Arnau Rodríguez Rubio

POST-SYNTHETIC MODIFICATIONS OF PHENYLALANINE CONTAINING PEPTIDES BY C-H OLEFINATION

TREBALL DE FI DE GRAU

dirigit pel Dr. Warren Cross

Grau de Química

UNIVERSITAT ROVIRA I VIRGILI

Nottingham

2016

Acknowledgements

I would like to thank Dr. Warren Cross for guiding me throughout all this project and his expert advice. The door of his office was always open whenever I ran into a trouble spot or had a question about my research or writing. Forming part of the Cross' group has been a priceless experience in both professional and personal meaning.

Secondly but not less important , I also want to thank Myles who helped me day-by-day on all my practical issues and made me improve so much as a chemist.

And how not to thank my family and friends who encouraged me to live this fulfilling experience, and also all the members of ERD 117, Ryan, Axel and Jonny who made me laugh with their constant jokes and made the work even better.

Finally, a massive gratitude must go to Ori, the one who always has been by my side, cheering me up during the toughest moments of the degree and without whose help this would have not been possible.

Table of contents

1 Abstract

The use of transition metals in catalysis has been studied during the last decades. However, it has been in the last few years when the most prevalent bond in organic chemistry, carbon-hydrogen, has become an object of study to researchers. This new method results in the formation of a highly desirable carbon-carbon or carbon-heteroatom bond.

The present dissertation has been about the synthesis of dipeptides and its olefination reactions through C-H activation methods. The previous work done for methyl acetylglycyl-*L*-phenylalaninate (Ac-Gly-Phe-OMe) served as a method for the trials with the newly proposed methyl acetyl-L-phenylalanylglycinate (Ac-Phe-Gly-OMe).

Different issues were found throughout all both synthetic and further olefination processes which are detailed and discussed later on as well as a proposal for the reaction mechanism and the transition states that are involved. Future work has been appointed in order to confirm such theory.

2 Introduction and background

For the better understanding of the following reading, the next section intends to contextualize the scope and the background of this dissertation. Some concepts are presented as well as the advantages over previous procedures.

2.1 Scope of work

The present work was held by the department of Chemistry and Forensic Science in Nottingham Trent University under the guidance of Professor Warren Cross. Its aim is about the functionalization of C-H bonds and the understanding of the reactions involved in the process.

Specifically, this work introduces the C-H functionalization as a novel route of modifying side chains of peptides containing phenylalanine residues. Although the application range of these molecules is not clear yet, as they are fluorescent under UV light they could serve as drug delivery systems.

Beyond an application for the products obtained, is worthy to think about the contribution to C-H activation knowledge and how this reactivity could be exploited. It appears as a new and promising strategy that could become the state of the art in synthetic chemistry.

2.2 Peptide synthesis and modification

A peptide is, basically, the molecule formed by the union of two or more amino acids by a peptide (amide) bond. These molecules are of great importance in biology and biochemistry as they play an important role in living organisms such as DNA replication, catalysing metabolic reactions, etc.

Peptides are chains of amino acids, small molecules containing amine and carboxylic acid functionalities, as well as a side-chain that differs from each amino acid. Structure of a general amino acid is shown in figure 1, note the chiral centre at the α carbon.

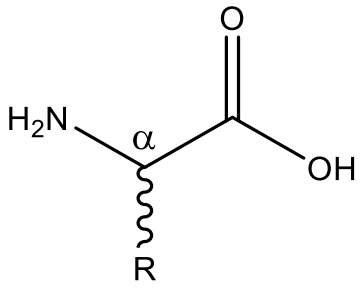
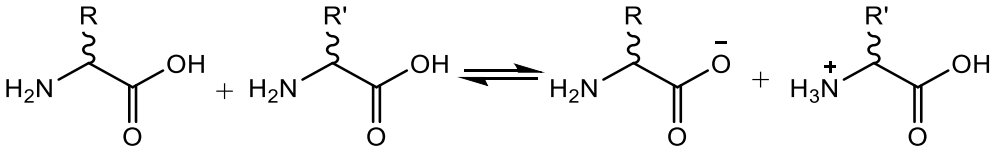


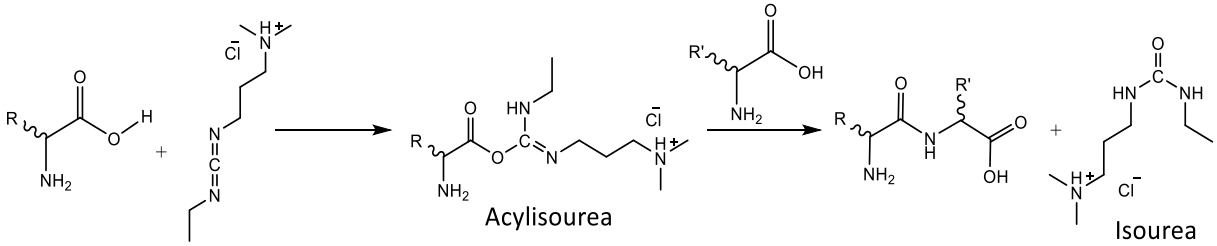
Figure 1. General structure for an amino acid.

Given that an amino acid has both basic and acid functionalities, the first problem arisen is to make them react through an addition-elimination at the carbonyl (also called nucleophilic substitution at the carbonyl) instead of an acid-base reaction as shown in scheme 1.



Scheme 1. Acid-base reaction between two amino acids.

A carboxylic acid is the less reactive functionality, or less electrophilic, for a nucleophilic substitution at the carbonyl, so a crosslinking agent is needed to catalyse the formation of the amide bond. This chemical, basically provides the molecule a better leaving group such as an acylisourea, so it reacts faster through the substitution. This method is called liquid phase or solution synthesis, mechanism is shown in scheme 2. However, this is a classic method and is only used for making dipeptides, for larger chains it is used what is called solid phase synthesis[1].



Scheme 2. Amide bond formation reaction mechanism using EDC as crosslinking agent.

Secondly, but not least important, a regioselectivity problem has to be spotted if is intended to react two different amino acids. Both terminus of each amino acid are likely to react with the equal one or the other amino acid, giving a mixture of 4 different products. Therefore,

the use of protected amino acids is a common routine in peptide synthesis to ensure the desired sequence.

Many natural products or drugs used as antibiotics or with other medicinal purposes have a peptide backbone e.g. Enkephalyn (figure2).

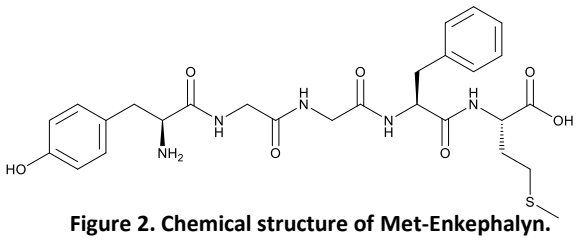
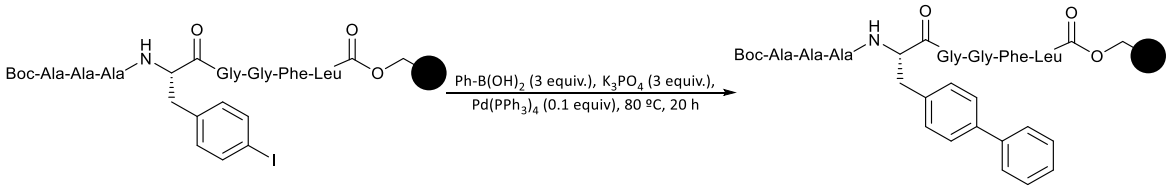


Figure 2. Chemical structure of Met-Enkephalyn.

Synthesis of these invaluable chemicals has been afforded previously modifying side chains of peptides using cross coupling reactions[2]. Doan et al. synthesized successfully Enkephalyn analogues functionalising tyrosine or phenylalanine residues using a Suzuki-Miyaura coupling[3], scheme 3.



Scheme 3. Suzuki-Miyaura for peptide functionalization.

However, these strategies require the use of unnatural amino acids that already have been halogenated or pre-functionalised. Novel reactions, as C-H activation, could stablish an alternative and more efficient synthetic route in terms of atom economy or environmental factor.

Although there is still a long way to go before applying such methods for these challenging compounds, due to the amount of C-H bonds present in them, C-H activation represents the state of the art in organic synthesis.

2.3 C-H activation and functionalization

To increase the complexity of organic structures in order to synthesize natural products or target molecules is an unending issue for chemists, as well as finding novel routes and methods to such purpose. The use of transition metal catalysed reactions has been proven as a promising approach to achieve these goals[4]. However, currently used methods require the pre-functionalization of the target site via halogenation, metalation, etc.

As an alternative, C-H activation avoids the need of previous functionalization and directly enables the formation of C-C or C-heteroatom bonds from a C-H bond, arguably the most unreactive bond in organic chemistry. Differently to cross coupling reactions, this novel route reduces the number of synthetic steps and the environmental impact minimizing the generation of by-products and waste.

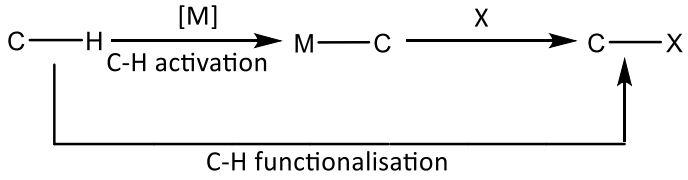
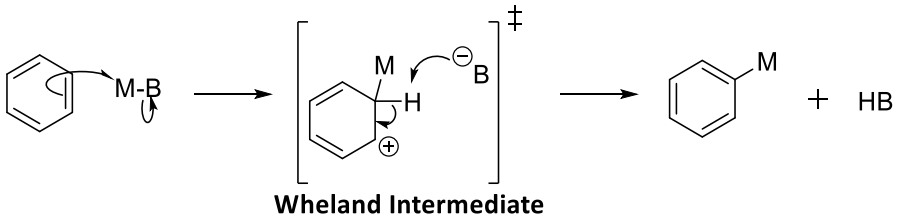


Figure 3. General scheme for C-H activation/functionalization.

2.3.1 Mechanisms for C-H activation

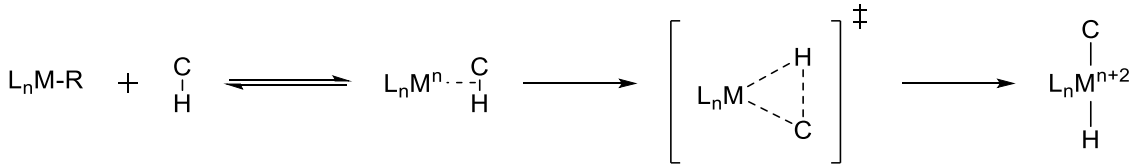
Four mechanisms have been proposed for C-H activation reactions: oxidative addition, σ-bond metathesis, electrophilic activation and Ambiphilic Metal Ligand Activation (AMLA) also known as Concerted Metalation-Deprotonation (CMD)[5–7].

As it is suggested by its name, in an electrophilic activation a metal atom is attacked by an electron rich carbon atom. Metalation occurs followed by the cleavage of a proton, scheme 4. Group 10 metals will follow this mechanism.



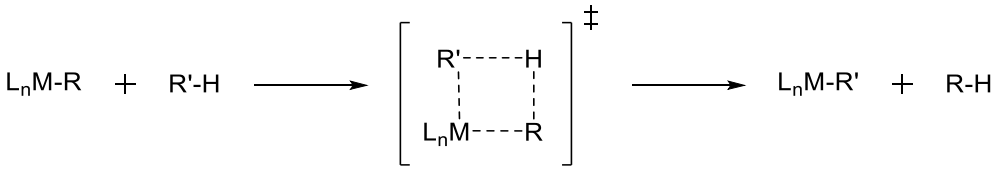
Scheme 4. Mechanism for electrophilic activation in C-H activation.

The oxidative addition mechanism occurs via an agostic interaction between the metal species and the target C-H bond. The reaction is concerted, cleaving the C-H bond at the same time C-M and M-H bonds are formed via a three membered ring as shown in scheme 5. This mechanism is typical from nucleophilic, or rich electron metals such as Ru(0) or Ir(I), both having d^8 electronic configuration. Note that the metals oxidation state is increased by two.



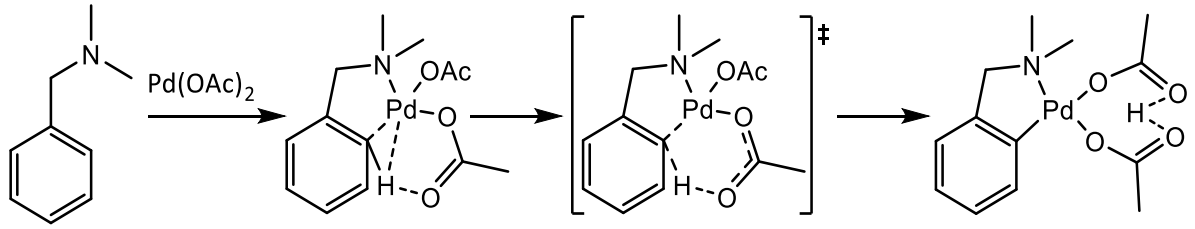
Scheme 5. Mechanism for oxidative addition in C-H activation.

On the other hand, σ-bond metathesis is held by electron deficient metals such as Zr(IV) or Rh(III) and exchanges one M-C bond per a new one, scheme 6. The mechanism is also concerted, forming two σ bonds whilst breaking others via a four membered ring transition state, so the metals oxidation state remains unchanged.



Scheme 6. Mechanism for σ-bond metathesis in C-H activation.

Recently proposed by Davies and Macgregor, scheme 7, 'Ambiphilic Metal Ligand Activation (AMLA)' describes a concerted process in which both the metal and the basic ligand synergistically cleave the C-H bond whilst the metalation takes place[8].



Scheme 7. Mechanism for AMLA.

2.3.2 Regioselectivity and directing groups

However, as the most abundant and inert bond in organic frameworks, affording the coordination of the metal at the desired site to cleave a C-H bond, becomes a challenging issue to overcome. The use of so called directing groups has given promising results to solve these problems, creating a more desired environment for the metal coordination and so for the target position.

Without the presence of a directing group, the regioselectivity of a C-H activation reaction between two different and non-equivalent C-H bonds would be difficult to control. However,

is assumable that the reaction will occur at the most reactive bond, in other words, the one that leads to the lowest energy transition state. According to Gorelsky et al., such bonds are the most acidic ones, leading to higher selectivity[9].

Although exploiting the different acidity on C-H bonds, or other properties such as the bulk of the ligands[10] or the use of a different catalyst[11], could be a strategy to obtain regiochemistry control. Nevertheless, the scope is limited by its specificity.

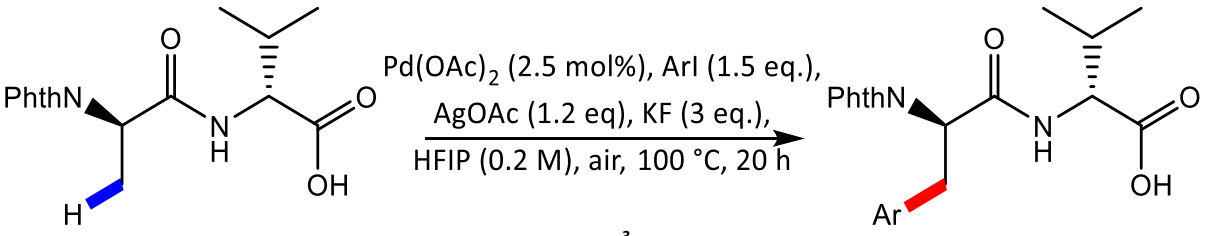
Directing groups have been proven to be a promising alternative strategy to control regioselectivity in C-H activation and further functionalization, although there is still a long way to go, so they are currently object of research.

Ideally, a directing group should be a moiety to be easily installed to then support metal coordination and finally be cleaved straightforwardly. To date, a vast amount of directing groups have been explored e.g. amides, imine, amine, carboxylic acids, hydroxyl groups, etc.

The directing group is, in fact a chelating group which binds covalently to the metal, controlling its position in space, thus ensuring the interaction with the targeted C-H bond.

They do have limitations, such as the installation and cleavage reactions, which add synthetic steps (main advantage for C-H functionalization above cross coupling). However, it could be the case of a molecule of study which already possesses a chelating group, i.e. peptides.

Previous work has been published about C(sp^3)-H activation on side chains of peptides[12] directed by the amide functionality. However the aim of the work by Yu et al. was the selective arylation of the side chain of alanine residues in different peptides.



Scheme 8. An example of a C(sp^3)- H arylation in a peptide.

The present work uses the amide functionality, distinctive from peptides, as a directing group in order to C(sp^2)-H activate and set up a novel route for modification of peptides as an alternative for cross coupling reactions.

