“New Multicomponent Strategies to Cyclic Lipopeptides”

Dissertation

zur Erlangung des Doktorgrades der Naturwissenschaften (Dr. rer. nat.)

der

Naturwissenschaftlichen Fakultät II
Chemie, Physik und Mathematik

der Martin-Luther-Universität
Halle-Wittenberg

vorgelegt von

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The work presented in this dissertation has been developed at the Leibniz-Institute of Plant Biochemistry (IPB) in cooperation with Martin-Luther Halle-Wittenberg University.

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“This dissertation is submitted as a cumulative thesis according to the guidelines provided by the PhD-program of Martin-Luther University Halle-Wittenberg. The thesis includes four original research papers (already published) and two published book chapters, which comprise the majority of author’s research work during the course of PhD.”

Micjel Chávez Morejón

1. Gutachter: Prof. Dr. Ludger Wessjohann

2. Gutachter: Prof. Dr. Stefan Bräse

Tag der Verteidigung: 31. Januar 2018

To my family and friends.

All is beautiful and constant,
all is music and reason,
and all, like the diamond,
before it is light is coal.

José Martí.

Acknowledgments

First and foremost I want to thank my advisors Daniel García Rivera and Ludger Wessjohann for their continuous support of my PhD studies. It has been an honor counting with their immense knowledge, and their guidance helped me in all the time of research and writing of this thesis. Professor Wessjohann guided me close in identifying and gathering appropriate resources to face scientific issues with accurate and wise comments. Daniel shared with me as a friend, the joy and enthusiasm he has for his research was contagious and motivational for me. They have taught me, both consciously and unconsciously, how good experimental chemistry is done. I appreciate all their contributions of time, ideas, and funding to make my PhD experience productive and stimulating. Besides my advisors, I would like to thank Prof. Dr. Stefan Bräse for his disposition in acts as consultant and second reviewer of my thesis.

The members of the Bioorganic Chemistry department have contributed immensely to my personal and professional time at the *Leibniz Institute of Plant Biochemistry* (IPB-Halle). The group has been a source of friendships as well as good advice and collaboration. I am especially grateful with Prof. Bernhard Westermann for his necessary advices in order to rise the quality of my thesis. My sincere thanks the former and current fellow labmates, Annegret, Angela, Ricardo, Alfred, Rainer, Sebastian Stark, Tiago, Ali Akbar, Nalin, Aldrin, Yanira, Manuel, Haider, David and Thomas for their enthusiasm and nice work atmosphere. I would like to place a special acknowledgement to Goran Kaluđerović and Ali N. Hmedat for the anticancer assay described in the chapter 2, and also to all the technical staff involved in the development of this thesis for their priceless contribution, Ms. Petra Majovsky (SPPS), Ms. Anja Ehrlich (HPLC), Mrs. Martina Lerbs (ESI-MS), Mrs. Gudrun Hahn (IR, OR, NMR), Ms. Rica Patzschke (NMR), Dr. Andrea Porzel (NMR), Dr. Jürgen Schmidt (HRMS).

I gratefully acknowledge the funding sources that made my PhD work possible. I was funded by MK-LSA, project “Lipopeptide”, for my first 2 years and the Leibniz Association for years 3 & 4. My work was also supported by the IPB and the University of Havana.

I would like to highlight the camaraderie and teamwork of Ricardo. We worked together along thousand and thousand hours in the lab, and I very much appreciated his enthusiasm, intensity, willingness to do and learn always something more, and his amazing ability to persevere even during tough times in the PhD pursuit. Without a doubt, our exciting scientific discussions and fruitful collaboration, were crucial for the success of this work. I would also like to express my gratitude to him for taking care of me and true friendship during all these years.

Last but not the least, I would like to thank my both, the Cuban and the German, families for all their love and encouragement. For my parents who raised me with a love of science and supported me in all my pursuits. For the presence of my brother Micjail in all special events of my life and for being an essential pillar for keeping our family strong and joined. And most of all, for my loving, supportive, encouraging, and patient wife Annegret whose faithful support during the final stages of this PhD is so appreciated. Thank you.

Micjel Chávez Morejón
Leibniz Institute of Plant Biochemistry (IPB)
Halle (Saale), June 2017

Table of contents

List of abbreviations

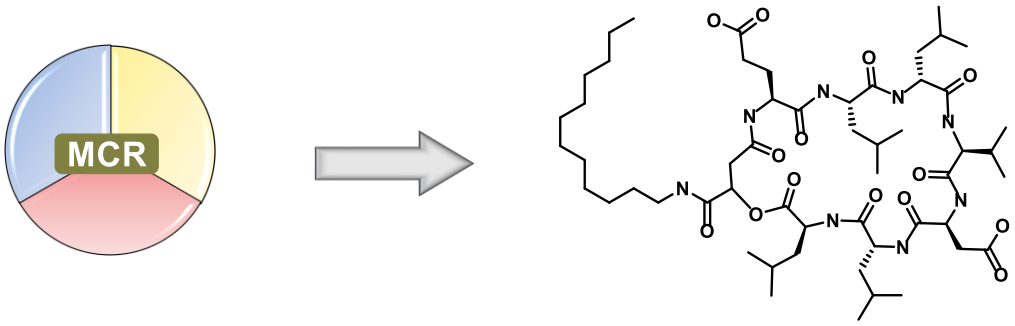
Ac	acetyl	Hz	Hertz
atm	atmosphere	*i*-	*Iso*-
Bn	benzyl	IC_{50}	median inhibitory concentration
Boc	*tert*-butoxycarbonyl	*i.e.*	*id est* (that is)
^{o}C	degrees Celcius (centigrade)	IMCR	Isocyanide multicomponent reaction
calcd	calculated	IPB	4-isocyanopermethylbutane-1,1,3-triol (or Leibniz-Institute of Plant Biochemistry)
Cbz	benzyloxycarbonyl	*J*	coupling constant (in NMR)
CFSE	5(6)-carboxyfluorescein diacetate *N*-succinimidyl ester	LC	liquid chromatography
CLPs	cyclic lipopeptides	M	molar
3CR	three-component reaction	m	mili
4CR	four-component reaction	m	multiplet (in NMR)
CSA	camphorsulfonic acid	MCR	multicomponent reaction
CV	crystal violet	MD	molecular dynamic
d	doublet in NMR	Me	methyl
DAPI	2-(4-amidinophenyl)-6-indolecarbamidine dihydrochloride	min	minutes
DHR	dihydrorhodamine	mp	melting point
DIC	*N,N'*-diisopropylcarbodiimide	MS	mass spectrometry
DIPEA	*N*,*N*-diisopropylethylamine		
DMF	*N*,*N*-dimethylformamide	MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
DMSO	dimethylsulfoxide	MW	microwave
DPBS	Dulbecco's phosphate-buffered saline	NBD	*N*-(3-azidopropyl)-7-nitro-benzo[*c*][1,2,5]oxadiazol-4-amine
d.r.	diastereomeric ratio		
EDC	*N,N'*-1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride		
EDTA	ethylenediaminetetraacetic acid	NMR	nuclear magnetic resonance
e.g.	*exempli gratia* (for example)	Nu	nucleophile
ESI	electrospray ionization	*p*-	*para*-
Et	ethyl	PBS	phosphate-buffered saline
et al.	*et alia* (and others)	P-3CR	Passerini three-component reaction
equiv	equivalent	PDA	photodiode array
FGC	functional group conversions	ppm	parts per milion
Fmoc	9-fluorenylmethoxycarbonyl	PyBOP	benzotriazol-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate
g	gram	q	quartet (in NMR)
h	hour(s)	RPMI	medium suitable for cell culture
HATU	1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate	R_t	retention time
HBTU	*O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate	r.t.	room temperature
HEPES	4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid	s	singlet (in NMR)
HOBt	hydroxybenzotriazole	SCR	single-component two-center reaction
HPLC	high performance liquid chromatography	SPPS	solid phase peptide synthesis
HRMS	high resolution mass spectrometry	*t*-	*tert*-(tertiary)

TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPS	triisopropylsilane
TLC	thin layer chromatography
TMS	tetramethylsilane
UDAC	Ugi-deprotection-activation-cyclization/condensation
UHPLC	ultra-high performance liquid chromatography

Chapter 1

Isocyanide-Based Multicomponent Reactions (IMCRs) as a Tool to Approach Macrocyclic Peptides

*Abstract**



Macrocycles comprise an important class of compounds since their widespread occurrence in nature and their intrinsic three-dimensional structures play an important role in chemistry, biology, and medicine.[1-4] Their outstanding success is based not only on the discovery of the macrocycles from natural sources, but also on the enormous challenge posed to chemists to design strategies for synthesizing these compounds.[5,6] The chapter summarizes basic concepts of the IMCR-based macrocyclization and its applications in the synthesis of cyclopeptide scaffolds.

* Parts of this chapter were published in: (a) Wessjohann, L. A.; Kaluđerović, G.; Neves Filho, R.A.W.; Morejon, M.C.; Lemanski, G.; Ziegler, T. in Multicomponent reactions 1 [Ed. Müller T.J.J.], *Science of Synthesis*, **2013**, 415-497. (b) Wessjohann, L. A.; Neves Filho, R. A.W.; Puentes, A. R.; Morejon, M. C. in Macrocycles from Multicomponent Reactions, *Multicomponent Reactions in Organic Synthesis* [Eds. Zhu, J.; Wang, Q.; Wang, M.-X.], Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, **2014**, 231–264.

1.1 Introduction[a]

Macrocycles are usually defined as molecules that bear at least one nonbridged 12-membered ring. This is not a fixed definition, however, and some authors consider smaller ring sizes (8-, 9-, 10-, and 11-membered rings) as macrocycles or define a higher limit at 13 or even 14 ring atoms.[4] Macrocycles have intrigued chemists in both the synthetic and the natural world. Early macrocycle syntheses mainly focused on homodi- or homooligomeric macrocycles, predominantly for host–guest chemistry. Meanwhile natural macrocycles fascinated chemists due to their great structural diversity and for their biological activity, for example, as antibiotics and membrane-active compounds.[7,5]

In the bioactive compound space, macrocycles occupy a privileged intermediate position, displaying the binding power of biologics, thereby at the same time retaining the availability of small molecules.[8] While medium-sized macrocycles in terms of molecular weight and number of H-bond donors and acceptors mostly fall into the limits of Lipinski's rule of five for oral availability,[9] larger rings have successfully challenged the rules.[10] Large macrocycles can "hide" excessive H-bond donors and acceptors and reduce conformational flexibility via transannular interactions or show Janus behavior by turning inside out and back either hydrophilic or lipophilic groups.[10] An example for an orally available drug that does not obey Lipinski's rule is cyclosporin A (**1**), a cycloundecapeptide used as immunosuppressant in organ transplantation.[11] Macrocyclic drug hits have also been found in fields where rational design is difficult.[12] A classical example is the glycopeptide antibiotic vancomycin (**2**), which was employed for many years as last line resort against very resistant strains.[13] The appearance of vancomycin-resistant organisms significantly reduces its use though (**Figure 1.1**).[14]

[a] Paragraphs 1.1-1.2 were partly taken from: (a) Wessjohann, L. A.; Kaluđerović, G.; Neves Filho, R.A.W.; Morejon, M.C.; Lemanski, G.; Ziegler, T. in Multicomponent reactions 1 [Ed. Müller T.J.J.], *Science of Synthesis*, **2013**, 415-497. (b) Wessjohann, L. A.; Neves Filho, R. A.W.; Puentes, A. R.; Morejon, M. C. in Macrocycles from Multicomponent Reactions, *Multicomponent Reactions in Organic Synthesis* [Eds. Zhu, J.; Wang, Q.; Wang, M.-X.], Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, **2014**, 231–264.

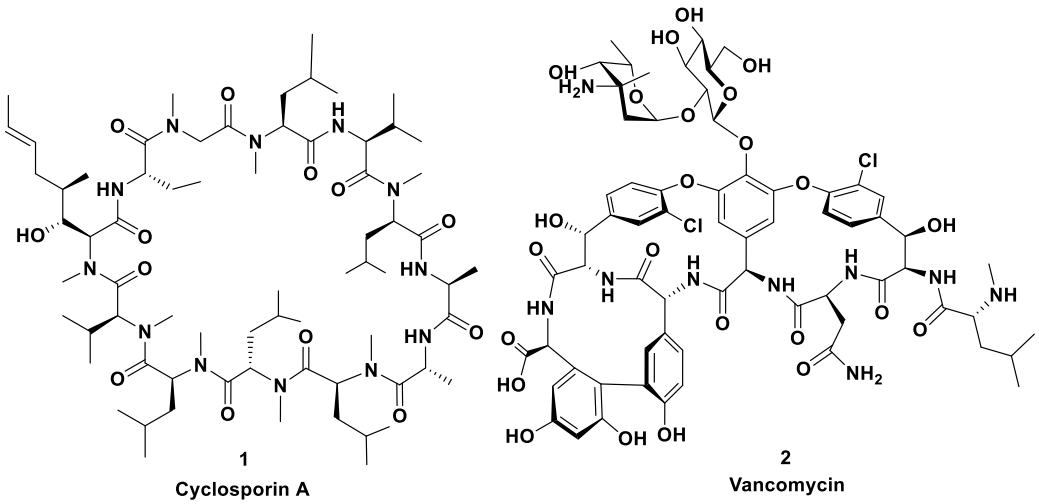


Figure 1.1* Selected structures of naturally occurring macrocyclic drugs.

Commonly, cyclization is used to anchor bioactive conformations as well as to study the structure - activity relationship of peptides. It is hypothesized that cyclization considerably reduces the conformational flexibility of the peptide backbone and provides the determination and the rational handling of the biologically important three-dimensional structures of active compounds.[15] In addition, these structures provide cyclopeptides a higher *in vivo* biological resistance against enzymatic degradation in comparison with their linear analogues. Besides their pharmacological potential, macrocycles are also applied in areas such as nanomaterials,[16] polymers,[17] and supramolecular chemistry.[18-20]

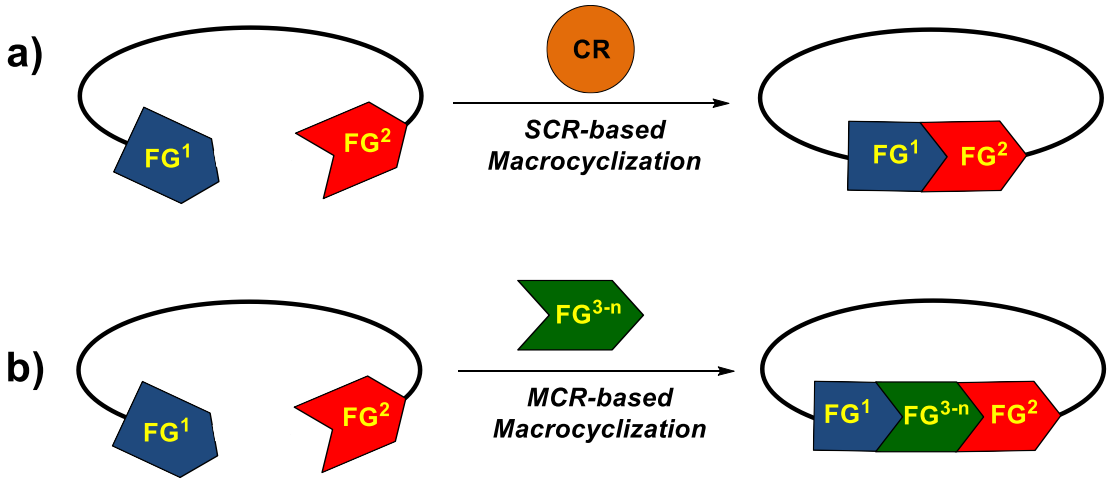
1.2 Cyclic peptides from multicomponent reactions

To deal with the synthesis of macrocyclic natural products or any designed macrocycles for a particular purpose, the ring closure is, naturally, the crucial step to determine the effectiveness of the overall synthetic strategy.[5, 21, 22] Most of the single-component reaction-based macrocyclizations start with the synthesis of a linear precursor carrying two joinable functional groups (FGs) attached to the head and tail, respectively, to allow for a tethered version of classical two-component reactions (**Scheme 1.1a**). The macrocyclization is then triggered by the addition of a coupling reagent (CR) under high- or pseudo-dilution conditions. Many reactions such as macrolactamizations,

* Taken from: Wessjohann, L. A.; Neves Filho, R. A.W.; Puentes, A. R.; Morejon, M. C. in Macrocycles from Multicomponent Reactions, *Multicomponent Reactions in Organic Synthesis* [Eds. Zhu, J.; Wang, Q.; Wang, M.-X.], Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, **2014**, 231–264.

macrolactonizations, ring-closing metathesis, cycloadditions, and cross-couplings have been successfully used in the past few years for cyclizing long linear molecules.[15] Using this methodology, the coupling reagent acts just as mediator of the macrocyclization process. Since its atoms are not present in the obtained structure, no further diversity can be added during the step. Sometimes the two terminals are connected under the loss of the activating or other moieties, further decreasing the atom economy of the process.

Another, so far less common, strategy is to perform the macrocyclization itself employing multicomponent reactions (MCRs) (**Scheme 1.1b**).[23] In these reactions, three or more reactive moieties – at least two of them tethered – condense to generate a product that contains most or all of the atoms involved in the process. Since all components contribute as a diversity input to the final product, the MCRs approach provides an ideal starting point to accomplish cyclic compounds in which diverse macrocyclic scaffolds are generated that display enough molecular complexity to resemble natural products.[23]

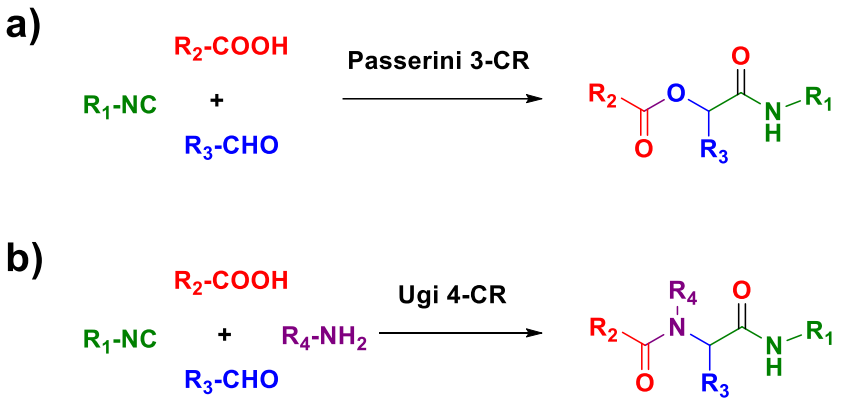


Scheme 1.1* Comparison of macrocyclization methodologies. (a) Single-component two-center reaction (SCR)-based approach with a condensation reagent. (b) MCR-based approach: the macrocycle carries the diversity delivered by the additional components FG^n (FG^{3-n}).

The development of MCRs has experienced a great boost in recent years, as the promise rapid, versatile and sustainable organic syntheses. A very prominent subgroup of MCRs is that involving isonitriles (or isocyanides), also abbreviated as IMCRs.[24] The research in this particular field has displayed a tremendous growth in the past few decades, in particular after the discovery of the Passerini three-component reaction (P-3CR)

* Taken from: Wessjohann, L. A.; Neves Filho, R. A.W.; Puentes, A. R.; Morejon, M. C. in Macrocycles from Multicomponent Reactions, *Multicomponent Reactions in Organic Synthesis* [Eds. Zhu, J.; Wang, Q.; Wang, M.-X.], Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, **2014**, 231–264.

(**Scheme 1.2a**) and Ugi four-component reaction (Ugi-4CR) (**Scheme 1.2b**),[25, 26] the latter one being the most explored IMCR, with great importance in the synthesis of heterocycles, peptidomimetics, and natural products.[27]



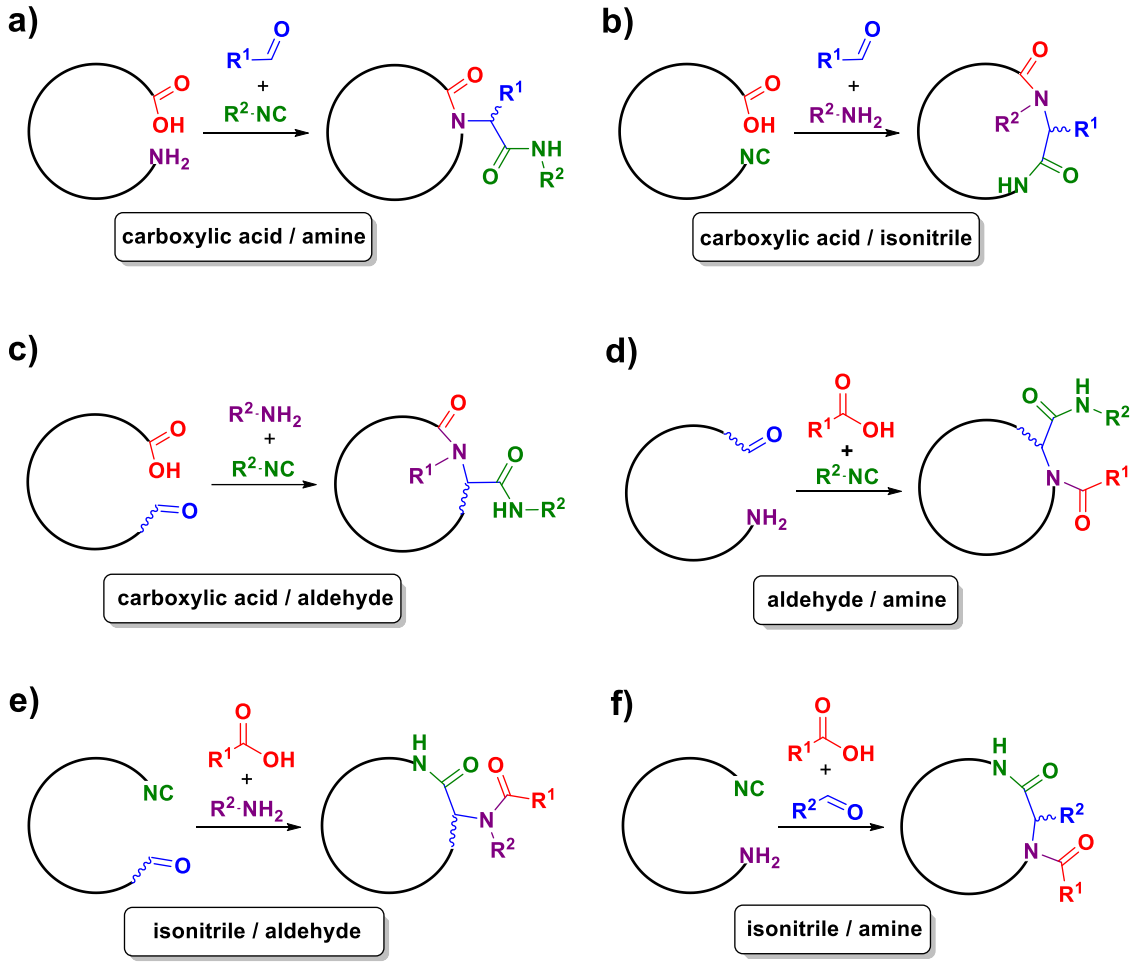
Scheme 1.2 a) The Passerini three component reaction (P3CR). b) The Ugi four component reaction (U4CR).

The diversity-generating ability of the Ugi-4CR has been explored in the synthesis of linear precursors of macrocycles with remarkable results, for example, in the work of the groups of Joullie and others on natural product-inspired ansacyclopeptides.[21,28] However, the use of IMCRs themselves for the ring-closing step remained almost untapped until the past decade.[23, 29]

A crucial condition for a cyclative IMCR to take place is that at least one of the building blocks must carry two reacting functionalities. If these two groups are connected through a linker consisting of at least 10 atoms, the obtained product, by definition, will be a macrocycle. For the most important IMCR, that is, the Ugi-4CR with its peptide backbone products, there are theoretically six different possible building block combinations (**Scheme 1.3**): (a) carboxylic acid/amine (peptide coupling type), (b) carboxylic acid/isonitrile, (c) carboxylic acid/aldehyde, (d) aldehyde/amine, (e) isonitrile/aldehyde, and (f) isonitrile/amine. In all cases, the atoms of the bifunctional building blocks remain endocyclic, while those from the additional reagents have varied degrees of appearing exocyclic (**Scheme 1.3**).[30]

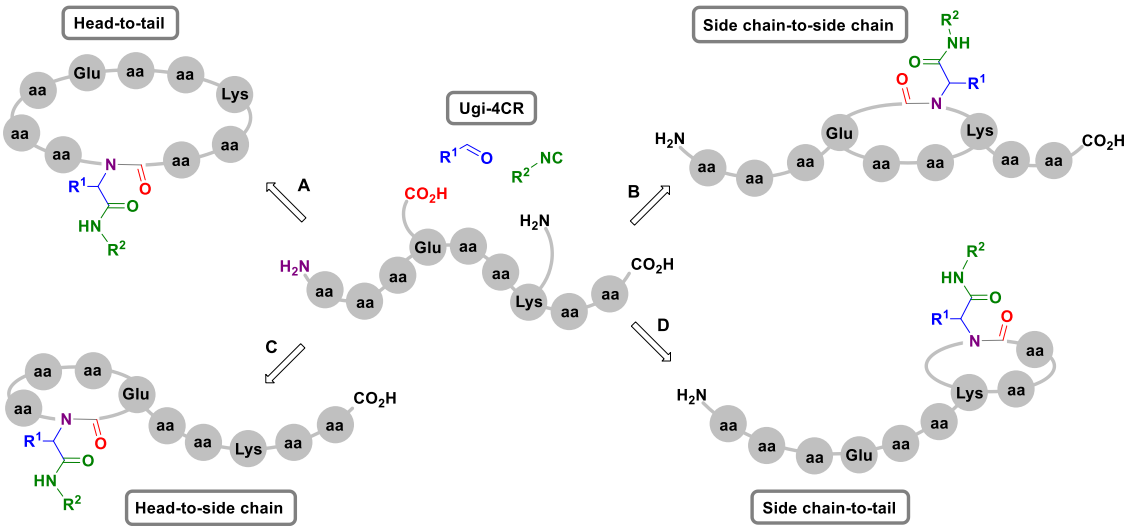
In spite of six possibilities, so far only the combination amine–carboxylic acid has been widely employed. Attempts of macrocyclizations involving other combinations have been made, but were less successful. For example, a carboxylic acid–isonitrile bifunctional building block (**Scheme 1.3b**) gave just traces of the desired product.[29] The authors suggest that instability of the bifunctional building block, originating from the

incompatibility of carboxylic acid and isonitrile functionalities under certain conditions, could be one reason behind the failed reaction.



Scheme 1.3 Ugi-4CR-based macrocyclizations of single bifunctional building blocks.

Additionally, as peptides may containing other residues which carry Ugi-reactive functionalities, e.g. Lys, Asp, Glu, etc., other macrocyclization possibilities like: head-to-side chain, side chain–to-head, side chain-to-side chain, and side-chain-to-tail are available (**Scheme 1.4**).

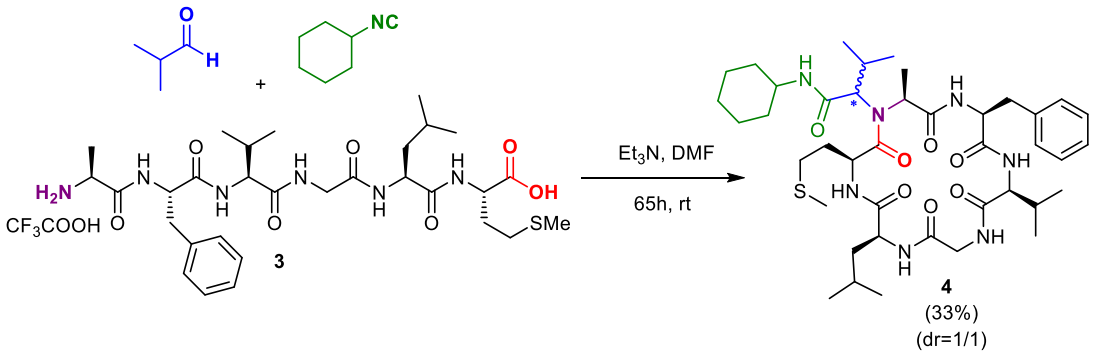


Scheme 1.4 Extended possibilities for Ugi-4CR macrocyclizations of peptides.

1.2.1 Synthesis of cyclopeptide analogues

A conceptually simple approach involves tying two out of four components and to perform an Ugi three-component, four-center condensation (U-3C-4CR).[31] In fact, the first example of a macrocyclization based on an Ugi reaction was reported in 1979 by the group of Failli (**Scheme 1.5**).[32]

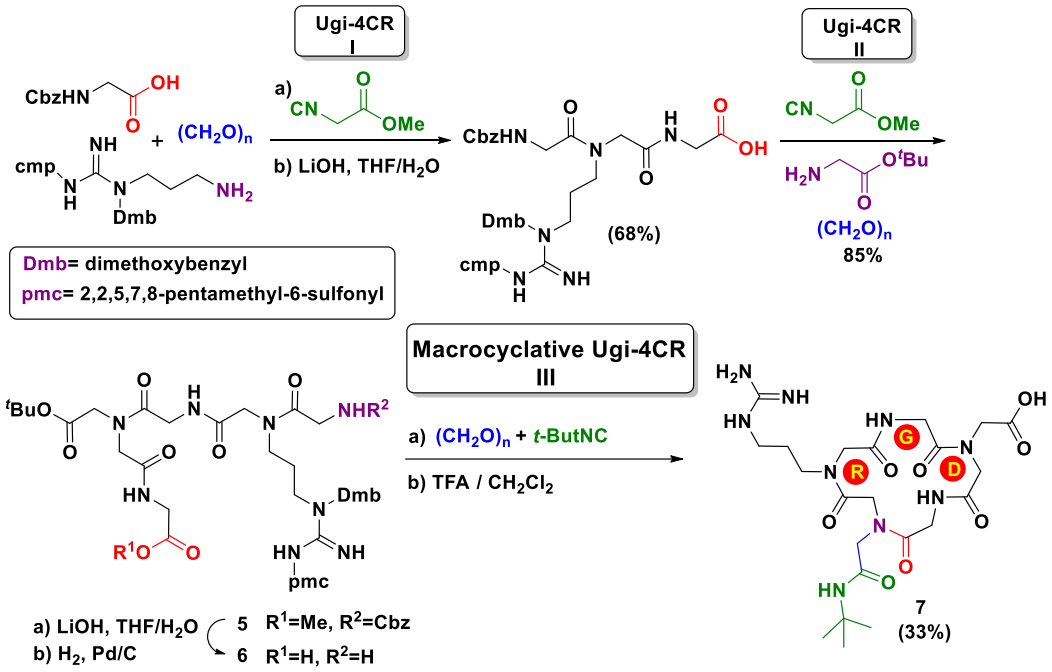
Scheme 1.5 illustrates the synthesis of cyclohexapeptide **4** as a mixture of two separable diastereomers. Since oligomerization is one of the major side reactions that influence the efficiency of any macrocyclization process, high-dilution conditions are generally applied to achieve the desired ring closure; for this reason, slow addition of **3** to the reaction mixture was applied in order to achieve pseudo-dilution conditions.



Scheme 1.5 Example of an U-3C-4CR macrocyclization.

1.2.2 Synthesis of cyclopeptoids

A similar approach has been used in the synthesis of cyclic RGD (arginine-glycine-aspartic acid) pentapeptoids by three consecutive Ugi-4CR including one for macrocyclization (**Scheme 1.6**).[33]



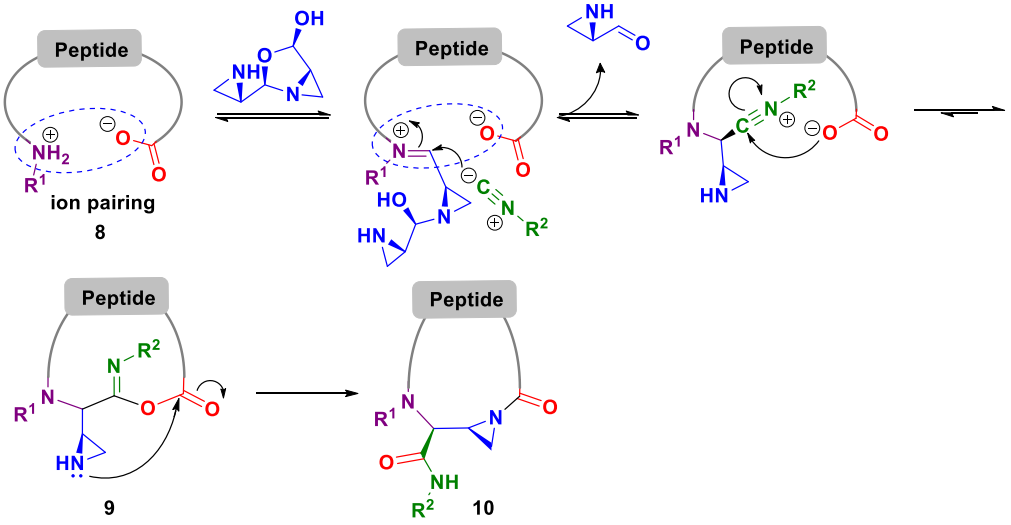
Scheme 1.6 Sequential elongation/cyclization in a Ugi-4CR-based approach to RGD cyclopeptoids **7**.

