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Enzymatic tools for peptide ligation and cyclization

Development and applications

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Addendum

List of Abbreviations

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List of Abbreviations

Abz	Aminobenzoic Acid
Ac ₂ O	Acetic Anhydride
acac	Acetylacetone
Acm	Acetamidomethyl
ACN	Acetonitrile
AEP	Aspraginy Endopeptidase
Aha	Homoazidoalanine
Aib	2-Aminoisobutyric Acid
aq.	Aqueous
Boc	tert-Butyloxycarbonyl
BSA	Bovine Serum Albumin
calc.	Calculated
Cam	Carboxamidomethyl
Cbz	Benzyloxycarbonyl
CEPS	Chemo-Enzymatic Peptide Synthesis
Cit	Citrulline
CLIPS	Chemical Linkage of Peptides onto Scaffolds
CTC	2-Chlorotrityl
CuAAC	Copper-catalyzed Alkyne Azide Cycloaddition
CV	Column Volume
DCM	Dichloromethane
DDE	Dead-End Elimination
Dde	1-(4,4-Dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl
DHCE	Double-Headed Cam-Ester
DIC	Diisopropylcarbodiimide
DIPEA	N,N-Diisopropylethylamine
DIPSI	Decoupling In the Presence of Scalar Interactions
DMAP	Dimethylaminopyridine
Dmb	N-(2,4-Dimethoxybenzyl)glycine
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
Dnp	2,4-Dinitrophenol
DRP	Doped Reversed Phase
DTT	Dithioerithreitol
EPL	Expressed Protein Ligation

Exn	Exenatide
exp.	Experimental
FITC	Fluorescein Isothiocyanate
Fmoc	Fluorenylmethyloxycarbonyl
FRET	Förster Resonance Energy Transfer
FXIIA	Coagulation Factor XIIa
Gdn.HCl	Guanidine Hydrochloride
GFP	Green Fluorescent Protein
GLP-1	Glucagon-Like Peptide I
GmPOPB	Prolyl Oligopeptidase B from <i>Galerina marginata</i>
GSH	Reduced Glutathione
GSSG	Oxidized Glutathione
HBTU	2-(1 <i>H</i> -Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HEPES	2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid
HFIP	Hexafluoroisopropanol
HMBA	Hydroxymethylbenzyl
HMBC	Heteronuclear Multiple Bond Correlation
HPLC	High Performance Liquid Chromatography
HSQC	Heteronuclear Single Quantum Coherence Spectroscopy
KAHA ligation	α -Ketoacid-Hydroxylamine ligation
kBI	Kalata B I
K _i	Inhibition Constant
LB	Luria Bertani
Lys(N3)	Azidolysine
MCA	4-Methyl-Coumaryl-7-Amide
MD	Molecular Dynamics
MMBA	Mercaptomethylbenzoic Acid
MPAA	Mercaptophenylacetic Acid
MS	Mass Spectrometry
MSA	Methanesulfonic Acid
MTBE	Methyl <i>tert</i> -Butyl Ether
Mtt	Methyltrityl
MW	Molecular Weight
MWCO	Molecular Weight Cut-Off
NCL	Native Chemical Ligation
NMR	Nuclear Magnetic Resonance

NOESY	Nuclear Overhauser Effect Spectroscopy
OGp	4-Guanidinophenyl
pAcF	<i>para</i> -Acetylphenylalanine
PAM	Peptide Amidase
PatGmac	Macrocyclase Domain of PatG
PBSL	Proximity-Based Sortase-Mediated Ligation
PDB	Protein Data Bank
PhAcm	Phenylacetamidomethyl
PITC	Phenylisothiocyanate
PPI	Protein-Protein Interaction
Ptl	Peptilgase
r.m.s.d.	Root Mean Square Deviation
RTD-I	Rhesus- θ -Defensin
S/H ratio	Synthesis/ Hydrolysis Ratio
SAL	Salicylaldehyde
SDS-PAGE	Sodium Dodecylsulfate Polyacrylamide Gel Electrophoresis
SML	Sortase-Mediated Ligation
SPAAC	Strain-Promoted Alkyne-Azide Cycloaddition
SPPS	Solid Phase Peptide Synthesis
StBu	<i>tert</i> -Butylthio
TATA	1,3,5-Triacryloyl-1,3,5-triazinane
TB	Terrific Broth
TBMB	1,3,5-Tris(bromomethyl)benzene
tBu	<i>tert</i> -Butyl
TCEP	Tris(2-carboxyethyl)phosphine
TFA	Trifluoroacetic Acid
TFE	Trifluoroethanol
TIS	Triisopropylsilane
Trt	Trityl
Trz	Triazole
UKI8	Urokinase Inhibitor 18
uPA	Urokinase Plasminogen Activator
UV	Ultraviolet
VIS	Visible

For the nomenclature of amino acids standard one and three-letter codes were used.