3 Aims

The aims of the following work were, as presented below:

- To trial the olefination reaction for the peptide Ac-Gly-Phe-OMe in order to learn about C-H activation.
- To synthesize suitable peptides of the type PG-Phe-AA-OMe to investigate the olefination reaction.
- To study the olefination reaction for the peptides mentioned, changing some parameters such as protecting groups on the N-terminus or the solvent.
- To characterize both peptides and products of olefination by ^{1}H and ^{13}C MNR spectroscopy.
- To learn about which effects control the reaction in order to be able to optimise it and set up a method.

4 Experimental Section

4.1 Materials

N-Acetyl-L-phenyalanine (Ac-Phe-OH), N-Acetyl-glycine (Ac-Gly-OH), Glycine methyl ester hydrochloride (H-Gly-OMe), Styrene, Glycine (H-Gly-OH), L-Phenylalanine (H-Phe-OH), L-Alanine (H-Ala-OH), L-Tyrosine (H-Tyr-OH), Thionyl chloride, Benzyl chloroformate (Cbz-Cl), N,N'-Dicyclohexylcarbodiimide (DCC), Filter agent Celite® 545, Methanol (MeOH), N,N-Dimethylformamide (DMF) and 1,2-Dichloroethane (DCE) were purchased from Sigma-Aldrich®.

N-(9-Fluorenylmethoxycarbonyl)-L-phenylalanine (Fmoc-Phe-OH), L-Leucine (H-Leu-OH), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), Potassium carbonate, Silver acetate, Palladium acetate, Triethylamine (NEt_3) and *tert*-Amyl alcohol (TAA) were purchased from Alfa-Aesar®.

L-Phenylalanine methyl ester hydrochloride (H-Phe-OMe), Acetic anhydride and Amberlyst® A-21, ion-exchange resin were purchased from Acros®.

Silicagel 60Å (40-63 micron) was purchased from Fluorochem®.

All other reagents and solvents were purchased from Fisher Scientific® unless specified.

4.2 Characterization

^{1}H NMR and ^{13}C NMR spectroscopy were recorded on a Jeol ECX-400 MHz instrument in $CDCl_3$ unless otherwise noted. Residual $CDCl_3$ was used as the internal standard for both ^{1}H NMR (δ=7.24 ppm) and ^{13}C NMR (δ=77.0 ppm). Spectra were processed using ACD Labs Spectrus Processor 2014.

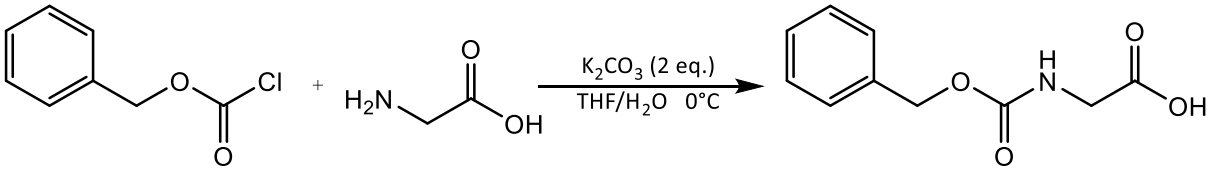
Analytical thin-layer chromatography (TLC) was performed on silica gel 60 plates with F_{254} indicator from Merck, visualized under UV-light either 254 nm or 365nm.

4.3 General procedures

When available, protected amino acids were purchased to those mentioned suppliers. Otherwise were synthesized prior to use.

4.3.1 Amino acid protection; N-terminus: Method A[13]

4.3.1.1 Product 24: Cbz-Gly-OH



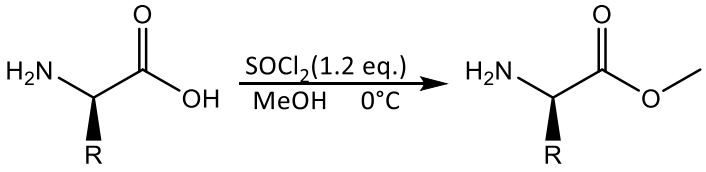
Scheme 9. Reaction for N-protection of H-Gly-OH, P24.

In a round bottom flask, H-Gly-OH (1.00 g, 13.32 mmol) and K_2CO_3 (3.68 g, 26.64 mmol) were dissolved in a mixture of THF/water (2.5:1, 17.5 mL). The solution was cooled to 0 ºC, then Cbz-Cl (2.09 mL, 14.65 mmol) was added dropwise. After 10 minutes stirring at low temperature, the mixture was allowed to warm to room temperature for half an hour.

The solution was filtered prior to reduce the solvent under vacuum to obtain an orange powder, which was dissolved in methanol in order to discard whatever was insoluble. Finally, the solution was reduced under vacuum to obtain a white solid (2.14 g, 77% yield).

^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 3.65 (s, 2H, C*H*$_2$[Gly]) 5.06 (s, 2H, C*H*$_2$[Cbz]) 7.30-7.36 (m, 5H, *aromatics*).

4.3.2 Amino acid protection; C-Terminus: Method B[14]



Scheme 10. Reaction for C-protection of a general amino acid.

The amino acid was dissolved in methanol in a round bottom flask prior to cool the solution to 0 ºC, then added thionyl chloride dropwise and left it stirring for half an hour in the cold bath. Subsequently, the reaction was heated to 80 ºC under reflux for 3 hours. Finally, the solvent was reduced from the crude mixture to obtain the crude product with no need of further purification.

4.3.2.1 Product 5: Tyr-OMe

Method B was used to synthesize H-Tyr-OMe. Reagents: H-Tyr-OH (1.00 g, 5.52 mmol), thionyl chloride (0.48 mL, 6.62 mmol) and 30 mL of methanol. A pale orange solid was obtained (1.26 g, 99% yield).

^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 3.00 (dd, 2H, ^{3}J=6.0 Hz, ^{2}J=14.5 Hz, CH_2) 3.79 (s, 3H, CO_2CH_3) 4.23 (t, 1H, ^{3}J=7.3 Hz, C*H*) 6.75-6.77 (t, 2H, ^{3}J=2.2 Hz, C2-*H*) 7.04-7.06 (t, 2H, ^{3}J=2.9 Hz, C1-*H*).

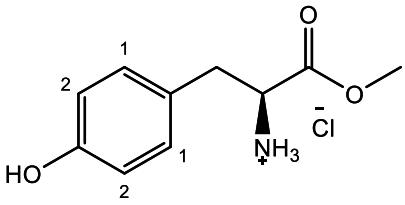


Figure 4. P5, H-Tyr-OMe.

4.3.2.2 Product 25: H-Phe-OMe

Method B was used to synthesize H-Phe-OMe. Reagents: H-Phe-OH (1.00 g, 6.05 mmol), thionyl chloride (0.53 mL, 7.26 mmol) and 30 mL of methanol. A white solid was obtained (1.30 g, 100% yield).

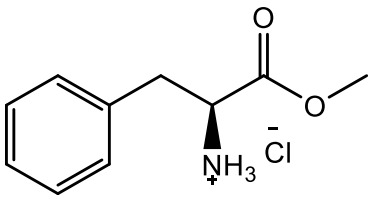


Figure 5. P25, H-Phe-OMe.

^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 3.20 (dd, 2H, ^{3}J=7.3 Hz, ^{2}J=14.3 Hz, CH_2) 3.79 (s, 3H, CO_2CH_3) 4.31 (t, 1H, ^{3}J=7.1 Hz, C*H*) 7.24-7.36 (m, 5H, *aromatics*).

4.3.2.3 Product 32:H- Leu-OMe

Method B was used to synthesize H-Leu-OMe. Reagents: H-Leu-OH (1.00 g, 7.62 mmol), thionyl chloride (0.67 mL, 9.15 mmol) and 30 mL of methanol. A white and wet solid was obtained.(1.48 g, 107% yield). The excess of yield is attributed to the presence of sulfuric acid. However, ^{1}H NMR spectrum showed good purity.

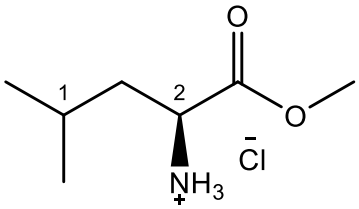
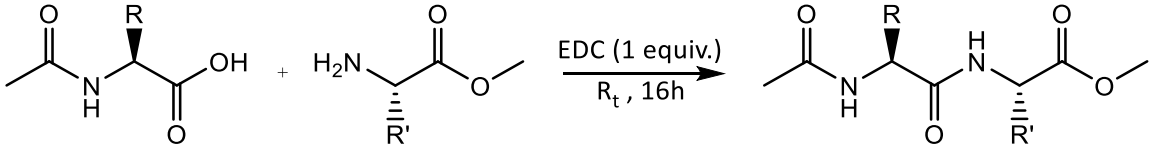


Figure 6. P32, H- Leu-OMe.

^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 0.98 (d, 6H, ^{3}J=6.1 Hz, CH(CH_3)$_2$) 1.67 (m, 1H, C1-*H*) 1.77 (m, 2H, CH_2) 3.82 (s, 3H, CO_2CH_3) 4.03 (t, 1H, ^{3}J=6.5 Hz, C2-*H*).

4.3.3 Peptide synthesis: Method C[15]



Scheme 11. Reaction for peptide coupling between two general amino acids.

The C-protected amino acid hydrochloride salt and potassium carbonate were dissolved in distilled water each in two different conical flasks. Mixed them and let the mixture react for 10 minutes stirring continuously. The crude mixture was extracted with diethyl ether three times and finally reduced the organic solvent under vacuum.

The N-protected amino acid and EDC were dissolved in dichloromethane (DCM) in a round bottom flask. Finally, added the free amine amino acid obtained in the first step and left it stirring overnight at room temperature.

Any precipitate was filtered prior to wash the crude mixture: once with hydrochloric acid 1M, three times with saturated $NaHCO_3$ and finally once with distilled water. Reduced the solvent under vacuum.

4.3.3.1 Product 1: Ac-Gly-Phe-OMe

Method C was used to synthesize Ac-Gly-Phe-OMe. Reagents: H-Phe-OMe·HCl (1.00 g, 4.60 mmol), Ac-Gly-OH (0.45 g, 3.80 mmol), EDC (0.73 g, 3.80 mmol), K_2CO_3 (0.95 g, 6.90 mmol) and 50 mL of DCM. A yellow powder was obtained (0.42 g, 39% yield).

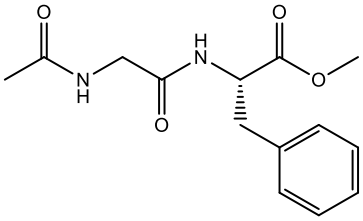


Figure 7. P1, Ac-Gly-Phe-OMe.

^{1}H NMR (400 MHz, CD_3Cl) δ (ppm) 2.02 (s, 3H, COC*H*$_3$) 3.12 (dd, 2H, ^3J=6.1 Hz, ^2J=13.8 Hz, C*H*$_2$[Phe]) 3.74 (s, 3H, CO_2CH_3) 3.90 (d, 2H, ^2J=5.2 Hz, C*H*$_2$[Gly]) 4.86 (app q, 1H, ^3J=7.9 Hz, C*H*) 6.19 (br s, 1H, N*H*) 6.39 (br s, 1H, N*H*) 7.10-7.30 (m, 5H, *aromatics*).

^{13}C NMR (400 MHz, CD_3Cl) δ (ppm) 23.0, 37.9, 43.2, 52.6, 53.3, 127.3, 128.7, 129.3, 135.7, 168.6, 170.7, 171.8.

4.3.3.2 Product 29: Cbz-Gly-Phe-OMe

Method C was used to synthesize Cbz-Gly-Phe-OMe. Reagents: H-Phe-OMe·HCl (0.62 g, 2.87 mmol), P24 (0.5 g, 2.39 mmol), EDC (0.46 g, 2.39 mmol), K_2CO_3 (0.63 g, 4.54 mmol) and 40 mL of DCM. A yellow oil was obtained (0.46 g, 52% yield).

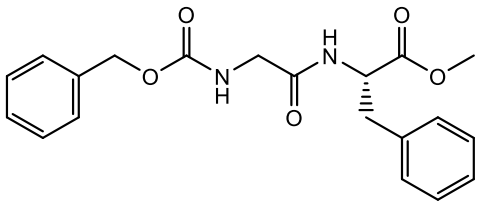


Figure 8. P29, Cbz-Gly-Phe-OMe.

^{1}H NMR (400 MHz, CD_3Cl) δ (ppm) 3.03 (dd, 2H, ^3J=6.1 Hz, ^2J=13.8 Hz, C*H*$_2$[Phe]) 3.59 (s, 3H, CO_2CH_3) 3.79 (br t, 2H, C*H*$_2$[Gly]) 4.84 (app q, 1H, ^3J=6.6 Hz, C*H*) 5.05 (s, 2H, C*H*$_2$[Cbz]) 6.16 (br s, 1H, N*H*) 7.08-7.29 (m, 10H, *aromatics*).

^{13}C NMR (400 MHz, CD_3Cl) δ (ppm) 8.8, 28.6, 36.4, 37.8, 44.3, 52.4, 53.4, 67.0, 71.1, 127.1, 128.1, 128.6, 129.3, 136.0, 136.4, 156.9, 169.5, 172.1.

4.3.3.3 Product 7: Ac-Phe-Gly-OMe

Method C was used to synthesize Ac-Phe-Gly-OMe. Reagents: H-Gly-OMe·HCl (1.00 g, 8.16 mmol), Ac-Phe-OH (1.41 g, 6.80 mmol), EDC (1.30 g, 6.80 mmol), K_2CO_3 (1.69 g, 12.24 mmol) and 50 mL of DCM.

A pale yellow oil was obtained, ^{1}H NMR spectrum showed it was EDC and Ac-Phe-OH. The procedure for freeing the amine of H-Gly-OMe failed because it is extremely soluble in water so the extraction did not work.

4.3.4 Peptide synthesis: Method D[15,16]

The C-protected amino acid hydrochloride salt was dissolved in chloroform and added triethylamine dropwise. The reaction was allowed to stir for 3 hours at room temperature, then increased the temperature to 80 ºC for 1 hour. The solution was filtered and washed with cold diethyl ether several times.

The solid obtained previously was dissolved in DCM prior to add the coupling agent and finally the N-protected amino acid. The reaction mixture was left stirring overnight at room temperature.

The crude mixture was worked-up as in method C.

4.3.4.1 Product 16: Ac-Phe-Gly-OMe, DCC coupling

Method D was used to synthesize Ac-Phe-Gly-OMe. Reagents: H-Gly-OMe·HCl (0.84 g, 6.72 mmol), triethylamine (0.94 mL, 6.72 mmol), Ac-Phe-OH (1.16 g, 5.60 mmol), DCC (1.16 g, 5.60 mmol) and 60 mL of DCM.

A yellow oil was obtained, ^{1}H NMR spectrum showed a mixture with starting material and N,N'-dicyclohexylurea (DCU). Impossible to achieve further purification.

4.3.4.2 Product 17: Fmoc-Phe-Gly-OMe, DCC coupling

Method D was used to synthesize Fmoc-Phe-Gly-OMe. Reagents: H-Gly-OMe·HCl (0.19 g, 1.55 mmol), triethylamine (0.22 mL, 1.55 mmol), Fmoc-Phe-OH (0.50 g, 1.29 mmol), DCC (0.27 g, 1.29 mmol) and 40 mL of DCM.

A colourless oil was obtained, ^{1}H NMR spectrum showed a mixture with unreacted DCC and DCU. Impossible to achieve further purification.

4.3.4.3 Product 11: Ac-Phe-Gly-OMe, EDC coupling

Method D was modified to synthesize Ac-Phe-Gly-OMe, ran the reaction using EDC as coupling agent. Reagents: H-Gly-OMe·HCl (0.84 g, 6.72 mmol), triethylamine (0.94 mL, 6.72 mmol), Ac-Phe-OH (1.16 g, 5.60 mmol), EDC (1.07 g, 5.60 mmol) and 60 mL of DCM. A white solid was obtained (0.28 g, 18% yield).

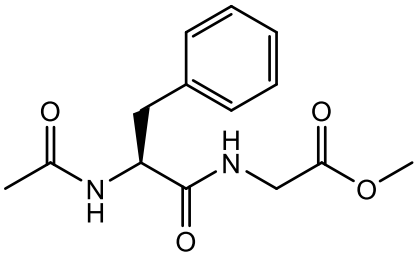


Figure 9. P11, Ac-Gly-Phe-OMe.

^{1}H NMR (400 MHz, CD_3Cl) δ (ppm) 1.96 (s, 3H, COC*H*$_3$) 3.06 (d, 2H, ^{2}J=5.5 Hz, C*H*$_2$[Gly]) 3.71 (s, 3H, CO_2C*H*$_3$) 3.96 (dd, 2H, ^{3}J=5.6 Hz, ^{2}J=18.8 Hz, C*H*$_2$[Phe]) 4.73 (app q, 1H, ^{3}J=7.2 Hz, C*H*) 6.34 (br s, 1H, N*H*) 6.62 (br s, 1H, N*H*) 7.13-7.30 (m, 5H, *aromatics*).