Thus, the amino acid **6**, the precursor for the cyclization, is obtained from protected peptide **5** by ester hydrolysis and benzyloxycarbonyl deprotection. Reaction of **6** with formaldehyde and *tert*-butyl isocyanide in methanol affords, after global deprotection under acidic conditions, the cyclopeptoid **7** in 33% yield over four steps. The macrocyclization step was achieved under pseudo-high-dilution conditions to avoid undesirable oligomerization side products (**Scheme 1.6**).

1.2.3 Macrocyclization using an aziridinecarbaldehyde

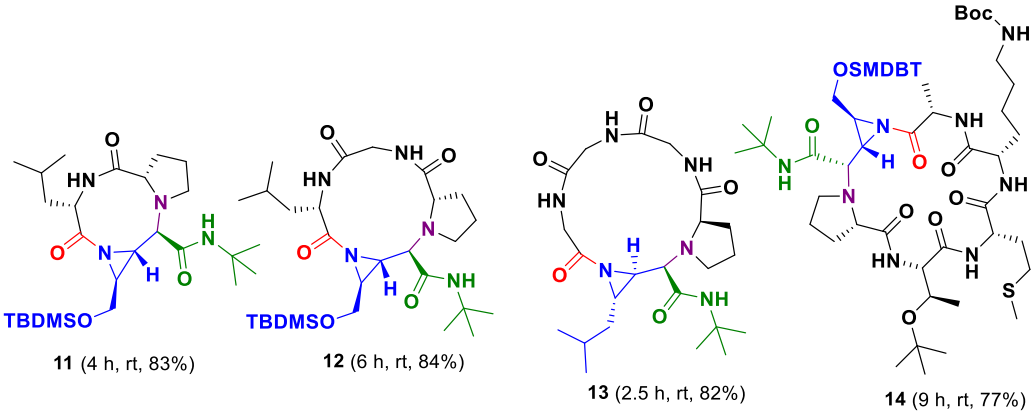
A variation of this methodology was reported in 2010 by Yudin and co-workers.[34] Remarkably, high-dilution or pseudo-dilution conditions are not used for the cyclization, and 12-, 15-, and 18-membered macrocycles, and even nine-membered medium-sized rings, are diastereoselectively synthesized in excellent yields. **Scheme 1.7** outlines the basic principle, in which a secondary-amine-terminated peptide **8**, an

aziridinecarbaldehyde (amphoteric amino aldehyde), and an isocyanide provide intermediate **9**, and eventually the cyclopeptide **10**.



Scheme 1.7* Ugi-4CR-based macrocyclizations of peptides involving amphoteric aziridine aldehydes.

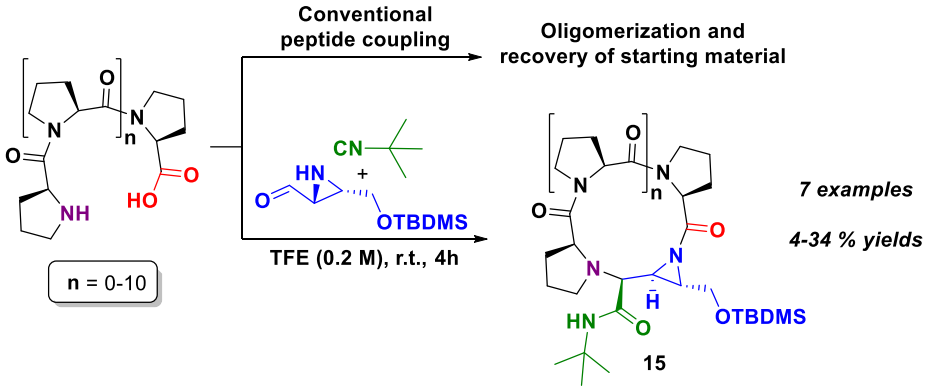
Conventionally, such an Ugi macrocyclization proceeds through a transannular process but in Yudin´s proposal the key ring-forming process is achieved by nucleophilic addition of the aziridine nitrogen situated exocyclic to the electrophilic imidate functionality (**Scheme 1.7**). It is assumed that the efficiency of the macrocyclization in this approach is due to the less hindered trajectory of attack of the aziridine nitrogen, which makes the final step of the process kinetically favorable. **Scheme 1.8** summarizes the yields and the reaction times of some macrocycles synthesized according to this methodology.



Scheme 1.8* Macrocycles synthesized via an aziridinecarbaldehyde.

* Taken from: Wessjohann, L. A.; Neves Filho, R. A.W.; Puentes, A. R.; Morejon, M. C. in Macrocycles from Multicomponent Reactions, *Multicomponent Reactions in Organic Synthesis* [Eds. Zhu, J.; Wang, Q.; Wang, M.-X.], Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, **2014**, 231–264.

The rigidity of homochiral oligoprolines is well documented in the literature to such an extent that these structures have been named “molecular rulers”.[35] Hypothesizing that electrostatic interactions between the *C*- and the *N*-terminus might be able to break up the helical structure of these peptides and create a loop that would enable macrocyclization, L-oligoprolines containing 2, 3, 4, 6, 8, 10, and 12 residues were submitted to a Ugi-4CR macrocyclization with aziridine aldehyde and *tert*-butyl isocyanide in TFE at 0.2 M for 4h.[36] In a controlled experiment, the macrocyclization of $(Pro)_6$, employing different coupling reagents and concentrations, was also attempted. Gratefully, all the Ugi-4CR-based macrocyclizations furnished the desired macrocycles **15** in good yields, while the conventional protocol resulted in undesired cyclo-oligomerization or recovery of the starting material. These encouraging results clearly highlight the usefulness of IMCR-based macrocyclizations even when using very rigid frameworks (**Scheme 1.9**).



Scheme 1.9* Synthesis of rigid homochiral oligoprolines and comparison with standard procedures.

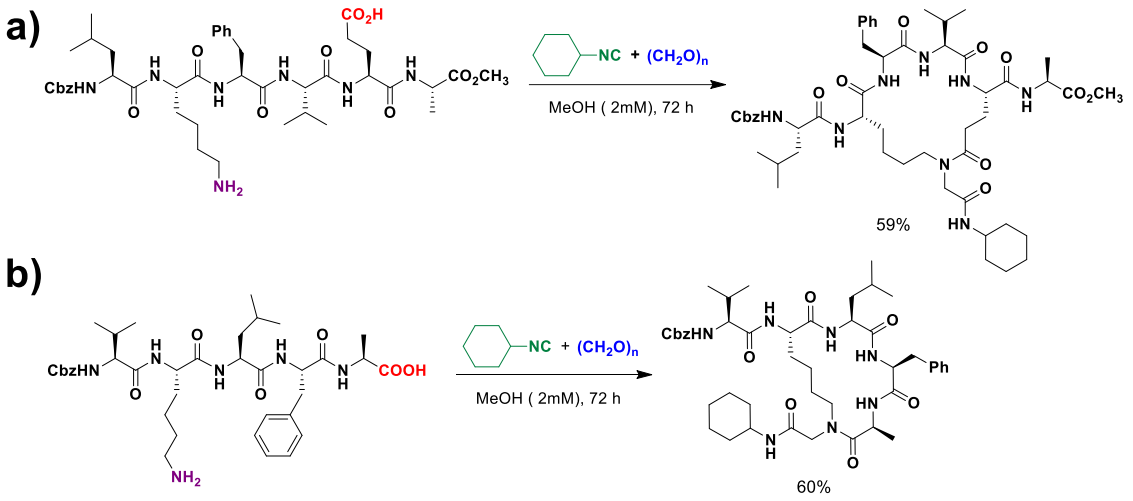
1.2.4 Macrocyclization of peptide side chains

Although many efforts have been directed towards the development of Ugi-4CR macrocyclization of peptides, these works focused solely into macrocyclizations involving the *N*- and *C*-termini, which are usually referred as head-to-tail cyclizations.

Recently, the Rivera and Wessjohann groups jointly investigated the latter two possibilities in order to access different folded peptide conformations. It was found that side chain-to-side chain macrocyclizations performed better than the head-to-side chain

* Taken from: Wessjohann, L. A.; Neves Filho, R. A.W.; Puentes, A. R.; Morejon, M. C. in Macrocycles from Multicomponent Reactions, *Multicomponent Reactions in Organic Synthesis* [Eds. Zhu, J.; Wang, Q.; Wang, M.-X.], Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, **2014**, 231–264.

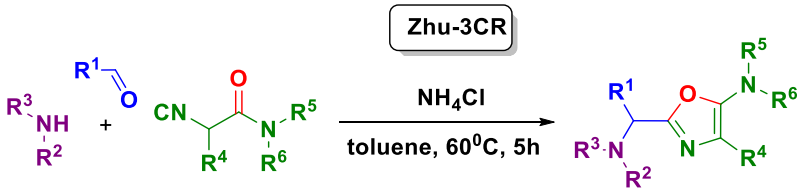
ones (**Scheme 1.10a**), but there were no great differences compared with the side chain-to-tail procedure (**Scheme 1.10b**); presumably, due to the higher flexibility of the Lys side chain amino group. Furthermore, NMR and MD analyses of the products revealed the stabilisation of folded structures like helical, alpha and beta turns.[37]



Scheme 1.10 a) Side-chain to side-chain macrocyclization. a) Side chain-to-head macrocyclization.

1.2.5 Zhu-3CR activation/macrocyclization cascade

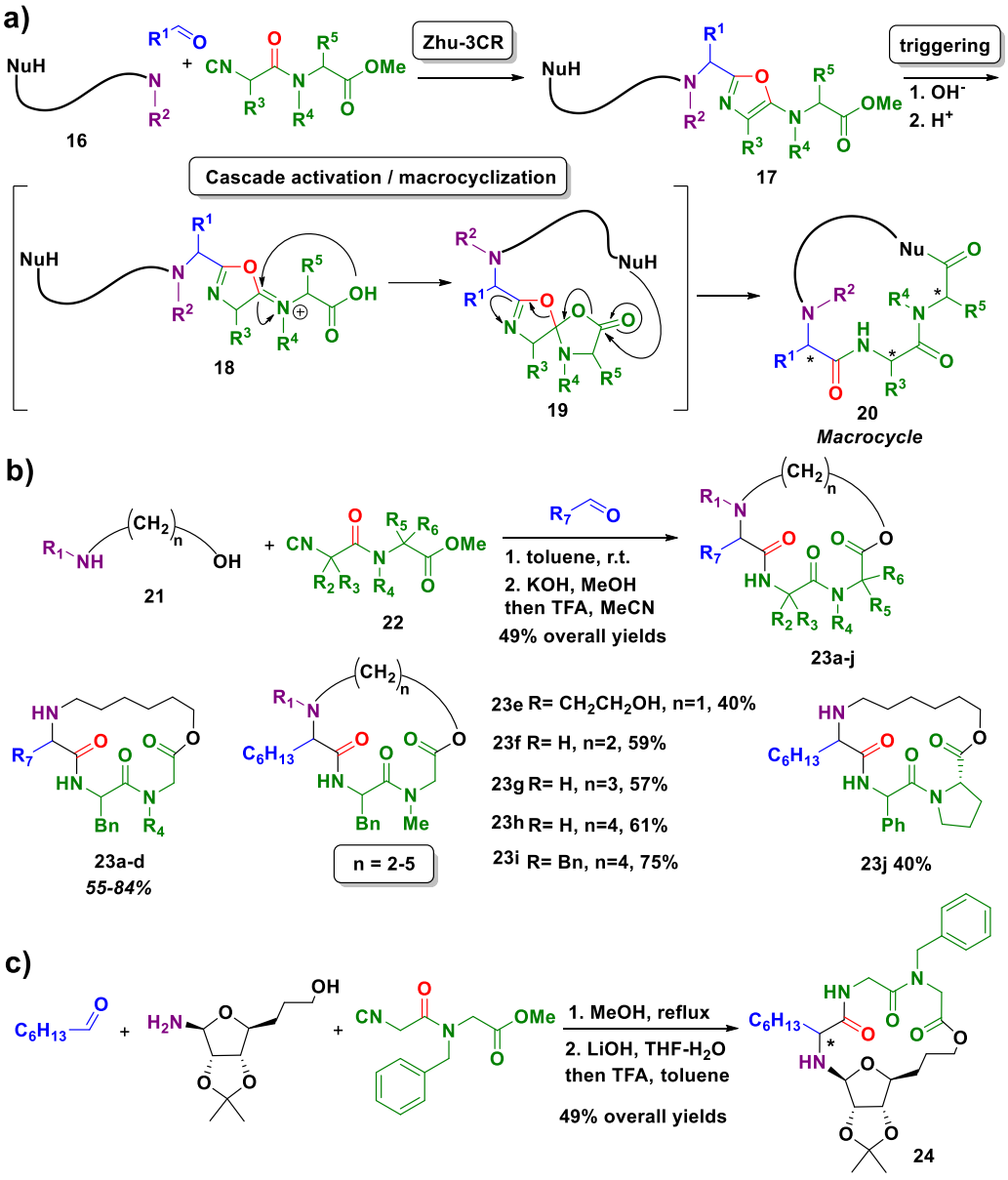
Although most of IMCR-based macrocyclizations involving single bifunctional building blocks rely on the use of Ugi-4CRs, other IMCRs have also proven useful to accomplish this task in a very elegant way. The condensation between aldehydes, amines and α-isocyanoacetamides developed by Zhu and co-workers in 2001 is a highly versatile method to yield 5-aminooxazoles and its potential has been explored in the synthesis of libraries of heterocyclic compounds (**Scheme 1.11**).[38, 39]



Scheme 1.11* Formation of 5-aminooxazoles by MCR between aldehydes, amines and α-isocyanoacetamides (Zhu-3CR).

* Taken from: Wessjohann, L. A.; Neves Filho, R. A.W.; Puentes, A. R.; Morejon, M. C. in Macrocycles from Multicomponent Reactions, *Multicomponent Reactions in Organic Synthesis* [Eds. Zhu, J.; Wang, Q.; Wang, M.-X.], Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, **2014**, 231–264.

The same group hypothesized that a bifunctional building block **16** carrying a nucleophilic moiety attached at the head and an acid at the end could be cyclized,[40] if a 5-aminooxazole moiety was conveniently placed between them (**Scheme 1.12**).[41, 43]



Scheme 1.12* Zhu-3CR/traceless activation/macrocyclization cascade. a) Proposed mechanism. b) Examples of synthesized depsicyclopeptides **23a-j**. c) Application in the synthesis of glycocyclodepsipeptides.

Mechanistically, the cascade process starts with the protonation of the oxazole ring in **17** to spring an eletrophilic iminium ion **18**. The nucleophilic attack of the acid on the iminium

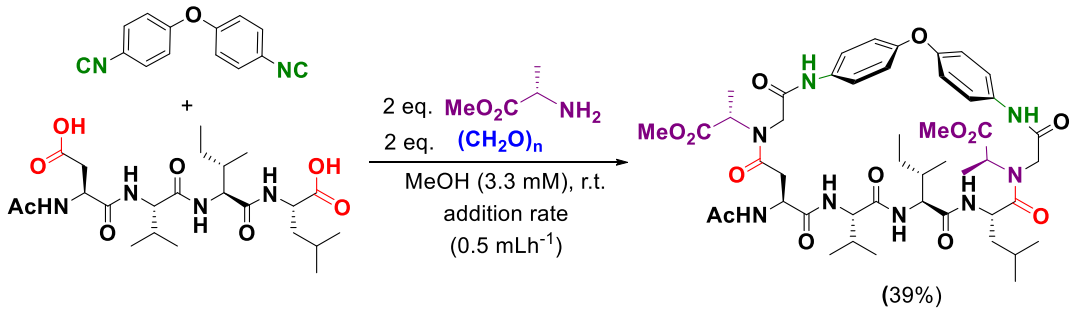
* Taken from: Wessjohann, L. A.; Neves Filho, R. A.W.; Puentes, A. R.; Morejon, M. C. in Macrocycles from Multicomponent Reactions, *Multicomponent Reactions in Organic Synthesis* [Eds. Zhu, J.; Wang, Q.; Wang, M.-X.], Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, **2014**, 231–264.

ion, favored by a 5-membered ring transition state, leads to spirolactone **19**. This latter constrained intermediate undergoes a nucleophilic attack by the tethered nucleophile to afford a macrocyclic structure **20**. Although the Zhu-3CR is not responsible for the ring-closure step itself, it generates the 5-aminooxazole moiety that acts at the same time as building block and carboxylic acid traceless activator. In order to demonstrate this concept, amino alcohols **21** reacted with aldehydes and α-isocyanoacetamides **22** to afford after saponification the suitable bifunctional building block. The cascade process is then triggered by a few equivalents of TFA to afford a macrocyclodepsipeptide **23** in good overall yields. The same approach was later employed in the synthesis of glycocyclodepsipeptide **24** with remarkable results (**Scheme 1.12**).[44]

1.2.6 Bidirectional macrocyclizations of peptides by double Ugi-4CR

In an attempt to further exploit MCRs in the field of cyclopeptides, Rivera´s group describe a bidirectional multicomponent approach for the synthesis of *N*-alkylated macrocyclic peptides of varied sequences and cross-linking positions. The process relies on the execution of two Ugi reactions between peptide diacids and diisocyanides. Varying the amino component enabled the installation of exocyclic elements of diversity, while skeletal diversity was created through different side chain and backbone cyclizations.[45]

Scheme 1.13 depicts an example, in which a peptide diacids bearing the side chain and terminal carboxylic groups was subjected to bidirectional macrocyclization with biaryl ether diisocyanide and a *C*-protected alanine as amino components. The double Ugi-4CR procedure, leads to the formation of two parallel *N*-substituted dipeptide backbones as tethers of the cross-linker.



Scheme 1.13 Side chain-to-backbone bidirectional macrocyclization of a peptide by double Ugi-4CR.

1.3 Remarks and future perspectives

Multicomponent reactions have revolutionized access to macrocycles in both major parts of their assembly: the synthesis of linear precursors, as well as the macrocyclization step itself. So far, they have been especially successful for the syntheses of macrocycles with pseudopeptidic, peptoid and some heterocyclic elements.

The following advantages are general for most MCRs so far utilized in macrocycle syntheses:

- high input of diversity
- high atom and step economy
- usually simple and efficient reaction conditions
- no condensing reagent required
- macrocyclizations with simultaneous introduction of functional elements

In the past, most studies were directed toward understanding the principles of using MCRs in macrocyclizations and to explore the scope of these reactions, especially of the valuable IMCRs. In the future, this will be extended to even more MCRs, which in themselves are only at the advent of being explored. More importantly, with the groundwork laid on how to efficiently use this methodology in macrocycle syntheses, near future work will concentrate even more on two topics of this yet underused concept: on the one hand, the generation of functional libraries for certain purposes, in medicinal chemistry or other, where rapid, easy variation is required; and, on the other hand, for the efficient synthesis of defined molecules with specific biological or other function.

1.4 Aims of this Ph.D. work

The present thesis is aiming the development of new macrocyclization strategies based on MCR towards functionalized cyclic peptides, by means of:

- Utilization of the Ugi and Passerini multicomponent reactions for the cyclization of peptides and oxo-peptides.
- Construction of a small library of analogues of the natural products mycosubtilin and surfactin A.
- Development of a new methodology for the solution and solid-phase macrocyclization of peptides based on Ugi-Smiles multicomponent reaction.
- Development of an on-resin turn-inducer mimic for the head-to-tail macrocyclization and functionalization of peptides in solid phase.
- Utilization of convertible isonitriles for the ligation and macrocyclization of peptides arising from isocyanide-based multicomponent reaction

1.5 References

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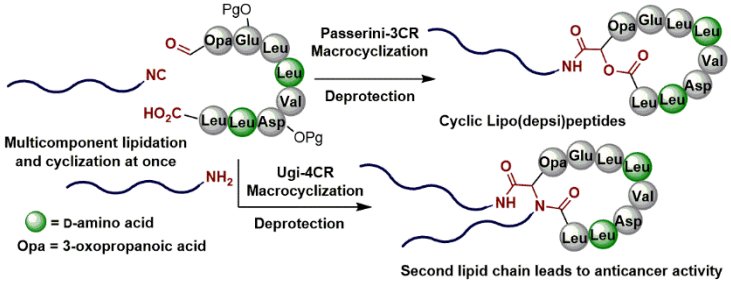
Chapter 2

A Multicomponent Macrocyclization Strategy to Natural Product-Like Cyclic Lipopeptides: Synthesis and Anticancer Evaluation of Surfactin and Mycosubtilin Analogues

*Abstract**



A multicomponent macrocyclization strategy towards cyclic lipopeptides is described. The approach relies on the utilization of the Ugi and Passerini multicomponent reactions for the cyclization of peptides and oxo-peptides and is employed for the construction of a small library of analogues of the natural products mycosubtilin and surfactin A. A key feature of this method is the simultaneous incorporation of either one or two exocyclic lipid tails along with the macrocyclic ring closure, which is only possible due to the multicomponent nature of the macrocyclization step. While the macrocyclic monolipidic surfactin analogues showed no significant activity, introduction of two lipid side chains leads to a more potent cytotoxicity on cancer cell lines. This is a new example of the multicomponent reaction potentials in rapidly producing macrocycles and natural product analogues for biological screening.

* This Chapter was published: Morejón, M. C.**; Laub, A.; Kaluđerović, G.; Puentes, A. R.; Hmedat, A. N.; Otero-González, A. J.; Rivera, D. G.; Wessjohann, L. A. *Org. Biomol. Chem.* **2017**, *15*, 3628–3637.

** Own contribution: Synthesis of Surfactin and Mycosubtilin analogues.

2.1 Introduction

Cyclic lipopeptides are microbial natural products exhibiting a wide diversity of biological applications, ranging from antibacterial, antifungal and anticancer activity to their employment as biosurfactants, ionophores and sequestering agents.[1] Mycosubtilin (**1**) and surfactin A (**2**) are two representatives of the lipopeptide family produced by *Bacillus subtilis*[2] and characterized by a cyclic oligopeptide skeleton with an exocyclic lipid tail (**Figure 2.1**). Mycosubtilin (**1**), belonging to the family of iturins, is endowed with a lipidic macrolactam scaffold, while surfactin is an acidic lipo-macrolactone featuring an amphipathic structure that makes it a powerful surfactant.[3] Surfactin has attracted considerable attention from the medicinal chemistry community due to its antiviral (including anti-HIV),[4] antibacterial[5] and anticancer[6] activities.

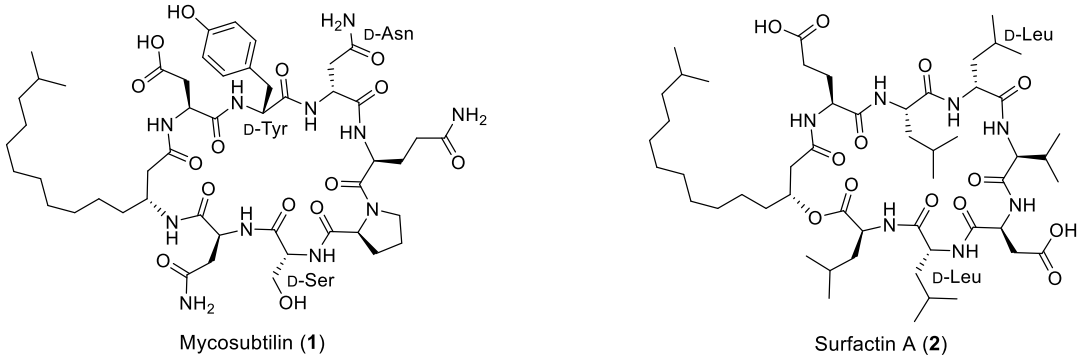


Figure 2.1 Structures of biologically relevant cyclic lipopeptide lead compounds for this work.

2.2 Synthetic Plan

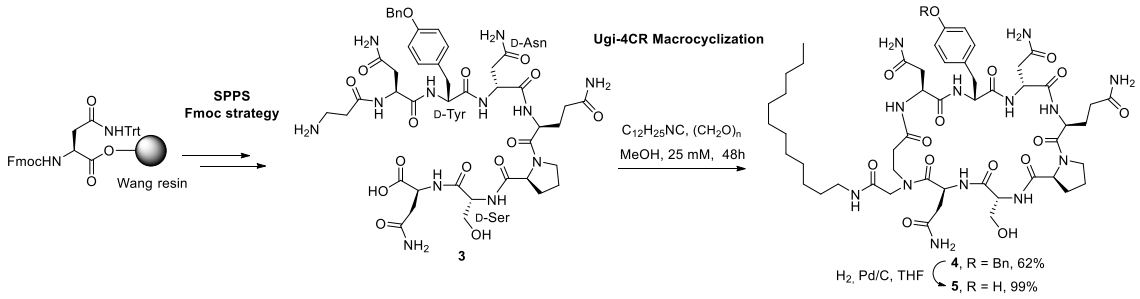
These cyclic lipopeptides have been mostly produced by fermentation methods,[7] although solid-phase peptide synthesis (SPPS) protocols have been also reported.[8] The synthetic strategies employed so far rely on the incorporation of either the amino- or hydroxy-fatty acid at the peptide *N*-terminus as final step of the peptide chain formation, followed by cleavage from resin and macrocyclization.[8]

Herein we report an alternative and powerful synthetic strategy towards cyclic lipopeptides. The approach comprises the utilization of isocyanide-based multicomponent reactions (IMCRs)[9] for the macrocyclization of the peptide skeleton and the simultaneous incorporation of the exocyclic lipid tail. A varity of IMCRs have been successfully employed for the macrocyclization of the peptide backbone[10] and the side chains.[11] Furthermore, we have proven the possibility of installing exocyclic functionalities at the time of the macrocyclization,[11a,12]which is only possible due to the multicomponent nature of the ring closing reaction. Here we further demonstrate this

concept utilizing Ugi and Passerini multicomponent macrocyclizations[13] for both the head-to-tail peptide cyclization and the concomitant lipidation of natural product-like peptides.

2.3 Synthesis of mycosubtilin analogue

An important feature of using of the Ugi four-component reaction (Ugi-4CR)[14] for cyclizing peptides is that the exocyclic appendage arising from the isocyanide component appears as substituent of the resulting tertiary amide.[11b,12] As depicted in **Scheme 2.1**, the utilization of this reaction was devised for the synthesis of mycosubtilin analogue **5**, in which the exocyclic lipidic moiety is located as *N*-substituent instead of at the β-position of the β-amino acid. For this, acyclic peptide **3** was produced by automated SPPS and next subjected to Ugi macrocyclization in the presence of paraformaldehyde and *n*-dodecyl isocyanide.



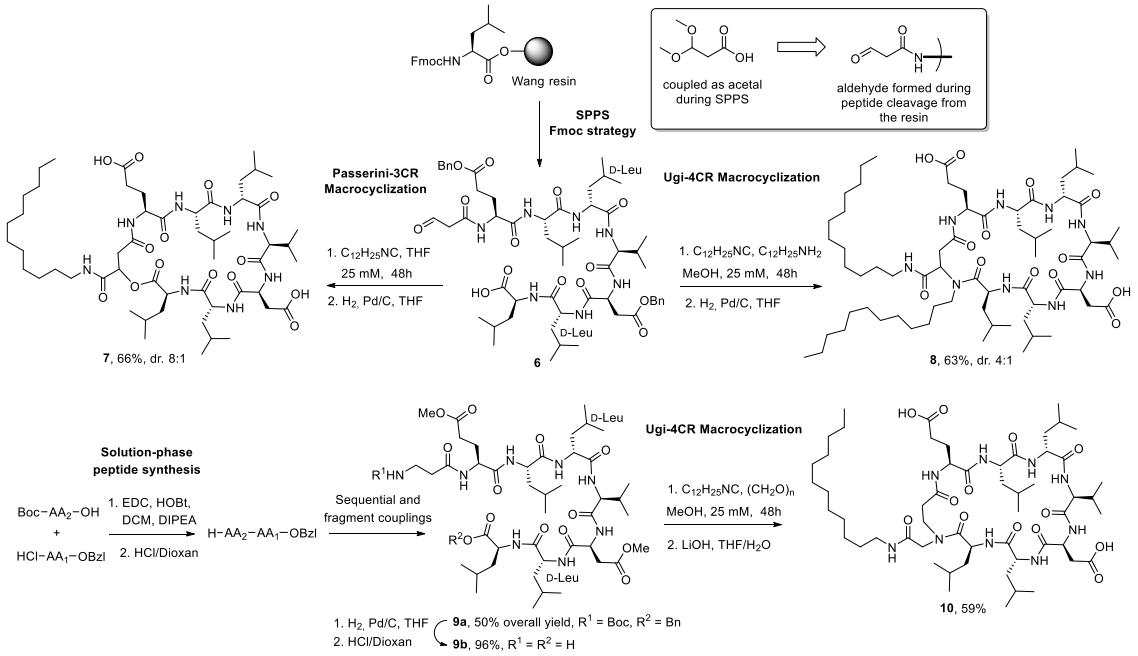
Scheme 2.1 Synthesis of a mycosubtilin analogue by Ugi-macrocyclization.

According to our experience, cyclodimerizations are frequent byproducts in Ugi macrocyclizations[15] unless very high dilution is employed. Nonetheless, because of the presence of reversed turns including D-amino acids in both mycosubtilin[16] and surfactin,[17] we envisioned that the likely bent structure of their folded acyclic precursors would facilitate the ring closure and avoid dimerization. To address this, a parallel study was carried out varying concentration and reaction time, which proved that 25 mM concentration and 48 h of reaction were suitable to ensure disappearance of the linear peptide without formation of the cyclodimer. Accordingly, protected cyclopeptide **4** was obtained in 62% yield after purification, and subsequently submitted to hydrogenation to produce cyclic lipopeptide **5** in pure form without requiring high- or pseudo-dilution. In this study, for reasons of comparability only *n*-C12 aliphatic chains are used, although in nature the length and branching (e.g., *iso*, *normal* and *anteiso*) of

the fatty acid moiety varies in the surfactin, iturin and fengycin families of cyclic lipopeptides.[1]

2.4 Synthesis of surfactin analogues

As surfactin is endowed with a lactone ring system encompassing the heptapeptide *C*-terminus and the hydroxyl of the *N*-terminal 3-hydroxy fatty acid, we envisioned that the Passerini three-component reaction (P-3CR) could be a suitable method to accomplish the macrolactone formation and the simultaneous incorporation of the exocyclic lipid tail. As shown in **Scheme 2.2**, side chain-protected oxo-peptide **6** was also produced by SPPS with the final coupling of 3,3-dimethoxypropanoic acid, followed by the standard acidic cleavage that generates the terminal oxo-functionality upon release from the resin. There are two previous reports of Passerini macrocyclizations,[18] although neither cyclizing peptides nor introducing a biologically relevant exocyclic moiety. In this work, stirring the oxo-peptide and the isocyanide component at 25 mM for 48 h proved also highly efficient, as full conversion to the lipopeptidic lactone was found by HPLC monitoring. The diastereoselectivity of the Passerini ring closure was 8:1 as determined by HPLC/ESI-MS analyses of the crude macrocyclic product. This latter compound was subsequently hydrogenated to deprotect the Asp and Glu side chains. Thus, the major diastereomer of cyclic lipodepsipeptide **7** was isolated in 99% purity and 66% yield over two steps, though the configuration of the newly formed stereocenter could not be determined.



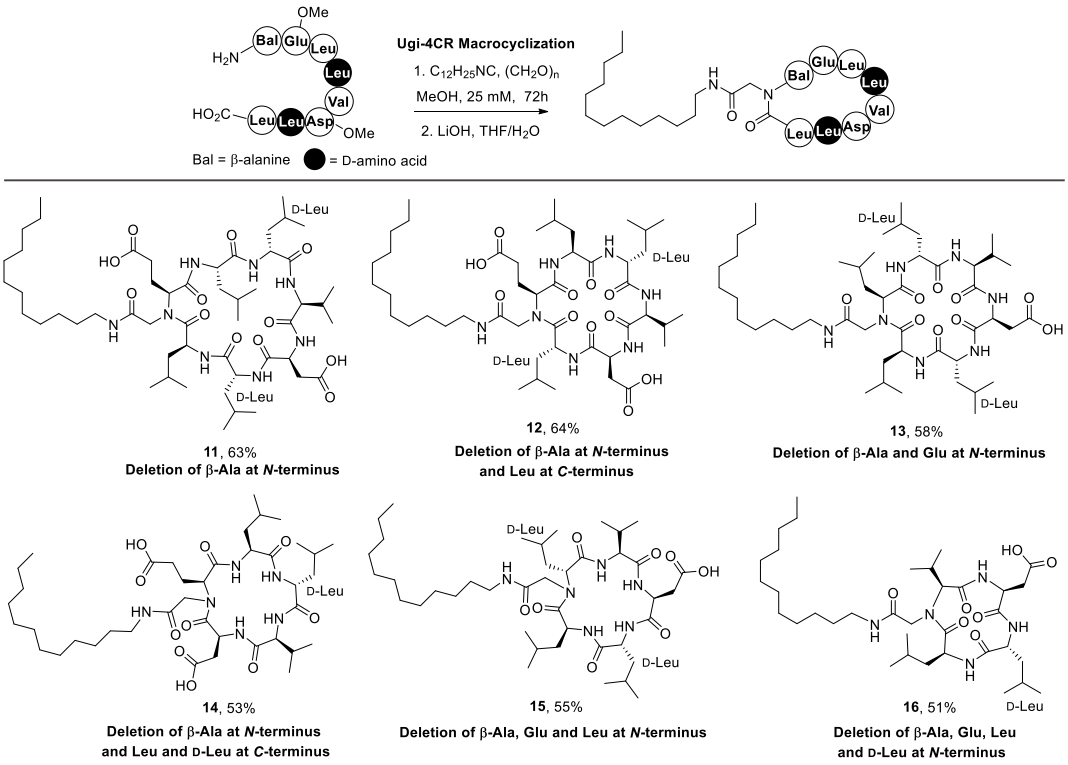
Scheme 2.2 Synthesis of surfactin analogues by Ugi and Passerini macrocyclizations.

