Appendices:

1. Academic CV
2. Academic achievements in the form of a list of publications with the impact factor (IF) in the year of publication and in the current year, number of citations, Hirsch index
3. Information on educational and organisational activities
4. Information on scientific activities and cooperation with institutional organisations in Poland and abroad
5. The summary of professional accomplishment is a monothematic description of a cycle of 14 publications
6. Statements of the co-authors and personal statements concerning publications with more than five co-authors
7. PhD degree diploma (copy)
8. Contact details
9. English version of the documents from 1 to 5

1. Academic CV of the applicant

Maciej, Marian Makowski
was born on the first of February nineteen fifty one (1 February 1951) in Muszyna where he finished Primary School in 1965

1965–1970:	Secondary School, Technical College in Tarnów–Mościce, ending in secondary school-leaving examinations
1971–1976:	Higher education at Powstańcy Śląscy Higher Pedagogical College, Faculty of Mathematics, Physics and Chemistry (two cycle studies, i.e.: 1971–1974: bachelor's degree, 1974–1976 – master's degree)
1976:	Master's degree with a distinction
1976–1978:	Assistant Lecturer, Department of Chemistry of Polymers, Institute of Chemistry, Higher Pedagogical College (head: prof. dr hab. inż. Maria Nowakowska)
1978–1986:	Assistant Lecturer, Senior Assistant Lecturer – Department of Organic Chemistry, Institute of Chemistry, Higher Pedagogical College (head: prof. dr hab. inż. Barbara Rzeszotarska)
1986:	PhD in Chemical Sciences, University of Warsaw, Faculty of Chemistry Doctoral thesis title: "Synthesis of Peptides with α,β–Dehydroamino Acids"

1990–1999: Assistant Professor, Department of Organic Chemistry, Higher Pedagogical College

1999–2017: Assistant Professor, Department of Analytical and Ecological Chemistry, Faculty of Chemistry, Opole University

Awards and distinctions:

1980 and 1982: Awards of the Vice–Chancellor of the Higher Pedagogical College in Opole for academic achievements

2015: Award of the Vice–Chancellor of Opole University for academic achievements

Maciej Makowski

2. Academic achievements:

1. M.L. Główka, G. Gilli, V. Bertolasi, **M. Makowski**
Structure of an α,β–Unsaturated Dipeptide, Racemic N–[(Phenylmetoxy)carbonyl] phenylalanyl–Δ(Z)phenylalanine
Acta Cryst.,**1989**, *C43*, 1403.

[IF 0.492 ; 0.403]

2. Z. Kubica, B. Rzeszotarska, **M. Makowski**, M. L.Główka, Z. Gałdecki
Synthesis of Peptides with α,β–Dehydroamino Acid, IV Crystal Structure of (E)–Trifluoroacetylglycyldehydrophenylalanine and the Assignment of the Geometry of Dehydrophenylalanine Residues
Pol. J. Chem., **1988**, *62*, 107.

[IF 0.422 ; 0.444]

3. K. Pawełczak, **M. Makowski**, M. Kempny, J. Dzik. M. Balińska, W. Rode
Sulfonamide Antifolates Inhibiting Thymidylate Synthase: Synthesis, Enzyme Inhibition and Cytotoxicity
Adv. Exp. Med. Biol. **1993**, *388*, 625–628.

[IF 1.825 ; 1.811]

4. T. Kowalik-Jankowska, H. Kozłowski, L. D. Pettit, K. Pawełczak, **M. Makowski**
Binding Ability of N–para–amino–phenylsulfonyl Derivatives of Amino Acids: Potentiometric and Spectroscopic Studies of Cu(II) Complexes
J. Inorg. Biochem. **1995**, *57*, 183–190.

[IF 1.478 ; 3.197]

5. T. Kowalik-Jankowska, H. Kozłowski, K. Pawełczak, **M. Makowski**
N-p-Amino and N–p–Nitrophenylsulfonyl Derivatives of Dipeptides: a New Family of Ligands for Cooper(II)
J. Chem. Soc. DALTON TRANS. **1995**, 2729–2733.

[IF 1.972 ; 2.820]

6. J. Cieśla, B. Gołos, M. Dzik, K. Pawełczak, M. Kempny, **M. Makowski**, M. Bretner, T. Kulikowski, B. Machnicka, B. Rzeszotarska, W. Rode
Thymidylate Synthases from *Hymenolepsis Dyminuta* and Regenerating Rat Liver: Purification, Properties and Inhibition by Substrate and Cofactor Analogues
Biochim. Biophys. Acta **1995**, *1294*, 127–136

[IF 2.507 ; 4.134]

7. J. Swiątek-Kozłowska, J. Brasuń, L. Chruściński, E. Chruścińska, **M. Makowski,** H. Kozłowski
Impact of α,β–dehydroamino Acids Residues on the Binding Abilities of Di–, Tri–, and Tetra– peptides
New J. Chem. , **2000,** *24*, 893–896

[IF 3.009 ; 2.966]

8. K. Ejsmont**, M. Makowski**, J. Zalewski
N–(tert-Butoxycarbonylglycyl–α,β–dehydrophenylalanylglycylphenylalanyl)–4–nitroaniline
Acta Crys. **2001,** *C57*, 205–207

[IF 0.570 ; 0.492]

9. **M. Makowski**, M. Pawełczak, R. Latajka, K. Nowak, P. Kafarski
Synthesis of Tetrapeptides p-nitrophenylanilides Containing Dehydroalanine and Dehydrophenylalanine, and Their Influence on Cathepsin C Activity
J. Peptide Sci., **2001**, *7*, 141–145

[IF 1.451 ; 2.071]

10. K. Pawełczak, **M. Makowski**, M. Kępny, J. M. Dzik, B. Gołos, W. Rode, B. Rzeszotarska
Sulfamide Antifolates Inhibiting Thymidylate Synthase: Synthesis, Enzyme Inhibition and Cytotoxicity
Acta Biochimica Polonica, **2002**, *49*, 407–420

[IF 0.600 ; 1.185]

11. J. Swiątek-Kozłowska, J. Brasuń, M. Łuczkowski, **M. Makowski**
Binding Abilities of Dehydropeptides towards Cu(II) and Ni(II) Ions: Impact of Z–E Isomerization on Metal Ion Binding
J. Inorg. Biochemistry, **2002**, *90*, 106–112

[IF 2.204 ; 3.197]

12. **M. Makowski,** K. Pawełczak, P. Kafarski, J. M. Dzik, B. Gołos, M. Balinska, W. Rode
Quinazoline Antifolate Thymidylate Synthase Inhibitors: Replacement of Glutamic Acid by Aminophosphonic Acids
Phosphorus, Sulphur, and Silicon, **2003** *178*, 1639–1651

[IF 0.323 ; 0.601]

13. J. Brasuń**, M. Makowski**, S. Ołdziej, J. Świątek-Kozłowska.
Coordination Ability of Pentapeptides with two Dehydro-amino Acid Residues Inserted into Their Sequences
J. Inorg. Biochemistry, **2004**, *98*, 1391–1398

[IF 2.225 ; 3.197]

14. **M. Makowski**, A. Brzuszkiewicz, M. Lisowski, T. Lis
N–[tert-Butoxycarbonylglycyl–(Z)–α,β–dehydrophenylalanylglycyl–(E)–α,β dehydrophenylalanylphenylalanyl]–4–nitroaniline Ethanol Solvate
Acta Cryst. **2005** *C61*, 0424-0426

[IF 0.777 ; 0.492]

16. J. Brasuń, M. Makowski, A. Janicka, J. Świątek-Kozłowska
Influence of the Position of Two Dehydro–amino AIDS Residues in the Oligopeptide Sequence the Binding Ability Aowards Cu(II) Ions
Polyhedron **2005** *24,* 1929–1936

[IF 1.957 ; 1.813]

15. R. Latajka, **M. Makowski**, M. Jegwinski, M. Pawełczak, H. Koroniak, P. Kafarski
Peptide p–nitroanilides Containing (E)-dehydrophenylalanine-synthesis, Structural Studies and Evaluation of their Activity towards Cathepsin C
New J .Chem., **2006,** *30,* 1009–1018

[IF 2.647 ; 2.966]

18. **M. Makowski**, M. Lisowski, A. Maciąg, T. Lis
N–[tert–Butoxycarbonylglycyl–(Z)–α,β–dehydrophenylalanylglycyl–(E)–α,β-dehydro–phenylalanyl]glycine Methyl Ester Dehydrate
Acta Cryst., **2006**, *E62*, 0807–0810

[IF 0.896 ; 0.552]

19. **M. Makowski**, M. Lisowski, I. Mikołajczyk, T. Lis
N–[tert-Butoxycarbonylglycyl–(E)–α,β–dehydrophenylalanylglycylglycyl–(E)–α,β dehydrophenylalanyl]glycine
Acta Cryst., **2007**, *E63*, 019–021

[IF 0.719 ; 0.552]

20. **M. Makowski,** M. Lisowski, I. Mikołajczk, T. Lis
N–[tert–Butoxycarbonylglycyl–(Z)–α,β–dehydrophenylalanylglycyl–(Z)–α,β–dehydrophenylalanyl]glicine Methyl Ester
Acta Cryst., **2007**, *E63*, 0989-0991

[IF 0.719 ; 0.552]

21. K. Ejsmont, R. Gajda, **M. Makowski**
Conformation of Tert–butoxycarbonylglycyl–dehydroalanyl–glyccine Methyl Ester in the Crystalline State and Calculated in the Gas Phase
Acta Cryst., **2007**, *C63*, 080–083

[IF 0.719 ; 0.432]

22. **M. Makowski**, M. Lisowski, I. Mikołajczyk, T. Lis
N–[Glycyl–(Z)–α,β–dehydrophenylalanylglycyl–(Z)–α,β-dehydrophenylalanyl]glycine trifluoroacetate Methanol Solvate
Acta Cryst., **2007,** *E63*, 02709–02710

[IF 0.719 ; 0.552]

23. R. Latajka, M. Jegwiński, **M. Makowski**, M. Pawelczak, T. Hubert, N. Sewald, P. Kafarski
Pentapeptides Containing Two Dehydrophenylalanine Residues: Synthesis, Structural Studies and Evaluation of their Activity towards Cathepsin C
J. Peptide Sci., **2008**, *14*, 1084–1095

[IF 1.828 ; 2.071]

24. R. Latajka, M. Jegwiński, **M. Makowski**, A. Krężel
Conformational Studies of Hexapeptides Containing Two Dehydroamino Acid Residues in Position 3 and 5 in Peptide Chain
J. Molecular Structure **2008,** *892*, 446–451

[IF 1.594 ; 1.404]

25. R. Latajka, M. Jegwinski, **M. Makowski**, A. Krężel, S. Paluch
Conformational Studies of Hexapeptides Containing Two Dehydroamino Acid Residues in Position 2 and 5 in Peptide Chain
Biopolymers, **2008**, *89*, 691–699

[IF 2.823 ; 2.879]

26. M. Lisowski, R. Latajka, B. Picur, T. Lis, I. Bryndal, M. Rospenek, **M. Makowski**, P. Kafarski
Combined Effect of the ΔPhe or ΔAla Residue and the p–Nitroanilide Group on a Dehydropeptides Conformation
Biopolymers, **2008**, *89*, 220–234

[IF 2.823 ; 2.879]

27. **M. Makowski**, M. Lisowski, A. Maciąg, M. Wiktor, A. Szlachcic, T. Lis
Two Pentadehydropeptides with Different Configuration of the ΔPhe Residues
Acta Cryst., **2010,** *C66*, 0119–0123.

[IF 0.403 ; 0.492]

28. M. Lisowski, Ł. Jaremko, M. Jaremko, A. Mazur, R. Latajka, **M. Makowski**
Effect of the ΔPhe Residue Configuration on a Dedehydropeptides Conformation: a Combined CD and NMR Study
Biopolymes, **2010**, *93*, 1055–1064.