Acknowledgments

The work described in this book was certainly not a job that would have been possible entirely on my own and I am deeply grateful for the people who made this an unforgettable journey and that created ample of pleasant memories.

Firstly, I wish to thank Dr. Timo Nuijens for the continuous support throughout the years. Dear **Timo**, it has been a long time, since I started at EnzyPep, at that time still a Master's student at the University of Bielefeld at that time. Almost a year earlier, autumn 2014, I was applying for several R&D internship in pharma and biotech companies. Whereas it took months to receive an invitation for an interview in case of some large pharma companies, it took less than 24 h from first application to a date for a solicitation at EnzyPep. I liked that agility. Shortly after, I travelled to Geleen and started the "EnzyPep-journey" as an intern in September 2015. What followed was an early extension of my contract to perform my Master thesis at EnzyPep, followed by the start of my PhD in autumn 2016. In the beginning my PhD project was coined by little clarity, but you early on guided me in the right directions. This eventually gave me the opportunity to follow many of my own ideas and enjoy the freedom in research I had. You always encouraged me to take advantage of the past three years. I have been given opportunities.... and taken advantage of them. It was a great privilege to visit numerous conferences and meetings around the world, not only presenting my own research, but also representing EnzyPep as a company. I pretty much enjoyed this and it always gave me new energy for the next steps. In addition, it was great to see how many projects, both academic and business, grew out of these contacts established at several occasions. To sum up, your continuous support and your willingness to immediately discuss any question or given problem gave me the freedom to grow, to develop lots of independence and, in particular, you taught me at the right points when to pivot or when to persevere. Thank you!

Equally thankful I am to Prof. Dr. Jan van Maarseveen. Dear **Jan**, it has been a fantastic time and you have been a superb scientific host during the past three years. Besides, me being located in the EnzyPep labs in Geleen in the south of the Netherlands and you being in Amsterdam, we managed to meet on a regular basis. I always enjoyed our discussions about science, gliding and about many other things.. I do not know anybody who as enthusiastic about what he is doing than you are! Your open nature and your enthusiasm are highly contagious, and I would wish you would have been one of my teachers in the early university days. You always believed in me, supported me and gave me a lot of scientific freedom as well as invaluable feedback, which I was luckily able to somehow pay back in the form of publications. Thank you!

Additionally to Jan, my sincere thanks also go to **Prof. dr. Tom N. Grossmann** and **Prof. dr. Francesco Mutti** for acting as my 2nd promotor and my co-promotor, respectively. In this respect, I would also like thank the members of my PhD defense committee: **Prof. dr. Dick B. Janssen**, **Prof. dr. Peter Timmerman**, **Prof. dr. Norbert Sewald**, **Prof. dr. Leendert Hamoen**, **Prof. dr. Gert-Jan Gruter** and **Prof. dr. Henk Hiemstra**.

During my time in the labs of EnzyPep there are several people I would like to thank for the good and inspiring time: **Michel, Mathijs, Elwira, Rowin, Linda, Ana, PQ, Rodney and Peter**. Thank you so much for all the good times and especially to the lab crew: thank you so much for helping out in so many instances. I very much appreciate all your efforts. Especially I would like to give my deep gratitude to **Ana**. You introduced me into the fabulous world of enzyme engineering and *in silico* modelling and always had an open ear for my questions, concerns and ideas. Thank you so much for so many of our good discussions. **PQ**, although you have only been mainly involved in the early stages of my research, you always had useful advices for me and you were always open for discussion and offered your help. Thank you so much that you "vacuum-cleaned" several of the draft manuscripts I prepared during my PhD. **Rodney**, I am deeply grateful for all the advices you had over the past three years. So often you have been a source of inspiration and I admire with how much energy and passion you still pursue projects. I learned so many things from you over the past three years and, again, I am deeply grateful for all the knowledge you shared with me and for slowly introducing me to the world of business development. I pretty much enjoyed our tour through California, US visiting university and companies in spring 2018. Finally, I would also like to thank you, **Peter**, for giving me the opportunity to do my PhD at EnzyPep and for giving me the financial freedom to pursue my projects and own ideas. Besides, I learned many extremely valuable lessons from you and enjoyed the long day trips we did together to Copenhagen and Milano last year. It was a pleasure to work together with you. To all of you: a big Thank You!