We next turned to a self-assembling reaction only possible by means of a four-component reaction, i.e., the incorporation not of one but two exocyclic lipid chains while simultaneously carrying out the peptide macrocyclization. To implement this, oxo-peptide **6** was reacted with *n*-dodecyl amine and *n*-dodecyl isocyanide under the Ugi macrocyclization conditions, followed by side chain deprotection and purification to furnish bilipidated cyclopeptide **8** in 63% yield. In this case, the diastereoselectivity of the Ugi-ring closure was 4:1, but the two diastereomers could not be separated by preparative HPLC.

A solution-phase peptide synthesis protocol – based on sequential and fragment coupling (see **Experimental Part**) – was used to produce orthogonally protected octapeptide **9a**. As shown in **Scheme 2.2**, deprotection of both termini followed by Ugi macrocyclization with *n*-dodecyl isocyanide and paraformaldehyde and subsequent saponification led to the surfactin aza-analogue[19] **10** in 59% yield. As mycosubtilin analogue **5**, cyclopeptide **10** bears the exocyclic lipid as *N*-substituent of the β-Ala used as surrogate of the β-amino or β-hydroxy fatty acid, so the lipid tail is shifted one position compared to the natural lipopeptides.

2.5 Synthesis of truncated derivatives of surfactin

Aiming at producing a series of cyclic lipopeptides for biological evaluation, we turned to the preparation of truncated derivatives of surfactin, i.e. cyclic lipopeptides in which some of the amino acids have been removed from the sequence. For this, various oligopeptides ranging from four to seven amino acids were prepared and subsequently macrocyclized by the Ugi-4CR seeking to assess the influence of the sequence truncation on the biological activity. As depicted in **Scheme 2.3**, the protocol encompassing the Ugi macrocyclization followed by side chain deprotection furnished the corresponding cyclic lipopeptides in moderate to good yields. We initially sought to produce truncated peptides derived from all possible amino acid-deleting combinations, but the disappointing biological results (*vide infra*) prompted us to not carrying out all combinations. In this regard, in some cases truncation comprised the deletion of amino acids at each terminus and in others the removal of amino acids in only one direction. Nonetheless, the outcome of this macrocyclization study is important, as this is the first comparison of the head-to-tail cyclization of octa-, hepta-, penta- and tetrapeptides by means of the classic Ugi-4CR.[20]



Scheme 2.3 Synthesis of cyclic lipopeptides based on the truncated sequence of surfactin.

The main difference between the macrocyclizations leading to octapeptides **8** and **10** and those producing the truncated derivatives, is that complete consumption of the acyclic precursors was not achieved after 48 h for the smaller cycles. Hence, the reaction time for macrocyclizations shown in **Scheme 2.3** was increased to 72 h, for which full conversion was attained for heptapeptide **11** but not for the smaller peptides. Accordingly, the yield of isolated product – after deprotection and purification – drops as the length of the peptide shortens. Whereas this is known to be a sequence related issue, it is an expected result as in most peptide cyclization approaches.[21] Despite the slow kinetics of the latter Ugi-macrocyclizations, an intriguing result is that no dimerization products were found either for pentapeptides **14** and **15**, or for tetrapeptide **16**.[22] This is rather unusual at least for tetrapeptides, which are known to be very difficult to cyclize unless they include tertiary amides derived from proline, pipecolic or *N*-methyl amino acids.[23,24] In our opinion, there are some structural features which can explain this result. Firstly, tetrapeptide **16** includes a D-amino acid, which in short sequences may favor folded conformations and aid engagement of the two reactive termini. Secondly, the intrinsic mechanism of the Ugi-4CR may enable the ring closure of short peptides, as the initial cyclization produces an intermediate (i.e. the α-adduct) that is three atoms larger than the final product.[25,26] Thus, the α-adduct (15-membered ring) evolves to the final cyclic tetrapeptide (12-membered ring) by an

intramolecular acylation (Mumm type rearrangement) comprising a ring contraction. A third reason is that cyclic peptides arising from Ugi-macrocyclizations have a *N*-substituted amide bond, which makes easier the access to the *s-cis* isomer ubiquitous in short cyclic peptides.[27] As partial summary, the Ugi-macrocyclization is an effective, although rather slow method for the head-to-tail cyclization of peptides ranging from 4 to 8 amino acids and having at least one D-configured residue. It remains to be proven whether *all*-L short peptides can be also cyclized without significant cyclodimerization. At least tetraglycine gives cyclo-dimers almost exclusively.[10g]

2.6 Evaluation of anticancer activity

All cyclic lipopeptides synthesized as well as surfactin were tested for their cytotoxic activity against B16F10 (mouse skin melanoma), HeLa (human cervix adenocarcinoma cells), HT-29 (colon adenocarcinoma cells) and PC3 (prostate cancer) cell lines using MTT and CV assays (72 h). At first, fast screening was performed by applying two different concentrations of the compounds (0.1 and 10 μM). Compounds were considered active when showing activity at least at 10 μM concentration after 72 h. Of all tested compounds, only cyclic peptide **8** was found to be active, selectively against B16F10 cells. Because of this, the IC_{50} values of **8** and surfactin were determined against mouse melanona B16F10 cell line (**Figure 2A**). MTT and CV results are in good agreement, pointing out that **8** does not affect cellular respiration. In addition, it was found that **8** (IC_{50}: 6.7 ± 0.8 μM, CV; 7.8 ± 0.9 μM, MTT) posesses 5 times higher cytotoxic effect against the selected cell line than natural cyclic lipopeptide surfactin (IC_{50}: 50.3 ± 0.6 μM, CV; 40.4 ± 0.3 μM, MTT). Thus, the introduction of second lipid chain appears to significantly improve the cytotoxicity of this class of compounds in the melanoma B16F10 cell line.

To investigate the mode of action of cyclopeptide **8**, treated B16F10 cells were analyzed by flow cytometry (**Figure 2.2 B**). Peptide **8** increases the cell number in the sub-G1 phase, which might indicate its involvement in apoptosis.

Autophagy is another anticancer mechanism that can be involved in various cell processes such as cell differentiation as well as in a cell death mechanisms including autophagosomic/lysosomal degradation of cellular components.[28] It was examined whether treatment with **8** would result in an induction of autophagy for the B16F10 cell line. However, the data acquired demonstrate that **8** is not a potential inducer of autophagy (**Figure 2.2 C**). The fluorescent cell staining CFSE dye can be used to

monitor cell proliferation due to the progressive halving of CFSE fluorescence within daughter cells following each cell division.

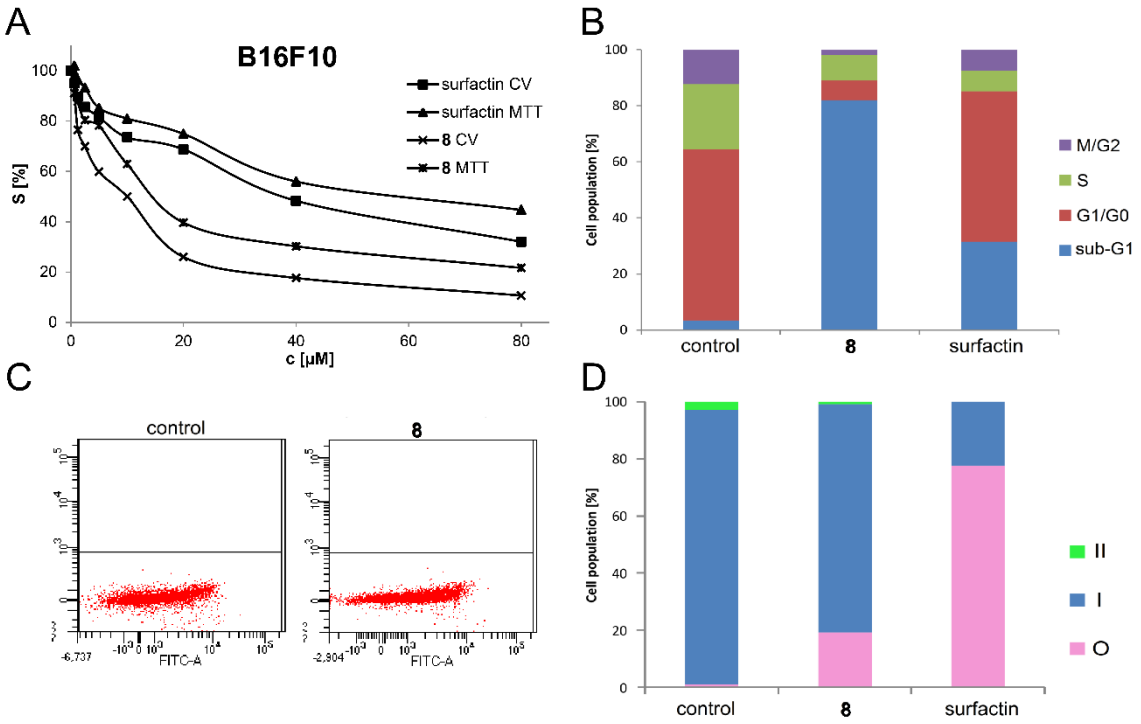


Figure 2.2 B16F10 cells treated with **8** and surfactin: A) dose-dependet survival (72 h); B) cell cycle distribution (72 h, IC_{50} doses); C) authophagy induction (48 h, $2\times IC_{50}$ doses) and D) proliferation (72 h; IC_{50} doses; O – undivided cells, I – 1st and II – 2nd cell division) of B16F10 cells.

An investigation of the growth potential of surviving clones in B16F10 cells exposed to **8** and surfactin was carried out in order to determine whether those compounds are able to block cell divisions of the cells under investigation. The results shown in **Figure 2.2 D** revealed a strong blockage in the first division (*ca.* 80%) upon treatment of B16F10 cells with surfactin. B16F10 cells treated with **8** showed only a moderate presence of undivided cells (*ca.* 20%).

2.7 Conclusions

In conclusion, it is clear that the additional lipid chain present in peptide **8** potentiates the cytotoxic activity up to five times in comparison to surfactin and changes the mode of action against the B16F10 cell line, though in general the cytotoxicity of both natural lead and analogues is rather low and may exclusively rely on the amphiphilic (biocidal) properties rather than on a specific toxic action. Most importantly, we could demonstrate that Ugi and Passerini macrocyclizations are suitable approaches for the

rapid preparation of natural-like cyclic lipopeptide analogues for property evaluations as surfactants or as bioactives. A key feature of this strategy is the integration of the lipid tail attachment and the macrocyclic ring closure, thus allowing rapid generation of exocyclic complexity. We believe this strategy shows promise for the, eventually on-resin, combinatorial creation of macrocyclic lipopeptide libraries for screening. A broad evaluation of other activities, e.g. antibacterial and antifungal properties of these compounds is under way.

2.8 Experimental Part

General remarks

Solid-phase peptide synthesis was carried out with a ResPep SL peptide synthesizer (Intavis Bioanalytical Instruments, Germany). Fmoc amino acids, Fmoc-Leu-Wang resin (100–200 mesh, loading 0.50–1.30 mmol/g resin), Fmoc-Asn(Trt)-Wang resin (100–200 mesh, loading 0.40 – 1.00 mmol/g resin) and PyBOP were supplied from Novabiochem (Germany). Boc amino acids were purchased from Carbolution Chemicals GmbH (Germany). Piperidine, acetic acid, formic acid, DIPEA, TFA and DMF were purchased from Sigma-Aldrich (Germany). All reagents and solvents were used as received, with the exception of DMF and DIPEA that were dried by distillation from CaH_2 under argon prior to use as reaction solvent, and DMF was stored over 4Å molecular sieves. Flash column chromatography was carried out using silica gel 60 (230-400 mesh) and analytical thin layer chromatography (TLC) was performed using silica gel on aluminum sheets. Analytical RP-HPLC analysis was performed with an Agilent 1100 Infinity system (binary pump, auto sampler, DAD; Agilent, USA) equipped with a reversed-phase (RP) C18 column (ODS-A, 4.6 × 150 mm internal diameter, 120 Å, 5 *µ*m, YMC, USA). A linear gradient from 5% to 100% of solvent B in solvent A over 15 min at a flow rate of 0.8 mL/min was used (solvent A: 0.1% (*v*/*v*) formic acid (FA) in water, solvent B: 0.1% (*v*/*v*) FA in acetonitrile). Detection was accomplished at 210, 215, or 254 nm. Preparative RP18 HPLC was carried out with a Knauer system equipped with a WellChrom K-1001 pump and a WellChrom K-2501 UV detector using a preparative column (polymeric RP, 9 × 250 mm internal diameter, 300 Å, 8 *µ*m, VYDAC, USA). ^{1}H NMR and ^{13}C NMR spectra were recorded in solutions on a Varian Mercury 400 spectrometer at 400 MHz and 101 MHz, respectively. Chemical shifts (δ) are reported in ppm relative to TMS (^{1}H NMR) and to the solvent signal (^{13}C NMR). The positive and negative ion high resolution ESI mass spectra were obtained with an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, Germany) equipped with a

HESI electrospray ion source (positive spray voltage 4.5 kV, negative spray voltage 3.5 kV, capillary temperature 275 °C, source heater temperature 250 °C, FTMS resolution 30000). The MS system was coupled to an ultra-high performance liquid chromatography (UHPLC) system (Dionex UltiMate 3000, Thermo Fisher Scientific) equipped with a RP18 column (Hypersil GOLD, 50 x 2.1 mm internal diameter, 1.9 µm, 175 Å, Thermo Fisher Scientific, column temperature 30 °C) and a photodiode array detector (190-400 nm, Thermo Fisher Scientific). The mobile phases were H_2O (A: Fluka Analytical, LC–MS Chromasolv®) and CH_3CN (B: Fluka Analytical, LC–MS Chromasolv®) with formic acid (0.1 %). A gradient system was used (0–15 min, 5–100% B; 15–18 min, 100% B; flow rate 0.150 mL/min). The instrument was externally calibrated using the Pierce LTQ Velos ESI positive ion calibration solution (product No. 88323) and the Pierce ESI negative ion calibration solution (product No. 88324) from Thermo Fisher Scientific. The data were evaluated using the software Xcalibur 2.2 SP1. Linear peptides **3** and **6** were produced by solid-phase peptide synthesis, while the truncated acyclic analogues were synthesized in solution by a peptide fragment coupling strategy as described in the Supporting Information. *n*-Dodecyl isocyanide was prepared as described previously.[29]

Digitonin (Riedel De Haen Seelze, Germany), surfactin, crystal violet, paraformaldehyde, MTT, acridine orange, DAPI (Sigma-Aldrich, Germany), RPMI medium 1640, tryban blue stain 0.4% (Life Technologies, Germany), DPBS, trypsin EDTA (PAN Biotech, Germany), fetal calf serum, penicillin/streptomycin, HEPES (PAA laboratories, Germany), DMSO (Duchefa Biochemie, Germany), acetic acid 33% (Roth, Germany), DHR and CFSE (DB Biosciences, USA) were obtained commercially. Plate reader (Spectramax from Molecular Devices) and FACSAria III (DB Biosciences) were used for the experiments.

Cell lines and cultivation

Mouse skin melanoma cells (B16F10) and colon adenocarcinoma cells (HT-29) were kindly provided by Prof. B. Seliger, Immunology Department, Martin-Luther-University Halle-Wittenberg, Germany. Human refractory prostate cancer cells (PC3) was purchased from the German Collection of Microorganisms and Cell Cultures (Leibniz-DSMZ, Germany). Human cervix adenocarcinoma cells (HeLa) were obtained from Ontochem GmbH (Halle, Germany).

Cells are regularly tested for mycoplasma infections and were cultured as follows. B16F10 and PC3: RPMI 1640 medium with L-glutamine and sodium hydrogen carbonate (Sigma-Aldrich, R8758), 10% fetal bovine serum (Sigma-Aldrich, F2442),

penicillin-streptomycin solution (Sigma-Aldrich, P4333, 10 000 units/mL penicillin and 100 mg/mL streptomycin). HT-29: Dulbecco's Modified Eagle's Medium with 1000 mg/L glucose, L-glutamine, and sodium hydrogen carbonate (Sigma-Aldrich D6046), 10% fetal bovine serum (Sigma-Aldrich, F2442), penicillin-streptomycin solution (Sigma-Aldrich, P4333, 10 000 units/mL penicillin and 100 mg/mL streptomycin). All cells were cultured at 37 °C in a humidified incubator with a 5% CO_2 atmosphere. In the flow cytometry experiments, each well in the 6-well plate was filled with 2 ml medium containing approx. 1×10^5 cells.

MTT and CV assays

For B16F10, HT-29, and HeLa cell lines, some 2000 cells/well were seeded. 6000 cells/well have been used for PC3 cell line. After 24 h from incubation, cells were treated with the tested compounds in various concentrations. Stock solutions of the investigated compounds were prepared at 20 mM concentration in DMSO. According to the experiments, suitable dilutions in the medium were prepared from the stock solution. MTT and CV assays were performed as described in the literature.[30] For the fast screening with MTT and CV assays, 2 concentrations have been used (0.1 and 10 µM) in quadruplicate. While for IC_{50} value determination a dilution series (80, 40, 20, 10, 5, 2.5, 1.25 and 0.625 µM) was prepared from the stock solution of the investigated compounds. Digitonin (125 µM) was used as control. In this study, the incubation time for all compounds with different cell lines was 72 h. IC_{50} values, defined as the concentrations of the compound at which 50% of cell growth inhibition occurs, were calculated using a four-parameter logistic function and presented as mean of three independent experiments. The absorbance of the dissolved dyes was measured in an automated microplate reader at 540 nm with a reference wavelength of 670 nm. The results are presented as a percentage of control values obtained from untreated cultures.

Cell cycle analysis

Cells (1×10^5 cells/well) were cultivated at the IC_{50} or $2\times IC_{50}$ doses of active compounds for 48 h and 72 h. Cells were washed with PBS and fixed in ethanol 70% at 4°C overnight, afterwards dyed with DAPI solution according to the literature[31] and then the distribution of cells in different cell cycle phases was determined with FACSAria III (DB Biosciences).

Autophagy detection

For this purpose cells were incubated with IC_{50} or $2\times IC_{50}$ doses of active compounds for 48 h and 72 h. Cells were washed and trypsinated, consequently collected and washed with PBS before staining in 10 µM acridine orange solution for 15 min at 37 °C in darkness.[32] Afterwards, cells were washed with PBS and the cell pellet was resuspended in 500 µL PBS for flow cytometry analysis with FACSAria III.

Synthesis

General procedure for the solid-phase peptide synthesis: Coupling reactions were carried out in an Automated Solid-Phase Peptide Synthesizer by stepwise Fmoc strategy using the amino acid-functionalized Wang resin in 0.10-0.30 mmol scale. The coupling cycle from the standard Intavis protocol was used (see **Attachment S33**). The intermediate linear peptides, further used in the solution-phase macrocyclization, were cleaved from the resin with the cocktail TFA/CH_2Cl_2/TIPS (49:49:2, *v*/*v*, 2 mL) and the purity was assessed by analytical RP-HPLC. Prior to solution-phase macrocyclization, the peptides were re-dissolved in 4 M HCl/dioxane, stirred at room temperature for 15 minutes and then dropped into cold diethyl ether. The precipitated product was centrifuged, then taken up in 1:2 acetonitrile/water and lyophilized to yield the corresponding peptide hydrochloride salt. Peptido-aldehyde **6** was obtained by coupling 3,3-dimethoxypropanoic acid at the last position of the *N*-terminus. Upon resin cleavage conditions, the acetal is deprotected to render the terminal free aldehyde. The modified peptide was precipitated into cold diethyl ether and the purity was assessed by analytical RP-HPLC.

General procedure for the solution-phase peptide synthesis. The Boc-protected amino acid (1.0 mmol, 1.0 equiv.), HOBt (168 mg, 1.1 mmol, 1.1 equiv.), EDC (210 mg, 1.1 mmol, 1.1 equiv.) and the amino acid benzyl ester hydrochloride (1.0 mmol, 1.0 equiv.) are dissolved either in dry CH_2Cl_2 (15 mL) or in a CH_2Cl_2/DMF mixture (15 mL, 3:1 *v*/*v*). Successively, Et_3N (0.15 mL, 1.1 mmol, 1.1 equiv.) is added in one portion and the resulting solution is stirred at room temperature overnight (~12 h). The reaction mixture is then diluted with 100 mL EtOAc, transferred to a separatory funnel and sequentially washed with 0.5 M aqueous solution of citric acid (2×50 mL), saturated aqueous solution of $NaHCO_3$ (2×50 mL) and brine (2×50 mL). The organic phase is dried over $MgSO_4$, filtered and concentrated under reduced pressure in a rotary evaporator.

General Boc removal procedure: The peptide is dissolved in a 4 M HCl solution in dioxane (2 mL) and the solution is stirred at room temperature. As the material

dissolved, gas evolution could be detected and the pressure that built up inside the reaction flask is slowly released by an adapted needle placed in the flask seal. After 30 min, usually no starting material is detected by thin layer chromatography and the reaction is concentrated under a stream of dry N_2. The volatiles are then fully removed by concentrating the resulting thick oily residue under reduced pressure in the rotary evaporator and then placing the flask under high vacuum for 2 h. The resulting salt was used forward assuming quantitative yield.

General procedure for the Ugi-macrocyclization: A suspension of either the terminal free peptide (0.10 mmol) and paraformaldehyde (0.10 mmol) or the peptide and the aliphatic amine in MeOH (4.0 mL) is stirred for 2 h at room temperature to enable imine formation. DIPEA (0.12 mmol, 1.2 equiv.) is added when the amine is employed as hydrochloride salt. *n*-Dodecylisocyanide (0.15 mmol, 1.5 equiv.) is added and the reaction mixture is stirred for 48 h at room temperature. The volatiles are then removed under reduced pressure in a rotary evaporator and the crude material is purified by flash column chromatography with EtOAc as eluent. The resulting product is subsequently used in the methyl/benzyl ester deprotection step.

Note: If unlike in this report, prefolding of the linear peptide is insufficient, pseudodilution can be employed to increase monomacrocyclization by very slowly (syringe pump) adding the peptide, or peptide and amine, to a diluted solution of the isonitrile.

General procedure for the Passerini-macrocyclization: A solution of the peptide-aldehyde (0.10 mmol) and *n*-dodecylisocyanide (0.15 mmol, 1.5 equiv.) in dry THF (4.0 mL) is stirred for 48 h at room temperature, and then the volatiles are removed under reduced pressure in a rotary evaporator. The crude material is purified by flash column chromatography with EtOAc as eluent. The resulting product is subsequently used in the benzyl ester deprotection step.

General procedure for the benzyl ester/ether deprotection: The peptide is dissolved in dry THF (4.0 mL) and the palladium catalyst supported on charcoal (Pd/C, 10wt %) is added. The mixture is subjected successively to vacuum and filling with hydrogen atmosphere and then stirred under hydrogen atmosphere for 12 h. The catalyst was removed by filtration over a pad of Celite and the filtrate was evaporated under reduced pressure.

For a pseudodilution variant see note above accordingly.

General procedure for the methyl ester deprotection: The peptide (0.10 mmol) is dissolved in THF/H_2O (2:1, 3.0 mL) and the mixture is cooled down to °0 C in an ice

bath. Then, LiOH is added (3 equiv.×-CO_2Me group) and the resulting mixture is stirred for 2-4 h whereby the conversion is checked by thin layer chromatography. The solution is brought to pH 3 by careful drop-wise addition of $NaHSO_4$ (1 M) and the product is extracted with EtOAc (3×20 mL) and the combined organic phases are transferred to a separatory funnel and washed with brine (2×20 mL). The organic phase is dried over $MgSO_4$, filtered and concentrated under reduced pressure in a rotary evaporator.

HCl·H-β-Ala-Asn-D-Tyr(Bzl)-D-Asn-Gln-Pro-D-Ser-Asn-OH (3): The linear peptide **3** was produced (93 mg, 0.09 mmol) in 91% purity according to the general protocol for solid-phase peptide synthesis using the Automated Solid-Phase Peptide Synthesizer starting from 0.10 mmol of Fmoc-Asn(Trt)-Wang resin. An analytical sample was purified by RP-HPLC to 97% purity (254 nm) for ESI-MS analysis. R_t = 8.11 min. HR-MS (ESI) *m/z*: 997.4382 $[M+H]^+$, calcd. for $[C_{44}H_{61}O_{15}N_{12}]^+$ 997.4374.

Cyclic lipopeptide 4: Peptide **3** (90 mg, 0.09 mmol), DIPEA (0.10 mmol), paraformaldehyde (2.7 mg, 0.09 mmol) and *n*-dodecylisocyanide (27 mg, 0.14 mmol) were reacted in MeOH (3.6 mL) for 48 h according to the Ugi-macrocyclization procedure to furnish the cyclic lipopeptide **4**. Preparative RP-HPLC purification produced **4** (67 mg, 62%) in 98% purity, according UHPLC (R_t = 15.32 min, PDA range: 190-400 nm). ^{1}H NMR (400 MHz, CD_3OD): δ = 8.44 (s, 1H), 7.65 (d, *J* = 7.9 Hz, 1H), 7.24 – 7.17 (m, 3H), 7.11 (dt, *J* = 8.8, 4.1 Hz, 1H), 7.03 – 6.96 (m, 2H), 6.93 (m, 1H), 6.70 (dd, *J* = 17.0, 8.4 Hz, 2H), 5.34 (t, *J* = 4.8 Hz, 1H), 5.26 (s, 1H), 5.18 (t, *J* = 7.4 Hz, 1H), 5.08 – 5.00 (m, 1H), 4.76 (t, *J* = 7.0 Hz, 1H), 4.71 – 4.61 (m, 2H), 4.56 – 4.49 (m, 1H), 4.47 – 4.41 (m, 1H), 4.39 – 4.30 (m, 3H), 4.28 – 4.22 (m, 1H), 4.20 – 4.13 (m, 2H), 3.99 – 3.79 (m, 4H), 3.79 – 3.55 (m, 5H), 3.48 (m, 1H), 3.26 – 3.19 (m, 2H), 3.16 – 3.03 (m, 3H), 3.02 – 2.93 (m, 1H), 2.92 – 2.81 (m, 2H), 2.78 – 2.64 (m, 3H), 2.63 – 2.47 (m, 3H), 2.45 – 2.41 (m, 1H), 2.39 – 2.27 (m, 3H), 2.23 – 2.05 (m, 4H), 1.92 – 1.79 (m, 2H), 1.62 – 1.45 (m, 4H), 1.39 – 1.13 (m, 18H), 0.89 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD): δ = 14.4 (*C*H_3), 22.5, 23.7, 26.2, 26.9, 28.1, 28.4, 29.4, 29.6, 30.3, 30.5, 30.7, 30.8, 33.1, 35.6, 36.3, 36.8, 37.7, 38.5, 46.3, 47.3, 49.7 (*C*H_2), 49.8, 49.9, 50.0, 50.4 (*C*H), 50.9 (*C*H_2), 54.8, 56.1, 60.6 (*C*H), 61.7, 69.7 (*C*H_2), 116.11, 116.14, 127.77, 127.79, 128.0, 128.1, 129.2 (*C*H), 129.8 (*C*), 130.5, 130.6 (*C*H), 135.7, 157.2 (*C*), 169.3, 169.6, 170.1, 170.5, 171.0, 171.3, 172.2, 172.7, 173.1, 173.3, 173.5, 173.8, 174.7 (*C*O). HRMS (ESI) *m/z*: 1202.6223 $[M-H]^-$, calcd. for $[C_{58}H_{84}O_{15}N_{13}]^-$ 1202.6215. Peptide **4** (60 mg, 0.05 mmol) is treated according to the general procedure for the benzyl ether deprotection to furnish the cyclic lipopeptide **5** (55 mg, 99%) in 98% purity,

according to UHPLC (R_t = 13.88 min, PDA range: 190-400 nm). HRMS (ESI) *m/z*: 1112.5737 $[M-H]^-$, calcd. for $[C_{51}H_{78}O_{15}N_{13}]^-$ 1112.5740.

$OCHCH_2CO$-Glu(OBzl)-Leu-D-Leu-Val-Asp(OBzl)-D-Leu-Leu-OH (6): The linear peptide **6** was produced in 78% purity according to the general protocol for solid-phase peptide synthesis using the Automated Solid-Phase Peptide Synthesizer starting from 0.30 mmol of Fmoc-Leu-Wang resin. Preparative RP-HPLC purification afforded peptide **6** (192 mg, 98% purity). R_t = 13.55 min. HRMS (ESI) *m/z*: 1064.5906 $[M+H]^+$, calcd. for $[C_{55}H_{82}O_{14}N_7]^+$ 1064.5914.

Cyclic lipopeptide 7: Peptide **6** (93 mg, 0.09 mmol) and *n*-dodecylisocyanide (27 mg, 0.14 mmol) were reacted in dry THF (3.6 mL) for 48 h according to the Passerini-macrocyclization procedure. The crude product was subjected to deprotection of the side chains by the benzyl ester removal procedure to furnish the cyclic lipopeptide **7** as a mixture of diastereomers (64 mg, 66%, dr. 8:1, R_t = 18.72, 19.08 min, analytical RP-HPLC). Preparative RP-HPLC purification afforded the major diastereomer of **7** (37 mg) in 99% purity, according to UHPLC (R_t = 11.24 min, PDA range: 190-400 nm). ^{1}H NMR (400 MHz, Acetone-d_6): δ = 5.35 (d, *J* = 10.1 Hz, 1H), 4.72 (t, *J* = 6.7 Hz, 1H), 4.45 – 4.36 (m, 2H), 4.34 – 4.26 (m, 3H), 4.09 (d, *J* = 5.2 Hz, 1H), 3.30 (m, 1H), 3.27 – 3.14 (m, 3H), 2.96 – 2.87 (m, 6H), 2.60 – 2.37 (m, 5H), 2.35 – 2.27 (m, 1H), 2.22 – 2.19 (m, 1H), 2.18 – 2.12 (m, 1H), 1.91 – 1.87 (m, 1H), 1.82 – 1.78 (m, 2H), 1.77 (m, 2H), 1.74 – 1.69 (m, 2H), 1.67 – 1.64 (m, 2H), 1.63 – 1.59 (m, 2H), 1.58 – 1.51 (m, 2H), 1.36 – 1.24 (m, 17H), 1.01 – 0.83 (m, 30H). ^{13}C NMR (100 MHz, Acetone-d_6): δ = 14.4, 18.0, 19.8, 21.4, 21.6, 22.0, 22.4, 23.1, 23.3, 23.4, 23.6 (CH_3), 25.3, 25.4, 25.5, 25.7 (*C*H), 27.6, 28.6, 29.7, 29.9, 30.1, 30.3, 30.5, 30.6, 31.4 (CH_2), 32.7 (*C*H), 36.6, 39.6, 39.8, 40.1, 40.3, 40.8, 41.7 (CH_2), 51.3, 52.3, 52.5, 52.8, 53.9, 55.1, 60.2, 73.2 (*C*H), 169.0, 170.61, 170.62, 172.0, 172.2, 172.3, 172.5, 174.2, 174.3, 175.28, 175.29 (*C*O). HRMS (ESI) *m/z*: 1077.6821 $[M-H]^-$, calcd. for $[C_{54}H_{93}O_{14}N_8]^-$ 1077.6817.

Cyclic lipopeptide 8: Peptide **6** (93 mg, 0.09 mmol), *n*-dodecylamine (17 mg, 0.09 mmol) and *n*-dodecylisocyanide (27 mg, 0.14 mmol) were reacted in MeOH (3.6 mL) for 48 h according to the Ugi-based macrocyclization procedure. The crude product was then subjected to deprotection of the side chains by the benzyl ester removal procedure to furnish the cyclic lipopeptide **8** as a mixture of diastereomers (71 mg, 63%). Preparative RP-HPLC purification produced **8** (56 mg) in 98% purity as a mixture of diastereomers. The two diastereomer peaks could be resolved in UHPLC (dr. 4:1, R_t = 12.95, 13.69 min, PDA range: 190-400 nm). ^{1}H NMR (400 MHz, CD_3OD): δ = 8.70 (d, *J* = 8.6 Hz, 1H), 8.59 (d, *J* = 8.6 Hz, 1H), 8.36 (s, 1H), 8.16 – 8.05 (m, 1H), 7.70 (s, 1H), 7.10 (s, 1H), 5.12 (d, *J* = 10.8 Hz, 1H), 5.00 (dd, *J* = 8.7, 5.3 Hz, 1H), 4.68

– 4.57 (m, 2H), 4.45 – 4.29 (m, 3H), 4.20 – 4.08 (m, 2H), 4.01 (dt, *J* = 9.2, 4.3 Hz, 1H), 3.70 – 3.44 (m, 4H), 3.11 – 2.94 (m, 2H), 2.81 – 2.60 (m, 3H), 2.53 – 2.42 (m, 2H), 2.21 – 2.12 (m, 1H), 2.11 – 2.02 (m, 2H), 2.01 – 1.90 (m, 2H), 1.80 – 1.45 (m, 12H), 1.44 – 1.12 (m, 36H), 1.05 – 0.83 (m, 30H). ^{13}C NMR (101 MHz, CD_3OD): δ = 14.5, 18.3, 20.2, 22.2, 22.7, 23.4, 23.4, 23.52, 23.54, 23.74, 23.75 (*C*H_3), 25.7, 25.8, 26.0, 26.3 (*C*H), 28.1, 28.2, 28.3, 28.4, 30.3, 30.4, 30.46, 30.49, 30.6, 30.65, 30.7, 30.80 (*C*H_2), 30.83 (*C*H), 30.87, 30.9, 31.6, 32.8, 33.1, 36.0, 39.5, 40.8, 41.3, 41.4, 41.6, 47.9 (*C*H_2), 48.9, 49.1, 49.3, 49.5, 49.7, 51.3, 52.6, 54.5 (*C*H), 165.5, 172.6, 173.1, 173.2, 173.3, 173.4, 174.4, 175.6, 175.7, 176.4 (*C*O). HRMS (ESI) *m/z*: 1244.8857 $[M-H]^-$, calcd. for $[C_{66}H_{118}O_{13}N_9]^-$ 1244.8855.