^{13}C NMR (400 MHz, CD_3Cl) δ (ppm) 23.2, 38.2, 41.2, 52.5, 54.3, 127.1, 128.7, 129.3, 136.6, 169.9, 170.4, 171.5.

4.3.4.4 Product 19: Fmoc-Phe-Gly-OMe, EDC coupling

Method D was modified to synthesize Fmoc-Phe-Gly-OMe, ran the reaction using EDC as coupling agent. Reagents: H-Gly-OMe·HCl (0.19 g, 1.55 mmol), triethylamine (0.22 mL, 1.55 mmol), Fmoc-Phe-OH (0.50 g, 1.29 mmol), EDC (0.25 g, 1.29 mmol) and 40 mL of DCM. A yellow-greensolid was obtained (0.44 g, 74% yield).

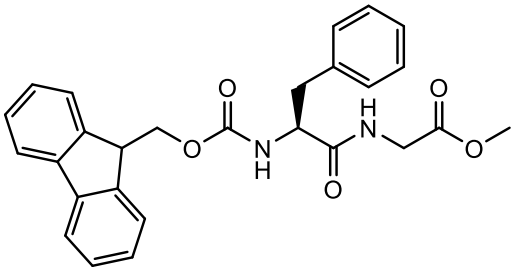


Figure 10. P19, Fmoc-Phe-Gly-OMe.

Although poor resolution probably due to impurities, the peptide backbone could slightly be observed. Not able to affirm the synthesis had worked.

4.3.5 Peptide synthesis: Method E[15,17]

The C-protected amino acid hydrochloride salt was dissolved in water and added Amberlyst® A-21, ion-exchange resin. The reaction mixture was allowed to stir for 15 minutes prior to filter the resin and reduce water under vacuum.

Proceeded as in method D with the solid or oil obtained.

4.3.5.1 Product 28: Ac-Phe-Gly-OMe

Method E was used to synthesize Ac-Phe-Gly-OMe. Reagents: H-Gly-OMe·HCl (0.50 g, 3.98 mmol), A-21 resin (5.00 g), Ac-Phe-OH (0.69 g, 3.32 mmol), EDC (0.64 g, 3.32 mmol) and 40 mL of DCM. A pale orange solid was obtained (0.15 g, 16% yield).

A white solid was found stuck to the walls of the flask, low yields were called to be a C-protected amino acid solubility problem. The solvent was changed to methanol for the following reactions.

^{1}H NMR (400 MHz, CD_3Cl) δ (ppm) 1.97 (s, 3H, COCH_3) 3.08 (d, 2H, ^{2}J=6.6 Hz, CH_2[Gly]) 3.72 (s, 3H, CO_2CH_3) 3.98 (dd, 2H, ^{3}J=5.5 Hz, ^{2}J=18.2 Hz, CH_2[Phe]) 4.71 (app q, 1H, ^{3}J=7.3 Hz, C*H*) 6.12 (br s, 1H, N*H*) 6.35 (br s, 1H, N*H*) 7.20-7.32 (m, 5H, *aromatics*).

4.3.5.2 Product 33: Ac-Phe-Leu-OMe

Method E was used to synthesize Ac-Phe-Leu-OMe. Reagents: P32 (0.50 g, 2.75 mmol), A-21 resin (5.00 g), Ac-Phe-OH (0.48 g, 2.29 mmol), EDC (0.44 g, 2.29 mmol) and 40 mL of MeOH. A colourless oil was obtained (0.17 g, 30% yield). ^{1}H NMR spectrum showed a mixture with starting material, however, the peaks of the peptide backbone could be spotted.

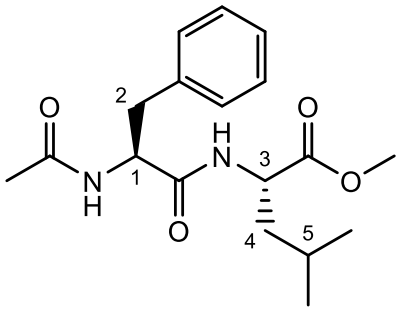
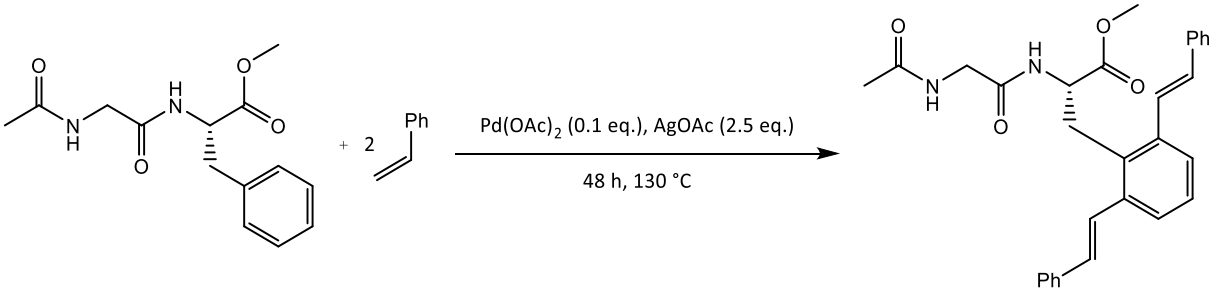


Figure 11. P33, Ac-Phe-Leu-OMe.

^{1}H NMR (400 MHz, CD_3Cl) δ (ppm) 0.83 (d, 6H, ^{3}J=5.5 Hz, CH(CH_3)$_2$) 1.46 (m, 1H, C5-*H*) 1.54 (m, 2H, C4-H_2) 1.86 (s, 3H, COCH_3) 2.98 (m, 2H, C2-H_2) 3.62 (s, 3H, CO_2CH_3) 4.48 (app q, 1H, ^{3}J=8.7 Hz , C1-*H*) 4.82 (t, 2H (due to overlapping, should be 1H), ^{3}J=6.9 Hz, C3-*H*) 6.67 (br s, 1H, N*H*) 7.05-7.40 (m, 18H (due to overlapping), *aromatics*).

^{13}C NMR (400 MHz, CD_3Cl) δ (ppm) 21.9, 22.8, 22.9, 24.8, 37.9, 38.5, 41.0, 51.0, 52.2, 52.3, 53.4, 54.3, 126.8, 127.1, 128.4, 128.6, 129.3, 129.5, 136.2, 136.9, 170.2, 170.4, 171.8, 172.6, 172.9.

4.3.6 Peptide olefination: Method F[18]



Scheme 12. Reaction scheme for C-H olefination of phenylalanine in a peptide chain.

To a dry and under N_2 atmosphere Schlenk tube palladium acetate, silver acetate and the dipeptide to functionalise were added. Then added styrene and finally the solvent. The reaction was allowed to stir during 48 hours in a paraffin bath at 130ºC.

The reaction was allowed to cool down to room temperature and then filtered through a plug of Celite®, before reducing the solvent under vacuum. The resulting residue was purified by column chromatography (Ethyl acetate, unless otherwise specified).

4.3.6.1 Product 4: Olefination of P1

Method F was used to modify the side chain of the phenylalanine residue of Ac-Gly-Phe-OMe. Reagents: P1 (133 mg, 0.48 mmol), $Pd(OAc)_2$ (11 mg, 0.05 mmol), AgOAc (199 mg, 1.19 mmol), Styrene (0.22 mL, 1.90 mmol), DMF (0.20 mL) and DCE (3.00 mL) as solvents. A brownish solid was obtained (18 mg, 8% yield).

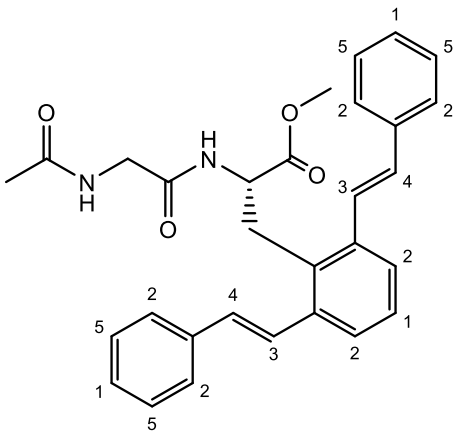


Figure 12. P4, diolefinated P1.

^{1}H NMR (400 MHz, CD_3Cl) δ (ppm) 1.83 (s, 3H, $COCH_3$) 3.49 (d, 2H, ^2J=7.0 Hz, CH_2[Gly]) 3.54 (s, 3H, CO_2CH_3) 3.73 (dd, 2H,^3J=5.2 Hz, ^2J=17.0 Hz, CH_2[Phe]) 4.78 (app q, 1H, ^3J=7.1 Hz, C*H*) 5.90 (br s, 1H, N*H*) 6.50 (br d, 1H, N*H*) 7.00 (d, 2H, ^3J=16.0 Hz, C3-*H*) 7.30 (app q, 3H, ^3J=7.7 Hz, C1-*H*) 7.38 (t, 4H, ^3J=7.3 Hz, C5-*H*) 7.46 (d, 2H, ^3J=16.0 Hz , C4-*H*) 7.57 (d, 6H, ^3J=7.8 Hz, C2-*H*).

^{13}C NMR (400 MHz, CD_3Cl) δ (ppm) 22.9, 31.1, 42.9, 52.8, 53.2, 125.9, 126.3, 126.8, 127.8, 128.1, 128.9, 131.7, 132.0, 137.3, 137.9, 168.4, 170.3, 171.8.

4.3.6.2 Product 8: repeating olefination of P1

Method F was used to modify the side chain of the phenylalanine residue of Ac-Gly-Phe-OMe. Reagents: P1 (100 mg, 0.36 mmol), $Pd(OAc)_2$ (10 mg, 0.05 mmol), AgOAc (150 mg, 0.90 mmol), Styrene (0.17 mL, 1.44 mmol), DMF (0.20 mL) and DCE (3.00 mL) as solvents.

It was intended to increase the yield by performing a longer column in order to avoid co-elution. However, nothing was observed on the TLC plate after having collected several fractions. Decided to elute the whole column changing the solvent to 100% MeOH. After reducing the solvent nothing was obtained, the product was lost.

4.3.6.3 Product 12: olefination of P11

Method F was used to modify the side chain of the phenylalanine residue of Ac-Phe-Gly-OMe. Reagents: P11 (100 mg, 0.36 mmol), $Pd(OAc)_2$ (8 mg, 0.04 mmol), AgOAc (150 mg, 0.90 mmol), Styrene (0.17 mL, 1.44 mmol), DMF (0.20 mL) and DCE (3.00 mL) as solvents. A brownish sticky solid was obtained (6 mg, 3% yield).

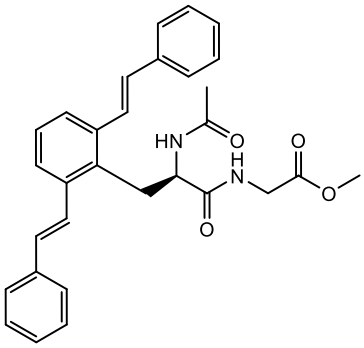


Figure 13. P12, diolefinated P11.

A doublet could be observed at δ=6.99 ppm J=15.4 Hz in the ^{1}H NMR spectrum. However, it was not pure and resolved enough to be able to affirm the reaction had worked.

4.3.6.4 Product 13: olefination of P11 under air atmosphere

Method F was used to modify the side chain of the phenylalanine residue of Ac-Phe-Gly-OMe. Reagents: P11 (100 mg, 0.36 mmol), $Pd(OAc)_2$ (10 mg, 0.05 mmol), AgOAc (150 mg, 0.90 mmol), Styrene (0.17 mL, 1.44 mmol), DMF (0.20 mL) and DCE (3.00 mL) as solvents. This time, no N_2 atmosphere was applied to the schlenk tube. A dark sticky solid was obtained (11 mg, 6% yield).

A doublet could be observed at δ=6.99 ppm J=15.4 Hz in the ^{1}H NMR spectrum. However, it was not pure and resolved enough to be able to affirm the reaction had worked.

4.3.6.5 Product 20: olefination of P11

Method F was used to modify the side chain of the phenylalanine residue of Ac-Phe-Gly-OMe. Reagents: P11 (78 mg, 0.28 mmol), $Pd(OAc)_2$ (7 mg, 0.03 mmol), AgOAc (117 mg, 0.70

mmol), Styrene (0.13 mL, 1.12 mmol), DMF (0.20 mL) and DCE (3.00 mL) as solvents. A brown solid was obtained (8 mg, 6% yield).

^{1}H NMR spectrum showed the fractions collected were just starting material . The reaction had not worked.

4.3.6.6 Product 21: olefination of P19

Method F was used to modify the side chain of the phenylalanine residue of Fmoc-Phe-Gly-OMe. Reagents: P19 (100 mg, 0.22 mmol), $Pd(OAc)_2$ (5 mg, 0.02 mmol), AgOAc (92 mg, 0.55 mmol), Styrene (0.10 mL, 0.87 mmol), DMF (0.20 mL) and DCE (3.00 mL) as solvents. Column chromatography was performed with a mixture of Petrol/Ethyl acetate (50:50). An orange solid was obtained (32 mg, 22% yield).

As long as the starting material was not what it was supposed to be, ^{1}H NMR spectrum showed no proof of olefinated product, reaction did not work.

4.3.6.7 Product 30: olefination of P28, changing the solvent

Method F was used to modify the side chain of the phenylalanine residue of Ac-Phe-Gly-OMe. Reagents: P28 (70 mg, 0.25 mmol), $Pd(OAc)_2$ (10mg, 0.03 mmol), AgOAc (110 mg, 0.63 mmol), Styrene (0.12 mL, 1.01 mmol), TAA (3.00 mL) as solvent.

A brown solid was obtained, ^{1}H NMR spectrum showed reaction had not worked.

4.3.6.8 Product 31: olefination of P29

Method F was used to modify the side chain of the phenylalanine residue of Cbz-Gly-Phe-OMe. Reagents: P29 (79 mg, 0.21 mmol), $Pd(OAc)_2$ (10mg, 0.02 mmol), AgOAc (88 mg, 0.53 mmol), Styrene (0.10 mL, 0.85 mmol), TAA (3.00 mL) as solvent. Column chromatography was performed with a mixture of Petrol/Ethyl acetate (50:50). A green solid was obtained (47 mg, 39% yield).

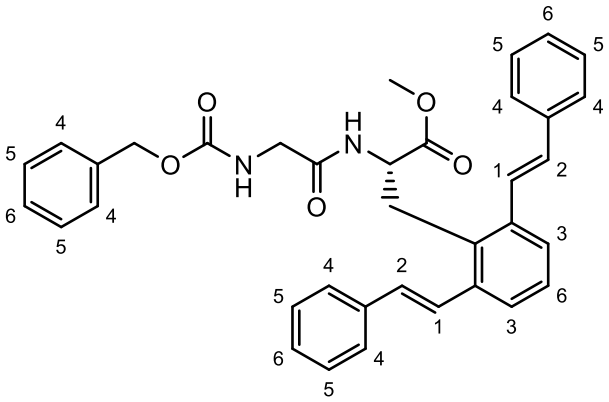


Figure 14. P31, diolefinated P29.

^{1}H NMR (400 MHz, CD_3Cl) δ (ppm) 3.46 (br s, 2H, C*H*$_2$[Phe]) 3.52 (s, 3H, CO_2C*H*$_3$) 3.71 (br t, 2H, C*H*$_2$[Gly]) 4.80 (app q, 1H, ^{3}*J*=4.82 Hz, C*H*) 5.04 (s, 2H, C*H*$_2$[Cbz]) 6.53 (br d, 1H, N*H*) 7.03 (d, 2H, ^{3}*J*=15.9 Hz, C1-*H)* 7.28 (d, 2H, ^{3}*J*=7.3 Hz, C3-*H*) 7.32 (br d, 3H, C6-*H*) 7.37 (t, 6H, ^{3}*J*=7.9 Hz, C5-*H*) 7.48 (d, 2H, ^{3}*J*=15.9 Hz, C2-*H*) 7.56 (d, 6H, ^{3}*J*=7.3 Hz, C4-*H*).

^{13}C NMR (400 MHz, CD_3Cl) δ (ppm) 31.5, 52.8, 53.2, 67.2, 125.9, 126.2, 126.8, 127.8, 128.1, 128.2, 128.6, 128.9, 131.8, 137.3, 137.9.

5 Results and discussion

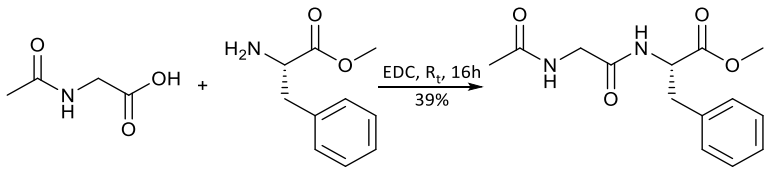
Previous investigations showed the olefination via C(sp^2)-H activation of the peptide Ac-Gly-Phe-OMe (P1) could be afforded in yields around 40%. So the question presented for the present work was trying the same reaction for the reverse peptide: Ac-Phe-Gly-OMe (P11) in order to learn about the effects that control the reaction.

