

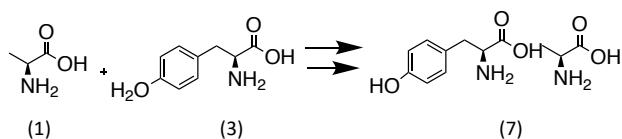
Dipeptide synthesis: Total synthesis of Alanine-Tyrosine dipeptide

Aaron D. Jones, Fiona Jordan, and Vrushabh Daga

Stonehill College, 320 Washington street,
Easton, MA-02357

Ajones6@students.stonehill.edu

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Abstract:

The synthesis of the dipeptide (**7**) was done in three steps (protecting, coupling, and deprotecting) using Alanine (**1**) and Tyrosine (**3**) as our starting amino acids. The protecting groups BOC and ethyl ester were added to Alanine (**1**) and Tyrosine (**3**) respectively, producing (**2**) and (**4**).

Compounds (**1**) and (**3**) were activated and then reacted together to produce the coupled (**5**) product. The C terminus was deprotected from (**5**) to produce compound (**6**), and then the N-terminus was deprotected from (**6**) producing the final product (**7**). All the intermediary products along with the final products were confirmed using H-NMR and IR spectral data.

Introduction:

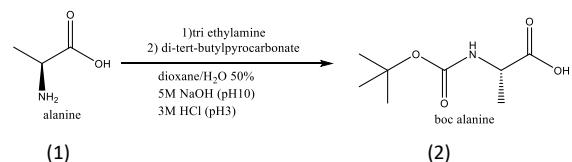
Peptides are short chains of covalently linked amino acid monomers, having each carbon connected by a peptide bond. Peptides are smaller versions of proteins (which may or may not have a biological function), for proteins can contain

upwards of hundreds of thousands of amino acids whereas peptides are under 50 amino acids in length. The properties and biological function of a peptide is determined by the amino acid residues connected to the alpha Carbon, which based on electrostatic interactions with other residues on the chain can cause it to form unique secondary structures. Peptides are very important in medicine and in the health/beauty supplement industry, being used in anything from skin care products and muscle enhancers to HIV or diabetes treatments.

The type of peptide we synthesized is a dipeptide, containing only two amino acid monomers. Our goal in lab was to link (**3**) to the C-terminus of (**1**), which involved multiple steps to protect the N-terminus of (**1**) and C-terminus of (**3**) to prevent unwanted reaction as well as deprotecting them when the reaction reached completion. Allowing the reaction to take place without protecting the N-terminus of (**1**) and C-terminus of (**3**) would lead to many unwanted products such as di-alanine's or di-tyrosine's, or other unwanted peptides containing more than two monomers. Protecting the ends involved the additions of what are known as "protective groups" to the necessary terminuses. The group t-butoxycarbonyl was covalently added to the N-terminus of (**1**) using the reagent di-tert-butylpyrocarbonate with triethylamine. The C-terminus on (**3**) was treated with thionyl chloride to produce an acyl halide which was then attacked by ethanol in solution producing ethyl ester. Both protective groups ultimately serve the same purpose of protecting the respective terminus from unwanted reaction. Once both protective groups were added to the respective amino acids, the carboxyl group of (**2**) was converted to an acyl chloride. (**2**) was then reacted with the (**4**), forming the peptide bond, linking the amino acids. Once the two

amino acids are bonded together to form (**5**), the protective groups must be removed from each of the terminuses to obtain (**7**). The final and intermediate products were characterized using H-NMR¹ and IR spectrometry². The experiment was followed closely to how it was written in the lab manual³.

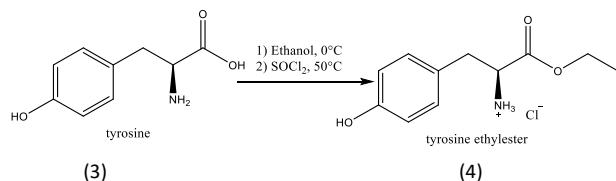
Scheme 1. Protection of Alanine



The protection of (**1**) was done through the addition of the t-butoxy carbonyl protective group on the N-terminus of alanine. (**1**) was reacted with triethylamine followed by di-tert-butylpyrocarbonate in a solution of dioxane/H₂O 50%, 5M NaOH and 3M HCl. This reaction replaced one of the N-H bonds of the amino group on (**1**) with an N-BOC bond. Adding this group reduces the nucleophilicity of Nitrogen, preventing it from attacking the C-terminus of other alanine or tyrosine molecules that it may encounter. The evidence that we successfully formed the protected alanine (**2**) comes from our ¹H-NMR results. Our ¹H-NMR reveals a doublet at 1.45 ppm having an integration of 12, indicating the presence of the BOC group in our molecule. This integration of 12 represents the hydrogens on the four methyl groups present in our

molecule (three of those coming from the BOC group). We yielded 0.850g of compound (**2**).