Boc-Val-Asp(OMe)-D-Leu-Leu-OBzl: *N*-Boc-D-Leu-OH (694 mg, 3.0 mmol) was coupled to HCl·Leu-OBzl (773 mg, 3.0 mmol) according to the peptide coupling procedure, following by deprotection of the *N*-terminus by Boc removal. The same protocol was employed for the sequential coupling of *N*-Boc-Asp(OMe)-OH (742 mg, 3.0 mmol) and *N*-Boc-Val-OH (652 mg, 3.0 mmol). Flash column chromatography purification (*n*-hexane/EtOAc 1:1) furnished the title tetrapeptide (1.41 g, 71%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37 – 7.30 (m, 5H), 7.10 – 6.96 (m, 2H), 5.19 – 5.08 (m, 3H), 4.83 (dt, *J* = 8.4, 5.3 Hz, 1H), 4.62 (td, *J* = 8.4, 4.9 Hz, 1H), 4.51 (ddd, *J* = 10.2, 7.8, 4.4 Hz, 1H), 4.01 – 3.95 (m, 1H), 3.69 (s, 3H), 3.13 (dd, *J* = 17.3, 4.3 Hz, 1H), 2.75 (dd, *J* = 17.4, 5.6 Hz, 1H), 2.18 – 2.11 (m, 1H), 1.76 – 1.69 (m, 1H), 1.67 – 1.55 (m, 5H), 1.43 (s, 9H), 0.99 – 0.94 (m, 3H), 0.93 – 0.85 (m, 15H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 17.8, 19.4, 21.5, 21.9, 22.9, 23.3 (CH_3), 24.7, 24.8 (CH), 28.4 (CH_3), 30.8 (CH), 36.0, 40.2, 41.1 (CH_2), 49.4 (CH), 50.9 (CH_3), 52.1, 52.2, 60.6 (CH), 66.8 (CH_2), 80.7 (C), 128.1, 128.3, 128.6 (CH), 135.7 (C), 156.5, 170.6, 171.8, 171.9, 172.5, 172.7 (CO). HRMS (ESI) *m/z*: 663.3974 $[M+H]^+$, calcd. for $[C_{34}H_{55}O_9N_4]^+$ 663.3969.

H-Val-Asp(OMe)-D-Leu-Leu-OH: Tetrapeptide Boc-Val-Asp(OMe)-D-Leu-Leu-OBzl (220 mg, 0.33 mmol) was subjected to deprotection of the *C*-terminus by the Bzl removal procedure and sequentially to deprotection of the *N*-terminus by the Boc removal procedure to furnish the title peptide (162 mg, 96%) in 94% purity (210 nm). R_t = 8.99 min. HRMS (ESI) *m/z*: 473.2965 $[M+H]^+$, calcd. for $[C_{22}H_{41}O_7N_4]^+$ 473.2970.

Boc-Glu(OMe)-Leu-D-Leu-OBzl: *N*-Boc-Leu-OH (462 mg, 2.0 mmol) was coupled to HCl·D-Leu-OBzl (516 mg, 2.0 mmol) according to the peptide coupling procedure, following by deprotection of the *N*-terminus by Boc removal. The same protocol was employed for the sequential coupling of *N*-Boc-Glu(OMe)-OH (523 mg, 2.0 mmol). Flash column chromatography purification (*n*-hexane/EtOAc 2:1) furnished the title

tripeptide (1.02 g, 88%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.31 – 7.20 (m, 5H), 7.00 – 6.92 (m, 1H), 6.78 – 6.68 (m, 1H), 5.54 – 5.46 (m, 1H), 5.12 – 4.97 (m, 2H), 4.51 (q, *J* = 7.4, 1H), 4.46 – 4.38 (m, 1H), 4.04 (q, *J* = 6.8 Hz, 1H), 3.59 (s, 3H), 2.44 – 2.28 (m, 2H), 2.07 – 1.97 (m, 1H), 1.92 – 1.81 (m, 1H), 1.69 – 1.45 (m, 6H), 1.35 (s, 9H), 0.91 – 0.73 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.7, 21.8, 22.9, 23.1 (CH_3), 24.8, 24.9 (CH), 27.2 (CH_2), 28.3 (CH_3), 30.4, 40.6, 40.8 (CH_2), 51.0 (CH_3), 51.8, 52.0, 54.4 (CH), 66.9 (CH_2), 80.5 (C), 128.2, 128.3, 128.6 (CH), 135.6 (C), 156.1, 171.8, 171.9, 172.7, 174.0 (CO). HRMS (ESI) m/z: 578.3444 $[M+H]^+$, calcd. for $[C_{30}H_{48}O_8N_3]^+$ 578.3441.

Boc-β-Ala-Glu(OMe)-Leu-D-Leu-OBzl: Tripeptide Boc-Glu(OMe)-Leu-D-Leu-OBzl (242 mg, 0.42 mmol) was subjected to deprotection of the *N*-terminus by the Boc removal procedure, followed by the coupling of *N*-Boc-β-Ala-OH (79 mg, 0.42 mmol). Flash column chromatography purification (CH_2Cl_2/EtOAc 3:2) furnished the title tetrapeptide (223 mg, 82%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.31 – 7.21 (m, 5H), 7.11 – 6.85 (m, 2H), 5.05 (q, *J* = 12.5 Hz, 2H), 4.61 – 4.47 (m, 2H), 4.45 – 4.35 (m, 1H), 4.18 – 4.06 (m, 1H), 3.59 (s, 3H), 3.37 – 3.21 (m, 2H), 2.48 – 2.29 (m, 4H), 2.08 – 1.87 (m, 2H), 1.71 – 1.63 (m, 1H), 1.62 – 1.47 (m, 5H), 1.36 (s, 9H), 1.25 – 1.15 (m, 1H), 0.90 – 0.76 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.7, 21.9, 22.9, 23.0 (CH_3), 24.9, 25.0 (CH), 27.2, 27.4 (CH_2), 28.5 (CH_3), 30.4, 36.3, 37.2, 40.8 (CH_2), 51.1 (CH_3), 51.7, 52.1, 53.5 (CH), 67.0 (CH_2), 79.5 (C), 128.2, 128.4, 128.6 (CH), 135.6 (C), 156.4, 171.2, 171.9, 172.7, 173.0, 174.2 (CO). HRMS (ESI) m/z: 649.3810 $[M+H]^+$, calcd. for $[C_{33}H_{53}O_9N_4]^+$ 649.3813.

Boc-β-Ala-Glu(OMe)-Leu-D-Leu-Val-Asp(OMe)-D-Leu-Leu-OBzl (9a): Tetrapeptide Boc-Val-Asp(OMe)-D-Leu-Leu-OBzl (99 mg, 0.30 mmol) was subjected to deprotection of the *N*-terminus by the Boc removal procedure to furnish HCl·Val-Asp(OMe)-D-Leu-Leu-OBzl. In parallel, tetrapeptide Boc-β-Ala-Glu(OMe)-Leu-D-Leu-OBzl (194 mg, 0.30 mmol) was submitted to deprotection of the *C*-terminus by the Bzl removal procedure and sequentially coupled to tetrapeptide HCl·Val-Asp(OMe)-D-Leu-Leu-OBzl. Purification by recrystallization (*n*-hexane/CH_2Cl_2/MeOH, 6:3:1) furnished the pure peptide **9a** (232 mg, 70%; 51% overall yield from HCl·Leu-OBzl) as a white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.31 – 8.23 (m, 2H), 8.12 (d, *J* = 7.9 Hz, 1H), 8.05 (d, *J* = 7.7 Hz, 1H), 7.99 (d, *J* = 7.6 Hz, 1H), 7.88 – 7.82 (m, 2H), 7.41 – 7.29 (m, 5H), 6.71 – 6.64 (m, 1H), 5.11 (s, 2H), 4.66 – 4.59 (m, 1H), 4.37 – 4.30 (m, 3H), 4.30 – 4.22 (m, 2H), 4.12 (dd, *J* = 8.5, 6.7 Hz, 1H), 3.57 (s, 6H), 3.16 – 3.07 (m, 2H), 2.75 – 2.66 (m, 2H), 2.35 – 2.25 (m, 4H), 2.06 – 1.96 (m, 2H), 1.95 – 1.84 (m, 2H), 1.80 – 1.68 (m, 2H), 1.59 – 1.51 (m, 4H), 1.49 – 1.42 (m, 4H), 1.37 (s, 9H), 1.21 – 1.10

(m, 1H), 0.90 – 0.76 (m, 30H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 13.9, 17.9, 19.2, 21.3, 21.3, 21.4, 21.7, 22.7, 22.9, 23.0 (CH_3), 23.1, 24.1, 24.2, 24.3 (CH), 27.1 (CH_2), 28.3 (CH_3), 29.9 (CH_2), 30.2 (CH), 35.6, 36.1, 36.7, 40.7, 40.8, 41.3 (CH_2), 49.7, 50.4 (CH), 51.0, 51.2 (CH_3), 51.3, 51.5, 51.6, 51.8, 57.8 (CH), 65.9 (CH_2), 77.6 (C), 127.8, 128.1, 128.5 (CH), 135.9 (C), 155.5, 169.8, 17057, 170.8, 171.0, 171.1, 171.9, 172.1, 172.2, 172.3, 172.8 (CO). HRMS (ESI) m/z: 1103.6607 $[M+H]^+$, calcd. for $[C_{55}H_{91}O_{15}N_8]^+$ 1103.6604.

H-β-Ala-Glu(OMe)-Leu-D-Leu-Val-Asp(OMe)-D-Leu-Leu-OH (9b): Peptide **9a** (200 mg, 0.18 mmol) was subjected to deprotection of the *C*-terminus by the Bzl removal procedure and sequentially to deprotection of the *N*-terminus by the Boc removal procedure to furnish peptide **9b** (165 mg, 96%) in 97% purity (210 nm). R_t = 10.89 min. HRMS (ESI) *m/z*: 913.5596 $[M+H]^+$, calcd. for $[C_{43}H_{77}O_{13}N_8]^+$ 913.5605.

Cyclic lipopeptide 10: Peptide **9b** (160 mg, 0.17 mmol), DIPEA (0.26 mmol), paraformaldehyde (5.1 mg, 0.17 mmol) and *n*-dodecylisocyanide (51 mg, 0.26 mmol) were reacted in MeOH (6.8 mL) for 48 h according to the Ugi-macrocyclization procedure. The crude product was subjected to deprotection of the side chains by the methyl ester removal procedure to furnish the cyclic lipopeptide **10**. Preparative RP-HPLC purification produced **10** (110 mg, 59%) in 92% purity, according to UHPLC (R_t = 10.44 min, PDA range: 190-400 nm). ^{1}H NMR (400 MHz, CD_3OD): δ = 5.49 (s, 1H), 4.74 – 4.54 (m, 3H), 4.52 – 4.34 (m, 3H), 4.32 – 4.19 (m, 2H), 4.17 – 4.08 (m, 1H), 4.07 – 3.92 (m, 2H), 3.71 – 3.61 (m, 4H), 3.59 – 3.54 (m, 1H), 3.39 – 3.33 (m, 1H), 3.26 – 3.12 (m, 2H), 2.92 – 2.73 (m, 2H), 2.55 – 2.42 (m, 2H), 2.37 – 2.26 (m, 2H), 2.18 – 2.06 (m, 1H), 2.02 – 1.94 (m, 1H), 1.89 – 1.80 (m, 1H), 1.71 – 1.52 (m, 9H), 1.40 – 1.21 (m, 23H), 1.04 – 0.76 (m, 30H). ^{13}C NMR (101 MHz, CD_3OD): δ = 14.5, 18.3, 19.8 (CH_3), 20.9 (CH_2), 21.4, 21.7, 21.8, 22.4, 23.4, 23.7, 23.8, 23.9 (CH_3), 25.5, 25.6, 25.7, 25.8 (CH), 25.9, 26.1, 26.3, 27.9, 28.02, 28.03, 28.2, 30.5, 30.6 (CH_2), 30.7 (CH), 30.8, 33.1, 36.3, 40.6, 40.8, 40.9, 41.0, 41.5, 42.0, 42.8 (CH_2), 54.8, 62.2, 71.1, 71.3, 71.4, 71.5, 71.63, 73.64 (CH), 171.1, 173.0, 173.1, 173.4, 173.1, 174.2, 174.4, 174.8, 175.1, 175.7, 175.8 (CO). HRMS (ESI) *m/z*: 1090.7140 $[M-H]^-$, calcd. for $[C_{55}H_{96}O_{13}N_9]^-$ 1090.7133.

Boc-D-Leu-Val-Asp(OMe)-D-Leu-Leu-OBzl: Tetrapeptide Boc-Val-Asp(OMe)-D-Leu-Leu-OBzl (220 mg, 0.33 mmol) was subjected to deprotection of the *N*-terminus by the Boc removal procedure, followed by the coupling of *N*-Boc-D-Leu-OH (76 mg, 0.33 mmol). Flash column chromatography purification (CH_2Cl_2/EtOAc 1:2) furnished the title pentapeptide (213 mg, 83%) as a white amorphous solid. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.35 – 7.32 (m, 5H), 7.12 – 6.96 (m, 2H), 5.17 – 5.10 (m, 2H), 5.08 – 5.02

(m, 1H) 4.80 (dt, *J* = 8.2, 5.2 Hz, 1H), 4.67 (td, *J* = 8.1, 4.4 Hz, 1H), 4.58 – 4.52 (m, 2H), 4.22 – 4.16 (m, 1H), 3.71 (s, 3H), 3.13 – 3.05 (m, 3H), 2.73 – 2.68 (m, 1H), 2.17 – 2.12 (m, 1H), 1.78 – 1.70 (m, 1H), 1.67 – 1.55 (m, 6H), 1.44 (s, 9H), 0.97 – 0.92 (m, 6H), 0.93 – 0.85 (m, 18H). ^{13}C NMR (100 MHz, CD_3OD): δ = 18.6, 19.2, 21.5, 21.9, 22.2, 22.7, 22.8, 23.1 (CH_3), 23.9, 24.6, 24.8 (CH), 28.3 (CH_3), 30.8 (CH), 36.1, 40.2, 40.5, 41.0 (CH_2), 49.9, 50.5 (CH), 51.9 (CH_3), 52.3, 52.9, 59.6 (CH), 66.6 (CH_2), 80.3 (C), 128.2, 128.3, 128.4 (CH), 135.6 (C), 156.4, 170.8, 171.6, 171.9, 172.2, 172.8, 173.6 (CO). HRMS (ESI) m/z: 776.4807 $[M+H]^+$, calcd. for $[C_{40}H_{66}O_{10}N_5]^+$ 776.4810.

H-D-Leu-Val-Asp(OMe)-D-Leu-Leu-OH: Pentapeptide Boc-D-Leu-Val-Asp(OMe)-D-Leu-Leu-OBzl (182 mg, 0.23 mmol) was subjected to deprotection of the *C*-terminus by the Bzl removal procedure and sequentially to deprotection of the *N*-terminus by the Boc removal procedure to furnish the title peptide (140 mg, 98%) in 97% purity (210 nm). R_t = 9.59 min. HRMS (ESI) *m/z*: 586.3807 $[M+H]^+$, calcd. for $[C_{28}H_{52}O_8N_5]^+$ 586.3810.

Boc-Leu-D-Leu-Val-Asp(OMe)-D-Leu-Leu-OBzl: Tetrapeptide Boc-Val-Asp(OMe)-D-Leu-Leu-OBzl (220 mg, 0.33 mmol) was subjected to deprotection of the *N*-terminus by the Boc removal procedure, followed by sequential coupling of *N*-Boc-D-Leu-OH (76 mg, 0.33 mmol) and *N*-Boc-Leu-OH (76 mg, 0.33 mmol). Flash column chromatography purification (CH_2Cl_2/EtOAc 1:1) furnished the title hexapeptide (232 mg, 79%) as a white amorphous solid. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.39 – 7.27 (m, 5H), 5.48 (s, 1H), 5.22 – 5.10 (m, 2H), 4.61 (dd, *J* = 8.2, 5.3 Hz, 1H), 4.52 (dd, *J* = 10.0, 4.4 Hz, 1H), 4.46 – 4.36 (m, 2H), 4.04 (t, *J* = 7.5 Hz, 1H), 3.98 (d, *J* = 6.5 Hz, 1H), 3.66 (s, 3H), 3.00 – 2.93 (m, 1H), 2.91 – 2.81 (m, 1H), 2.18 (h, *J* = 6.8 Hz, 1H), 1.81 – 1.69 (m, 3H), 1.68 – 1.56 (m, 7H), 1.55 – 1.49 (m, 2H), 1.44 (s, 9H), 1.00 – 0.85 (m, 30H). ^{13}C NMR (100 MHz, CD_3OD): δ = 18.9, 19.6, 21.5, 21.9, 22.0, 22.1, 23.3, 23.4, 23.5, 23.7 (CH_3), 25.8, 25.91, 25.98 (CH), 28.8 (CH_3), 30.8 (CH), 36.3, 41.2, 41.5, 41.6, 41.8 (CH_2), 52.0 (CH_3), 52.3, 52.5, 53.1, 53.4, 54.8, 61.4 (CH), 67.9 (CH_2), 80.7 (C), 129.1, 129.2, 129.3, 129.6 (CH), 137.2 (C), 157.9, 172.5, 172.7, 173.8, 173.9, 174.8, 175.3, 176.1 (CO). HRMS (ESI) m/z: 889.5652 $[M+H]^+$, calcd. for $[C_{46}H_{77}O_{11}N_6]^+$ 889.5650.

H-Leu-D-Leu-Val-Asp(OMe)-D-Leu-Leu-OH: Hexapeptide Boc-Leu-D-Leu-Val-Asp(OMe)-D-Leu-Leu-OBzl (200 mg, 0.22 mmol) was subjected to deprotection of the *C*-terminus by the Bzl removal procedure and sequentially to deprotection of the *N*-terminus by the Boc removal procedure to furnish the title peptide (159 mg, 98%) in 95% purity (210 nm). R_t = 10.83 min. HRMS (ESI) *m/z*: 699.4642 $[M+H]^+$, calcd. for $[C_{34}H_{63}O_9N_6]^+$ 699.4651.

Boc-Glu(OMe)-Leu-D-Leu-Val-Asp(OMe)-OBzl: *N*-Boc-Val-OH (91 mg, 0.42 mmol) was coupled to HCl·Asp(OMe)-OBzl (115 mg, 0.42 mmol) according to the peptide coupling procedure, following by deprotection of the *N*-terminus by Boc removal to furnish HCl·Val-Asp(OMe)-OBzl. In parallel, tripeptide Boc-Glu(OMe)-Leu-D-Leu-OBzl (242 mg, 0.42 mmol) was subjected to deprotection of the *C*-terminus by the Bzl removal procedure and sequentially coupled to dipeptide HCl·Val-Asp(OMe)-OBzl. Flash column chromatography purification (CH_2Cl_2/EtOAc 2:1) furnished the title pentapeptide (261 mg, 77%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.39 – 7.29 (m, 5H), 7.18 (d, *J* = 7.6 Hz, 1H), 7.14 – 7.00 (m, 3H), 5.62 (d, *J* = 6.7 Hz, 1H), 5.25 – 5.13 (m, 2H), 4.88 – 4.80 (m, 1H), 4.50 – 4.41 (m, 2H), 4.25 (dd, *J* = 8.5, 5.7 Hz, 1H), 4.17 – 4.10 (m, 1H), 3.63 (s, 3H), 3.61 (s, 3H), 2.99 – 2.94 (m, 2H), 2.46 (t, *J* = 6.8 Hz, 2H), 2.22 – 2.13 (m, 1H), 2.11 – 2.01 (m, 1H), 2.00 – 1.91 (m, 2H), 1.80 – 1.71 (m, 2H), 1.67 – 1.53 (m, 4H), 1.40 (s, 9H), 0.97 – 0.84 (m, 18H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 17.8, 19.4, 21.9, 22.0, 23.0, 23.1 (CH_3), 24.8, 25.0 (CH), 27.3 (CH_2), 28.4 (CH_3), 30.3 (CH), 36.2, 39.3, 40.0 (CH_2), 49.1 (CH), 51.8, 52.0 (CH_3), 52.1, 52.2, 54.5, 59.1 (CH), 67.9 (CH_2), 80.6 (C), 128.4, 128.6, 128.7 (CH), 135.1 (C), 156.2, 171.0, 171.3, 171.4, 172.0, 172.2, 173.1, 174.4 (CO). HRMS (ESI) m/z: 806.4555 $[M+H]^+$, calcd. for $[C_{40}H_{64}O_{12}N_5]^+$ 806.4551.

H-Glu(OMe)-Leu-D-Leu-Val-Asp(OMe)-OH: Pentapeptide Boc-Glu(OMe)-Leu-D-Leu-Val-Asp(OMe)-OBzl (210 mg, 0.26 mmol) was subjected to deprotection of the *C*-terminus by the Bzl removal procedure and sequentially to deprotection of the *N*-terminus by the Boc removal procedure to furnish the title peptide (160 mg, 94%) in 96% purity (210 nm). R_t = 9.05 min. HRMS (ESI) *m/z*: 616.3547 $[M+H]^+$, calcd. for $[C_{28}H_{50}O_{10}N_5]^+$ 616.3552.

Boc-Glu(OMe)-Leu-D-Leu-Val-Asp(OMe)-D-Leu-OBzl: *N*-Boc-Asp(OMe)-OH (104 mg, 0.42 mmol) was coupled to HCl·D-Leu-OBzl (108 mg, 0.42 mmol) according to the peptide coupling procedure, following by deprotection of the *N*-terminus by Boc removal. The same protocol was employed for the sequential coupling of *N*-Boc-Val-OH (91 mg, 0.42 mmol), following by deprotection of the *N*-terminus by Boc removal to furnish HCl·Val-Asp(OMe)-D-Leu-OBzl. In parallel, tripeptide Boc-Glu(OMe)-Leu-D-Leu-OBzl (242 mg, 0.42 mmol) was subjected to deprotection at the *C*-terminus by the Bn removal procedure and sequentially coupled to tripeptide HCl·Val-Asp(OMe)-D-Leu-OBzl. Flash column chromatography purification (CH_2Cl_2/EtOAc 1:1) furnished the title hexapeptide (266 mg, 69%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.41 – 7.32 (m, 5H), 7.17 (d, *J* = 7.4 Hz, 1H), 7.10 – 7.03 (m, 3H), 5.60 – 5.66 (m, 2H), 5.25 – 5.13 (m, 2H), 4.87 – 4.82 (m, 2H), 4.49 – 4.40 (m, 2H), 4.20 (dd, *J* = 8.3,

5.5 Hz, 1H), 4.16 – 4.11 (m, 1H), 3.65 (s, 3H), 3.59 (s, 3H), 3.01 – 2.97 (m, 2H), 2.47 – 2.40 (m, 2H), 2.21 – 2.16 (m, 1H), 2.15 – 2.00 (m, 1H), 1.97 – 1.90 (m, 2H), 1.80 – 1.78 (m, 4H), 1.65 – 1.50 (m, 4H), 1.42 (s, 9H), 0.95 – 0.85 (m, 24H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 17.8, 19.4, 21.5, 21.9, 22.1, 22.6, 23.2, 23.4 (CH_3), 24.4, 24.7, 25.1 (CH), 26.8 (CH_2), 28.5 (CH_3), 30.1 (CH_2), 30.3 (CH), 36.7, 38.8, 39.5, 40.5 (CH_2), 49.7, 50.1 (CH), 51.6, 51.9 (CH_3), 52.1, 52.0, 54.8, 59.0 (CH), 67.3 (CH_2), 80.4 (C), 127.8, 128.1, 128.3 (CH), 135.9 (C), 156.7, 171.0, 171.2, 171.3, 171.5, 172.1, 172.3, 173.4, 174.0 (CO). HRMS (ESI) m/z: 919.5389 $[M+H]^+$, calcd. for $[C_{46}H_{75}O_{13}N_6]^+$ 919.5392.

H-Glu(OMe)-Leu-D-Leu-Val-Asp(OMe)-D-Leu-OH: Hexapeptide Boc-Glu(OMe)-Leu-D-Leu-Val-Asp(OMe)-D-Leu-OBzl (200 mg, 0.22 mmol) was subjected to deprotection of the *C*-terminus by the Bzl removal procedure and sequentially to deprotection of the *N*-terminus by the Boc removal procedure to furnish the title peptide (163 mg, 97%) in 94% purity (210 nm). R_t = 9.90 min. HRMS (ESI) *m/z*: 729.4387 $[M+H]^+$, calcd. for $[C_{34}H_{61}O_{11}N_6]^+$ 729.4393.

Boc-Glu(OMe)-Leu-D-Leu-Val-Asp(OMe)-D-Leu-Leu-OBzl: Tetrapeptide Boc-Val-Asp(OMe)-D-Leu-Leu-OBzl (220 mg, 0.33 mmol) was subjected to deprotection of the *N*-terminus by the Boc removal procedure to furnish HCl·Val-Asp(OMe)-D-Leu-Leu-OBzl. In parallel, tripeptide Boc-Glu(OMe)-Leu-D-Leu-OBzl (191 mg, 0.33 mmol) was submitted to deprotection of the *C*-terminus by the Bzl removal procedure and sequentially coupled to tetrapeptide HCl·Val-Asp(OMe)-D-Leu-Leu-OBzl. Flash column chromatography purification (CH_2Cl_2/MeOH 10:1) furnished the title heptapeptide (238 mg, 70%) as a white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.21 – 8.15 (m, 2H), 8.10 – 8.07 (m, 1H), 8.06 – 8.01 (m, 1H), 7.88 – 7.82 (m, 2H), 7.41 – 7.29 (m, 5H), 6.65 – 6.59 (m, 1H), 5.11 (s, 2H), 4.68 – 4.65 (m, 1H), 4.39 – 4.35 (m, 2H), 4.34 – 4.16 (m, 3H), 4.08 (dd, *J* = 8.5, 6.7 Hz, 1H), 3.61 (m, 6H), 3.15 – 3.08 (m, 2H), 2.75 – 2.66 (m, 2H), 2.45 – 2.35 (m, 2H), 2.00 – 1.95 (m, 2H), 1.94 – 1.83 (m, 2H), 1.77 – 1.64 (m, 2H), 1.54 – 1.45 (m, 2H), 1.46 – 1.42 (m, 4H), 1.39 (s, 9H), 1.21 – 1.10 (m, 1H), 0.92 – 0.74 (m, 30H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 14.1, 17.7, 19.5, 21.1, 21.3, 21.4, 21.7, 22.8, 22.9, 23.2 (CH_3), 23.5, 24.2, 24.3, 24.5 (CH), 27.3 (CH_2), 28.5 (CH_3), 29.9 (CH_2), 30.6 (CH), 35.5, 36.5, 36.7, 40.9 (CH_2), 49.9, 50.7 (CH), 51.1, 51.2 (CH_3), 51.4, 51.5, 51.7, 51.9, 57.6 (CH), 65.8 (CH_2), 79.5 (C), 127.8, 128.1, 128.3 (CH), 136.1(C), 155.9, 169.1, 170.7, 170.8, 171.3, 171.7, 172.0, 172.6, 172.8, 172.9 (CO). HRMS (ESI) m/z: 1032.6230 $[M+H]^+$, calcd. for $[C_{52}H_{86}O_{14}N_7]^+$ 1032.6233.

H-Glu(OMe)-Leu-D-Leu-Val-Asp(OMe)-D-Leu-Leu-OH: Heptapeptide Boc-Glu(OMe)-Leu-D-Leu-Val-Asp(OMe)-D-Leu-Leu-OBzl (200 mg, 0.19 mmol) was subjected to deprotection of the *C*-terminus by the Bzl removal procedure and sequentially to

deprotection of the *N*-terminus by the Boc removal procedure to furnish the title peptide (162 mg, 97%) in 96% purity (215 nm). R_t = 10.97 min. HRMS (ESI) *m/z*: 842.5228 $[M+H]^+$, calcd. for $[C_{40}H_{72}O_{12}N_7]^+$ 842.5233.

Cyclic lipopeptide 11: Peptide H-Glu(OMe)-Leu-D-Leu-Val-Asp(OMe)-D-Leu-Leu-OH (150 mg, 0.17 mmol), DIPEA (0.26 mmol), paraformaldehyde (5.1 mg, 0.17 mmol) and *n*-dodecylisocyanide (51 mg, 0.26 mmol) were reacted in MeOH (6.8 mL) for 48 h according to the Ugi-macrocyclization procedure. Then, the crude product was subjected to deprotection of the side chains by the methyl ester removal procedure to furnish the cyclic lipopeptide **11**. Preparative RP-HPLC purification produced **11** (109 mg, 63%) in 98% purity, according to UHPLC (R_t = 9.50 min, PDA range: 190-400 nm). ^{1}H NMR (400 MHz, CD_3OD): δ = 4.94 (dd, *J* = 9.2, 4.8 Hz, 1H), 4.76 (dd, *J* = 11.0, 3.7 Hz, 1H), 4.51 – 4.34 (m, 5H), 4.25 (d, *J* = 5.0 Hz, 1H), 3.80 (s, 2H), 3.23 – 3.10 (m, 4H), 2.68 (dd, *J* = 13.1, 3.7 Hz, 2H), 2.51 – 2.34 (m, 3H), 2.26 – 2.19 (m, 1H), 1.95 – 1.86 (m, 1H), 1.75 – 1.64 (m, 5H), 1.56 – 1.44 (m, 5H), 1.36 – 1.24 (m, 20H), 1.01 – 0.86 (m, 33H). ^{13}C NMR (100 MHz, CD_3OD): δ = 14.4, 18.1, 19.5 (*C*H_3), 21.6 (*C*H_2), 22.2, 22.4, 22.5, 23.1, 23.2, 23.4, 23.6, 23.7 (*C*H_3), 25.9, 26.0, 26.2, 26.3 (*C*H), 26.6, 28.0, 28.1, 30.45, 30.48, 30.53, 30.6, 30.7, 30.8 (*C*H_2), 30.9 (*C*H), 31.7, 33.1, 39.0, 40.4, 40.7, 40.9, 42.4, 42.8 (*C*H_2), 47.7, 52.5, 53.2, 53.3, 54.6, 59.6, 61.3 (*C*H), 170.5, 171.6, 172.2, 173.4, 173.6, 173.7, 174.2, 175.2, 175.6, 175.9 (*C*O). HRMS (ESI) *m/z*: 1019.6768 $[M-H]^-$, calcd. for $[C_{52}H_{91}O_{12}N_8]^-$ 1019.6762.

Cyclic lipopeptide 12: Peptide H-Glu(OMe)-Leu-D-Leu-Val-Asp(OMe)-D-Leu-OH (156 mg, 0.20 mmol), DIPEA (0.30 mmol), paraformaldehyde (6.0 mg, 0.20 mmol) and *n*-dodecylisocyanide (59 mg, 0.30 mmol) were reacted in MeOH (8.0 mL) for 48 h according to the Ugi-macrocyclization procedure. The crude product was then subjected to deprotection of the side chains by the methyl ester removal procedure to furnish the cyclic lipopeptide **12**. Preparative RP-HPLC purification produced **12** (116 mg, 64%) in 99% purity, according to UHPLC (R_t = 10.17 min, PDA range: 190-400 nm). ^{1}H NMR (400 MHz, CD_3OD): δ = 4.94 – 4.88 (m, 1H), 4.72 (dd, *J* = 9.7, 3.5 Hz, 1H), 4.49 (q, *J* = 7.6 Hz, 1H), 4.38 – 4.31 (m, 3H), 4.12 (t, *J* = 7.8 Hz, 1H), 3.72 (dd, *J* = 9.6, 5.7 Hz, 1H), 3.67 – 3.61 (m, 1H), 3.23 (dd, *J* = 6.6 Hz, 2H), 2.95 (dd, *J* = 16.6, 3.6 Hz, 1H), 2.71 (dd, *J* = 16.7, 9.7 Hz, 1H), 2.46 – 2.37 (m, 2H), 2.19 – 2.09 (m, 2H), 2.08 – 2.01 (m, 1H), 2.00 – 1.92 (m, 1H), 1.89 – 1.80 (m, 2H), 1.79 – 1.72 (m, 2H), 1.67 – 1.57 (m, 4H), 1.57 – 1.49 (m, 4H), 1.37 – 1.23 (m, 19H), 1.06 – 0.83 (m, 27H). ^{13}C NMR (100 MHz, CD_3OD): δ = 14.4, 19.5, 19.9 (*C*H_3), 21.2 (*C*H_2), 22.3, 22.8, 23.2, 23.6, 23.7, 23.8 (*C*H_3), 24.9, 25.5, 26.0 (*C*H), 26.1, 28.1, 29.8, 29.9, 30.3, 30.5, 30.6, 30.7, 30.8 (*C*H_2), 31.5 (*C*H), 33.1, 36.7, 40.3, 40.8, 41.2, 41.8, 41.9 (*C*H_2), 51.7, 52.8, 52.9,

53.0, 63.3, 63.4, 63.9 (*C*H), 164.7, 171.1, 172.4, 173.9, 173.9, 174.2, 174.6, 175.7, 175.8 (*C*O). HRMS (ESI) *m/z*: 906.5924 $[M-H]^-$, calcd. for $[C_{46}H_{80}O_{11}N_7]^-$ 906.5921.