Although the synthesis of the starting material P1 was simple and easily carried out by method C, as is discussed later the synthesis of the peptide P11 led to unexpected issues that slowed down the study of the olefination reaction.

5.1 N-PG-Glycine-Phenylalanine-Methyl ester synthesis

The first step of the research process was to synthesize the starting materials for the olefination reactions. As is presented in section 2.2, to afford these compounds a coupling agent is needed as well as protected amino acids in order to ensure the peptide sequence.

The first synthesis attempted was the one for Ac-Gly-Phe-OMe, shown in scheme 13.

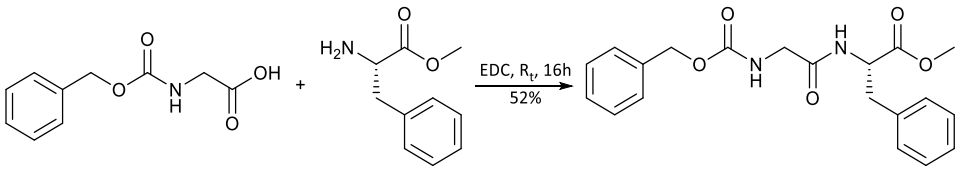


Scheme 13. Synthesis of peptide P1.

As said in section 4.3.3.1 method C was used to make this peptide in 39% yield. The work up procedure worked really well for this peptide and both ^1H and ^{13}C NMR spectrums displayed a pure and well-defined peptide backbone.

In order to try the olefination reaction changing the protecting group at the N-terminus, Cbz-Gly-Phe-OMe was synthesized by method C as well. Prior to this reaction, N-protected amino acid was synthesized due to unavailability of it.

Section 4.3.1.1 describes the procedure carried out to synthesize Cbz-Gly-OH (P24), reaction conditions are shown at scheme 9. P24 was later made react with H-Phe-OMe to make the peptide P29, as is shown in scheme 14.



Scheme 14.Synthesis of peptide P29.

The reaction led to a higher yield than for the acetyl protected reaction, 52%. It was characterised by both ^{1}H and ^{13}C NMR spectroscopy, which demonstrated the reaction had worked.

5.2 N-PG-Phenylalanine-Glycine-Methyl ester synthesis

First attempt tried to synthesize the reverse peptide P7 using method C failed. Initially, was thought this method could be useful but, H-Gly-OMe is not soluble in organic solvents as is H-Phe-OMe (due to its benzylic side chain), but it is extremely soluble in water.

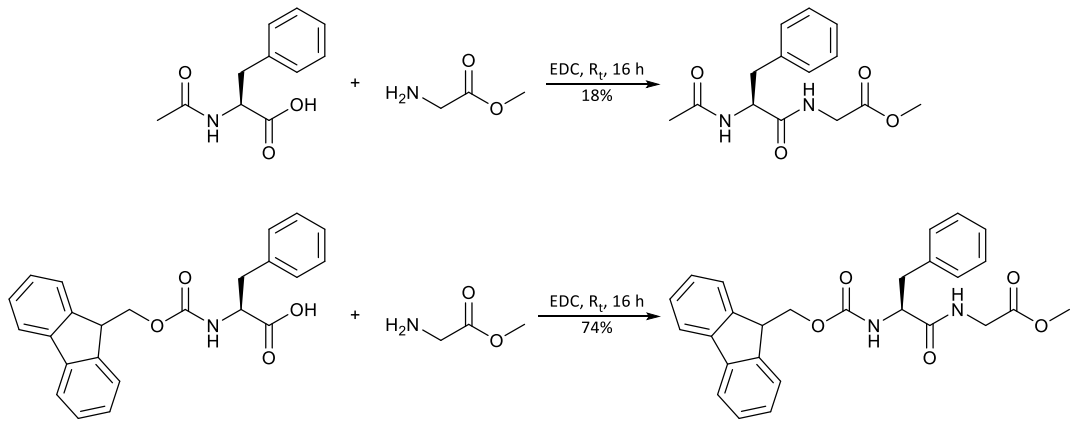
After making the salt react with potassium carbonate was intended to extract the aqueous solution with diethyl ether, so the amino acid methyl ester would migrate to the organic layer. However, because of this solubility issue, the first step of the method: freeing the amine from the hydrochloride salt, did not work.

Consequently, P7 was a failure finding out there was just Ac-Phe-OH and EDC in the reaction mixture because all C-protected amino acid was discarded with the aqueous layer.

Different method might be used to afford the reverse peptide coupling, avoiding the presence of water. So method D was designed in such purpose although, as it was discovered later, this method led to triethylammonium salts as by-products which are very difficult to get rid of.

Attempting to free the amine in chloroform and triethylamine was found to be an alternative to avoid water. Unfortunately, by this time EDC was not available in the laboratory so a DCC coupling was attempted.

Purification of products P16 and P17 was impossible to achieve due to DCU by-product low solubility in water, so the work up did not eliminate it all. When using EDC as coupling agent, both products P11 and P19 were obtained method D.



Scheme 15. Synthesis of peptides P11 (top) and P19 (bottom).

As it is said before, method D afforded successfully the free amine C-protected amino acid, as well as high quantities of triethylammonium salts. This was thought to be a possible cause for the low yields obtained in peptide couplings. So the use of an ionic-exchange resin was proposed, leading to method E.

The results obtained were not better than the ones registered for the previous method, but it was more convenient because of no need of triethylamine or chlorinated solvents as chloroform.

5.3 N-PG-Glycine-Phenylalanine-Methyl ester olefinations

Initially, the olefination reaction was trialled with P1 in order to apply the same reaction conditions to P11. Although the results were not as good as expected in terms of yield (8% yield), further analysis by ^{1}H NMR spectrum showed that the product had been successfully afforded. The loss of yield is attributed to an experimental defect, as co-elution or loss of the product at any stage.

^{1}H NMR spectrum showed a well resolved backbone for the peptide as well as a better splitting than for the starting material in the aromatic region which allow to confirm the olefination occurred at the *ortho* position. Two new peaks, doublets at 7.00 and 7.46 ppm for the alkenes, were the proof for the diolefinated product. Additionally, ^{3}J values for those doublets showed a *trans* stereoisomerism, which also proofed the *syn* elimination step.

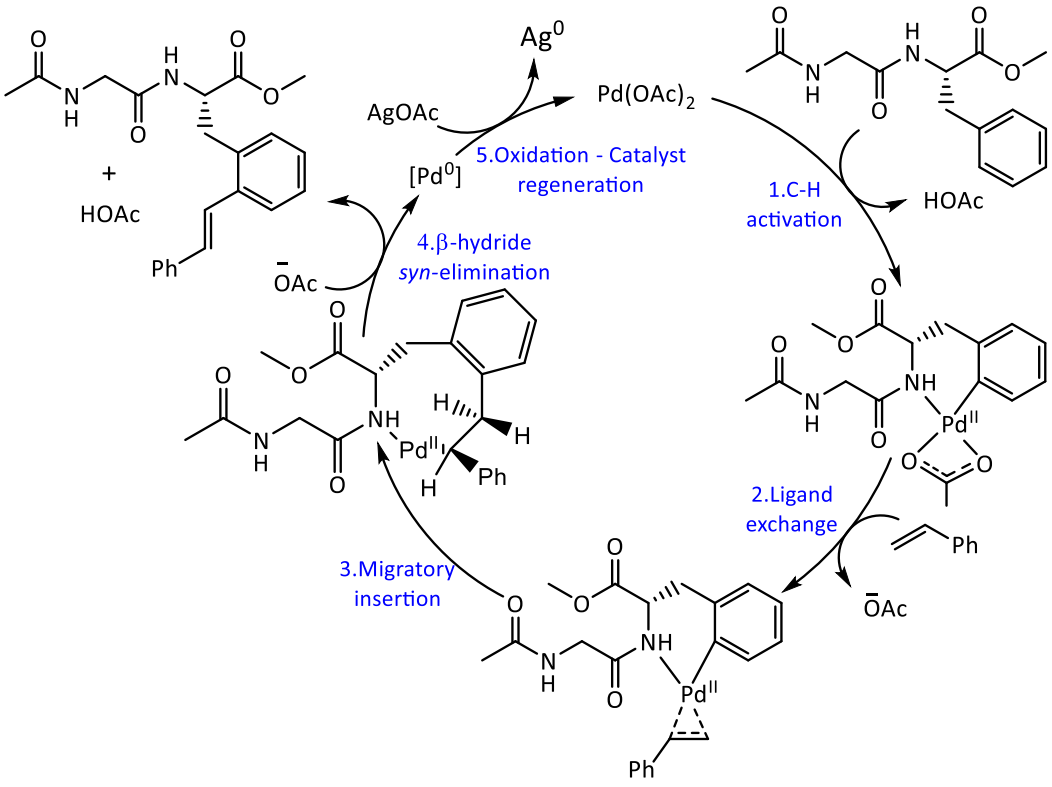
Later fractions collected during the column chromatography were analysed as well in order to see what was left in the crude mixture and elucidate if there were any side reactions that competed with the olefination one. In the ^{1}H NMR spectrum it could be observed some of

the starting material and traces of the mono-olefinated product. Thus, was noticed that the C-H activation did not led to the di-olefinated product selectively.

The same amino acid sequence but with a different protecting group on the N-terminus (carboxybenzyl instead of acetyl) was trialled to olefinate.

The yield of the olefination reaction was in the same range as the olefinations reactions for the acetyl protected amino acid, around 40%. Although the ^{1}H NMR spectra was poor resolved in some regions, probably because of the low concentration of the product and the high amount of DCM present in it, the peptide backbone could be observed as well as the representative peaks for the alkenes. It was proofed that the reaction did work for P29.

The mechanism of the catalytic process can be summarised as a conventional Heck reaction where the coordination step cleaves a C-H bond instead a C-heteroatom bond. The reaction was supposed to proceed through the following catalytic cycle presented in scheme 16.



Scheme 16. Catalytic cycle for C-H olefination.

As the mostly obtained product was the diolefinated one, means that the majority of the molecules go through the catalytic cycle twice. Note the reaction is selective for the *ortho*

product due to the six membered ring transition state, against less likely seven or eight membered rings that would led to *meta* and *para* products.

5.4 N-PG-Phenylalanine-Glycine-Methyl ester olefinations

Once P11 was successfully synthesized, olefination reaction was attempted. As well as for P4 the yield was very low (3% yield). ^{1}H NMR spectrum showed a doublet for two protons integration at 6.99 ppm shift, but the spectra was not resolved enough to be able to affirm the reaction had worked successfully.

According to Li et al.[19], O_2 could help the regeneration of the catalyst, so the reaction would achieve higher yields. Intending this, P13 olefination reaction was ran under air atmosphere. Although the yield was higher (6% yield), it was not a such big difference with P12 so it could not be attributed just to the presence of oxygen, i.e. the packing of the column or a less loss of product during the filtration through the Celite® plug. However, it was proofed that no N_2 atmosphere was not beneficial nor harmful for the catalyst, so the following C-H functionalizations were ran under air atmosphere.

As for P12 ^{1}H NMR spectrum, a doublet for two protons appeared at 6.99 ppm shift, but the resolution was not good enough to confirm the reaction had worked.

The protecting group on the N-terminus was taken into account, given that it could affect the nucleophilicity of the amide, thus the coordination to palladium. So instead of an acetyl, a carbamate (Fmoc-Phe-OH) was used in order to change the nuclephilicity of the N-terminus. This election was not trivial, Fmoc-protected amino acids are the ones used in solid phase synthesis, so the applicability of the method in presence of this protecting group was of big interest.

In order to be sure of the reproducibility of the experiments, an olefination of Ac-Phe-Gly-OMe, P20, was performed in parallel with the Fmoc-Phe-Gly-OMe, P21. Thus, ensuring the method applied to both was exactly the same.

Unfortunately, both reactions: coupling of the peptide and olefination reaction for P19 were ran consecutively. So ^{1}H NMR spectroscopy was performed to both samples at the same time and it was noticed that the peptide coupling had not obtained the desired product,

thus in the spectra no peptide backbone could be observed. Obviously, C-H activation did not take place because the starting material was not what it was supposed to be.

Further investigations on N-terminus protecting groups were not trialled. In fact carbamates are less nucleophile than amides, so it was expected a less reactivity by the Fmoc-protected peptide.

The last trial for the olefination of Ac-Phe-Gly-OMe was changing the solvent. According to Yu et al.[20] different solvents could increase the yield of C-H activation reaction. For the first C-H activation studied, P4, it was proven that the use of *tert*-amyl alcohol increased the yield.

However, it was not successful and the results for this reaction were equal to the ones obtained before in DCE/DMF as solvents, no olefinated product was obtained or such a low amount that would not make the reaction worth.

It was planned to try an olefination reaction for P33, it was impossible because of the lack of time. However, this and other experiments that were planned have not been let down and are part of the future work that is intended.

5.5 Approaching the causes for the results

After all these trials and assuming the reaction for the reverse peptide did not work, a theory was proposed to explain why the C-H activation occurs when phenylalanine residue is at C-terminus but not at N-terminus.

A different transition state to the one that was thought at the beginning might be the cause for this effect. For P1, it may be the case of palladium binding to both nitrogen atoms of the peptide, forming a six and five membered rings, acting as a bidentate directing group. As for P11, the metal complex would form a bicycle as is shown in figure 15.

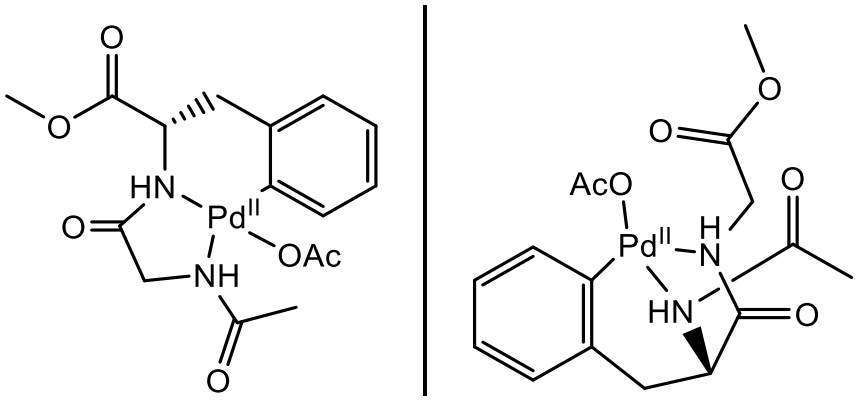


Figure 15. Proposed TS for both peptides P1(left) and P11(right).

It is noteworthy reminding that Pd (II) tends to adopt square planar (D_{4h}) geometries, so whatever out of this would not be likely to exist nor proceed through a catalytic cycle. Whether the TS for P1 would allow palladium to adopt the mentioned geometry, the one for P11 would force the chain of the peptide to bend in order to coordinate as a bidentate ligand resulting in a non-square planar geometry.

Following the previous statement, a phenylalanine residue in the N-terminus of a peptide would not lead to a D_{4h} geometry, in other words: palladium coordination and further C-H activation. Nevertheless, a phenylalanine residue wherever else in a peptide chain would proceed to coordination of palladium and the following C-H activation.

So the necessary condition to be fulfilled in order to C-H activate the peptide would not to be at the C-terminus, but not to be at the N-terminus, because there is no chelating group close enough to coordinate to, forming a D_{4h} complex.

Previous work by Yu et al.[12] on C(sp^3)-H activation in peptides also showed similar transition states to the ones proposed above.

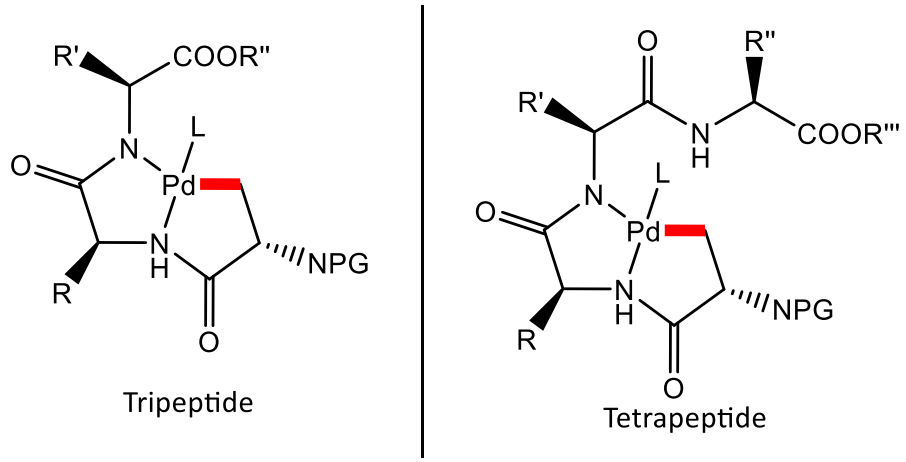


Figure 16. Proposed TS for a C(sp3)-H activation in different peptides.

