

**SYNTHESIS OF PEPTOID SUBMONOMER
MIMICS OF ARGININE**

By

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ABSTRACT

Peptides identified through natural product or high-throughput screening are becoming attractive therapeutics. However, many potential peptide therapeutics have poor proteolytic stability and short in vivo half-lives. To address these issues, many researchers use peptoid compounds, which are peptide mimics, as therapeutics. Because they are completely resistant to proteolysis, peptoids make excellent candidates for a range of different biomedical applications. The present study supports the growing field of research into peptoid therapeutics by developing peptoid submonomer mimics of common amino acids. The peptide that was used in this research is the affinity tag AviD, which has unique amino acids that can be studied by peptoid submonomer substitution. The main focus of this work is on arginine. The overall goal of this study is to determine which peptoid submonomer best mimics these amino acids and maintains peptomer secondary structure in AviD. The present research will aid researchers in developing peptoid mimics of useful peptide therapeutics.

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CHAPTER I: INTRODUCTION

1.1 AMINO ACIDS AND PROTEINS

Proteins are a vital component of the body, regulating a number of important functions in the body. The amino acids are an essential part of the protein structure in all living organisms. Amino acids are defined as the organic substances that contain both amino and carboxylic acid groups. More than 300 kinds of amino acids are present in nature but among them only 20 alpha amino acids are the common building blocks of proteins. Most proteins, either from unicellular bacteria or complex multicellular organisms, are built from the same set of 20 amino acids, linking covalently in distinctive linear sequences. The protein can also be hydrolyzed to their constituent amino acids by various methods. Among the 20 amino acids, asparagine was first discovered in 1806.¹⁻³ Beside these 20 amino acids, non-protein alpha amino acids (e.g., ornithine, homocysteine, and citrulline) and non-alpha amino acids (e.g., β -alanine and taurine) also play vital roles in cell functions.⁴

All of the amino acids have common or trivial names. Some amino acids names are derived from their origin sources, for example, asparagine is an amino acid which was first isolated from asparagus, and glutamate in wheat gluten. Glycine's name was given due to its sweet taste (Greek *glykos*, "sweet"). Tyrosine was isolated from cheese, so its name is derived from it (the Greek *tyros*, "cheese").³

1.1.1 General structure of amino acids:

All 20 common amino acids which are building blocks of proteins are α -amino acids. They have a carboxylic acid group, and an amino group bonded covalently to the

same carbon atom (the α -carbon) (Figure1). The difference between various amino acids is due to their side chains or R groups. These groups have differences in structure, size, and electric charge. These differences have great effects on the solubility of these amino acids in water and function in proteins.⁴

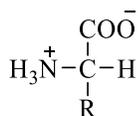


Figure 1: Structure of a general amino acid. This structure is common to all amino acids but one of the *alpha*-amino acids (proline, a cyclic amino acid, is an exception).

The α -carbon atom present in all amino acids is chiral except in glycine, so all amino acids can exist in at least two stereoisomeric forms but the amino acids with “L” absolute configuration are naturally present in proteins as they are derived from naturally occurring L-glyceraldehyde.

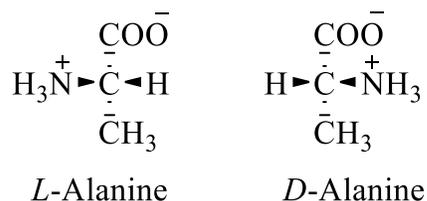


Figure 2: Stereoisomerism in α -amino acids.

1.1.2 Classification of amino acids:

Amino acids can be classified into the following five categories on the basis of their polarity and the charge (at pH 7) of their side chain (R groups). A brief introduction and their structures are described as follows:

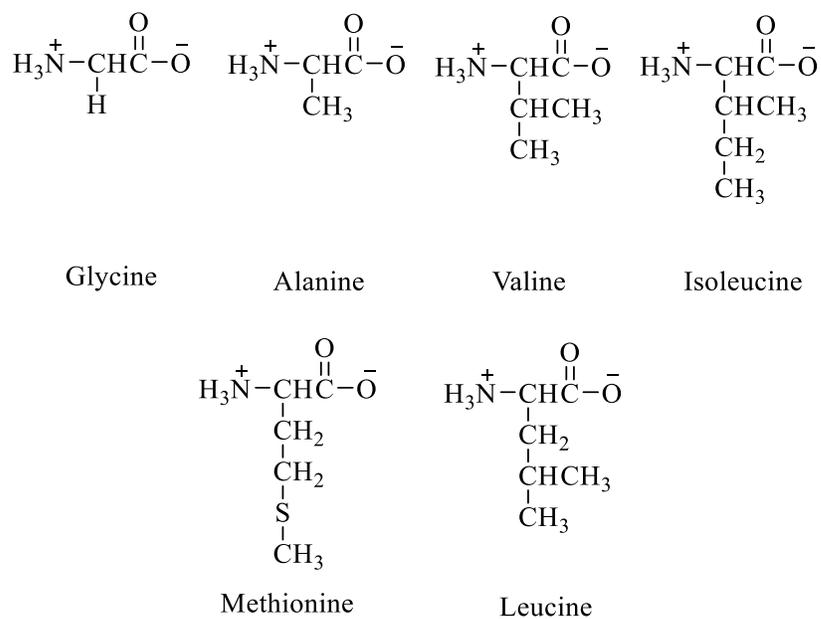


Figure 3: Amino acids having nonpolar, aliphatic R groups.

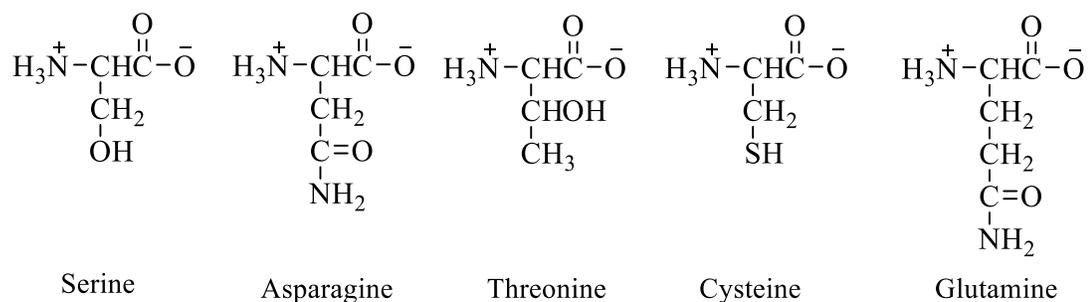


Figure 4: Amino acids having polar uncharged R group.

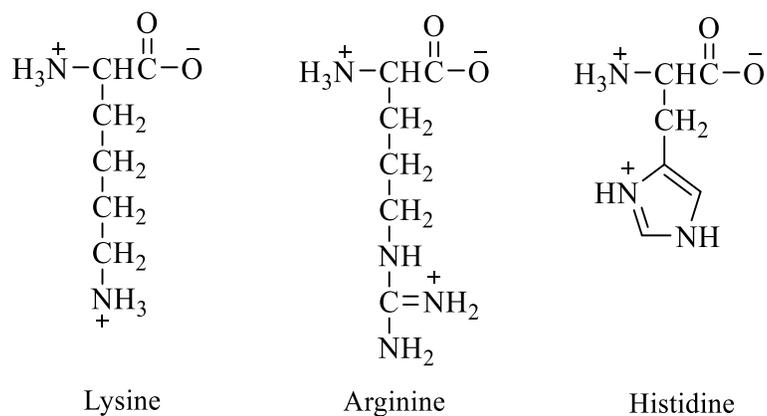


Figure 5: Amino acids having positively charged R groups.

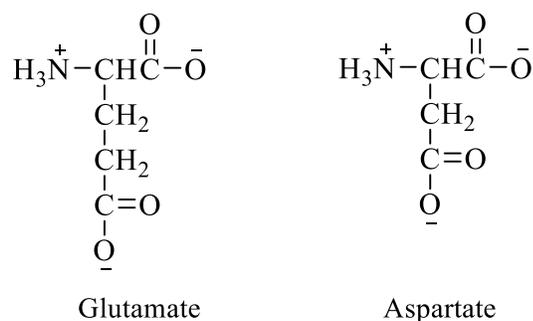


Figure 6: Amino acids having negatively charged R groups.

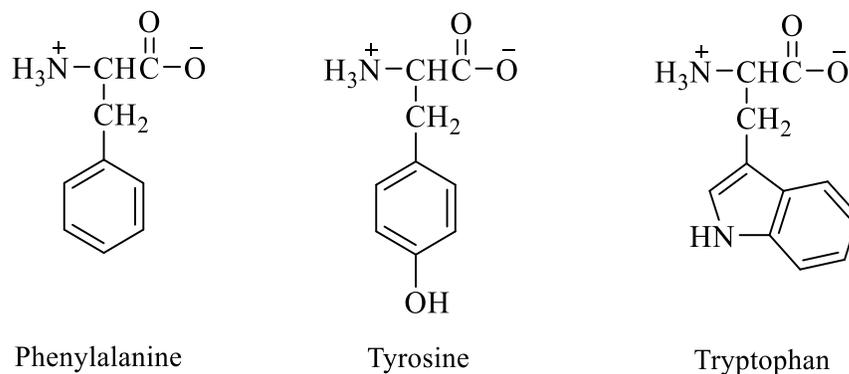


Figure 7: Amino acids having aromatic R groups.

1.1.3 Importance of amino acids:

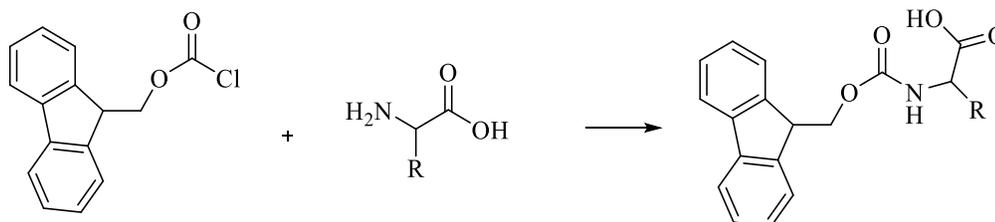
According to modern research, proteins are composed of all 20 naturally occurring amino acids. During digestion, proteins are broken down into amino acids, which are distributed throughout the body and play an important role in the formation of significant tissue proteins and many non-protein nitrogenous components of the cells. Therefore, the amino acids have a unique position in our nutrition and are a source of building blocks for the synthesis of important compounds in our body.⁵

1.2 PROTECTING GROUPS IN SOLID PHASE PEPTIDE SYNTHESIS (SPPS)

Peptides are polymers of amino acids that make up proteins. During peptide synthesis, chemists must ensure that the amino acids that make up peptides are added in the correct order to prevent unwanted side reactions. Amino acids contain an amino group at one end called the N-terminus and a carboxylic acid group at the other end called the C-terminus. To prevent the amino acid from reacting with itself, one group of the amino acid must be protected to prevent unwanted side products. The protected amino acid is then added to another amino acid attached to the solid phase support to start building the peptide chain. There are different protecting groups used in SPPS.

1.2.1 Fluorenylmethoxycarbonyl (Fmoc) group:

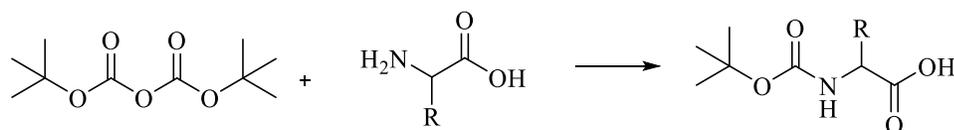
Fluorenylmethoxycarbonyl (Fmoc) is the most common protecting group for the amino group, particularly in SPPS.⁶ It is easily introduced by coupling an amine with an activated fluorenyl methyl carbonate mainly Fmoc-Cl and Fmoc-OSu.^{7,8} The major advantage of the Fmoc protecting group is its stability towards acidic conditions, which allows the selective removal of other protecting groups like Boc.⁹



Scheme 1: Using Fmoc-Cl to protect an amino acid.

1.2.2 *Tert*-butyloxycarbonyl (Boc) group:

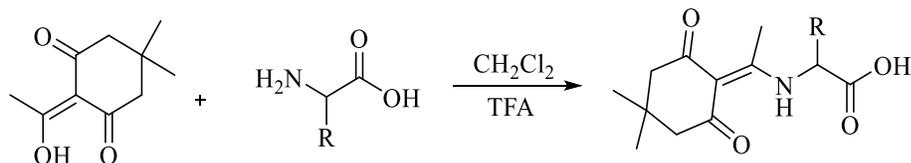
Tert-butyloxycarbonyl (Boc) protecting groups are used to protect the terminal amine in the presence of sodium carbonate as a base. The protected Boc group can be removed by the use of strong acids such as trifluoroacetic acid and hydrochloric acid. Boc anhydride is often used to protect α -amino acids or side chain amino groups.^{10,11,12}



Scheme 2: Using Boc anhydride to protect an amino acid.

1.2.3 1-(4,4-dimethyl-2,6-dioxacyclohexylidene)ethyl (DDE) group:

The DDE group is an orthogonal protecting group often used to protect side chain amino groups in SPPS. The DDE group is installed using 2-acetyldimedone with trifluoroacetic acid (TFA) in CH₂Cl₂, and it can be removed by 2% hydrazine in dimethylformamide (DMF).¹³



Scheme 3: Using 2-acetyldimedone to protect an amino acid.

1.2.4 *Tert*-butyl ester (OtBu) group:

The *t*-butyl ester groups (OtBu) of amino acids are beneficial for the protection of carboxyl and hydroxyl groups in peptide synthesis. Strong acids will remove the OtBu group readily from the amino acids.¹⁴

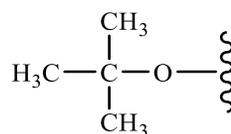
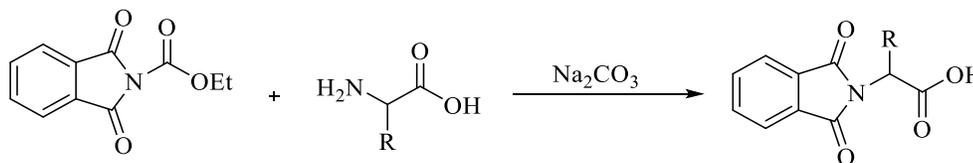


Figure 8: Structure of OtBu group.

1.2.5 Phthalimide group:

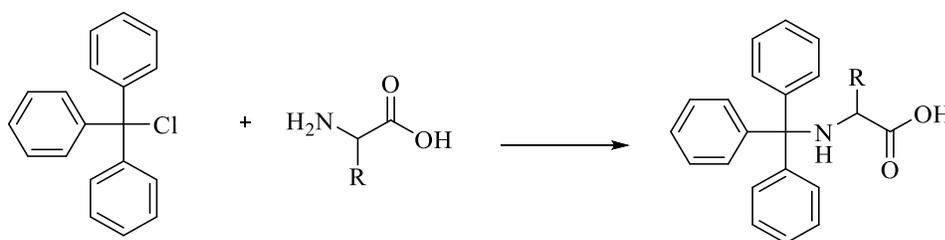
Phthalimide protecting groups are used to protect the α -amino group under mild conditions by using phthaloylating agent, e.g., *N*-(ethoxycarbonyl) phthalimide and monoethyl phthalate.¹⁵



Scheme 4: Using *N*-(ethoxycarbonyl) phthalimide to protect the amino acid.

1.2.6 Triphenylmethyl (Trt) group:

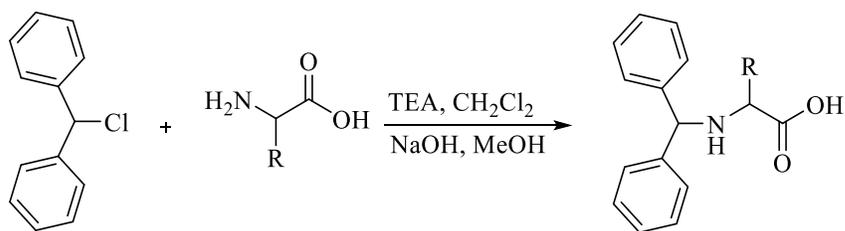
Triphenylmethyl chloride derivative is used in protecting the α -amino group in SPPS. *N*-Trt-amino acid is prepared by reacting amino acid methyl ester with Trt-chloride which is then followed by alkaline hydrolysis of the ester.^{16,17}



Scheme 5: Using Trt-Chloride to protect the amino acid.

1.2.7 Benzhydryl group:

N-benzhydryl groups are used in synthesis to make side chains less susceptible to side reaction. Benzhydryl group is more stable to acid than the Trt group and also offers less steric crowding in peptide coupling.¹⁸



Scheme 6: Using benzhydryl chloride to protect the amino acid.

1.2.8 Diphenylphosphine (DPP) group:

Amino-DPP protected amino acids are prepared by treating amino acid methyl ester with diphenyl phosphonic acid chloride (DPP-Cl).¹⁹

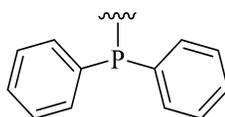


Figure 9: Structure of DPP group.

1.2.9 Allyloxycarbonyl (Alloc) Group:

Allyl base protection is another orthogonal protecting group which is selectively removed by Pd(0)-catalyzed hydrogenation under milder reaction conditions. The alloc group is introduced using allyl chloroformate or diallyl dicarbonate.^{20,21}

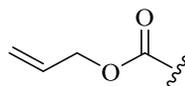
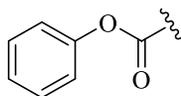


Figure 10: Structure of Alloc group.

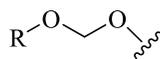
Apart from these protecting groups, vinyl, sulfonyl, sulfanyl and many other groups are also used in amino and side chain protection in SPPS.

1.2.10 Carboxyl group protection of amino acid:

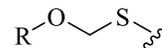
The most important and exclusive method for carboxyl group protection is esterification.^{22,23,24}



Phenacyl (Pac)



Methoxymethyl (Mom)



Methylthiomethyl (Mtm)

Figure 11: Structure of various carboxyl group protections.

Substituted benzyl ester and tertiary butyl ester are also used in protecting carboxyl groups in peptide synthesis.²⁵

1.3 PEPTIDES AND PEPTOIDS

Peptides are found throughout nature, having a large variety of roles. They influence significant functions such as immune defense, metabolism, reproduction and respiration by acting as extracellular messenger hormones, neuromodulators and

neurotransmitters in animals as well as in plants. They also perform intracellular functions, for example, the antioxidant and transport tripeptide glutathione.²⁶

Kitts and Weiler defined bioactive peptides as particular protein fragments that have a constructive effect on body functions or body conditions and may eventually influence health.²⁷ Fungi, bacteria, marine sponges, tunicates and other lower animal forms are enriched in biologically active peptides.²⁸ These active peptides show various activities, including antimicrobial, insecticidal, antiviral, tumor promotive, antitumor, immunosuppressive and anti-inflammatory actions. Some of these peptides have served as drugs or as lead molecules in drug development, while others have been useful in the study of biochemical pathways.^{29,30} As therapeutic compounds about, 60 peptides are approved in the US and also currently about 130 peptides are in the clinical pipeline.^{31,32} Global sales topped \$13 billion in 2010 for these compounds, making peptide therapeutics of particular interest to large pharmaceutical companies.³³ This wide spectrum of activity of peptides has attracted much attention from medicinal, bioorganic, and polymer chemists.

Besides the above-mentioned biological activities of peptides, they are generally considered to be poor drug candidates because they are rapidly degraded by proteases, resulting in very low oral bioavailability. Some peptides are frequently immunogenic. These characteristics have inspired researchers to design non-natural peptide mimics having fundamental molecular aspects of peptides, including *N*-substituted glycine oligomers (peptoids), peptide nucleic acids, β -peptides, and γ -peptides.^{34,35}

Peptoids are a class of peptidomimetic oligomers which are composed of *N*-substituted glycine units. Bartlett and co-workers first developed peptoids in the early

1990's and considered them as peptide backbone modified peptidomimetics.³⁶ Initially, they were proposed as lead compounds that could be used to identify potential drugs. Previous to the work of Bartlett and co-workers, the term peptoid as a peptidomimetic was introduced by Farmer and Ariens as any compound that could mimic a peptide's biological function, however, peptoids did not show resemblance structurally. Eventually, Bartlett and co-workers defined peptoid as oligomeric *N*-substituted glycines so they opened the field of research for peptoids studies.^{37,38}

Although peptides and peptoids have a similar amide backbone, the only major difference in structure is that, in peptoids, the side chain is attached to the α -nitrogen of the amide backbone while in peptides, the side chain is attached to the α -carbon (Figure 12).^{34,39}

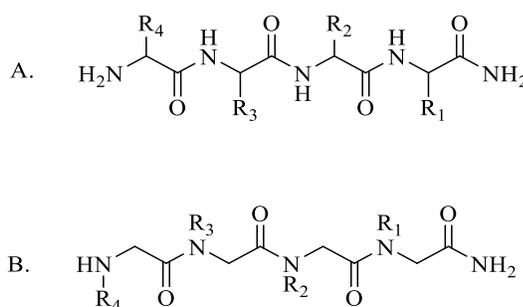


Figure 12: (A.) peptides structure (B.) peptoids structure.

Significant interest has been shown by different research groups in peptoids as potential pharmaceuticals because they can be easily synthesized, so they are more cost effective than peptides. They have good biocompatibility and also they have excellent stability towards strong acidic conditions and proteases.^{32,35} Tripeptoid ligands for the

R1-adrenergic receptors and opiate and also high-affinity di and tripeptoid ligands for the G-protein coupled receptors were recognized by screening combinatorial peptoid libraries. These tripeptoid libraries have been monitored for antimicrobial activity.⁴⁰ Among these innovative applications of peptoids, the recent research of Zuckermann, DeYoreo, and co-workers has broad potential particularly in the fields of materials science and nanotechnology.⁴¹ Peptoid bilayers, which form two-dimensional sheets, have been made using an adaptable synthetic platform for adding numerous functions with a particular order to the nanostructures. Additionally, these non-natural biomimetic polymers have also been shown to mimic the peptides and proteins in the mineralization of CaCO_3 .^{41,42}

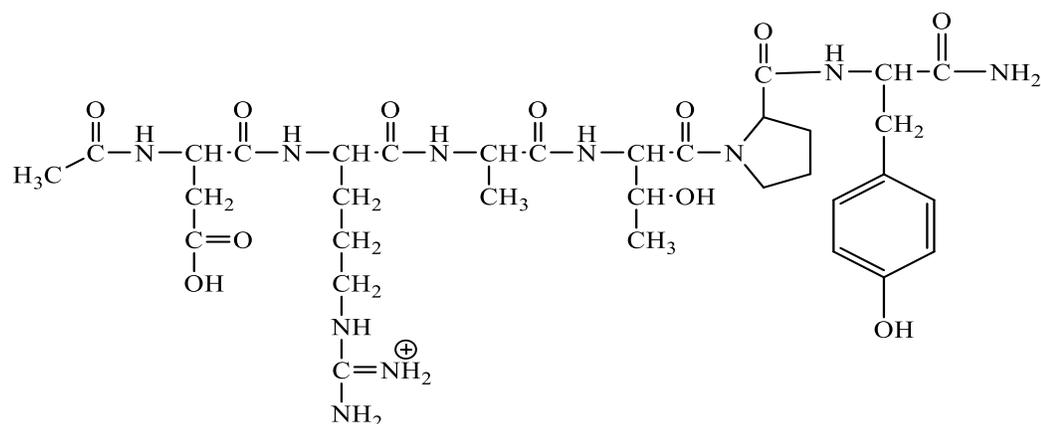
Spatial orientation of the peptoid side chains and secondary structure of the peptoid mimic are often different from the original peptide. A peptoid which lacks these properties may not perform the same functions as the peptide counterpart, so detailed studies need to be done on peptoid building blocks which afford the best peptide amino acid mimics. Such studies for arginine, a particularly interesting and important amino acid, will be the focus of the work discussed herein because it serves as important residues in many therapeutic peptides, such as Cetrorelix, which is used to control hormone levels in breast and prostate cancers.⁴³

This research seeks to support the various emergent fields of research into peptoid therapeutics. In addition, a number of synthetic methods to make peptide/peptoid hybrids (known as peptomers) and peptoids have been studied here. As described in the next sections, this work focuses on identifying peptoid mimics for amino acids in an affinity

tag peptide. Peptide affinity tags (e.g., AviD, Strep-Tag, myc-Tag, Flag-Tag) bind tightly and specifically with certain proteins, making them an excellent platform for quantifying the effect a peptoid submonomers mimic has on overall compound structure and function. Various affinity tag peptides contain unique amino acids that can be selectively studied by peptoid submonomer substitution to the further identify the most effective amino acid mimic. The focus of this study is on arginine. This work will ultimately be expanded to study all natural amino acids.