[IF 2.691 ; 2.879]

29. P. Lenartowicz, **M. Makowski**
Inhibitory katepsyny C [Cathepsin C Inhibitors]
Na pograniczu chemii i biologii [On the Borderline between Chemistry and Biology] (**2011**) *Vol. XXVII*, 253–261.

30. K. Małek, **M. Makowski**, A. Królikowska, J. Bukowska
Comparative Studies on IR, Raman and Surface Enhanced Raman Scattering: Spectroscopy of Dipeptides Containing ΔAla and ΔPhe
J. Phys. Cem. B **2012**, *116*, 1414–1425.

[IF 3.702 ; 3.607]

31. K. Małek, **M. Makowski**
The Infrared and Raman Spectra of Solid Tirdehydropeptides: Influence of ΔAla and ΔPhe on the Spectral Profile
Vibrational Spectroscopy **2012**, *60* 73–78.

[IF 1.978 ; 1.747]

32. B. Oszywa, **M. Makowski**, M. Pawełczak
Purification and Partial Characterisation of Aminopeptidase from Barley (Hordeum vulgare L.) Seeds
Plant Phisiology and Biochemisry **2013**, *63* 75–80.

[IF 2.775]

33. M. Jegwiński, R. Latajka, A. Krezel, K. Haremza, **M. Makowski**, P. Kafarski
Influence of Solvents on Conformation of Dehydropeptides
Journal of Molecular Structure **2013**, *1035*, 129–139.

[IF 1.404]

34. A. Brzuszkiewicz, **M. Makowski**, M. Lisowski, E. Lis, M. Otreba, T. Lis
Two Phosphonodehydrotripeptides:Boc–Gly–Δ(Z)Phe–α–AbuPO$_3$Me$_2$, Boc–Gly–Δ(Z)Phe–α–NvaPO$_3$Et$_2$
Acta Cryst., **2013,** *C69*, 277–281.

[IF 0.492]

35. A. Buczek, **M. Makowski**, M. Jegwiński, R. Latajka, T. Kupka, M.A. Broda
Toward Engineering Efficient Peptidomimetics: Screening Conformational Landscape of Two Modified Dehydroamino Acids
Biopolymers **2013 DOI** 10.1002/bip.22264

[IF 2.879]

36. M. Jaremko, Ł. Jaremko, A. Mazur, **M. Makowski**, M. Lisowski
Enhanced β–turn conformational stability of tripeptides containing ΔPhe in *cis* over *trans* Configuration
Amino Acids **2013**, *45*, 865–875

[IF 3.913]

37. P. Lenartowicz, **M. Makowski**, B. Zarychta, K. Ejsmont
Crystal Structure of N–(tert–butoxycarbonyl)glycyl–(Z)–β-bromodehydroalanine Methyl Ester [Boc-Gly– (β)Br)$^{(Z)}$ΔAla–OMe]
Acta Cryst. **2014,** *E70*, 596–598

[IF 0.347]

38. P. Lenartowicz, **M. Makowski**, B. Zarychta, K. Ejsmond
Crystal Structure of N–(tert-butoxycarbonyl)–phenylalanyldehydroalanine Isopropyl Ester (Boc-Phe–ΔAla–OiPr)
Acta Cryst. **2014**, *70E,* 599–602

[IF 0.350]

39. M. Jegwiński, J. Krzciuk-Gula, **M. Makowski**, R. Latajka, P. Kafarski
Conformation of Dehydropentapeptides Containing Four Achiral Amino Acids Residues: Controlling the Role of L-Valine
Beilstain J. Org. Chem., **2014,** 10, 660–666

[IF 2.801]

40. **M. Makowski**, P. Lenartowicz, B. Oszywa, M. Jegwiński, M. Pawełczak, P. Kafarski
Synthesis of Dehydrodipeptide Esters and Their Evaluation as Inhibitor of Cathepsin C
Med. Chem. Res. **2015,** 24 (8), 3157–3165

[IF 1.402]

41. **M. Makowski**, M. Jegwiński, J. Hurek, A. Paliwoda, P. Kafarski
Kinetics of Photochemical Isomerization of TFA–Gly–^{Z}ΔPhe into TFA–Gly–^{E}ΔPhe
Arkivoc **2017**, part iv, 88–94

[IF 1.096]

The *Total Impact Factor* of the scientific publications as per the list in the *Journal Citation Reports* (JCR), according to the year of publication: **69.212**

Number of citations of the publications according to the *Web of Science* (WOS) database:
total – **362**
without self-citations – **248**

Hirsch index of the publications according to the *Web of Science* database: **12**

Maciej Makowski

Complete achievements (scientific output) of the author include as follows:

— 49 publications, including 48 in the *Journal Citation Reports* database
— 43 conference presentations
— Participation in six research projects
— Grants in 1992–1998, KBN [State Committee for Scientific Research] No. 6 6253 92 03/02 and No. 6 6254 92 03, "Synthesis, Reactivity and Citostaticity of Quinazoline Inhibitors of Thymidylate Synthase" – author and main author
— Own grant in 2005–2008 No. 0474/P04/2005/29: „Design and Reactivity of Low-Molecular Gingipain Inhibitors" – main author
— Own grant in 2005–2008 No. 2 P04B 020 29 "Inhibitors and Substrates of Cathepsin C" – main author
— Own grant in 2009–2011 No. NN 204 333 037 "Molecular Structure and Manner of Adsorption of Dehydropeptides in Vibrational Spectroscopy" – main author
— 2010–2014: grant No. 01.01.02–02–003/08WCB/5/I/2010 "Therapy of Diseases of Affluence – Innovative Anti-cancer and Anti-osteoporosis Drugs" – main author

3. Educational and organisational activities

Academic supervision over students – activities connected with designing, providing guidance and assisting in writing of master's theses

Academic supervision over mgr Paweł Lenartowicz, a doctoral student, as assistant thesis supervisor from 1 October 2010 to September 2016; title of the PhD thesis:

"Synthesis of Peptide Inhibitors of Cathepsin C"

Organisational unit responsible for education of the doctoral student:

Wrocław University of Technology, Faculty of Chemistry

Organisational unit under which the doctoral thesis was written:

Opole University, Faculty of Chemistry, Department of Analytical and Ecological Chemistry

Planning and preparation of appropriate pyrotechnic materials and demonstrations at the Opole Science Festivals

Paweł Kafarski

4. Scientific activities and cooperation with institutional organisations in Poland and abroad

It concerns the former and present cooperation with persons from the following research centres:

Wrocław University of Technology, Faculty of Chemistry, Institute of Bioorganic Chemistry
– Prof. Paweł Kafarski
– Dr hab. Rafał Latajka
– Dr Michał Jegwiński

Structural Research Laboratory:
– Dr Sławomir Paluch

University of Wrocław, Faculty of Chemistry:
– Prof. Henryk Kozłowski
– Prof. Tadeusz Lis
- Dr hab. Marek Lisowski
-

Faculty of Biotechnology:
- Prof. Artur Krężel

Jagiellonian University, Faculty of Chemistry:
– Dr Kamila Małek

Medical University, Faculty of Basic Medical Examinations, Wrocław
– Prof. Jolanta Światek-Kozłowska
– Dr hab. Justyna Brasuń

University of Gdańsk, Faculty of Chemistry:
– Dr hab. Stanisław Ołdziej

Nencki Institute, Experimental Biology, Warszawa:
– Prof. Wojciech Rode
– Dr hab. Małgorzata Balińska
– Dr hab. Jolanta M. Dzik

Adam Mickiewicz University, Faculty of Chemistry, Poznań:
– Prof. Henryk Koroniak

University of Bielefeld, Department of Chemistry, Germany:
– Prof. Norbert Sewald

Maciej Makowski

Habilitation work entitled:

"Synthesis, Structure and Biological Activity of Peptides with α,β-Dehydroamino Acids"

The habilitation work is based mainly on the following publications:

1. J. Brasuń, **M. Makowski**, S. Ołdziej, J. Świątek-Kozłowska
Coordination Ability of Pentapeptides with Two Dehydro–amino Acid Residues Inserted into Their Sequences
J. Inorg. Biochemistry, **2004**, *98*, 1391–1398

[IF 2.225 ; 3.197]

2. J. Brasuń, **M. Makowski**, A. Janicka, J. Świątek-Kozłowska
Influence of the Position of Two Dehydro-amino Acids Residues in the Oligopeptide Sequence on the Binding Ability towards Cu(II) Ions
Polyhedron **2005,** *24,* 1929–1936

[IF 1.957 ; 1.813]

3. R. Latajka, **M. Makowski**, M. Jegwinski, M. Pawełczak, H. Koroniak, P. Kafarski
Peptide p–nitroanilides Containing (E)–dehydrophenylalanine–synthesis: Structural Studies and Evaluation of Their Activity towards Cathepsin C
New J. Chem., **2006**, *30,* 1009–1018

[IF 2.647 ; 2.966]

4.R. Latajka, M. Jegwinski, **M. Makowski**, M. Pawelczak, T. Huber, N. Sewald, P. Kafarski
Pentapeptides Containing Two Dehydrophenylalanine Residues: Synthesis, Structural Studies and Evaluation of Their Activity towards Cathepsin C
J. Peptide Sci., **2008**, *14*, 1084–1095

[IF 1.828 ; 2.071]

5. R. Latajka, M. Jegwiński, **M. Makowski**, A. Krężel
Conformational Studies of Hexapeptides Containing Two Dehydroamino Acid Residues in Position 3 and 5 in Peptide Chain
J. Molecular Structure, **2008,** *892,* 446–451

[IF 1.594 ; 1.404]

6. R. Latajka, M. Jegwinski, **M. Makowski**, A. Krężel, S. Paluch
Conformational Studies of Hexapeptides Containing Two Dehydroamino Acid Residues in Position 2 and 5 in Peptide Chain
Biopolymers, **2008**, *89*, 691–699

[IF 2.823 ; 2.879]

7. M. Lisowski, R. Latajka, B. Picur, T. Lis, I. Bryndal, M. Rospenk, **M. Makowski**, P. Kafarski
Combined Effect of the ΔPhe or ΔAla Residue and the p-Nitroanilide Group on a Dehydropeptides Conformation
Biopolymers, **2008**, *89*, 220–234

[IF 2.823 ; 2.879]

8. M. Lisowski, Ł. Jaremko, M. Jaremko, A. Mazur, R. Latajka, **M. Makowski**
Effect of the ΔPhe Residua Configuration on a Didehydropeptides Conformation: a Combined CD and NMR Study
Biopolymers, **2010,** *93*, 1055–1064.

[IF 2.691 ; 2.879]

9. A. Buczek, **M. Makowski**, M. Jegwiński, R. Latajka, T. Kupka, M. A. Broda
Towards Engineering Efficient Peptidomimetics: Screening Conformational Landscape of Two Modified Dehydroamino Acids
Biopolymers, **2013,** 101, 28–40

[IF 2.879]

10. M. Jaremko, Ł. Jaremko., A. Mazur, **M. Makowski**, M. Lisowski
Enhanced β-turn Conformational Stability of Tripeptides Containing ΔPhe in *cis* over *trans* Configuration
Amino Acids **2013**, *45*, 865–875

[IF 3.196]

11. M. Jegwiński, J. Krzciuk-Gula, **M. Makowski**, R. Latajka, P. Kafarski
Conformation of Dehydropentapeptides Containing Four Achiral Amino Acids Residues: Controlling the Role of L-Valine
Beilstain J. Org. Chem., **2014,** 10, 660–666

[IF 2.801]

12. **M. Makowski**, P. Lenartowicz, B. Oszywa, M. Jegwiński, M. Pawełczak, P. Kafarski
Synthesis of Dehydrodipeptide Esters and Their Evaluation as Inhibitor Cathepsin C
Med. Chem. Res. **2015,** 24 (8), 3157–3165

[IF 1.436]

13. **M. Makowski**, M. Jegwiński, J. Hurek, A. Paliwoda, P. Kafarski

Kinetics of Photochemical Isomerization of TFA–Gly–^{Z}ΔPhe into TFA–Gly–^{E}ΔPhe
Arkivoc **2017**, part iv, 88–94

14. Unpublished data.

5. Summary of Professional Accomplishments

The habilitation concerns syntheses of dehydropeptides and phosphonodehydroalanine, which were designed for the purposes of investigation of their properties:

- Biochemical – activity in relation to cathepsin C
- Structural, in particular the impact of dehydroamino acids residues on conformations of dehydropeptides in comparison with classical peptides
- Responsible for complex formation of heavy metal ions, in particular Cu(II) and Ni(II)
- Isomerisation of isomer (E) into (Z) and (Z) into (E)
- Using reactivity of the double bond: synthesis of peptidomimetics which are potential inhibitors of cathepsin C

Contrary to what it would seem, the synthesis of peptides with α,β-dehydroamino acids residues is not trivial.