Scheme 2. Protection of Tyrosine



The protection of Tyrosine (**3**) was accomplished by reacting the hydroxyl group of the C-terminus of (**3**) with thionyl chloride. This converted it into a better leaving group, making the alpha carbon of the C-terminus open to nucleophilic attack from the electrons on the OH group of ethanol (solvent). The purpose of this protection was to prevent the unwanted reaction of the C terminus of (**3**) with the N-terminus of either alanine or other tyrosine molecules. The evidence that our protection was successful comes from the ¹H-NMR and IR spectral data. The evidence from the IR spectra comes from the presence of a strong peak at 1737.76 cm⁻¹, indicating the presence of an ester group in our molecule. This presence of an ester group in our compound suggests that the ethyl ester has been successfully added to the C-terminus. The ¹H-NMR data also suggest the presence of the ethyl ester protecting group in our molecule. The triplet present at 1.18 ppm with an

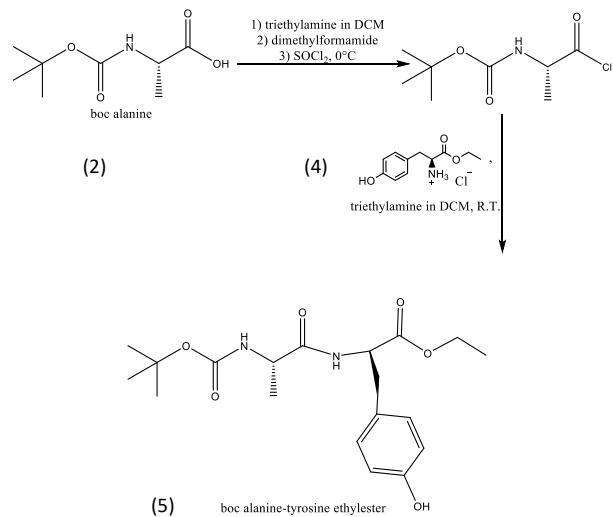
¹ Hornback, J. M. *Organic chemistry*; Brooks/Cole: Belmont, CA, 2006.

² IR Spectrum Table & Chart.
<https://www.sigmaaldrich.com/technical-documents/articles/biology/ir-spectrum-table.html> (accessed Dec 7, 2018).

³ Liotta, L. J. *DESIGN AND CARRY OUT THE SYNTHESIS OF A DIPEPTIDE*; rep.

Integration of 3 represents the hydrogens on the primary carbon at the end of the ethyl ester protecting group. This confirms that the conversion of our compound (**3**) to the protected form (**4**) was successful. We yielded 0.840g of compound (**4**).