In general, the goal of this proposed work is to identify the most effective and applicable peptoid submonomer mimic of arginine. This work will be accomplished by synthesizing a range of peptide or peptoid hybrids (known as peptomers) of the peptide AviD, in which the amino acids of interest are substituted with a number of different peptoid submonomer mimics (Figure 13). AviD is selected here because it contains both types of the amino acids to be studied and is itself an affinity tag peptide. AviD binds selectively to NeutrAvidin.⁴⁴

A .



B .

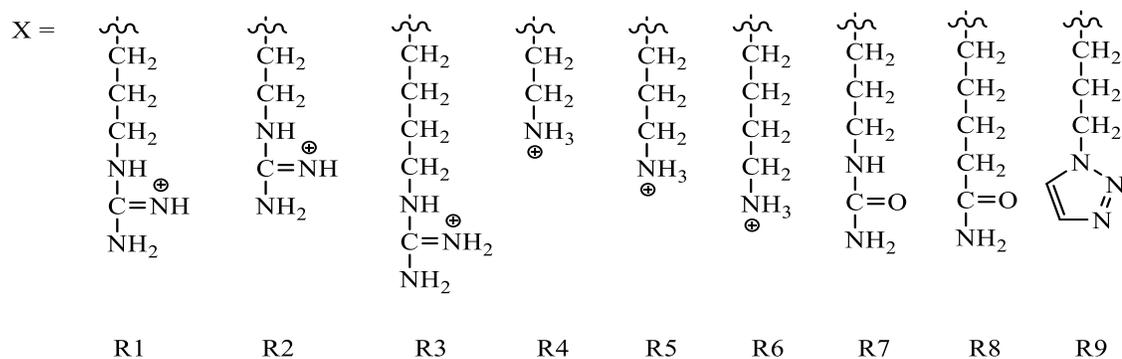
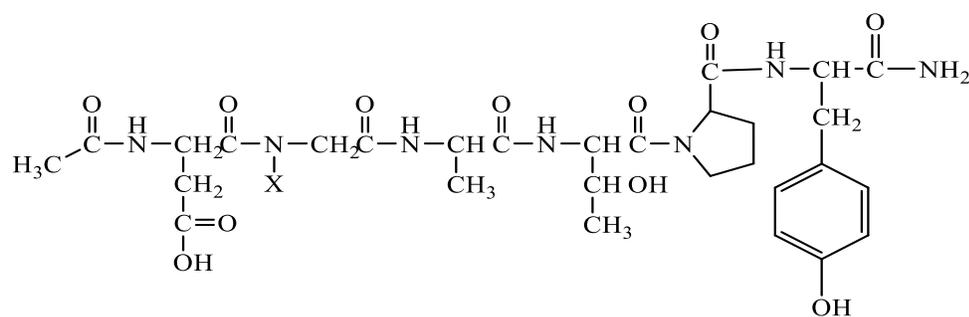


Figure 13: (A.) Structure of AviD peptide. (B.) Structure of various arginine peptomers of AviD.

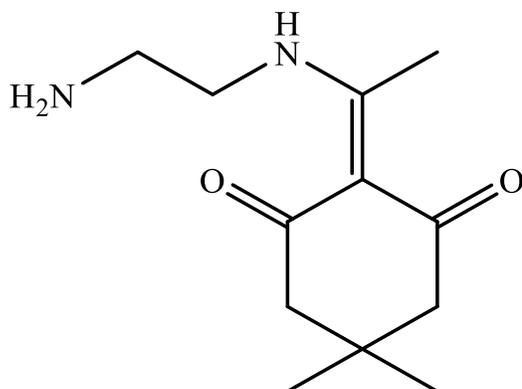
CHAPTER II: EXPERIMENTAL METHODS

2.1 MATERIALS

All the reagents and solvents used in the experiments were from AnaSpec, Inc (Fremont, CA), Novabiochem (City, State), Alfa Aesar (Ward Hill, MA), Bachem (Torrance, CA), Fisher Scientific (Pittsburgh, PA), EMD (City, State), Creosalus (Louisville, KY), and BDH (City, State). The ^1H NMR that was used in this research was JEOL 500 MHz. The MS's results were from Waters Synapt HDMS QToF with Ion Mobility and LTQ XLTM Linear Ion Trap Mass Spectrometer. All peptide and peptomer syntheses were carried out in a 10 mL and 20 mL vials and purified by reverse phase HPLC using a C18 column and a gradient of H_2O + 0.05% TFA to acetonitrile + 0.05% TFA.

2.2 SUBMONOMERS SYNTHESIS

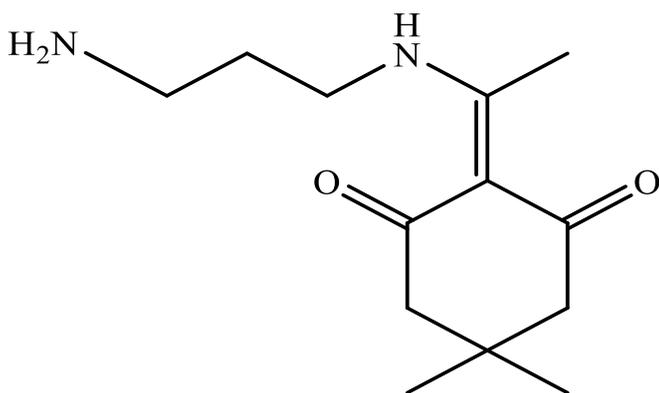
2.2.1 Mono-DDE-diaminoethane (NDab-DDE)



To begin 4.0 g (22.0 mmol) of 2-acetyldimedone was dissolved in 200 mL of dichloromethane (CH_2Cl_2) and transferred to a slow addition funnel. Next, 5.9 mL (5.3 g, 88 mmol) of ethylenediamine in 10 mL of CH_2Cl_2 was added into a round bottom flask before addition of 0.20 mL (2.20 mmol) of trifluoroacetic acid (TFA) with stirring. The reaction was started by slowly adding (1 drop/sec) 2-acetyldimedone solution and the reaction was stirred 36 h at room temperature. After completion of the reaction, the reaction was concentrated under vacuum. Next, the dissolved compound in a separating funnel was combined with 50 mL of 2 M NaHCO_3 as a base, and 50 mL of CH_2Cl_2 . The bottom organic layer was collected, and then dried with anhydrous CaCl_2 . CH_2Cl_2 was removed under vacuum to provide crude product. The crude product was dissolved in 5 mL of 0.05% TFA in H_2O and purified by reverse phase HPLC. The desired product was

obtained after lyophilization (1.35 g; 27.5%). ^1H NMR (500 MHz, CDCl_3): δ 5.31 (s, 1H), 3.45 (q, 5.7 Hz, 2H), 3.03 (m, 2H), 2.58 (s, 3H), 2.37 (m, 4H), 1.03 (s, 6H). ESI-MS [M+H] expected: 225.16 g/mol observed: 225.1 g/mol

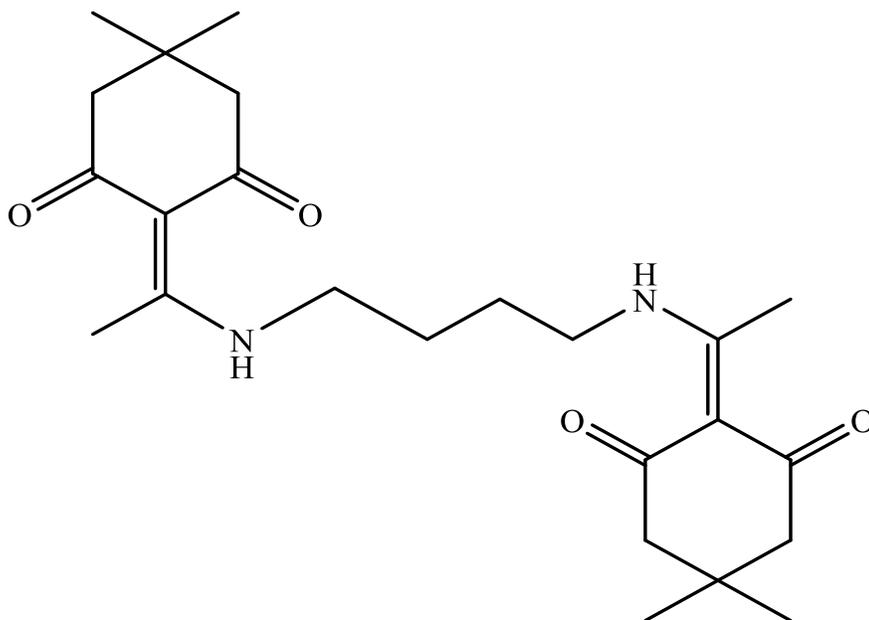
2.2.2 Mono-DDE-diaminopropane (NOrn-DDE)



To begin 4.0 g (22.0 mmol) of 2-acetyldimedone was dissolved in 200 mL of dichloromethane (CH_2Cl_2) and transferred to a slow addition funnel. Next, 7.3 mL (6.5 g, 88 mmol) of 1,3-propanediamine in 10 mL of CH_2Cl_2 was added into a round bottom flask before addition of 0.20 mL (2.20 mmol) of trifluoroacetic acid (TFA) with stirring. The reaction was started by slowly adding (1 drop/sec) 2-acetyldimedone solution and the reaction was stirred 36 h at room temperature. After completion of the reaction, the reaction was concentrated under vacuum. Next, the dissolved compound in a separating funnel was combined with 50 mL of 2 M NaHCO_3 as a base, and 50 mL of CH_2Cl_2 . The bottom organic layer was collected, and then dried by with CaCl_2 . CH_2Cl_2 was removed under vacuum to provide crude product. The crude product was dissolved in 5 mL of 0.05% TFA in H_2O and purified by reverse phase HPLC. The desired product was obtained after lyophilization (1.6 g; 30%). ^1H NMR (500 MHz, CDCl_3): δ 5.32 (s, 1H),

3.52 (m, 2H), 2.86 (m, 2H), 2.58 (s, 3H), 2.36 (s, 4H), 1.84 (q, J = 6.9 Hz, 2H), 1.04 (s, 6H). ESI-MS [M+H] expected: 239.18 g/mol observed: 239.2 g/mol

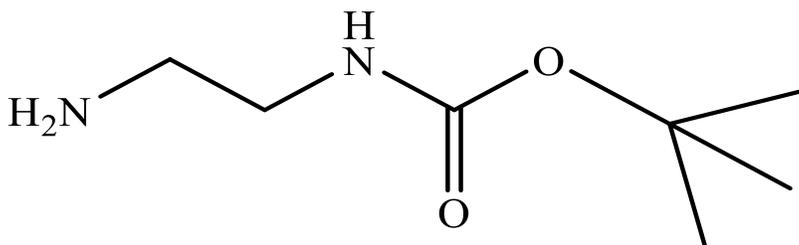
2.2.3 Di-DDE-diaminobutane (NLys-(DDE)₂)



To begin 4.0 g (22.0 mmol) of 2-acetyldimedone was dissolved in 200 mL of dichloromethane (CH_2Cl_2) and transferred to a slow addition funnel. Next, 8.9 mL (7.8 g, 88 mmol) of 1,4-butanediamine in 10 mL of CH_2Cl_2 was added into a round bottom flask before addition of 0.20 mL (2.20 mmol) of trifluoroacetic acid (TFA) with stirring. The reaction was started by slowly adding (1 drop/sec) 2-acetyldimedone solution and the reaction was stirred 36 h at room temperature. After completion of the reaction, the reaction was concentrated under vacuum. Next, the dissolved compound in a separating funnel was combined with 50 mL of 2 M NaHCO_3 as a base, and 50 mL of CH_2Cl_2 . The bottom organic layer was collected, and then dried by with CaCl_2 . CH_2Cl_2 was removed

under vacuum to provide crude product. The crude product was dissolved in 5 mL of 0.05% TFA in H₂O and purified by reverse phase HPLC, and the results indicated that only diprotected product was isolated. ¹H NMR (500 MHz, CDCl₃): δ 5.31 (s, 1H), 3.46 (m, 2H), 2.71 (m, 2H), 2.56 (s, 3H), 2.37 (s, 4H), 1.83 (m, 2H), 1.47 (q, J = 3.45 Hz, 2H), 1.02 (s, 6H). ESI-MS [M+H] expected: 253.19 g/mol observed: 417.3 g/mol, which is diprotected diamine.

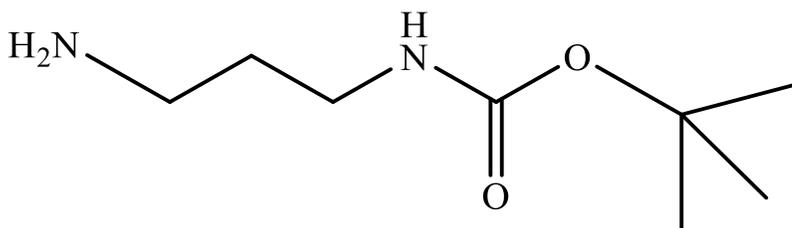
2.2.4 Mono-Boc-diaminoethane (NDab-Boc)



In a beaker, 0.777 mL (31.8 mmol) of concentrated hydrochloric acid (HCl) was added very slowly to 5 mL of methanol (MeOH). This mixture was stirred for 15 min at room temperature. In an ice bath, 0.623 mL (0.56 g, 9.1 mmol) of ethylenediamine was added to a round bottom flask, and then the HCl/MeOH was added very slowly with stirring. Next, the mixture was stirred for 15 min at room temperature. After that, 1.60 mL of H₂O was added to the round bottom flask and stirred for 30 min at room temperature. In another beaker, 3.21 mL (3.05 g, 14 mmol) of Boc anhydride was added with 6.70 mL of MeOH as solvent. The Boc anhydride solution was added very slowly to the ethylenediamine solution and the reaction was stirred about 1 h at room temperature. The reaction was concentrated under vacuum and washed (2x) with diethyl ether. The resulting solid was dissolved in 10 mL of 2 M NaOH and added to a separatory funnel. Next, 15 mL of CH₂Cl₂ was added to the separatory funnel, and the bottom layer was collected (3x), and then dried with CaCl₂. CH₂Cl₂ was removed under vacuum to provide crude product. The crude product was dissolved in 5 mL of 0.05% TFA in H₂O and

purified by reverse phase HPLC. The desired product was obtained after lyophilization (1 g; 69%). ^1H NMR (500 MHz, CDCl_3): δ 4.91 (s, 1H), 2.84 (t, $J = 5.7$ Hz, 2H), 3.21 (m, 2H), 1.45 (s, 9H), 1.29 (s, 2H).

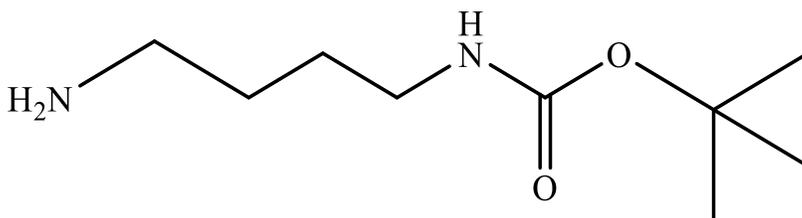
2.2.5 Mono-Boc-diaminopropane (NOrn-Boc)



In a beaker, 0.777 mL (31.8 mmol) of concentrated hydrochloric acid (HCl) was added very slowly to 5 mL of methanol (MeOH). This mixture was stirred for 15 min at room temperature. In an ice bath, 0.776 mL (0.69 g, 9.3 mmol) of 1,3-propanediamine was added to a round bottom flask, and then the HCl/MeOH was added very slowly with stirring. Next, the mixture was stirred for 15 min at room temperature. After that, 1.60 mL of H₂O was added to the round bottom flask and stirred for 30 min at room temperature. In another beaker, 3.21 mL (3.05 g, 14 mmol) of Boc anhydride was added with 6.70 mL of MeOH as solvent. The Boc anhydride solution was added very slowly to the 1,3-propanediamine solution and the reaction was stirred about 1 h at room temperature. The reaction was concentrated under vacuum and washed (2x) with diethyl ether. The resulting solid was dissolved in 10 mL of 2 M NaOH and added to a separatory funnel. Next, 15 mL of CH₂Cl₂ was added to the separatory funnel, and the bottom layer was collected (3x), and then dried with CaCl₂. CH₂Cl₂ was removed under vacuum to provide crude product. The crude product was dissolved in 5 mL of 0.05% TFA in H₂O and purified by reverse phase HPLC. The desired product was obtained after

lyophilization (0.747 g; 46%). ^1H NMR (500 MHz, CDCl_3): δ 3.28 (m, 2H), 2.80 (m, 2H)
1.65 (m, 2H), 1.44 (s, 9H).

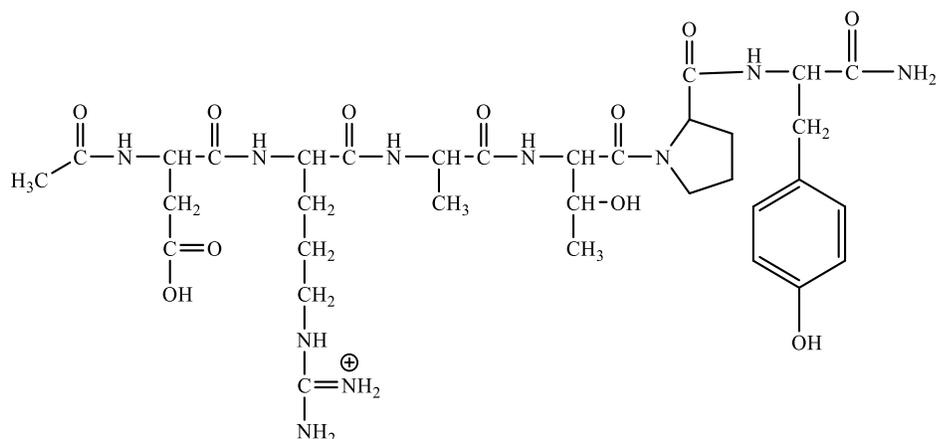
2.2.6 Mono-Boc-diaminobutane (NLys-Boc)



In a beaker, 0.777 mL (31.8 mmol) of concentrated hydrochloric acid (HCl) was added very slowly to 5 mL of methanol (MeOH). This mixture was stirred for 15 min at room temperature. In an ice bath, 0.935 mL (0.82 g, 9.3 mmol) of 1,4-butanediamine was added to a round bottom flask, and then the HCl/MeOH was added very slowly with stirring. Next, the mixture was stirred for 15 min at room temperature. After that, 1.60 mL of H₂O was added to the round bottom flask and stirred for 30 min at room temperature. In another beaker, 3.21 mL (3.05 g, 14 mmol) of Boc anhydride was added with 6.70 mL of MeOH as solvent. The Boc anhydride solution was added very slowly to the 1,4-butanediamine solution and the reaction was stirred about 1 h at room temperature. The reaction was concentrated under vacuum and washed (2x) with diethyl ether. The resulting solid was dissolved in 10 mL of 2 M NaOH and added to a separatory funnel. Next, 15 mL of CH₂Cl₂ was added to the separatory funnel, and the bottom layer was collected (3x), and then dried with CaCl₂. CH₂Cl₂ was removed under vacuum to provide crude product. The crude product was dissolved in 5 mL of 0.05% TFA in H₂O and purified by reverse phase HPLC. The desired product was obtained after

lyophilization (0.690 g; 39%). ^1H NMR (500 MHz, CDCl_3): δ 3.13 (m, 2H), 2.87 (m, 2H), 1.62 (s, 2H), 1.52 (s, 2H), 1.44 (s, 9H).