The presence of double bonds in the side chain of these peptides causes vulnerability of this fragment of peptide to radical reactions and addition reactions, both nucleophilic and electrophilic. Thus, they become *locus minoris resistantiae*. This is a fundamental reason why there is no fully documented information in the literature or in my publications on isolation and identification of many by–products, which are created during syntheses of dehydropeptides whose presence is confirmed by thin-layer chromatography (TLC) and high–performance liquid chromatography (HPLC). Therefore, what becomes an important future challenge is to take up research concerning this area of knowledge which is so much uninvestigated.

π electron couplings of a fragment of dehydroamino acid with electrons of a peptide bond effectively deactivate the amino and carboxyl groups, in particular in peptides with alkyl α,β–dehydroamino acids residues.

This type of coupling has a much smaller impact on the deactivation of α,β–dehydroaminophenylalanine residue due to the presence of an aromatic ring.

Consequently, this deactivation has a major impact on the efficiency of the peptide bond created in the synthesis reaction and is a reason stimulating an optimum choice and selection of appropriate activators, i.e. such activators which would make it possible to achieve a large (high) yield of the synthesised dehydropeptides with a minimum amount of accompanying by–products. This should make their purification and extraction (isolation) considerably easier.

Hence, I believe that the fact of diverse adaptation of activators, selected by myself and presented in the further part of the discussion, was fully justified. The criteria of their selection were as follows:

— Yield of the coupling reaction
— Quantity and type of accompanying by-products
— Avoiding difficulties associated with isolation of the obtained dehydropeptides
— Toxicity of the activator used (to a lesser extent)

Substrates for the Syntheses of Dehydropeptides: Synthesis of Dehydropeptide Esters

TFA–Gly–$\Delta^{(E)}$Phe was obtained in condensation reaction of TFA–Gly–NH_2 with phenylpyruvic acid in benzene and crystallisation from ethyl acetate and hexane (a yield of 10%) or as a result of photoisomerisation of TFA–Gly–$\Delta^{(Z)}$Phe. A solution of dehydrodipeptide and benzophenone in acetone and benzene was being exposed to radiation with a wavelength of 366 nm for 80 hours. Isomer (E) was obtained with a yield of 94%. However, as a result of exposure to radiation with a wavelength of 254 nm, after 9 days, isomer (E) was obtained with a yield of 61%.[14] Deprotection of trifluoroacetyl protecting group with ammonia and locking of the amino group with *tert*–butyloxycarbonyl (Boc–) protecting group makes it possible to obtain **Boc–Gly–$\Delta^{(E)}$Phe** used in further syntheses.

Dehydropeptide esters were obtained in standard reactions also used in classical synthesis of peptides. Reduced reactivity of the carboxyl residue required selection of appropriate methods of synthesis, in particular in the case when they concerned a synthesis of unusual, reactive esters, designed as potential inhibitors of cathepsin C.

1. TFA*Gly–ΔAla–OMe was obtained in the reaction of caesium chloride of Boc–Gly–ΔAla–OH with CH_3I in dichloromethane (DCM) after standard deprotection of Boc– protecting group with trifluoroacetic acid (TFA) with addition anisole (5%v/v) preventing the oligomerization of this dehydrodipetide. **Diagram 1**[12]

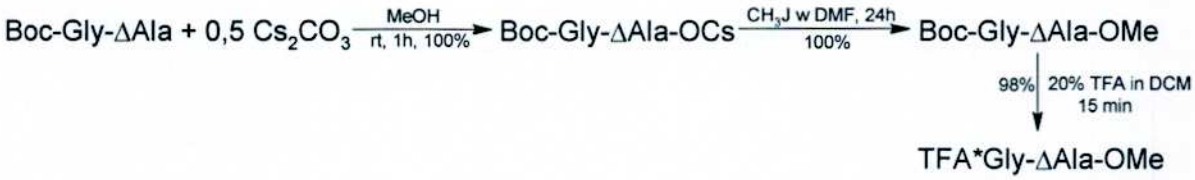


Diagram 1

This method was used for syntheses of allyl (–All), propargyl (–Prg) and glycidyl (–Gdl) esters of dehydropeptides:

[In the case of this method of synthesis of dehydropeptides with C–terminal residue of isomer (E) of dehydrophenylalanine, its isomerisation into isomer (Z) is avoided.]

From **Boc–Gly–ΔAal–OAll**, after deprotection of Boc– protecting group, **Tos *Gly–ΔAal–OAll** was obtained (72%).[12]

Boc–Gly–ΔAla–OPrg Tos*Gly–ΔAla–OPrg 71%[12]

Boc–Gly–$\Delta^{(E) \text{ and } (Z)}$Phe–OAll 99% **TFA* Gly-$\Delta^{(E) \text{ and } (Z)}$Phe–OAll** 88%[12]
Boc–Gly–$\Delta^{(E) \text{ and } (Z)}$Phe–OPrg 96% **TFA*Gly-$\Delta^{(E) \text{ and } (Z)}$Phe–OPrg** 92%[12]
Boc–Gly–$\Delta^{(Z)}$Phe–OGdl(R)[12]
Boc–Gly–$\Delta^{(Z)}$Phe–OGdl(S)[12]

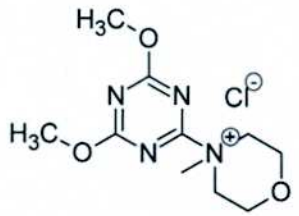
In deprotection reaction of Boc– protecting group from the above dehydrodipeptides using trifluoroacetic acid, opening of the oxirane ring takes place with the creation of 2,3–dihydroxypropyl ester [TFA* Gly-$\Delta^{(Z)}$Phe–$OCH_2CH(OH)CH_2OH$] (~50%).

In contrast, deprotection of HCl in methanol leads to the creation of 2–hydroxy–3–chloropropyl ester [HCl* Gly–$\Delta^{(Z)}$Phe–$OCH_2CH(OH)CH_2Cl$] (50%).

The suspected reason for the existence of such a large fraction of by–products was the presence of water in the reaction environment.

Esterification using DMTMM

DMTMM (Fig. 1) is not normally used in the synthesis of peptide esters. Nevertheless, its use made it possible to obtain the desired esters, albeit with moderate yields. The mechanism of this reaction was shown in Figure 2.



4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride

DMTMM

Fig. 1

Mechanism of reaction of esterification of dehydropeptides with the participation of DMTMM **(Diagram 2)**

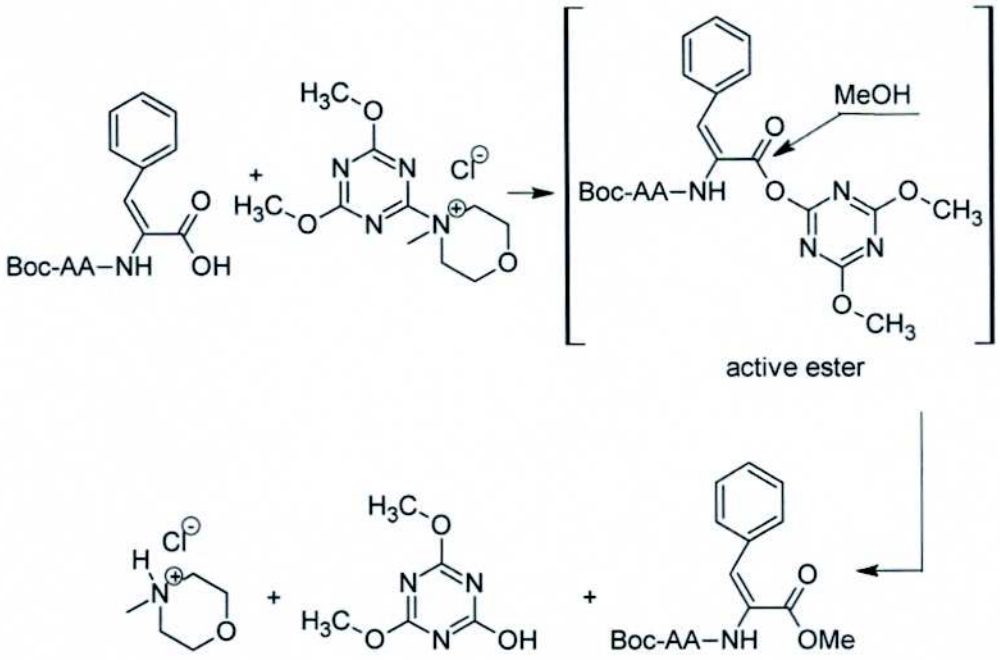


Diagram 2

During esterification reaction using the DMTMM method, with Boc–Gly–$\Delta^{(E)}$Phe as a substrate, its partial isomerisation into isomer (Z) is observed **(Diagram 3).** Isomers were divided on a chromatographic column with type H60 silica gel packing, eluting by means of a solution of ethyl acetate in benzene with increasing concentration gradient of ethyl acetate (EtOAc): from 2.5% to 20%.

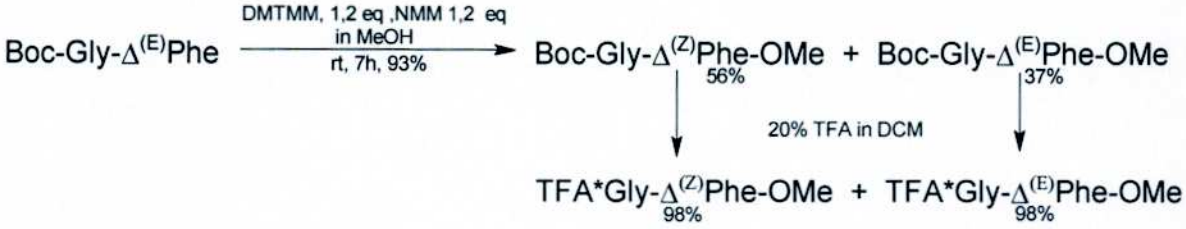


Diagram 3

2. TFA*Gly–$\Delta^{(Z)}$Phe–OMe was obtained after unlocking Boc– protecting group using trifluoroacetic acid from the obtained Boc–Gly–$\Delta^{(Z)}$Phe–OMe [a model making it possible to distinguish isomer (Z) from (E) on the basis of chromatography of TLC].

3. TFA*Gly–Δ$^{(E)}$Phe–OMe was obtained after deprotection of Boc– protecting group using trifluoroacetic acid, after division on a chromatographic column of methyl esters (E) and (Z) of dipeptides obtained in a reaction of Boc–Gly–Δ$^{(E)}$Phe with DMTMM (**Fig. 1**) (**Diagram 3**). Crystallisation with i–Pro: EtOAc(1/1v/v)/hexane[13].

Methods of creation of a peptide bond

A variety of methods of activation were used in the synthesis of dehydropeptides, whereas their selection depended on specific conditions described in detail below. What follows are the employed activations with the specification of their advantages and disadvantages.