Moreover, I would like to thank all our lab neighbors from InnoSyn B.V., especially **Jan** and **Timo**. Timo, it was a great time together and looking back, I am sad when I realize that you left InnoSyn already two years ago. **Jan**, thank you for all the coffee sessions and good discussions, also late in the afternoon or in the evening. I am happy that we became close friends over the years.

And of course, also a big thank you to all the students that came to EnzyPep to work on projects together with me for 4-6 months each: **Nienke, Krit, Marciano, Wessel, Jurgen, Maxime, Laura and Steffen**. You all delivered very good work and without you I would have clearly had significantly less results.

Gaston and Dieuwertje, it was a pleasure working together with you on our collaborative project. In a short period of time we were able to gather decent amounts of data that we were able to shape up in two nice publications. Thank you very much for all your efforts. I wish you all the best for your future career! In this respect of course also a big thank you goes to **Peter** (Timmermann). Thank you so much for continuously supporting the collaborative projects with Gaston and Dieuwertje, bringing in so many great ideas and suggestions and last, but not least thank you for all the efforts you put into the manuscripts to help them get the good quality they finally had. In this context also many thanks to **Tilman** and **Hans** from CARIM. Thank you very much for your help in 3D structure determination using NMR. Hans, you are truly an NMR genius! **Dick, Henriette, Hein and Eduardo**: I would like to thank you for your continuous support in protein modelling

and X-ray crystallography. It was a pleasure working together with you and I am happy that we could shape up our mutual research in two nice publications. **Crystal** and **David**, I think it has been the longest distance collaboration one could imagine – approx. 14.000 km (Geleen – Brisbane). Nevertheless, we shipped a lot of peptides back and forth and I am deeply grateful for the NMR measurement and bioassays you performed for me, Crystal. It was a pleasure working together with you and I am happy that we could publish a collaborative article together. David, thank you for always believing in our enzymatic ligation technology and offering support from your side so many times. I am looking forward to continue working together with you in the future.

Finally, I can of course not thank everybody in person, but to all the great people I met during conferences and meetings around the world: all the in-depth discussions with wine and beer as a solid inspirational source as well as all the non-scientific fun was definitely extremely life-enhancing. Thank you!

Besides all the people already mentioned, this journey of course would not be complete with all the friends that made and still make my stay in Aachen (and first in Maastricht) so great: **Stan, Fabi, Luisa, Jan, Vanessa, Stukki, Holle, Henni, Ingar, Sebastian, Anna, Tim** and many more. Also all the friends that visited me regularly: my brother **Andi, Linda, Ann-Kris** etc. and of course my former fellow students from Bielefeld **Fabian, Philipp, Lisa, Thomas and Hedwig**. Thank you so much for having been part of this journey!

Last, but not least I want to give a very big thank you to the most wonderful woman on this planet: **Viktoria**. Liebe Vicky, du hast innerhalb der letzten Jahre ganz entscheidend dazu beigetragen die richtigen Entscheidungen zu treffen. Bei dir kann ich immer Ich sein! Du hast mich immer unterstützt meine Träume zu leben und Ideen zu realisieren – und dazu zählt unter anderem dieses PhD Projekt, an dem du einen ganz entscheidenden Anteil hast. Du hast immer toleriert, wenn ich des Öfteren „mal eben“ eine E-Mail schreiben, etwas zu Ende bringen oder etwas recherchieren wollte und aus dem „mal eben“ schnell Stunden geworden sind – ob spät abends oder am Wochenende. Danke für diese Freiheit. Danke, dass du da bist. Danke für Alles.

Finally, the very last words I would like to dedicate to two very special persons: **Mama und Papa**. Ohne euch wäre ich nicht hier und hätte niemals die Möglichkeit bekommen dieses Buch zu verfassen. Ich bin euch unendlich dankbar für Alles in den zurückliegenden 30 Jahren. Ihr habt immer zu mir gehalten, in tollen Zeiten mit mir gefeiert und mich in schweren Zeiten ununterbrochen unterstützt. Ihr habt mir immer ein Zuhause gegeben, ein Zuhause, wie es sein soll und wo ich auch heute noch genauso gerne bin früher. Danke für Alles!