2.3 PEPTIDE SYNTHESIS (Avid)

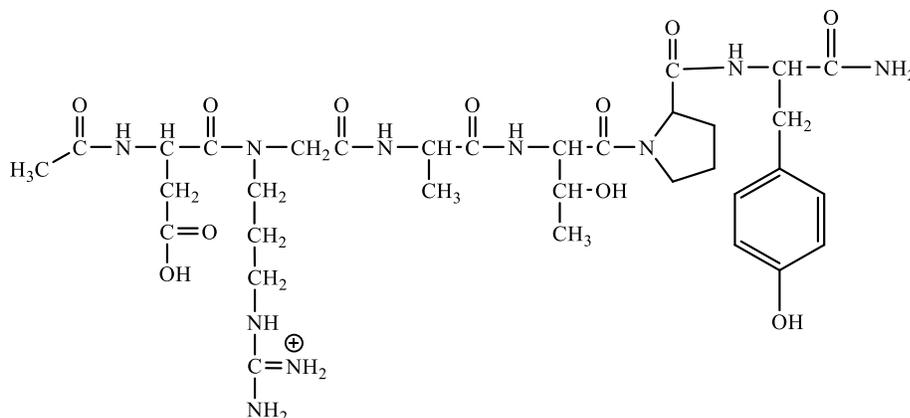


Avid was synthesized using traditional Fmoc strategies. Briefly, 0.172 g (0.0655 mmol) of Rink Amide resin was placed in a synthesis vial with 15 mL of DMF. After rocking for 30 min at room temperature, the solution was removed by aspiration. For deprotection of Fmoc groups, the resin was treated with 7 mL of 20% piperidine in DMF (v/v) (2x) 10 min each. After that, DMF was used to wash the resin (3x). 0.12 g (0.0655 mmol) of Fmoc-Tyr(tBu)-OH was activated with 0.10 g (0.0655 mmol) of HBTU in 5 mL of 5% N-methylmorpholine (NMM) in DMF 10 min. This mixture was added to the resin and reacted 1 h at room temperature. To remove the Fmoc protecting groups, 7 mL of 20% piperidine in DMF (v/v) was added (2x) 10 min each and washed (3x) by DMF. For the second amino coupling, the same steps were used as for the first amino, but 0.088 g (0.0655 mmol) of Fmoc-Pro-OH was used instead of Fmoc-Tyr(tBu)-OH. For the third coupling, 0.10 g (0.0655 mmol) of Fmoc-Thr(tBu)-OH was used. For the fourth coupling,

0.08 g (0.0655 mmol) of Fmoc-Ala-OH was used. For the fifth coupling, 0.17 g (0.0655 mmol) of Fmoc-Arg(Pbf)-OH was used. For the sixth coupling, 0.11 g (0.0655 mmol) of Fmoc-Asp(OtBu)-OH was used. After each coupling step, reaction completion was confirmed colorimetrically by Kaiser test. To add the acetyl group, 6.65 mL of CH₂Cl₂ and 0.35 mL of acetic anhydride (5% v/v) were added, and the vial was placed on the rocker about 1 h at room temperature. Then, the resin was washed by DMF (3x). This compound was cleaved using 95:2.5:2.5 TFA:TIS:H₂O (3x) for 1 h at room temperature. The crude product was dissolved in 5 mL of 0.05% TFA in H₂O and purified by reverse phase HPLC. The desired product was obtained after lyophilization (0.069g; 40%). ESI-MS [M+H] expected: 763.37 g/mol observed: 763.3 g/mol

2.4 PEPTOMER SYNTHESIS

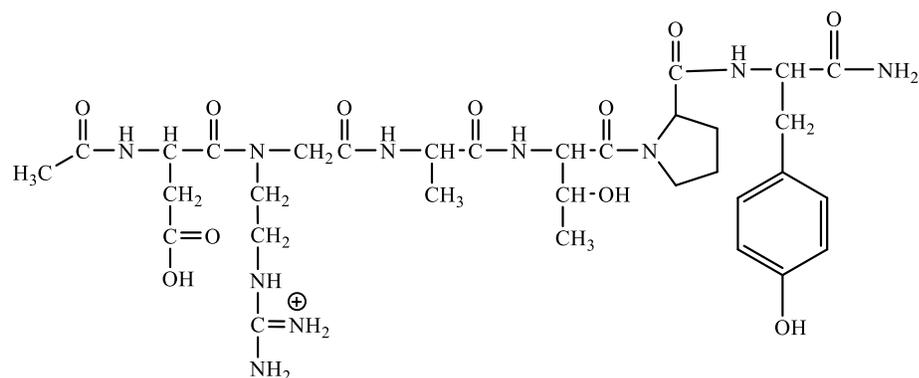
2.4.1 PEPTOMER “R1”



The initial peptide portion of R1 (which is Tyr, Pro, Thr, and Ala) was synthesized as previously described for AviD. In a new tube, 0.42 g (3 mmol) of bromoacetic acid was dissolved in 1.5 mL of anhydrous DMF. In another tube, 0.76 mL (4.8 mmol) of N,N'-diisopropylcarbodiimide (DIC) was mixed with 0.74 mL of anhydrous DMF. Those tubes were combined together then added to the resin and the vial was placed in a microwave at 10% power (100 kw) for 30 sec, and then reacted 15 min at room temperature. After that, DMF was used to wash the resin (3x). 0.72 g (3 mmol) of mono-DDE-diaminopropane (NOrn-DDE) was dissolved in 3 mL of anhydrous DMF. This mixture was added to the resin and the vial was placed in a microwave at 10% power (100 kw) for 30 sec, and then reacted 15 min at room temperature. After that, DMF was used to wash the resin (3x). After completion of these reactions, 0.11 g (0.0655

mmol) of Fmoc-Asp(OtBu)-OH was activated with 0.11 g (0.0655 mmol) of COMU in 5 ml of 5% NMM in DMF 10 min. This mixture was added to the resin and reacted 1 h at room temperature. To remove the Fmoc protecting groups, 7 mL of 20% piperidine in DMF (v/v) was added (2x) 10 min each and washed (3x) by DMF. To add the acetyl group, 6.65 mL of CH₂Cl₂ and 0.35 mL of acetic anhydride (5% v/v) were added, and the vial was placed on the rocker about 1 h at room temperature. Then, the resin was washed by DMF (3x). For deprotection of DDE groups, resin was treated with 0.35 mL of hydrazine monohydrate in 6.65 mL of DMF (5% v/v). After that, DMF was used to wash the resin (3x). To add the guanidinium group, 0.513 g (3.5 mmol) of pyrazole carboxamide was dissolved in 0.39 mL of NMM in DMF (5% v/v) and 0.80 mg of DMAP was added as a catalyst. This mixture was added to the resin and reacted overnight at room temperature. Then, the resin was washed by DMF (3x). This compound was cleaved using 95:2.5:2.5 TFA:TIS:H₂O (3x) for 1 h at room temperature. The crude product was dissolved in 5 mL of 0.05% TFA in H₂O and purified by reverse phase HPLC. The desired product was obtained after lyophilization (0.0038 g; 7%). ESI-MS [M+H] expected: 763.38 g/mol observed: 763.4 g/mol

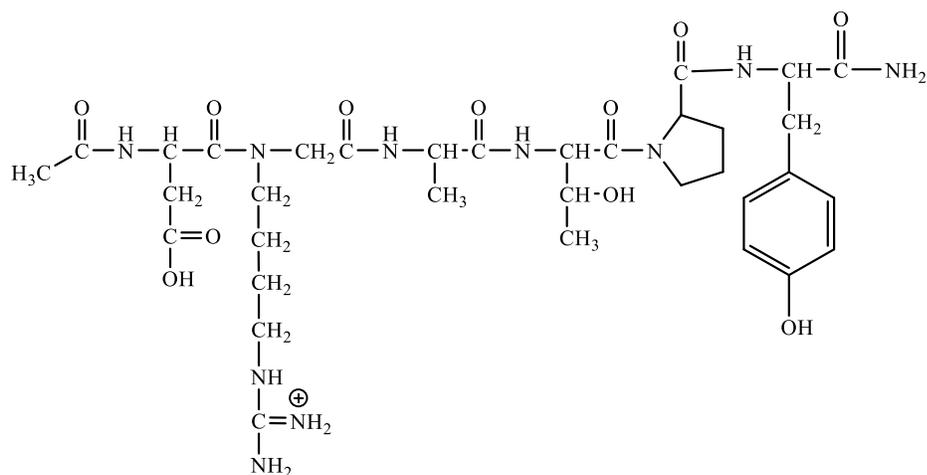
2.4.2 PEPTOMER "R2"



R2 was synthesized as previously described for R1, but 0.67 g (3 mmol) of mono-DDE-diaminoethane (NO_{rn}-DDE) was used instead of mono-DDE-diaminopropane (NO_{rn}-DDE). The desired product was obtained after lyophilization (0.0062 g; 12.7%).

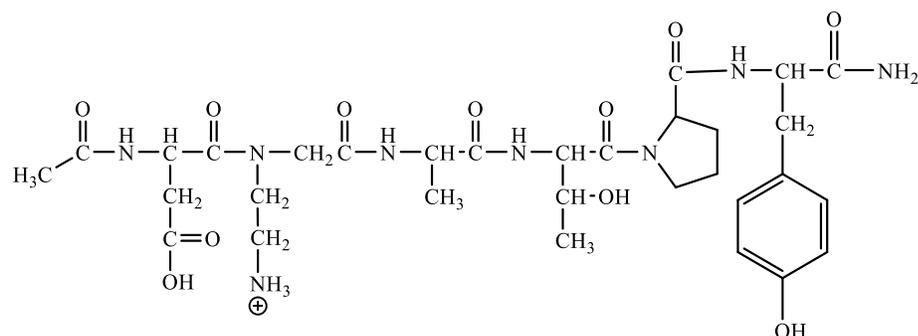
ESI-MS [M+H] expected: 749.36 g/mol observed: 749.59 g/mol

2.4.3 PEPTOMER "R3"



R3 was synthesized as previously described for R1, but 1.35 g (3 mmol) of Mono-DDE-diaminobutane (NLys-DDE) was used instead of mono-DDE-diaminopropane (NOrn-DDE). Due to impurity of NLys-DDE, synthesis of this product was not successful and is being repeated.

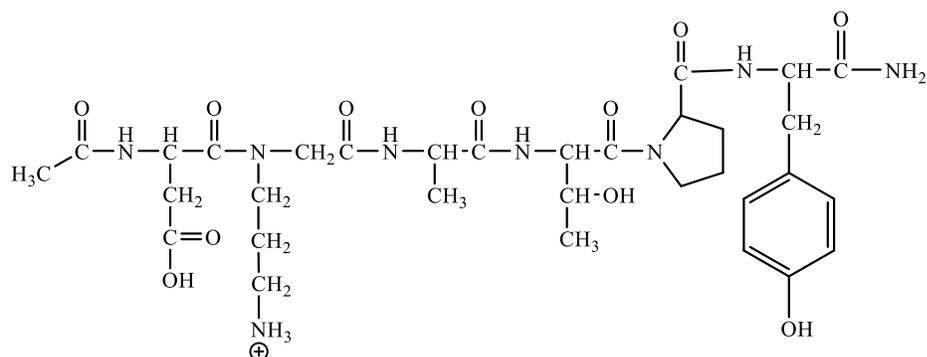
2.4.4 PEPTOMER “R4”



The initial peptide portion of R4 (which is Tyr, Pro, Thr, and Ala) was synthesized as previously described for AviD. In a new tube, 0.42 g (3 mmol) of bromoacetic acid was dissolved in 1.5 mL of anhydrous DMF. In another tube, 0.76 mL (4.8 mmol) of N,N'-diisopropylcarbodiimide (DIC) was mixed with 0.74 mL of anhydrous DMF. Those tubes were combined together then added to the resin and the vial was placed in a microwave at 10% power (100 kw) for 30 sec, and then reacted 15 min at room temperature. After that, DMF was used to wash the resin (3x). 0.48 g (3 mmol) of mono-Boc-diaminoethane (NDab-Boc) was dissolved in 3 mL of anhydrous DMF. This mixture was added to the resin and the vial was placed in a microwave at 10% power (100 kw) for 30 sec, and then reacted 15 min at room temperature. After that, DMF was used to wash the resin (3x). After completion of these reactions, 0.11 g (0.0655 mmol) of Fmoc-Asp(OtBu)-OH was activated with 0.11 g (0.0655 mmol) of COMU in 5 ml of 5% NMM in DMF 10 min. This mixture was added to the resin and reacted 1 h at

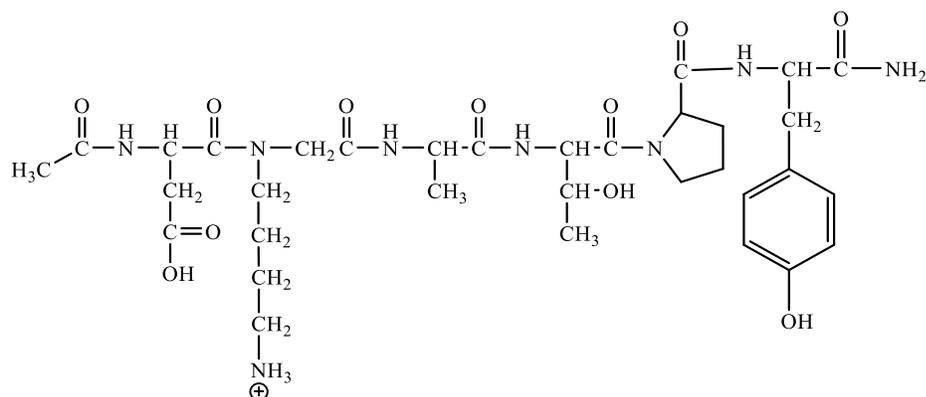
room temperature. To remove the Fmoc protecting groups, 7 mL of 20% piperidine in DMF (v/v) was added (2x) 10 min each and washed (3x) by DMF. To add the acetyl group, 6.65 mL of CH₂Cl₂ and 0.35 mL of acetic anhydride (5% v/v) were added, and the vial was placed on the rocker about 1 h at room temperature. Then, the resin was washed by DMF (3x). This compound was cleaved using 95:2.5:2.5 TFA:TIS:H₂O (3x) for 1 h at room temperature. When the peptomer was cleaved, the Boc groups were cleaved. The crude product was dissolved in 5 mL of 0.05% TFA in H₂O and purified by reverse phase HPLC. The desired product was obtained after lyophilization (0.0097 g; 20.9%). ESI-MS [M+H] expected: 707.34 g/mol observed: 707.301 g/mol

2.4.5 PEPTOMER "R5"



R5 was synthesized as previously described for R4, but 0.87 g (3 mmol) of mono-Boc-diaminopropane (NOrn-Boc) was used instead of mono-Boc-diaminoethane (NDab-Boc). The desired product was obtained after lyophilization (0.0324 g; 39.5%). ESI-MS [M+H] expected: 721.35 g/mol observed: 721.21 g/mol

2.4.6 PEPTOMER "R6"



R6 was synthesized as previously described for R4, but 0.94 g (3 mmol) of mono-Boc-diaminobutane (NLys-Boc) was used instead of mono-Boc-diaminopropane (NOrn-Boc). The desired product was obtained after lyophilization (0.0378 g; 33.8%). ESI-MS [M+H] expected: 735.37 g/mol observed: 735.3 g/mol

CHAPTER III: RESULTS AND DISSECTIONS

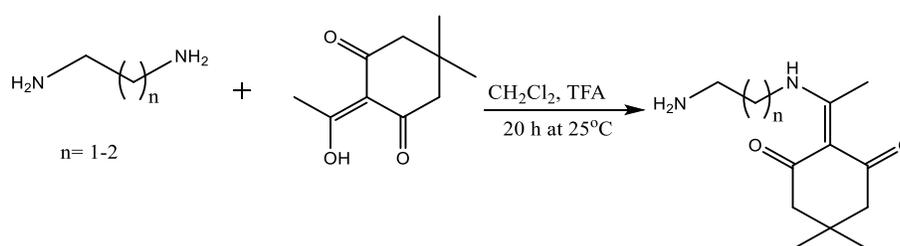
3.1 SUBMONOMERS

In general, the goal of this proposed work is to identify the most effective and applicable peptoid submonomer mimic of the peptide amino acid arginine. This work will be accomplished by synthesizing a range of peptide or peptoid hybrids (known as peptomers) of the peptide AviD, in which the amino acids of interest are substituted with a number of different peptoid submonomer mimics. As shown in (Table 1), the synthesis of submonomers b1, b2, and b3 that have Boc groups, gave better yields than the synthesis of submonomers a1, a2, and a3 which have DDE groups. All mono-protected diamine submonomers were synthesized using slow-addition methods but different protecting groups. Protection of a1, a2, and a3 was done with 2-acetyldimedone in CH₂Cl₂ with a TFA catalyst and extended reactions times (Scheme 7). On the other hand, Protection of b1, b2, and b3 was done with Boc anhydride in MeOH with an HCl catalyst and brief reaction times (Scheme 8). After completion of a1, a2, and a3 reactions, the mono protection was checked by ¹H NMR and the initial results were good (See Appendices, Figures A1, A4, and A7). However, later testing indicted that residual diamine remained, resulting in impure peptomers. Therefore, a1, a2, and a3 were purified by HPLC with an acidic water/acetonitrile gradient and a1 and a2 eluted around 27 min (Figures A2 and A5), but a3 did not (Figure A8). After that, the molecular weights of a1 and a2 were confirmed by MS analysis at 225.1 g/mol and 239.2 g/mol, respectively (Figures A3 and A6). For a3, the molecular weight should be observed at 253.19 g/mol by MS analysis but it was not confirmed (Figure A9). It was observed that

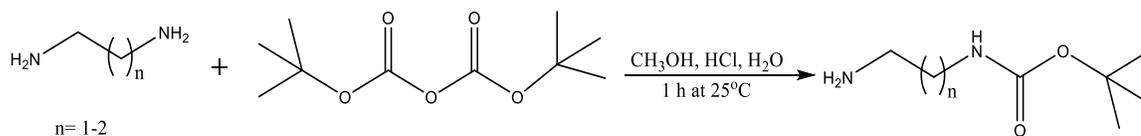
the major product here was diprotected butanediamine. This work is being repeated to complete the synthesis of R3. After synthesis of b1, b2, and b3, the structure of Boc mono-protected diamines was confirmed by ^1H NMR (Figures A10, A11, and A12). Boc mono-protected diamines were used without further purification because work-up procedures effectively removed unreacted diamine.

Table 1: A summary of diamine mono-protection with DDE and Boc anhydride.

Name	Id	Substrate	Protecting group	Solvent	Temp.	Yield
NDab-DDE	a1	ethylenediamine	2-acetyldimedone	CH_2Cl_2	25 °C	27.5%
NOrn-DDE	a2	1,3-propanediamine	2-acetyldimedone	CH_2Cl_2	25 °C	30%
NLys-DDE	a3	1,4-butanediamine	2-acetyldimedone	CH_2Cl_2	25 °C	N/A
NDab-Boc	b1	ethylenediamine	Boc anhydride	MeOH	25 °C	69%
NOrn-Boc	b2	1,3-propanediamine	Boc anhydride	MeOH	25 °C	46%
NLys-Boc	b3	1,4-butanediamine	Boc anhydride	MeOH	25 °C	39%



Scheme 7: Synthesis of a1, a2, and a3.



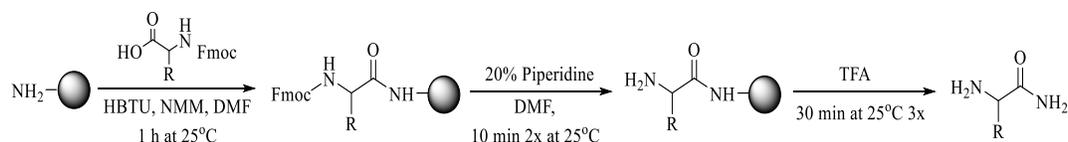
Scheme 8: Synthesis of b1, b2, and b3.

3.2 PEPTIDE AND PEPTOMERS

Having synthesized the needed submonomers, we turned our attention towards the synthesis of AviD and peptomers R1 to R6. Several compounds had to be synthesized multiple times due to issues with synthesis and purification. Table 2 shows the yields of AviD and peptomers R1 to R6 after HPLC purification and lyophilization. AviD was synthesized on the solid phase using common Fmoc protecting group strategies (Scheme 9). Then, the compound was purified by HPLC with an acidic water/acetonitrile gradient and the product eluted around 18 min (Figure A13). After that, the molecular weight of AviD was confirmed by MS analysis at 763.3 g/mol (Figure A14) and the yield was good for this type of synthesis.

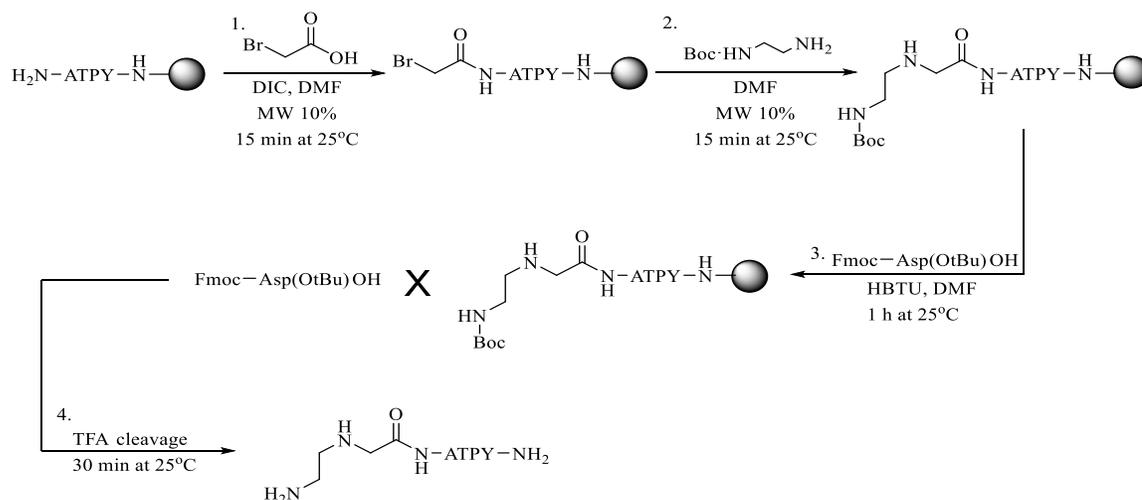
Table 2: The percent yield for each synthesis of AviD and submonomers R1 to R6.

Name	Temperature	Yield
AviD	25 °C	40%
R1	25 °C	7%
R2	25 °C	12.7%
R3	25 °C	N/A
R4	25 °C	20.9%
R5	25 °C	39.5%
R6	25 °C	33.8%

**Scheme 9:** General peptide synthesis procedures used to synthesis AviD.

For synthesis of peptomers R1 to R6, the first peptide portion (ATPY) was synthesized using the conditions that were mentioned previously (Scheme 9). During R4 synthesis which was the first peptomer synthesized, there was an issue with aspartic coupling after adding b1. Because the alpha-nitrogen of a peptoid is a secondary amine, it is not as nucleophilic as the primary amine present during peptide synthesis. After activating the aspartic acid with HBTU and coupling this to the peptomer, we observed

that the aspartic acid was not completely coupled as shown in Scheme 10. The expected molecular weight of this step should be observed at 887.39 g/mol and without aspartic acid would be observed at 550.30 g/mol (Figure 14). This figure clearly shows a mixture of peptomer with and without aspartic acid coupled.



Scheme 10: Synthesis of R4 using HBTU as an activating agent with aspartic acid. This led to incomplete coupling of aspartic acid.