Activation using DMTMM

Coupling with the participation of this reagent was used for the synthesis of Boc–Gly–Δ$^{(E)}$Phe–Gly–Phe–pNA dehydropeptide (**Diagram 4**) in type [2 + 2] condensation. After a 28–hour reaction, the desired product in the form of a mixture of stereoisomers was obtained with a total yield of 92%[4].

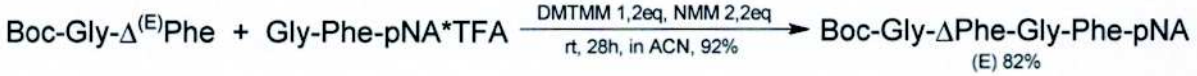


Diagram 4

In this reaction, behaviour resembling to a great extent that of the configuration (E) of C–terminal dehydrophenylalanine was observed, i.e. creation of a negligible amount of dehydropeptide, containing products of its isomerisation – (Z)-dehydrophenylalanine. It was a great advantage of this method. Unfortunately, too little reproducibility of coupling of C–terminal residue of (E)–dehydroalanine with other amino agents did not allow its wider application.

Adaptation of acidic (acyl) fluorides to syntheses of dehydropeptides

The use of acyl fluorides as a acylating agent was studied more extensively using Boc–Gly–Δ$^{(Z)\text{ and }(E)}$Phe–F as the substrate. The mechanism of reaction of creation of acidic fluorides was shown in (**Diagram 5**).

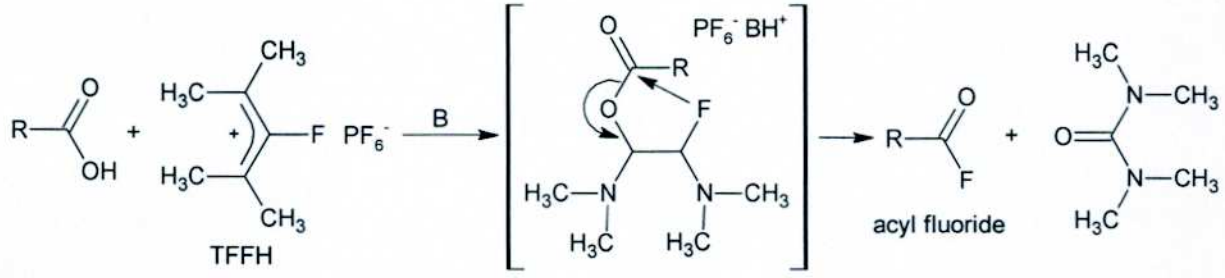
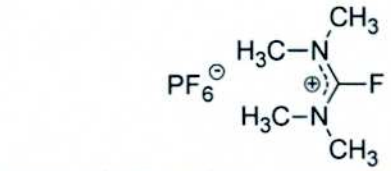


Diagram 5

This technique proved to be particularly useful in the case of syntheses of phosphonodehydrotripeptides and tetradehydropeptides containing two dehydrophenylalanine residues in positions 2 and 4 of the peptide chain. These reactions were accompanied by minute amounts of by–products. Fluorides are obtained in an *in situ* reaction with TFFH (**Fig. 2**) in the presence of diisopropylethylamine (DPEA). These are solids of a considerably greater stability than chlorides, which is of great practical importance.

The synthesis of fluorides of dipeptides with C–terminal dehydrophenylalanine was shown in **Diagram 6**.



tetramethylfluoroformamidinium hexafluorophosphate

(TFFH)

Fig. 2

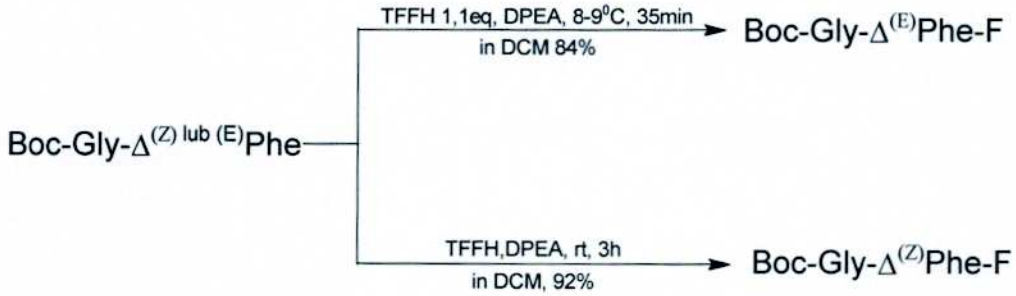


Diagram 6

An alternative method of obtaining acidic fluorides is the use of cyanuric fluoride (2,4,6–trifluoro–1,3,5–triazine). This was shown in **Diagram 7**.

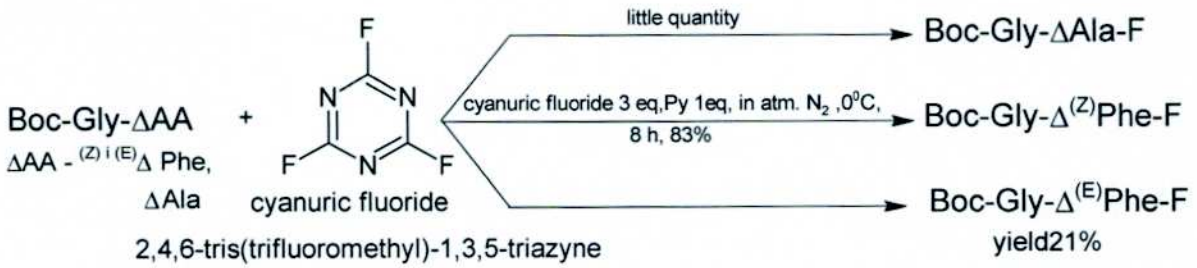


Diagram 7

Synthesis of fluorides using this method requires anaerobic conditions (the reaction is conducted in the atmosphere of nitrogen and argon), and the used cyanuric fluoride is a lacrimator! Moreover, isolation of products through crystallisation is more difficult and it is required to wash the post-reaction mixture in organic solvents which do not mix with water or basic/acidic water solutions. This causes hydrolysis of acidic fluorides up to as much as 80%. A disadvantage of this method is a low yield of the obtained isomer (E) (21%), i.e. considerable isomerisation of isomer (E) into isomer (Z). Isomer (E) was obtained through fractional crystallisation from three-ingredient set of solvents (DCM : Et_2O : hexane = 1:10: 5). Division of isomers on a chromatographic column was not possible due to a very large probability of anchoring of fluorides on the silica gel bed.

It was not possible to obtain acidic fluoride of dipeptide with C-terminal dehydroalanine residue. **Boc–Gly–ΔAla**, reacting with such donors of fluorine as TFFH and cyanuric fluoride, did not yield the expected effect (**Diagram 7**). What was established chromatographically (TLC and HPLC) was only a minute amount of fluoride in the post-reaction mixture with the by–products dominant in it. Most likely, those were oligomers created as a result of oligomerisation of dehydroalanine residue.

The efficiency of acidic fluorides in the synthesis of peptides was verified by obtaining phosphonic mimetics of dehydropeptides – **Boc–Gly–$\Delta^{(Z)}$PheAAPO(OR)$_2$**.[14] **(Fig. 3)**

In the case of these derivatives, all the other activation methods described in the summary of professional accomplishments were not efficient enough in order to ensure a sufficiently high yield of their synthesis and eliminate the considerable workload put into their isolation.

Using this method, peptides were obtained containing C–terminal phosphonic analogues of:

alanine	—	**AlaPO(OEt)$_2$**	**a yield of 69%**
aminopropionic acid	—	**AbuPO(OMe)$_2$**	**a yield of 58%**
aminobutyric acid	—	**n-BuPO(OMe)$_2$**	**a yield of 42%**
glycine	—	**GlyPO(OEt)$_2$**	**a yield of 53%**
phenylglycine	—	**PhgPO(OEt)$_2$**	**a yield of 46%**
valine	—	**ValPO(OMe)$_2$**	**a yield of 59%**

Fig. 3

The synthesis of amides of **Boc–Gly–Δ $^{(Z)\text{ and }(E)}$ Phe–N(CH$_3$)$_2$** and **Boc–Gly-Δ$^{(Z)\text{ and }(E)}$Phe–NHCH$_3$**, with the use of Boc–Gly-**Δ$^{(Z)}$Phe-F**, was conducted by barbotating the reaction mixture with dimethylamine or methylamine. In this case, a completely unexpected result was obtained. Isomer (Z) isomerised into isomer (E) in such reaction conditions[14]. Once the isomers had been divided using a chromatographic column, what was obtained was as follows:

Boc–Gly–Δ $^{(Z)}$ Phe–N(CH$_3$)$_2$ 82.5%
Boc–Gly–Δ $^{(E)}$ Phe–N(CH$_3$)$_2$ 13%
which gave a preference (Z)/(E) = 6.3:1[9]

and a pair of isomers **Boc–Gly-Δ$^{(Z)}$Phe–NHCH$_3$ 3%**
Boc–Gly-Δ$^{(E)}$Phe–NHCH$_3$ 89%
which gave a preference (Z)/(E) = 1:30[14]

This result is highly unexpected, since there is no information in the literature on this kind of unusual isomerisation of isomer (Z) into (E). Isomerisation was also observed in the synthesis of amides (described below) using the method of mixed anhydrides.

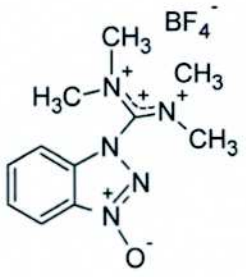
Creation of a peptide bond using TBTU

The use of TBTU [O–(Benzotriazol–1–yl) –N,N,N',N'–tetramethyluronium tetrafluoroborate] (**Fig. 4**) makes it possible to obtain high yields of the coupling products and low epimerisation. The presence of HOBt in the molecule, combined with tetramethylurea, makes this activator dangerous due to its explosive properties.

In the model coupling reaction, Boc–Gly–Δ$^{(E)}$Phe and Boc–Gly–ΔAla with methyl glycinate were used **(Diagram 8)**. During the reaction, isomer (E) isomerised whereby the total yield of reaction (72%) consisted of partial yields of two tripeptides containing isomer (E) (34%) and isomer (Z) of dehydrophenylalanine (38%).

Merck H60 chromatographic column with silica gel was used to divide the isomers by eluting with ethyl acetate in benzene under increasing concentration gradient from 2.0% to 15%.

In the course of the reaction of TBTU with the C–terminal carboxyl group of N–blocked amino acids or peptides, two active esters with N,N,N',N'–tetramethylurea (TMM) and 1–hydroxybenzotriazole (HOBt) were temporarily created, intensifying the efficiency of the coupling reaction with N–terminal esters of amino acids or peptides (**Diagram 9**).



TBTU structure

Fig. 4

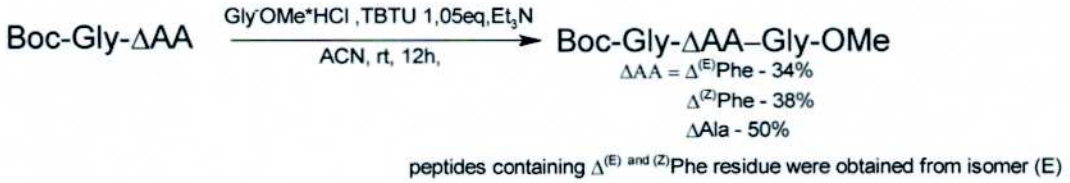


Diagram 8

After a standard deprotection of N–terminal Boc- protecting group using trifluoroacetic acid or C–terminal –OMe protecting group using 1M NaOH, amino and carboxyl substrates for further syntheses were obtained.

