.07
3.80
3.79
3.77
3.76
3.31
3.30
3.28
3.27
3.14
3.12
3.11
3.10
2.47
2.44
2.42
2.02
2.00
1.98
1.97
1.96
1.94
1.41
1.33
0.83
0.82
0.77
0.75
0.00


G_140.002.esp
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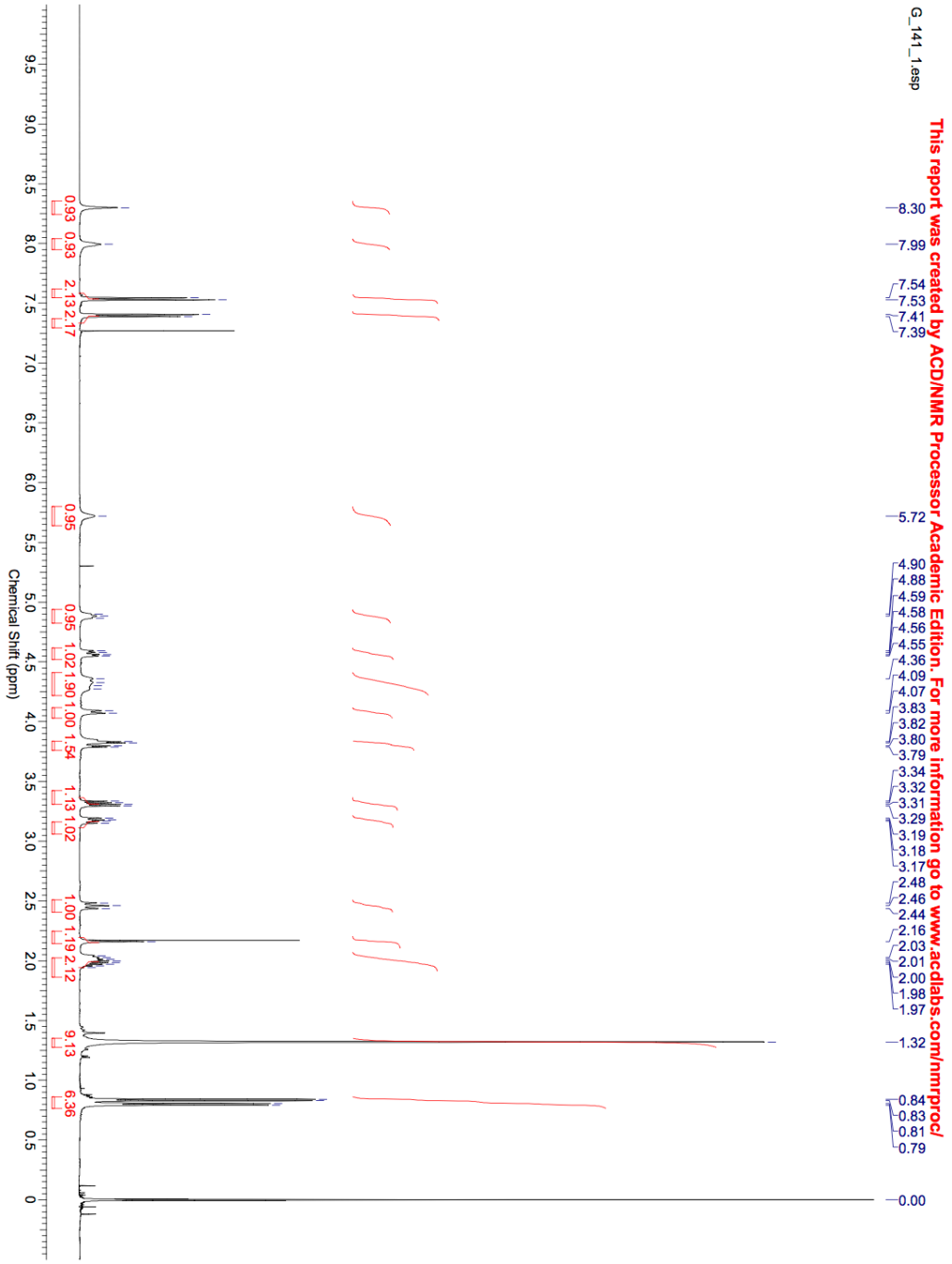
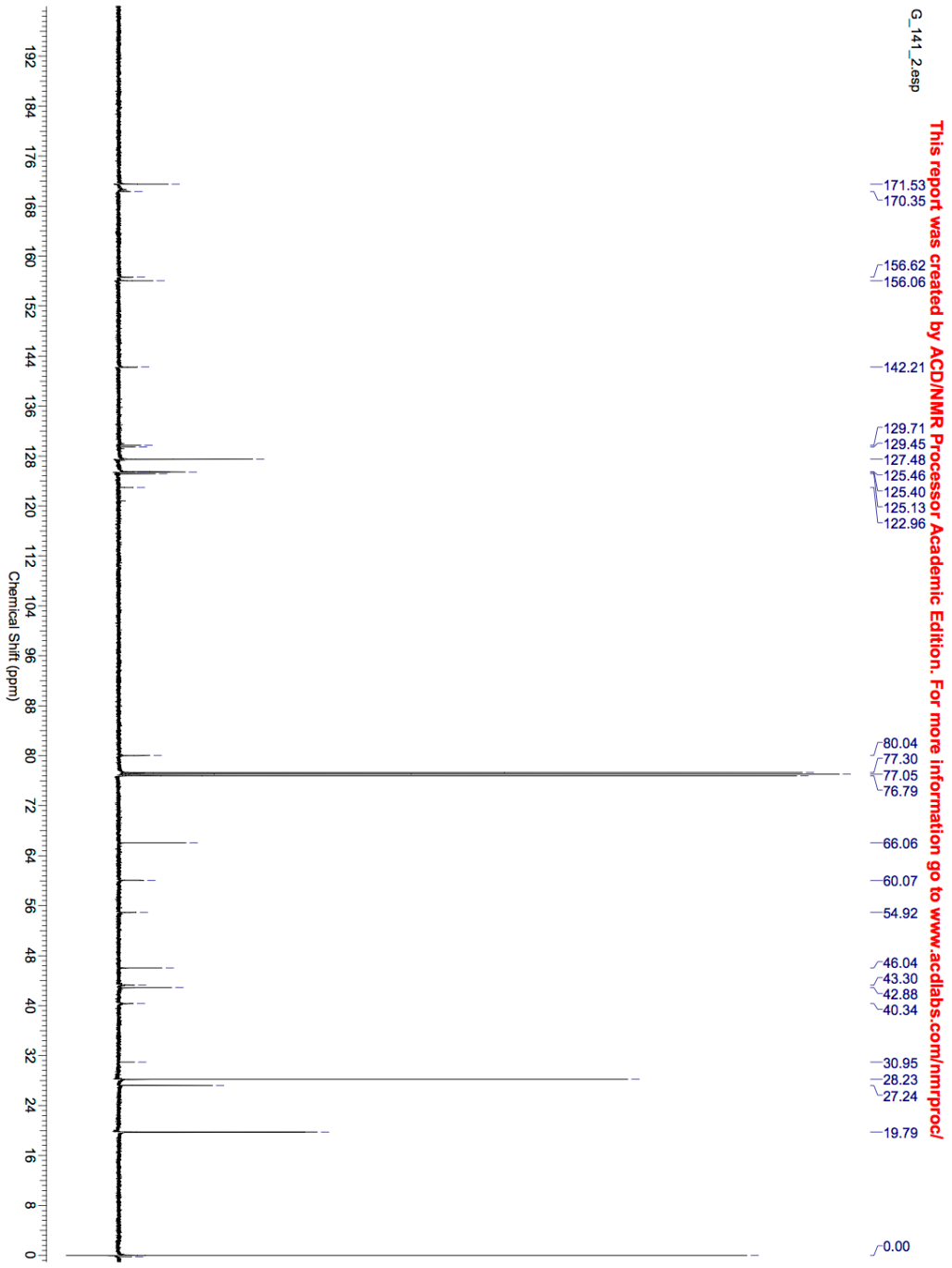
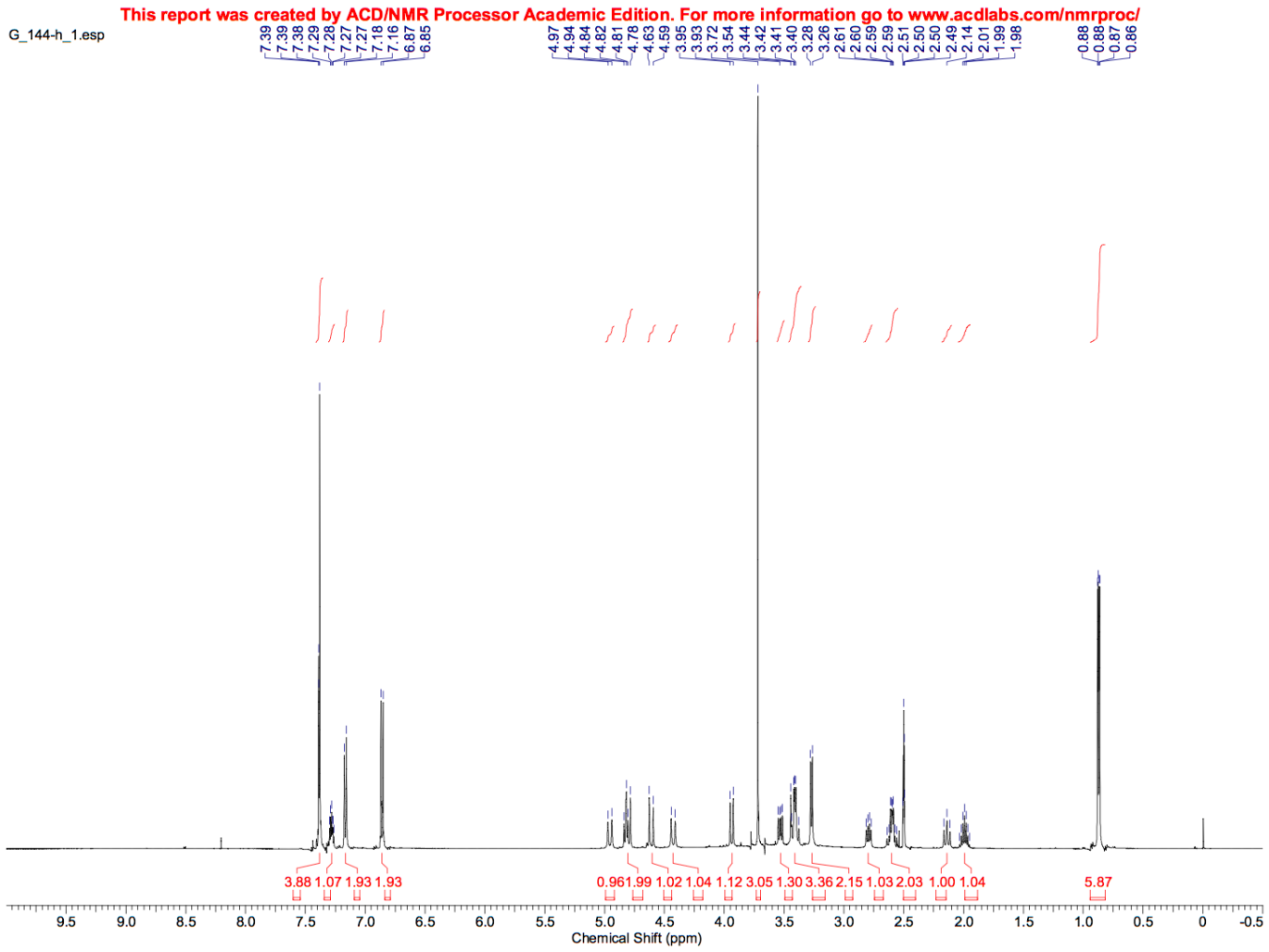
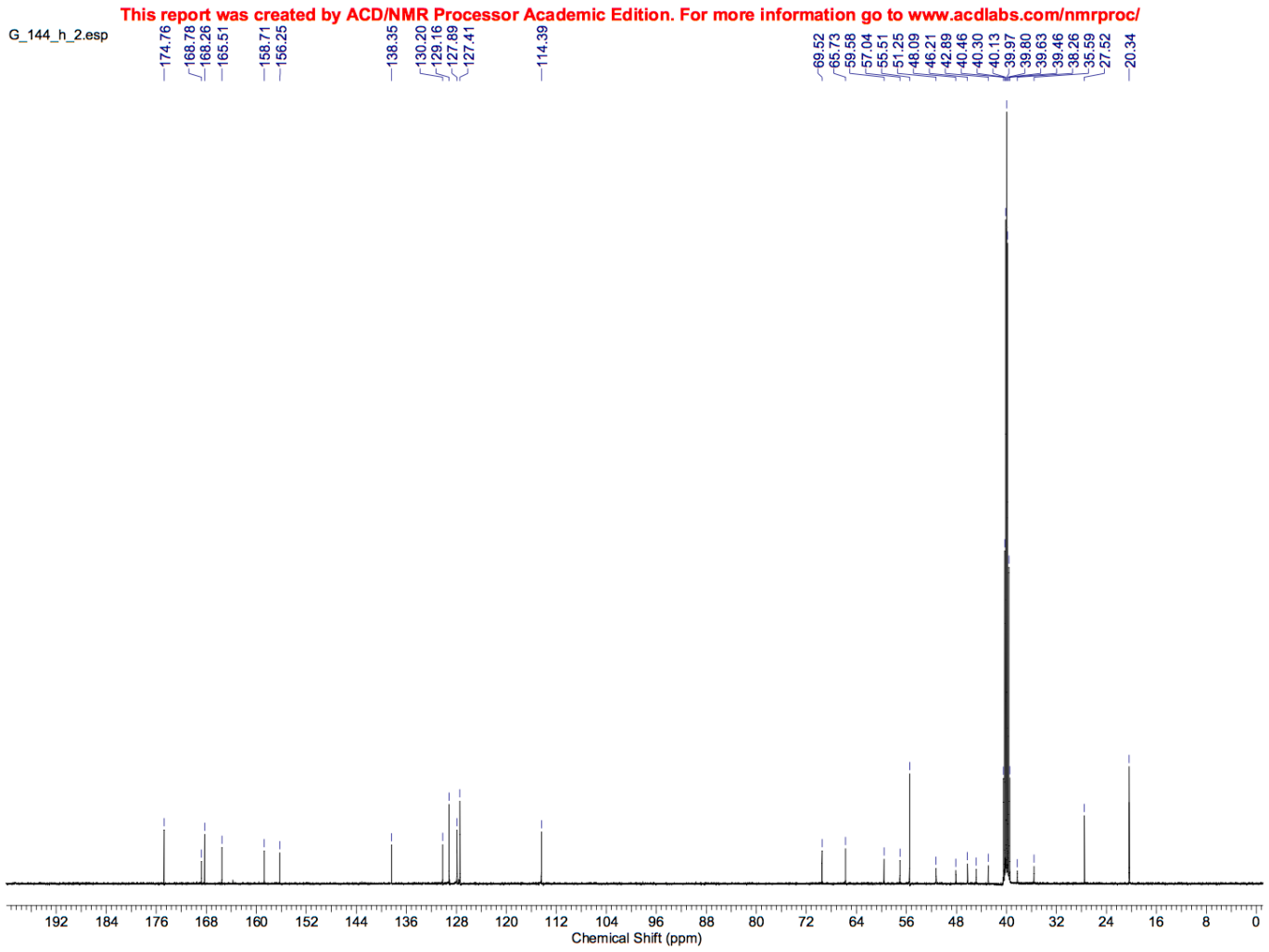
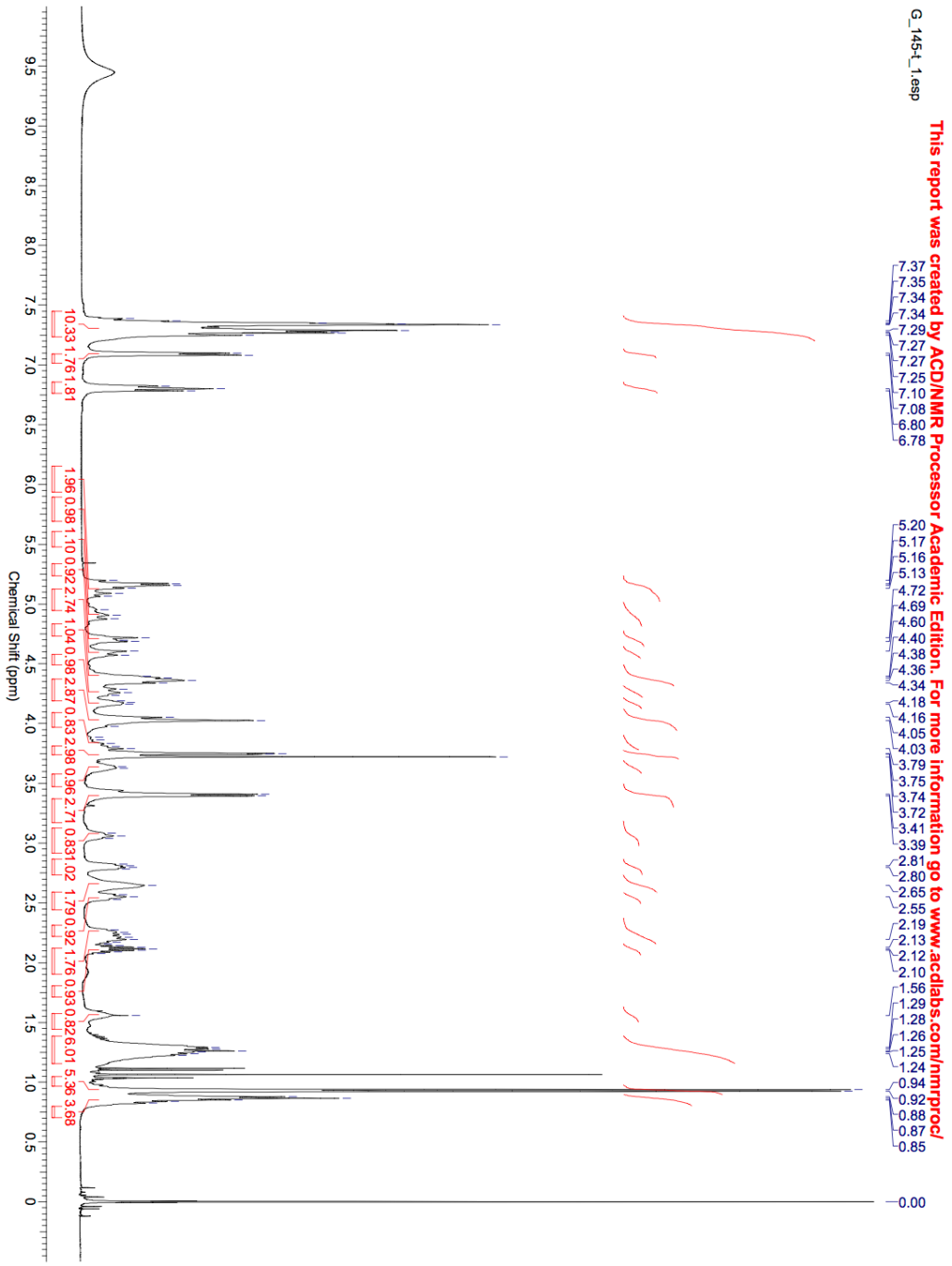
170.73
169.90
156.46
156.02
137.97
128.51
127.31
127.27
79.67
77.37
77.11
76.86
66.12
59.88
54.48
45.82
43.32
43.13
40.71
28.24
27.14
25.28
19.81
19.66
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/

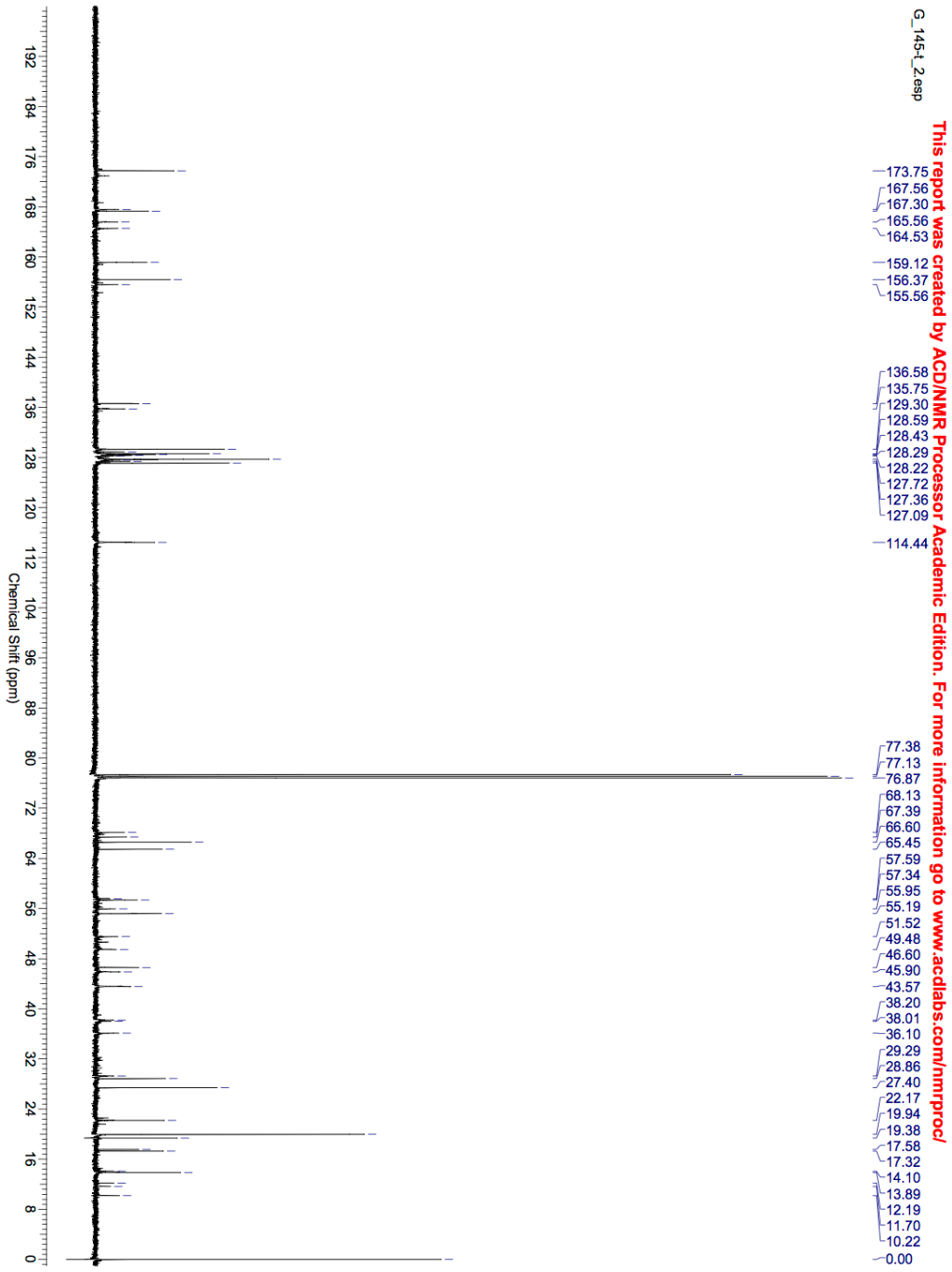

G_141_1.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
Chemical Shift (ppm)
8.30
7.99
7.54
7.53
7.41
7.39
5.72
4.90