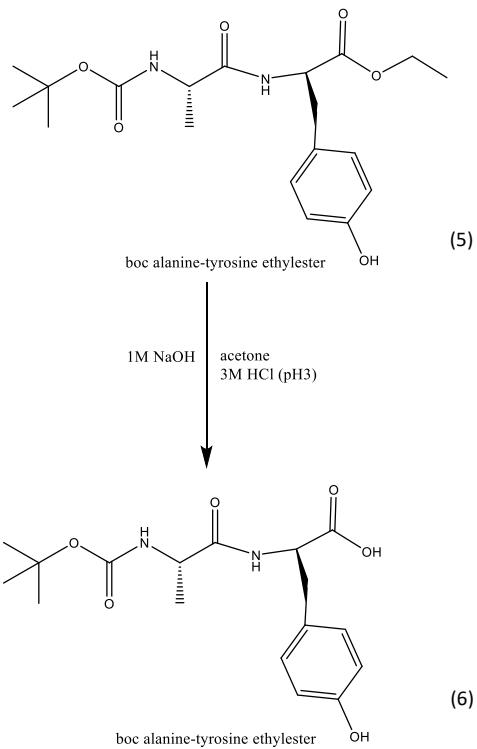
Scheme 3. Activation and coupling



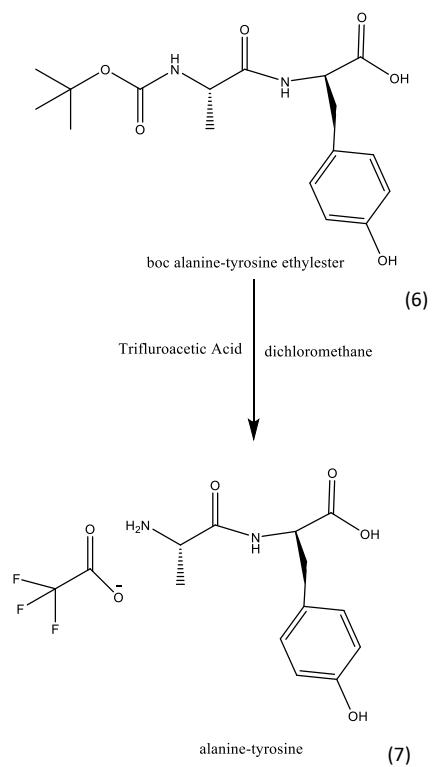
The coupling of compounds (**2**) and (**4**) began with the activation of the C-terminus of (**2**). This was done by reacting the hydroxyl group of the C-terminus of (**2**) with triethylamine in DCM, dimethylformamide, and thionyl chloride sequentially. This served the purpose of converting the OH into the better chloride leaving group, making the alpha carbon of the C-terminus more subject to nucleophilic attack. The activated (**2**) was then reacted with compound (**4**) in a solution of triethylamine and DCM. The electrons of the N-terminus Nitrogen of (**4**) undergo an SN₂ reaction with the activated C-terminus of (**2**), joining the two molecules together. The success of our reaction was confirmed with data obtained from ¹H-NMR. The presence of a

multiplet with an integration of 15 at 1.45ppm indicates the presence of the BOC protecting group in the compound. The Integration of 15 comes from the Hydrogens of the three methyl groups of the BOC protecting group (9 H) the methyl group of the alanine (3 H) and the hydrogens on the primary carbon of alanine's ethyl ester protecting group (3 H). The second piece of spectral evidence is the presence of a singlet at 6.9ppm with an integration of 2 and a doublet at 6.7ppm with an integration of 2. These two peaks represent the two sets of two equivalent hydrogens found on the benzene ring of tyrosine. The presence of both the Benzene ring and the BOC protecting group indicate that both groups are present in the same compound, confirming that our coupling reaction to produce (**5**) was successful. We yielded 0.14g, giving us a percent yield of 11%.

Scheme 4. Deprotection of C-terminus



Scheme 5. Deprotection of N-terminus



Our deprotection of the N and C-terminuses was done stepwise: deprotecting the C-terminus of compound (5) [Scheme 4] followed by the deprotection of its N-terminus [Scheme 5]. Deprotection of the C-terminus was done by converting the ethyl-ester group back into a hydroxyl group through reaction with NaOH in acetone, followed by reaction with 3M HCl in the workup. This returns the C-terminus to a carboxylic acid. Once this product (6) was obtained, the N-terminus was deprotected through treatment with trifluoroacetic acid in DCM, which converted the BOC protecting group into an amine group. Both of these subsequent deprotections produced our desired compound (7). The evidence for our reaction's success comes from $^1\text{H-NMR}$ spectral data. The absence of 12 hydrogens from the integration value for the peak at 1.45ppm indicates that the BOC protective group has been successfully removed. The integration value of 3 for this peak at 1.45ppm confirms the presence of the methyl group of alanine, whereas the doublets at 7.1ppm and 6.7ppm with integration of 2 each support the presence of the benzene ring of tyrosine present in our molecule. There is an impurity that manifests at a singlet at

5.0ppm that can be attributed to drying agent contaminating our final product. The overall data confirm the structure of the coupled and deprotected di-peptide product (**7**) lining up with the predicted ¹H-NMR peaks in Chem-draw. We obtained a final yield of 0.16g, giving us a percent yield of 14.5%.

Experimental:

Protection of Alanine:

8mmols were dissolved in a 16ml 1:1 dioxane/water mixture by first dissolving the amino acid in water first and then adding the dioxane to a 50ml Erlenmeyer with a magnetic stir bar. Pre-distilled triethylamine (2.4 g, 3.4 mL, 24 mmoles) and then di-tert-butylpyrocarbonate (2.0 g, 9.2 mmoles) were added while stirring. The mixture was stirred in a 40 °C water bath for 1 hour. After cooling to room temperature, the reaction mixture was transferred to a large test tube and 5M sodium hydroxide was added dropwise while stirring using a glass stir rod until the pH was 10.0 by pH paper. 2 mL of water was added to dissolve excess solid. The resulting solution was washed three times with 5 mL of dichloromethane. The aqueous layer was adjusted to pH 3.0 with concentrated HCl. The resulting acidified solution was extracted with five 5mL portions of ethyl acetate. Each portion of ethyl acetate was mixed to dissolve the product at the bottom of the tube. The ethyl acetate layers were then combined in a large test tube, and then washed with saturated sodium chloride. The aqueous layer was removed, and the ethyl acetate was dried over magnesium sulfate and then was filtered and evaporated to dryness. Our

resulting product (**2**) was left over night to dry in the drawer. We then obtained NMR using CDCl₃ to prepare the sample. The final yield was 0.85g. ¹H-NMR (300MHz, CDCl₃): δ 4.35 (s, 1H), 1.45 (d, 12H).

Protection of Tyrosine:

4mmol (**3**) in 25mL absolute ethanol was chilled to 0 °C in an ice bath and stirred using a stir bar. 4ml thionyl chloride was added dropwise to the solution. The mixture was heated in a 50 °C water bath for an hour while being stirred. The mixture was then evaporated by heating on a hot plate while blowing air on it. The residue was dissolved in 5ml absolute ethanol and then evaporated again to help remove residual thionyl chloride. The crystals were then dissolved in about 1.5ml of warmed reagent ethanol and then rewarmed to dissolve the solid product. The product was then precipitated with 20mL ether. The ethyl ester of (**3**) was then collected by suction filtration and dried. The product (**4**) was allowed to dry for a day before characterizing. An NMR was prepared from the dried sample. An IR was then collected. The final yield was 0.84g. IR V_{max} (cm⁻¹): 3340.38, 3192.76, 3017.85, 2914.81, 2864.78, 1737.76, 1612.77, 1591.51, 1513.68, 1241.28: ¹H-NMR (300MHz, D₂O): δ 7.1 (d, 2H), 6.84 (d, 2H), 4.23 (m, 3H), 3.17 (m, 2H), 1.19 (t, 4H)

Activation and coupling:

3mmoles of (**2**) were dissolved in 5.6mL dichloromethane containing triethylamine (660 mg, 0.9 mL, 6.6 mmoles). A drop of dimethylformamide was added and then the solution was cooled to 0 °C in an ice bath. 0.3mL Thionyl chloride was

added to the chilled solution while being stirred. The mixture was left to stir for 15 minutes while a suspension of 3mmoles of (**4**) was prepared in 2.4mL dichloromethane containing triethylamine (660 mg, 0.9 mL, 6.6 mmoles). (**4**) was added to the cold acid chloride solution dropwise. The solution was then left to stir at room temperature for 45 minutes. The mixture was transferred to another test tube and the original flask was rinsed with 1 mL of dichloromethane. The dichloromethane layer was washed with 7mL water, 7mL of 1 M hydrochloric acid, two separate 7mL portions of 0.5 M sodium bicarbonate, and another 7mL portion of water. The dichloromethane solution was then dried over anhydrous sodium sulfate, separated from the sodium sulfate, and was then evaporated. The resulting product (**2**) was left to dry in our drawer. An NMR sample was prepared using CDCl_3 . The final yield was 0.12g (11% yield). $^1\text{H-NMR}$ (300MHz, CDCl_3): δ 6.9 (s, 2H), 6.7 (d, 2H) 4.7 (s, 1H), 4.2 (s, 2H), 3.0(m, 2H), 1,4 (m, 15H)

Deprotection of C-terminus:

(**5**) was then dissolved in a mixture of 2mL of 1 M sodium hydroxide and 0.8 mL of acetone. The resulting mixture was set aside at room temperature for an hour. The flask was then weighed. The acetone was evaporated when the appropriate weight is lost to account for the 0.8 mL of acetone. The mixture is acidified to pH 3.0 using 3 M hydrochloric acid. The product (**6**) was then extracted into 5mL ethyl acetate and dried over anhydrous sodium sulfate. The resulting mixture was then evaporated to dryness.

Deprotection of N-terminus:

(**6**) was dissolved in 1mL trifluoroacetic 3mL of dichloromethane. The reaction was then stirred at room temperature for an hour, and then evaporated to dryness using a 40° C water bath. The residue was then dissolved twice in 1ml of methanol and evaporated to remove residual trifluoroacetic acid. An NMR was then obtained. The final yield was 0.516 (14% yield). $^1\text{H-NMR}$ (300MHz, CD_3OD): δ 7.08 (d, 2H), 6.70 (d, 2H), 4.6 (q, 1H), 3.95 (m, 1H), 2.9 (m, 1H), 1.5 (d, 3H), 1.25 (t, 3H).