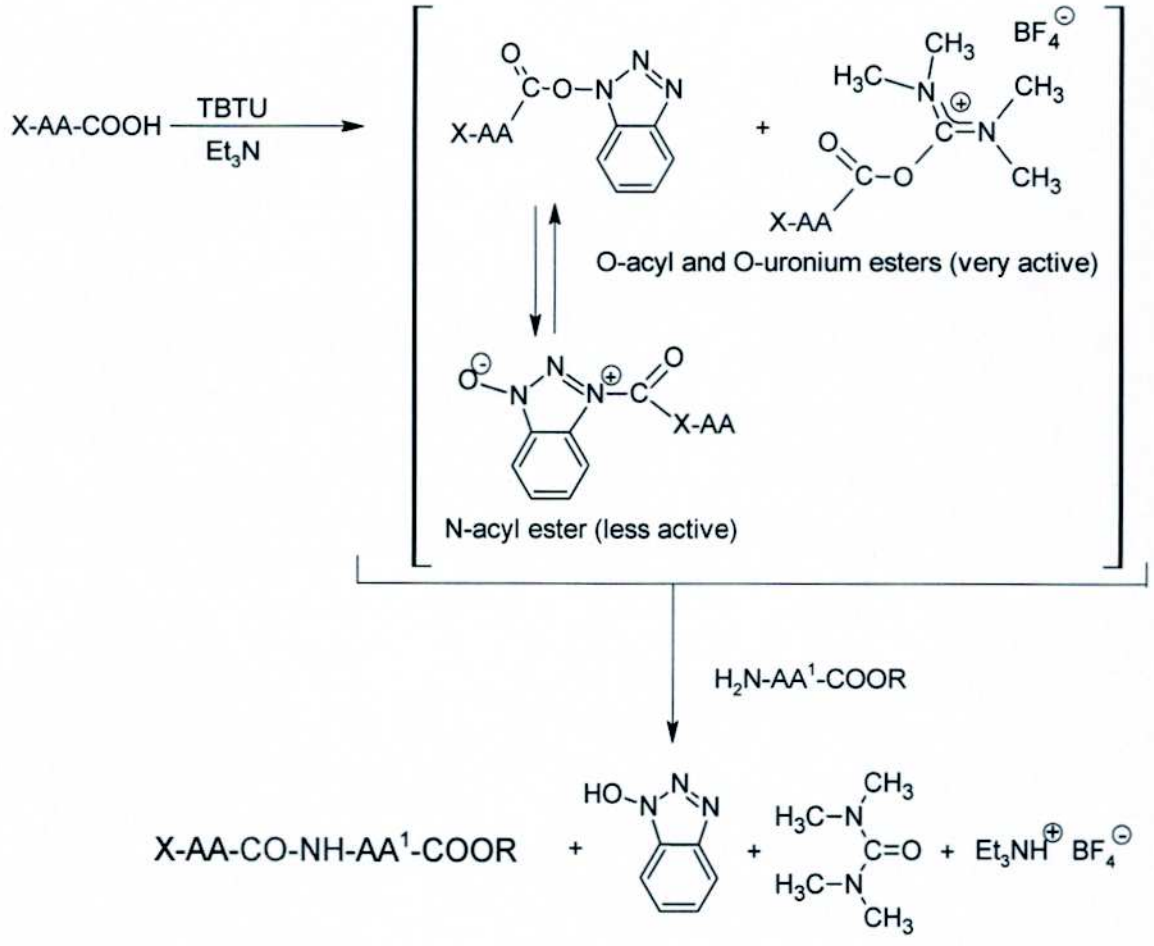


Diagram 9

The method using TBTU was the most frequently employed one by me in the synthesis of dehydropeptides. Equimolar amounts of substrates are used to which, once they have been dissolved (most often in THF or DCM), a tertiary amine is added (Et_3N or N–

methylmorpholine) as well as a small excess (5–10%) of TBTU. Depending on the type of coupled peptide fragments, reactions are carried out at room temperature for 3–24 hours. Great efficiency of the method, yields within the range of 60–95%, a small amount of by–products, which definitely makes it easier to isolate the main products of syntheses, and very low epimerisation, when using optically active amino acids for syntheses, make it possible to regard this method as effective. However, there are sometimes difficulties in eliminating 1–hydroxybenzotriazole (HOBt) from the post-reaction mixture.

By using this method, I obtained what follows:

1. glycidyl esters of **Boc–Gly–ΔPhe–OGdl** – (S) configuration ester with a yield of 89%/ (R) configuration ester with a yield of 92% [14]
2. **Boc–Gly–$\Delta^{(Z)}$Phe–Gly–Phe–pNA** [2 + 2] – a yield of 68%[4]
3. **Boc–Gly–Gly–ΔAla–Phe–pNA** [1 + 3] – a yield of 77%[7]
4. **Boc–Gly–Gly–ΔPhe–Phe–pNA** [1 + 3] – a yield of 96%[4]
5. **Boc–Gly–ΔPhe–Gly–Gly–ΔPhe–Gly–OMe** [3 + 3]
   where isomer (E)(E) – a yield of 96%
   and isomer (Z)(Z) – a yield of 97%[2,6]
6. **Boc–Gly–ΔAla–Gly–Gly–ΔAla–Gly–OMe** [3 + 3] – a yield of 65%[2,6]
7. **Boc–Gly–Gly–ΔAla–Gly–ΔAla–Phe–pNA** [1 + 5] – 74%[6]
8. **Boc–Gly–ΔPhe–Phe–Gly–ΔPhe–PheOMe** [3 + 3]
   where isomer (Z)(Z) – a yield of 93%
   and isomer (E)(E) – a yield of 87% respectively[7]
9. **Boc–Gly–$\Delta^{(Z)}$Phe–Gly–Phe–OMe** [3 + 1] – a yield of 91%[6,7]
10. **Boc–Gly-$\Delta^{(E)}$Phe–Gly–Phe–OMe** [3 + 1] – a yield of 92%[6]
11. **Boc–Gly–ΔAla–Gly–Phe–OMe** [3 + 1] – a yield of 87%[6]
12. **Boc–Gly–Gly–ΔPhe–Gly–ΔPhe–Phe–pNA** [1 + 5]
    where the isomers were obtained with the following yields:
    (Z)(Z) – 71%,
    (Z)(E) – 72%
    (E)(Z) – 81%
    (E)(E) – 76%[5]

Using the TBTU method, peptides whose general formula was **Boc–AA–NHCH(CF_3)CH_2–$PO(OEt)_2$**, were also obtained. They contained C-terminal, fluorinated β-aminophosphonate. In the case when the N–terminal amino acid was glycine, the yield of reaction was 75%; alanine – 75%, valine – 90%, isoleucine – 73%, proline – 89% and phenylalanine – 79%. Furthermore, **Boc–Gly–ΔPhe–NHCH(CF_3)CH_2–$PO(OEt)_2$** hybrid tripeptide was also obtained by means of this technique (a yield of 63%), containing in its molecule both dehydroamino acid residue and aminophosphonate.[14]

The TBTU method was employed to synthesise p-nitroanilide amides in the form of **Boc–Gly–ΔAal–PhepNA**[8] and **Boc–Gly-$\Delta^{(Z)\text{ and }(E)}$Phe–Phe–pNA** [4]. Isomerisation of the C–terminal residue (E) of dehydrophenylalanine forced a division of isomers (Z) and (E) on a chromatographic column with H60 silica gel packing, elating the products with ethyl acetate

in benzene under increasing concentration gradient of acetate from 10% to 60% (**Diagrams 10a, b**).

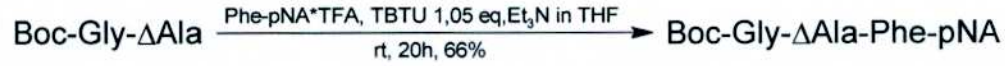


Diagram 10a

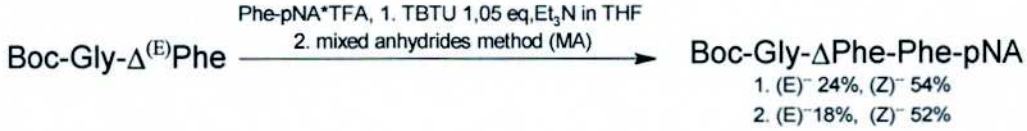


Diagram 10b

Mixed anhydrides method (MA)

It is a very popular method in the synthesis of peptides. Various chloroformates are used in it, most frequently alkyl chloroformates (saturated or unsaturated), e.g. isobutyl chloroformate, isopropen–2–yl chloroformate, isopropyl chloroformate and many others (**Fig. 5**), depending on the type of synthesised dehydropeptides. Despite that these reagents are lacrimators, their popularity is determined by a relatively low price and uncomplicated procedure of synthesis of peptides.

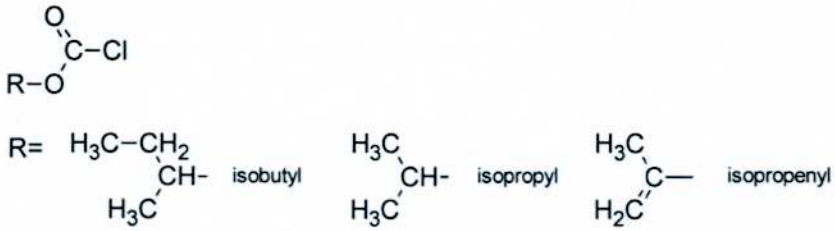


Fig. 5 Chloroformates

By–products created as a result of false opening of a mixed anhydride – urethanes, sometimes effectively hinder isolation of the main product of synthesis through crystallisation, this being a disadvantage of this method (**Diagram 11**). In order to obtain a pure product, it is necessary to employ preparative column chromatography which is a time and material–consuming as well as labour intensive method. The efficiency of the method depends mainly on the steric structure of the coupling substrates. A significant and important fact, which cannot be passed over as it impacts the yield of the process, is the spatial structure of chloroformate alkyl

residue. Agents with a large steric obstacle react more slowly, but they make it possible to avoid the creation of urethanes (false opening products).

Stereochemistry of substrates also has a substantial impact on the course of a reaction, particularly the chemistry of amino acids which are located the closest to the reaction centre. A small inconvenience is the fact of conducting the first stage of synthesis in order to obtain a smaller anhydride at a temperature below zero from -10°C to -20°C.

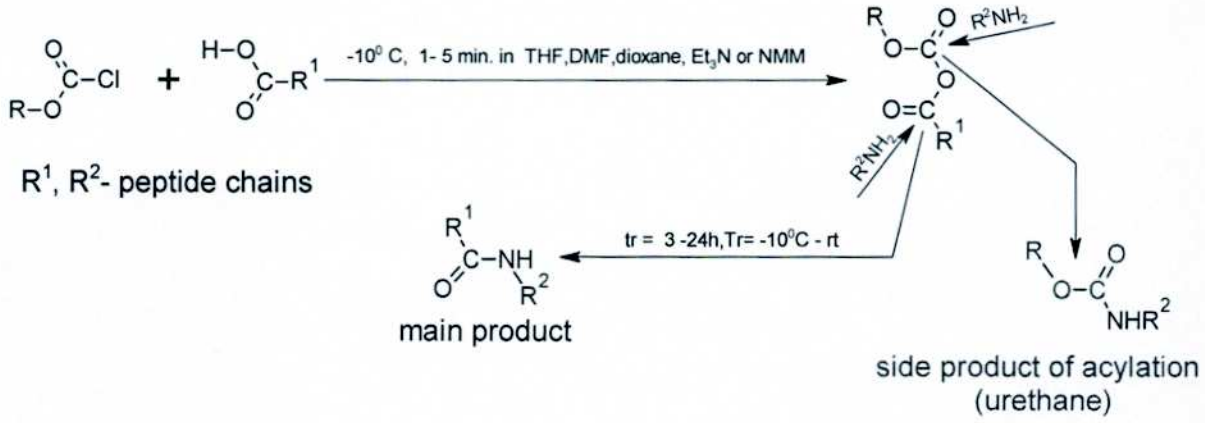


Diagram 11

Peptides synthesised using this method:

1. **(Boc)$_2$–His–Gly–ΔPhe–OMe** [1 + 2] from isomer (E) – a yield of 86%
   from isomer (Z) – a yield of 92%[14]