4.88
4.59
4.58
4.56
4.55
4.36
4.09
4.07
3.83
3.82
3.80
3.79
3.34
3.32
3.31
3.29
3.19
3.18
3.17
2.48
2.46
2.44
2.16
2.03
2.01
2.00
1.98
1.97
1.32
0.84
0.83
0.81
0.79
0.00


G_141_2.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
171.53
170.35
156.62
156.06
142.21
129.71
129.45
127.48
125.46
125.40
125.13
122.96
80.04
77.30
77.05
76.79
66.06
60.07
54.92
46.04
43.30
42.88
40.34
30.95
28.23
27.24
19.79
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0


This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
G_144-h_1.esp
7.39 7.39 7.38 7.29 7.28 7.27 7.27 7.18 7.16 6.87 6.85
4.97 4.94 4.84 4.82 4.81 4.78 4.63 4.59 3.95 3.93 3.72 3.54 3.44 3.42 3.41 3.40 3.28 3.26 2.61 2.60 2.59 2.59 2.51 2.50 2.50 2.49 2.14 2.01 1.99 1.98
0.88 0.88 0.87 0.86
3.88 1.07 1.93 1.93
0.96 1.99 1.02 1.04 1.12 3.05 1.30 3.36 2.15 1.03 2.03 1.00 1.04
5.87
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0 -0.5
Chemical Shift (ppm)


This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
G_144_h_2.esp
174.76
168.78
168.26
165.51
158.71
156.25
138.35
130.20
129.16
127.89
127.41
114.39
69.52
65.73
59.58
57.04
55.51
51.25
48.09
46.21
42.89
40.46
40.30
40.13
39.97
39.80
39.63
39.46
38.26
35.59
27.52
20.34
192 184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 16 8 0
Chemical Shift (ppm)


G_145-t_1.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
Chemical Shift (ppm)
10.33 1.76 1.81 1.96 0.98 1.10 0.92 2.74 1.04 0.98 2.87 0.83 2.98 0.96 2.71 0.83 1.02 1.79 0.92 1.76 0.93 0.82 6.01 5.36 3.68
7.37 7.35 7.34 7.34 7.29 7.27 7.27 7.25 7.10 7.08 6.80 6.78