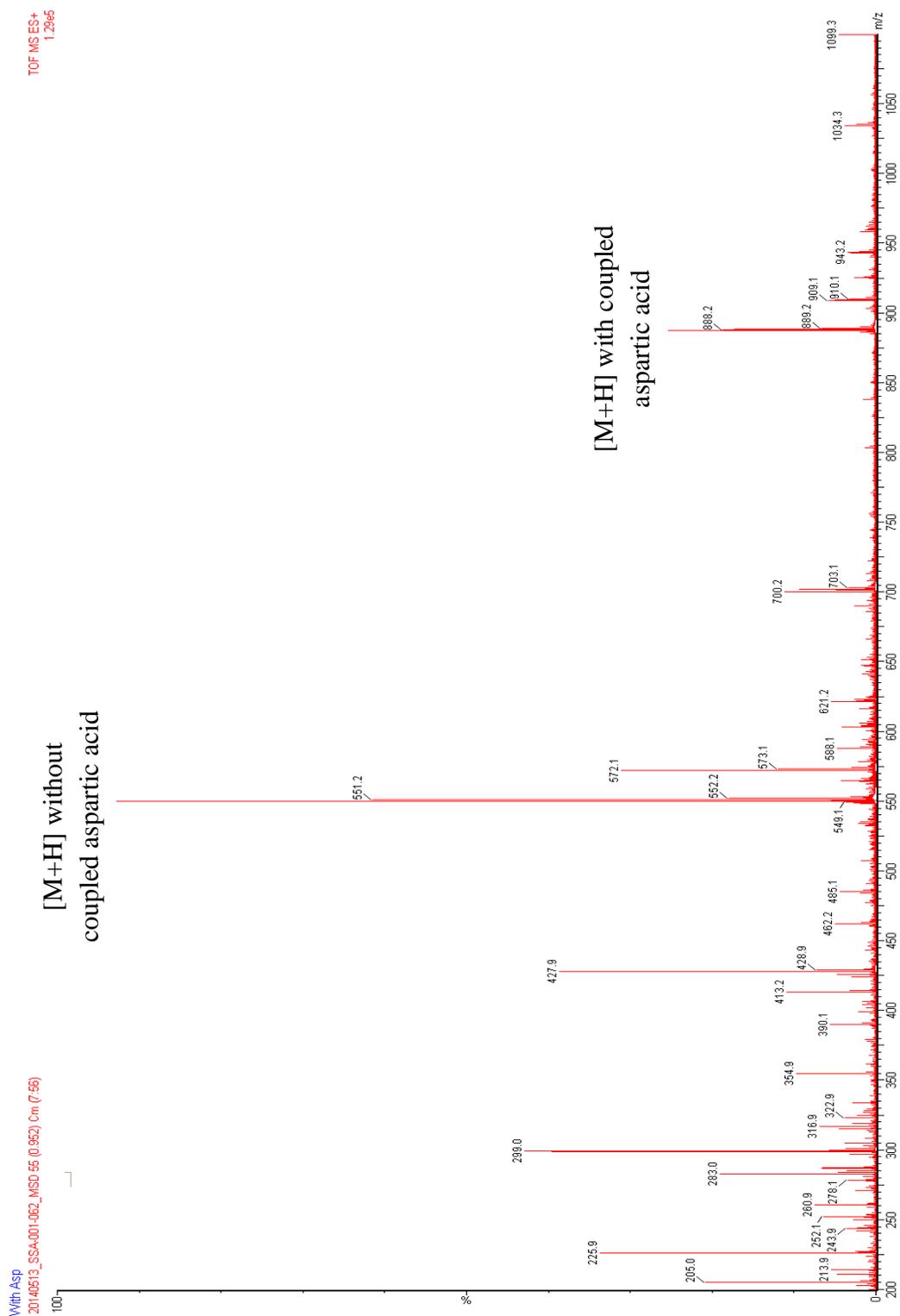
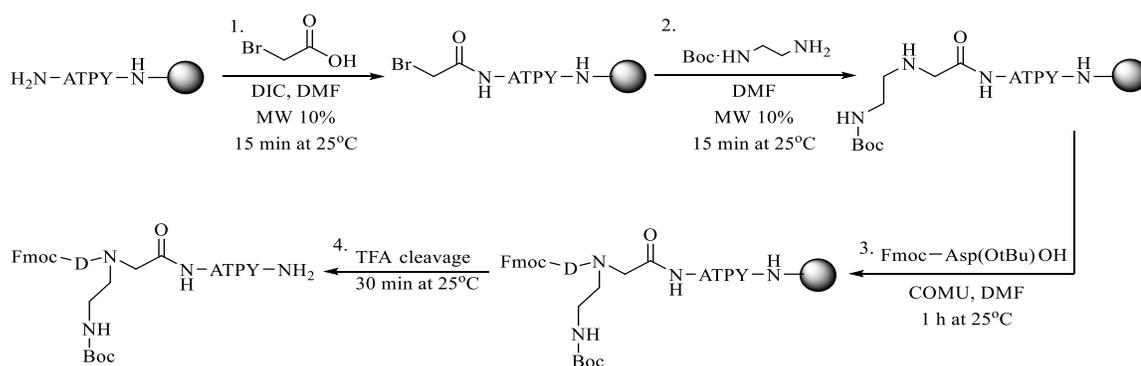


Figure 14: The MS identifying of R4 with aspartic acid coupling using HBTU.

This issue was solved by using COMU instead of HBTU as shown in (Scheme 11), because COMU is a stronger, more efficient activate agent with the secondary amine compared to HBTU. Then, the molecular weight observed during MS analysis was at 888 g/mol which is with the aspartic acid completely coupled to the peptomer (Figure 15). COMU was therefore used during the synthesis of the other peptomers for aspartic acid coupling.



Scheme 11: Synthesis of R4 using COMU as an activating agent with aspartic acid. This provided complete coupling of aspartic acid with the peptomer.

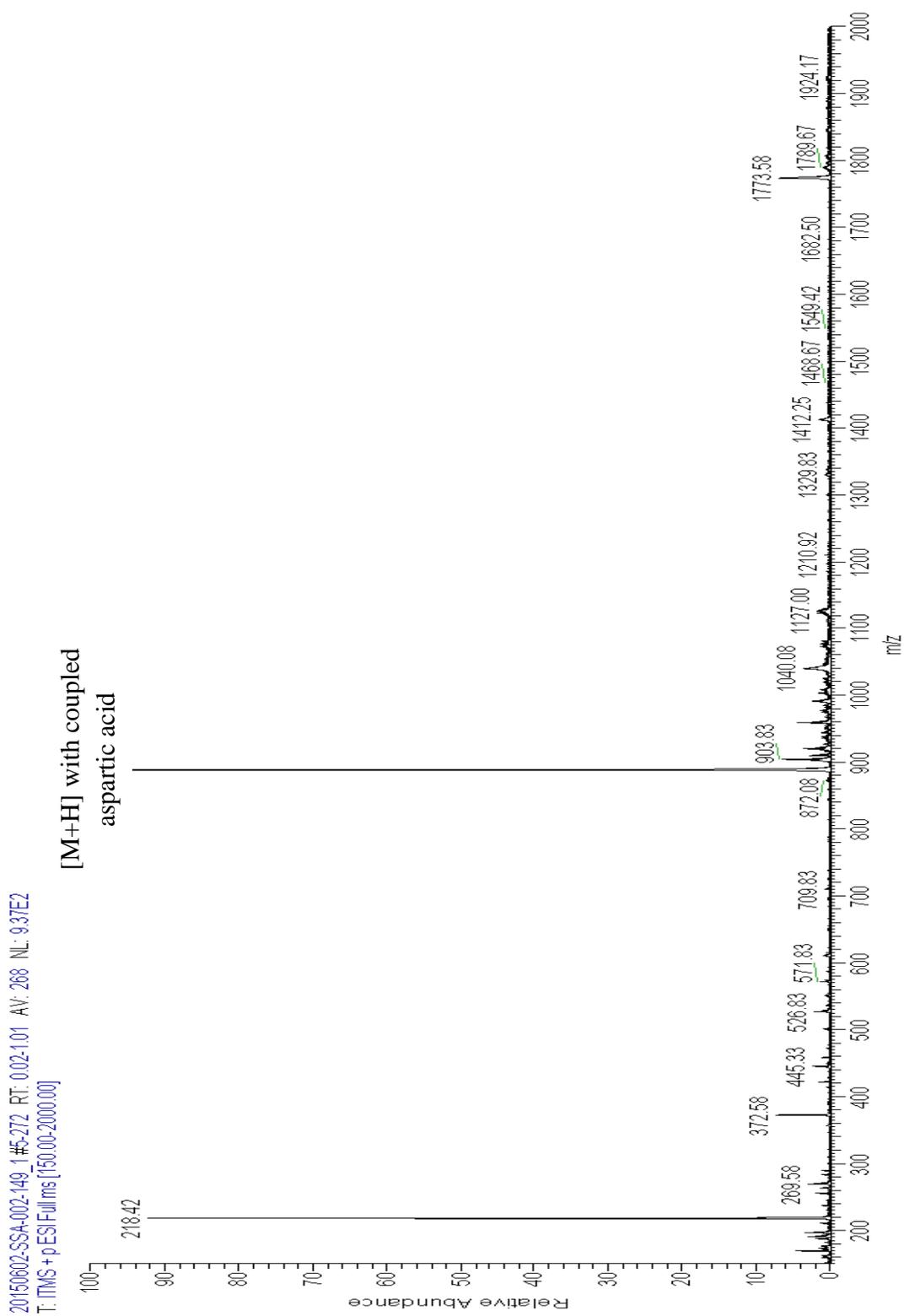
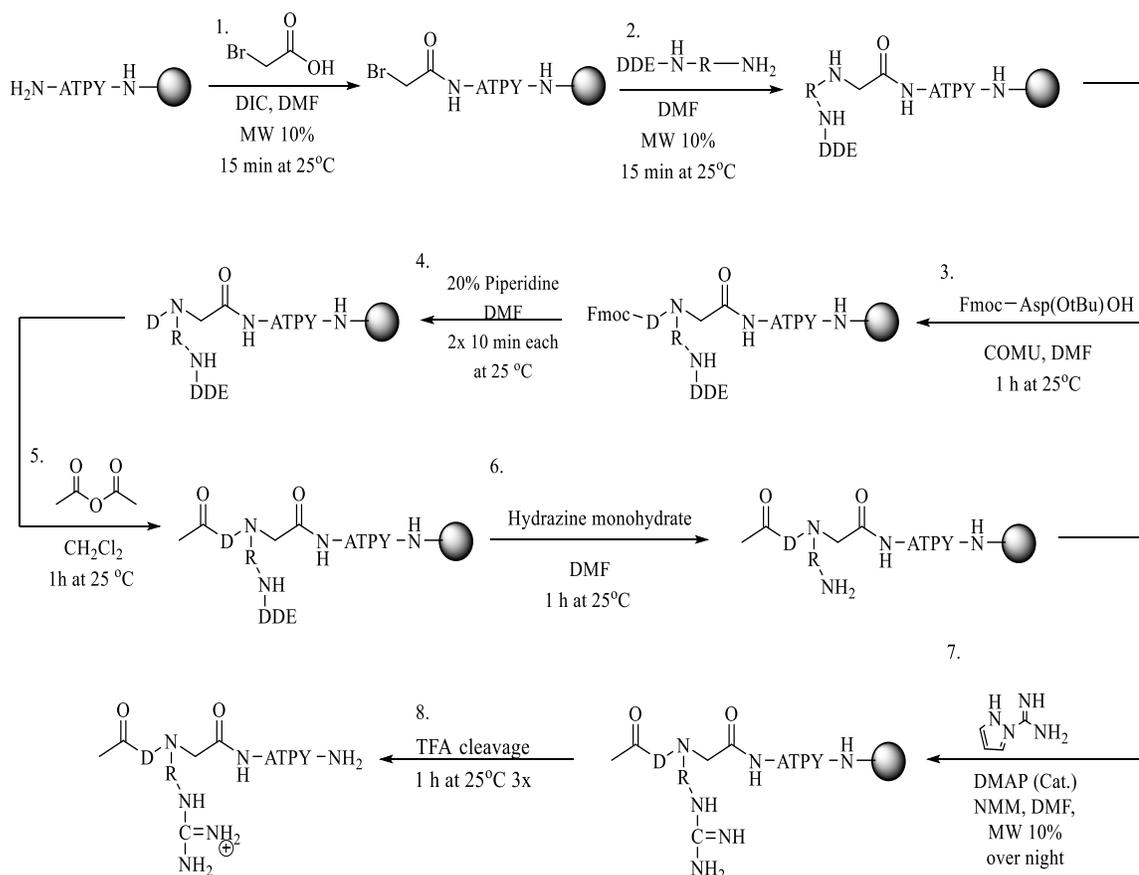


Figure 15: The MS identifying of R4 with aspartic acid coupling using COMU.

However, R1, R2, and R3 have guanidinium groups. After deprotecting of DDE groups using hydrazine monohydrate in DMF, the guanidinium groups were installed using pyrazole carboxamide with DMAP as a catalyst (Scheme 12). After completion of the peptomers, all compounds, except R3, were purified by HPLC (Figures A15 and A17). The molecular weight of R1 was confirmed by MS analysis at 763.4 g/mol (Figure A16) and R2 at 749.59 g/mol (Figure A18).



Scheme 12: Synthesis of peptomers R1 through R3. Note that only the amine used in the step 2 changes from one peptomer to another.

Due to an impurity in the submonomer a3 (NLys-DDE), there was branching during the synthesis of R3 as shown in Scheme 13. During a3 synthesis, we observed three compounds as shown in (Figure 16). This resulted in our desired product (777.5 g/mol; Figure 17) and a branched product (892.5 g/mol; Figure 17) during R3 synthesis.

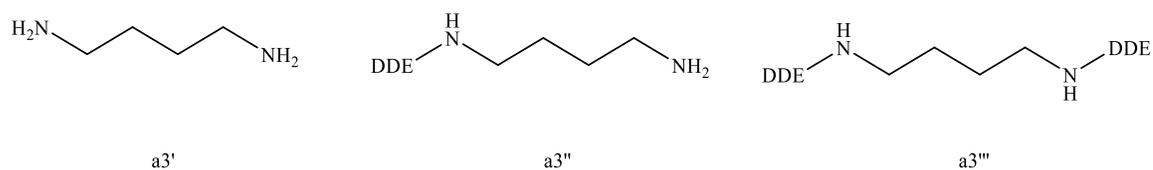
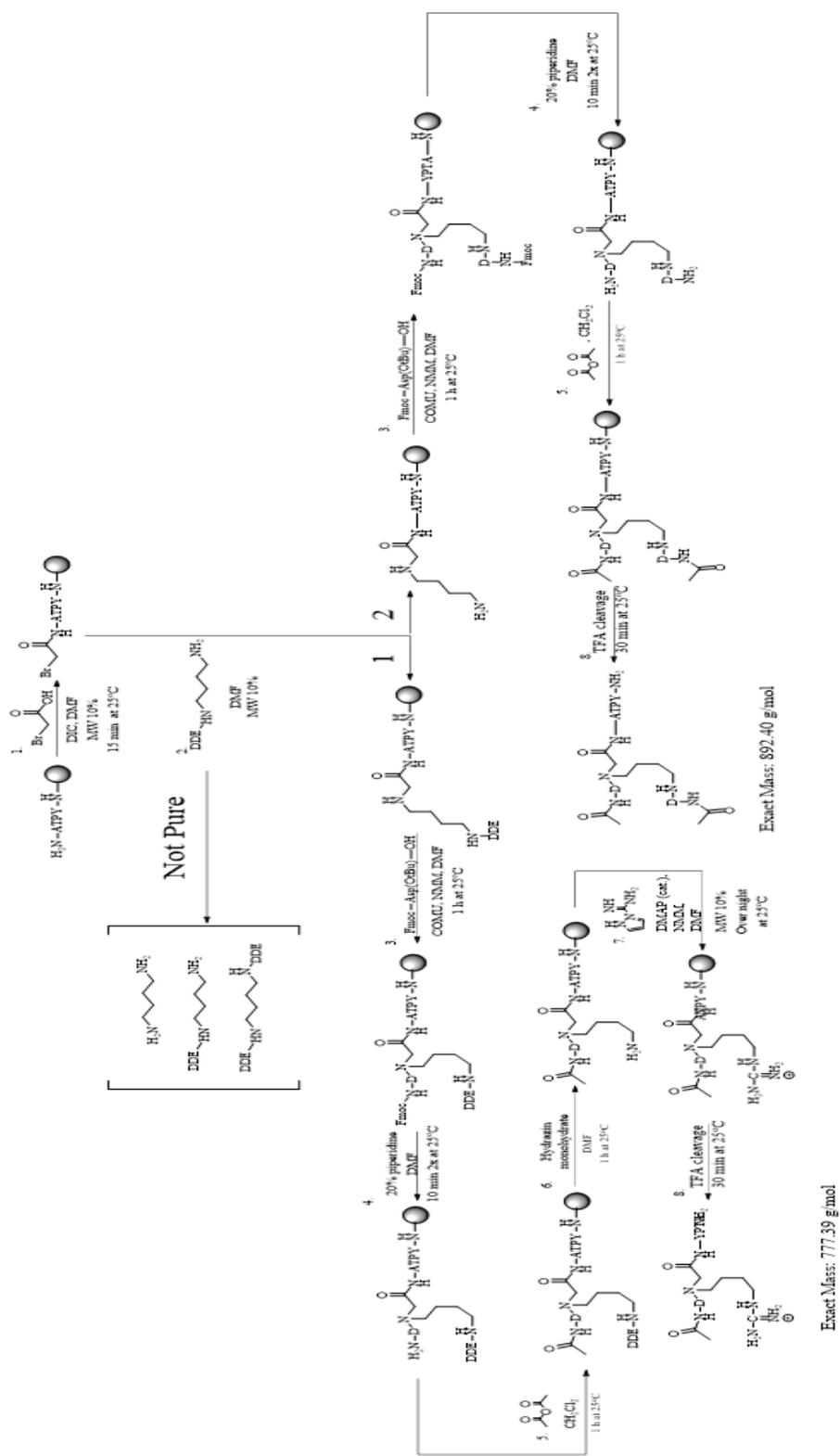


Figure 16: (a3') Structure of 1,4-butanediamine without DDE group. (a3'') Structure of NLys-DDE. (a3''') Structure of NLys with Diprotected.



Scheme 13: Synthesis of R3 with branching.

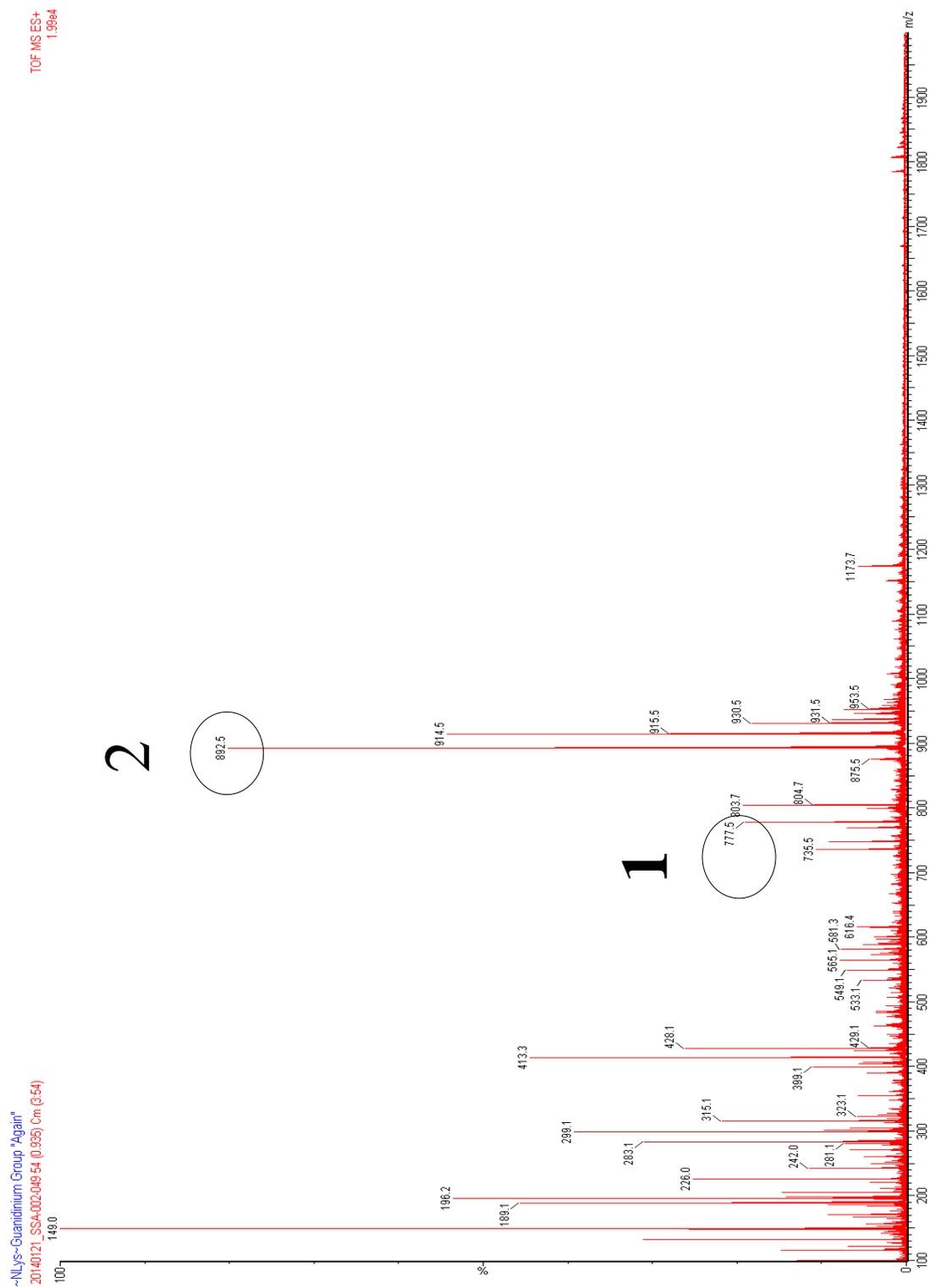
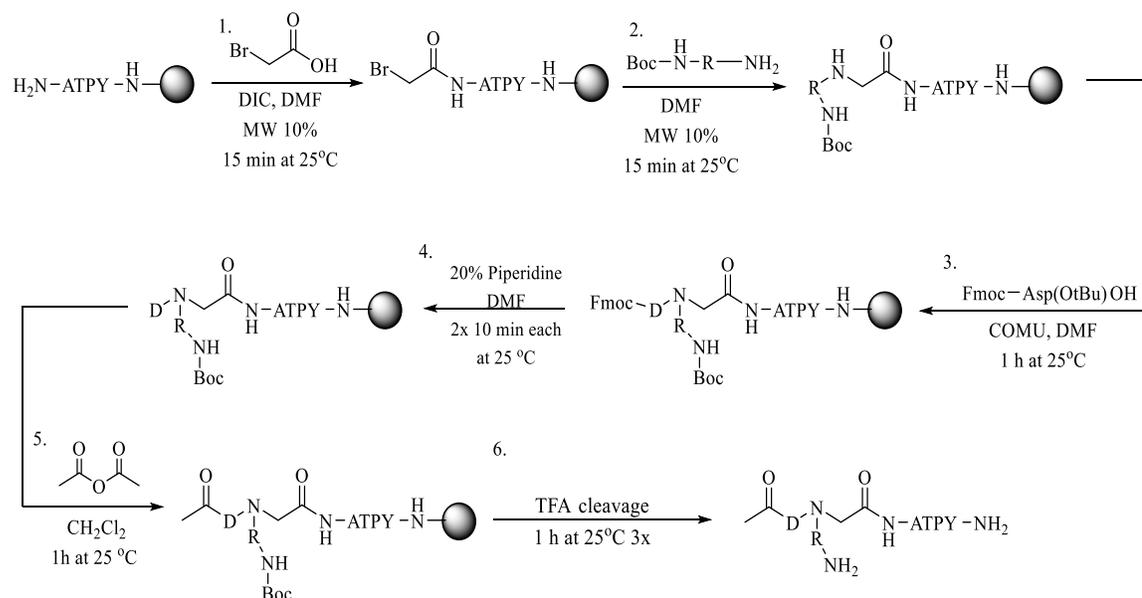


Figure 17: The MS identifying of R3 with branching.

On the other hand, R4, R5, and R6 were reacted with b1, b2, and b3, respectively. When those peptomers were cleaved, the Boc groups were also removed (Scheme 14). After completion of those peptomers, R4 to R6 were purified by HPLC (Figure A19, A21, and A23). The molecular weight of R4 was confirmed by MS analysis at 707.34 g/mol (Figure A20), R5 at 721.21 g/mol (Figure A22), and R6 at 735.3 g/mol (Figure A24).



Scheme 14: Synthesis of peptomers R4 through R6. Note that only the amine used in the step 2 changes from one peptomer to another.

Briefly, the yields of R1 and R2, which have guanidinium groups, are lower than for R4 to R6 (Table 2). This is presumably due to the extra synthetic steps needed to install the guanidinium group on R1 and R2.