In short...it was a pleasant journey over the past years and I thank everybody for having been part of it!

Marcel

Curriculum Vitae

Marcel Schmidt was born on December 5th 1988 in Brilon, Germany. After completing secondary school (classical gymnasium, Abitur) in 2008 he started an apprenticeship as a biological lab assistant at the Max-Planck-Institute of Molecular Physiology in Dortmund, Germany. During this time he received the Trainee Award of the Max-Planck Society and was awarded the best lab assistant (biology) trainee in all of Germany by the DIHK. In 2011 Marcel started studying biochemistry at the University of Bielefeld and graduated in 2014 after performing his B.Sc. thesis in the lab of Prof. T.N. Grossmann at the Chemical Genomics Institute of the Max-Planck Society. During his M.Sc. program in chemical biology at the University of Bielefeld he conducted research in the lab of Prof. Dr. N. Sewald (University of Bielefeld) and in the lab of Prof. M. Schmeing at the McGill University in Montreal, CA, supported by scholarships from the *Studienstiftung des deutschen Volkes* and a RISE scholarship of the German Academic Exchange Service (DAAD). Towards the end of his M.Sc. program, Marcel joined EnzyPep B.V. in Geleen, the Netherlands, as a M.Sc. student, where he developed a novel chemo-enzymatic approach for the greener and more cost-efficient synthesis of thymosin- α_1 . Subsequently, after graduating from the University of Bielefeld in 2016, Marcel worked as a Lead Chemist at EnzyPep B.V. and pursued his PhD in collaboration with the University of Amsterdam (group of Jan H. van Maarseveen). During his research he focused on the development of novel enzymatic methodologies for peptide ligation and, in particular, its application to the synthesis of complex multicyclic peptides.

After completion of his PhD, Marcel continues his career at EnzyPep B.V.

List of Publications

- [1] C. Stiller, D. M. Krüger, N. Brauckhoff, **M. Schmidt**, P. Janning, H. Salamon, T. N. Grossmann, Translocation of an Intracellular Protein via Peptide-Directed Ligation, *ACS Chem. Biol.* 2017, 12, 2, 504-509.
- [2] **M. Schmidt**, A. Toplak, P. J. L. M. Quaedflieg, H. Ippel, G. J. J. Richelle, T. M. Hackeng, J. H. Van Maarseveen, T. Nuijens, Omniligase-I : A Powerful Tool For Peptide Head-to-Tail Cyclization, *Adv. Synth. Catal.* 2017, 359, 2050–2055. (*VIP-paper + cover feature*)
- [3] T. Nuijens, A. Toplak, M. B. A. C. Van De Meulenreek, **M. Schmidt**, M. Goldbach, P. J. L. M. Quaedflieg, Improved Solid Phase Synthesis of Peptide Carboxyamidomethyl (Cam) Esters for Enzymatic Segment Condensation, *Tetrahedron Lett.* 2016, 57, 3635–3638.
- [4] T. Nuijens, A. Toplak, M. B. A. C. van de Meulenreek, **M. Schmidt**, M. Goldbach, P. J. L. M. Quaedflieg, D. B. Janssen, Chemo-Enzymatic Synthesis of Linear- and Head-to-Tail Cyclic Peptides using Omniligase-I, *Chem. Today* 2016, 34, 16–19.
- [5] **M. Schmidt**, A. Toplak, P. JLM Quaedflieg, J. H. van Maarseveen, T. Nuijens, Enzyme-Catalyzed Peptide Cyclization, *Drug Discov. Today Technol.* 2017, 26, 11–16.
- [6] **M. Schmidt**, A. Toplak, P. J. Quaedflieg, T. Nuijens, Enzyme-Mediated Ligation Technologies for Peptides and Proteins, *Curr. Opin. Chem. Biol.* 2017, 38, 1–7.
- [7] **M. Schmidt**, A. Toplak, H. J. Rozeboom, H. J. Wijma, P. J. L. M. Quaedflieg, J. H. Van Maarseveen, D. B. Janssen, T. Nuijens, Design of a Substrate-Tailored Peptiligase Variant for the Efficient Synthesis of Thymosin- α_1 , *Org. Biomol. Chem.* 2018, 16, 609–618.
- [8] G. J. J. Richelle[§], **M. Schmidt**[§], H. Ippel, T. M. Hackeng, J. H. van Maarseveen, T. Nuijens, P. Timmerman, A One-Pot “Triple-C” Multicyclization Methodology for the Synthesis of Highly Constrained Isomerically Pure Tetracyclic Peptides, *ChemBioChem* 2018, 19, 1934–1938. (*+ cover feature*)
- [9] **M. Schmidt**, Y-H. Huang, E.F.T. de Oliveira, A. Toplak, H.J. Wijma, D.B. Janssen, J.H. van Maarseveen, D.J. Craik, T. Nuijens, Efficient Enzymatic Cyclization of Disulfide-rich Peptides using Peptiligases, *ChemBioChem*, 2019, DOI: 10.1002/cbic.201900033.
- [10] **M. Schmidt**, T. Nuijens, Chemo-Enzymatic Synthesis of Linear- and Head-to-Tail Cyclic Peptides using Omniligase-I, in *Enzym. Ligation Methods* (Eds.: M. Schmidt, T. Nuijens), Springer Nature, 2019, *accepted*.