Cyclic lipopeptide 13: Peptide H-Leu-D-Leu-Val-Asp(OMe)-D-Leu-Leu-OH (150 mg, 0.20 mmol), DIPEA (0.30 mmol), paraformaldehyde (6.0 mg, 0.20 mmol) and *n*-dodecylisocyanide (59 mg, 0.30 mmol) were reacted in MeOH (8.0 mL) for 48 h according to the Ugi-based macrocyclization procedure. The crude product was subjected to deprotection of the side chains by the methyl ester removal procedure to furnish the cyclic lipopeptide **13**. Preparative RP-HPLC purification produced **13** (103 mg, 58%) in 99% purity, according to UHPLC (R_t = 12.01 min, PDA range: 190-400 nm). ^{1}H NMR (400 MHz, CD_3OD): δ = 4.96 (t, *J* = 7.3 Hz, 1H), 4.68 (dd, *J* = 8.5, 1.6 Hz, 2H), 4.42 (dd, *J* = 11.9, 2.9 Hz, 1H), 4.38 – 4.32 (m, 2H), 4.17 (dd, *J* = 11.6, 2.5 Hz, 1H), 3.94 (d, *J* = 17.6 Hz, 2H), 3.50 – 3.42 (m, 2H), 3.14 – 3.10 (m, 1H), 3.08 – 2.99 (m, 2H), 2.88 (dd, *J* = 14.5, 1.6 Hz, 1H), 2.42 (dd, *J* = 14.5, 8.3 Hz, 2H), 2.15 (dq, *J* = 14.2, 7.1 Hz, 2H), 2.02 (ddd, *J* = 14.3, 8.7, 5.3 Hz, 2H), 1.88 – 1.80 (m, 2H), 1.78 – 1.74 (m, 1H), 1.73 – 1.68 (m, 3H), 1.67 – 1.65 (m, 2H), 1.65 – 1.60 (m, 3H), 1.60 – 1.57 (m, 1H), 1.55 – 1.52 (m, 2H), 1.51 – 1.46 (m, 1H), 1.37 – 1.22 (m, 18H), 1.02 – 0.83 (m, 30H). ^{13}C NMR (100 MHz, CD_3OD): δ = 14.4, 19.2, 19.5 (*C*H_3), 21.1 (*C*H_2), 22.7, 22.9, 23.3, 23.6, 23.7, 23.9, 24.0 (*C*H_3), 25.4, 26.0, 26.1, 28.2 (*C*H), 30.3, 30.4, 30.5, 30.6, 30.7 (*C*H_2), 30.8 (*C*H), 32.9, 33.1, 38.5, 41.0, 41.1, 41.4, 41.5, 42.6 (*C*H_2), 52.3, 52.7, 52.8, 53.5, 54.3, 59.6, 63.4 (*C*H), 170.9, 173.1, 173.3, 173.4, 173.5, 174.1, 174.7, 174.8 (*C*O). HRMS (ESI) *m/z*: 890.6335 $[M-H]^-$, calcd. for $[C_{47}H_{84}O_9N_7]^-$ 890.6336.

Cyclic lipopeptide 14: Peptide H-Glu(OMe)-Leu-D-Leu-Val-Asp(OMe)-OH (152 mg, 0.23 mmol), DIPEA (0.35 mmol), paraformaldehyde (6.9 mg, 0.23 mmol) and *n*-dodecylisocyanide (68 mg, 0.35 mmol) were reacted in MeOH (9.2 mL) for 72 h according to the Ugi-macrocyclization procedure. Then, the crude product was subjected to deprotection of the side chains by the methyl ester removal procedure to furnish the cyclic lipopeptide **14**. Preparative RP-HPLC purification produced **14** (97 mg, 53%) in 99% purity, according to UHPLC (R_t = 11.11 min, PDA range: 190-400 nm). ^{1}H NMR (400 MHz, CD_3OD): δ = 5.49 (s, 1H), 5.09 (dd, *J* = 8.9, 5.5 Hz, 1H), 4.61 – 4.48 (m, 1H), 4.47 – 4.40 (m, 1H), 4.38 – 4.27 (m, 1H), 4.21 – 4.08 (m, 1H), 4.06 – 3.88 (m, 2H), 3.70 – 3.57 (m, 1H), 3.27 – 3.15 (m, 2H), 3.02 – 2.82 (m, 2H), 2.65 – 2.49 (m, 2H), 2.47 – 2.36 (m, 1H), 2.28 (h, *J* = 7.6, 7.1 Hz, 1H), 2.18 (h, *J* = 7.6, 6.6 Hz, 1H), 2.10 – 1.98 (m, 1H), 1.95 – 1.77 (m, 1H), 1.76 – 1.42 (m, 8H), 1.40 – 1.20 (m, 18H), 1.02 – 0.84 (m, 18H). ^{13}C NMR (100 MHz, CD_3OD): δ = 14.5, 18.3, 19.7 (*C*H_3), 22.4 (*C*H_2), 22.8, 22.9, 23.1, 23.7 (*C*H_3), 25.7, 26.0 (*C*H), 26.2, 28.1, 30.2, 30.4, 30.5, 30.74, 30.76, 30.78, 30.81 (*C*H_2), 30.82 (*C*H), 31.3, 33.1, 40.8, 40.9, 41.4, 41.8 (*C*H_2), 52.2,

53.4, 55.0, 56.9, 61.6, 69.4 (*C*H), 171.4, 172.8, 173.2, 173.6, 173.9, 174.3, 174.9, 175.6 (*C*O). HRMS (ESI) *m/z*: 793.5085 $[M-H]^-$, calcd. for $[C_{40}H_{69}O_{10}N_6]^-$ 793.5081.

Cyclic lipopeptide 15: Peptide H-D-Leu-Val-Asp(OMe)-D-Leu-Leu-OH (134 mg, 0.22 mmol), DIPEA (0.33 mmol), paraformaldehyde (6.6 mg, 0.22 mmol) and *n*-dodecylisocyanide (64 mg, 0.33 mmol) were reacted in MeOH (8.8 mL) for 72 h according to the Ugi-macrocyclization procedure. The crude product was subjected to deprotection of the side chains by the methyl ester removal procedure to furnish the cyclic lipopeptide **15**. Preparative RP-HPLC purification produced **15** (94 mg, 55%) in 97% purity, according to UHPLC (R_t = 12.79 min, PDA range: 190-400 nm). ^{1}H NMR (400 MHz, CD_3OD): δ = 5.49 (s, 1H), 5.09 (s, 1H), 4.77 – 4.68 (m, 1H), 4.67 – 4.61 (m, 1H), 4.56 – 4.47 (m, 2H), 4.38 – 4.28 (m, 1H), 4.22 – 4.15 (m, 1H), 4.15 – 4.06 (m, 1H), 3.38 – 3.32 (m, 1H), 3.24 – 3.12 (m, 2H), 2.96 – 2.82 (m, 1H), 2.77 – 2.61 (m, 1H), 2.21 – 2.07 (m, 1H), 1.71 – 1.60 (m, 4H), 1.55 – 1.46 (m, 4H), 1.34 – 1.24 (m, 20H), 1.04 – 0.79 (m, 27H). ^{13}C NMR (100 MHz, CD_3OD): δ = 14.5, 18.1, 19.9 (*C*H_3), 22.0 (*C*H_2), 22.4, 22.6, 23.2, 23.3, 23.6, 23.7 (*C*H_3), 25.9, 26.0, 26.1 (*C*H), 28.0, 28.07, 28.1, 30.3, 30.5, 30.6, 30.7, 30.8 (*C*H_2), 31.9 (*C*H), 33.1, 37.7, 39.3, 40.2, 40.7, 41.2 (*C*H_2), 47.5, 52.0, 52.1, 54.8, 56.7, 60.3 (*C*H), 170.4, 172.2, 173.4, 173.5, 174.1, 175.9, 176.2 (*C*O). HRMS (ESI) *m/z*: 777.5497 $[M-H]^-$, calcd. for $[C_{41}H_{73}O_8N_6]^-$ 777.5495.

Cyclic lipopeptide 16: Peptide H-Val-Asp(OMe)-D-Leu-Leu-OH (156 mg, 0.31 mmol), DIPEA (0.47 mmol), paraformaldehyde (9.3 mg, 0.31 mmol) and *n*-dodecylisocyanide (92 mg, 0.47 mmol) were reacted in MeOH (12.4 mL) for 72 h according to the Ugi-macrocyclization procedure. The crude product was subjected to deprotection of the side chains by the methyl ester removal procedure to furnish the cyclic lipopeptide **16**. Preparative RP-HPLC purification produced **16** (105 mg, 51%) in 98% purity, according to UHPLC (R_t = 10.58 min, PDA range: 190-400 nm). ^{1}H NMR (400 MHz, CD_3OD): δ = 4.73 (dd, *J* = 8.8, 6.4 Hz, 1H), 4.66 – 4.55 (m, 1H), 4.54 – 4.42 (m, 2H), 4.13 – 3.96 (m, 2H), 3.64 – 3.45 (m, 1H), 3.28 – 3.17 (m, 2H), 2.80 (dd, *J* = 14.6, 3.7 Hz, 1H), 2.74 – 2.57 (m, 2H), 1.67 – 1.56 (m, 4H), 1.55 – 1.44 (m, 4H), 1.39 – 1.25 (m, 18H), 1.06 – 0.79 (m, 21H). ^{13}C NMR (100 MHz, CD_3OD): δ = 14.4, 19.4 (*C*H_3), 22.3 (*C*H_2), 22.5, 22.8, 23.0, 23.1(*C*H_3), 23.2, 23.7 (*C*H_2), 25.8, 26.1 (*C*H), 28.1, 28.2, 30.1, 30.5 (*C*H_2), 30.6 (*C*H), 30.7, 30.8, 33.1, 37.6, 38.1, 41.1, 42.8 (*C*H_2), 48.2, 51.6, 52.3, 54.9, 71.8 (*C*H), 170.9, 171.9, 172.3, 172.8, 173.5, 175.5 (*C*O). HRMS (ESI) *m/z*: 664.4655 $[M-H]^-$ calcd. for $[C_{35}H_{62}O_7N_5]^-$ 664.4655.

2.9 References

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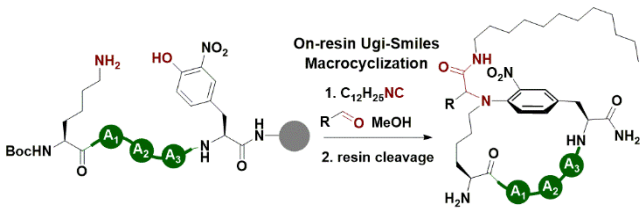
Chapter 3

Solution and Solid-Phase Macrocyclization of Peptides by the Ugi-Smiles Multicomponent Reaction: Synthesis of *N*-Aryl-Bridged Cyclic Lipopeptides

*Abstract**



A new multicomponent methodology for the solution and solid-phase Macrocyclization of peptides is described. The approach comprises the utilization of the Ugi-Smiles reaction for the cyclization of 3-nitrotyrosine-containing peptides either by the *N*-terminus or the lysine side chain amino groups. Both the on-resin and solution cyclizations took place with good to excellent efficiency in the presence of an aldehyde and a lipidic isocyanide, while the use of paraformaldehyde required an aminocatalysis-mediated imine formation prior to the on-resin Ugi-Smiles ring closure. The introduction of a turn motif in the peptide sequence facilitated the cyclization step, shortened reaction times and delivered crude products with > 90% purity. This powerful method provided a variety of structurally novel *N*-aryl-bridged cyclic lipopeptides occurring as a single atropisomer.

* This Chapter was published: Morejón, M. C.; Laub, A.; Westermann, B.; Rivera, D. G.; Wessjohann, L. A. *Org. Lett.* **2016**, *18*, 4096–4099.

3.1 Introduction

Peptide cyclization stands among the most effective ways of introducing conformational constraints in peptide sequences used either as protein ligands or mimetics of protein epitopes.[1] Such a covalent modification is also known to improve membrane permeability[2] and metabolic resistance of peptides,[3] while it can enhance the binding affinity to biological targets, compared to their acyclic analogues.[4] Currently, macrocyclizations based on peptide coupling,[1,5] olefin metathesis[6] and click reactions[7] are the most common methods to seek cyclic peptides with improved activity or pharmacological properties.

Isocyanide-based multicomponent reactions[8] (IMCRs) have lately risen as powerful tools for the synthesis of cyclic peptides and peptidomimetics.[9,10] The Ugi four-component reaction (Ugi-4CR)[9] – and a highly diastereoselective variant based on an amphoteric aziridine-aldehyde component[10] – have been the methods employed to cyclize linear peptides either by their termini or side chains. Besides of being highly efficient and diversity-oriented processes, MCRs provide a strategic benefit not inherent in traditional methods, i.e., the simultaneous incorporation of additional exocyclic moieties during the macrocyclic ring closure. In cyclic peptides, such *exo* fragments may be crucial for biological activity, and may vary from functional appendages[11] for conjugation or fluorescent labeling to peptide bond *N*-alkylations and exocyclic amides capable to either control the conformation[12] or modulate the membrane permeability.[13] Encouraged by the possibilities provided by IMCRs, we pursued the development of new multicomponent macrocyclization approaches capable to increase the diversity of cyclic peptide scaffolds.

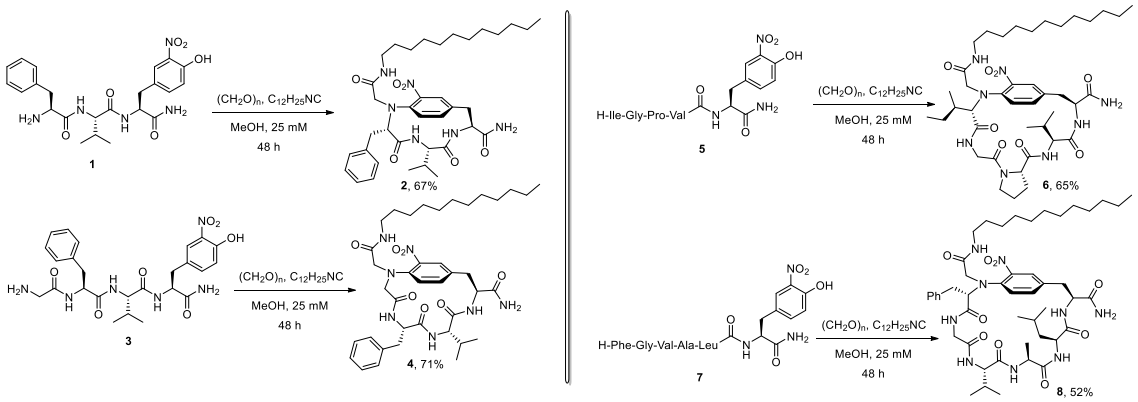
3.2 Synthetic Plan

This work describes a novel peptide cyclization method based on the Ugi-Smiles[14] multicomponent reaction. Besides being the first report describing this IMCR for macrocyclization procedures, this article provides two innovations: 1) the employment of a lipidic isocyanide to incorporate an exocyclic lipidic tail while closing the macrocyclic ring, and 2) the implementation of both solution- and solid-phase multicomponent cyclization protocols enabling the efficient synthesis of structurally unique *N*-aryl-bridged cyclic lipopeptides. Aryl-bridged cyclic peptides are among the most relevant bioactive macrocycles, including members like K-13 and vancomycin.[15]

3.3 Development of Ugi-Smiles macrocyclization methodology in solution-phase

The Ugi-Smiles reaction[14] is a remarkable variant of the classic Ugi-4CR,[8] where the carboxylic acid component is replaced by electron-poor phenols like 2- or 4-nitrophenols upon condensation with a primary amine, an aldehyde and an isocyanide, giving rise to tertiary nitroanilines. The substrate scope at the acidic component of this reaction is remarkable, spanning beyond electron-poor phenols to hydroxyl-heterocycles and conjugated enols.[16] However, we are not aware of applications in the covalent modification of peptides.

To provide a reliable synthetic tool to the repertoire of peptide cyclization methods, we endeavored the macrocyclization of 3-nitrotyrosine-containing peptides by reaction with both *N*-terminal and side chain amino groups. As depicted in **Scheme 3.1**, a variety of oligopeptides bearing the 3-nitrotyrosine residue at the *C*-terminus were subjected to Ugi-Smiles macrocyclizations in the presence of paraformaldehyde and *n*-dodecylisocyanide[17] to provide *N*-aryl-bridged cyclic lipopeptides. Peptides used in the solution-phase macrocyclizations were produced by automated solid-phase synthesis as described in the **Experimental Part**.



Scheme 3.1 Solution-phase synthesis of *N*-aryl-bridged cyclic lipopeptides by Ugi-Smiles macrocyclizations.

Parallel studies were carried out with peptides **1** and **5**, in which both concentrations (2 mM, 10 mM and 25 mM) and reaction times (12 h to 96 h) were varied to assess the best macrocyclization conditions. HPLC monitoring showed that the peptidic substrates were fully consumed after 48 h of reaction at both 10 mM and 25 mM, while there was no difference in the macrocyclization efficiency (derived from HPLC conversion) at both concentrations and no oligomeric products were detected by HPLC/ESI-MS analysis.

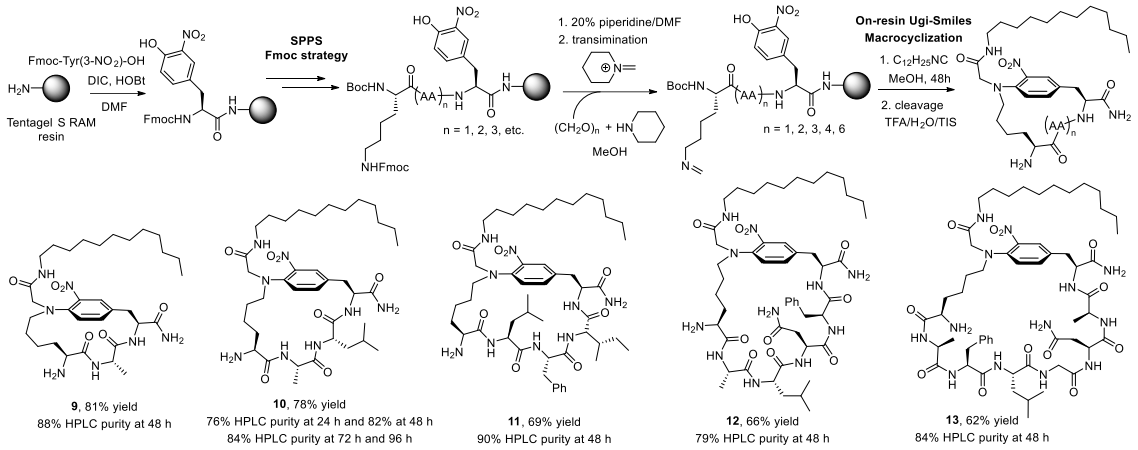
Although 25 mM may be seen as a rather high concentration for peptide cyclization, this is in agreement with previous reports of IMCR-based cyclizations of linear peptides. [10] The ionic mechanism of this reaction goes *via* a zwitterionic iminium-phenolate intermediate, which may aid bringing closer together the two reactive termini upon addition to the isocyanide and the subsequent Smiles rearrangement. Also, differently from the head-to-tail cyclization of short peptides where access to *cis*-peptide bonds may be energetically costly and thus detrimental to cyclization yield, the current method comprises a head-to-side chain cyclization that proved efficient even for short peptide **1**. Consequently, all four cyclic peptides, incorporating from three to six amino acids, were obtained in good yields of isolated product.

3.4 Development of on-resin Ugi-Smiles macrocyclization protocol

After proving the success of the Ugi-Smiles macrocyclization in solution, we turned to the development of the on-resin protocol, as this shows great promise in parallel combinatorial synthesis and high-throughput screening. **Scheme 3.2** shows the first solid-phase protocol implemented, which comprises the initial incorporation of 3-nitrotyrosine to the Tentagel S RAM resin, followed by typical Fmoc/*t*Bu strategy for the peptide elongation. Finally, Boc-Lys(Fmoc)-OH is installed at the *N*-terminus followed by removal of the Fmoc group at the Lys side chain, thus leaving the two reactive functional groups free for the side chain-to-side chain macrocyclization. A key step in Ugi-type reactions is the imine formation, which is typically accomplished prior to addition of the isocyanide component to avoid the competing Passerini reaction. However, on-resin imine formation with paraformaldehyde turned out to be inefficient even at the reactive amino group of a Lys side chain. The solution to this problem was the implementation of an aminocatalysis-mediated transimination protocol recently developed to enable on-resin Ugi-4CR on peptide *N*-termini.[18] The procedure comprises the addition to the resin-bound peptide of a piperidinium ion arising from reaction of paraformaldehyde and piperidine. After shaking 20 minutes, complete transimination was achieved at the Lys side chain according to a ninhydrin test.[18]

A relatively low resin loading (0.23 mmol/g) was used to provide *pseudo*-dilution conditions minimizing cyclodimerization processes. As shown in **Scheme 3.2**, peptides from three to eight amino acids were efficiently cyclized under these conditions, providing macrocyclic peptides in good yield and 79% to 90% purity after cleavage from the resin, as determined by HPLC analysis after 48 h. To check for an optimum

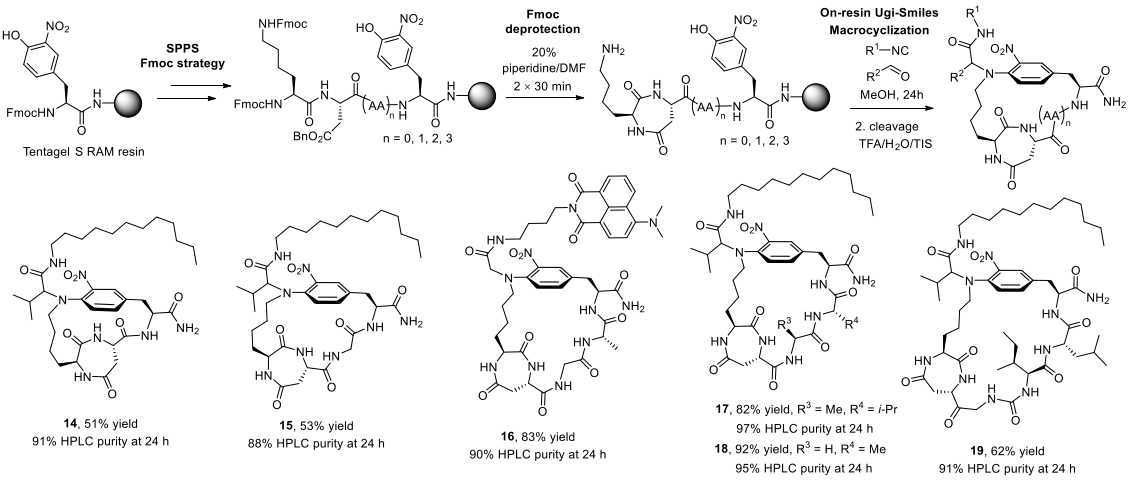
reaction time, synthesis of cyclic peptide **10** was taken as model, performing partial cleavages at 24, 48, 72 and 96 h and HPLC/ESI-MS analysis (see the **Experimental Part**). This proved that cyclization efficiency was already high after 48 h. An analytical sample of all cyclic peptides was purified by semi-preparative RP-HPLC for NMR and HR-MS characterization. We believe that on-resin head-to-side chain macrocyclizations can be as efficient as in solution, since the transimination step has proven to be very effective at the *N*-terminus as well.[18]



Scheme 3.2 Solid-phase synthesis of side chain *N*-aryl cross-linked peptides by on-resin Ugi-Smiles macrocyclization.

This approach creates cyclic peptides featuring an *N*-aryl bridge between the Tyr(NO_2) side chain and either the *N*-terminus or the Lys side chain. This class of covalent linkage has no equivalent in any known natural or synthetic cyclopeptide fragment, but it is related with ansa-cyclopeptides.[15] Due to the tertiary nature of the aniline and the presence of an *ortho*-nitro substituent at the phenyl ring, it is likely that these compounds show atropisomerism due to the high rotational energy barrier at the N–C(sp^2) single bond. As previously described,[19] sterically congested anilines with bulky *ortho*-aryl substituents provide stereoisomers that can be isolated by chromatographic purification and with different NMR spectra (upon existence of other chirality elements). In this regard, HPLC and NMR analyses of compounds in **Schemes 3.1** and **3.2** suggest the presence of a single atropisomer, although the fast interconversion cannot be ruled out at this point. Depending on how high the rotational barrier is, dynamic NMR and HPLC experiments at either low or elevated temperatures could provide a useful insight into the interconversion process around the N–C(sp^2) bond of these cyclic peptides.[19] Whereas such an study has been previously carried out for Ugi-4CR-derived compounds,[20] it is beyond the goal of this report.

Despite of the high conversion achieved in the on-resin Ugi-Smiles macrocyclization at 48 h, we pursued shortening the reaction time by the introduction of structural motifs capable to facilitate the ring closing step. For this, we devised a solid-phase synthetic route comprising the incorporation of the dipeptide fragment Fmoc-Lys(Fmoc)-Asp(OBzl) at the peptide *N*-terminus. As shown in **Scheme 3.3**, a 1,4-diazepane-2,5-dione ring was readily formed upon treatment of the resin-bound peptide with the mixture 20% piperidine in DMF employed for Fmoc removal, thus capping the *N*-terminus and deprotecting the Lys side chain for the subsequent multicomponent cyclization. 1,4-Diazepanediones, and their analogous 1,4-diazepines, are known mimics of ß-turns and have proven merit in peptidomimetic medicinal chemistry.[21] Our rationalization for the installation of such a moiety at the end of the peptide sequence was to provide a turn motif capable to bring the reacting side chains closer. For this, a set of oligopeptides were produced on solid phase and subjected to diazepane-ring closure followed by Ugi-Smiles macrocyclization.



Scheme 3.3 On-resin Ugi-Smiles macrocyclization attended by a ß-turn mimic.

To our delight, HPLC/ESI-MS analysis proved in most cases that the linear peptides were fully consumed after 24 h, thus enabling to reduce the reaction time required to complete the multicomponent macrocyclizations to a half. In addition, the crude cyclic peptides arising from cleavages showed a high purity, in most cases over 90% and up to 97% and 95% for compounds **17** and **18**, respectively. Indeed, the high purity of the crude products resulting from on-resin cyclizations shown in **Scheme 3.2** and **3.3** is a desired characteristic for solid-phase protocols intended to be used in the creation of combinatorial libraries for biological screening. An advantage of the on-resin approach is that the peptide side chains can be protected during cyclization, which enables the use of all amino acids in the peptide sequence. Even various Lys can be present if a

three-dimensional orthogonal strategy is employed, i.e., the use of Alloc at a specific Lys side chain to enable orthogonal deprotection without affecting other Boc-protected Lys side chains.

To prove wider synthetic scope, we also aimed at varying the nature of the lipidic and aldehyde components. Accordingly, isobutyraldehyde was employed as alternative oxo-component, which led to an efficient on-resin imine formation and thus avoided the use of a previous transimination step required with paraformaldehyde. Cyclic peptides derived from isobutyraldehyde were obtained as a mixture of two diastereomers of variable ratio, due to the poor stereoselectivity of this reaction. Alternatively, synthesis of fluorescently labeled cyclic peptide **16** demonstrated the effectiveness of this methodology in the production of tagged cyclic peptides. This conveys one of the greatest advantages over other methodologies, as it allows for the cyclopeptide labeling at the same time as the ring closure. As known from previous Ugi-Smiles reports,[14] the scope of the isocyanide component is great and it may enable the incorporation of further exocyclic fragments.

3.5 Conclusions

In conclusion, we have developed a robust methodology for the cyclization of linear peptides by means of the Ugi-Smiles reaction. To our knowledge, this is the first application of this multicomponent reaction in the synthesis of cyclic peptides, and in macrocyclization approaches in general. Macrocycles reported herein are structurally novel, as they integrate a cyclopeptide skeleton cross-linked – either at the side chains or head-to-side chain – by an *N*-aryl bridge having a lipidic (or fluorescent) tail arising from the isocyanide component. HPLC and NMR evidence confirmed the presence of a single diastereomer of compounds produced with paraformaldehyde, i.e., either a single atropisomer is formed or atropisomers rapidly interconvert. Additionally, we demonstrated the feasibility of this multicompoment method in both solution and solid-phase cyclization protocols. For the use of paraformaldehyde as oxo-component in the on-resin protocol, an aminocatalytic-mediated transimination step was required prior to the addition of the isocyanide. Finally, the installation of a 1,4-diazepanedione moiety (ß-turn mimic) in the resin-bound peptide shortened the reaction time, likely by bringing the two reactive ends of the peptide precursor closer together.

3.6 Experimental Part

General remarks

Solid-phase peptide synthesis was carried out with a ResPep SL peptide synthesizer (Intavis Bioanalytical Instruments, Germany). Fmoc amino acids and PyBOP were supplied from Novabiochem (Germany). Fmoc-Lys(Fmoc)-OH, Fmoc-Asp(Bzl)-OH and TentaGel-S-RAM resin were purchased from Iris Biotech GmbH (Germany). Piperidine, acetic acid, formic acid, DIPEA, TFA and DMF were purchased from Sigma-Aldrich (Germany). All reagents and solvents were used as received, with the exception of DMF and DIPEA that were dried by distillation from CaH_2 under argon prior to use as reaction solvent, and DMF was stored over 4Å molecular sieves. Flash column chromatography was carried out using silica gel 60 (230-400 mesh) and analytical thin layer chromatography (TLC) was performed using silica gel on aluminum sheets. HPLC analysis was performed with an Agilent 1100 Infinity system (binary pump, auto sampler, DAD; Agilent, USA) equipped with a reversed-phase (RP) C18 column (ODS-A, 4.6× 150 mm internal diameter, 120 Å, 5 *µ*m, YMC, USA). A linear gradient from 5% to 100% of solvent B in solvent A over 15 min at a flow rate of 0.8 mL/min was used (solvent A: 0.1% (*v*/*v*) formic acid (FA) in water, solvent B: 0.1% (*v*/*v*) FA in acetonitrile). Detection was accomplished at 210, 215 or 254 nm. Preparative RP18 HPLC was carried out with a Knauer system equipped with a WellChrom K-1001 pump and a WellChrom K-2501 UV detector using a preparative column (polymeric RP, 8 *µ*m, 300 Å, 250 x 9 mm internal diameter, VYDAC, USA). ^{1}H NMR and ^{13}C NMR spectra were recorded in solutions on a Varian Mercury 400 spectrometer at 400 MHz and 101 MHz, respectively. Chemical shifts (δ) are reported in ppm relative to TMS (^{1}H NMR) and to the solvent signal (^{13}C NMR). The positive- and negative-ion high-resolution ESI mass spectra were obtained with an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, Germany) equipped with a HESI electrospray ion source (positive spray voltage 4.5 kV, negative spray voltage 3.5 kV, capillary temperature 275 °C, source heater temperature 250 °C, FTMS resolution 30000). The MS system was coupled to an ultra-high performance liquid chromatography (UHPLC) system (Dionex UltiMate 3000, Thermo Fisher Scientific) equipped with a RP18 column (Hypersil GOLD, 50 x 2.1 mm internal diameter, 1.9 µm, 175 Å, Thermo Fisher Scientific, column temperature 30 °C) and a photodiode array detector (190-400 nm, Thermo Fisher Scientific). The mobile phases were H_2O (A: Fluka Analytical, LC–MS Chromasolv®) and CH_3CN (B: Fluka Analytical, LC–MS Chromasolv®) with formic acid (0.2 %). A gradient system was used (0–15 min, 5–100% B; 15–18 min, 100% B; 13–16 min; flow rate 0.150 mL/min). The instrument was externally calibrated using the

Pierce LTQ Velos ESI positive ion calibration solution (product No. 88323) and the Pierce ESI negative ion calibration solution (product No. 88324) from Thermo Fisher Scientific. The data were evaluated using the software Xcalibur 2.2 SP1.

General protocol for solid-phase peptide synthesis: Coupling reactions were carried out in an Automated Solid-Phase Peptide Synthesizer by stepwise Fmoc strategy using the TentaGel S RAM resin (200–400 mesh, loading 0.26 mmol/g resin) in 0.10–0.30 mmol scale. The first coupling was made with Fmoc-protected 3-nitro-tyrosine (Fmoc-Tyr(3-NO_2)-OH) and the coupling cycle from the standard Intavis protocol was used (see **Attachment S33**). The intermediate linear peptides further used in the solution-phase macrocyclization were cleaved from the resin with the cocktail TFA/TIPS/H_2O (95:2.5:2.5, *v*/*v*, 2 mL) and the purity was assessed by analytical RP-HPLC. Prior to solution-phase macrocyclization, the peptides were re-dissolved in 4 M HCl/dioxane, stirred at room temperature for 15 minutes and then the mixture was dropped into cold diethyl ether. The precipitated product was centrifuged, then taken up in 1:2 acetonitrile/water and lyophilized to yield the corresponding peptide hydrochloride salt.