Conclusion:

Based on the spectral evidence collected through $^1\text{H-NMR}$ and IR, we can confirm that the protection of Alanine and Tyrosine, activation and coupling, and deprotection were all successful. The final $^1\text{H-NMR}$ supports our final deprotected dipeptide, containing only one impurity which manifests at 5ppm which can be attributed to drying agent. Our final yield for our desired product came out to be 0.16g to give us a percent yield of 14.5%. It is important to note however, that this final yield includes the mass of the drying agent that contaminated our final product. The true percent yield is expected to be slightly lower than 14.5%

References:

1. Liotta, L. J. DESIGN AND CARRY OUT THE SYNTHESIS OF A DIPEPTIDE; rep.
2. IR Spectrum Table & Chart.
<https://www.sigmaaldrich.com/technical-documents/articles/biology/ir-spectrum-table.html> (accessed Dec 7, 2018).

3.Hornback, J. M. Organic chemistry;
Brooks/Cole: Belmont, CA, 2006.

experimental procedure. I would also like to thank my lab partners Vrushabh Daga and Fiona Jordan for all of the dedication and time they invested in order to make this experiment work

Acknowledgements

I would like to thank Stonehill College, Professor Kristin Bushell, the lab assistant Kim, and the TA Elizabeth for helping and supporting us through this

Supplemental:

diPeptide Sythesis Protected Alanine FJ, VD, AJ

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2029.089

1680.758

1308.747

1244.519

1122.756

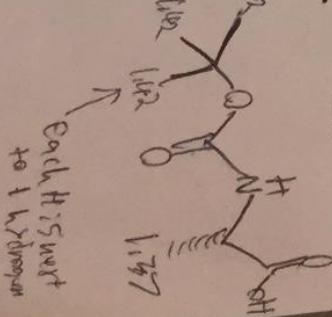
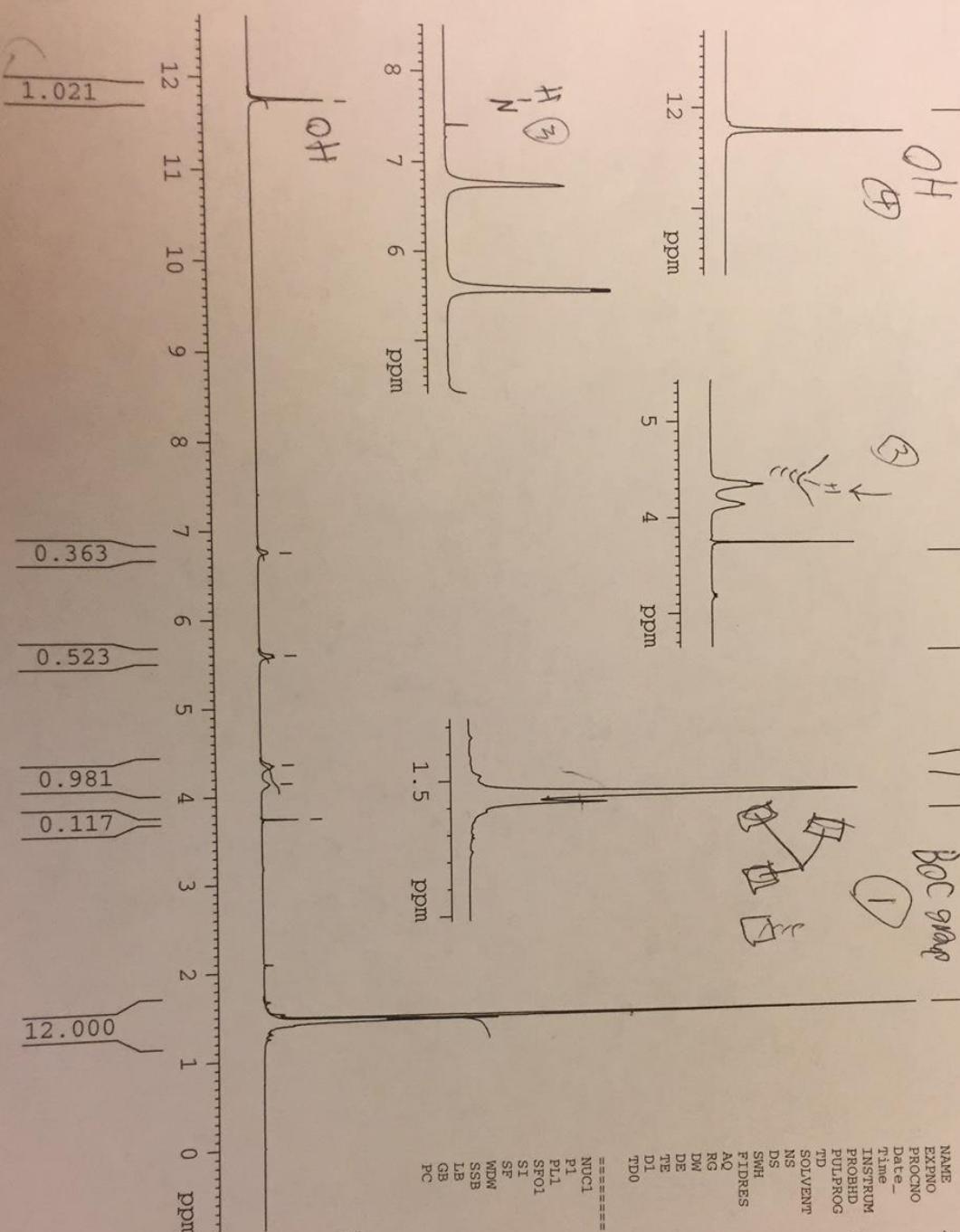
436.239