CHAPTER IV: CONCLUSION

This research seeks to achieve the overall goals which are to identify the most effective and applicable peptoid submonomer mimic of the peptide amino acid arginine and was accomplished by synthesizing a range of peptide or peptoid hybrids (known as peptomers) of the peptide AviD, in which the amino acids of interest are substituted with a number of different peptoid submonomer mimics. To begin the project, the submonomers which are (NDab-DDE “a1”, NORn-DDE “a2”, NLys-DDE “a3”, NDab-Boc “b1”, NORn-Boc “b2”, and NLys-Boc “b3”) were synthesized using slow-addition methods but different protecting groups. Protection of a1, a2, and a3 was done with 2-acetyldimedone (DDE), and b1, b2, and b3 was done with Boc anhydride. All mono protection was checked by ¹H NMR and the initial results were good. Submonomers a1, a2, and a3 were purified by HPLC and confirmed by MS analysis. All results of HPLC and MS of a1 and a2 were good, but a3 was not, because it was observed that the major product here was diprotected butanediamine. Boc mono-protected diamines were used without further purification because work-up procedures effectively removed unreacted diamine. The submonomers b1, b2, and b3 that have Boc groups, gave better yields than the synthesis of submonomers a1, a2, and a3 which have DDE groups. AviD was synthesized using traditional Fmoc strategies. During R4 synthesis, there was an issue with aspartic coupling activating with HBTU after adding b1. This issue was solved by using COMU instead of HBTU, because COMU is better activating agent with the secondary amine. Therefore, COMU was used during the synthesis of the other peptomers for aspartic acid coupling. The guanidinium groups were installed, which was

using pyrazole carboxamidine with DMAP as a catalyst, to complete the synthesis of the peptomers R1, R2, and R3. After completion of the peptomers, all compounds, except R3, were purified by HPLC and confirmed by MS analysis. As we mentioned previously, due to impurity of submonomer a3 (NLys-DDE), there was branching during the synthesis of R3. For others peptomers (R4, R5, and R6) synthesis was completed using b1, b2, and b3, respectively. When those peptomers were cleaved, the Boc groups were also removed. All compounds were purified by HPLC and confirmed by MS analysis and the all results were good. Finally, the yields of peptomers R1 and R2 are lower than for R4 to R6, because they had an extra synthetic step to install the guanidinium group.

For future study, we will continue the synthesis of peptoid mimics of arginine (R7, R8, and R9). Aspartic acid will also be studied in this research to identify the most effective and applicable peptoid submonomer mimic of aspartic acid (Figure 18). After successful completion of the synthesis of peptomers R1 to R9 and D1 to D7, the two-dimensional NMR and circular dichroism (CD) analysis will be used to identify the most effective peptoid submonomer mimic of these amino acids. The NMR analysis will be used to study the cis/trans equilibrium ($K_{cis/trans}$) of the amides around substituted peptoid submonomers. The CD analysis will be used to study the secondary structure and thermal stability of the AviD peptomers. Also, the fluorescence polarization (FP) assay will be used to determine the binding properties of AviD peptomers to the protein NeutrAvidin.

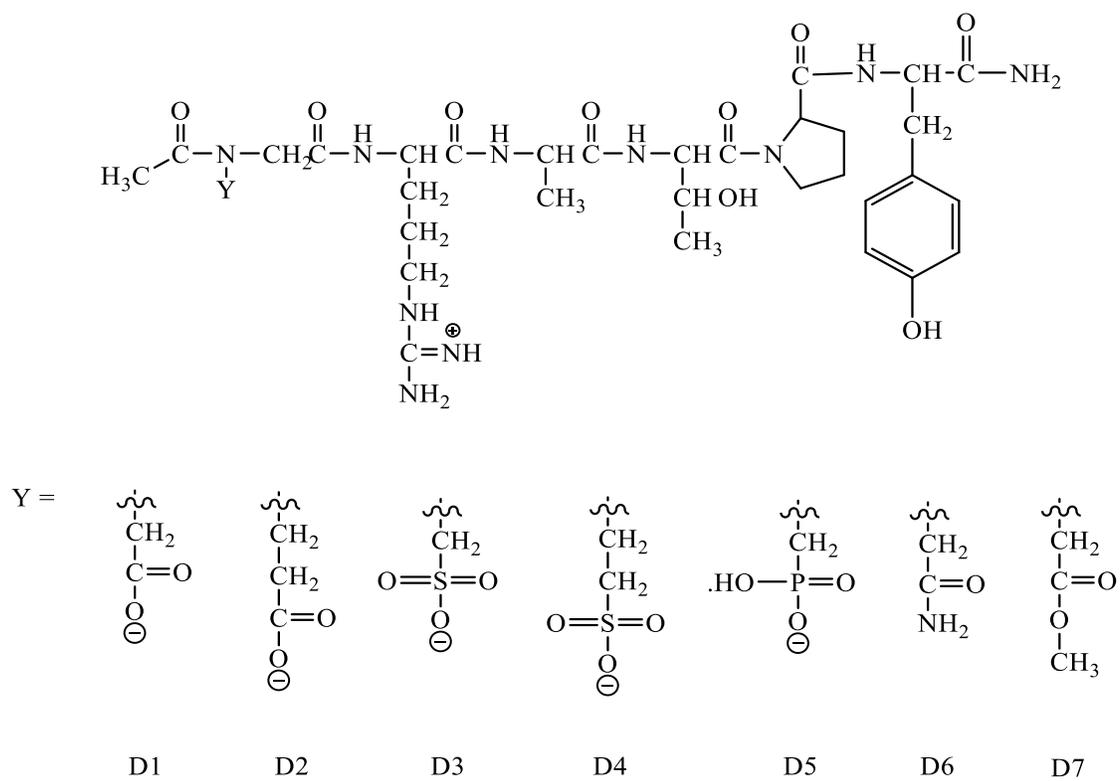


Figure 18: Structure of various aspartic acid peptomers of AviD.

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APPENDICES

APPENDIX A: Spectra

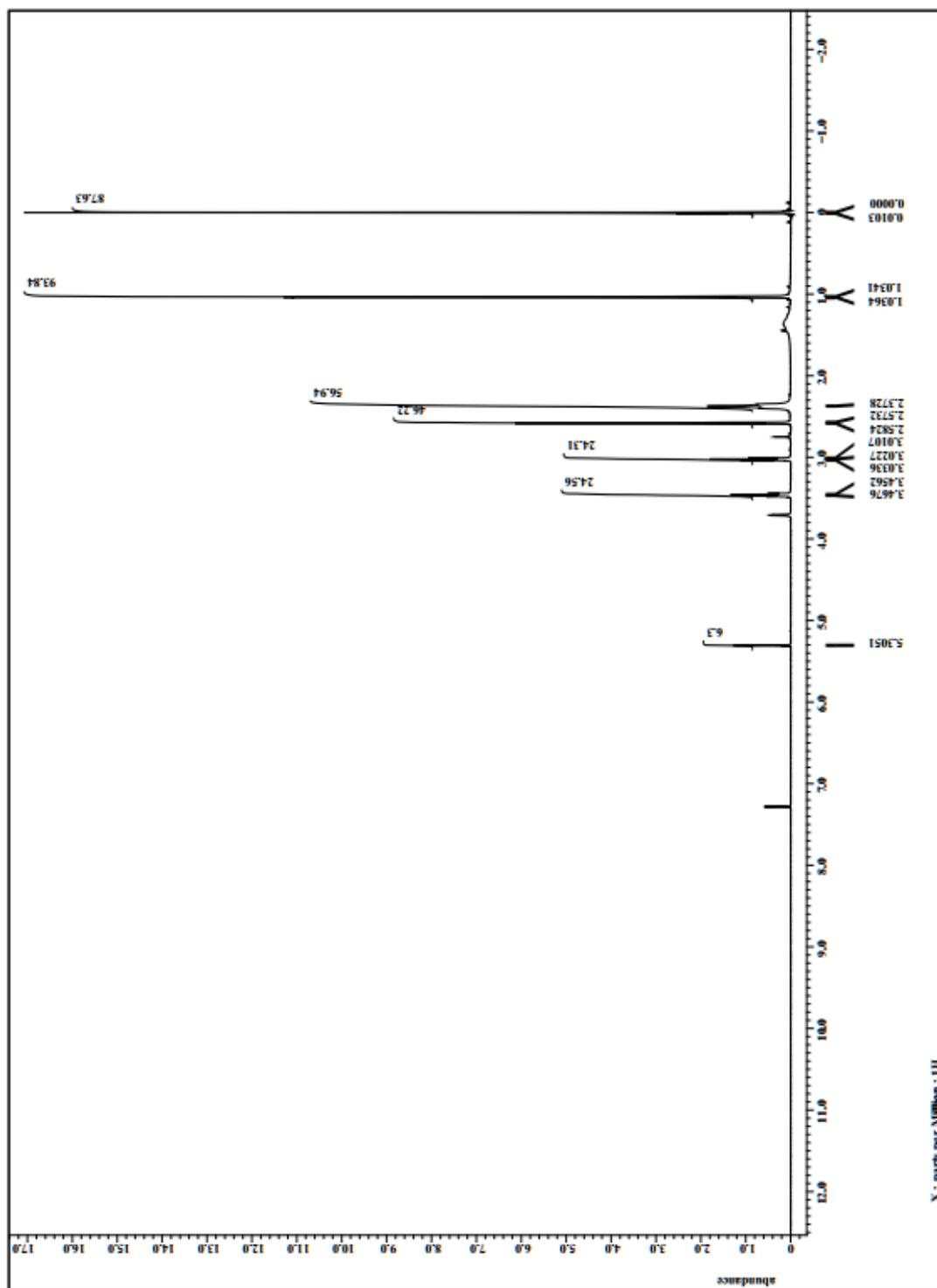
Figure A1: ^1H NMR for NDab-DDE.

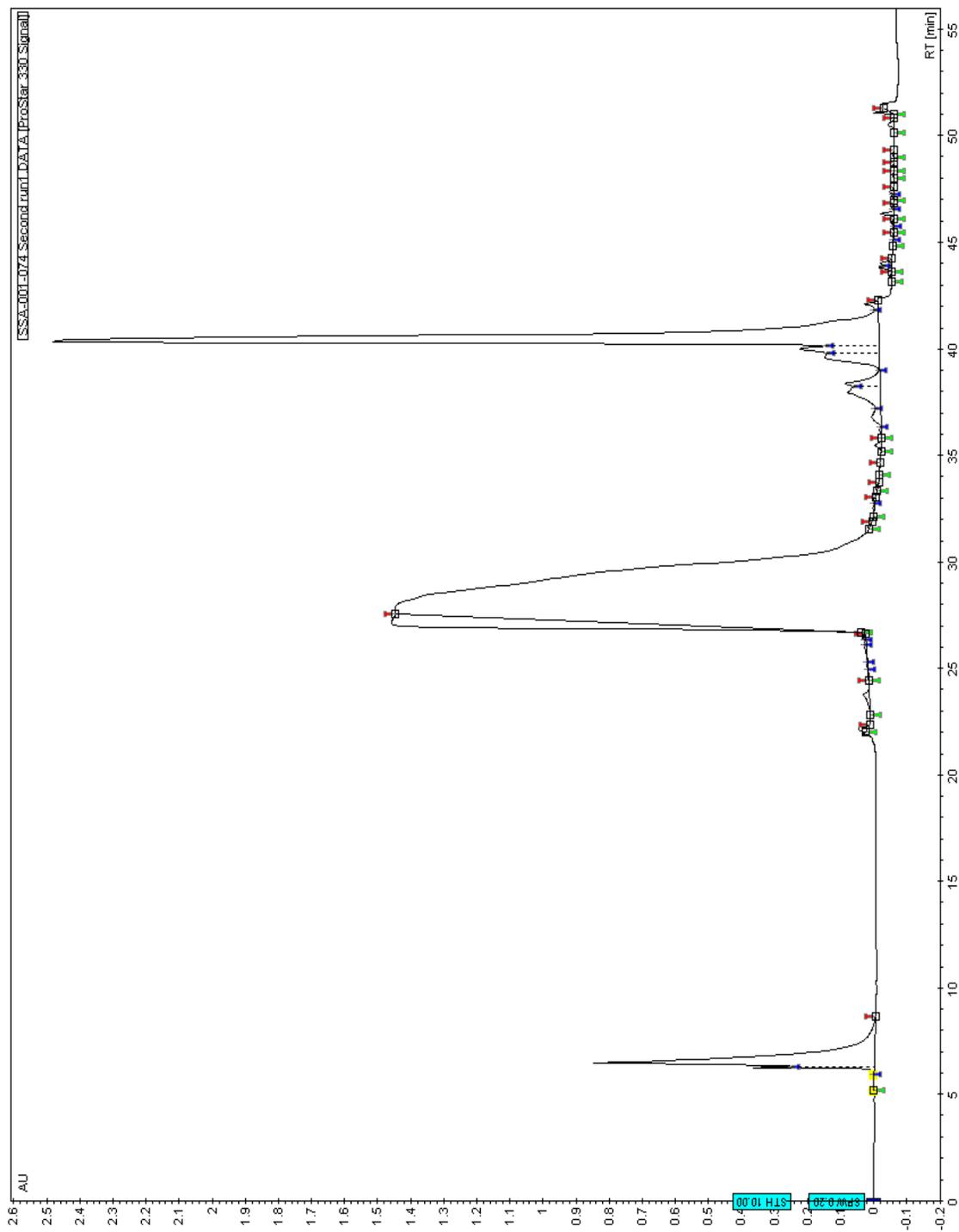
Figure A2: NDab-DDE purification.

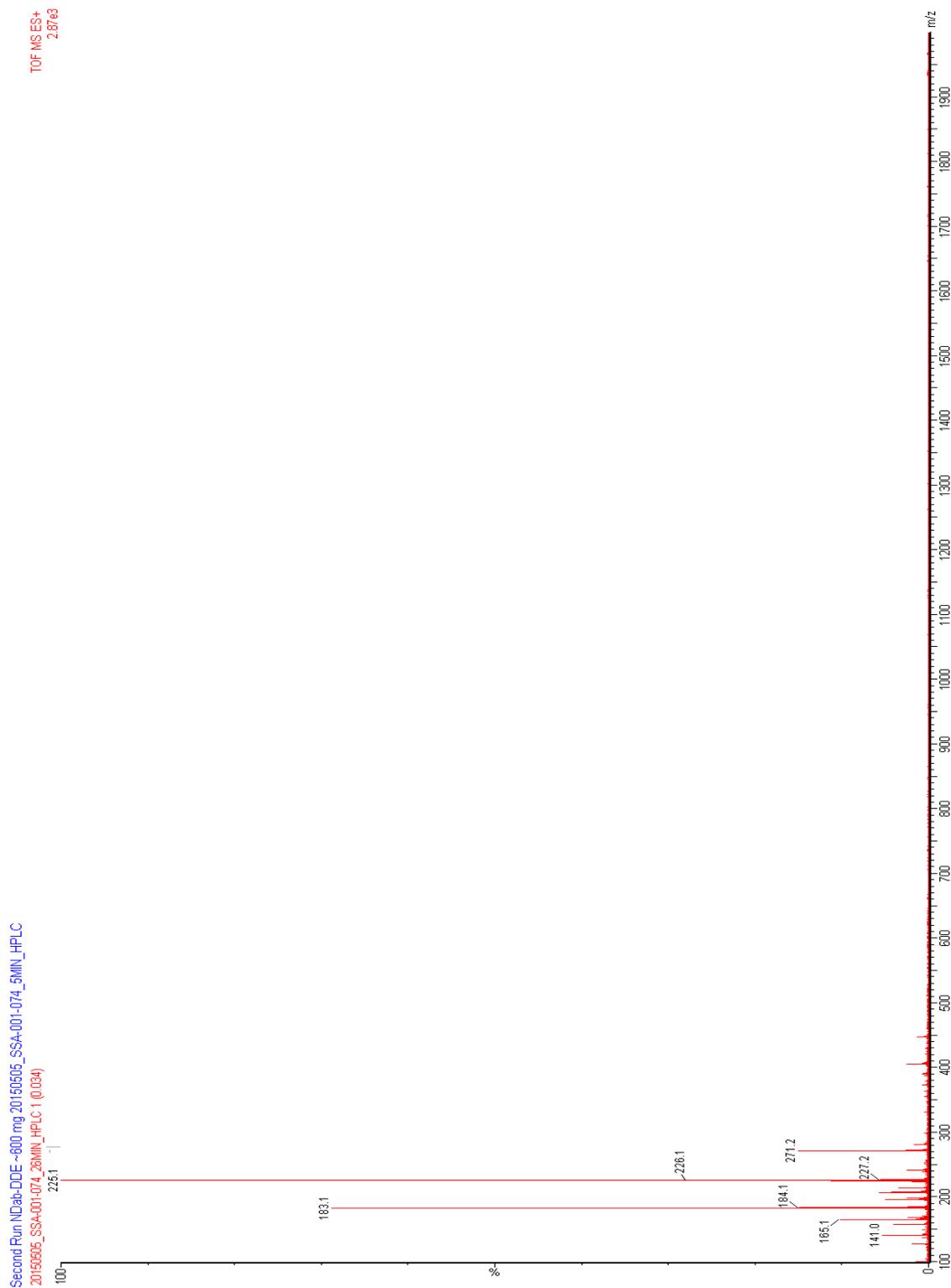
Figure A3: The MS identifying of NDab-DDE.

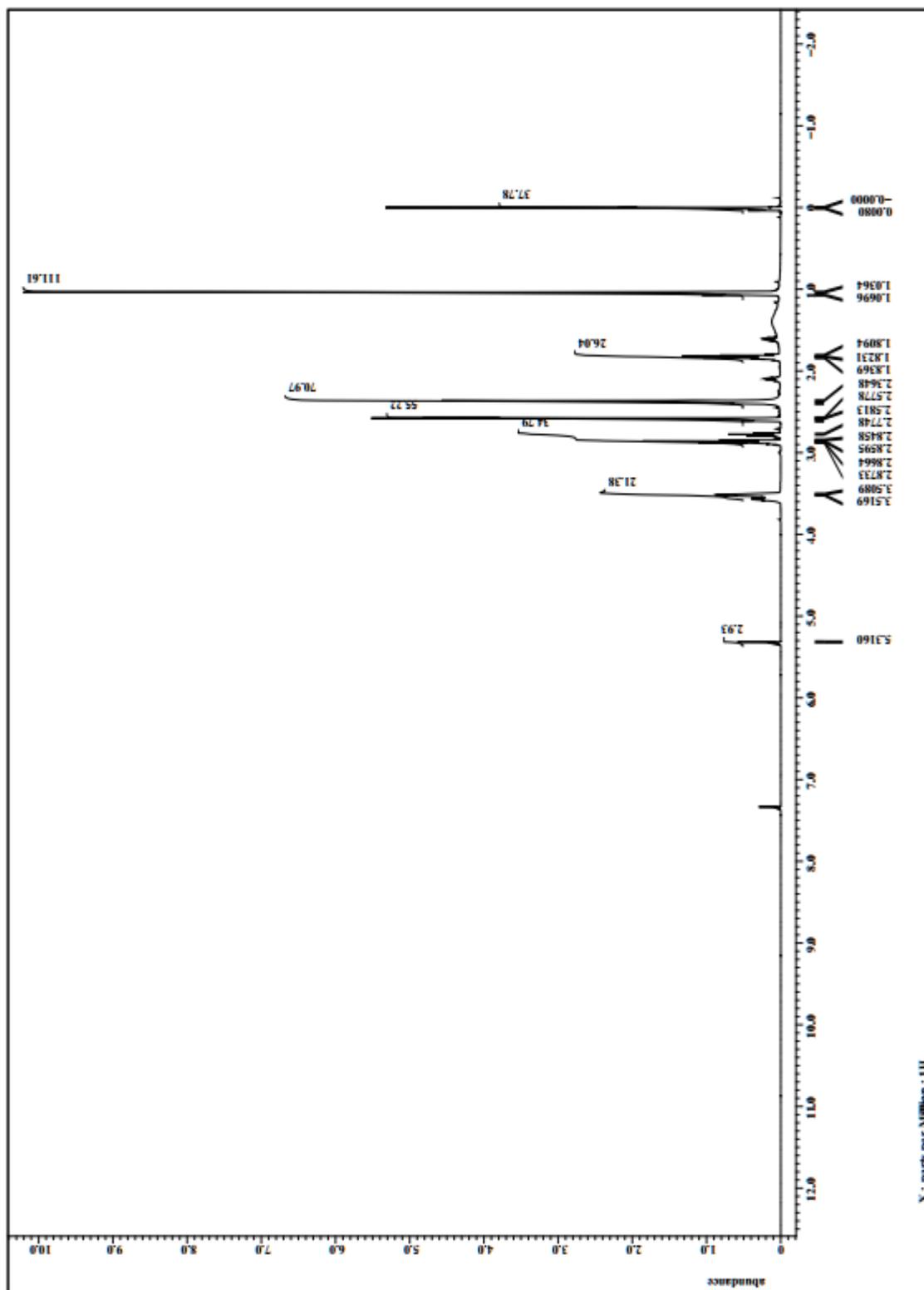
Figure A4: ^1H NMR for NO₂-DDE.

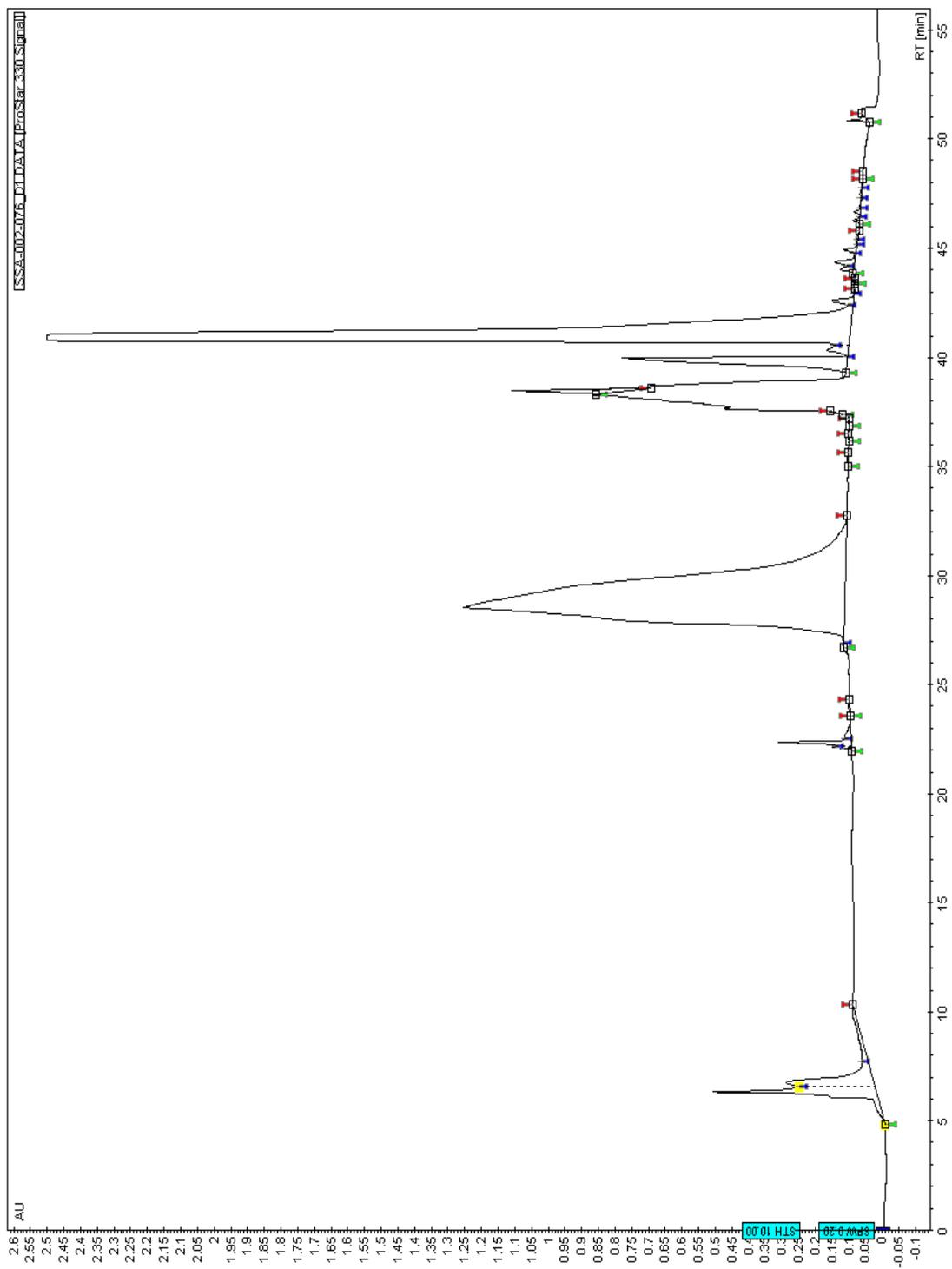
Figure A5: NO₂-DDE purification.