5.20 5.17 5.16 5.13 4.72 4.69 4.60 4.40 4.38 4.36 4.34 4.18 4.16 4.05 4.03 3.79 3.75 3.74 3.72 3.41 3.39 2.81 2.80 2.65 2.55 2.19 2.13 2.12 2.10 1.56 1.29 1.28 1.26 1.25 1.24 0.94 0.92 0.88 0.87 0.85
0.00


G_145-t_2.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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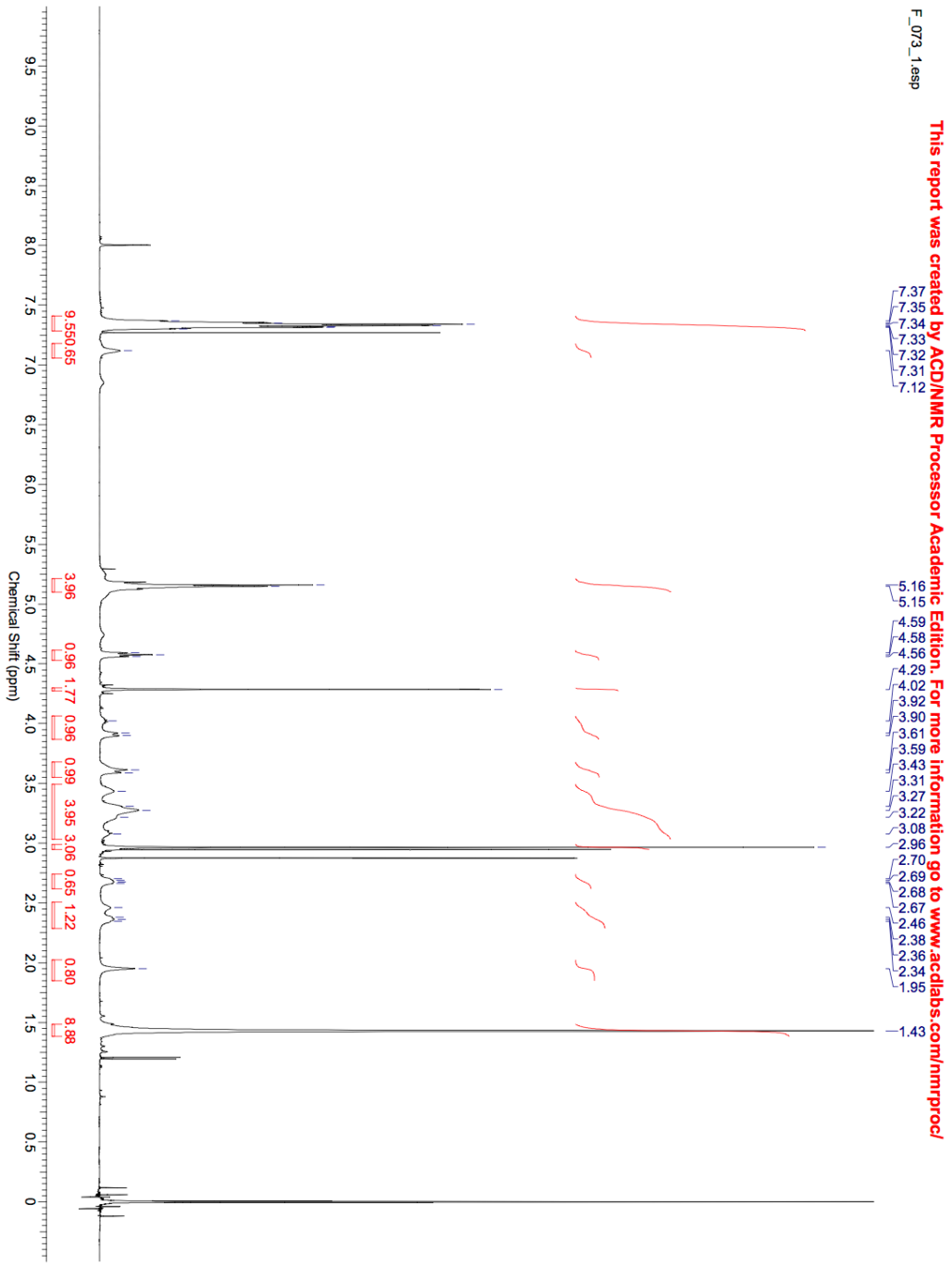
167.56
167.30
165.56
164.53
159.12
156.37
155.56
136.58
135.75
129.30
128.59
128.43
128.29
128.22
127.72
127.36
127.09
114.44
77.38
77.13
76.87
68.13
67.39
66.60
65.45
57.59
57.34
55.95
55.19
51.52
49.48
46.60
45.90
43.57
38.20
38.01
36.10
29.29
28.86
27.40
22.17
19.94
19.38
17.58
17.32
14.10
13.89
12.19
11.70
10.22
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0