Procedure for the synthesis of 1,4-diazepane-2,5-dione-containing peptides:[22] The resin-bound peptide (0.10 mmol) having the *N*-terminal sequence Fmoc-Lys(Fmoc)-Asp(OBzl), as arising from the automated solid-phase peptide synthesizer, is treated with a 20% piperidine solution in DMF (*ca.* 3 mL) and swirled for 30 min. This procedure is repeated once to ensure complete diazepane-2,5-dione formation, which is confirmed by cleavage of a sample portion and ESI-MS. The resin is washed successively with CH_2Cl_2, MeOH and THF prior to the Ugi-Smile macrocyclization.

General procedure for the solution-phase Ugi-Smiles macrocyclization: To a stirred solution of the free *N*-terminal 3-nitro-tyrosine-containing peptide (0.10 mmol, hydrochloride salt) in MeOH (4.0 mL) is added the aldehyde (0.10 mmol, 1.0 equiv.) and DIPEA (0.15 mmol, 1.5 equiv.). The mixture is stirred for 4 h to enable formation of the imine and then the *n*-dodecylisocyanide (0.15 mmol, 1.5 equiv.) is added. The reaction mixture is stirred for 48 h and the volatiles are then removed under reduced pressure in a rotary evaporator. The crude material is dissolved in the mixture MeCN/H_2O (1:1, *v*/*v*, 30 mL) and the solution phase is transferred to a separatory funnel and extracted with (3×10 mL). The organic phase is dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure in a rotary evaporator. The crude product is finally purified by preparative RP-HPLC.

General procedure for the solid-phase Ugi-Smiles macrocyclization: The 3-nitro-tyrosine-containing peptide (0.10 mmol) bound to the TentaGel S RAM resin (arising from the automated solid-phase peptide synthesizer, loading 0.26 mmol/g resin) is washed successively with CH_2Cl_2, MeOH and THF. The resin is pre-swelled with THF during 5 minutes and then submitted for one of the following Ugi-Smiles macrocyclization procedures.

Method A (transimination-mediated): A suspension of paraformaldehyde (0.4 mmol, 4 equiv.) and piperidine (0.4 mmol, 4 equiv.) in THF/MeOH (1:1, *v*/*v*, 2 mL) is added to the resin-bound peptide (0.1 mmol) and the reaction mixture is shaken for 30 minutes. The excess of reagent is removed by washing the beads with THF (4×1 mL). The isocyanide (0.4 mmol, 4 equiv.) dissolved in THF/MeOH (1:1; *v*/*v*, 2 mL) is added to the on-resin preformed imine and the mixture is swirled for a period of time indicated by RP-HPLC monitoring after partial cleavages. The resin is washed sequentially with DMF, THF and CH_2Cl_2. The final resin bound Ugi-Smiles macrocycle products are cleaved from the resin with the cocktail TFA/TIPS/H_2O (95:2.5:2.5, *v*/*v*, 2 mL), precipitated from frozen diethyl ether, centrifuged, washed twice with frozen diethyl ether, lyophilized and purified by preparative RP-HPLC to provide the pure Ugi-Smiles macrocycle product.

Method B: The aliphatic aldehyde (0.4 mmol, 4 equiv.) dissolved in THF/MeOH (1:1; 2 mL) is added to the resin-bound peptide and the reaction mixture is shaken for 4 h. The remaining solvent is filtered and the resin is washed sequentially with DMF, CH_2Cl_2, MeOH and THF. The isocyanide (0.4 mmol, 4 equiv.) dissolved in THF/MeOH (1:1; *v*/*v*, 2 mL) is added to the resin-bound peptidic imine and the mixture is swirled for a period of time indicated by RP-HPLC monitoring after mini-cleavage. The resin is washed sequentially with DMF, THF and CH_2Cl_2. The final resin-bound Ugi-Smiles products are cleaved from the resin with the cocktail TFA/TIPS/H_2O (95:2.5:2.5, *v*/*v*, 2 mL), precipitated from frozen diethyl ether, washed twice with frozen diethyl ether, centrifuged, lyophilized and purified by preparative RP-HPLC to provide the Ugi-Smiles macrocycle product.

HCl·H-Phe-Val-Tyr(NO_2)-NH_2 (1): The linear peptide **1** was produced (142 mg, 0.28 mmol) in 95% purity according to the general protocol for solid-phase peptide synthesis using the Automated Solid-Phase Peptide Synthesizer starting from 0.30 mmol of TentaGel S RAM resin. An analytical sample was purified by RP-HPLC to 99% purity (254 nm) for ESI-MS analysis. R_t = 6.97 min. HRMS (ESI) *m/z*: 470.2045 $[M-H]^-$, calcd. for $C_{23}H_{28}O_6N_5$ 470.2040.

Cyclic Lipopeptide 2: Peptide **1** (91 mg, 0.18 mmol), DIPEA (0.27 mmol), paraformaldehyde (0.27 mmol, 8.1 mg) and *n*-dodecylisocyanide (53 mg, 0.27 mmol) were reacted in MeOH (7.2 mL) for 48 h according to the solution-phase Ugi-Smiles macrocyclization procedure to furnish the cyclic lipopeptide **2**. Preparative RP-HPLC purification produced **2** (82 mg, 67%) in 98% purity (254 nm). R_t = 9.99 min. ^{1}H NMR (400 MHz, CD_3OD): δ = 8.01 – 7.88 (m, 1H), 7.54 – 7.43 (m, 1H), 7.42 – 7.10 (m, 5H), 7.08 – 6.99 (m, 1H), 4.71 – 4.62 (m, 1H), 4.30 – 4.21 (m, 1H), 4.14 – 4.04 (m, 1H), 3.75 – 3.52 (m, 2H), 3.49 – 3.37 (m, 1H), 3.27 – 3.12 (m, 3H), 3.11 – 2.99 (m, 2H), 2.98 – 2.81 (m, 2H), 2.42 – 2.30 (m, 1H), 2.04 – 1.85 (m, 1H), 1.36 – 1.26 (m, 21H), 0.93 – 0.55 (m, 9H). ^{13}C NMR (101 MHz, CD_3OD): δ = 14.4, 19.1, 19.4 (*C*H_3), 23.7, 28.0, 28.4, 30.3, 30.4, 30.5, 30.7, 30.8, 30.8 (*C*H_2), 30.8 (*C*H), 33.1, 37.2, 37.8, 39.9, 54.6 (*C*H_2), 57.3, 62.2, 67.4, 120.8, 126.7 (*C*H), 127.7 (*C*), 129.3, 129.5, 130.4, 130.7, 131.1, 135.6 (*C*H), 138.8, 139.1, 154.1 (*C*), 170.5, 171.7, 173.5, 175.0 (*C*O). HRMS (ESI) *m/z*: 677.4028 $[M-H]^-$, calcd. for $C_{37}H_{53}O_6N_6$: 677.4027.

HCl·H-Gly-Phe-Val-Tyr(NO_2)-NH_2 (3): The linear peptide **3** (57 mg, 0.10 mmol) was produced in 83% purity according to the general protocol for solid-phase peptide synthesis using the Automated Solid-Phase Peptide Synthesizer starting from 0.10 mmol of TentaGel S RAM resin. An analytical sample was purified by RP-HPLC to 97% purity (254 nm) for ESI-MS analysis. R_t = 8.24 min. HRMS (ESI) *m/z*: 527.2260 $[M-H]^-$, calcd. for $C_{25}H_{31}O_7N_6$ 527.2254.

Cyclic Lipopeptide 4: Peptide **3** (51 mg, 0.09 mmol), DIPEA (0.135 mmol), paraformaldehyde (0.135 mmol, 4.1 mg) and *n*-dodecylisocyanide (26 mg, 0.135 mmol) were reacted in MeOH (4 mL) for 48 h according to the solution-phase Ugi-Smiles macrocyclization procedure to furnish the cyclic lipopeptide **4**. Preparative RP-HPLC purification produced **4** (47 mg, 71%) in 99% purity (254 nm). R_t = 9.47 min. ^{1}H NMR (400 MHz, CD_3OD): δ = 8.10 – 7.90 (m, 3H), 7.85 – 7.65 (m, 1H), 7.59 – 7.42 (m, 1H), 7.35 – 7.03 (m, 3H), 4.68 – 4.51 (m, 1H), 4.48 – 4.38 (m, 1H), 4.27 – 4.16 (m, 1H), 4.05 – 3.91 (m, 1H), 3.82 – 3.63 (m, 1H), 3.24 – 3.09 (m, 6H), 2.44 – 2.29 (m, 1H), 1.91 (s, 1H), 1.52 – 1.47 (m, 4H), 1.30 (m, 26H), 0.90 (m, 9H). ^{13}C NMR (101 MHz, CD_3OD): δ = 14.5, 18.8, 19.16 (*C*H_3), 23.7, 27.5, 27.9, 30.3, 30.4, 30.5, 30.6, 30.7, 30.8, 30.9, 32.2 (*C*H_2), 33.1 (*C*H), 39.0, 40.5, 43.0, 54.1, 54.4 (*C*H_2), 57.8, 59.0, 59.4, 127.8, 127.9, 129.6, 129.9, 130.0, 130.3 (*C*H), 135.7 (*C*), 136.5, 137.0 (*C*H), 138.0, 141.5, 147.1 (*C*), 163.7, 167.2, 173.1 (*C*O). HRMS (ESI) *m/z*: 734.4244 $[M-H]^-$, calcd. for $C_{39}H_{56}O_7N_7$: 734.4241.

HCl·H-Ile-Gly-Pro-Val-Tyr(NO_2)-NH_2 (5): The linear peptide **5** (62 mg, 0.098 mmol) was produced in 91% purity according to the general protocol for solid-phase peptide

synthesis using the Automated Solid-Phase Peptide Synthesizer starting from 0.10 mmol of TentaGel S RAM resin. An analytical sample was purified by RP-HPLC to 96% purity (254 nm) for ESI-MS analysis. R_t = 7.84 min. HRMS (ESI) *m/z*: 590.2944 $[M-H]^-$, calcd. for $C_{27}H_{40}O_8N_7$: 590.2938.

Cyclic Lipopeptide 6: Peptide **5** (0.096 mmol), DIPEA (0.144 mmol), paraformaldehyde (0.144 mmol, 4.3 mg) and *n*-dodecylisocyanide (28 mg, 0.144 mmol) were reacted in MeOH (4 mL) for 48 h according to the solution-phase Ugi-Smiles macrocyclization procedure to furnish the cyclic lipopeptide **6**. Preparative RP-HPLC purification produced **6** (50 mg, 65%) in 97% purity (254 nm). R_t = 10.54 min. ^{1}H NMR (400 MHz, CD_3OD): δ = 8.09 (s, 1H), 7.86 (t, *J* = 5.9 Hz, 1H), 7.72 (m, 1H), 7.61 (d, *J* = 7.3 Hz, 1H), 7.29 (d, *J* = 8.3 Hz, 1H), 7.23 – 7.15 (m, 1H), 5.12 (m, 1H), 4.50 – 4.39 (m, 1H), 4.14 (dd, *J* = 9.2, 4.4 Hz, 1H), 4.08 – 3.90 (m, 2H), 3.73 – 3.58 (m, 2H), 3.57 – 3.40 (m, 2H), 3.25 – 3.12 (m, 2H), 3.11 – 3.03 (m, 1H), 2.78 (dd, *J* = 14.0, 4.9 Hz, 1H), 2.30 – 2.15 (m, 2H), 2.05 – 1.90 (m, 2H), 1.86 – 1.64 (m, 2H), 1.57 – 1.42 (m, 2H), 1.30 (m, 22H), 1.16 – 0.80 (m, 15H). ^{13}C NMR (101 MHz, CD_3OD): δ = 11.6, 14.5, 15.8, 19.8, 20.3 (*C*H_3), 23.8, 25.7, 25.9, 27.6, 28.1, 30.0, 30.2 (*C*H_2), 30.5 (*C*H), 30.6, 30.7, 30.8, 30.9, 33.1 (*C*H_2), 34.7 (*C*H), 37.1, 39.9, 42.1, 48.0, 50.8, 53.4 (*C*H_2), 62.0, 62.6, 63.4, 75.2, 124.9 (*C*H), 128.4 (*C*), 135.5, 136.0 (*C*H), 142.1, 147.9 (*C*), 164.7, 170.2, 171.2, 173.0, 174.1, 175.3 (*C*O). HRMS (ESI) *m/z*: 797.4929 $[M-H]^-$, calcd. for $C_{41}H_{65}O_8N_8$: 797.4925.

HCl·H-Phe-Gly-Val-Ala-Leu-Tyr(NO_2)-NH_2 (7): The linear peptide **7** (135 mg, 0.18 mmol) was produced in 93% purity according to the general protocol for solid-phase peptide synthesis using the Automated Solid-Phase Peptide Synthesizer starting from 0.20 mmol of TentaGel S RAM resin. An analytical sample was purified by RP-HPLC to 98% purity (254 nm) for ESI-MS analysis. R_t = 8.99 min. HRMS (ESI) *m/z*: 711.3467 $[M-H]^-$, calcd. for $C_{34}H_{47}O_9N_8$: 711.3466.

Cyclic Lipopeptide 8: Peptide **7** (120 mg, 0.16 mmol), DIPEA (0.24 mmol), paraformaldehyde (0.24 mmol, 7.2 mg) and *n*-dodecylisocyanide (47 mg, 0.24 mmol) were reacted in MeOH (6.4 mL) for 48 h according to the solution-phase Ugi-Smiles macrocyclization procedure to furnish the cyclic lipopeptide **8**. Preparative RP-HPLC purification produced **8** (77 mg, 52%) in 97% purity (254 nm). R_t = 10.34 min. ^{1}H NMR (400 MHz, CD_3OD): δ = 8.02 – 7.94 (m, 1H), 7.61 (m, 1H), 7.57 – 7.46 (m, 1H), 7.38 – 7.16 (m, 5H), 7.07 (dd, *J* = 8.6, 3.4 Hz, 1H), 4.67 (dd, *J* = 7.1, 4.3 Hz, 1H), 4.57 (dt, *J* = 11.9, 5.9 Hz, 1H), 4.46 – 4.33 (m, 1H), 4.28 – 4.15 (m, 2H), 4.12 – 4.04 (m, 1H), 4.01 – 3.90 (m, 1H), 3.89 – 3.79 (m, 1H), 3.72 – 3.60 (m, 2H), 3.56 – 3.45 (m, 1H), 3.25 – 3.15 (m, 3H), 3.14 – 3.02 (m, 2H), 2.99 – 2.88 (m, 1H), 2.41 – 2.29 (m, 1H), 2.26 – 2.14 (m,

1H), 2.13 – 1.97 (m, 2H), 1.93 – 1.80 (m, 1H) 1.66 – 1.55 (m, 2H), 1.54 – 1.45 (m, 2H), 1.35 – 1.20 (m, 20H), 1.07 – 0.80 (m, 15H). ^{13}C NMR (101 MHz, CD_3OD): δ = 14.5, 17.5, 18.7, 19.5 (*C*H_3), 21.7 (*C*H_2), 23.6, 23.8 (*C*H_3), 25.8 (*C*H), 27.8, 30.2, 30.3, 30.5, 30.6, 30.7, 30.8 (*C*H_2), 30.9 (*C*H), 33.1, 36.6, 39.0, 40.1, 44.3, 51.1 (*C*H_2), 53.9 (*C*H), 54.4 (*C*H_2), 61.7, 62.3, 70.6, 71.6, 120.9, 127.9 (*C*H), 129.5 (*C*), 129.6, 130.0, 130.3, 130.4, 130.7, 135.5 (*C*H), 136.1, 138.3, 142.9 (*C*), 163.7, 172.1, 172.7, 173.5, 174.7, 174.7, 174.8 (*C*O). HRMS (ESI) *m/z*: 918.5452 $[M-H]^-$, calcd. for $C_{48}H_{72}O_9N_9$: 918.5453.

Cyclic lipopeptide 9: The resin-bound peptide Boc-Lys(Fmoc)-Ala-Tyr(NO_2)-NH (0.10 mmol) was subjected to Fmoc-deprotection at the Lys side chain using the standard protocol of 20% piperidine in DMF. The resulting peptide was submitted to transimination by treatment with paraformaldehyde (12.0 mg, 0.40 mmol) and piperidine (0.40 mmol) in THF/MeOH (1:1, *v*/*v*, 2 mL), and next reacted for 48 h with *n*-dodecylisocyanide (78 mg, 0.40 mmol) according to the solid-phase Ugi-Smiles macrocyclization method A. After cleavage from the resin, cyclic lipopeptide **9** (55 mg, 81%) was obtained in 88% purity as determined by analytical RP-HPLC (254 nm). A sample was purified by preparative RP-HPLC to 99% purity for characterization. R_t = 12.73 min. ^{1}H NMR (400 MHz, CD_3OD): δ = 8.49 (s, 1H), 7.98 (d, *J* = 7.7 Hz, 1H), 7.89 – 7.69 (m, 2H), 7.57 – 7.46 (m, 1H), 7.44 – 7.31 (m, 1H), 7.28 – 7.14 (m, 1H), 4.69 – 4.55 (m, 1H), 4.46 – 4.35 (m, 1H), 3.88 – 3.78 (m, 1H), 3.77 – 3.68 (m, 2H), 3.63 (s, 3H), 3.26 – 3.11 (m, 4H), 2.70 (t, *J* = 12.9 Hz, 1H), 1.74 – 1.64 (m, 1H), 1.62 – 1.54 (m, 1H), 1.46 – 1.37 (m, 2H), 1.35 – 1.07 (m, 20H), 1.01 – 0.93 (m, 2H), 0.90 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD): δ = 14.5, 19.7 (*C*H_3), 23.7, 27.7, 30.2, 30.3, 30.5, 30.6, 30.7, 30.8, 30.9, 32.5, 33.1, 40.1 (*C*H_2), 54.1, 55.1 (*C*H), 71.5 (*C*H_2), 127.6 (*C*H), 129.2 (*C*), 130.6, 133.0 (*C*H), 143.0, 144.6 (*C*), 169.0, 172.3, 173.2, 175.2 (*C*O). HRMS (ESI) *m/z*: 632.4122 $[M+H]^+$, calcd. for $C_{32}H_{54}O_6N_7$: 632.4136.

Cyclic lipopeptide 10: The resin-bound peptide Boc-Lys(Fmoc)-Ala-Leu-Tyr(NO_2)-NH (0.10 mmol) was subjected to Fmoc-deprotection at the Lys side chain using the standard protocol of 20% piperidine in DMF. The resulting peptide was submitted to transimination by treatment with paraformaldehyde (12.0 mg, 0.40 mmol) and piperidine (0.40 mmol) in THF/MeOH (1:1, *v*/*v*, 2 mL), and next reacted for 48h with *n*-dodecylisocyanide (78 mg, 0.40 mmol) according to the solid-phase Ugi-Smiles macrocyclization method A. After cleavage from the resin, cyclic lipopeptide **10** (62 mg, 78%) was obtained in 82% purity as determined by analytical RP-HPLC (254 nm). A sample was purified by preparative RP-HPLC to 99% purity for characterization. In a parallel experiment under the same conditions, mini-cleavages and RP-HPLC analysis at 24 h, 48 h, 72 h and 96 h were made to assess the best reaction time.

^{1}H NMR (400 MHz, CD_3OD): δ = 8.00 – 7.88 (m, 1H), 7.79 (t, *J* = 5.8 Hz, 1H), 7.70 (d, *J* = 2.1 Hz, 1H), 7.65 – 7.58 (m, 1H), 7.55 – 7.48 (m, 1H), 7.44 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.30 (d, *J* = 8.4 Hz, 1H), 7.10 – 7.02 (m, 1H), 4.69 (dd, *J* = 11.7, 3.5 Hz, 1H), 4.30 (q, *J* = 7.2 Hz, 1H), 4.10 (dd, *J* = 9.6, 5.3 Hz, 1H), 3.84 (s, 2H), 3.75 (dd, *J* = 7.5, 4.5 Hz, 1H), 3.63 (s, 2H), 3.25 – 3.15 (m, 3H), 3.11 (t, *J* = 6.8 Hz, 2H), 2.98 – 2.86 (m, 2H), 1.76 – 1.66 (m, 2H), 1.63 – 1.56 (m, 2H), 1.55 – 1.49 (m, 2H), 1.48 – 1.41 (m, 3H), 1.38 (d, *J* = 7.2 Hz, 3H), 1.34 – 1.17 (m, 20H), 0.99 – 0.86 (m, 9H). ^{13}C NMR (101 MHz, CD_3OD): δ = 14.4, 18.0 (*C*H_3), 21.9 (*C*H_2), 22.8, 23.5 (*C*H_3), 23.8 (*C*H_2), 25.8 (*C*H), 27.6, 27.8, 30.2, 30.3, 30.5, 30.6, 30.7, 30.8, 30.9, 32.3, 33.1, 37.5, 40.2, 41.4 (*C*H_2), 50.5 (*C*H), 53.6 (*C*H_2), 54.0, 54.4 (*C*H), 55.4 (*C*H_2), 56.9, 124.8 (*C*H), 127.2 (*C*), 134.0, 135.5 (*C*H), 143.5, 145.5 (*C*), 169.8, 172.4, 173.5, 174.9, 175.8 (*C*O). HRMS (ESI) *m/z*: 743.4823 $[M-H]^-$, calcd. for $C_{38}H_{63}O_7N_8$: 743.4820.

Cyclic lipopeptide 11: The resin-bound peptide Boc-Lys(Fmoc)-Leu-Phe-Ile-Tyr(NO_2)-NH (0.10 mmol) was subjected to Fmoc-deprotection at the Lys side chain using the standard protocol of 20% piperidine in DMF. The resulting peptide was submitted to transimination by treatment with paraformaldehyde (12.0 mg, 0.40 mmol) and piperidine (0.40 mmol) in THF/MeOH (1:1, *v*/*v*, 2 mL), and next reacted for 48h with *n*-dodecylisocyanide (78 mg, 0.40 mmol) according to the solid-phase Ugi-Smiles macrocyclization method A. After cleavage from the resin, cyclic lipopeptide **11** (68 mg, 69%) was obtained in 90% purity as determined by analytical RP-HPLC (254 nm). A sample was purified by preparative RP-HPLC to 99% purity for characterization. R_t = 13.85 min. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.61 (m, 1H), 7.44 (dd, *J* = 8.4, 2.2 Hz, 2H), 7.31 – 7.18 (m, 7H), 4.70 (d, *J* = 3.5 Hz, 1H), 4.67 (d, *J* = 3.5 Hz, 1H), 4.64 (dd, *J* = 8.6, 5.1 Hz, 2H), 4.57 (m, 2H), 4.32 – 4.27 (m, 2H), 4.16 (d, *J* = 7.3 Hz, 1H), 3.81 (d, *J* = 12.8 Hz, 2H), 3.76 – 3.71 (m, 2H), 3.34 (s, 3H), 3.24 – 3.21 (m, 2H), 3.20 – 3.17 (m, 2H), 2.99 (t, *J* = 8.0 Hz, 2H), 2.91 – 2.83 (m, 3H), 1.82 – 1.74 (m, 3H), 1.64 – 1.55 (m, 2H), 1.47 – 1.40 (m, 5H), 1.32 – 1.21 (m, 17H), 1.20 – 1.15 (m, 2H), 0.95 – 0.82 (m, 15H). ^{13}C NMR (101 MHz, CD_3OD): δ = 11.7, 14.4, 15.9 (*C*H_3), 21.5 (*C*H_2), 23.1, 23.4 (*C*H_3), 23.7, 25.7 (*C*H_2), 25.9 (*C*H), 27.7, 27.8, 30.2, 30.4, 30.5, 30.6, 30.7, 30.8, 30.9, 32.7, 33.1 (*C*H_2), 37.7 (*C*H), 38.2, 40.1, 41.6, 53.5, 54.4 (*C*H_2), 54.5, 55.0, 56.8, 57.2, 58.9 (*C*H), 71.6 (*C*H_2), 125.1, 127.1 (*C*H), 127.7 (*C*), 129.5, 130.6, 134.7, 135.4 (*C*H), 138.5, 143.5, 146.0 (*C*), 172.4, 172.9, 173.3, 174.1, 175.4 (*C*O). HRMS (ESI) *m/z*: 934.6116 $[M+H]^+$, calcd. for $C_{50}H_{80}O_8N_9$: 934.6130.

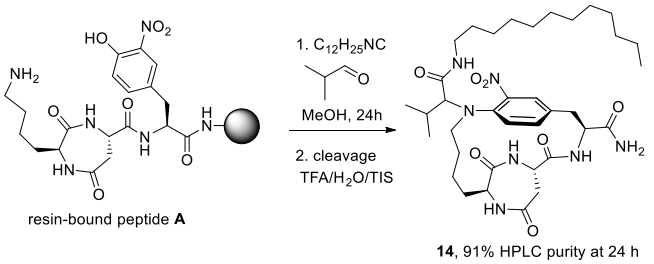
Cyclic lipopeptide 12: The resin-bound peptide Boc-Lys(Fmoc)-Ala-Leu-Asn-Phe-Tyr(NO_2)-NH (0.10 mmol) was subjected to Fmoc-deprotection at the Lys side chain using the standard protocol of 20% piperidine in DMF. The resulting peptide was

submitted to transimination by treatment with paraformaldehyde (12.0 mg, 0.40 mmol) and piperidine (0.40 mmol) in THF/MeOH (1:1, *v*/*v*, 2 mL), and next reacted for 48 h with *n*-dodecylisocyanide (78 mg, 0.40 mmol) according to the solid-phase Ugi-Smiles macrocyclization method A. After cleavage from the resin, cyclic lipopeptide **12** (69 mg, 66%) was obtained in 79% purity as determined by analytical RP-HPLC (254 nm). A sample was purified by preparative RP-HPLC to 99% purity for characterization. R_t = 13.26 min. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.99 (m, 1H), 7.73 (m, 1H), 7.63 – 7.58 (m, 1H), 7.57 – 7.52 (m, 1H), 7.35 (d, *J* = 8.5 Hz, 1H), 7.28 – 7.22 (m, 2H), 7.21 – 7.16 (m, 2H), 7.15 – 7.09 (m, 2H), 7.02 (d, *J* = 7.5 Hz, 1H), 4.61 – 4.56 (m, 4H), 4.46 (t, *J* = 5.6 Hz, 1H), 4.27 (t, *J* = 7.5 Hz, 1H), 4.21 (dd, *J* = 8.3, 6.6 Hz, 1H), 3.83 – 3.71 (m, 3H), 3.70 – 3.64 (m, 1H), 3.63 (s, 2H), 3.35 (s, 3H), 3.21 – 3.15 (m, 2H), 3.07 (t, *J* = 7.3 Hz, 2H), 3.04 – 2.98 (m, 2H), 2.97 – 2.92 (m, 1H), 2.79 – 2.67 (m, 4H), 1.83 – 1.74 (m, 2H), 1.70 – 1.62 (m, 3H), 1.49 (d, *J* = 7.3 Hz, 3H), 1.46 – 1.34 (m, 5H), 1.32 – 1.20 (m, 15H), 1.04 – 0.86 (m, 9H). ^{13}C NMR (101 MHz, CD_3OD): δ = 14.5, 18.3 (*C*H_3), 22.5 (*C*H_2), 22.6, 23.2 (*C*H_3), 23.7 (*C*H_2), 25.8 (*C*H), 25.9, 28.0, 30.3, 30.4, 30.5, 30.6, 30.7, 30.8, 30.9, 32.2, 33.1, 35.5, 36.9, 38.4, 40.2, 41.5, 43.4 (*C*H_2), 50.2, 52.8 (*C*H), 54.1 (*C*H_2), 55.2, 55.4, 57.8, 58.1 (*C*H), 71.6 (*C*H_2), 124.6, 127.2 (*C*H), 129.6 (*C*), 130.1, 130.3, 134.7, 135.7 (*C*H), 138.3, 143.0, 145.7 (*C*), 172.2, 173.3, 173.6, 174.7, 174.9, 175.5, 175.6, 175.8 (*C*O). HRMS (ESI) *m/z*: 1006.6062 $[M+H]^+$, calcd. for $C_{51}H_{80}O_{10}N_{11}$: 1006.6090.

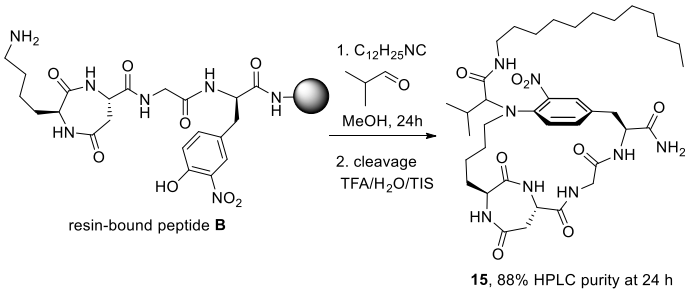
Cyclic lipopeptide 13: The resin-bound peptide Boc-Lys(Fmoc)-Ala-Phe-Leu-Gly-Asn-Ala-Tyr(NO_2)-NH (0.10 mmol) was subjected to Fmoc-deprotection at the Lys side chain using the standard protocol of 20% piperidine in DMF. The resulting peptide was submitted to transimination by treatment with paraformaldehyde (12.0 mg, 0.40 mmol) and piperidine (0.40 mmol) in THF/MeOH (1:1, *v*/*v*, 2 mL), and next reacted for 48 h with *n*-dodecylisocyanide (78 mg, 0.40 mmol) according to the solid-phase Ugi-Smiles macrocyclization method A. After cleavage from the resin, cyclic lipopeptide **13** (73 mg, 62%) was obtained in 84% purity as determined by analytical RP-HPLC (254 nm). A sample was purified by preparative RP-HPLC to 99% purity for characterization. R_t = 13.55 min. ^{1}H NMR (400 MHz, CD_3OD): δ = 8.42 (m, 1H), 7.69 – 7.61 (m, 1H), 7.58 – 7.51 (m, 1H), 7.47 (s, 1H), 7.35 (dd, *J* = 8.5, 1.9 Hz, 1H), 7.29 – 7.14 (m, 6H), 5.49 (s, 1H), 4.73 – 4.60 (m, 4H), 4.05 – 3.99 (m, 1H), 3.99 – 3.90 (m, 2H), 3.87 – 3.80 (m, 1H), 3.79 – 3.73 (m, 1H), 3.65 – 3.60 (m, 4H), 3.35 (s, 1H), 3.24 – 3.14 (m, 3H), 3.13 – 3.04 (m, 3H), 3.04 – 2.91 (m, 4H), 2.86 – 2.79 (m, 2H), 1.65 – 1.48 (m, 6H), 1.37 – 1.19 (m, 21H), 1.17 – 1.10 (m, 4H), 1.09 – 1.03 (m, 2H), 0.95 – 0.78 (m, 9H). ^{13}C NMR (101 MHz, CD_3OD): δ = 14.5, 18.4, 19.2 (*C*H_3), 21.6 (*C*H_2), 22.6, 23.7 (*C*H_3),

23.8 (*C*H_2), 25.4 (*C*H), 27.7, 28.6, 30.2, 30.3, 30.5, 30.6, 30.7, 30.8, 32.3, 33.1, 37.8, 38.1, 39.2, 40.1, 40.4, 44.3 (*C*H_2), 50.2, 50.8, 51.5 (*C*H), 53.6 (*C*H_2), 54.2, 55.0, 55.4, 56.3 (*C*H), 71.6 (*C*H_2), 124.1, 127.5 (*C*H), 127.8 (*C*), 129.6, 130.7, 133.5, 136.1 (*C*H), 138.2, 143.6, 145.1 (*C*), 171.7, 172.1, 173.1, 173.2, 174.3, 174.4, 174.5, 174.6, 174.8 (*C*O). HRMS (ESI) *m/z*: 1134.6656 $[M+H]^+$, calcd. for $C_{56}H_{88}O_{12}N_{13}$: 1134.6675.