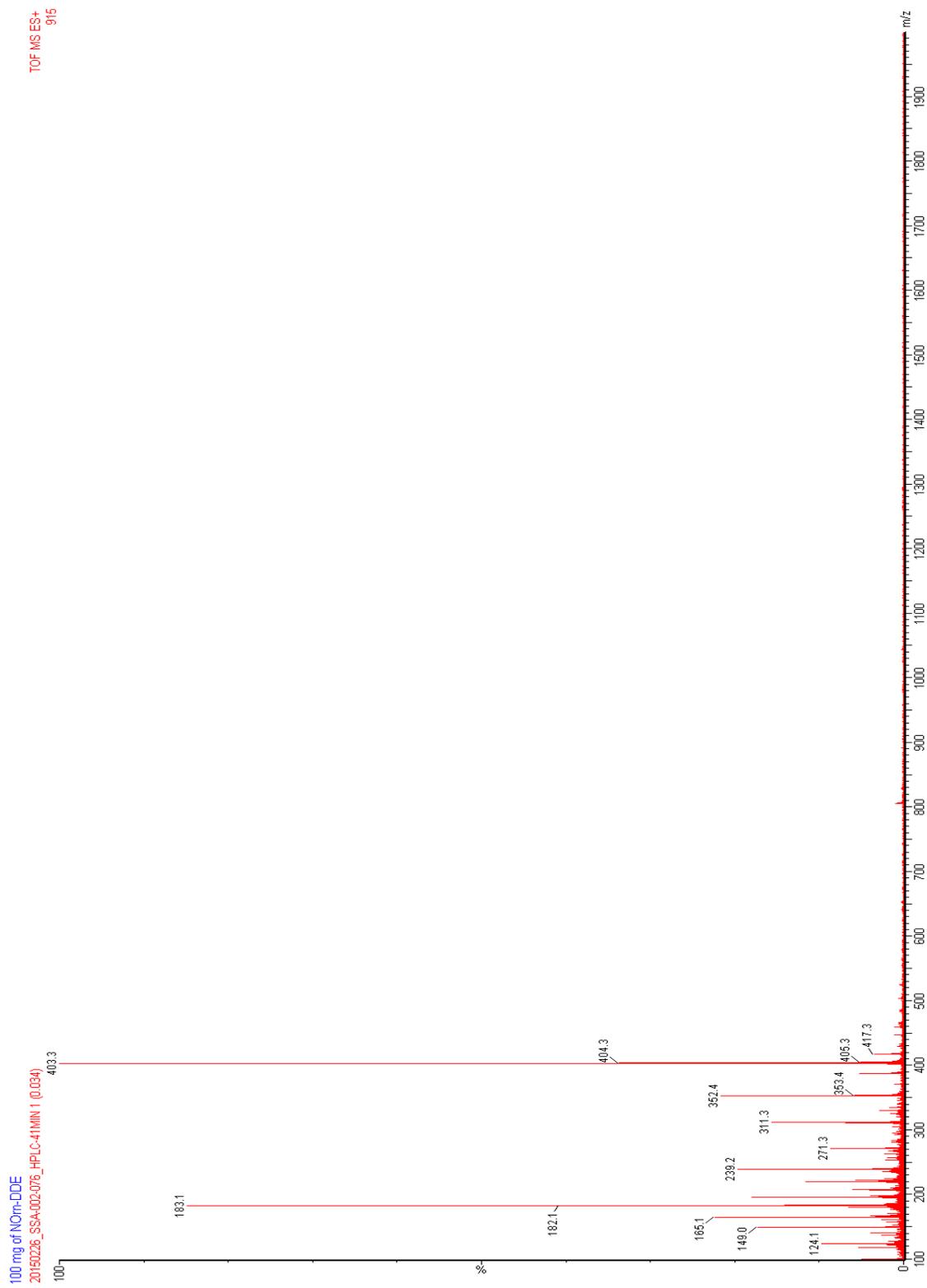
Figure A6: The MS identifying of NOrn-DDE.

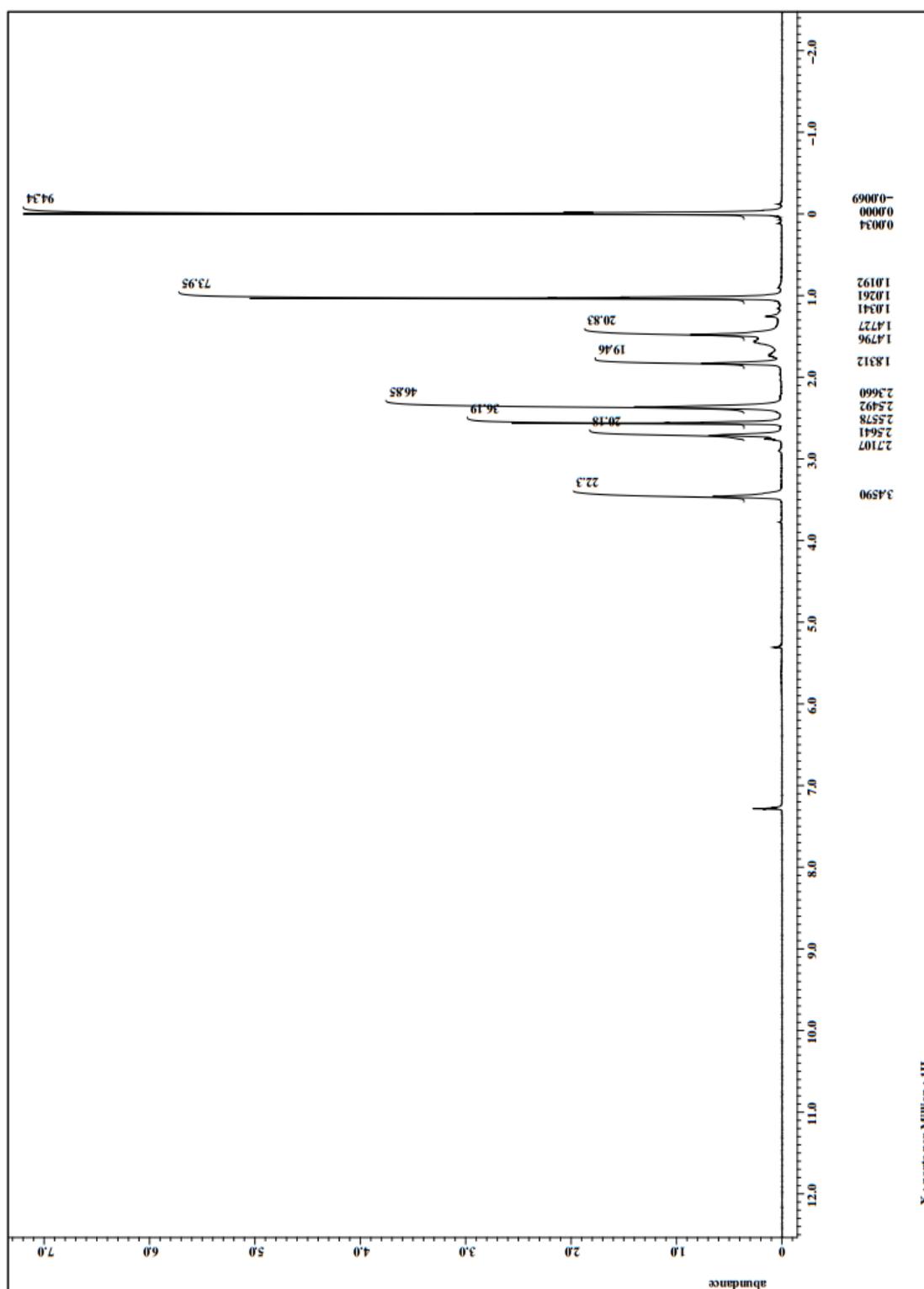
Figure A7: ^1H NMR for impure NLys-DDE.

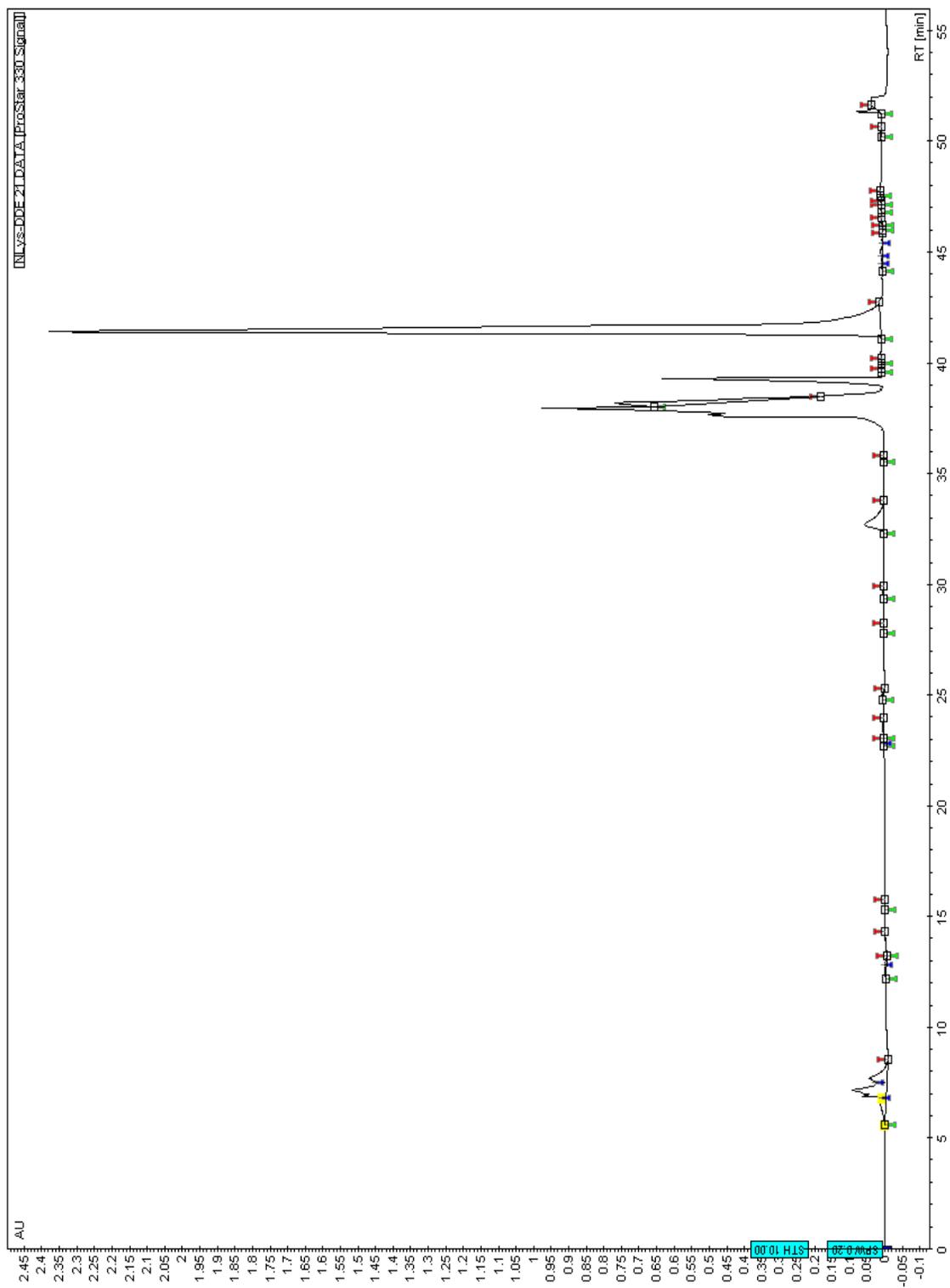
Figure A8: Nlys-DDE purification.

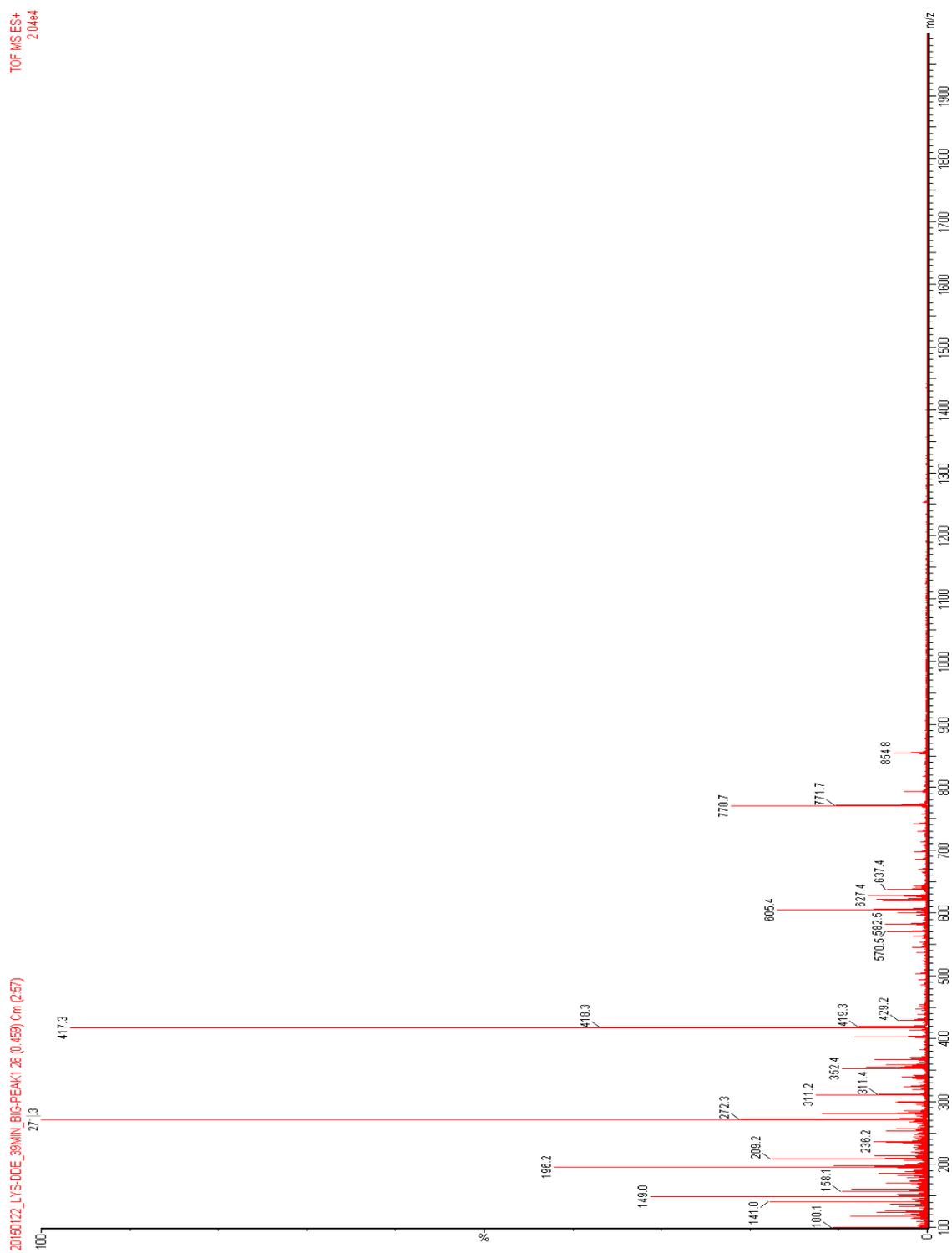
Figure A9: The MS identifying of impure NLys-DDE.

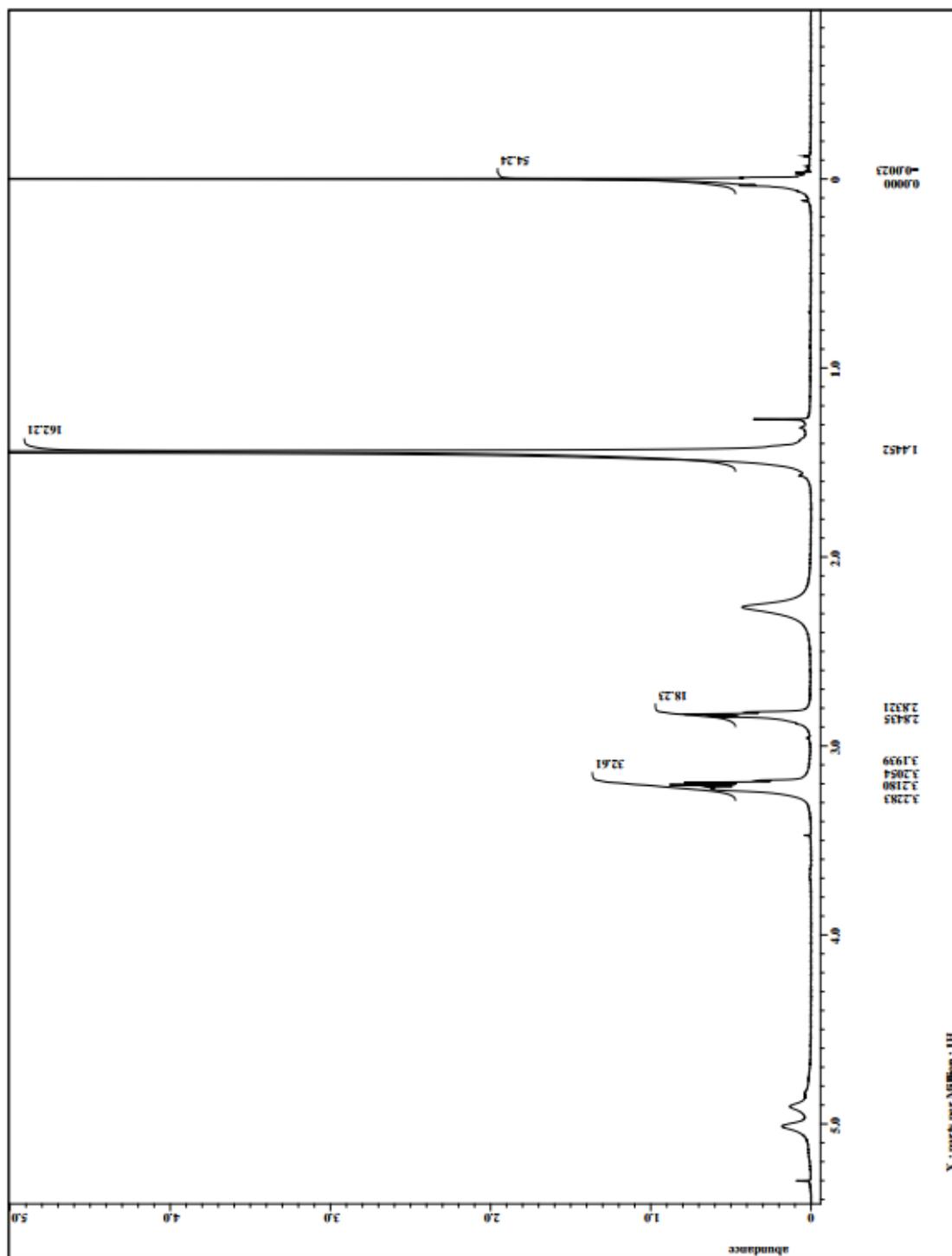
Figure A10: ^1H NMR for NDab-Boc.

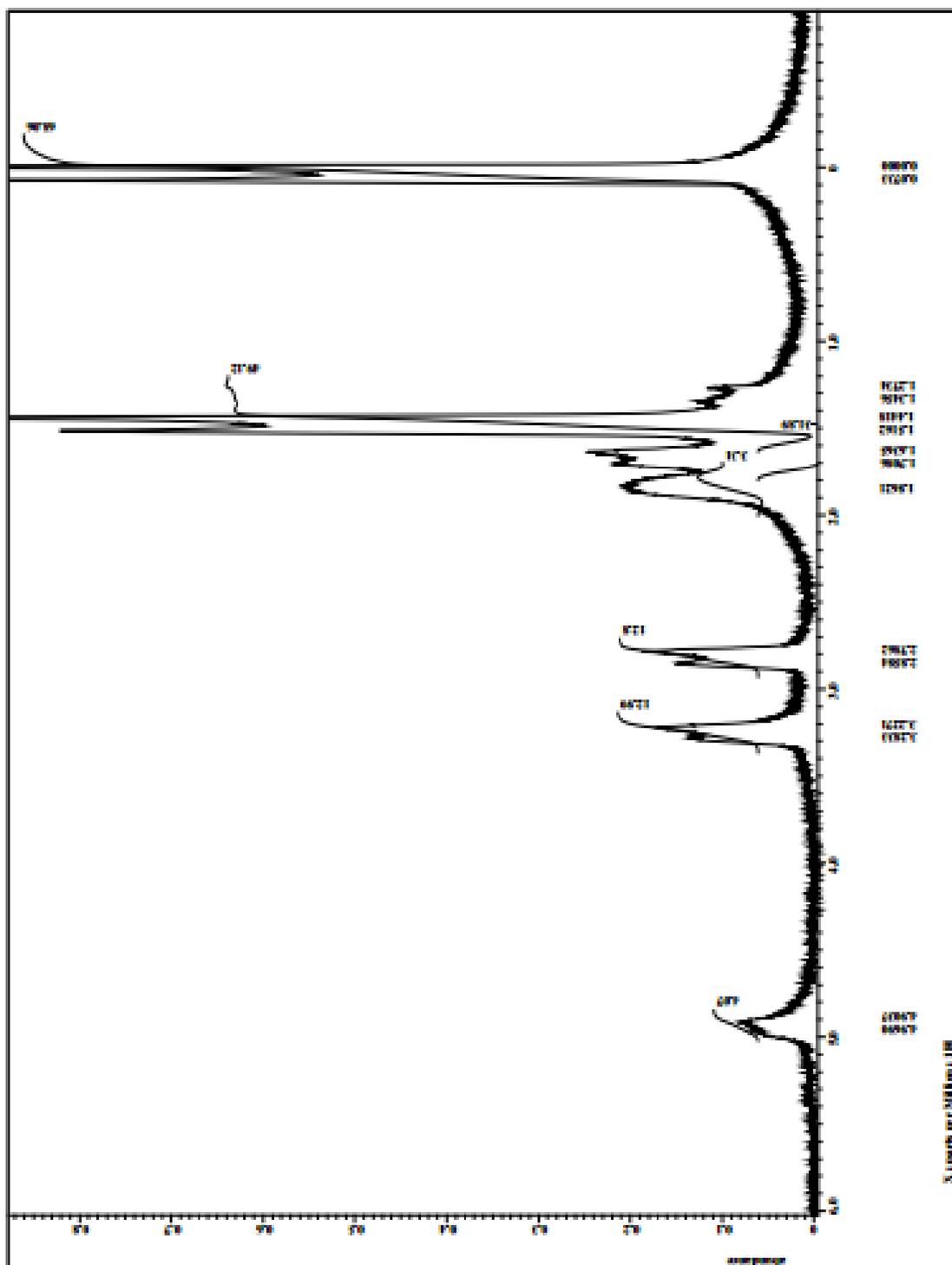
Figure A11: ^1H NMR for NOm-Boc.