F_073_1.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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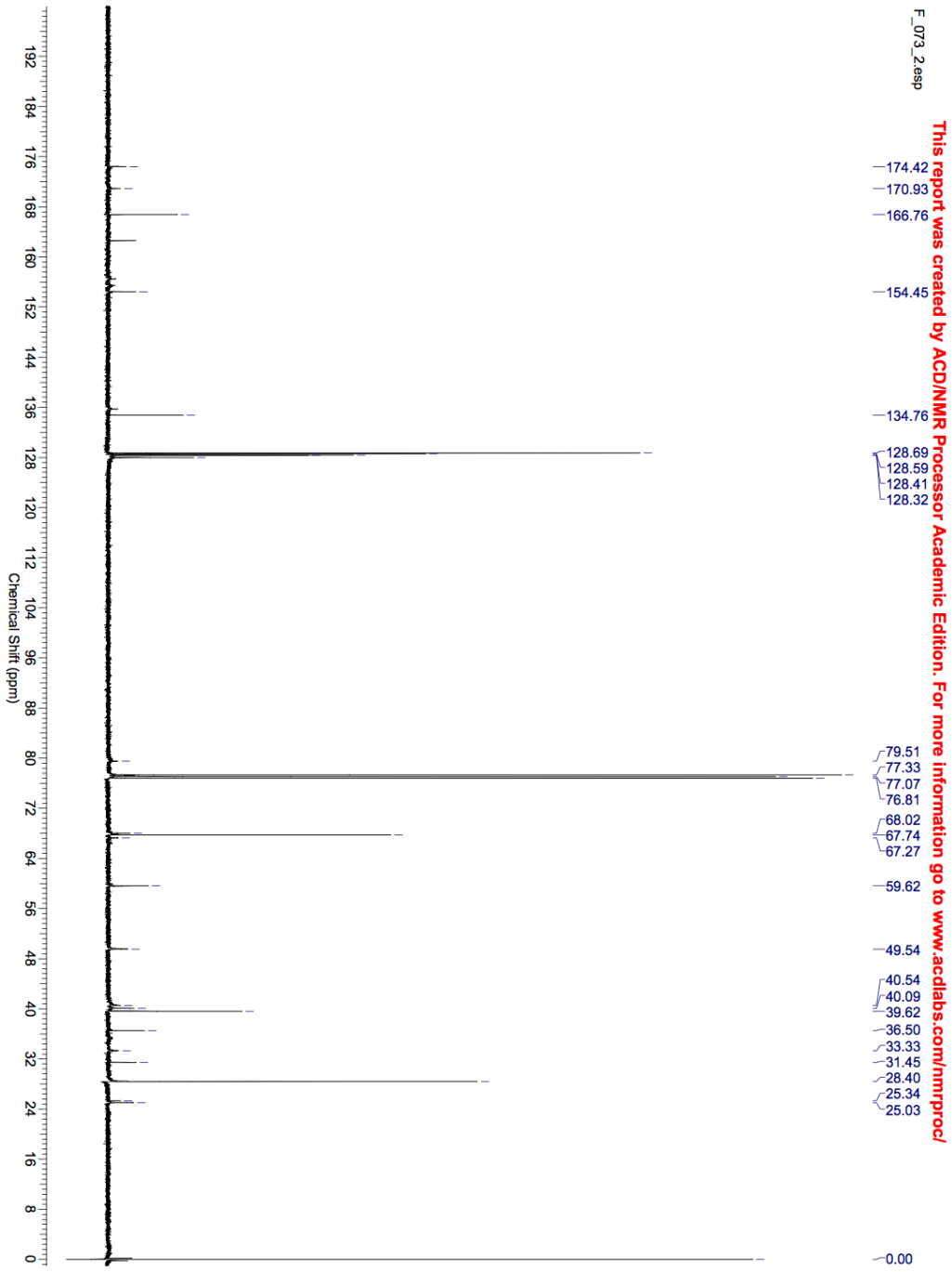
7.35
7.34
7.33
7.32
7.31
7.12
5.16
5.15
4.59
4.58
4.56
4.29
4.02
3.92
3.90
3.61
3.59
3.43
3.31
3.27
3.22
3.08
2.96
2.70
2.69
2.68
2.67
2.46
2.38
2.36
2.34
1.95
1.43
9.55
0.65
3.96
0.96
1.77
0.96
0.99
3.95
3.06
0.65
1.22
0.80
8.88
Chemical Shift (ppm)


F_073_2.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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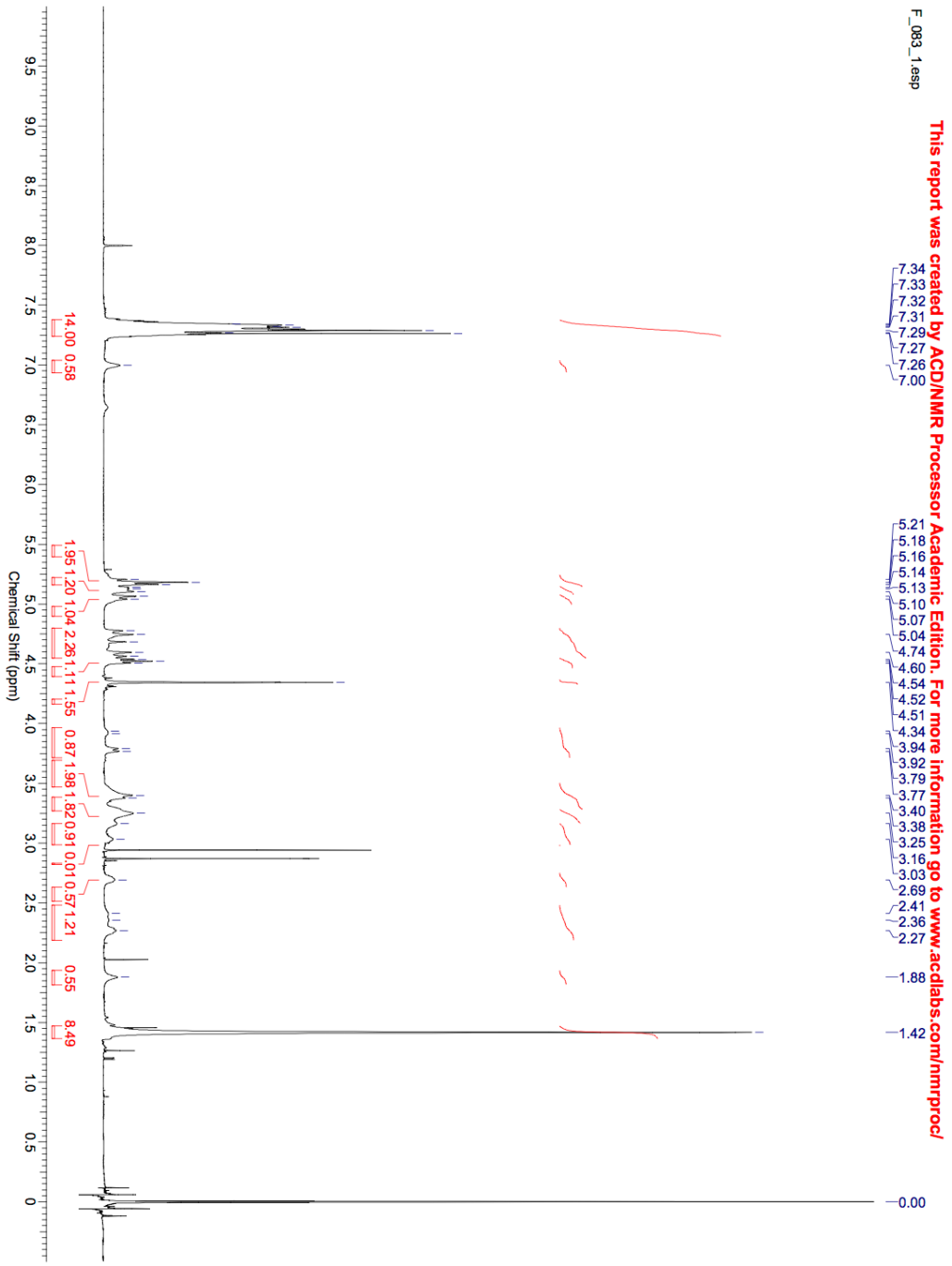
170.93
166.76
154.45
134.76
128.69
128.59
128.41
128.32
79.51
77.33
77.07
76.81
68.02
67.74
67.27
59.62
49.54
40.54
40.09
39.62
36.50
33.33
31.45
28.40
25.34
25.03