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- [11] D.E. Streefkerk[§], **M. Schmidt**[§], H. Ippel, T.M. Hackeng, T. Nuijens, P. Timmermann, J.H. van Maarseveen, Synthesis of Constrained Tetracyclic Peptides by Consecutive CEPS, CLiPS and Oxime Ligation, *Org. Lett.*, 2019, 21, 7, 2095-2100.
- [12] G.J.J. Richelle, **M. Schmidt**, J.H. van Maarseveen, P. Timmermann, Tricyclic Peptides via T3 CLIPS Ligation-Cyclizations and CuAAC Ring Closure, *manuscript in preparation*.
- [13] G.J.J. Richelle, H. Bieraugel, **M. Schmidt**, H. Hiemstra, J.H. van Maarseveen, P. Timmermann, General and Facile Route to Highly Complex Penta- and Hexacyclic Peptides via One-Pot T6 CLIPS/CuAAC Ligation-Multicyclizations, *manuscript in preparation*.

[§] Authors contributed equally.

Book Edit

Marcel Schmidt, Timo Nuijens, Enzyme-mediated ligation methods, *Methods in Molecular Biology*, Springer Nature, 2019, *submitted*.

Internal Symposia

Oral Presentations

1. 34th European and International Peptide Symposium 2016, Leipzig: **M. Schmidt**, T. Nuijens, A. Toplak, P.J.L.M. Quaedflieg, A Novel and Scalable Chemo-Enzymatic Route for the Synthesis of the Pharmaceutically Active Peptide Thymosin- α_1 : Engineering of a Specific Ligase.
2. European Health Science Match 2016, Heidelberg, Germany: **M. Schmidt**, A. Toplak, P.J.L.M. Quaedflieg, J.H. van Maarseveen, T. Nuijens, CEPS Technology – A Greener Route to Future Peptide Drugs.
3. Modern Solid Phase Peptide Synthesis Symposium 2017, Frazer Island, Australia: **M. Schmidt**, A. Toplak, T. Nuijens, Chemoenzymatic Peptide Synthesis (CEPS) using Peptiligase Variants: A More Efficient and Greener Route for the Synthesis of Long Peptides, Proteins and Conjugates. **Invited Presentation.**
4. 12th Australian Peptide Conference 2017: **M. Schmidt**, A. Toplak, J.H. van Maarseveen, T. Nuijens: Omniligase- I: A Powerful Tool for Peptide Head-to-Tail Cyclization.
5. 13th German Peptide Symposium 2017, Erlangen, Germany: **M. Schmidt**, T. Nuijens, A. Toplak, P.J.L.M. Quaedflieg, Synthesis of Thymosin- α_1 using CEPS: A Novel and Scalable Process.
6. 7th Chemical Protein Synthesis Meeting, Haifa, Israel: **M. Schmidt**, A. Toplak, R. Lax, T. Nuijens, Use of Subtilisin Variants to Assemble Long Peptides, Proteins and Conjugates.
7. Belgian Peptide Group Meeting 2018, Brussels, Belgium: **M. Schmidt**, A. Toplak, J.H. van Maarseveen, T. Nuijens, Omniligase- I: A Powerful Tool for Peptide Head-to-Tail Cyclization.
8. CHI's Drug Discovery Chemistry 2018, San Diego, USA: **M. Schmidt**, T. Nuijens, Chemo-enzymatic Synthesis of Highly Constrained Multicyclic Peptides.
9. German Biotech Days 2018, Berlin, Germany: **M. Schmidt**, A. Toplak, T. Nuijens, Use of Peptiligase Variants to Assemble Long Peptide, Proteins and Conjugates.
10. Dutch Peptide Symposium 2018, Maastricht, the Netherlands: **M. Schmidt**, Gaston J.J. Richelle, A. Toplak, P. Timmermann, J.H. van Maarseveen, T. Nuijens, Chemo-Enzymatic Synthesis of Highly Constrained Multicyclic Peptides.
11. EuroPEPTIDES 2018, Amsterdam, the Netherlands: **M. Schmidt**, A. Toplak, J.H. van Maarseveen, T. Nuijens, Application of Chemo-Enzymatic Peptide Synthesis (CEPS) to Large Scale Manufacture. **Invited Presentation & Conference Chair.**
12. 22nd ACS Green Chemistry & Engineering Conference 2018, Portland, USA: **M. Schmidt**, A. Toplak, J.H. van Maarseveen, T. Nuijens, CEPS Technology: A More Efficient and Greener Route to Peptide Drugs. **Invited Presentation.**
13. Basellife 2018, Basel, Switzerland: **M. Schmidt**, G.J.J. Richelle, A. Toplak, P. Timmermann, J.H. van Maarseveen, T. Nuijens, Chemo-Enzymatic Synthesis of Highly Constrained Multicyclic Peptides.