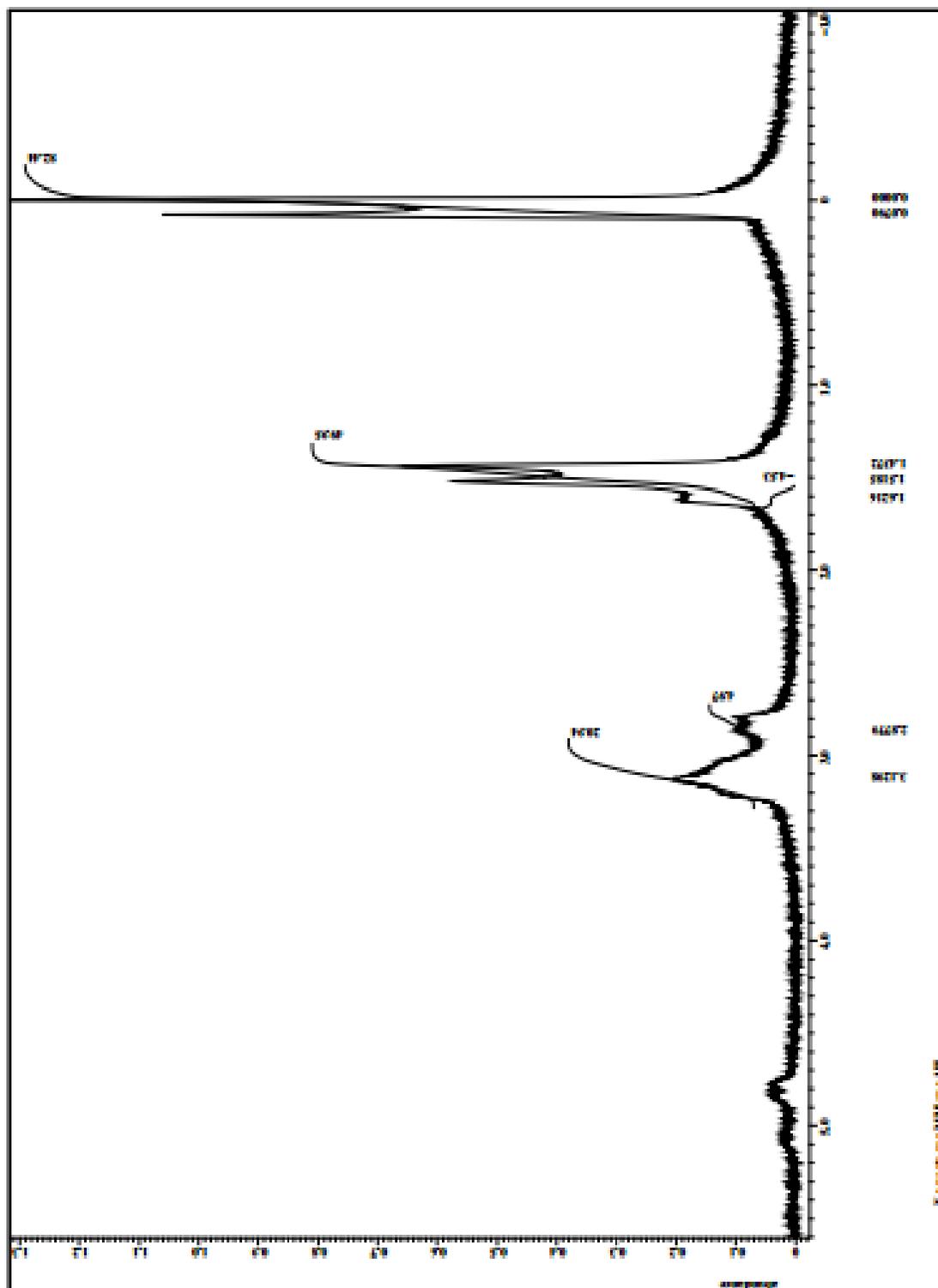
Figure A12: ^1H NMR for NLys-Boc.

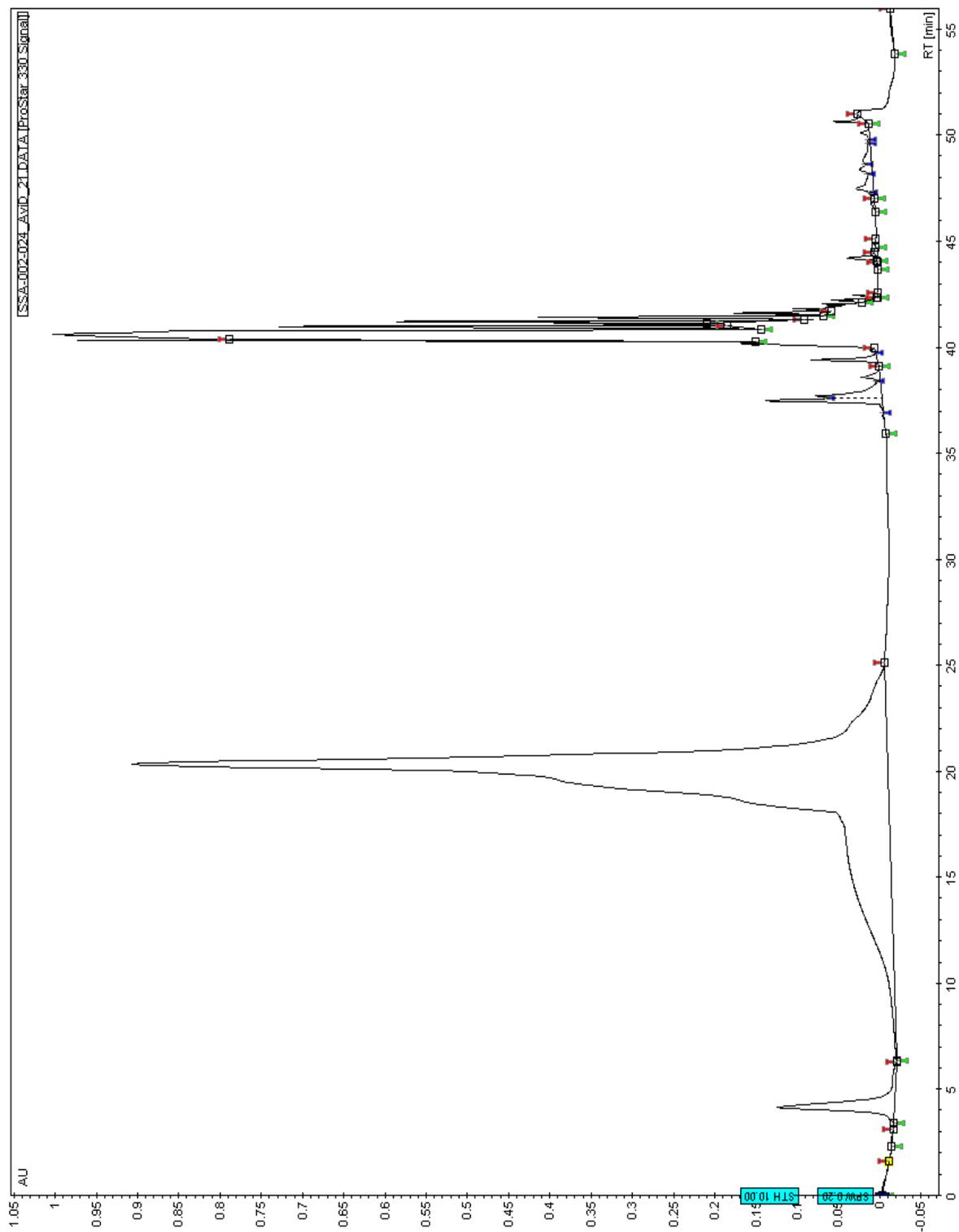
Figure A13: Avid purification.

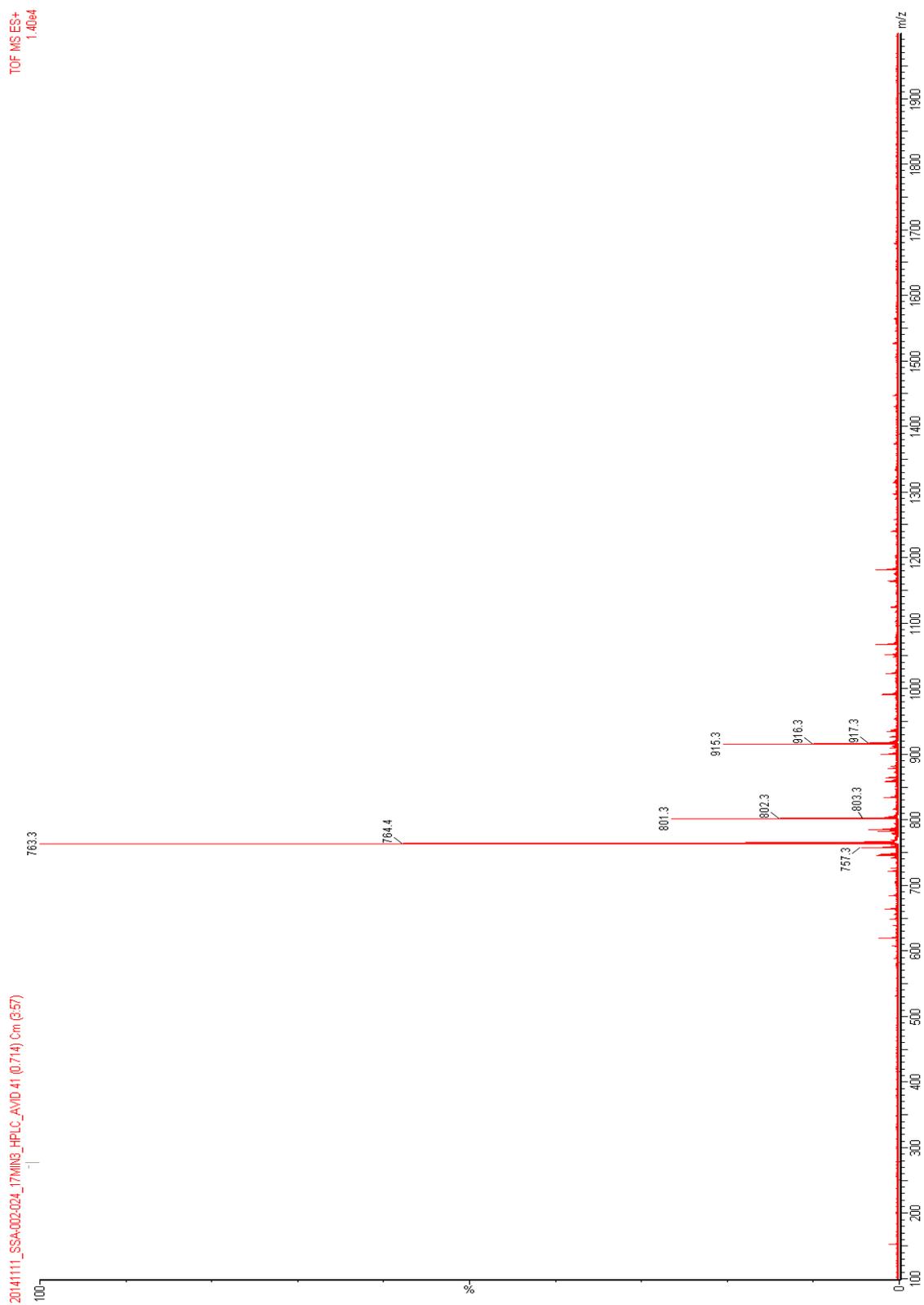
Figure A14: The MS identifying of Avid.

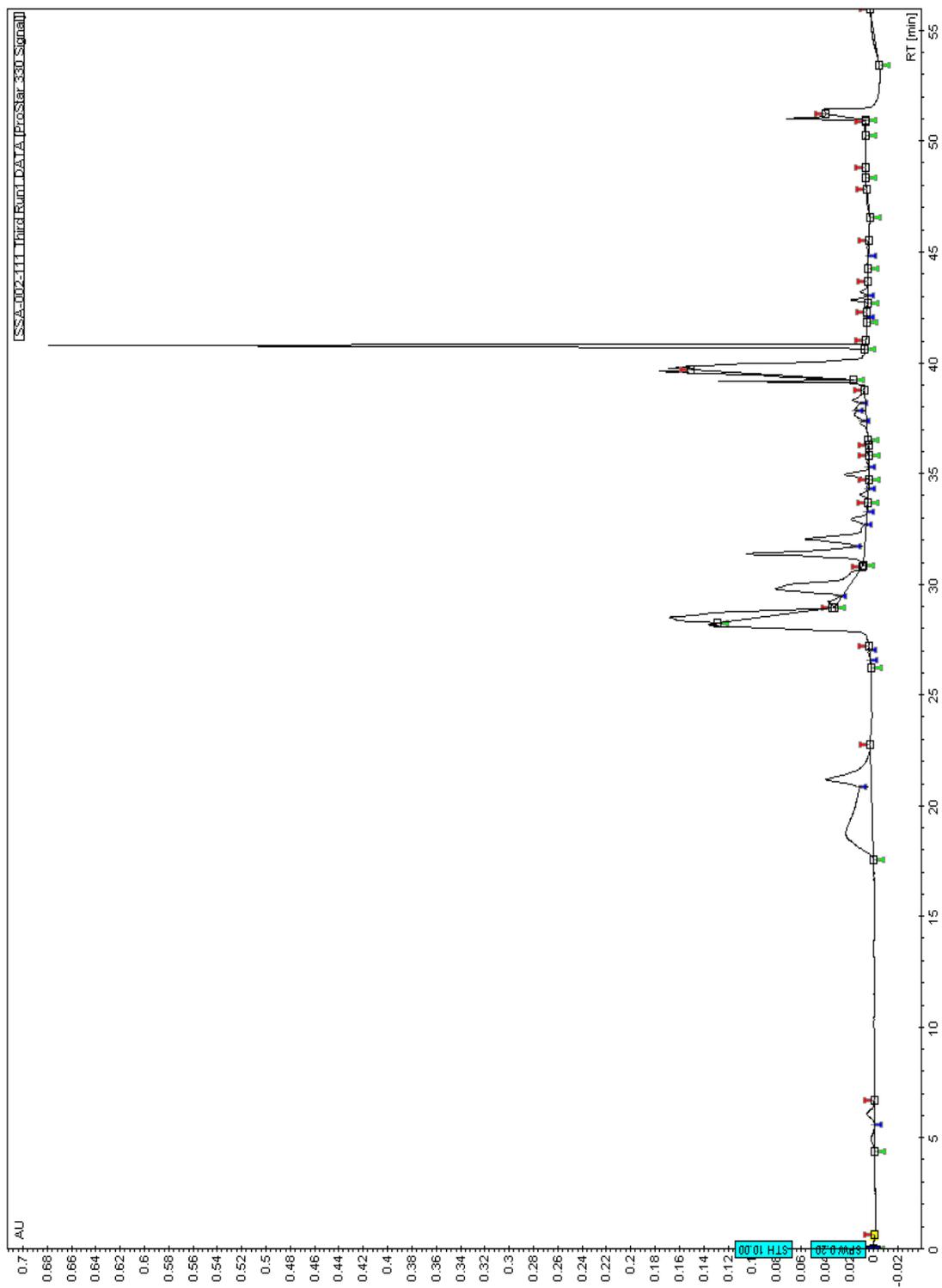
Figure A15: R1 purification.

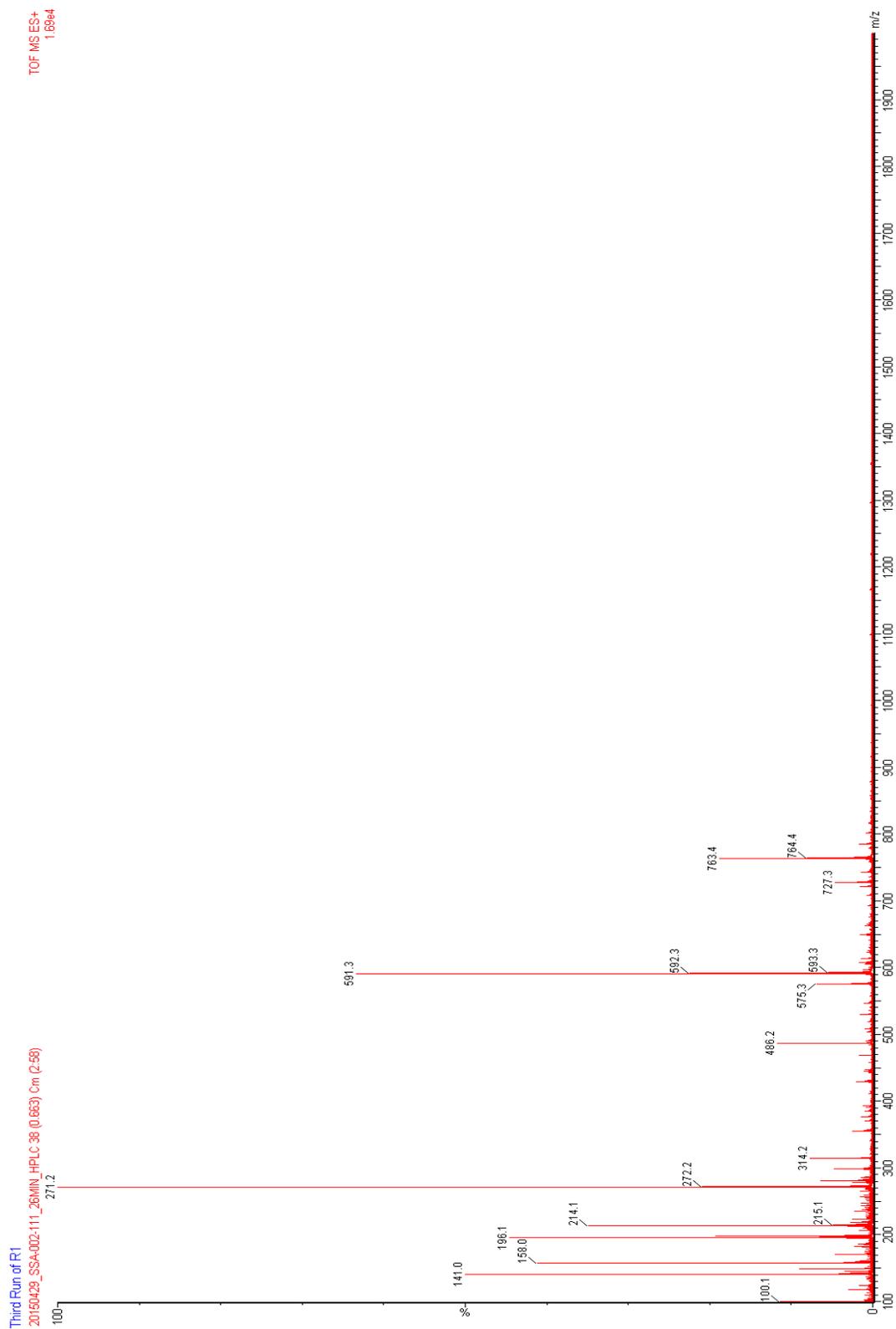
Figure A16: The MS identifying of R1.

Figure A17: R2 purification.