2. **Boc–Gly–Δ$^{(E) \text{ and } (Z)}$Phe–Gly–OMe** [2 + 1] from isomer (E), (E) – a yield of 34%
   from isomer (Z) – a yield of 38%[3]
   Division of the isomers on the column

3. **Boc–Gly–Δ$^{(E) \text{ and } (Z)}$Phe–Phe–pNA** [2 + 1] from isomer (E), (E) – a yield of 18%
   from isomer (Z) – a yield of 52%[3]
   Division of isomers on the column

4. **Boc–Gly–ΔAla–Gly–$^{(Z)}$ΔPhe–Gly–OMe** [2+3] – a yield of 37%[1]

5. **Boc–Gly–ΔAlavGly–$^{(E)}$ΔPhe–GlyvOMe** [2+3] – a yield of 48%[1]

6. **Boc–Gly–$^{(E)}$ΔPhe–Gly–$^{(E)}$ΔPhe–GlyvOMe** [2+3]
   a yield of 83%, including:
   from isomer (E, E) – a yield of 28%
   from isomer (Z, E) – a yield of 55%

Division of isomers on the column:[5]

7. **Boc–Gly–$^{(Z)}$ΔPhe–Gly–$^{(E)}$ΔPhe–Gly–OMe** [2+3] – a yield of 80%[4]

8. **Boc–Gly–$^{(Z)}$ΔPhe–Gly–$^{(Z)}$ΔPhe–Gly–OMe** [2+3] – a yield of 75%[4]

9. **Boc–Gly–ΔAla–Gly–ΔAla–Phe–pNA** [2+3] – a yield of 41%[5]

10. **Boc–Gly–$^{(E)}$ΔPhe–Gly–$^{(Z)}$ΔPhe–Phe–pNA** [2+3]
    a yield of 72%, including from isomers (E)(Z) – a yield of 21%
    from isomers (Z, Z) – a yield of 51%
    Division of isomers on the column[5]

11. **Boc–Gly–$^{(E)}$ΔPhe–Gly–$^{(E)}$ΔPhe–Phe–pNA** [2+3]
    80% including from isomers (E, E) – a yield of 27%
    from isomers (Z, E) – a yield of 53%
    Division of isomers on the column[4]

12. **Boc–Gly–ΔPhe–O(S) and (R)Gdl (glycidyl esters)** (S) – a yield of 90%
    (R) – a yield of 96%[12]

However, when using this method to synthesise butyloxycarbonyl –glycine–dehydrophenylalanine N,N–dimethylamide (**Boc–Gly–ΔPhe–N(CH$_3$)$_2$**[12]) in THF and barbotating the reaction mixture with dimethylamine during the reaction, a mixture of isomers (E) and (Z) was obtained from isomer (Z). The yield of the reaction was 83%, including **68% for isomer (E)**, which gave a preference **(E)/(Z) = 4,5:1**. In a similarly conducted synthesis of **Boc–Gly–ΔPhe–NHCH$_3$**[14], a mixture of isomers (E) and (Z) was also obtained from isomer (Z). The yield of the reaction was 87%, including **81% for isomer (E)**, which gave a preference **(E)/(Z) = 13,5:1**.

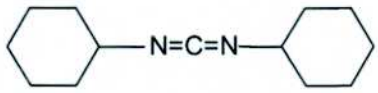
Isomerisation of isomer (Z) into (E) to such a significant extent is not known and has not been described in the literature.

Carbodiimides as mediators in the creation of a peptide bond

Carbodiimides are a wide range of compounds with diverse structure of substituents (R,R^1) near imide atoms of nitrogen of the carbodiimide group R–N=C=N–R^1. According to general assessments, these compounds belong to a group of very toxic substances due to very effective dehydrating properties and, in the case of contact with the skin, they are the cause of

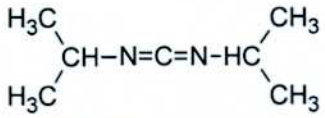
eczemas, which are very hard to heal, and open, suppurating wounds which sometimes are the origin of gangrene. Inhalation of vapours of these compounds is particularly dangerous.

In the 1970s, this was a commonly used method of activation in the synthesis of peptides. Two types of these activators are used: insoluble in water (DCC, DPCD) **(Figs. 6, 6a)** and soluble in water (WSC). **(Figs. 7, 7a)**



N,N'-dicyclohexylcarbodiimide (DCC)

Fig. 6



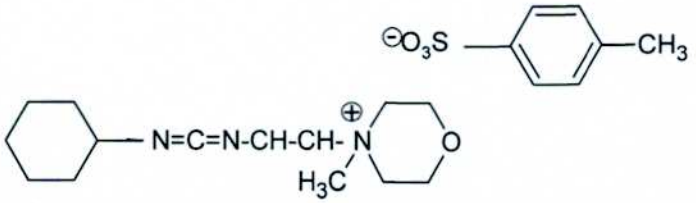
N,N'-diisopropylcarbodiimide(DPCD)

Fig. 6a

CH_3-CH_2-N=C=N-CH_2-CH_2-CH_2-$N(CH_3)_2$ * HCl

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

Fig. 7



N-Cyclohexyl-N'-(2-morpholinoethyl)carbodiimide metho-p-toluenesulfonate (CMC)

Fig. 7a

The reason of such structural diversity of carbodiimides is mainly the problems with easy and fast isolation of a peptide from the post-reaction mixture. For instance, the commonly used DCC (**Fig. 6**) entails issues concerning elimination of the created dicyclohexylurea from the post-reaction mixture. There is even a saying according to which "it is not soluble in any solvent, but it is present in large quantities everywhere". In order to obtain a chromatographically homogenous product, it is necessary to use a chromatographic column. Furthermore, to avoid the difficulties associated with the division of products, polymeric carbodiimide can be used, anchored on an insoluble carrier. An example of such an activator is polyhexamethylene carbodiimide (**Fig. 8.**).

$$-(CH_2)_6-N=C=N-[-(CH_2)_6-N=C=N-]_n-(CH_2)_6$$

Fig. 8

Diisopropylcarbodiimide (DPCD) (Fig. 6a), no less effective than DCC, is – similarly to diisopropylurea created in the reaction – soluble in DMF, DCM, CHL, AcOEt and many other solvents which makes isolation of the product through, say, crystallisation much easier. The two other popular activators soluble in water [(EDC) (Fig. 7) and (CMC) (Fig. 7a)] facilitate high yield coupling reactions in water environment as well as in organic solvents. An advantage of these activators is that the derivatives of urea created during the reaction are water-soluble and, washing the post–reaction mixture in a solvent which does not mix with water or basic/acidic water solutions easily gets rid of the by–products. Adding carbodiimide to a solution of N–blocked amino acid or peptide causes the creation of an intermediate, protonated, imine form of carbodiimide, followed by the creation of very active O–acylurea which, in the presence of nucleophiles which are amino substrates in the form of esters of amino acids/peptides or HOBt (an additive which supports the reaction), makes the creation of a peptide bond easier (**Diagram 12**).

The reaction environment has a fundamental impact on the efficiency of a reaction, whereas the reaction rate increases along with the increase in concentration of substrates. Large polarity of aprotic solvents is conducive to reactions of rearrangement of O-acylurea to an inactive by–product of N–acylurea, effectively hindering isolation of the main reaction product. High reactivity of O-acylurea may be the cause of creation of azlactones (Fig. 9, Diagram 12), especially when the N–terminal protecting group is an acetyl group. Boc–, Z–, Bpoc– and Fmoc– protecting groups limit this secondary reaction to a very large extent.

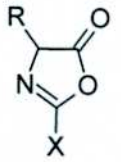


Fig. 9 Azlactones

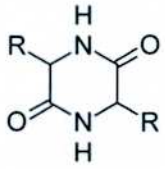


Fig. 10 2,5-dioxopiperazines

As standard, a 2.5–time excess of X–AA (N–blocked amino acid or peptide) and carbodiimide in relation to H_2N–AA–X (C–protected amino acid or peptide) is used.

When using carbodiimides, there is a large probability of creation of 2,5–dioxopiperazine if the third amino acid residue joins the peptide. This is true particularly in the case of syntheses of peptides with the N–terminal Boc- protecting group (**Fig. 10**).

Syntheses of dehydropeptides, which are longer than dehydrotripeptides, using this method did not end successfully due to very large amounts of by-products created in reactions. Hence, the use of this method was abandoned.

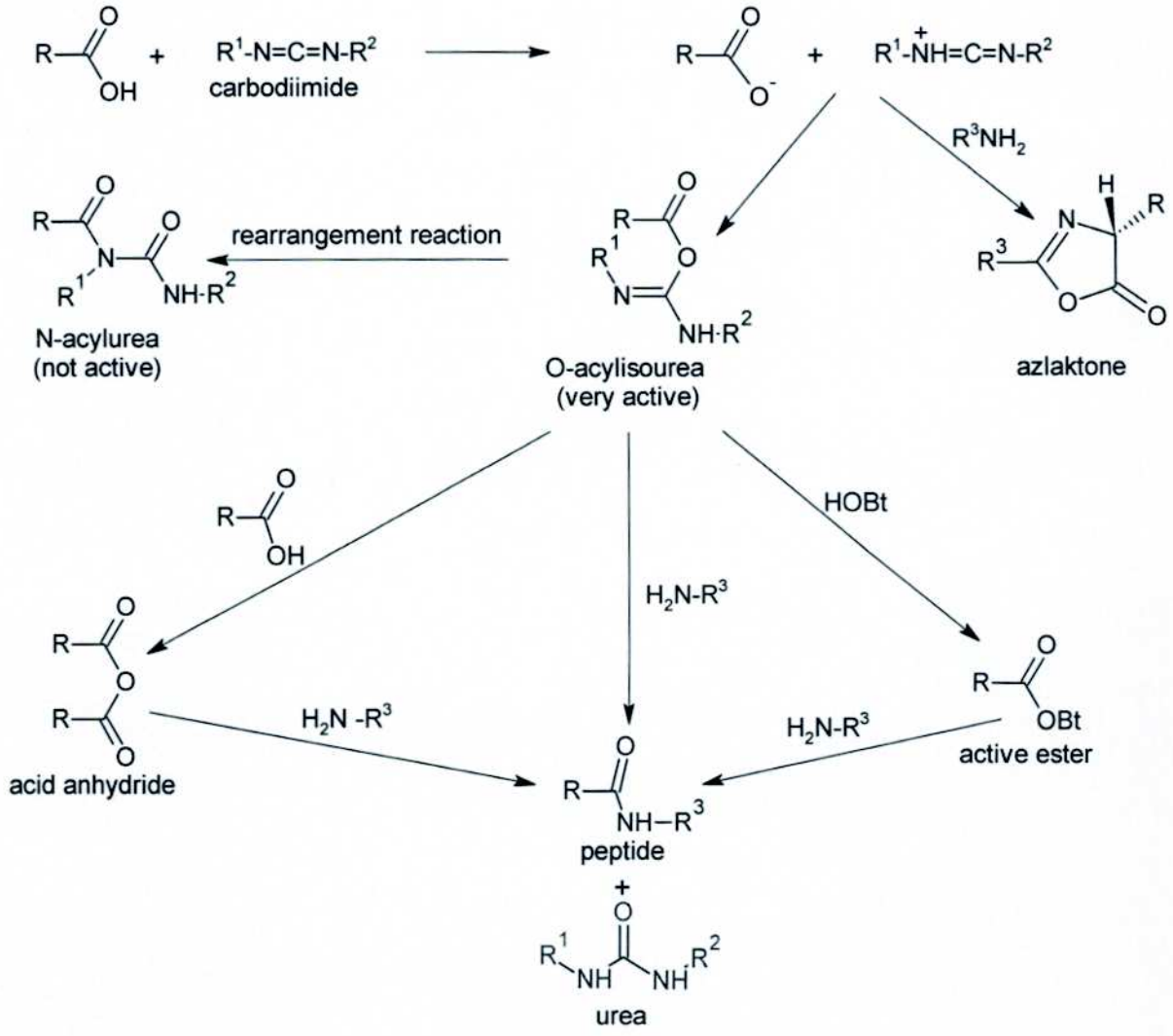


Diagram 12

1–propylphosphonic acid cyclic anhydride (T3P)

Models of dipeptides, which were necessary to identify isomers containing residue (E) of dehydrophenylalanine, were obtained using activation of T3P (1–propylphosphonic acid anhydride). Previously discussed Boc–Gly$\Delta^{(E)}$Phe–OMe was such a peptide. It was obtained with a yield of 85% and contaminated in 13.5% with isomer (Z).