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0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This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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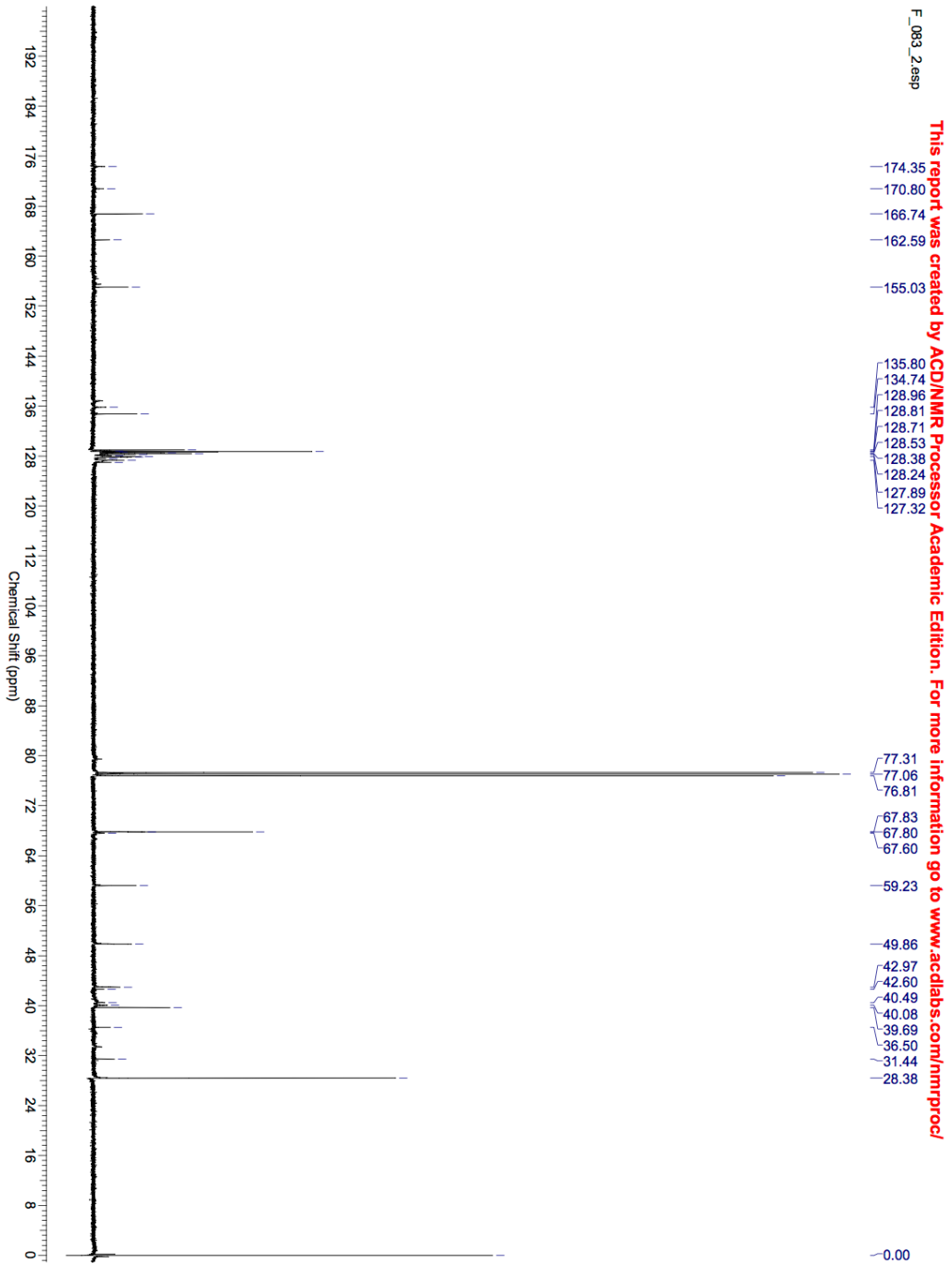
7.32
7.31
7.29
7.27
7.26
7.00
5.21
5.18
5.16
5.14
5.13
5.10
5.07
5.04
4.74
4.60
4.54
4.52
4.51
4.34
3.94
3.92
3.79
3.77
3.40
3.38
3.25
3.16
3.03
2.69
2.41
2.36
2.27
1.88
1.42
0.00
14.00 0.58
1.95 1.20 1.04 2.26 1.11 1.55 0.87 1.98 1.82 0.91 0.01 0.57 1.21 0.55 8.49
Chemical Shift (ppm)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155.03
135.80
134.74
128.96
128.81
128.71
128.53
128.38
128.24
127.89
127.32
77.31
77.06
76.81
67.83
67.80
67.60
59.23
49.86
42.97
42.60
40.49
40.08
39.69
36.50
31.44
28.38
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
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32
24
16
8