Relating to all these premises, further investigations on this proposal are going to be held. Synthesis of tripeptides e.g. Ac-Gly-Phe-Gly-OMe, is intended to proof the reason why the olefinations of the reverse peptide did not work is, in fact because the phenylalanine residue was at the N-terminus and the reaction can work either when it is not just at the C-terminus. Computational studies would provide the relative energy values for these transition states. So they can be compared with the ones that were assumed at the beginning (monodentate directing group) and demonstrate the likeliness of these structures.

6 Conclusions

The first of the aims was achieved in terms of knowledge, but also demonstrate the sensibility of the method used. Because of the scale of the trials, the accuracy and meticulousness of work at every single step of the synthesis was crucial in order to obtain reproducible results.

The aim of synthesizing different target peptides resulted trickier than expected because of the different nature of each amino acid. This fact end up in several changes on the method stablished and aimed the development of new ones.

Regarding the third point of the aims, it was not possible to set up a method for the olefination of the reverse peptide. But, despite the lack of progress in the optimization of the olefination reaction, according to the final aim of the project, this work has been useful in order to know more about the details of C-H activation in peptide frameworks.

Finally, a reasonable theory has been proposed to justify such results and it has resulted into future work that will continue providing useful information for future investigations in this field.

7 References

1 R. B. Merrifield, John Wiley & Sons, Inc., 2006, pp. 221–296.

2 H. Nakamura, M. Fujiwara and Y. Yamamoto, *Bull. Chem. Soc. Jpn.*, 2000, **73**, 231–235.

3 N. Doan, S. Bourgault, M. Létourneau and A. Fournier, *J. Comb. Chem.*, 2008, **10**, 44–51.

4 C. C. C. Johansson Seechurn, M. O. Kitching, T. J. Colacot and V. Snieckus, *Angew. Chemie - Int. Ed.*, 2012.

5 J. A. Labinger and J. E. Bercaw, *Nature*, 2002, **417**, 507–14.

6 D. Balcells, E. Clot and O. Eisenstein, *Chem. Rev.*, 2010, **110**, 749–823.

7 L. Ackermann, *Chem. Rev.*, 2011, **111**, 1315–1345.

8 D. L. Davies, S. M. A. Donald and S. A. Macgregor, *J. Am. Chem. Soc.*, 2005, **127**, 13754–13755.

9 S. I. Gorelsky, D. Lapointe and K. Fagnou, .

10 Y. H. Zhang, B. F. Shi and J. Q. Yu, *J. Am. Chem. Soc.*, 2009, **131**, 5072–5074.

11 W. B. Cross, S. Razak, K. Singh and A. J. Warner, *Chem. - A Eur. J.*, 2014, **20**, 13203–13209.

12 W. Gong, G. Zhang, T. Liu, R. Giri and J. Q. Yu, *J. Am. Chem. Soc.*, 2014, **136**, 16940–16946.

13 F.-T. Tsai, Y. Wang and D. J. Darensbourg, *J. Am. Chem. Soc.*, 2016, **138**, 4626–4633.

14 J. Li and Y. Sha, *Molecules*, 2008, **13**, 1111–1119.

15 M. Bodanszky and A. Bodanszky, in *The Practice of Peptide Synthesis*, Springer Berlin Heidelberg, Berlin, Heidelberg, 1994, pp. 118–126.

16 J. D. McKerrow, J. M. A. Al-Rawi and P. Brooks, *Synth. Commun.*, 2010, **40**, 1161–1179.

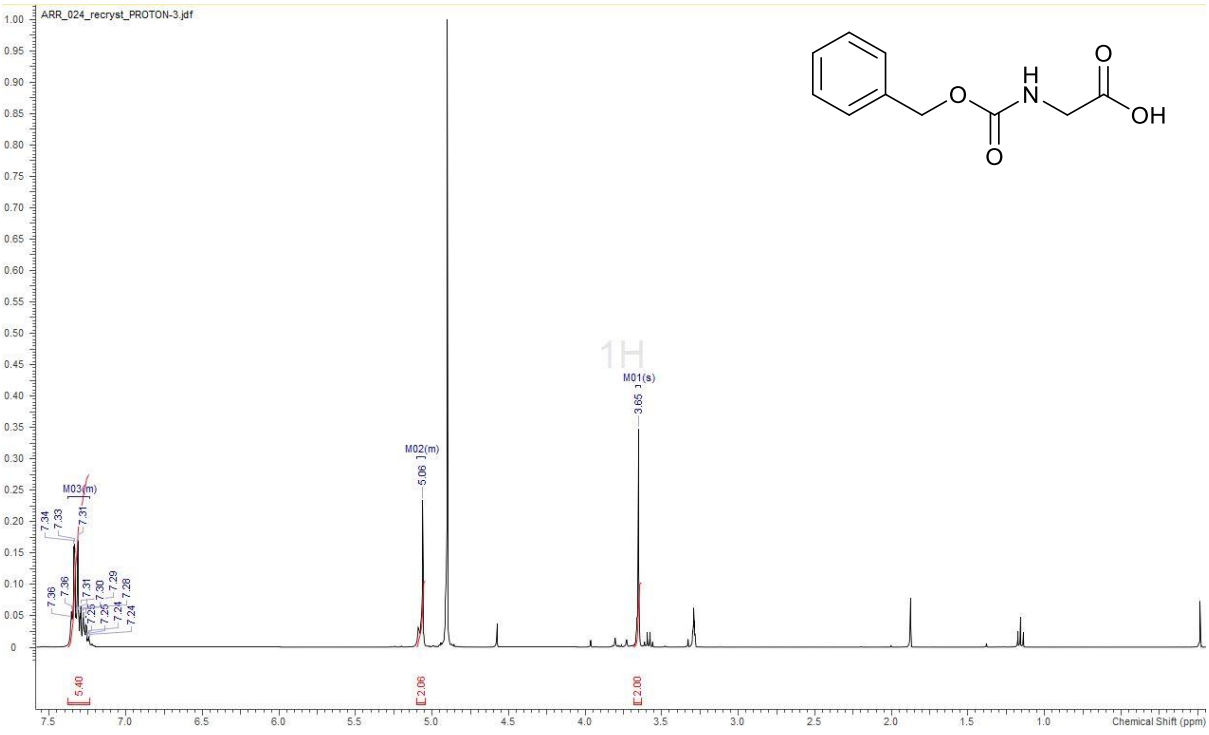
17 R. Vallakati and J. A. May, *J. Am. Chem. Soc.*, 2012, **134**, 6936–6939.

18 J.-J. Li, T.-S. Mei and J.-Q. Yu, *Angew. Chemie Int. Ed.*, 2008, **47**, 6452–6455.

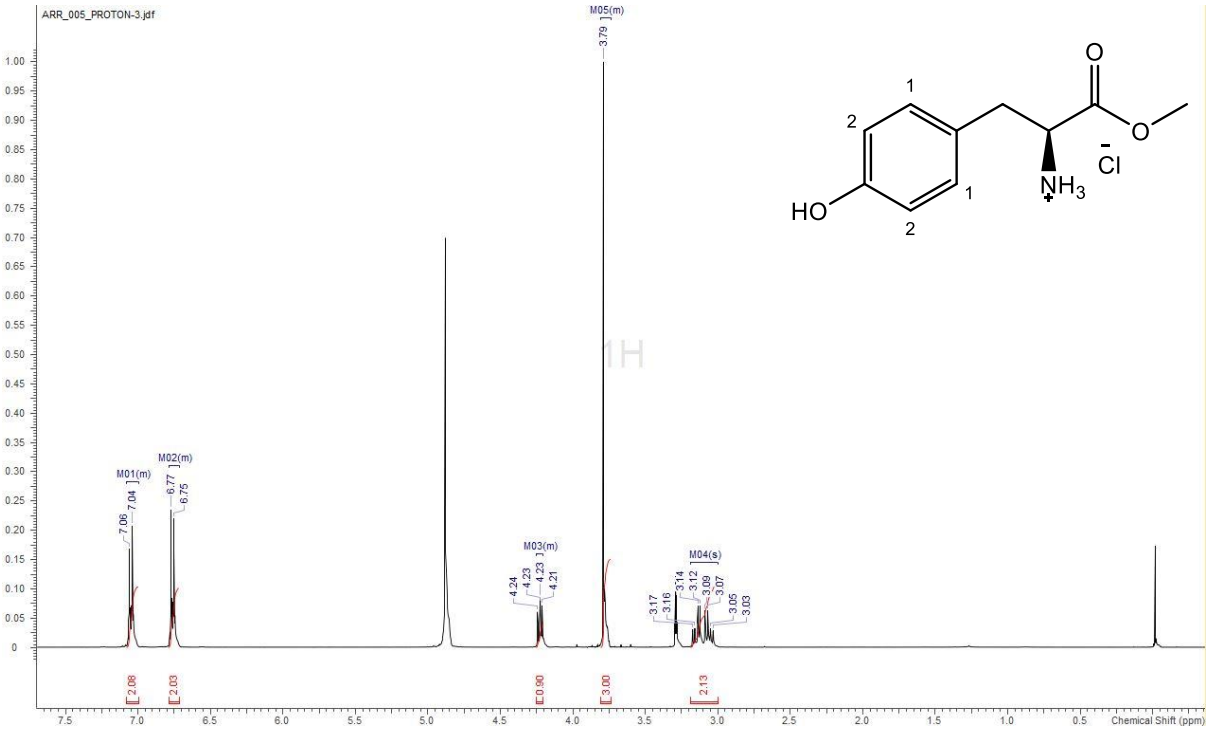
19 S. Li, L. Cai, H. Ji, L. Yang and G. Li, *Nat. Commun.*, 2016, **7**, 10443.

20 N. Dastbaravardeh, T. Toba, M. E. Farmer and J.-Q. Yu, *J. Am. Chem. Soc.*, 2015, **137**, 9877–84.

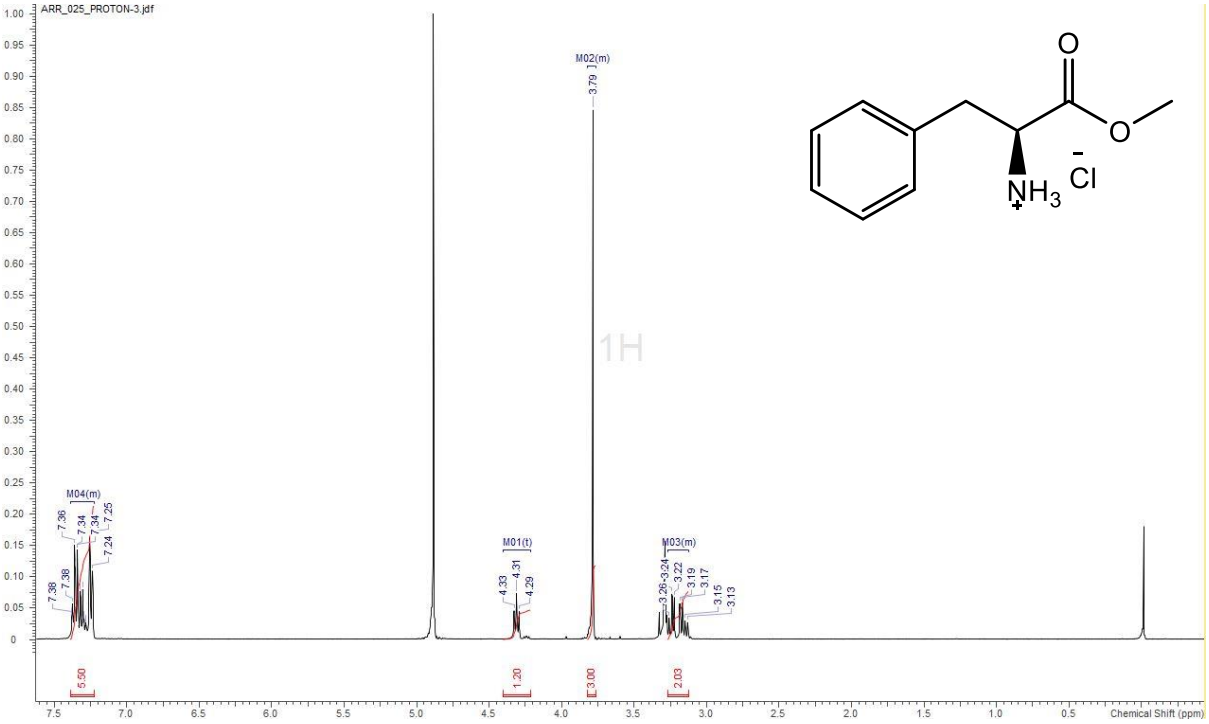
Annexes and supporting information



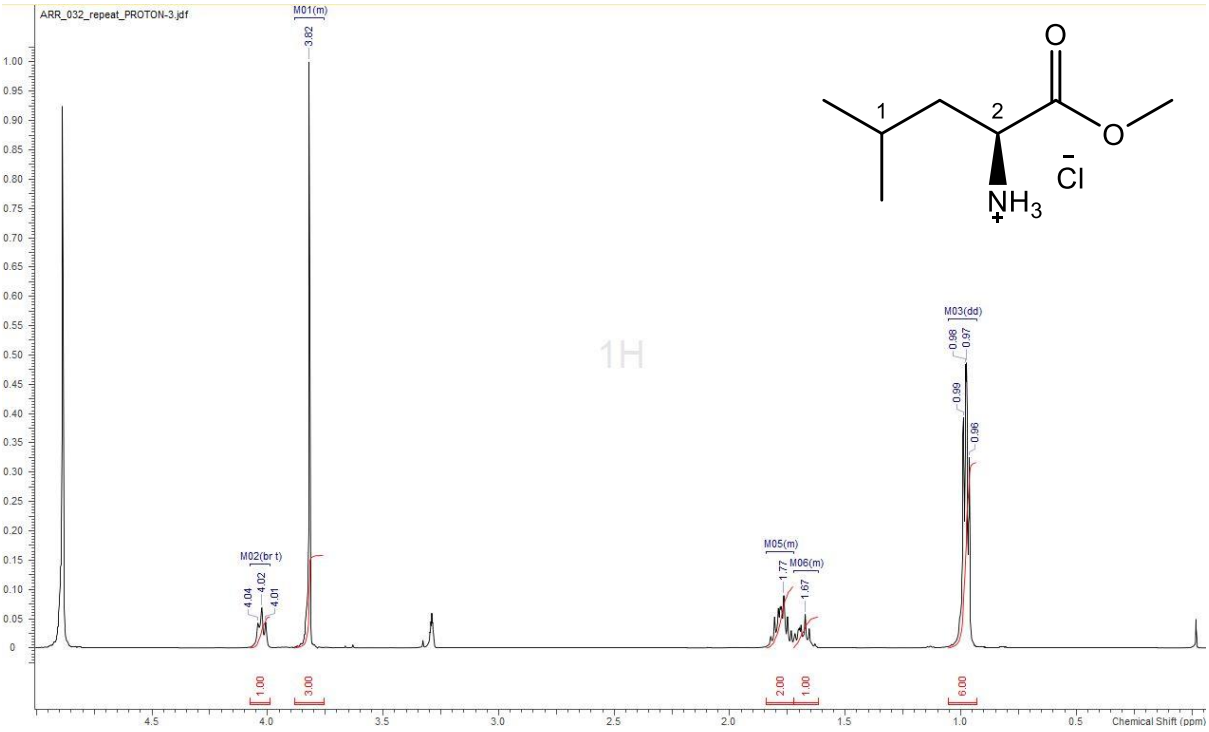
Annex 1. ^{1}H NMR spectrum of P24, Cbz-Gly-OH.



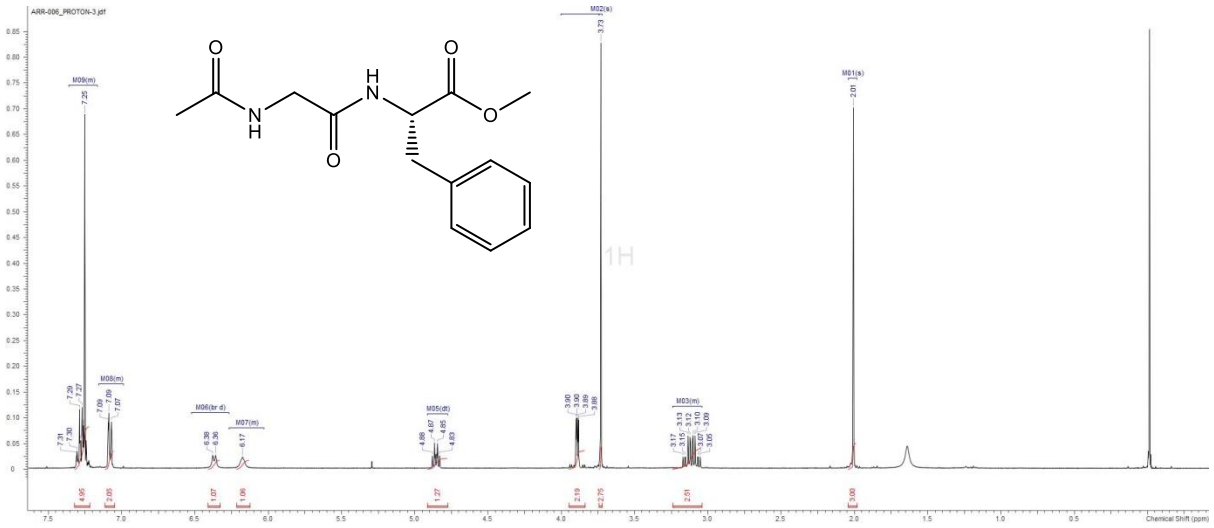
Annex 2. ^{1}H NMR spectrum of P5, H-Tyr-OMe.