BRUKER

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TD	65536
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DW	64
DE	81.000 usec
TE	6.50 usec
D1	297.5 K
TDO	1.0000000 sec

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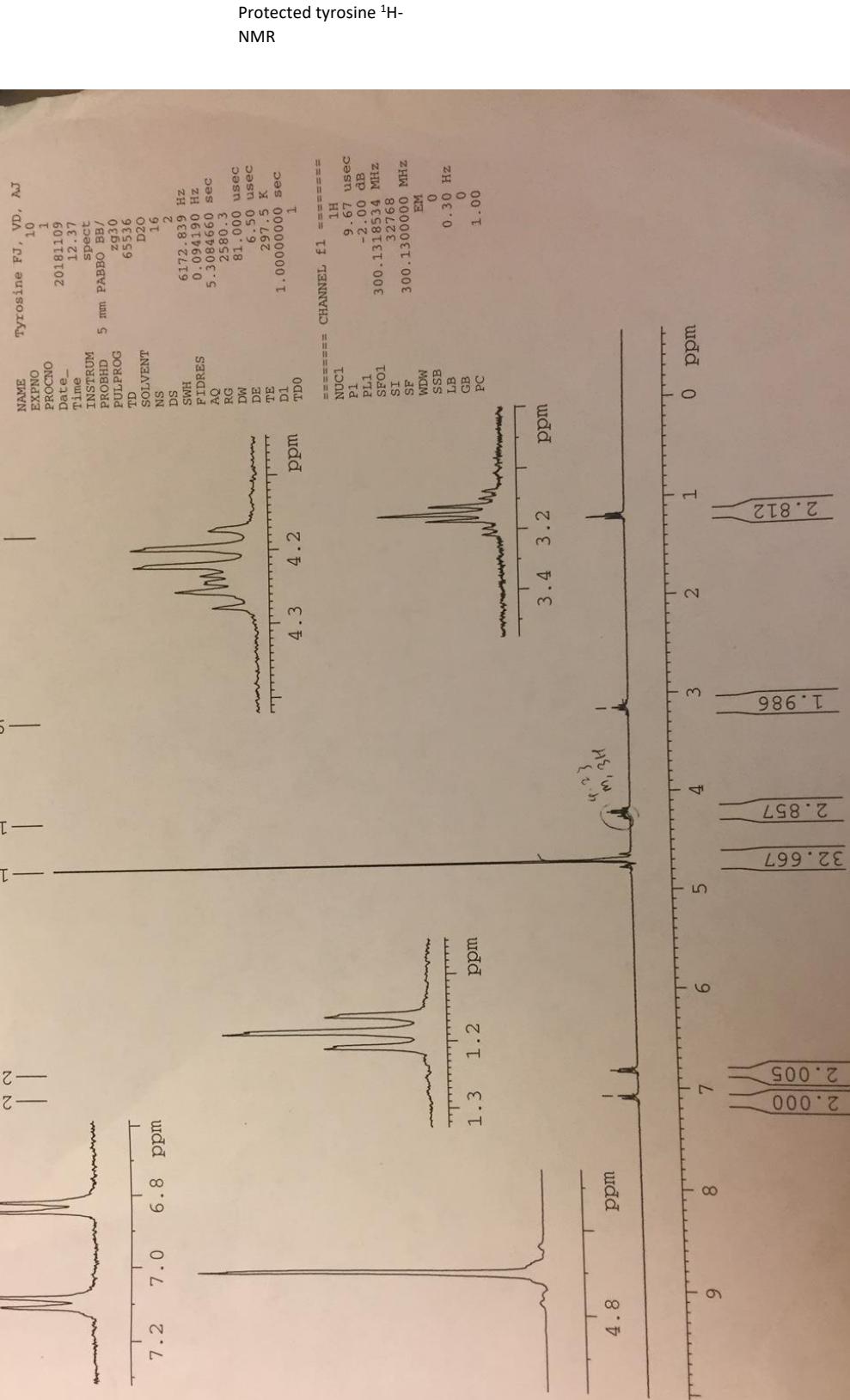