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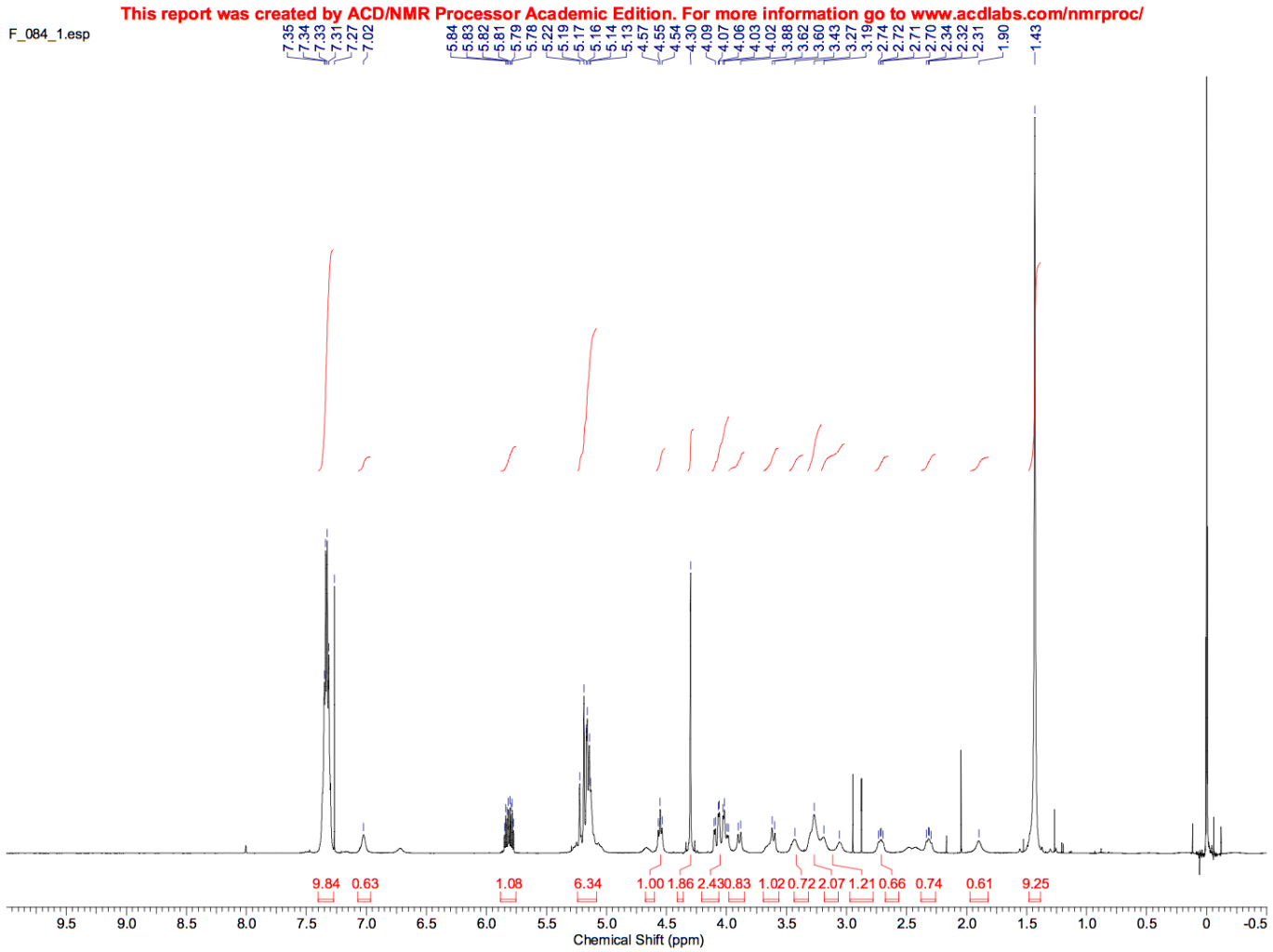
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/


This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
F_084_1.esp
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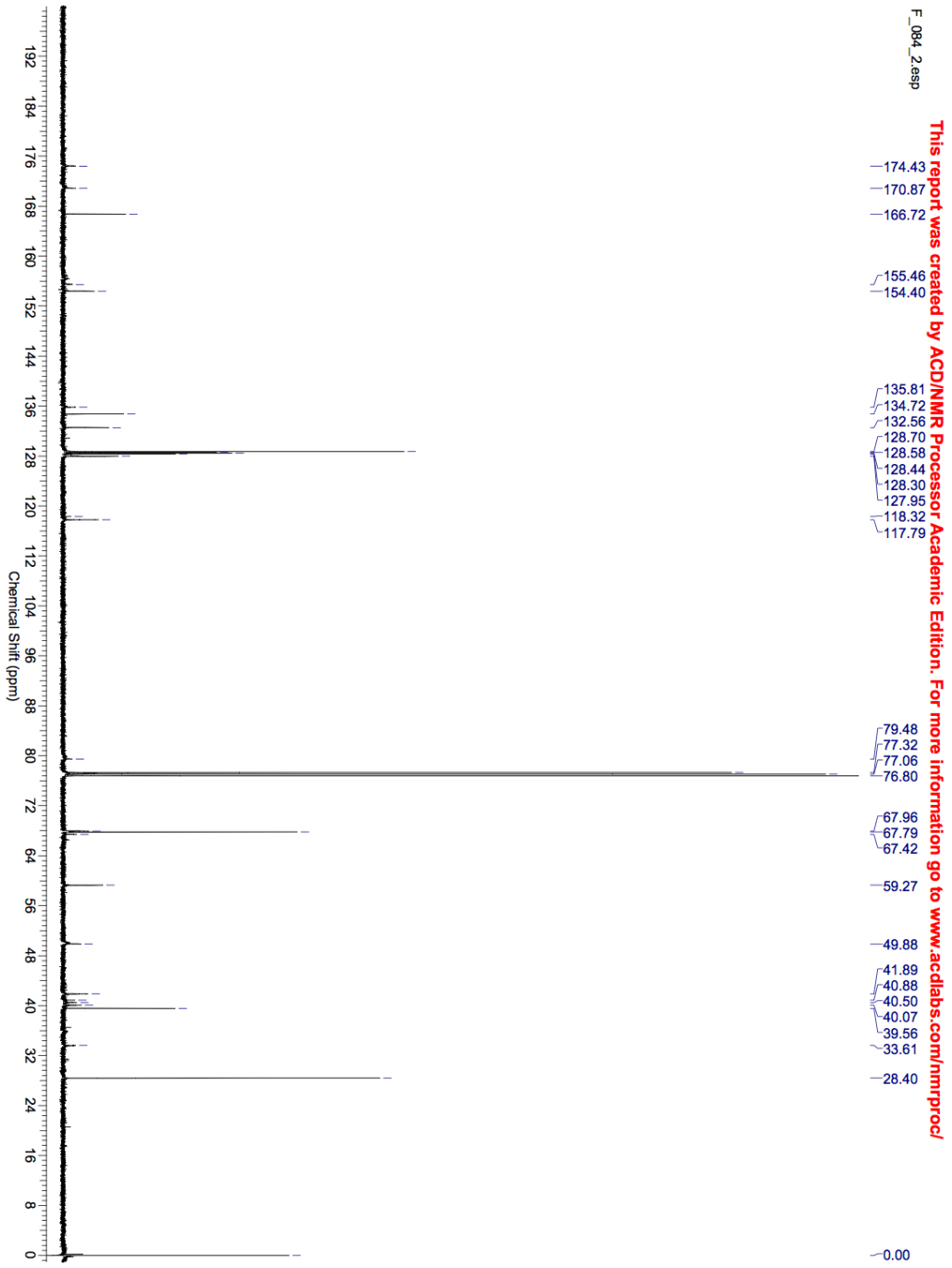
9.84 0.63 1.08 6.34 1.00 1.86 2.43 0.83 1.02 0.72 2.07 1.21 0.66 0.74 0.61 9.25
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0 -0.5
Chemical Shift (ppm)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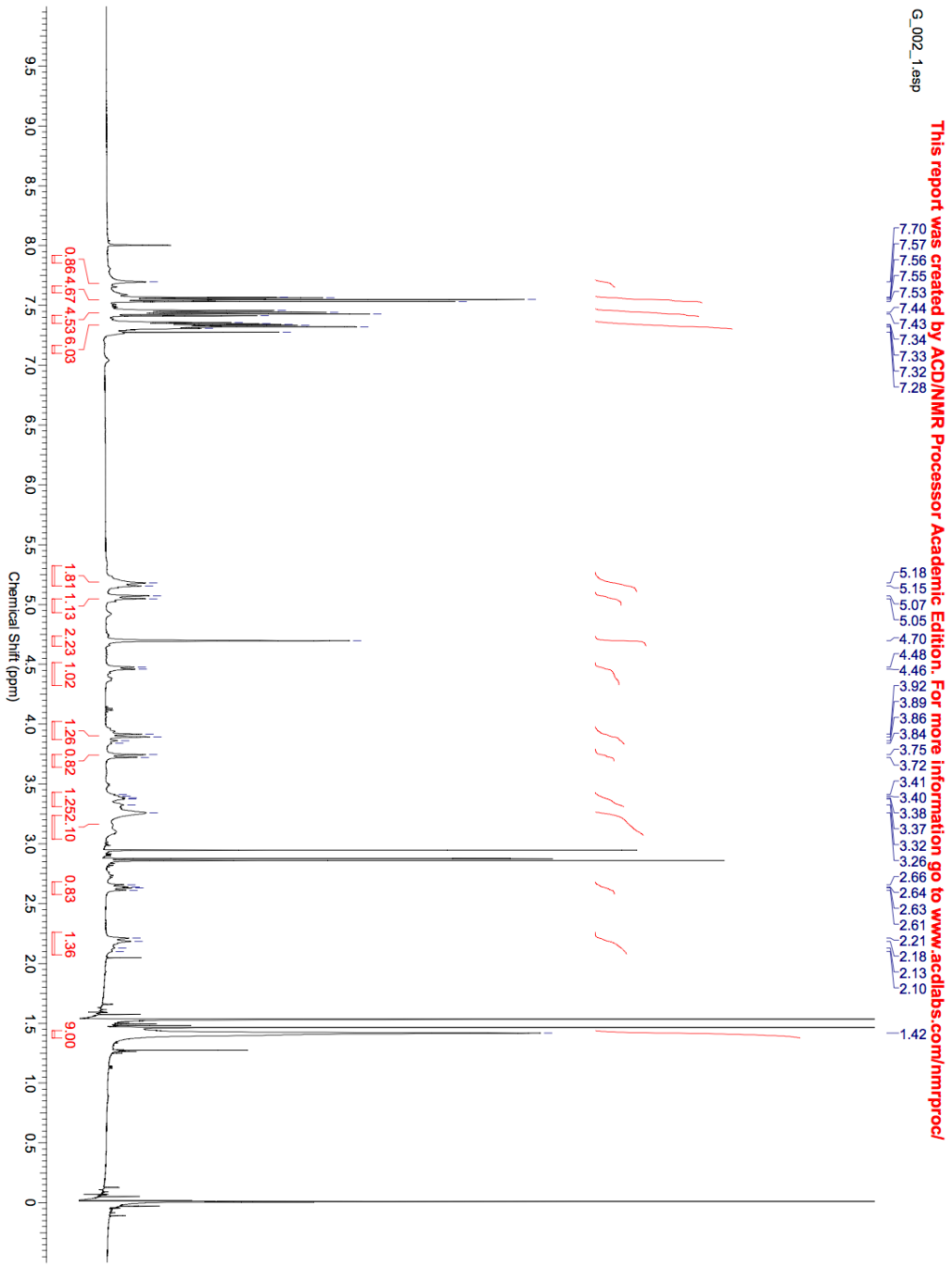
135.81
134.72
132.56
128.70
128.58
128.44
128.30
127.95
118.32
117.79
79.48
77.32
77.06
76.80
67.96
67.79
67.42
59.27
49.88
41.89
40.88
40.50
40.07
39.56
33.61
28.40
0.00
Chemical Shift (ppm)
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/