Poster Presentations

1. 34th European and International Peptide Symposium 2016, Leipzig: **M. Schmidt**, T. Nuijens, A. Toplak, P.J.L.M. Quaedflieg, A Novel and Scalable Chemo-Enzymatic Route for the Synthesis of the Pharmaceutically Active Peptide Thymosin- α_1 : Engineering of a Specific Ligase.
2. EuroPEPTIDES 2016, Berlin, Germany: **M. Schmidt**, T. Nuijens, A. Toplak, P.J. L. M. Quaedflieg, Development of a Novel and Scalable Chemo-enzymatic Route for the Synthesis of the Pharmaceutical Peptide Thymosin- α_1 .
3. 12th Australian Peptide Conference 2017: **M. Schmidt**, A. Toplak, J.H. van Maarseveen, T. Nuijens: Omniligase-1: A Powerful Tool for Peptide Head-to-Tail Cyclization.
4. Dutch Peptide Symposium 2017, Eindhoven, the Netherlands: **M. Schmidt** A. Toplak, P.J.L.M. Quaedflieg, J.H. van Maarseveen, T. Nuijens, Use of Subtilisin Variants for Assembling Peptide Head-to-Tail Macrocycles.
5. 4th Annual Peptides Congress 2017, London, UK: **M. Schmidt**, A. Toplak, P.J.L.M. Quaedflieg, T. Nuijens, Synthesis of Tymosin- α_1 using CEPS: a Novel and Scalable Process.
6. CHAINS 2017, Veldhoven, the Netherlands: **M. Schmidt**, A. Toplak, J.H. van Maarseveen, T. Nuijens, Omniligase-1: A Powerful Tool for Peptide Head-to-Tail Cyclization.
7. 7th Chemical Protein Synthesis Meeting 2017, Haifa, Israel: **M. Schmidt**, A. Toplak, R. Lax, T. Nuijens, Use of Subtilisin Variants to Assemble Long Peptides, Proteins and Conjugates.
8. HRSMC Summer School Organic Chemistry 2017, Vaeshartelt, the Netherlands: **M. Schmidt**, A. Toplak, J.H. van Maarseveen, T. Nuijens, Omniligase-1: A Powerful Tool for Peptide Head-to-Tail Cyclization.
9. HRSMC Symposium 2018, Leiden, the Netherlands: **M. Schmidt**, G.J.J. Richelle, P Timmermann, J.H. van Maarseveen, T. Nuijens, Omniligase-1: A Powerful Tool for the Synthesis of (Multi-)Cyclic Peptides.

Department Lectures

1. University of Ghent, Department of Organic and Macromolecular Chemistry, Belgium, 2016.
2. University of Queensland, Institute of Molecular Bioscience, Australia, 2017.