DiPeptide Synthesis Protected Tyrosine FJ, VD, AJ

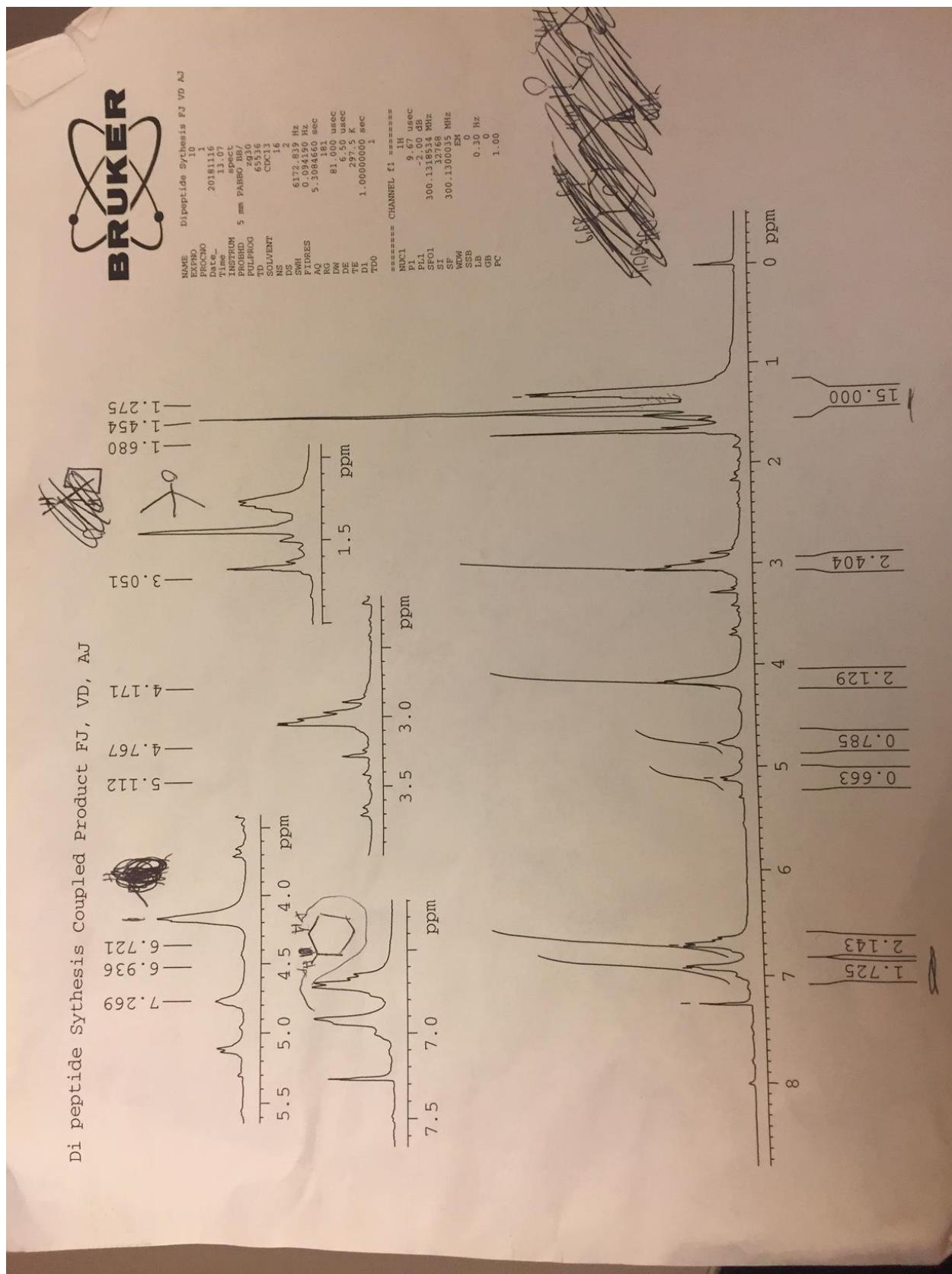


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