G_002_1.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
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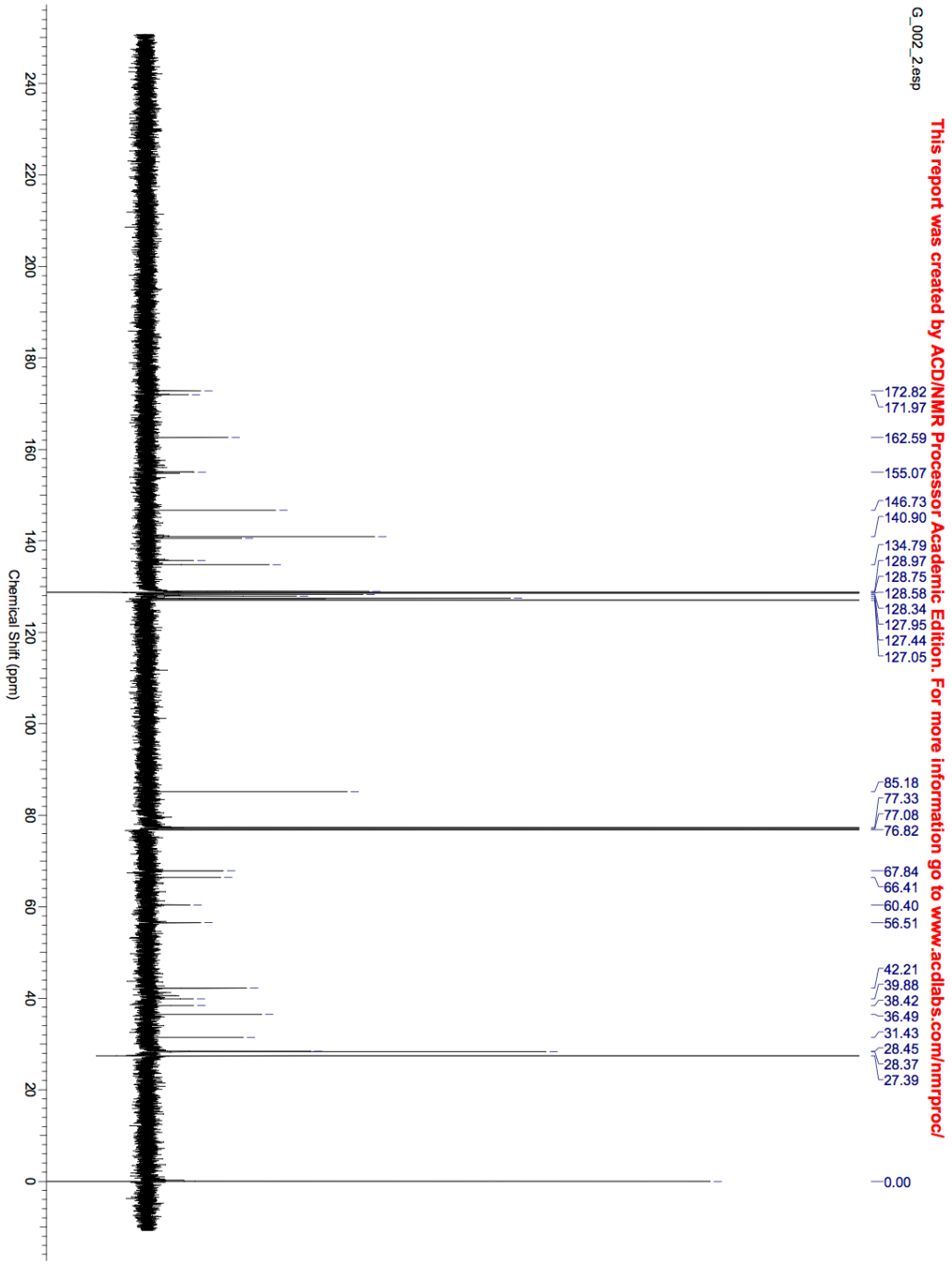
7.57
7.56
7.55
7.53
7.44
7.43
7.34
7.33
7.32
7.28
5.18
5.15
5.07
5.05
4.70
4.48
4.46
3.92
3.89
3.86
3.84
3.75
3.72
3.41
3.40
3.38
3.37
3.32
3.26
2.66
2.64
2.63
2.61
2.21
2.18
2.13
2.10
1.42
0.86 4.67 4.53 6.03
1.81 1.13 2.23 1.02
1.26 0.82 1.25 2.10
0.83
1.36
9.00
Chemical Shift (ppm)


G_002_2.esp
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
Chemical Shift (ppm)
240
220
200
180
160
140
120
100
80
60
40
20
0
172.82
171.97
162.59
155.07
146.73
140.90
134.79
128.97
128.75
128.58
128.34
127.95
127.44
127.05
85.18
77.33
77.08
76.82
67.84
66.41
60.40
56.51
42.21
39.88
38.42
36.49
31.43
28.45
28.37
27.39
0.00