T3P is a forgotten, original and very rarely used activator in the synthesis of peptides (**Fig. 11**).

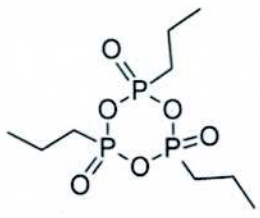


Fig. 11 T3P

It is very convenient to use and is characterised by excellent selectivity in a variety of reactions, does not cause epimerisation (usually below 2%), and high yields of reactions and a small or insignificant amount of by-products obtained are its additional positive features. It can certainly be classified as 'green' reagent, as it is called. It is neither allergenic nor irritating, whereas the created by–products are non–toxic, completely water–soluble salts which, through washing with acidic or basic solutions, can be completely removed from the post–reaction mixture. Most commonly, 50% T3P solutions in ethyl acetate, acetone, toluene, THF, DCM or DMF are used.

It has a very broad spectrum of applications (**Diagram 13**). In addition to creation of a peptide bond or use in reactions of cyclisation of peptides, it is employed as a reagent in the following:

— Esterification reactions
— Oxidisation of alcohols to aldehydes under very mild conditions
— Lossen rearrangement
— Obtaining nitriles from amides
— Obtaining aldehydes from carboxylic acids
— Syntheses of β-lactams
— Dehydration of secondary alcohols to alkenes

Therefore, it is justified to state that this activator is to a large extent universal.

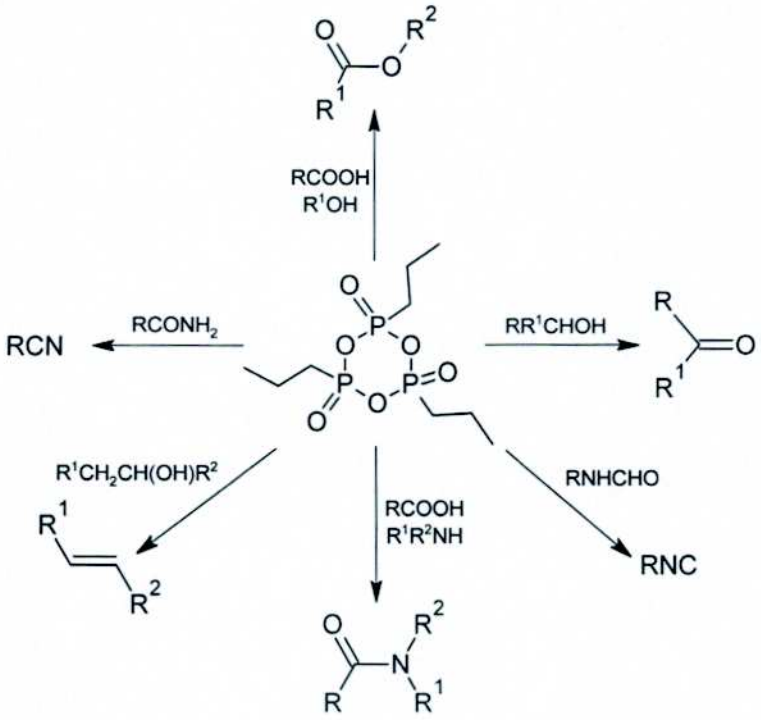


Diagram 13. Some of the examples of uses of T3P

The synthesis of peptides is most often conducted in THF at room temperature. 1.2 of an equivalent of a solution of T3P gets dropped into a solution of substrates and triethylamine. The reaction usually lasts from 1 to 12 hours, and less often up to 24 hours, resulting in obtaining products with a yield of 70–85%.

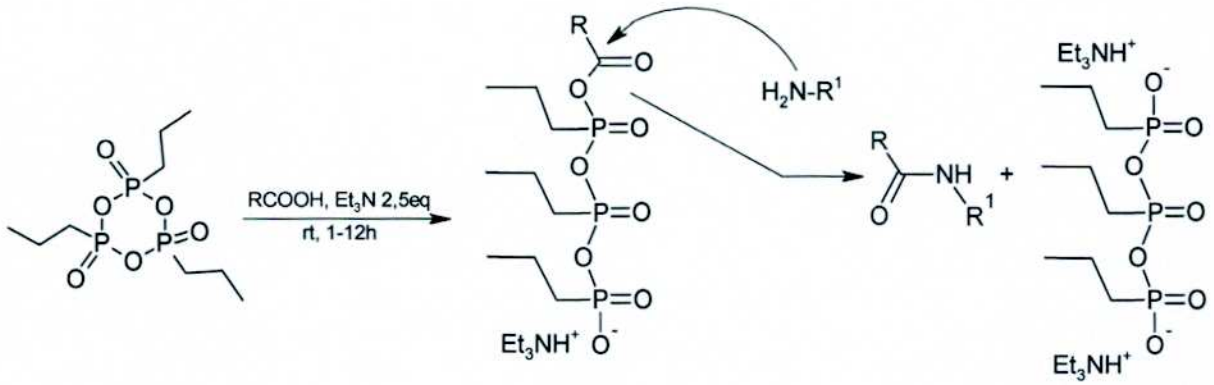


Diagram 13

Very high efficiency of T3P was documented by synthesising Z–MeLeu–MeValOMe dipeptide.

(**Diagram 14**). Large steric obstacles of neither amino acid had an essential impact on the reduction of yield of reaction.

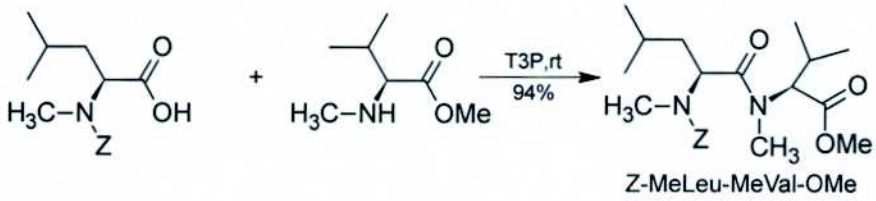


Diagram 14

Using T3P as an activator, the following dehydropeptides were obtained, with reactions lasting from 4.5 hours to 3 days:

1. **Boc–Gly–ΔAla–N(CH$_3$)$_2$**[9] – a yield of 73%
2. **Boc–Gly–ΔAla–NHCH$_3$**[14] – a yield of 77%
3. **Boc–Gly–ΔPhe–Val–OMe** [2 + 1][11] – a yield of 54%
4. **Boc–Gly–Val–ΔPhe–OMe** [1 + 2][11] – a yield of 75%
5. **Boc–Val–ΔPhe–Gly–Gly–ΔAla–OMe** [3 + 2][11] – a yield of 67%
6. **Boc–Gly–Val-ΔPhe–Gly–ΔAla–OMe** [3 + 2][11] – a yield of 70%
7. **Boc–Gly–ΔAla–Val–ΔPhe–Gly–O^{t}Bu** [2 + 3][11] – a yield of 74%
8. **Boc–Gly–ΔAla–Gly–Val–ΔPhe–OMe** [3 + 2][11] – a yield of 70%
9. **Boc–Gly–Δala–Gly–ΔPhe–Val–OMe** [2 + 3][11] – a yield of 78%
10. **(Boc)$_2$–His–Gly–ΔPhe–Gly–ΔPhe–His–OMe** [3 + 3][14] – a yield of 65%
11. **(Boc)$_2$–His–Gly–ΔPhe–His–Gly–ΔPhe–OMe** [3 + 3][14]

isomer (E)(E) was obtained with – a yield of 75%
and isomer (Z)(Z) with – a yield of 94%

12. **Boc–Gly–ΔPhe–His–His–Gly–ΔPhe–OMe** [3 + 3][14]
isomer (Z)(Z) was obtained with – a yield of 56%
and isomer (Z)(E) – a yield of 56%
13. **Boc–Gly–ΔPhe–His–Gly–ΔPhe–His–OMe** [3 + 3][14] – a yield of 63%

Verification of procedures of synthesis of dehydropeptides – obtaining isomeric octapeptides containing four dehydrophenylalanines of different configuration

Diagram 15 shows the synthesis of octapeptides with four dehydrophenylalanine residues in positions 2, 4, 6 and 8.

The Diagram shows the path of synthesis of the hardest to obtain peptide which would contain dehydroamino acids of (E) configuration only. The end octapeptide was obtained with a yield of 67% in the form of isomers (E)(Z)(E)(E) and (E)(E)(E)(E). Isomerisation of C–terminal (E) –dehydrophenylalanine, occurring during the creation of a peptide bond, was the biggest problem in this synthesis as the mixture of isomers had to be divided on a chromatographic column after each stage of coupling.

Thus, the following peptides of the following configuration of isomers were obtained:

1. **(Z)(Z)(Z)(Z)** – a yield of 88%
2. **(Z)(Z)(Z)(E)** – a yield of 70%
3. **(Z)(Z)(E)(E)** – a yield of 80%
4. **(Z)(Z)(E)(Z)** – a yield of 80%
5. **(E)(Z)(E)(E)** – a yield of 83%
6. **(E)(Z)(E)(Z)** – a yield of 67%
7. **(Z)(E)(Z)(E)** – a yield of 67% in the form of a mixture of isomers (Z)(Z)(Z)(E) and (Z)(E)(Z)(E)
8. **(Z)(E)(E)(Z)** – a yield of 75% in the form of a mixture of isomers (Z)(Z)(E)(Z) and (Z)(E)(E)(Z)
9. **(E)(E)(E)(E)** – a yield of 67% in the form of a mixture of isomers (E)(Z)(E)(E) and (E)(E)(E)(E)[14]

Three peptides were obtained in the form of a mixture of isomers. It was not possible to divide them, using chromatographic methods due to too small differences in polarity between the individual pairs of isomers and close affinity to the bed.