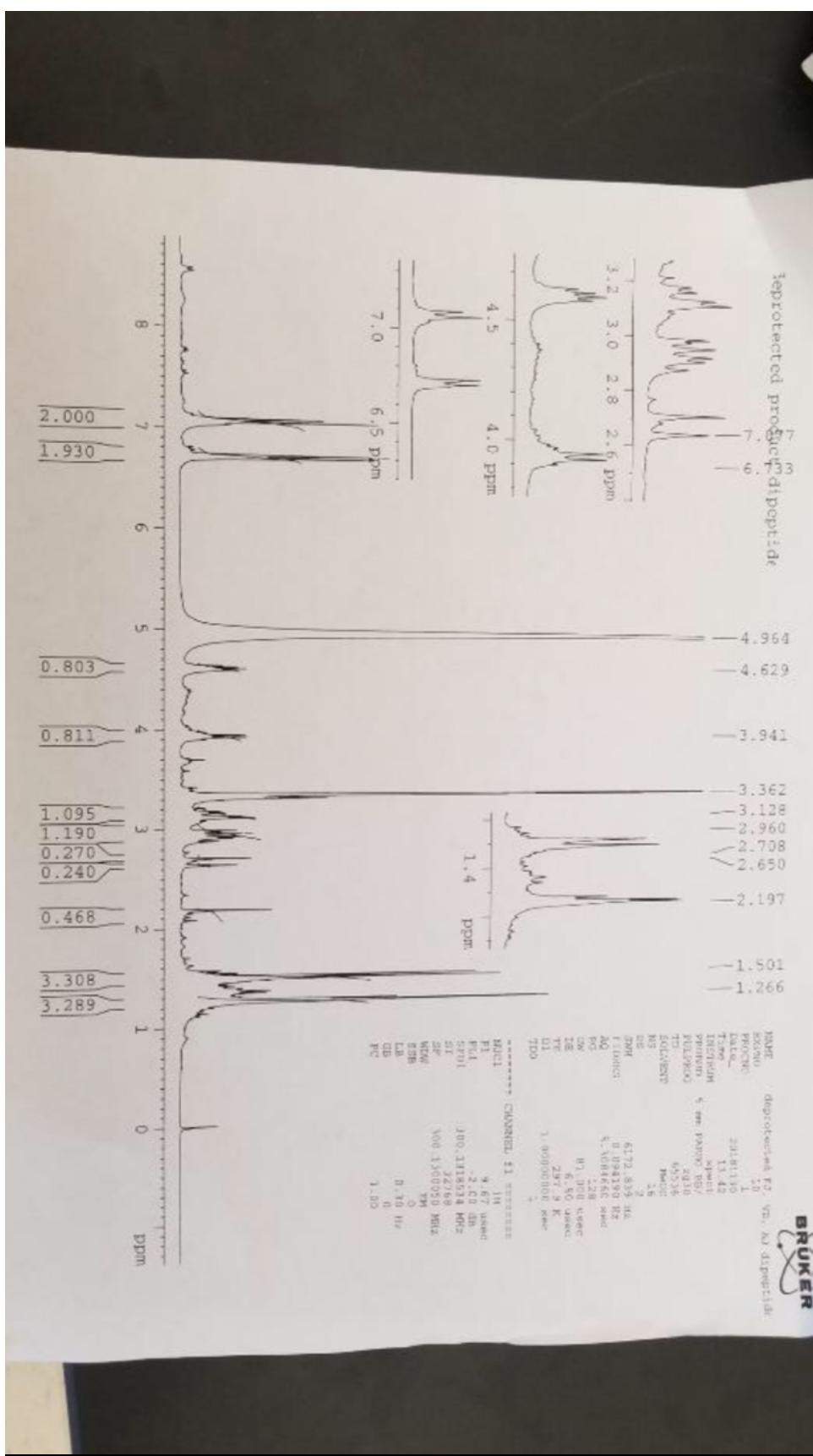
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Activation and coupling
¹H-NMR



Final dipeptide ^1H -NMR



Protected tyrosine IR