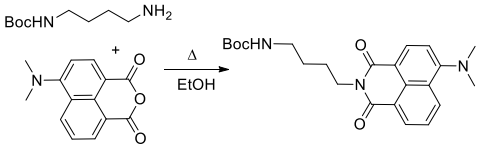
Cyclic lipopeptide 14: The resin-bound peptide **A** (0.05 mmol) was reacted with isobutyraldehyde (14.5 mg, 0.20 mmol) and *n*-dodecylisocyanide (39 mg, 0.20 mmol) for 24 h according to the solid-phase Ugi-Smiles macrocyclization method B. After cleavage from the resin, cyclic lipopeptide **14** (17.5 mg, 51%, mixture of diastereomers, dr. 4.6:1) was obtained in 91% purity as determined by analytical RP-HPLC (254 nm). A sample was purified by preparative RP-HPLC to 99% purity for characterization. R_t = 16.11; 16.51 min. ^{1}H NMR (400 MHz, CD_3OD): δ = 8.41 (s, 1H), 7.80 (s, 1H), 7.52 (s, 2H), 7.40 – 7.26 (m, 4H), 7.20 (d, *J* = 8.2 Hz, 1H), 6.52 – 6.45 (m, 1H), 4.58 (s, 1H), 4.53 (s, 1H), 4.20 (d, *J* = 11.1 Hz, 2H), 4.01 – 3.85 (m, 4H), 3.79 (d, *J* = 11.4 Hz, 2H), 3.68 – 3.59 (m, 1H), 3.48 (m, 1H), 3.44 – 3.36 (m, 2H), 3.16 – 3.11 (m, 2H), 3.09 – 2.94 (m, 6H), 2.92 – 2.82 (m, 3H), 2.72 (t, *J* = 13.1 Hz, 3H), 2.24 – 2.09 (m, 3H), 2.07 – 1.94 (m, 4H), 1.90 – 1.75 (m, 4H), 1.70 – 1.56 (m, 3H), 1.28 (m, 36H), 1.19 – 1.06 (m, 6H), 1.00 (m, 3H), 0.92 – 0.87 (m, 6H), 0.86 – 0.83 (m, 3H). HRMS (ESI) *m/z*: 700.4388 $[M+H]^+$, calcd. for $C_{36}H_{58}O_7N_7$: 700.4398.



Cyclic Lipopeptide 15: The resin-bound peptide **B** (0.05 mmol) was reacted with isobutyraldehyde (14.5 mg, 0.20 mmol) and *n*-dodecylisocyanide (39 mg, 0.20 mmol) for 24 h according to the solid-phase Ugi-Smiles macrocyclization method B. After cleavage from the resin, cyclic lipopeptide **15** (20 mg, 53%, mixture of diastereomers, dr. 2.3:1) was obtained in 88% purity as determined by analytical RP-HPLC (254 nm). An analytical sample was purified by preparative RP-HPLC to 98% purity for characterization. R_t = 16.18; 16.44 min. ^{1}H NMR (400 MHz, CD_3OD): δ = 8.19 (s, 1H), 7.74 – 7.63 (m, 3H), 7.50 (m, 1H), 7.48 – 7.43 (m, 2H), 7.40 – 7.31 (m, 4H), 4.64 – 4.53 (m, 5H), 4.36 – 4.30 (m, 1H), 4.29 – 4.19 (m, 3H), 4.09 (d, *J* = 17.1 Hz,

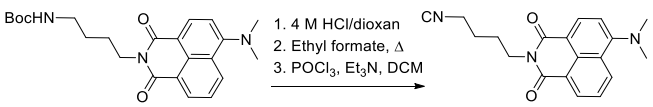


1H), 3.97 (d, *J* = 17.1 Hz, 1H), 3.89 – 3.82 (m, 3H), 3.80 – 3.76 (m, 2H), 3.75 – 3.69 (m, 3H), 3.68 – 3.66 (m, 1H), 3.65 – 3.60 (m, 1H), 3.59 – 3.56 (m, 1H), 3.50 – 3.46 (m, 1H), 3.34 (s, 3H), 3.17 – 3.03 (m, 8H), 2.79 – 2.75 (m, 1H), 2.75 – 2.71 (m, 1H), 2.71 – 2.65 (m, 2H), 2.62 (dd, *J* = 8.7, 3.7 Hz, 1H), 2.57 (dd, *J* = 9.0, 5.8 Hz, 2H), 2.54 – 2.50 (m, 1H), 2.24 – 2.12 (m, 4H), 2.03 (m, 1H), 1.90 – 1.84 (m, 2H), 1.79 (q, *J* = 7.2 Hz, 3H), 1.50 – 1.20 (m, 36H), 1.17 (t, *J* = 7.1 Hz, 3H), 1.02 – 0.94 (m, 5H), 0.92 – 0.87 (m, 5H), 0.86 – 0.80 (m, 5H). HRMS (ESI) *m/z*: 757.4601 $[M+H]^+$, calcd. for $C_{38}H_{61}O_8N_8$: 757.4612.



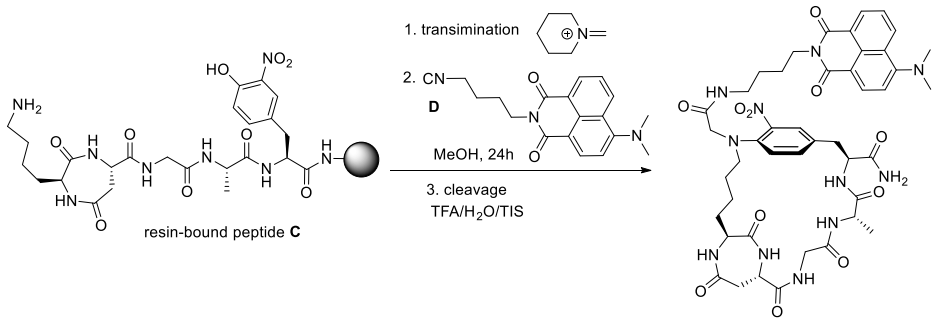
4-(4-Dimethylamino-1,8-naphthalimido)butyl-*N*-Boc-amine: 4-(dimethylamino)-1,8-naphthalic anhydride (1.45 g, 6.00 mmol) and 4-*N*-Boc-amino-butane-1-amine (1.24 g, 6.59 mmol) were refluxed in absolute ethanol (75 mL) for 30 min. The solution was cooled to room temperature and the solvent removed by rotary evaporation. The resulting crude product was purified by flash column chromatography (EtOAc) to yield the pure 4-(4-dimethylamino-1,8-naphthalimido)butyl-*N*-Boc-amine as an orange solid (2.08 g, 5.05 mmol, 84%). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.55 (d, *J* = 7.3, 1H), 8.46 (d, *J* = 8.2 Hz, 1H), 8.43 (d, *J* = 8.5, 1H), 7.65 (dd, *J* = 8.4, 7.3 Hz, 1H), 7.11 (d, *J* = 8.2 Hz, 1H), 4.68 (m, 1H), 4.19 – 4.16 (m, 2H), 3.22 –3.16 (m, 2H), 3.10 (s, 6H), 1.80 – 1.73 (m, 2H), 1.65 – 1.56 (m, 2H), 1.43 (s, 9H). ^{13}C NMR (151 MHz, $CDCl_3$): δ = 25.6, 27.6 (*C*H_2), 28.5 (*C*H_3), 39.8, 40.4 (*C*H_2), 44.9 (*C*H_3), 79.1 (*C*), 113.4, 115.0 (*C*H), 123.1, 125.0, 125.4 (*C*), 130.3, 131.1 (*C*H), 131.3 (*C*), 132.8 (*C*H), 156.0 (*C*), 157.1, 164.1, 164.7 (*C*O). HRMS (ESI) *m/z*: 411.2160 $[M+H]^+$, calcd. for $C_{23}H_{29}O_4N_3$: 411.2158.

4-(4-dimethylamino-1,8-naphthalimido)butyl isocyanide: 4-(4-Dimethylamino-1,8-naphthalimido)butyl-*N*-Boc-amine (1.40 g, 3.40 mmol) was submitted to the Boc group removal in 4 M HCl/dioxan solution using standard protocol. The resulting amine hydrochloride salt was dissolved in ethylformate (40 mL) and was added Et_3N (0.71 mL, 5.11 mmol). The mixture was stirred at reflux for 20 h. The volatiles were removed under reduced pressure in a rotary evaporator to furnish the corresponding formamide. This crude product was dissolved in dry CH_2Cl_2 (120 mL), the solution was cooled in an ice-bath and treated with Et_3N (1.98 mL, 14.28 mmol). A solution of phosphorous oxychloride (0.51 mL; 5.44 mmol; dissolved in 5 mL of dry CH_2Cl_2) was added dropwise under nitrogen atmosphere to the stirred mixture. After the bath was removed, the reaction was stirred at room temperature for 1 h. A 0.5 M solution of sodium carbonate (10 mL)



was then added slowly to the reaction mixture, which was then stirred for an additional 30 min. The organic phase was separated, washed once with brine, dried over anhydrous sodium sulphate, filtered and concentrated. The product was purified by flash chromatography (EtOAc/n-Hex (1:1)) and isolated as a crystalline orange solid (940 mg, 2.92 mmol, 86 %). IR (KBr, cm^{-1}) v_{max}: 2857, 2148, 1685, 1637, 1582, 1355, 1318. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.56 (d, *J* = 7.3, 1H), 8.49 – 8.42 (m, 2H), 7.66 (dd, *J* = 8.5, 7.3 Hz, 1H), 7.12 (d, *J* = 8.2 Hz, 1H), 4.22 (t, *J* = 6.9 Hz, 2H), 3.52 – 3.44 (m, 2H), 3.11 (s, 6H), 1.96 – 1.86 (m, 2H), 1.84 – 1.75 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 25.3, 26.8, 39.0, 41.3 (*C*H_2), 44.9 (*C*H_3), 113.4, 114.8 (*C*H), 123.0, 125.0, 125.4 (*C*), 130.4, 131.3 (*C*H), 131.5 (*C*), 132.9 (*C*H), 156.2 (*C*), 157.2 (N*C*), 164.2, 164.8 (*C*O).

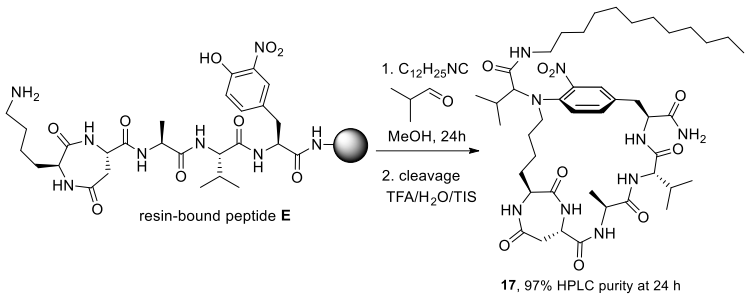
Cyclic fluorescently labeled peptide 16:



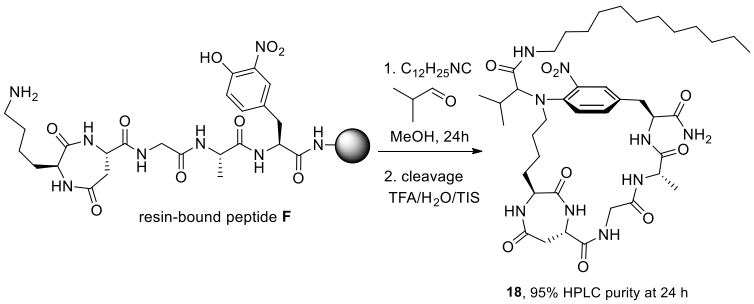
The resin-bound peptide **C** (0.10 mmol) was subjected to transimination by treatment with paraformaldehyde (12.0 mg, 0.40 mmol) and piperidine (0.40 mmol) in THF/MeOH (1:1, *v*/*v*, 2 mL), and next reacted for 24 h with the fluorescent-labeled isocyanide **D** (97 mg, 0.30 mmol) according to the solid-phase Ugi-Smiles macrocyclization method A. After cleavage from the resin, cyclic lipopeptide **16** (75 mg, 83%) was obtained in 90% purity as determined by analytical RP-HPLC (254 nm). A sample was purified by preparative RP-HPLC to 99% purity for characterization. R_t = 10.91 min. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.52 (d, *J* = 8.5 Hz, 1H), 8.46 (d, *J* = 7.2 Hz, 1H), 8.39 – 8.32 (m, 1H), 8.06 (m, 1H), 8.00 – 7.92 (m, 2H), 7.87 (s, 1H), 7.76 (t, *J* = 7.9 Hz, 1H), 7.61 (s, 1H), 7.35 – 7.26 (m, 2H), 7.25 – 7.13 (m, 2H), 4.46 – 4.38 (m, 1H), 4.28 – 4.21 (m, 1H), 4.05 – 3.96 (m, 3H), 3.85 – 3.76 (m, 2H), 3.71 – 3.57 (m, 3H), 3.56 – 3.46 (m, 4H), 3.10 (s, 6H), 2.77 (dd, *J* = 14.7, 11.0 Hz, 1H), 2.66 (dd, *J* = 15.1, 5.1 Hz, 1H), 2.58 (d, *J* = 6.3 Hz, 1H), 2.54 (d, *J* = 6.3 Hz, 1H), 1.68 – 1.62 (m, 2H), 1.60 – 1.52 (m, 2H), 1.45 – 1.37 (m, 2H), 1.33 – 1.21 (m, 4H), 1.16 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 17.6 (*C*H_3), 21.6, 25.2, 26.4, 26.6, 31.7, 35.7, 37.8, 38.0, 38.2, 42.2 (*C*H_2), 44.4 (*C*H_3), 48.7 (*C*H), 51.4 (*C*H_2), 52.3, 53.0 (*C*H), 53.9 (*C*H_2), 55.75, 113.0, 113.3 (*C*H), 122.3 (*C*), 123.1, 124.2, 125.0, 125.5 (*C*H), 129.6, 130.6, 131.6 (*C*), 131.8,

132.3 (*C*H), 133.6, 142.1, 142.4, 156.6 (*C*), 163.0, 163.6, 167.5, 167.6, 168.6, 169.6, 171.9, 172.7 (*C*O). HRMS (ESI) *m/z*: 910.3856 $[M-H]^-$, calcd. for $C_{44}H_{52}O_{11}N_{11}$: 910.3848.

Cyclic Lipopeptide 17: The resin-bound peptide **E** (0.10 mmol) was reacted with isobutyraldehyde (29 mg, 0.40 mmol) and *n*-dodecylisocyanide (78 mg, 0.40 mmol) for 24 h according to the solid-phase Ugi-Smiles macrocyclization method B. After cleavage from the resin, cyclic lipopeptide **17** (71 mg, 82%, mixture of diastereomers, dr. 1.3:1) was obtained in 97% purity as determined by analytical RP-HPLC (254 nm). An analytical sample was purified by preparative RP-HPLC to 99% purity for characterization. R_t = 13.35; 13.63 min. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.47 (m, 1H), 7.42 – 7.39 (m, 5H), 7.38 – 7.34 (m, 1H), 7.33 – 7.31 (m, 1H), 7.29 – 7.27 (m, 1H), 7.26 – 7.24 (m, 1H), 4.71 (dd, *J* = 9.5, 2.9 Hz, 1H), 4.42 – 4.36 (m, 1H), 4.35 – 4.30 (m, 1H), 4.19 (q, *J* = 7.4 Hz, 1H), 4.11 (d, *J* = 7.6 Hz, 1H), 3.98 (d, *J* = 8.1 Hz, 1H), 3.93 – 3.88 (m, 1H), 3.79 (d, *J* = 11.5 Hz, 1H), 3.74 – 3.67 (m, 1H), 3.66 – 3.61 (m, 1H), 3.52 – 3.46 (m, 1H), 3.20 – 3.06 (m, 4H), 2.69 – 2.55 (m, 2H), 2.28 – 2.16 (m, 2H), 2.13 – 1.98 (m, 2H), 1.39 (d, *J* = 7.5 Hz, 2H), 1.28 (m, 19H), 1.02 – 0.83 (m, 15H). ^{13}C NMR (101 MHz, CD_3OD): δ = 14.4, 16.9, 19.7, 19.9 (*C*H_3), 20.1, 23.7 (*C*H_2), 28.10 (*C*H), 29.0, 30.2, 30.4, 30.5 (*C*H_2), 30.7 (*C*H), 30.8, 33.1, 36.5, 38.0, 40.0 (*C*H_2), 51.6, 53.3 (*C*H), 54.1 (*C*H_2), 54.4, 62.6, 75.3, 125.7 (*C*H), 128.4 (*C*), 132.1, 136.2 (*C*H), 142.1, 150.1 (*C*), 171.9, 172.2, 172.8, 173.3, 174.2, 174.8, 176.1 (*C*O). HRMS (ESI) *m/z*: 870.5449 $[M+H]^+$, calcd. for $C_{44}H_{72}O_9N_9$: 870.5453.

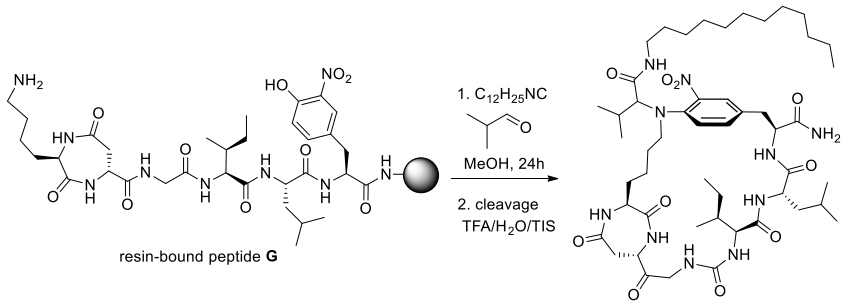


Cyclic Lipopeptide 18: The resin-bound peptide **F** (0.10 mmol) was reacted with isobutyraldehyde (29 mg, 0.40 mmol) and *n*-dodecylisocyanide (78 mg, 0.40 mmol) for 24 h according to the solid-phase Ugi-Smiles macrocyclization method B. After cleavage from the resin, cyclic lipopeptide **18** (76 mg, 92%, mixture of diastereomers, dr. 1.5:1) was obtained in 95% purity as determined by analytical RP-HPLC (254 nm).



An analytical sample was purified by preparative RP-HPLC to 99% purity for characterization. R_t = 16.06; 16.28 min. ^{1}H NMR (400 MHz, CD_3OD): δ = 8.28 – 8.22 (m, 1H), 7.80 – 7.71 (m, 3H), 7.54 (m, 1H), 7.50 – 7.44 (m, 1H), 7.37 – 7.31 (m, 4H), 7.31 – 7.24 (m, 2H), 4.72 – 4.63 (m, 2H), 4.44 – 4.36 (m, 2H), 4.32 – 4.24 (m, 2H), 3.95 (s, 1H), 3.90 (m, 1H), 3.86 (s, 1H), 3.83 (s, 1H), 3.79 (t, *J* = 5.7 Hz, 1H), 3.75 (s, 1H), 3.70 (s, 1H), 3.66 (s, 1H), 3.60 – 3.52 (m, 2H), 3.49 – 3.40 (m, 4H), 3.38 – 3.33 (m, 2H), 3.22 – 3.11 (m, 5H), 3.10 – 2.99 (m, 6H), 2.76 – 2.56 (m, 6H), 2.29 – 2.16 (m, 3H), 2.10 – 1.95 (m, 3H), 1.44 (d, *J* = 7.4 Hz, 4H), 1.39 (d, *J* = 7.4 Hz, 3H), 1.37 – 1.32 (m, 6H), 1.28 (s, 30H), 0.99 (d, *J* = 6.6 Hz, 6H), 0.92 – 0.83 (m, 12H). ^{13}C NMR (101 MHz, CD_3OD): δ = 14.5, 17.1, 17.5, 19.2, 19.6 (*C*H_3), 20.1, 22.1, 23.8, 24.1, 26.7, 27.1 (*C*H_2), 28.1, 28.2 (*C*H), 28.8, 30.1, 30.2, 30.3, 30.4, 30.5, 30.7, 30.8, 33.1, 36.3, 38.3, 38.4, 39.9, 40.0, 40.1, 44.4, 44.6 (*C*H_2), 46.2, 46.7 (*C*H), 51.8, 51.9 (*C*H_2), 53.1, 53.3, 53.5, 53.9, 54.5, 54.8, 75.6, 76.0, 124.0, 125.7 (*C*H), 128.3, 128.7 (*C*), 131.6, 133.8, 136.6 (*C*H), 141.0, 141.9, 150.1, 150.6 (*C*), 171.5, 171.6, 171.7, 171.8, 172.1, 172.5, 172.7, 173.1, 173.2, 173.2, 173.5, 174.6, 175.3, 175.6 (*C*O). HRMS (ESI) *m/z*: 828.4979 $[M+H]^+$, calcd. for $C_{41}H_{66}O_9N_9$: 828.4983.

Cyclic Lipopeptide 19:



The resin-bound peptide **G** (0.10 mmol) was reacted with isobutyraldehyde (29 mg, 0.40 mmol) and *n*-dodecylisocyanide (78 mg, 0.40 mmol) for 24 h according to the solid-phase Ugi-Smiles macrocyclization method B. After cleavage from the resin, cyclic lipopeptide **19** (61 mg, 62%, mixture of diastereomers, dr. 1.1:1) was obtained in 91% purity as determined by analytical RP-HPLC (254 nm). An analytical sample was purified by preparative RP-HPLC to 97% purity for characterization. R_t = 13.65; 13.93 min. ^{1}H NMR (400 MHz, CD_3OD): δ = 8.27 (d, *J* = 6.6 Hz, 1H), 8.21 (s, 1H), 8.01 (s, 1H), 7.66 – 7.58 (m, 2H), 7.51 (m, 1H), 7.35 – 7.28 (m, 2H), 4.65 – 4.57 (m, 2H), 4.39 – 4.32 (m, 2H), 4.25 – 4.19 (m, 1H), 4.16 (d, *J* = 17.0 Hz, 2H), 4.0 – 3.94 (m, 1H), 3.65 (d, *J* = 16.9 Hz, 1H), 3.42 – 3.36 (m, 1H), 3.34 (s, 1H), 3.27 – 3.17 (m, 6H), 3.12 – 3.04 (m, 3H), 3.00 – 2.92 (m, 2H), 2.72 (dd, *J* = 16.3, 10.2 Hz, 2H), 2.29 – 2.18 (m, 2H),

1.93 – 1.85 (m, 2H), 1.65 – 1.58 (m, 3H), 1.55 – 1.48 (m, 3H), 1.44 – 1.37 (m, 2H), 1.31 – 1.26 (m, 26H), 1.08 (d, *J* = 6.6 Hz, 3H), 0.98 (d, *J* = 6.8 Hz, 3H), 0.94 (d, *J* = 6.0 Hz, 3H), 0.92 – 0.91 (m, 3H), 0.90 – 0.89 (m, 6H), 0.88 – 0.87 (m, 3H). ^{13}C NMR (101 MHz, CD_3OD): δ = 11.7, 14.4, 16.1, 19.7, 20.3 (*C*H_3), 21.9 (*C*H_2), 23.0, 23.3 (*C*H_3), 23.7 (*C*H_2), 25.9 (*C*H), 27.9 (*C*H_2), 28.1 (*C*H), 28.6, 28.9, 30.2, 30.4, 30.5, 30.6, 30.7, 30.8, 30.9, 33.1, 34.1 (*C*H_2), 37.3 (*C*H), 39.0, 39.3, 40.0, 40.6, 40.7 (*C*H_2), 44.3 (*C*H), 53.1 (*C*H_2), 54.1, 54.7, 55.8, 58.8, 75.7, 127.0 (*C*H), 128.4 (*C*), 133.4, 135.9 (*C*H), 142.2, 150.2 (*C*), 163.7, 168.7, 169.0, 172.0, 173.3, 173.9, 174.3, 174.7 (*C*O). HRMS (ESI) *m/z*: 981.6143 $[M-H]^-$, calcd. for $C_{50}H_{81}O_{10}N_{10}$: 981.6137.

3.7 References

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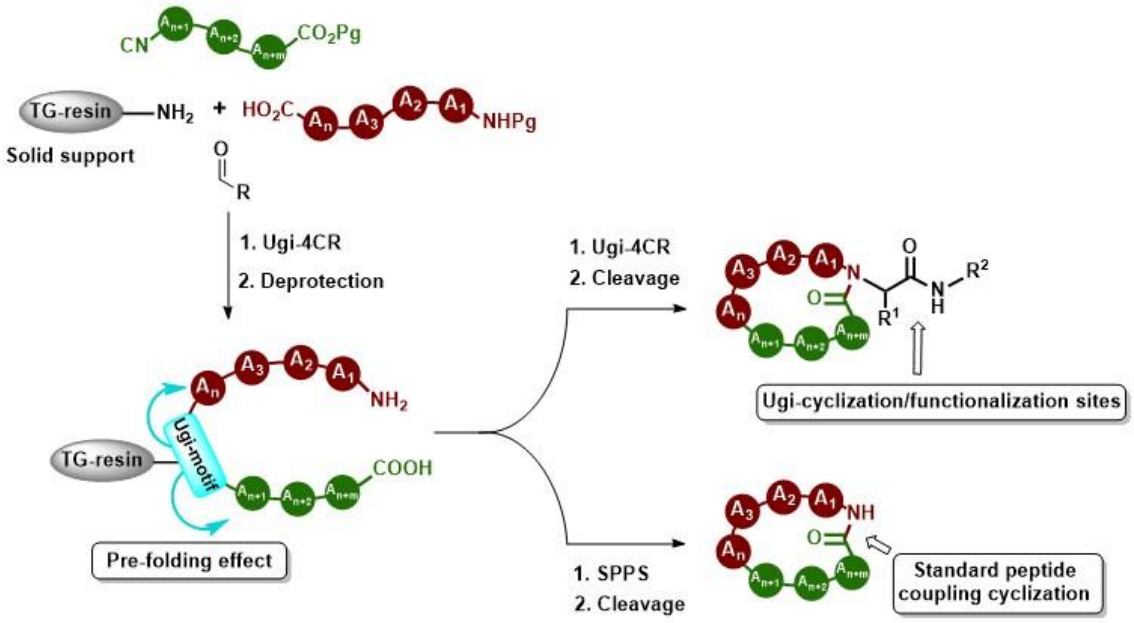
Chapter 4

Peptide Macrocyclization Assisted by a Traceless Turn-Inducer Derived from Ugi Peptide Ligation with a Resin-Linked Amine

*Abstract**



This chapter focuses on the development of a new methodology for peptide head-to-tail cyclization on solid phase. It combines the versatility of multicomponent reactions with the advantages of solid phase synthesis. The strategy relies on the insertion of a turn-inducer mimic by means of an Ugi-4CR, which bends the linear peptide structure, thereby proximising its *C*- and *N*-termini to favor eventual cyclization. Further functionalization may be reached by using an additional Ugi-4CR for the second, ring closuring step.

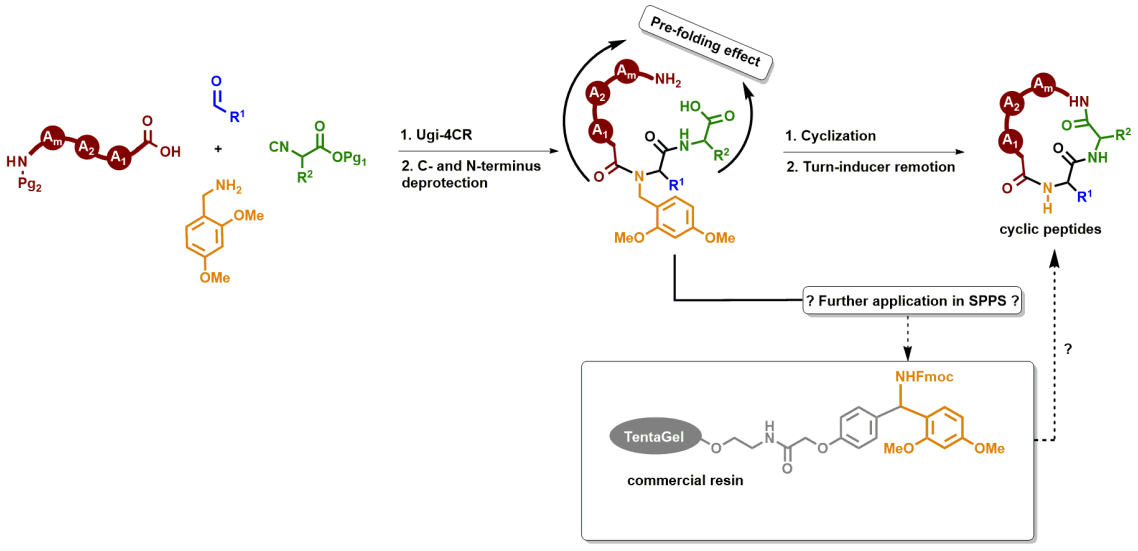
* Part of his Chapter was published: Morejon, M. C.**; Puentes, A. R.; Rivera, D.G.; Wessjohann, L. A. *Org. Lett.* **2017**, *19*, 4022–4025.

** Own contribution: Applications of the turn-inducer mimic in solid phase.

4.1 Introduction

The synthesis of cyclic peptides and derivatives increasingly attract the attention of the scientific community due to potential applications in diverse areas of chemistry and pharmacology. Currently, cyclization of peptides are employed for improving biological activity, bioavailability and metabolic stability compared to their linear counterparts.[1] Indeed, cyclic peptides display a higher resistance to proteolysis[2] and enzymatic degradation, as well as entropy advantage and larger binding affinities towards enzymatic receptors when compared to their more flexible linear counterparts.[3,4]

In addition, the restricted conformational flexibility of the cyclic peptides compels their functional groups to adopt a spatially well-defined position and this structural bias constitutes a powerful tool to get an insight into bioactive protein conformations.[5] Unfortunately, the head-to-tail cyclization of a linear peptide is usually associated with undesired drawbacks such as slow reaction, low yields and by-products of dimerization and sometimes epimerization.[6] Recently, we have developed a new strategy based on multicomponent reactions for peptide cyclization, which employs a removable turn-inducer to assist the ring closure process.[7] As depicted in **Scheme 4.1**, the use of an Ugi-4CR allows for inserting a 2,4-dimethoxybenzyl moiety as substituent of a tertiary amide, forcing the linear peptide structure to bend, and adopt a conformation that has an improved cyclization propensity, especially in the *s*-cis conformation.[8,9]



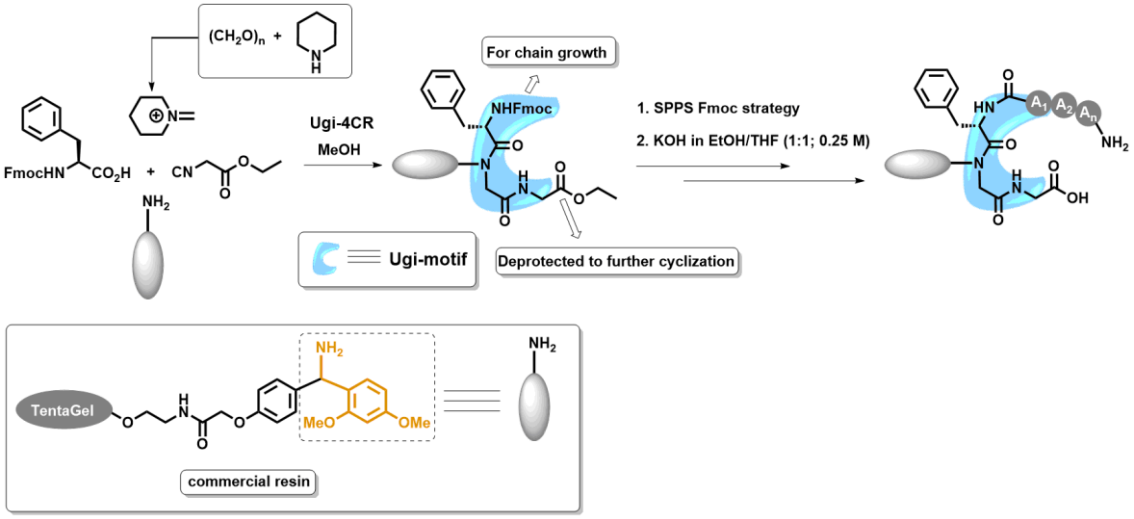
Scheme 4.1 Applications of a turn-inducer in SPPS towards the synthesis of cyclic peptide scaffolds.

The approach was successfully applied in the synthesis of different cyclic peptides with sizes ranging from the strained tetrapeptide ring to cyclic heptapeptides; including the total synthesis of the cyclic heptapeptide natural product crassipin B.[7,10]

Aiming at extending the scope of the new strategy, we turned to the development of an on-resin version of our turn-inducer mimic and explore its potential for the solid phase synthesis of cyclic peptides and lipid derivatives. The combined versatility of the multicomponent reactions with the advantages of solid phase synthesis will allow an automated process. E.g. if applied on arrays or in microtiter plates (MTPs), which provide a spatial assignment, it can be an ideal tool for discovery libraries and experiments.

4.2 Synthetic Plan

All commercial resins based on a Rink amide linker share a structural feature with our previous turn-inducer mimic developed in solution phase.[7] Fortunately, the 2,4-dimethoxybenzyl moiety, also present in Rink-based resins (see **Scheme 4.1**, highlighted in orange), was the perfect starting point to explore our approach on a supported matrix. **Scheme 4.2** depicts the concept, in which the commercial solid support is modified by a MCR process in order to outfit the resin with a bifunctional substrate, allowing on-resin head-to-tail cyclization.



Scheme 4.2 Development of the supported version for the turn-inducer mimic.

The turn-inducer mimic is loaded on the resin also by means of the Ugi-4CR. Tentagel S RAM resin was chosen as the amino component. This resin has a PEGylated linker which results in a relatively good swelling behavior in methanol, which is the solvent commonly used to carry out Ugi reactions. In order to avoid the competing Passerini reaction, prior to addition of the ethyl isocyanoacetate as isocyanide component and glycine equivalent, and Fmoc-Phe-OH as acid component, the imine was pre-formed on-resin by implementing an aminocatalysis-mediated transimination protocol recently

developed in the Rivera group.[11] The procedure comprises the addition to the resin of a piperidinium ion arising from reaction of paraformaldehyde and piperidine. After shaking for 30 minutes, complete transimination was achieved at the amino extreme of the solid support according to a ninhydrin test.[11] The efficiency of the on-resin Ugi reaction was assessed by UHPLC/ESI-MS analysis after the cleavage of an analytical sample, in which the protecting groups at *C*- and *N*-termini were previously removed. The corresponding Ugi-product (peptide H-Phe-Gly-Gly-OH) is obtained in 82% of purity from the crude reaction (**Figure 4.1**).