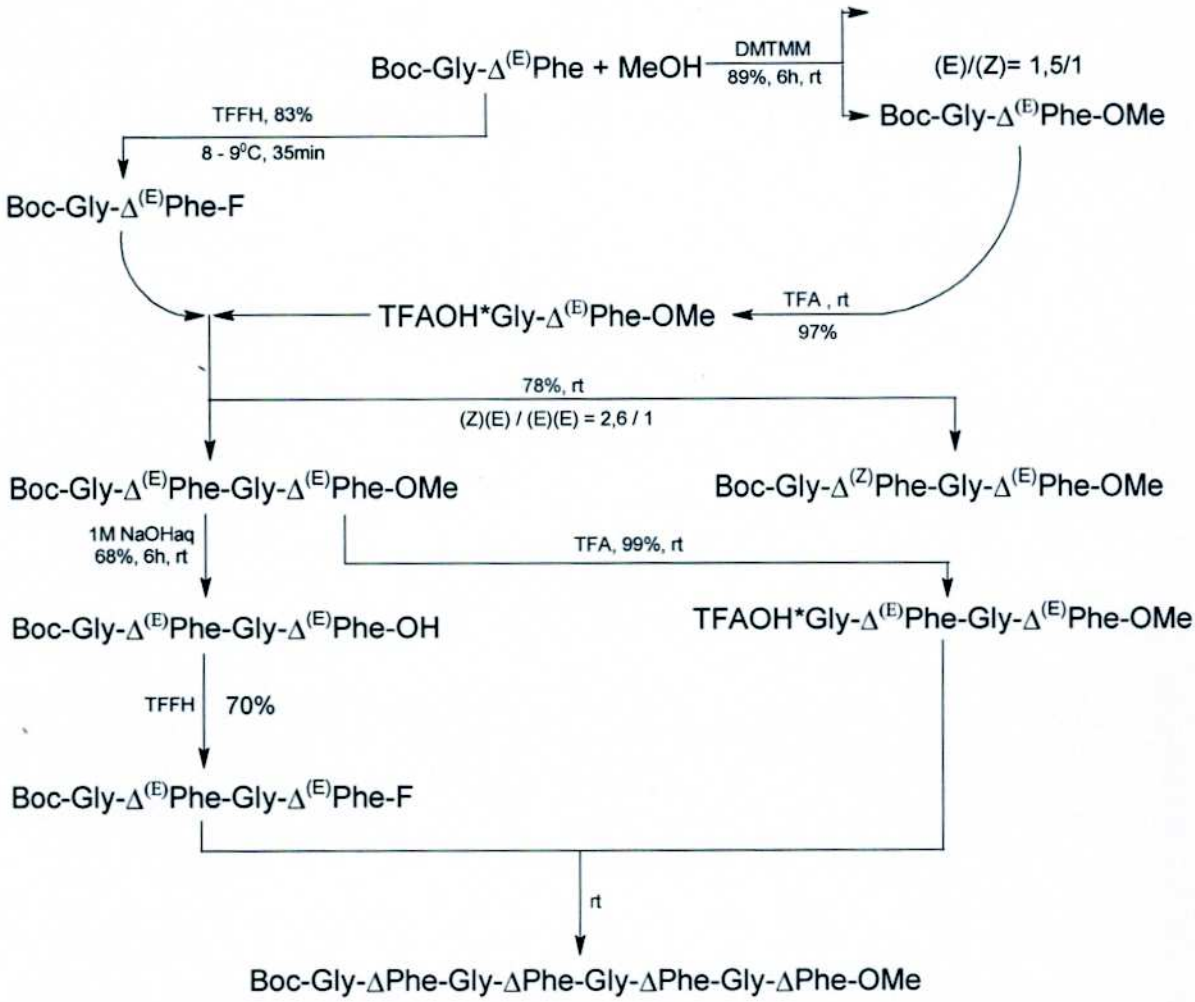


Diagram 15

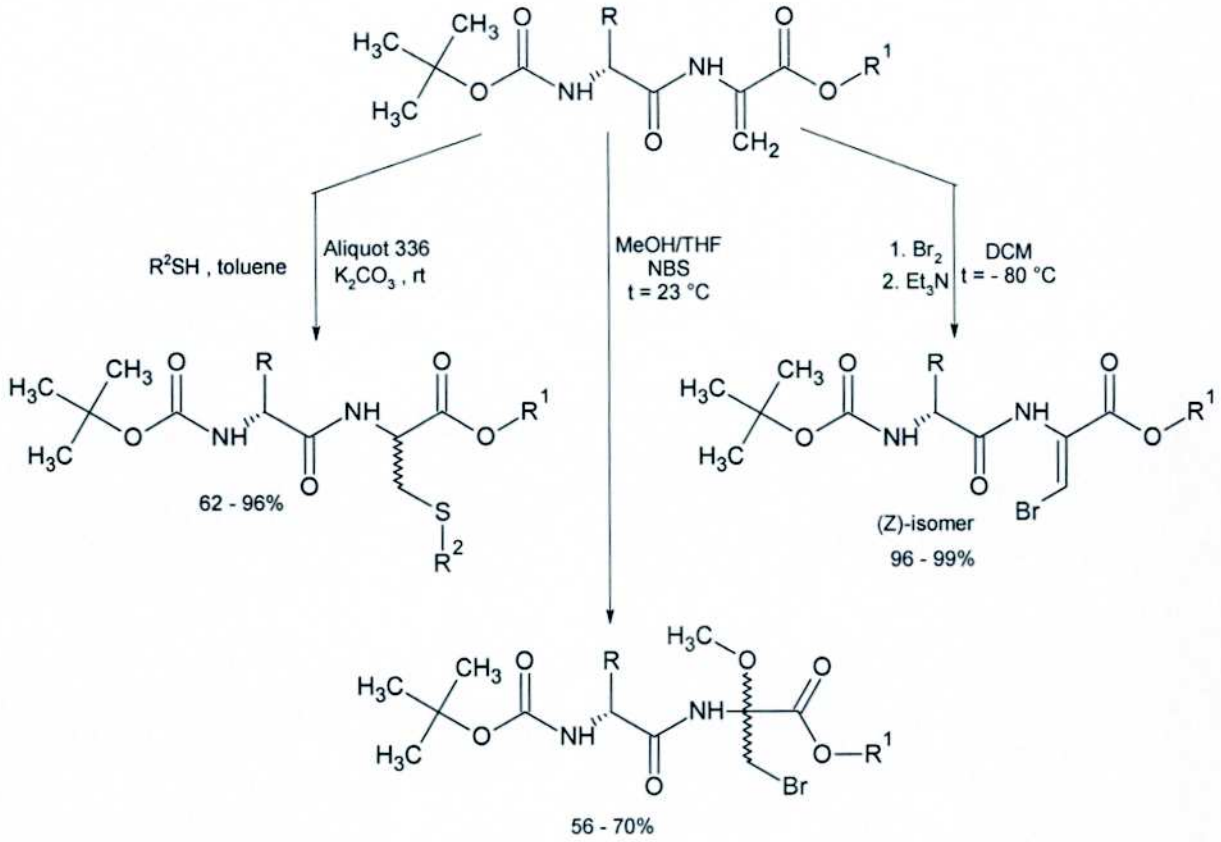
Addition of nucleophiles to the double bond of dehydroalanine in a peptide chain

Taking advantage of a strong tendency of the double bond of dehydroalanine residue in dehydrodipeptides to undergo electrophilic and nucleophilic addition reactions, dipeptides containing different, non–protein, C–terminal amino acids were synthesised (**Diagram 16**). The aim of these syntheses was to obtain and investigate how a modification of C-terminal amino acid would affect the inhibiting activity of those dipeptides in relation to cathepsin C. The results of these studies will be used in order to develop syntheses of new peptidomimetics, potential inhibitors of this important enzyme.

Addition of thiols to the double bond in the presence of a phase-transfer catalyst (Aliquat 336) in the presence of potassium carbonate made it possible to obtain β–thiol derivatives in the form of mixtures of stereoisomers, since a new stereogenic centre on carbon C^{α} was created in the reaction. One of the peptides containing a fragment of thiol–adamantane was divided into stereoisomers by means of preparative HPLC. This compound proved to be an interesting, micromolar inhibitor of cathepsin C[14].

Addition of a bromine atom to the double bond with the participation of NBS (N–bromosuccinimide) in a mixture of methanol and THF (1:1v/v) causes the creation of an α–methoxy–β–bromo derivative of the dipeptide[14].

In contrast, in the addition reaction of bromine (Br_2) in the presence of Et_3N at a temperature of -80^0C in DCM, creation of β–bromo–(Z)-dehydrodipeptide is preferred.



R = H, $-C_6H_5CH_3$
R^1 = $-CH_3$, $-C_2H_5$, $-CH(CH_3)_2$
R^2 = $-C_2H_5$, C_6H_{10}, $-C_6H_5$, p–C_6H_4F , p–C_6H_4Cl , p–C_6H_4Br , p–$C_6H_4NH_2$, –adamantane, Boc–Cys–OMe

Diagram 16
Protecting groups of amino and carboxyl groups of amino acids and peptides as well as their deprotection – brief discussion

The majority of protecting groups of α-amino groups of amino acids belong to the type of urethanes:

RO–CO–NH–R^1, e.g.:

Boc – (*tert*–butyloxycarbonyl); deprotection using a 2–20% solution of TFAOH acid in DCM
Z – (benzyloxycarbonyl); HBr/CH_3COOH deprotection, Na/liquid NH_3, H_2/Pd/C

Fmoc – (fluorenyl–9–methoxycarbonyl); NH_3,$C_2H_5NH_2$ deprotection, morpholine, piperidine
Bpoc – [2–(p–biphenyl)–2,2–dimethylmetoxycarbonyl]; CH_3COOH deprotection
Z(OMe) – p–methoxybenzyloxycarbonyl; deprotection similarly to Boc–

Acyl protecting groups, such as **TFA**– (trifluoroacetyl) and NH_3 water solution deprotection, are used less frequently.

The most frequently used protecting groups of the carbonyl group are as follows:

— **OMe, –OEt;** 1M LiOH, NaOH, KOH water solution deprotection
— **O^{t}Bu;** deprotection by means of a 2–10% TFAOH solution in DCM
— **OBzl;** Na/liquid NH_3, HF deprotection

A peptide synthesis strategy requires such a selection of a type of amino and carboxyl protecting groups in order, after selective deprotection of any of them, to be able to continue further expansion of peptides, either from the N- or C-terminal side.

The synthesis of dehydropeptides forces the use of protecting groups which can be removed under mild conditions in order not to cause side reactions, this being very easily done in the case of peptides with dehydroamino acids residues.

That is why **Boc**– and **–OMe** protecting groups met our requirements. Furthermore, since they were characterised by universal features, they were used in syntheses of dehydropeptides.

The Boc– protecting group is very easily removed in acidic conditions, mostly using an approx. 20% solution of trifluoroacetic acid (TFAOH) in DCM. The deprotection reaction lasts no more than 15 minutes at room temperature, an important fact being the absence of side reactions. The obtained dehydropeptide trifluoroacetates are crystalline in the majority of cases which in turn makes it easier to obtain these substances with very high degree of purity. Yields of the deprotection reaction are very high (97–99%).

The –OMe protecting group gets removed in a hydrolysis reaction in a 1M water solution of NaOH at room temperature. The reaction duration (usually from 2 to 8 hours) depends to a great extent on the size of a dehydropeptide and is extended along with the increase in the amount of amino acids in a peptide chain.

As a result of hydrolysis reaction, crystalline peptides with yields in the range of 80–95% are predominantly obtained. No side reactions were observed, the reason for a yield of 80% having been not completely conducted substrate hydrolysis.

The N–terminal **Fmoc**– protecting group requires protecting of a carboxyl group of a C–terminal peptide with a *tert*-butyl group (**–OtBu**).

Chemical similarity of **Bpoc**– to **Boc**– could suggest the use of the first one in the previously presented syntheses. In both cases, its use was given up due to, *inter alia*, economic reasons. The use of the Z– protecting group was also abandoned because of radical conditions of the deprotection reaction.

Addition of hydrogen bromide (HBr) present in the reaction environment or hydrogen in the presence of a palladium catalyst to the double bonds of dehydroamino acid residues excluded its use.

It is possible to deprotect this protecting group using a 100% trifluoroacetic acid with the addition of anisole (scavenger), but the reaction lasts for a long period of time – even up to 48 hours – and is accompanied by side reactions despite the presence of anisole. The created by-products effectively hinder isolation of the main product.

Final remarks

Synthetic effects of the employed methods of activation vary and are completely unpredictable and that is why, especially in the case of α,β–dehydropeptides, it is necessary to consider very carefully their choice before making a decision. This applies in particular to the C–terminal –COOH group of ΔAla residue due to the previously mentioned, unfortunate coupling of π electrons of the double bond and the carbonyl group causing its deactivation.

A rational interpretation of the results of biological tests and reasonable planning of synthesis of new dehydropeptides requires specific knowledge of relationships between their structure and activity. For the most part, this is true for the stream of research on synthesis of drugs, focussed on obtaining short, potentially effective peptidomimetics through a modification of the peptide chain aimed at potential increase of its biological activity.

Extremely high diversity and abundance of the identified peptides with α,β–dehydroamino acids residues in the world around us is a fact. However, the role and functions they serve still remain not fully understood, since filling up of so vast gaps in knowledge requires the involvement of very extensive material resources and human determination.

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Maciej Makowski