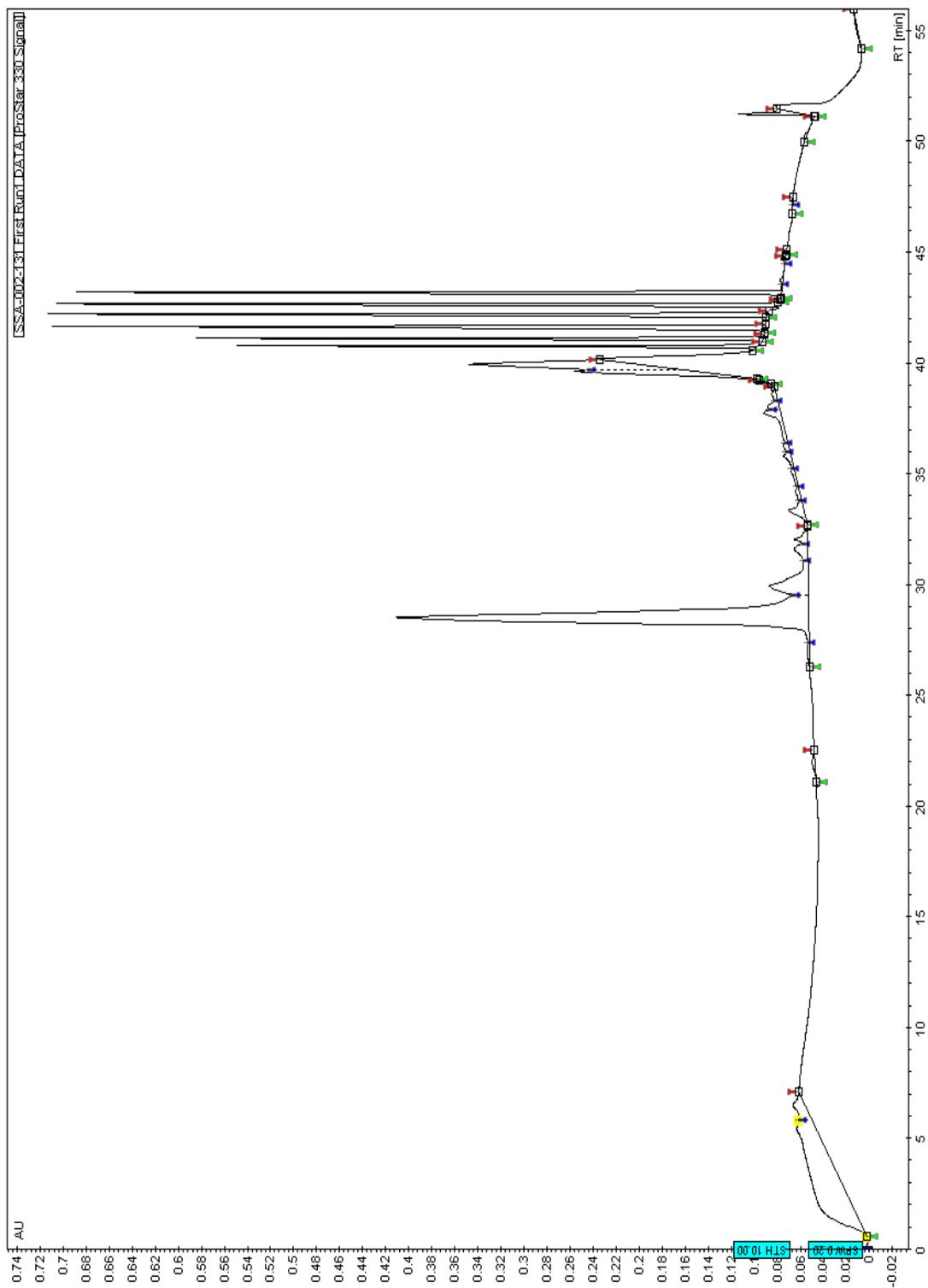


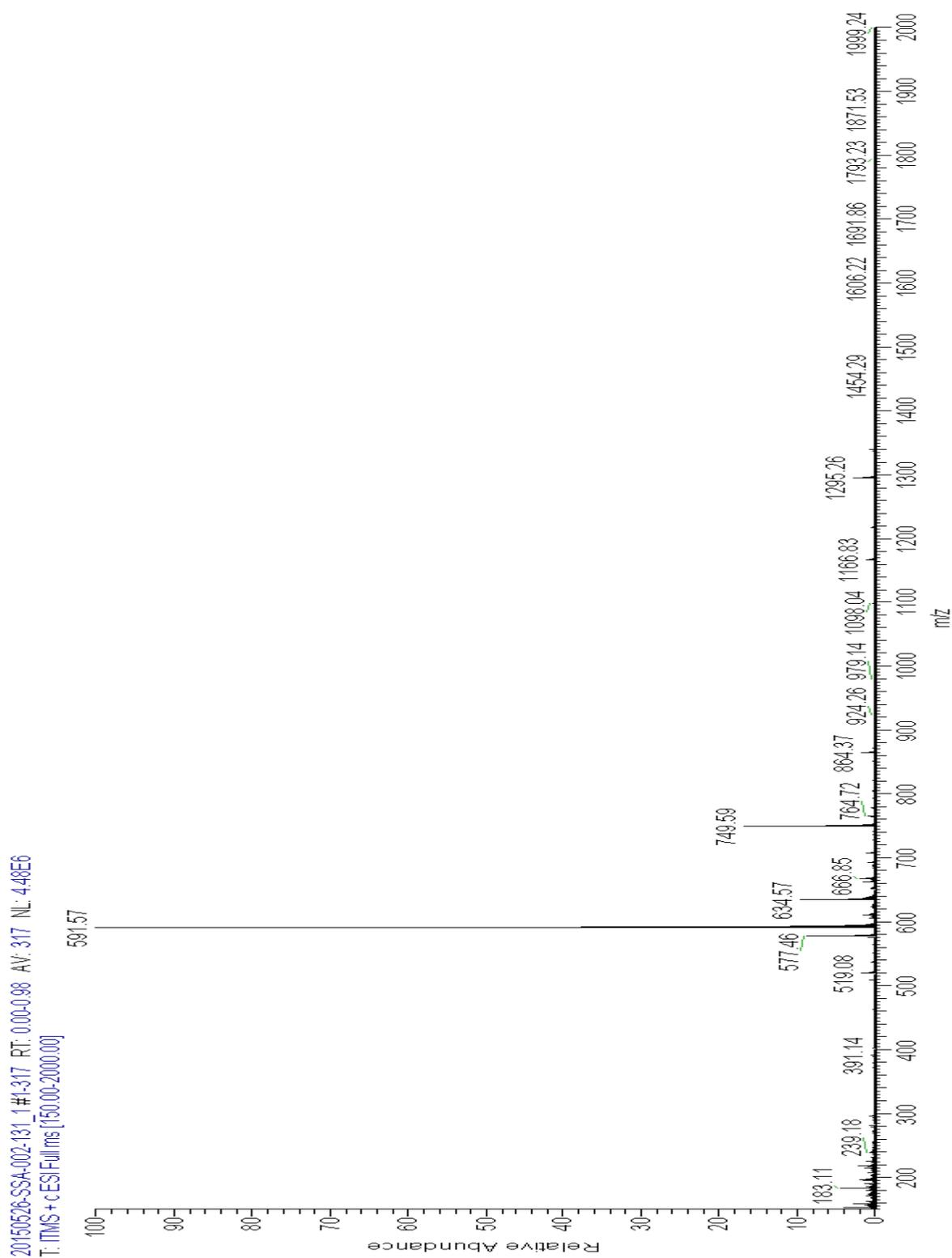
Figure A18: The MS identifying of R2.

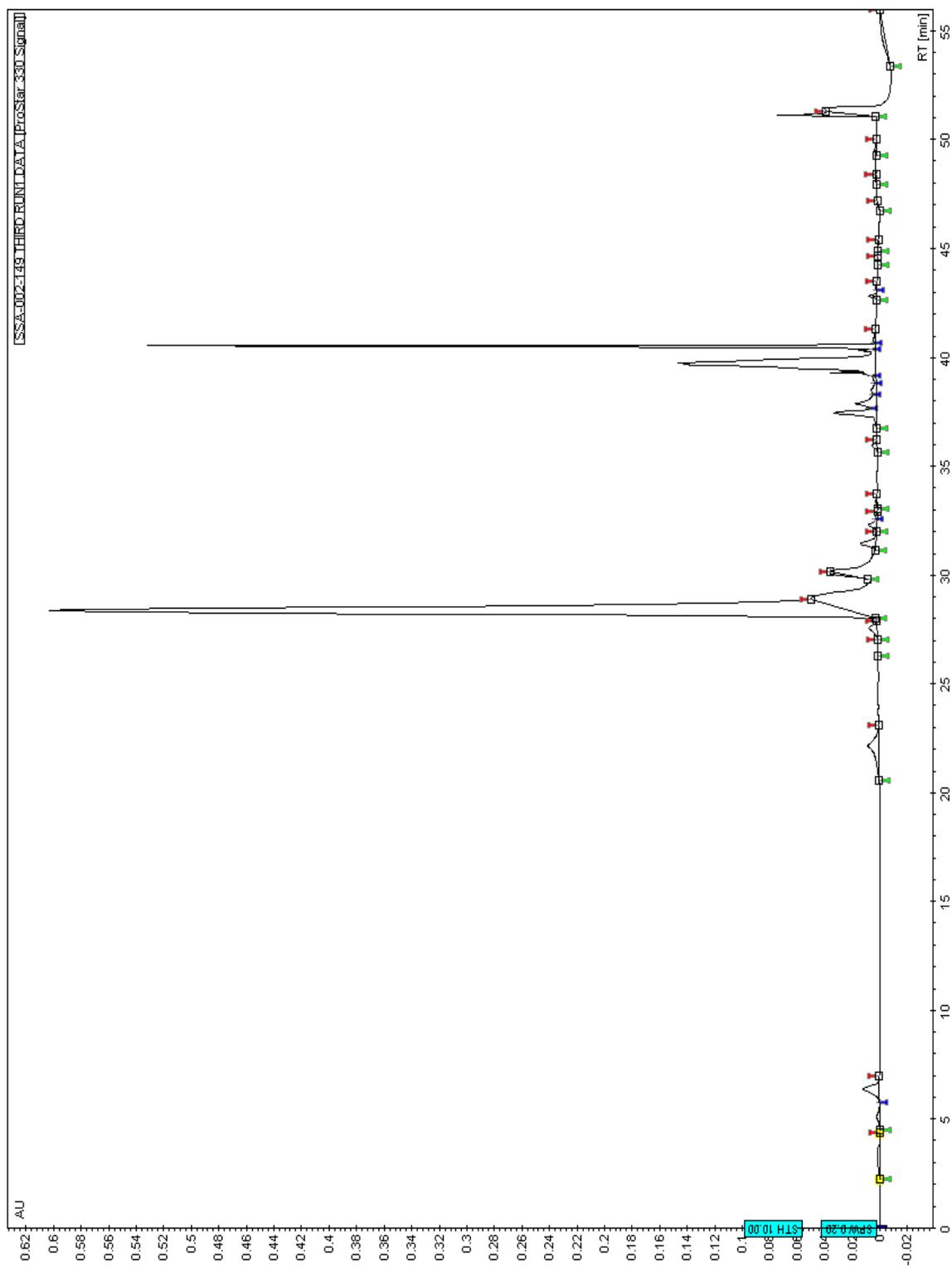
Figure A19: R4 purification.

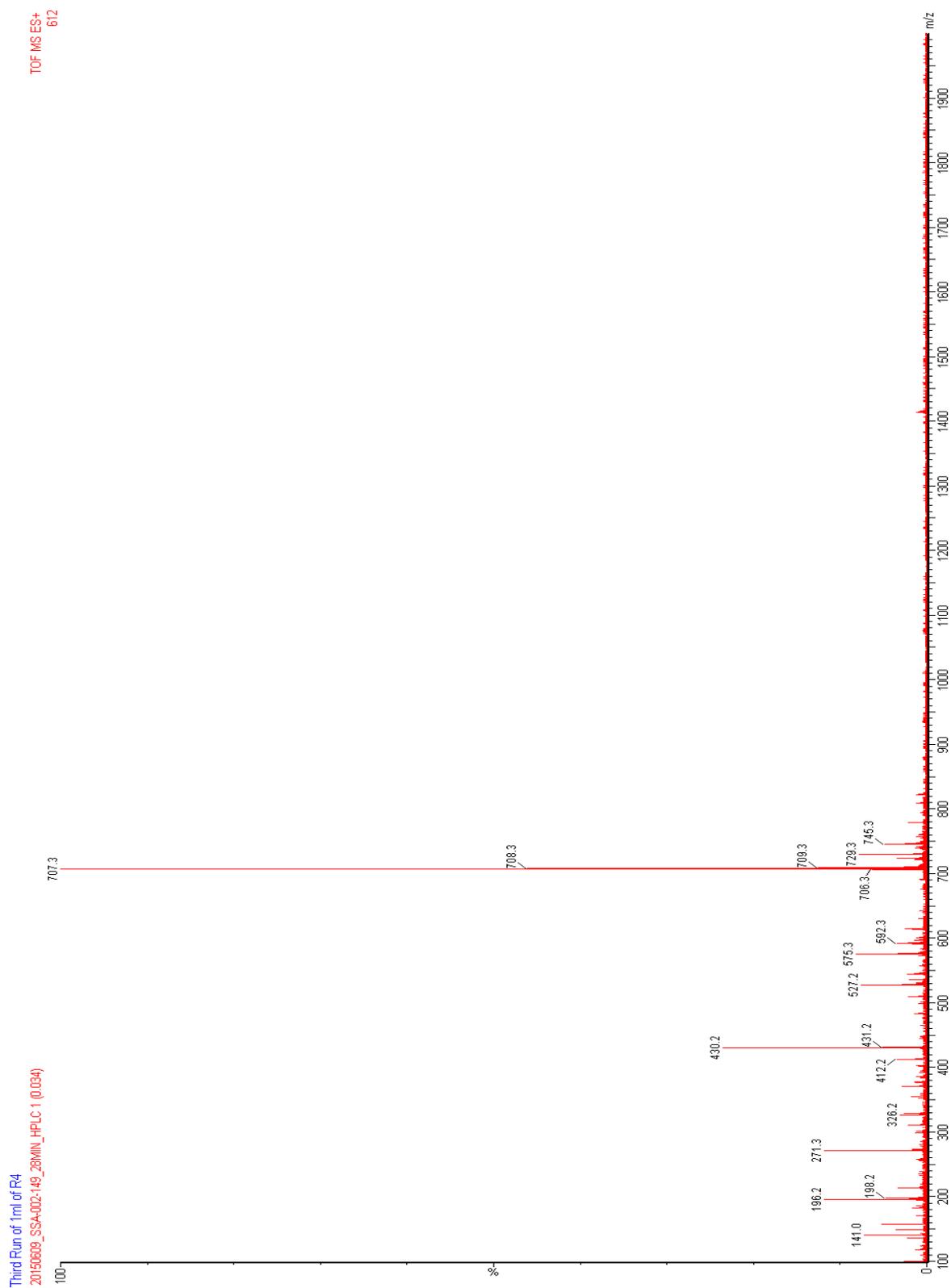
Figure A20: The MS identifying of R4.

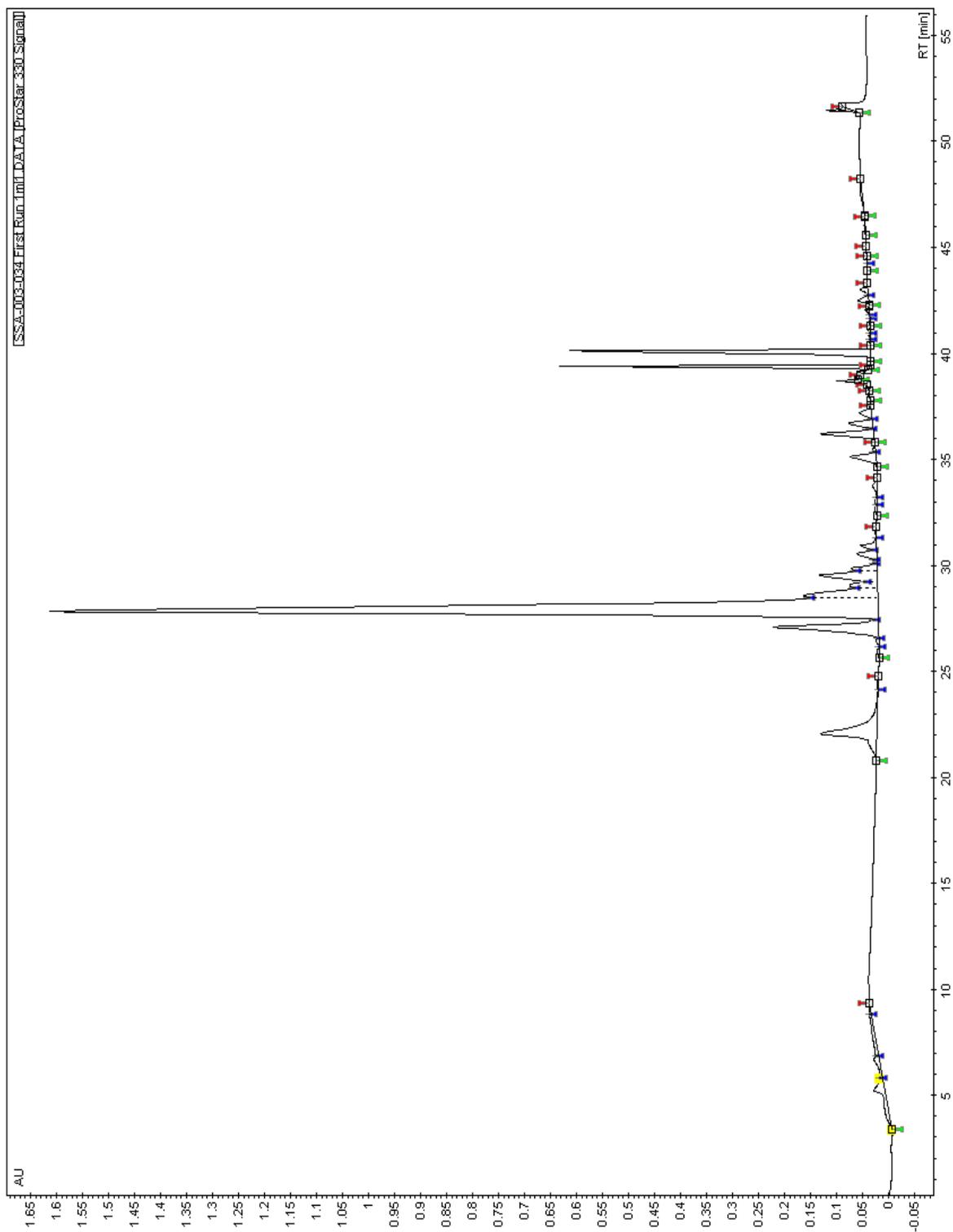
Figure A21: R5 purification.

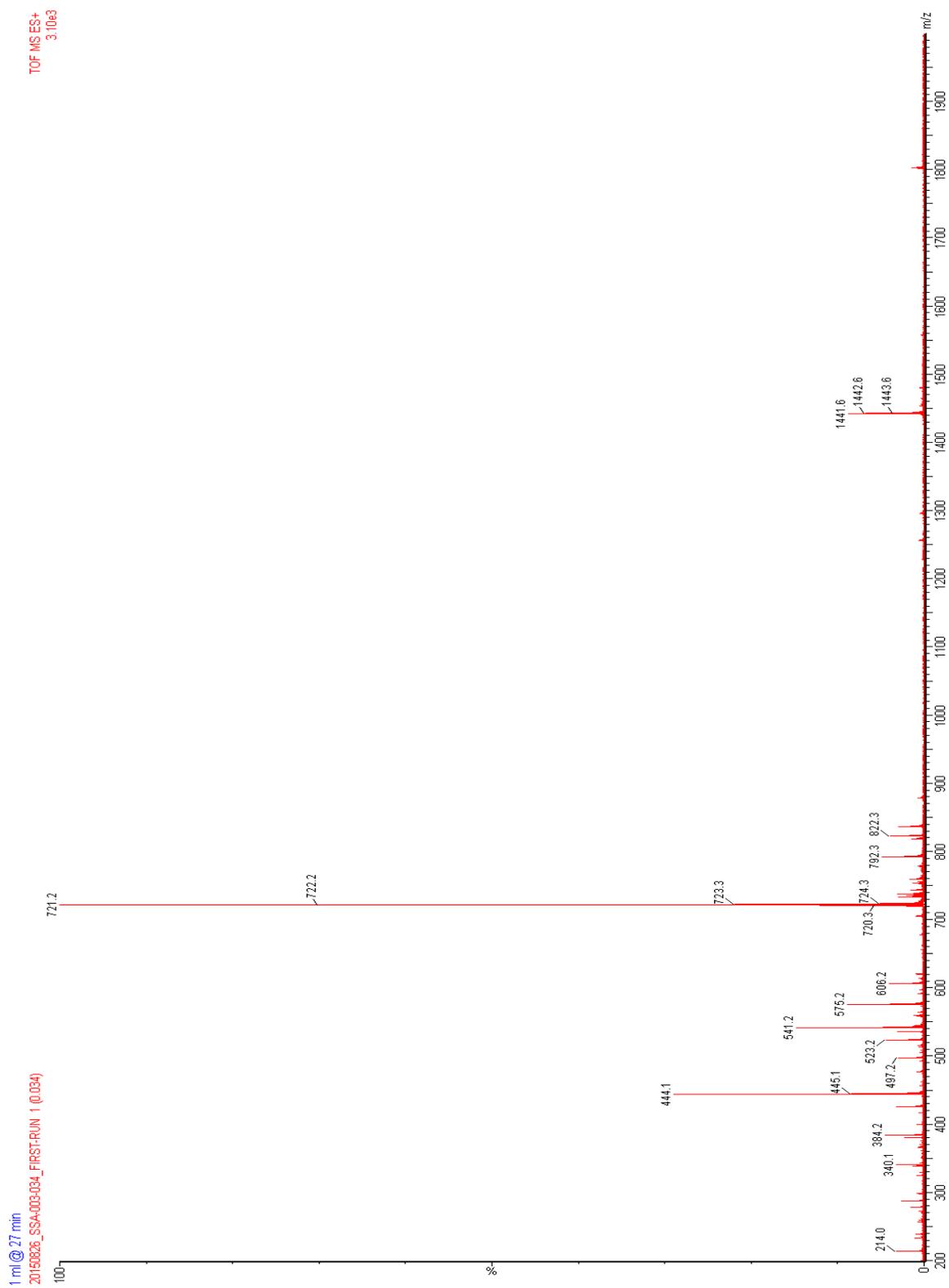
Figure A22: The MS identifying of R5.

Figure A23: R6 purification.

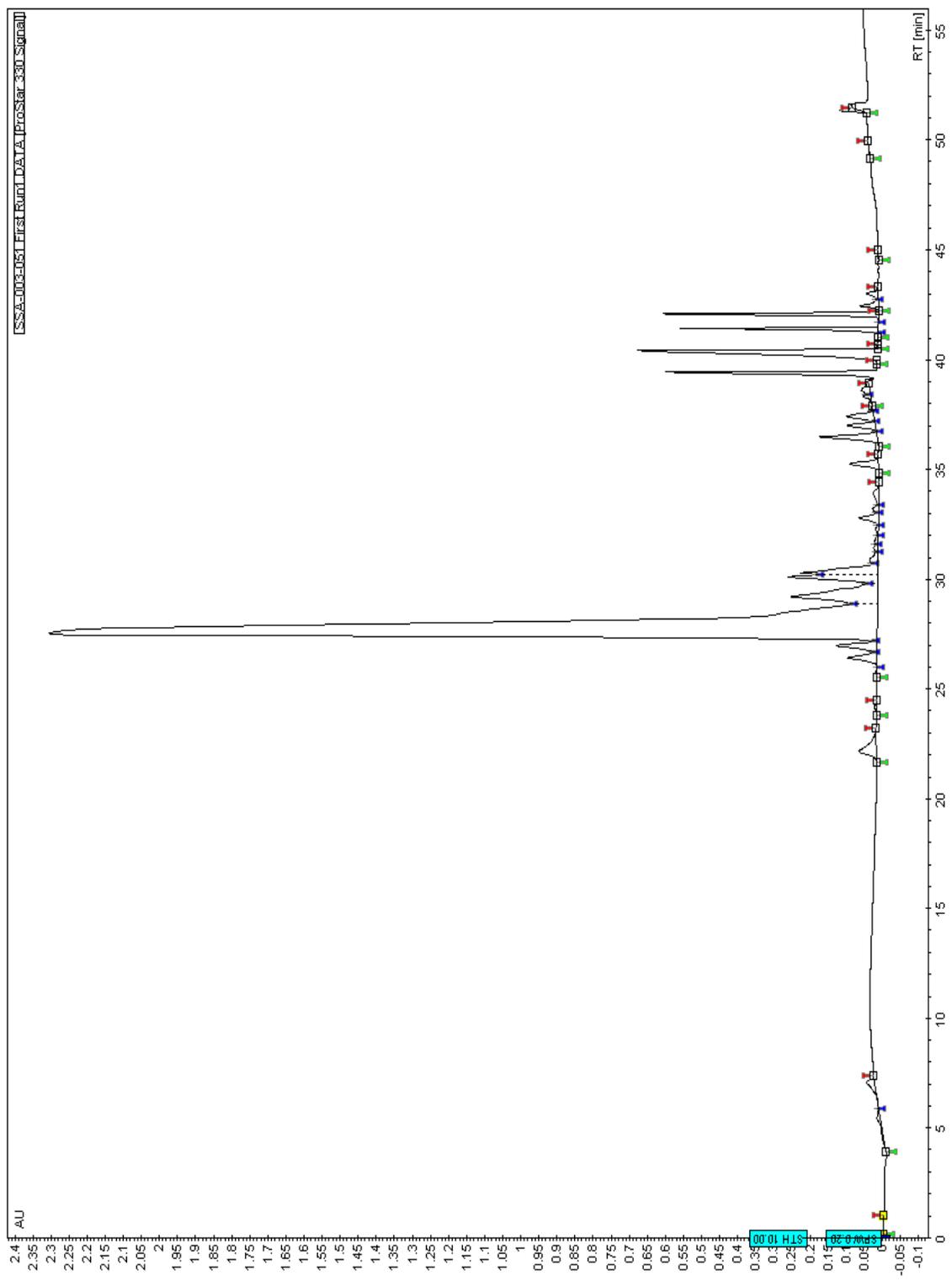


Figure A24: The MS identifying of R6.