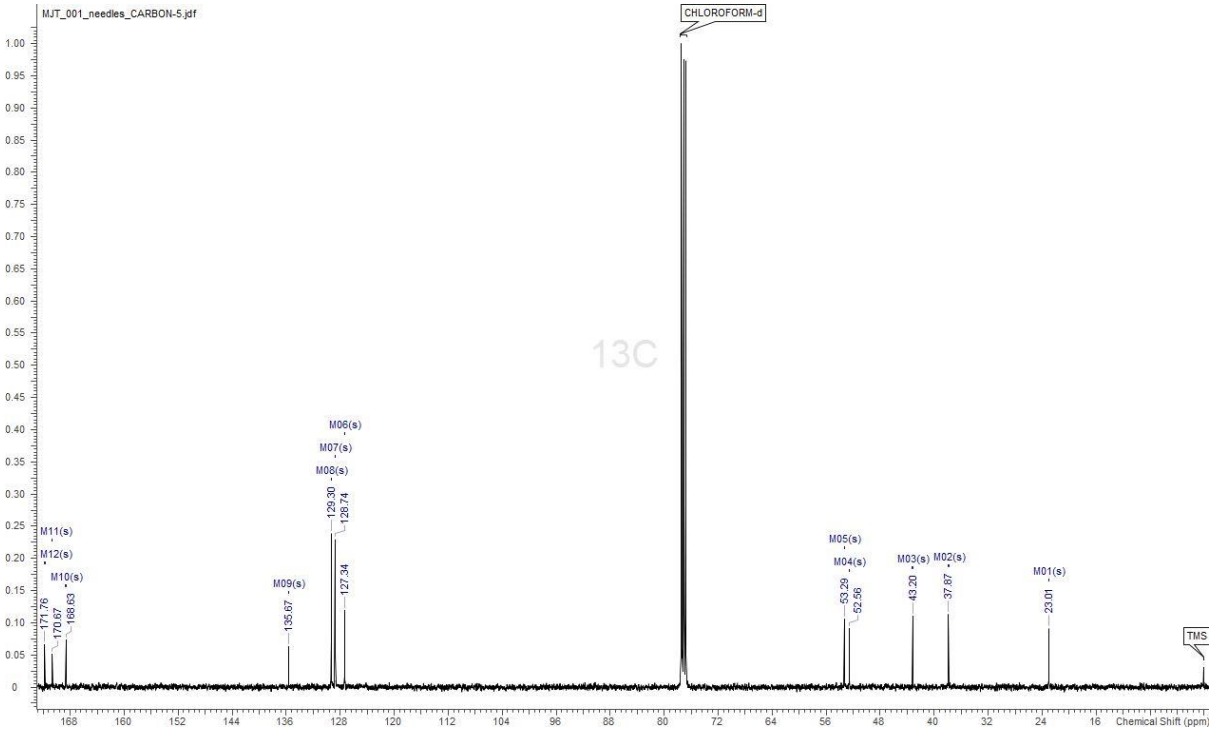
Annex 3. ^{1}H NMR spectrum of P25, H-Phe-OMe.



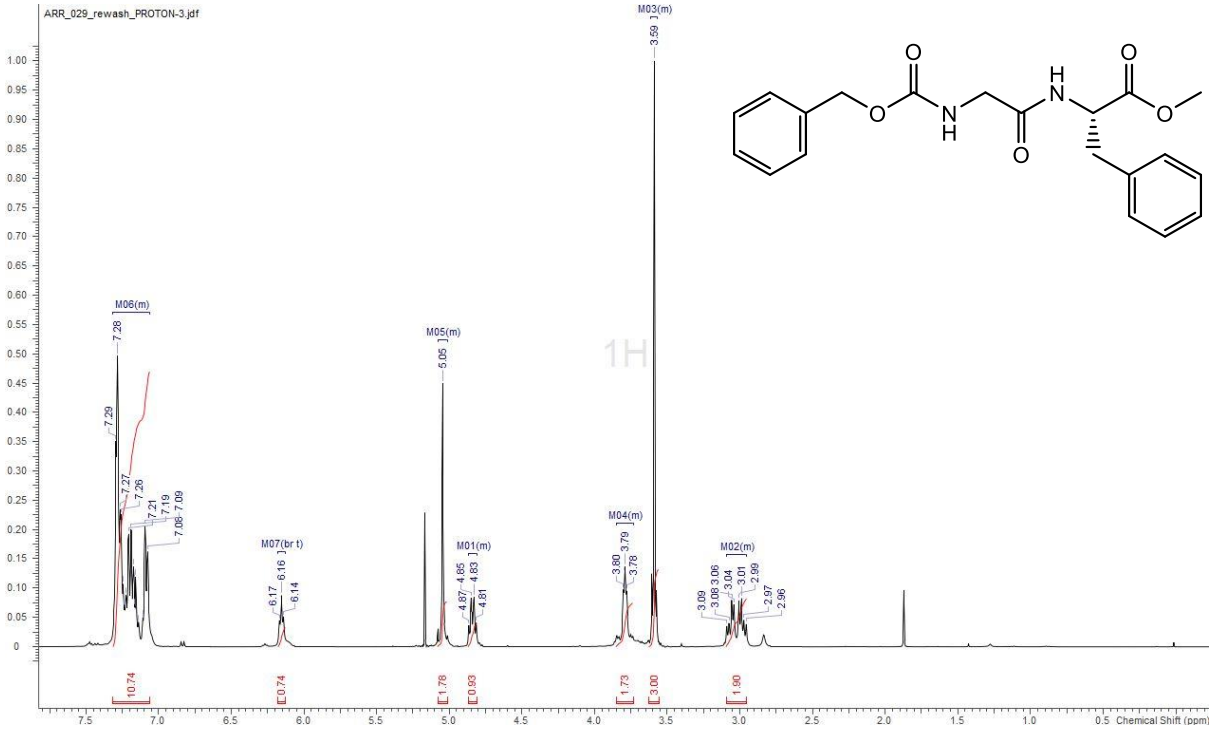
Annex 4.^{1}H NMR spectrum of P32, H-Leu-OMe.



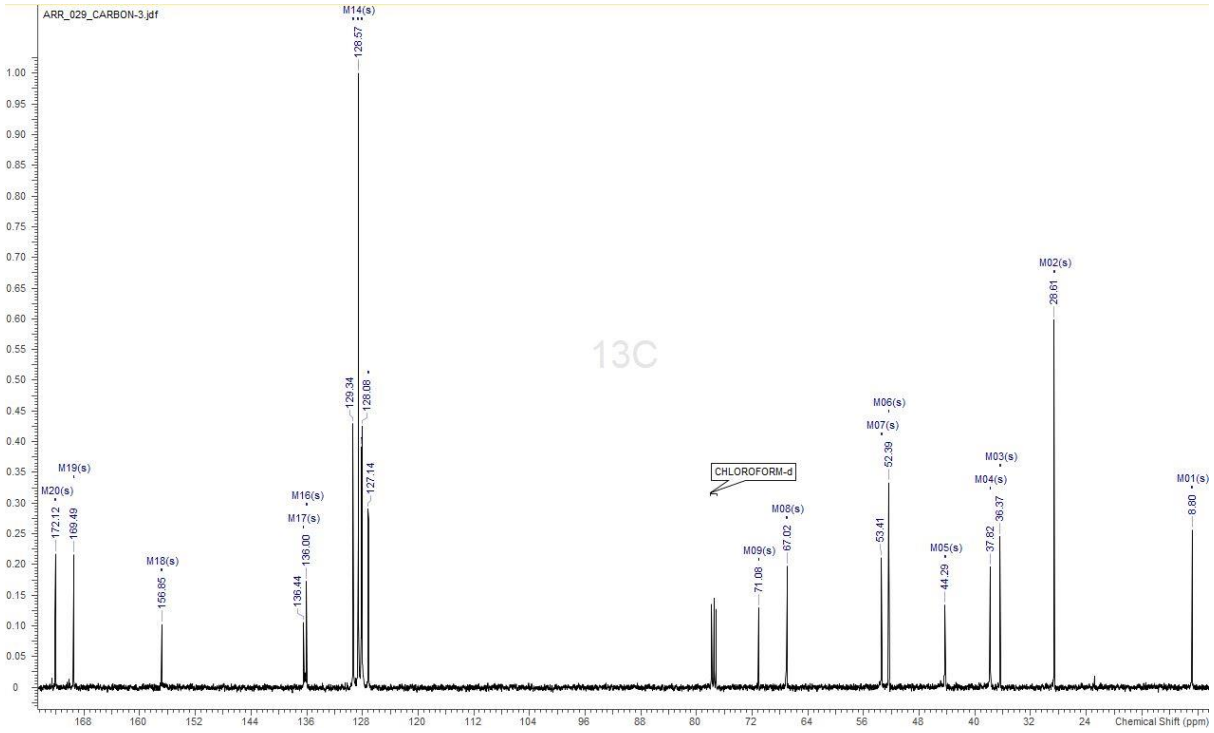
Annex 5. ^{1}H NMR spectrum of P1, Ac-Gly-Phe-OMe.



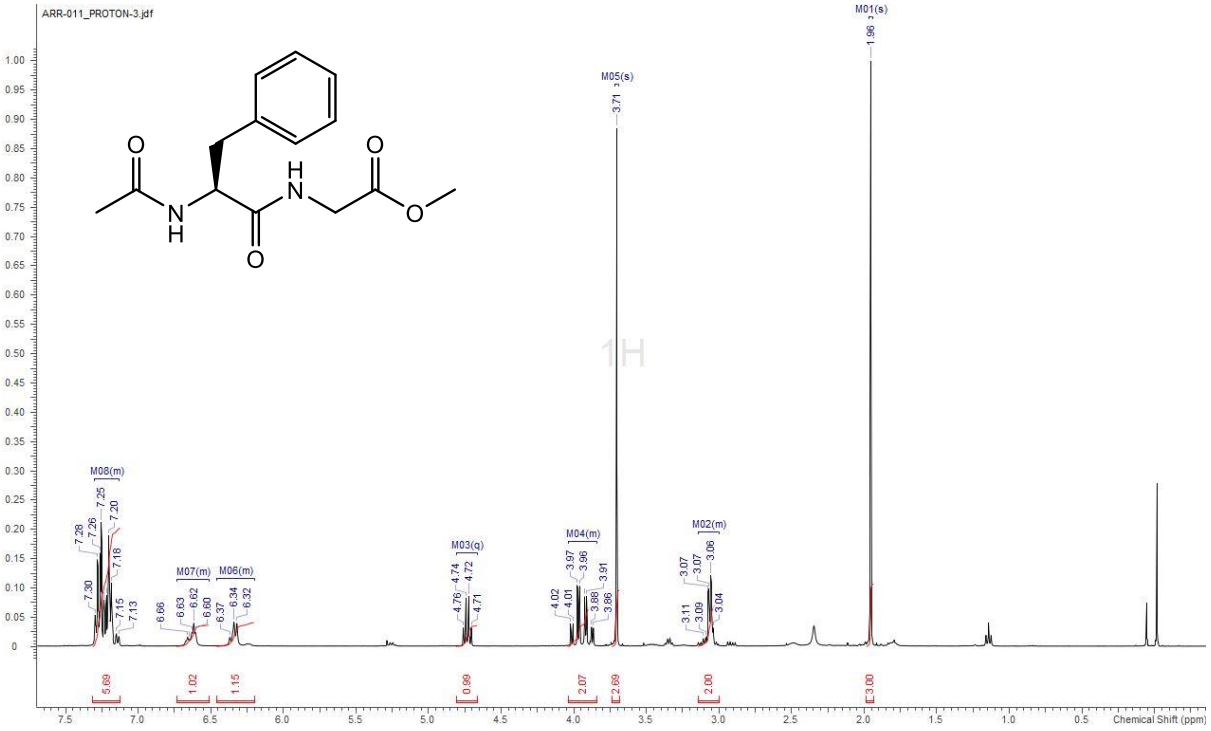
Annex 6. ^{13}C NMR spectrum of P1, Ac-Gly-Phe-OMe.



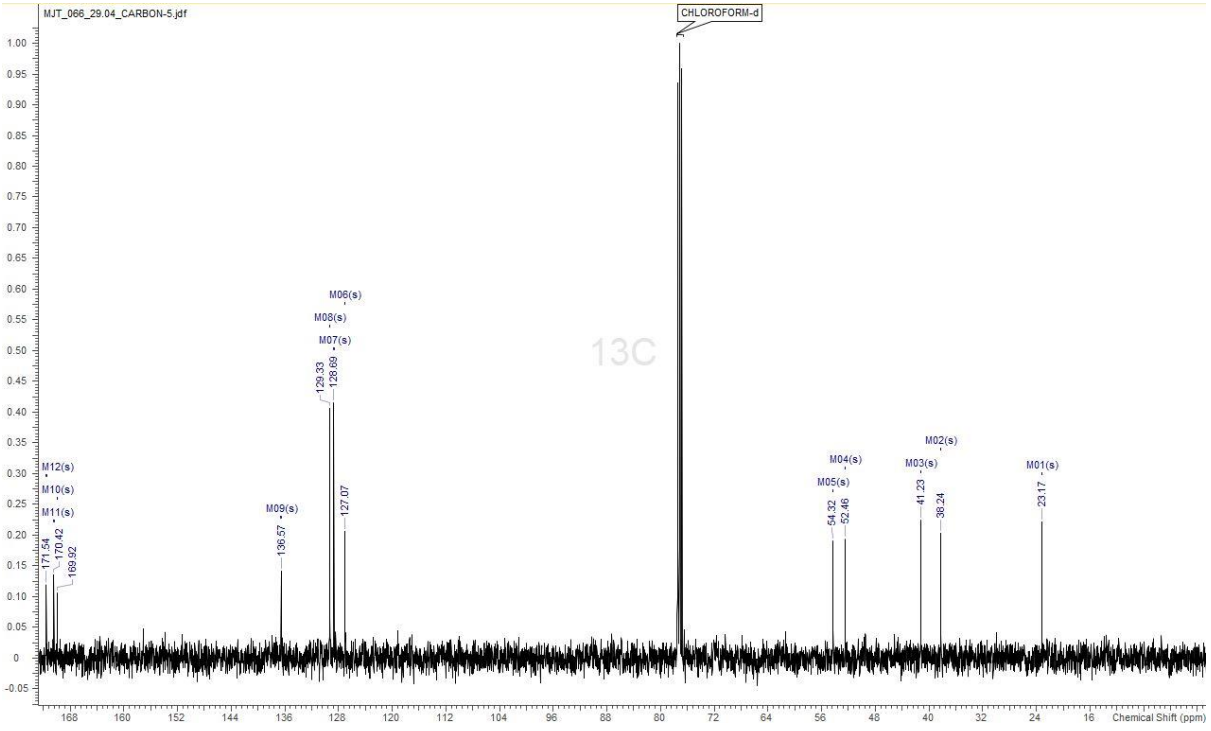
Annex 7. ^{1}H NMR spectrum of P29, Cbz-Gly-Phe-OMe.



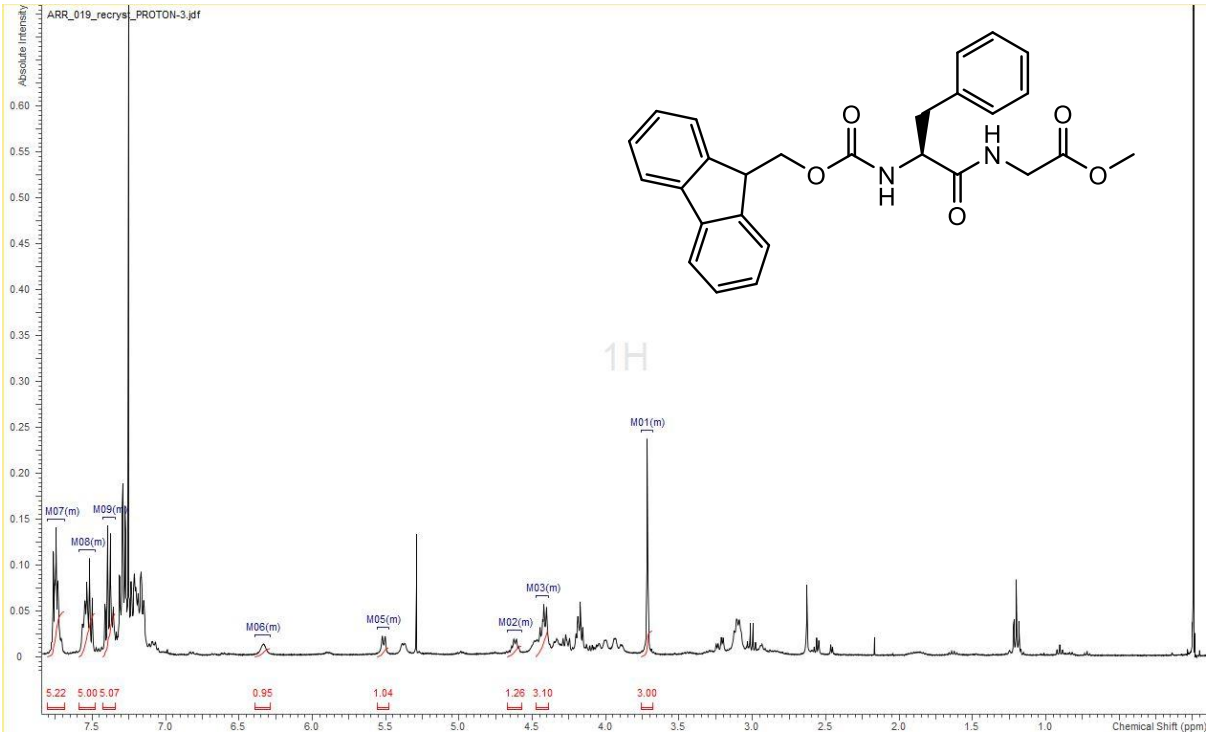
Annex 8. ^{13}C NMR spectrum of P29, Cbz-Gly-Phe-OMe.



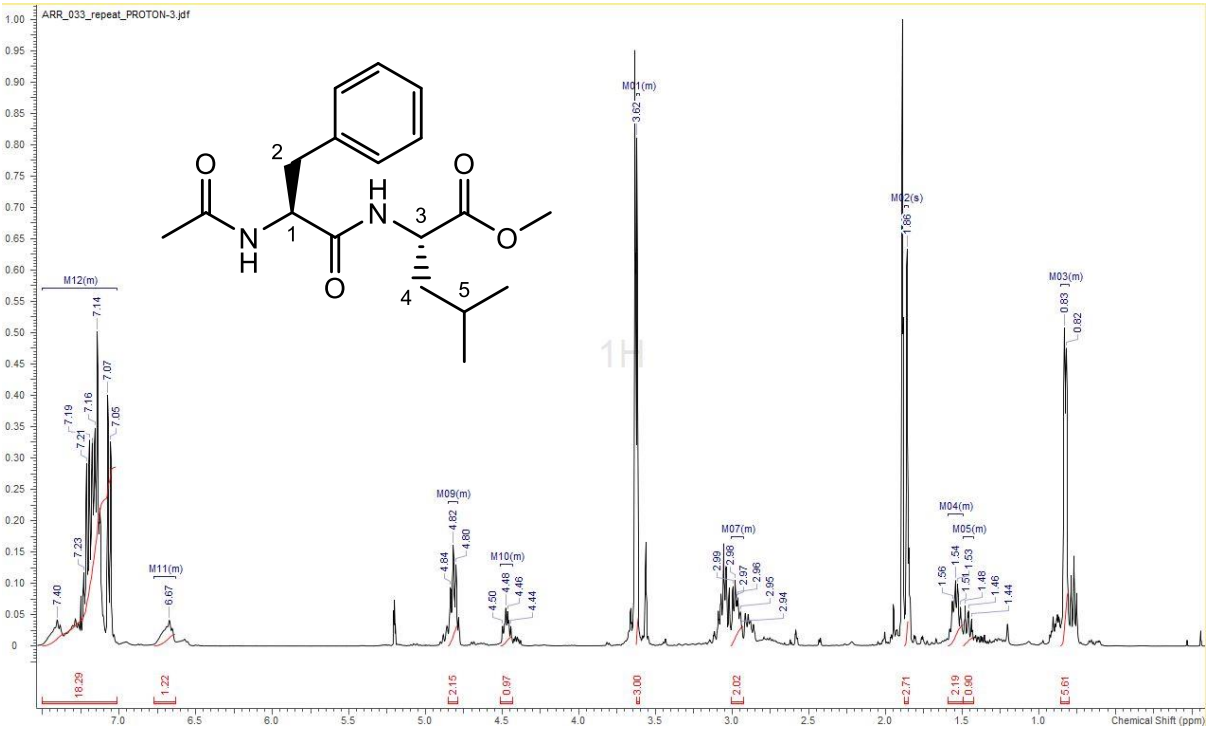
Annex 9. ^{1}H NMR spectrum of P11, Ac-Phe-Gly-OMe.



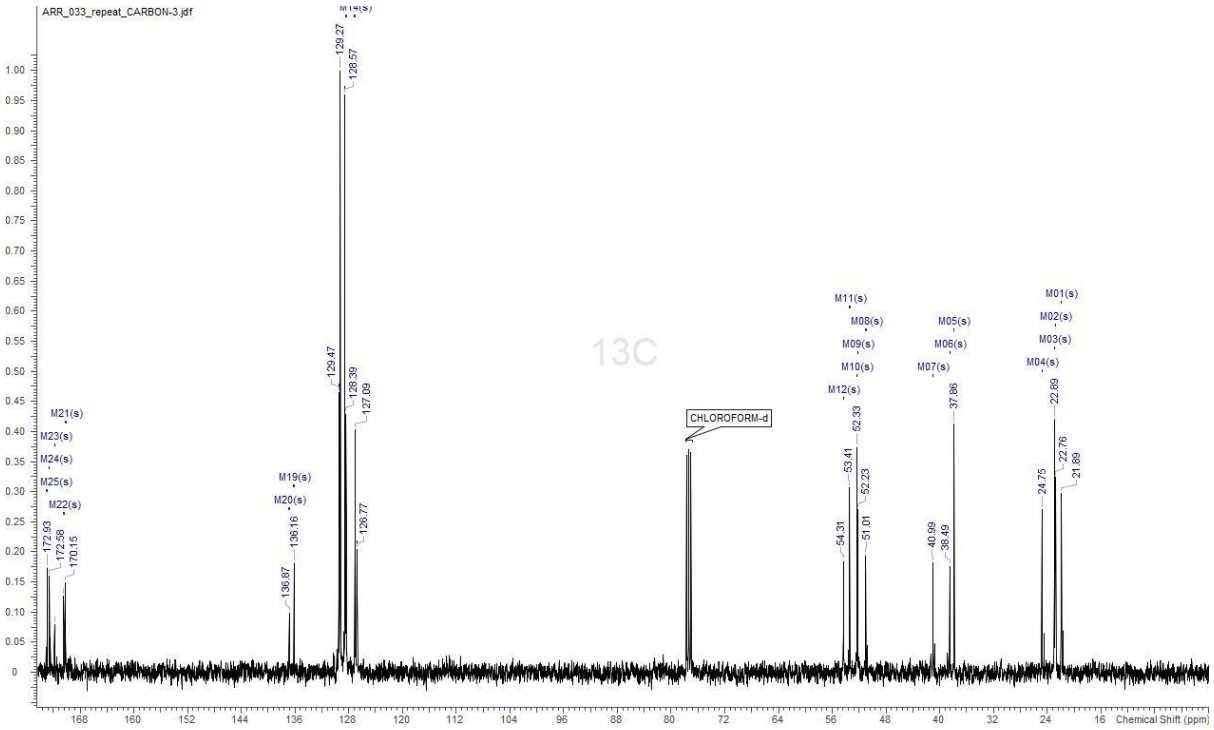
Annex 10. ^{13}C NMR spectrum of P11, Ac-Phe-Gly-OMe.



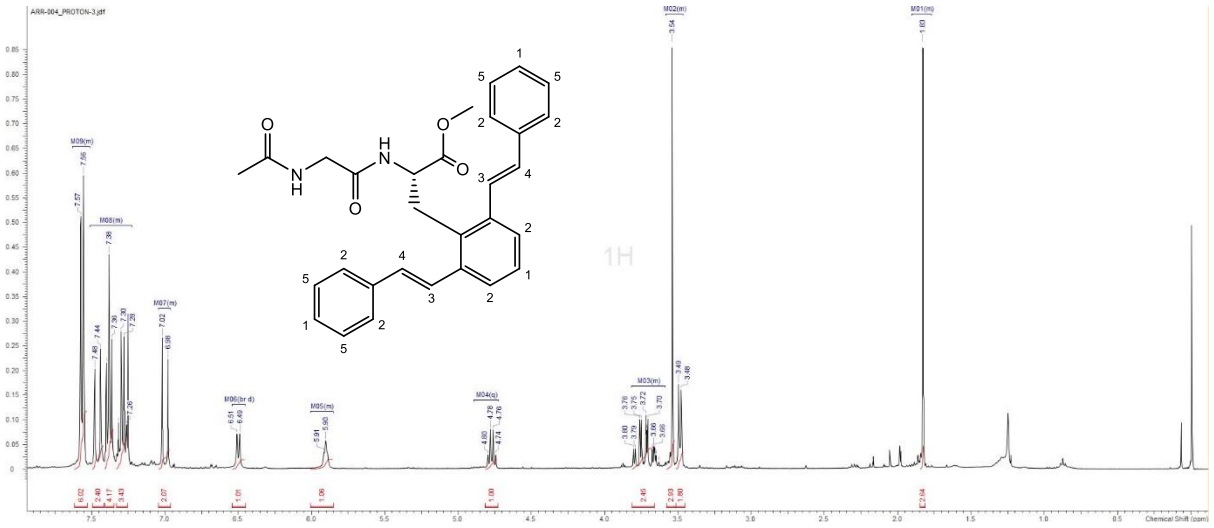
Annex 11. ^{1}H NMR spectrum of P19, Fmoc-Phe-Gly-OMe.



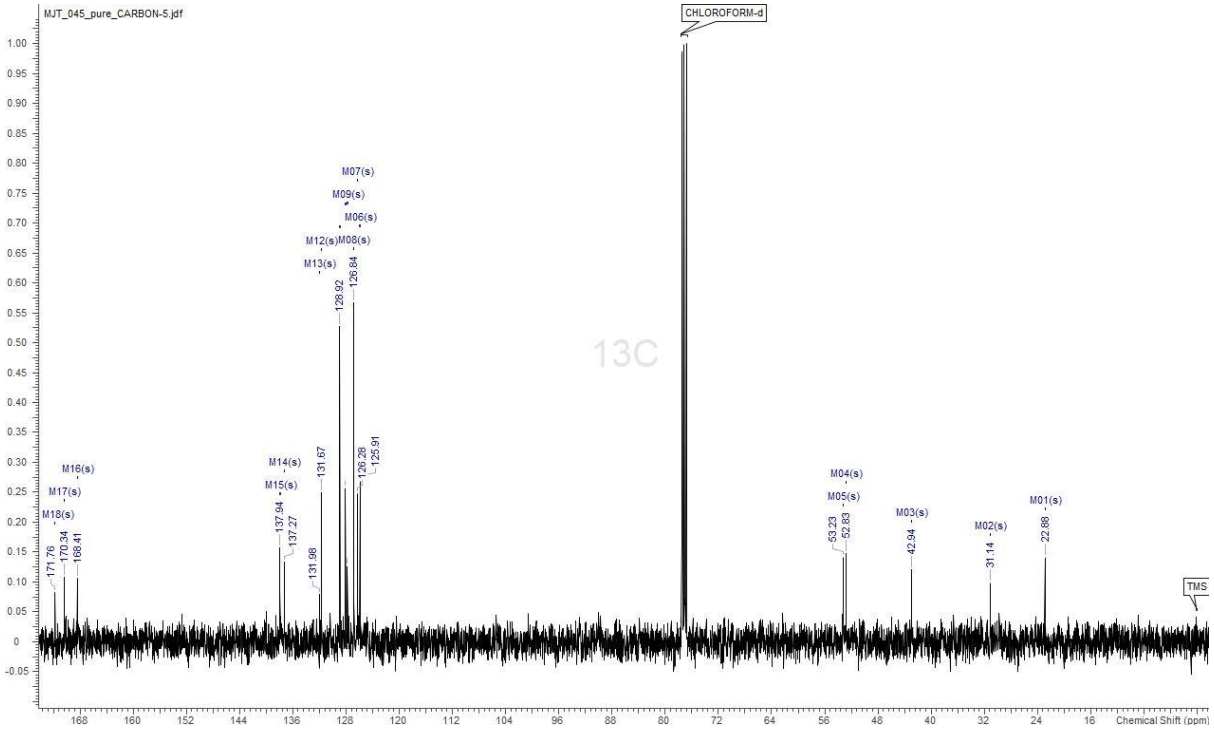
Annex 12. ^{1}H NMR spectrum of P33, Ac-Phe-Leu-OMe.



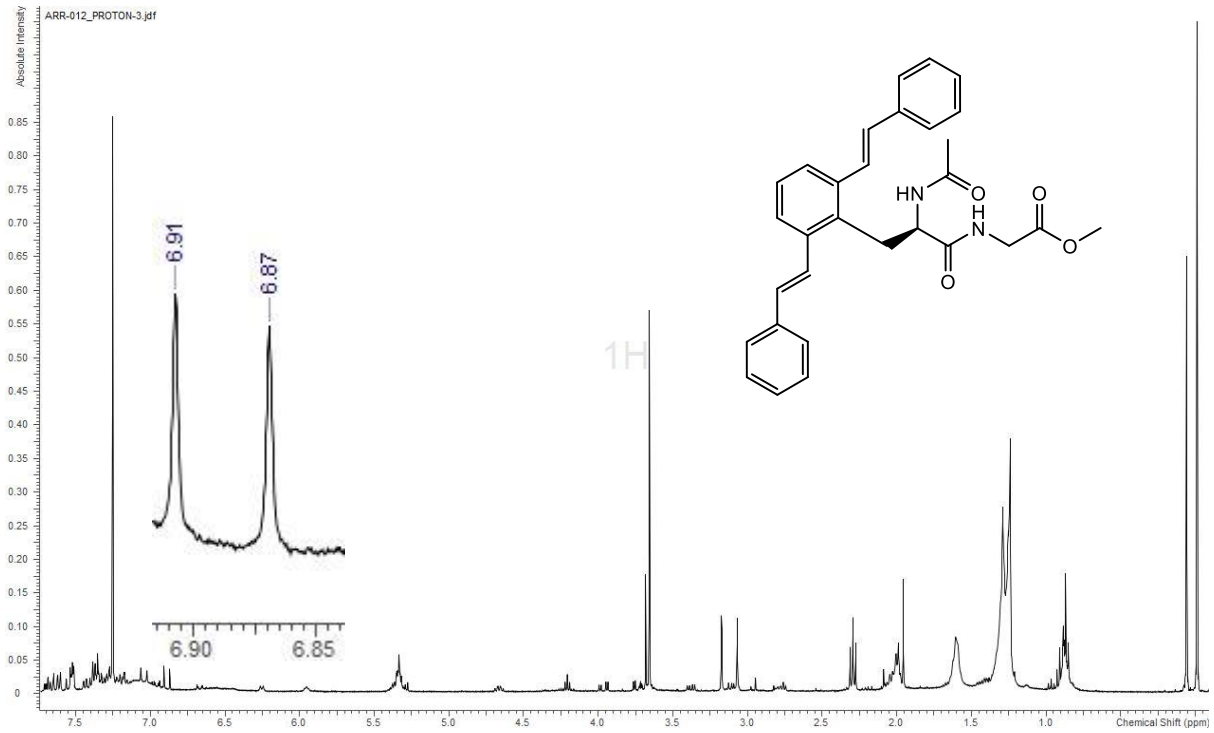
Annex 13. ^{13}C NMR spectrum of P33, Ac-Phe-Leu-OMe.



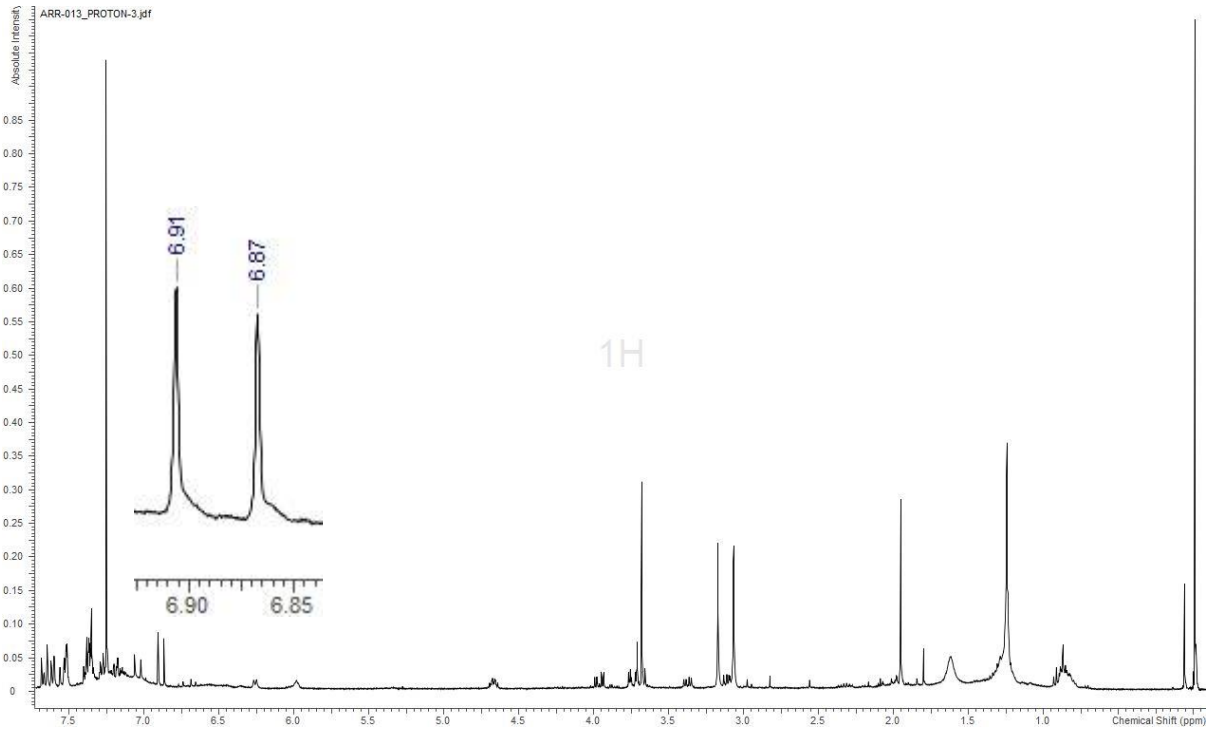
Annex 14. ^{1}H NMR spectrum of P4, diolefinated Ac-Gly-Phe-OMe.



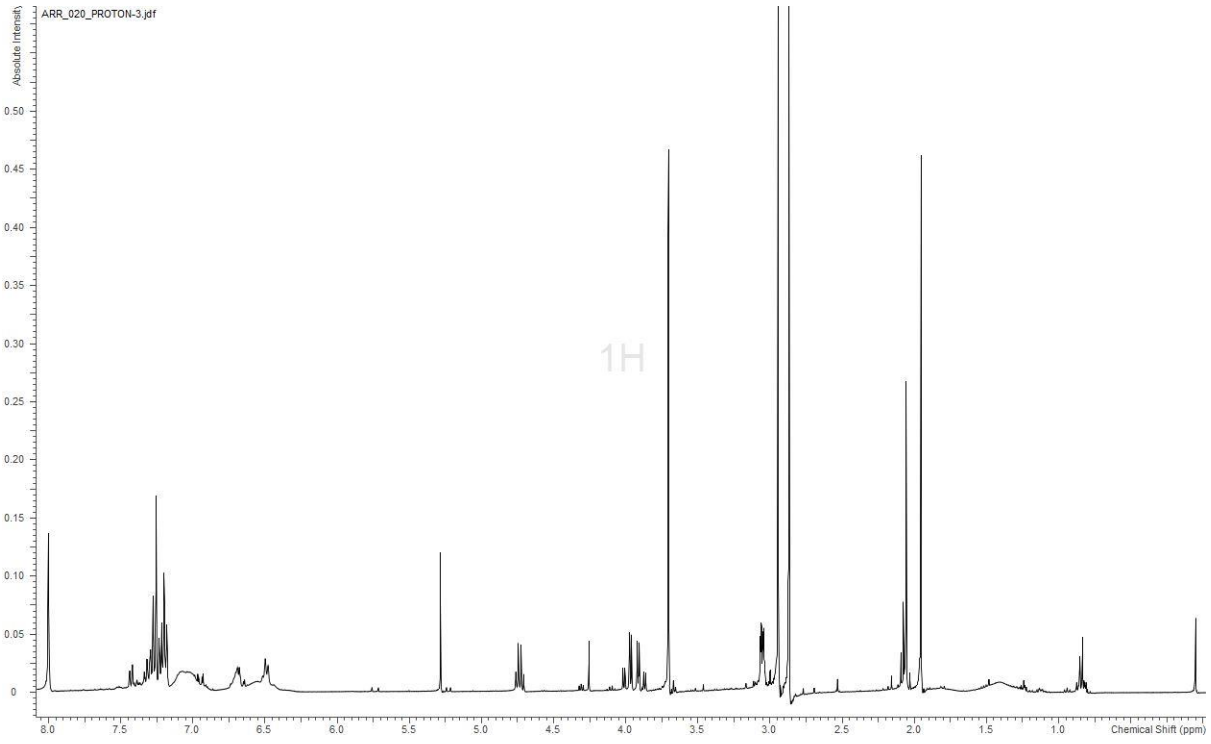
Annex 15. ^{13}C NMR spectrum of P4, diolefinated Ac-Gly-Phe-OMe.



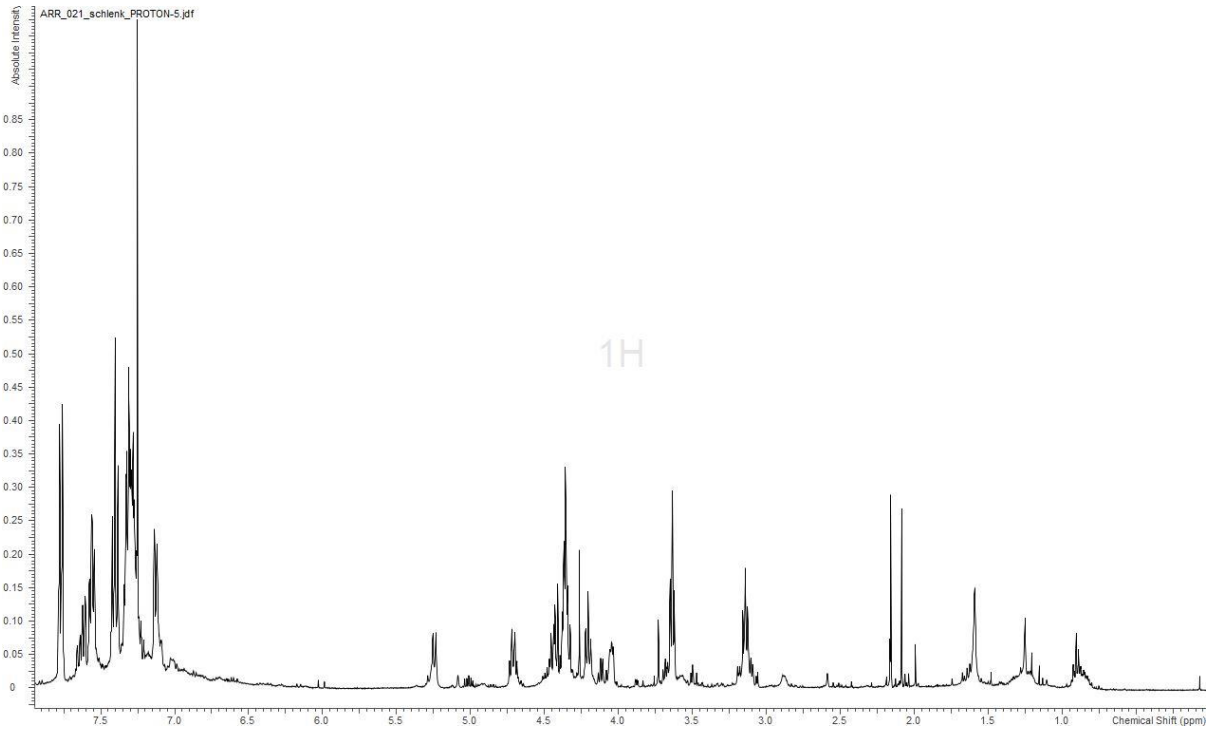
Annex 16. ^{1}H NMR spectrum of P12.



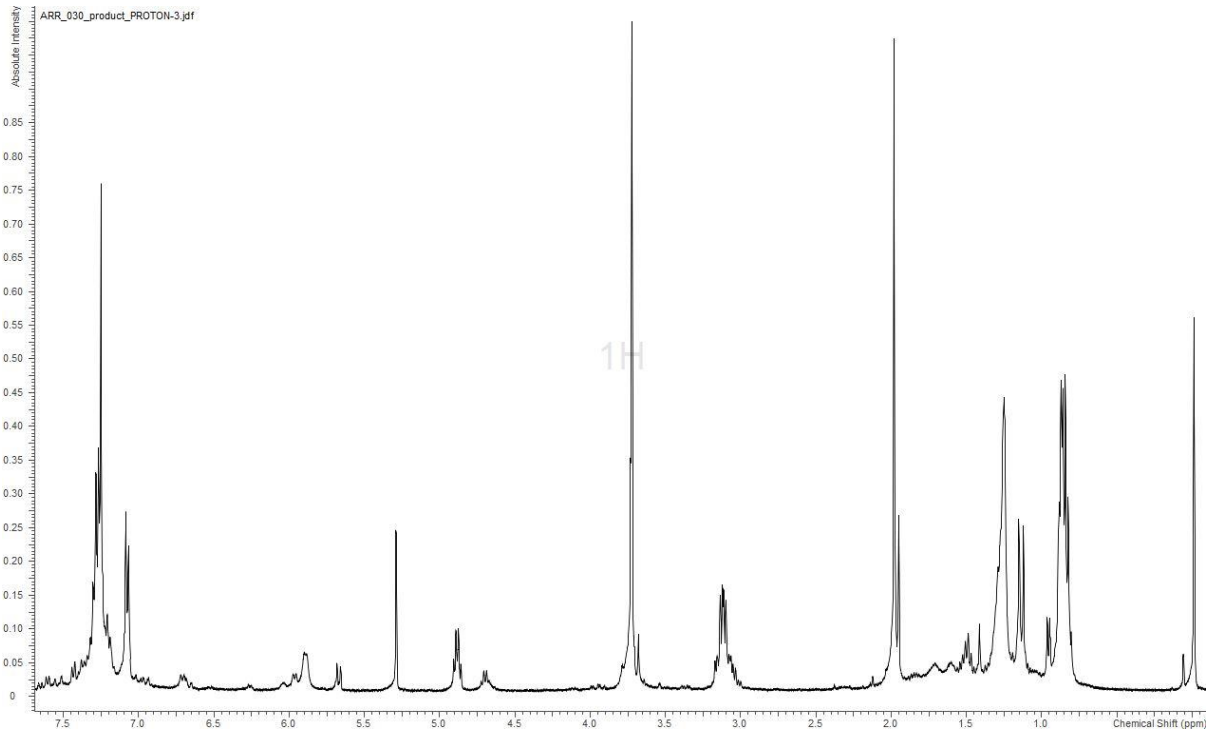
Annex 17. ^{1}H NMR spectrum of P13.



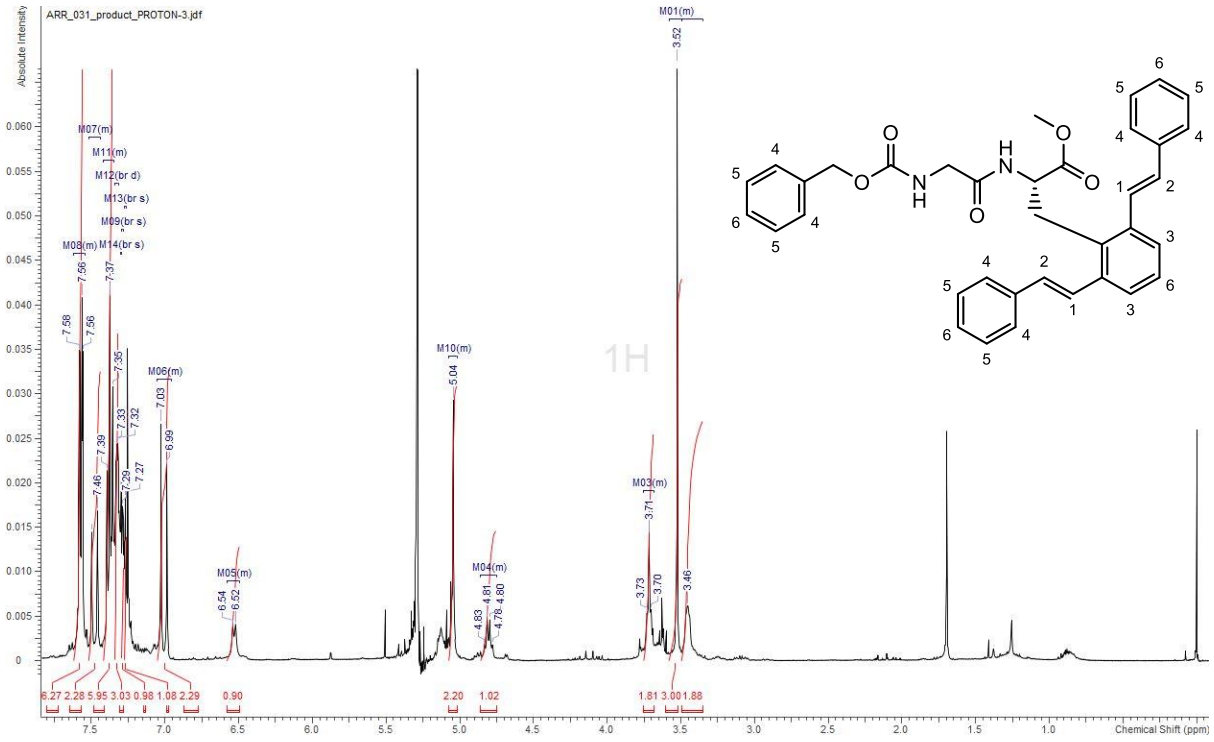
Annex 18. ^{1}H NMR spectrum of P20.



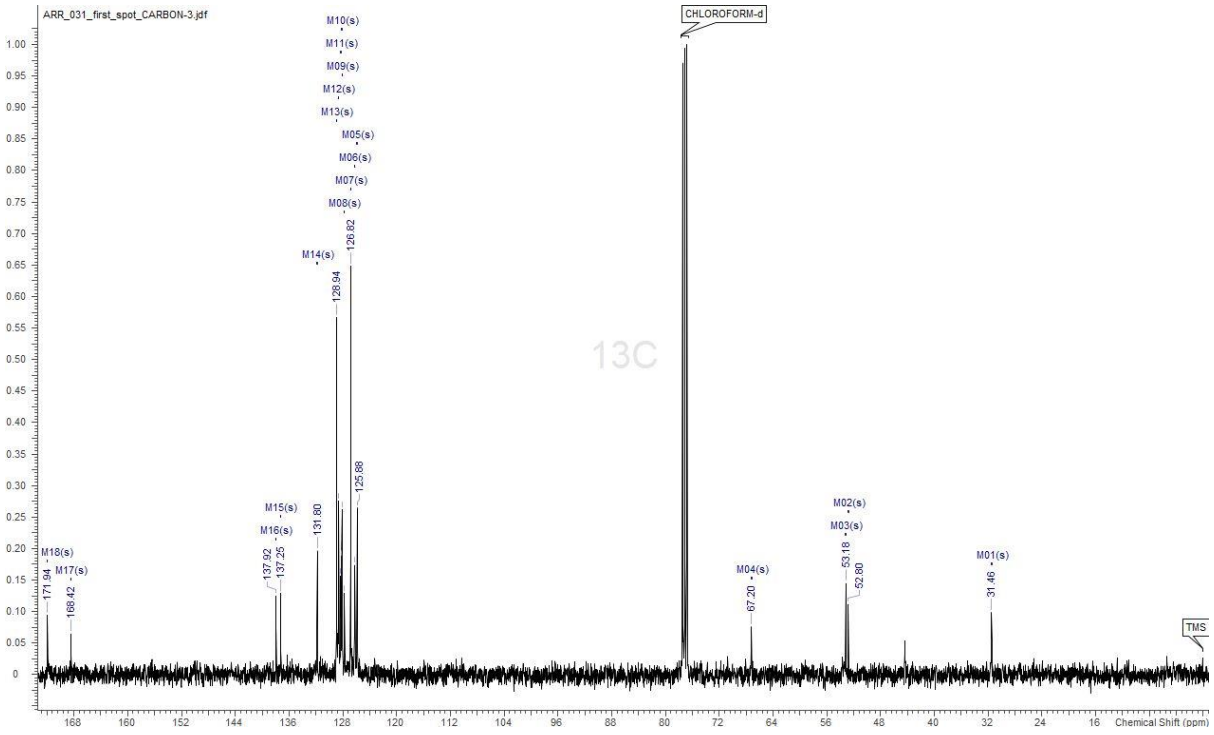
Annex 19. ^{1}H NMR spectrum of P21.



Annex 20. ^{1}H NMR spectrum of P30.



Annex 21. ^{1}H NMR spectrum of P31, diolefinated Cbz-Gly-Phe-OMe.



Annex 22. ^{13}C NMR spectrum of P31, diolefinated Cbz-Gly-Phe-OMe.