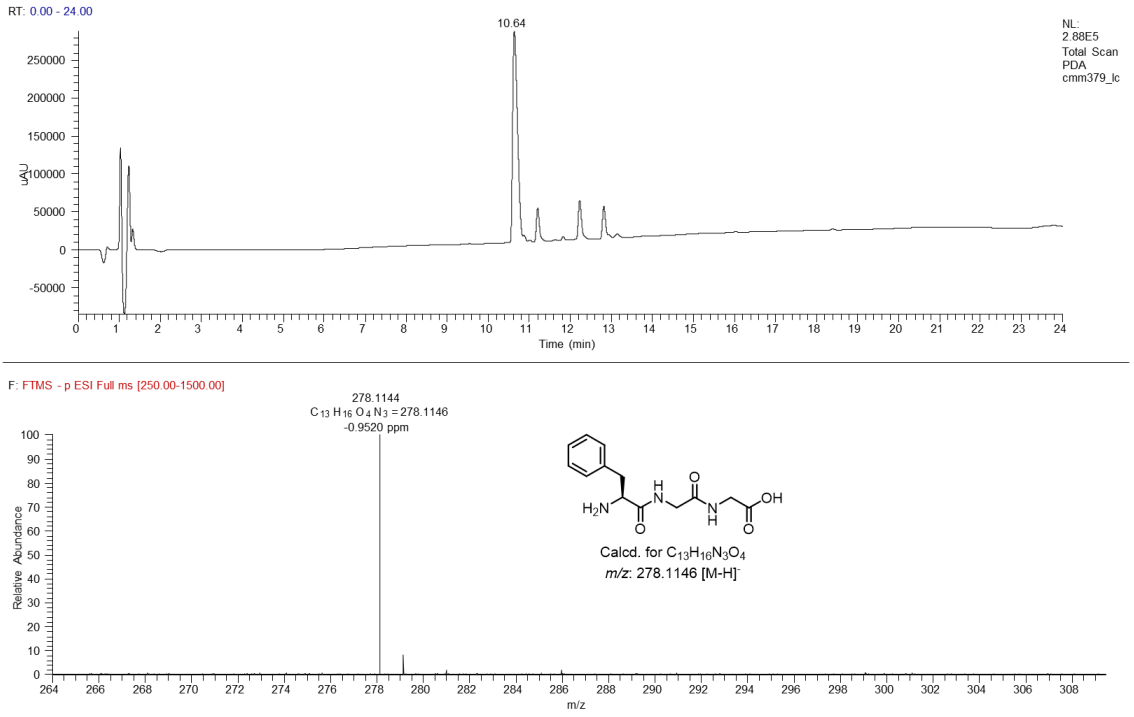


Figure 4.1 RP-UHPLC chromatogram and ESI-HRMS of crude peptide H-Phe-Gly-Gly-OH after resin cleavage.

Thus, first the automated synthesis of the suitable linear sequence is carried out following the typical Fmoc/*t*Bu strategy. In sequence, the cyclization is performed on-resin to afford the desired cyclic peptides either by classical methods, or a multicomponent reaction again. The latter allows to accomplish the macrocyclization and a functionalization, here exemplarily lipid insertion, in a single step. The obtained cyclic lipopeptides are then released from the resin and purified.

It is noteworthy to remark, as this work is primarily a proof of concept, that the same Ugi-motif is used as supported turn-inducer in all the cases (**Scheme 4.2**, element highlighted in blue). However, intrinsic to the multicomponent concept, also different oxo- and acid-components can be used, as well as diverse isocyanides if orthogonality with the resin cleavage conditions is considered. Thus the structural diversity of the turn-inducer position in the final cyclic peptides is owed to the Ugi-4CR reactants.

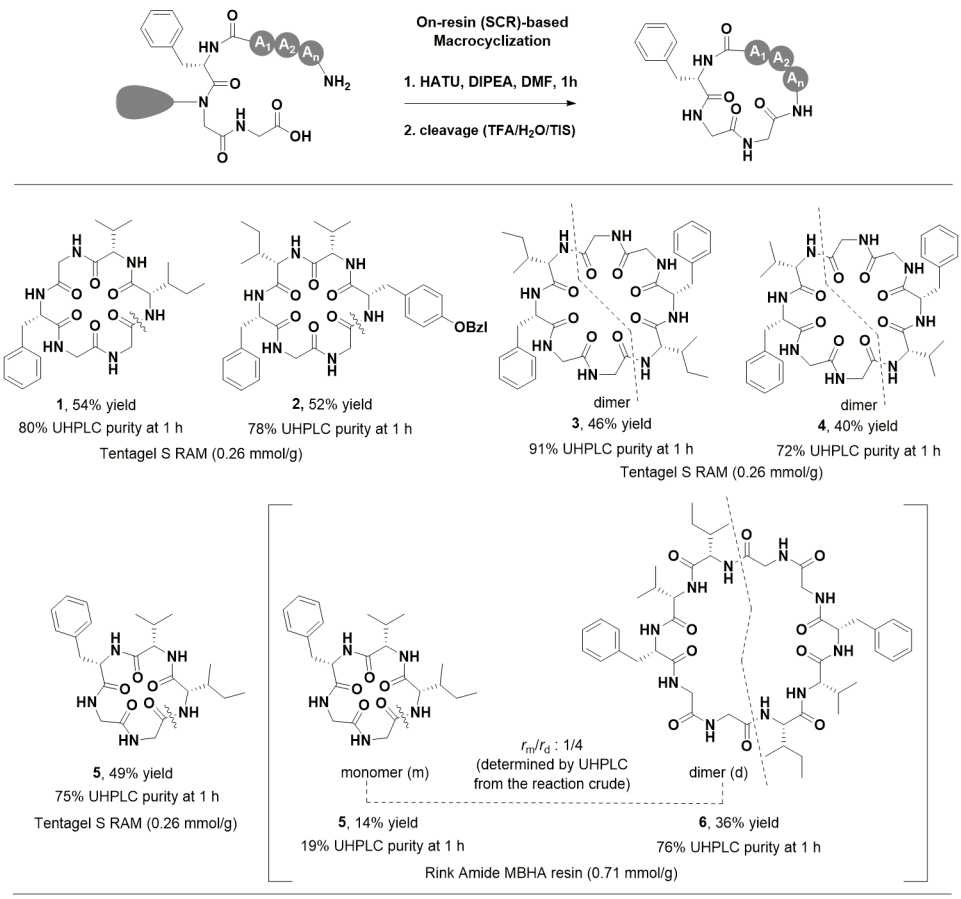
4.3 On-resin (SCR)-based macrocyclization assisted by the supported turn-inducer anchor *N*-substituted peptide

After the introduction of the bifunctional building block to the solid phase by an Ugi reaction, a typical Fmoc/tBu strategy is used for the peptide elongation in an automated synthetic process (see the **Experimental Part**). Finally, the Fmoc group is removed followed by the ethyl ester deprotection to provide unprotected termini (as in **Scheme 4.2**). As the tertiary amide generated during the Ugi reaction induces β-turn like characteristics,[9] it is expected that the solid support will act as a temporary loop-inducer, which bends the linear peptide structure (via Thorpe-Ingold or proline effect), approximate *C*- and *N*-terminus, facilitating the final macrocyclization (**Scheme 4.3**).

In order to verify the best reaction conditions for the on-resin cyclization, the synthesis of cyclic peptide **1** was taken as model, of four different coupling reagents: PyBOP, HBTU, HATU and oxyma/DIC; the first three gave complete conversion within 1 h. It was not possible to detect the intermediate open peptide after resin cleavage. The purity of cyclic peptide **1** in the corresponding reaction crudes was around 80% for all three coupling reagents as determined by UHPLC/ESI-MS analysis (see the **Experimental Part**). On the other hand, the use of oxyma/DIC was ineffective since after 1 h of reaction, the main product is the intermediate open peptide and the formation of the desired cyclic peptide was not detected. Based on these results, the use of HATU as coupling reagent and 1 h of reaction time were chosen for the standard conditions to perform on-resin macrocyclizations (**Scheme 4.3**).

As shown in **Scheme 4.3**, hexapeptides were efficiently cyclized under these conditions, providing the corresponding macrocycles **1** and **2** in good yield as well as high purity after cleavage from the resin, as determined by UHPLC analysis after 1 h of reaction (80% and 78% purity, respectively). However, even when a relatively low resin loading (0.26 mmol/g) was used to provide pseudo-dilution conditions, the cyclization of the tetrapeptides resulted in the selective formation of dimers (cyclic peptides **3** and **4**, **Scheme 4.3**). A detailed analysis of the reaction crudes using UHPLC/ESI-MS, shows

that the expected cyclic tetrapeptides were formed in a ratio lower than 1:5 respect their corresponding dimers.



Scheme 4.3 Solid-phase synthesis of cyclic peptides assisted by the supported turn-inducer mimic. Purities refer to crude after cleavage, yields refer to purified products.

Characterization of compounds **3** and **4** was carried out meticulously considering the structural symmetry of the dimers. NMR techniques may throw false results, because, the chemical environment of the atoms in both halves of the dimer is the same. It may result in signal overlapping by the matching of the chemical shift of the atoms with its related mirror image inside of the same molecule. The final result is that the NMR spectra of monomer and dimer are absolutely indistinguishable. The hint to solve this problematic comes with the use of MS/MS experiments. The fragmentation pattern in the MS^2 spectra of **3** and **4** acts as a finger print that allows for differentiating unequivocally the dimer from the monomer.

A good example to distinguish between monomer and dimer is highlighted with the associated structures **5** and **6** (**Scheme 4.3**). When a Tentagel S RAM resin with a loading of 0.26 mmol/g was used, only the cyclic pentapeptide (monomer) **5** was isolated in good overall yield. Nevertheless, the outcome in the attempt to cyclize the

same pentapeptide using Rink Amide MBHA resin with a higher loading (0.71 mmol/g), was a mixture of the monomeric cyclic pentapeptide (**5**) and its corresponding dimer (**6**) in a ratio 1:4 as examined by UHPLC. Surprisingly, the ^{1}H and ^{13}C-NMR spectra of both compounds are identical, sharing the same number of signals at the same chemical shifts (see **Experimental Part**). In order to provide a correct structural assignment for cyclic peptides **5** and **6**, MS/MS experiments were performed.

In **Figure 4.2 A** the MS^2 spectrum in the positive mode for the molecular ion of compound **5** is presented. For most cyclic peptides, fragmentation follows protonation of the peptide bond to open the ring and give an open-chain acylium ion or isomeric equivalent. In the case of compound **5** the fragmentation pattern has as root three (a-c, **Figure 4.2 A**) of the five theoretically possible molecular ions due to the ring opening caused by the collision induced dissociation. By the sequential assignment of the characteristic fragments obtained from the MS^2 experiment, it is clearly possible to determine that **5** corresponds to the cyclic pentapeptide.

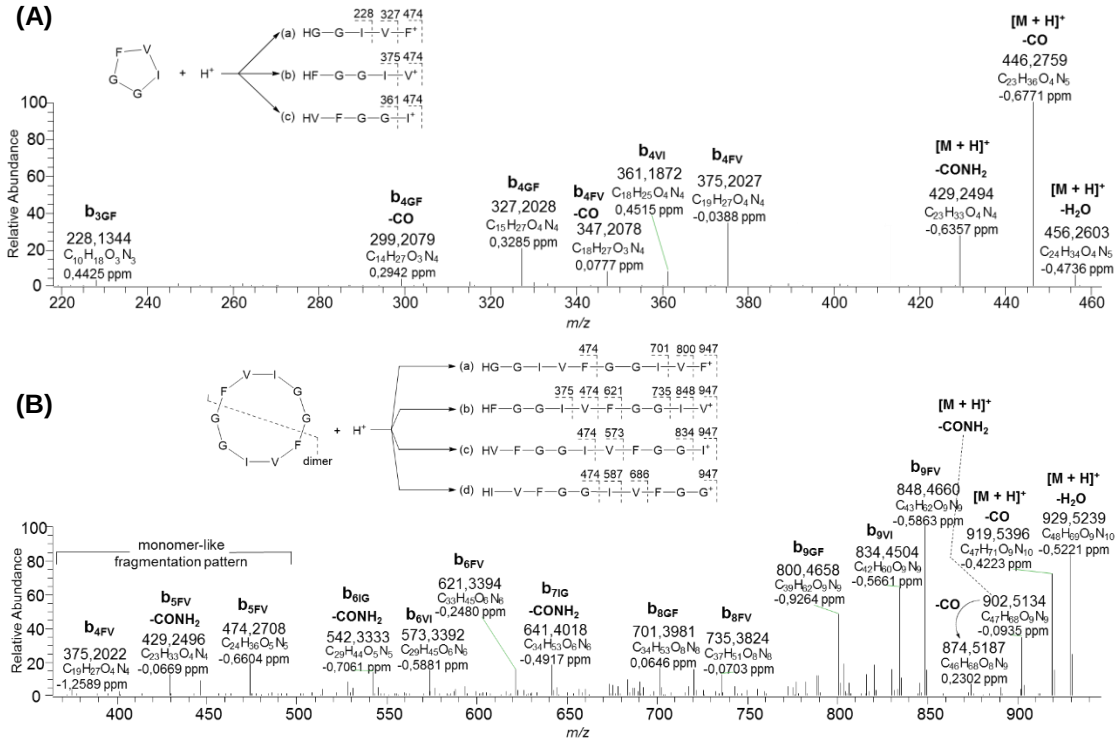


Figure 4.2 Positive ion HR-ESI-MS^2 spectrum of $[M+H]^+$ of: (A) cyclic peptide **5** (monomer). (B) cyclic peptide **6** (dimer).

Alternatively, analysis of the MS^2 spectrum shown in **Figure 4.2 B** demonstrated that compound **6** is, indeed, a dimer generated by the union of covalent bonds. The fragmentation of the molecular ion of **6**, triggered by the collision induced dissociation, shows higher relationship *m/z* values than the expected ones for the monomer,

confirming that the $[M+H]^+$ ion observed in the HR-MS spectrum it is not an adduct of the monomer created during ionization. By mapping the fragmentation pattern for the molecular ion (isomeric equivalent ions a-d, **Figure 4.2 B**) generated in the MS/MS experiment, it was established that the cyclic peptide **6** is the related dimer of the compound **5**. Additionally, the presence of typical fragments associated with the primary structure of the monomer confirms this hypothesis.

Originally, peptide cyclization on solid support aimed to avoid linear polymerization and cyclo-oligomerization by sufficient spatial distance on the support. Nevertheless, intersite reactions on resins have previously been reported, suggesting that the resin, at least definitely tentagel, is not rigid enough to preclude intersite contacts,[12, 13] e.g., intersite disulfide bond formation of resin-bound cysteine peptides[14], or attempted head-to-tail peptide cyclizations.[15] Indeed, examples of dimeric cyclic peptide formation have been described.[16] The extent of the reaction between adjacent sites in the solid support may be mediated by the degree of loading, and by factors which affect polymer mobility, such as temperature, the extent of cross-linking and the swelling capacity of the solvent used.[13] The specific conformation of the precursor structure of the resin-bound peptide also has an influence.

Thus, the antiparallel cyclic dimers **3**, **4** and **6** (**Scheme 4.3**) have been formed through an intersite reaction between two adjacent peptide chains on the resin. At least for the tetrapeptides, this outcome might be expected, considering an intersite reaction favored over the slow intramolecular cyclization to afford a small tensioned ring.

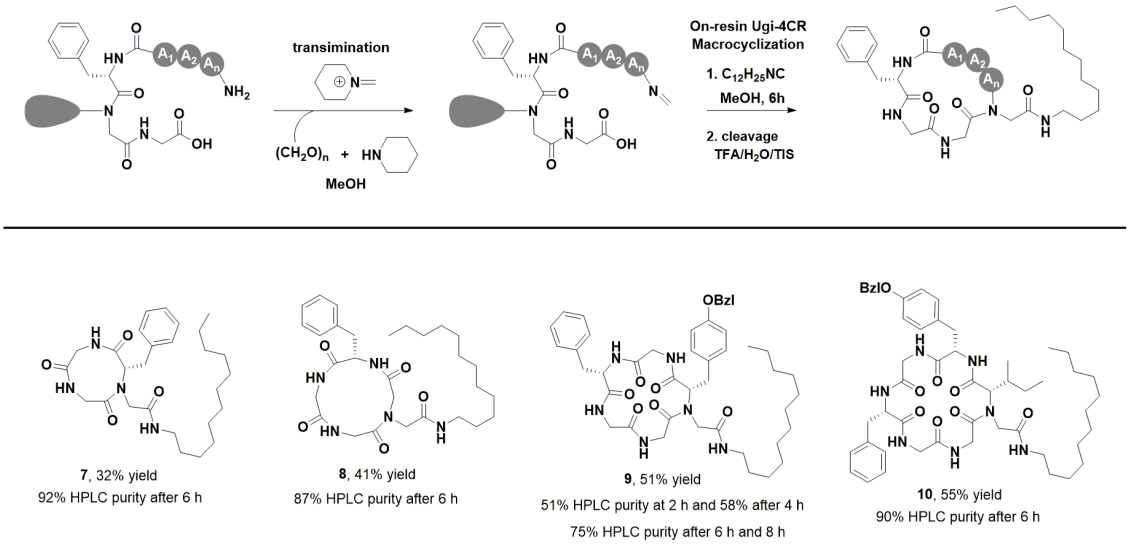
Since we know from previous work,[7] that cyclic tetrapeptides with a peptoid moiety can be formed, other resins and conditions not sought here might deliver the lower monomeric cyclopeptides. However, we know that the Ugi-4CR is much more useful to form such small cyclopeptides, and thus in the following we rather concentrated on this cyclization method.

4.4 On-resin IMCR-based macrocyclization assisted by the supported turn-inducer anchor *N*-substituted peptide

Looking for further applications of the supported loop-inducer in the synthesis of natural-like cyclic peptides, we took a glance in the cyclic lipopeptides (CLPs), a class of bacterial secondary metabolites.[17] CLPs are widely found in nature and present a broad scope of biological activities, such as antibiosis against bacteria, fungi, protozoa, nematodes and human tumor cell lines.[18,19] They also have potential as candidates for

the bio-control of plant pathogens, as well as in stimulating the host defense mechanism.[20-27] Interestingly, resistance is not commonly described for cyclic lipopeptides (CLPs).[28]

Despite the promising antimicrobial effect of cyclic lipopetides, the lack of versatile techniques to synthesize these compounds in reasonable amounts has hampered further studies. The main challenges in the synthesis of cyclic lipopeptides are the installation of the lipidic moiety and the macrocyclization of the linear peptide chain. In order to address these issues, we decided to assess the reliability of the supported turn-inducer mimic as synthetic tool to synthesize CPLs. The strategy is to employ the resin-immobilized loop mimic carrying on a suitable peptide sequence along with a lipidic isocyanide in a multicomponent reaction to 'trap' the lipidic moiety on the solid phase, thereby accomplishing the macrocyclization, lipidation and purification simultaneously in a single chemical operation as unreacted reagents can be washed out (**Scheme 4.4**). With the Ugi reaction not only cyclotetrapeptides form, even cyclotripeptides are possible in reasonable yield.



Scheme 4.4 Solid-phase synthesis of CLPs assisted by the supported turn-inducer mimic.

In order to assess the best reaction time to carry out the MCR-macrocyclization on solid support mediated by the loop inducer moiety, cyclic lipopeptide **9** was taken as model, quenching the chemical process after 2, 4, 6 and 8 h. The HPLC/ESI-MS analysis proved that cyclization yield was already high after 6 h. Once again, a relatively low resin loading (0.26 mmol/g) was used to provide pseudo-dilution conditions trying to minimize cyclodimerization processes. As shown in **Scheme 4.4**, all desired cyclic lipopeptides were already obtained in high to good purity, after cleavage from resin. Final purification was achieved by semi-preparative RP-HPLC.

Regarding the yield, the structural dependence to ring sizes to be formed is evident. However, it is a remarkable result that even for the extremely strained cyclic tripeptide, no formation of cyclodimer was detected in the reaction crudes by HPLC/ESI-MS analysis.[29] A plausible explanation for this improved performance of the Ugi vs. classical cyclization might be related to the intrinsic mechanistic features.[30] First, the Ugi-4CR may enable the ring closure of short peptides, as the initial cyclization produces an intermediate (i.e., the α-adduct) that is three-members larger than the final product. Thus, the α-adduct (12-membered ring) evolves to the final cyclic tripeptide (9-membered ring) by an intramolecular acylation (Mumm rearrangement) comprising a ring contraction. A second reason is that the cyclic peptides arising from Ugi-macrocyclizations have a *N*-substituted amide bond, which makes easier the access to the *S-cis* isomer ubiquitous in short cyclic peptides. Third, *N*- and *C*-terminus ion pairing (-NH_3^+ ^-O_2C-) as preorganizing feature is not disrupted by Ugi-4CR, other than in classical carboxylate activation by coupling reagents.

4.5 Conclusions

In summary, a new methodology to peptide head-to-tail cyclization in solid phase was successfully developed. The approach makes use of an Ugi-4CR to functionalize a commercial solid support endowing it with a bifunctional building block that acts as a loop-inducer mimic favoring the final on-resin cyclization. Two strategies were implemented in order to afford the ring closure.

In the first one, the cyclization is triggered by the classical coupling reagent HATU. This route is characterized by a fast reaction (in 1 h all the starting material is consumed) and shows high cyclization efficiency in the synthesis of a cyclic pentapeptide (macrocycle **5**, 75% purity), and cyclic hexapeptides (macrocycles **1** and **2**, 80% and 78% purity, respectively) and presumably also for longer peptide sequences. However, its ability to reach smaller peptide rings is limited. In the attempted synthesis of cyclic tetrapeptides, only the corresponding cyclodimers were obtained selectively. The loading of the used resin plays also an important role in the cyclization process, as it was exemplified in the synthesis of macrocycle **5**, where to increase the resin loading at to 0.71 mmol/g it results in a mixture of the cyclic pentapeptide (**5**) and its associated dimer (**6**) in a ratio of 1:4 as assessed by UHPLC.

On the other hand, the second strategy made use of an IMCR to perform the macrocyclization on-resin. It allows efficient ring closure and lipid moiety insertion in a single reaction step to produce cyclic lipopeptides. An outstanding result of this

cyclizing approach is the possibility to synthesize cyclic peptide scaffolds ranging from three to six (and likely more) residues of amino acids in good cyclization efficiency and without evasive prior oligomerization can be noted.

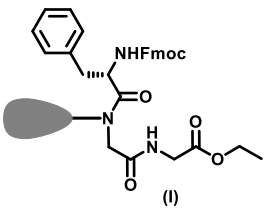
4.6 Experimental Part

General remarks

Solid-phase peptide synthesis was carried out with a ResPep SL peptide synthesizer (Intavis Bioanalytical Instruments, Germany). Fmoc amino acids, PyBOP and HATU were supplied from Novabiochem (Germany). TentaGel-S-RAM resin was purchased from Iris Biotech GmbH (Germany). Piperidine, acetic acid, formic acid, DIPEA, TFA and DMF were purchased from Sigma-Aldrich (Germany). All reagents and solvents were used as received, with the exception of DMF and DIPEA that were dried by distillation from CaH_2 under argon prior to use as reaction solvent, and DMF was stored over 4Å molecular sieves. HPLC analysis was performed with an Agilent 1100 Infinity system (binary pump, auto sampler, DAD; Agilent, USA) equipped with a reverse-phase (RP) C18 column (ODS-A, 4.6× 150 mm internal diameter, 120 Å, 5 *µ*m, YMC, USA). A linear gradient from 5% to 100% of solvent B in solvent A over 15 min at a flow rate of 0.8 mL/min was used (solvent A: 0.1% (*v*/*v*) formic acid (FA) in water, solvent B: 0.1% (*v*/*v*) FA in acetonitrile). Detection was accomplished at 210, 215 or 254 nm. Preparative RP18 HPLC was carried out with a Knauer system equipped with a WellChrom K-1001 pump and a WellChrom K-2501 UV detector using a preparative column (polymeric RP, 8 *µ*m, 300 Å, 250 x 9 mm internal diameter, VYDAC, USA). ^{1}H NMR and ^{13}C NMR spectra were recorded in solutions on a Varian Mercury 400 spectrometer at 400 MHz and 101 MHz, respectively. Chemical shifts (δ) are reported in ppm relative to TMS (^{1}H NMR) and to the solvent signal (^{13}C NMR). The positive- and negative-ion high-resolution ESI mass spectra were obtained with an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, Germany) equipped with a HESI electrospray ion source (positive spray voltage 4.5 kV, negative spray voltage 3.5 kV, capillary temperature 275 °C, source heater temperature 250 °C, FTMS resolution 30000). The MS system was coupled to an ultra-high performance liquid chromatography (UHPLC) system (Dionex UltiMate 3000, Thermo Fisher Scientific) equipped with a RP18 column (Hypersil GOLD, 50 x 2.1 mm internal diameter, 1.9 µm, 175 Å, Thermo Fisher Scientific, column temperature 30 °C) and a photodiode array detector (190-400 nm, Thermo Fisher Scientific). The mobile phases were H_2O (A: Fluka Analytical, LC–MS Chromasolv®) and CH_3CN (B: Fluka Analytical, LC–MS

Chromasolv®) with formic acid (0.2 %). A gradient system was used (0–15 min, 5–95% B; 15–18 min, 100% B; flow rate 0.150 mL/min). The instrument was externally calibrated using the Pierce LTQ Velos ESI positive ion calibration solution (product No. 88323) and the Pierce ESI negative ion calibration solution (product No. 88324) from Thermo Fisher Scientific. The data were evaluated using the software Xcalibur 2.2 SP1.

Synthesis of the supported turn-inducer mimic (I): TentaGel S RAM resin (1.92 g, loading 0.26 mmol/g resin) is treated with a 20% piperidine solution in DMF (*ca.* 5 mL) and swirled for 30 min in order to remove the Fmoc group attached to the resin. Then, the resin is washed successively with CH_2Cl_2, MeOH and THF prior to the Ugi reaction. A suspension of paraformaldehyde (2.0 mmol, 4 equiv.) and piperidine (2.0 mmol, 4 equiv.) in THF/MeOH (1:1, *v*/*v*, 4 mL) is added to the resin (0.5 mmol) and the reaction mixture is shaken for 30 minutes. The excess of reagent is removed by washing the beads with THF (4×2 mL). Ethyl isocyanoacetate (2.0 mmol, 4 equiv.) dissolved in MeOH (2 mL) and Fmoc-Phe-OH (2.0 mmol, 4 equiv.) dissolved in MeOH (2 mL) are added to the on-resin preformed imine and the mixture is swirled for 24 h. The resin is washed sequentially with DMF, THF and CH_2Cl_2.



(I)

The modified resin (**I**) is dried and used in the further solid phase peptide synthesis assuming full conversion of the Ugi reaction. Coupling reactions were carried out in an Automated Solid-Phase Peptide Synthesizer by stepwise Fmoc strategy in 0.04 mmol scale and the coupling cycle from the standard Intavis protocol was used (see **Attachment S33**).

General procedure for one-step Fmoc and ethyl ester deprotection: The turn-inducer containing peptide bound to the TentaGel S RAM resin (arising from the automated solid-phase peptide synthesizer) is washed successively with DMF, CH_2Cl_2 and DMF. The resin is treated with a 0.25 M solution of KOH in EtOH/THF (1:1) for 2 h. The solution is filtered and the excess of reagent is washed out with H_2O (2×2 mL), EtOH (2×2 mL) and CH_2Cl_2 (2×2 mL). Finally, the resin is soaked with an aqueous saturated solution of NaHSO4 during 15 minutes, washed with H_2O (2×2 mL), EtOH (2×2 mL), CH_2Cl_2 (2×2 mL) and dried.

General procedure for the solid-phase macrocyclization: The turn-inducer containing peptide (0.10 mmol) bound to the TentaGel S RAM resin (arising from the *N*- and *C*-termini deprotection, loading 0.26 mmol/g resin) is washed successively with DMF, CH_2Cl_2 and DMF. The resin is pre-swelled with DMF for 5 minutes. Then, HATU

(0.40 mmol, 4 equiv.) and DIPEA (1.20 mmol, 12 equiv.) dissolved in DMF (4 mL) are added to the resin and the mixture is swirled for 1 h. Afterwards the solid support is washed sequentially with DMF and CH_2Cl_2. The final resin bound cyclic peptides are cleaved from the resin with the cocktail TFA/TIPS/H_2O (95:2.5:2.5, *v*/*v*, 5 mL), precipitated from cold diethyl ether, centrifuged, washed twice with cold diethyl ether, lyophilized and purified by preparative RP-HPLC to provide the pure macrocycle product.

General procedure for the solid-phase Ugi-4CR macrocyclization: The turn-inducer containing peptide (0.10 mmol) bound to the TentaGel S RAM resin (arising from the *N*- and *C*-termini deprotection, maximal theoretical loading 0.26 mmol/g resin) is washed successively with CH_2Cl_2, MeOH and THF. The resin is pre-swelled with THF for 5 minutes. A suspension of paraformaldehyde (0.40 mmol, 4 equiv.) and piperidine (0.40 mmol, 4 equiv.) in THF/MeOH (1:1, *v*/*v*, 4 mL) is added to the resin-bound peptide (0.10 mmol) and the reaction mixture is shaken for 30 minutes. Excess of reagent is removed by washing the beads with THF (4×2 mL). The isocyanide (0.40 mmol, 4 equiv.) in MeOH (4 mL) is added to the imine preformed on-resin. The mixture is swirled for a period of time indicated by RP-HPLC monitoring after mini-cleavages. The resin is washed sequentially with DMF, THF and CH_2Cl_2. The final Ugi-cyclization products are cleaved from the resin with the cocktail TFA/TIPS/H_2O (95:2.5:2.5, *v*/*v*, 5 mL), precipitated from cold diethyl ether, centrifuged, washed twice with cold diethyl ether, lyophilized and purified by preparative RP-HPLC to provide the pure Ugi-4CR macrocycles.

Cyclic peptide 1: The resin-bound peptide Fmoc-Ile-Val-Gly-Phe-Gly-Gly-OEt (0.04 mmol) was subjected to one-step Fmoc/ethyl ester deprotection according to the general procedure described above. The resulting peptide was submitted to ring closure process by treatment with HATU (0.16 mmol, 4 equiv.) and DIPEA (0.48 mmol, 12 equiv.) in DMF (2 mL) according to the general procedure for the solid-phase macrocyclization. After cleavage from the resin, cyclic peptide **1** was obtained in 80% purity as determined by UHPLC (PDA range: 190-400 nm). Preparative RP-HPLC purification produced **1** (12 mg, 54%) in 99% purity, according to UHPLC (R_t = 12.48 min, PDA range: 190-400 nm). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.65 – 8.60 (m, 1H), 8.46 – 8.41 (m, 1H), 7.96 (d, *J* = 5.9 Hz, 1H), 7.89 (d, *J* = 7.6 Hz, 1H), 7.84 (d, *J* = 9.0 Hz, 1H), 7.26 (dd, *J* = 7.9, 6.5 Hz, 2H), 7.23 – 7.18 (m, 1H), 7.18 – 7.12 (m, 2H), 4.50 – 4.42 (m, 1H), 4.13 – 4.07 (m, 1H), 3.99 – 3.92 (m, 1H), 3.88 – 3.80 (m, 1H), 3.80 – 3.73 (m, 2H), 3.73 – 3.63 (m, 2H), 3.50 – 3.43 (m, 1H), 3.07 (dd, *J* = 14.0, 5.4 Hz, 1H), 2.93 (dd, *J* = 13.9, 7.8 Hz, 1H), 2.07 (hept, *J* = 6.3, 5.8 Hz, 1H), 1.80 – 1.71 (m,

1H), 1.36 – 1.26 (m, 1H), 1.05 – 0.92 (m, 1H), 0.88 (d, *J* = 6.8 Hz, 6H), 0.75 (t, *J* = 7.4 Hz, 3H), 0.70 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.6, 170.9, 170.8, 169.3, 168.9, 168.8, 137.2, 129.1, 128.1, 126.4, 59.6, 57.8, 54.3, 43.3, 42.5, 41.9, 37.1, 36.4, 29.5, 23.9, 19.4, 18.0, 15.4, 10.7. HRMS (ESI) *m/z*: 531.2922 $[M+H]^+$, calcd. for $C_{26}H_{39}O_6N_6$: 531.2926.

Cyclic peptide 2: The resin-bound peptide Fmoc-Tyr(Bzl)-Val-Ile-Phe-Gly-Gly-OEt (0.04 mmol) was subjected to one-step Fmoc/ethyl ester deprotection according to the general procedure above described. The resulting peptide was submitted to ring closure process by treatment with HATU (0.16 mmol, 4 equiv.) and DIPEA (0.48 mmol, 12 equiv.) in DMF (2 mL) according to the general procedure for the solid-phase macrocyclization. After cleavage from the resin, cyclic peptide **2** was obtained in 78% purity as determined by UHPLC (PDA range: 190-400 nm). Preparative RP-HPLC purification produced **2** (15 mg, 52%) in 99% purity, according to UHPLC (R_t = 15.56 min, PDA range: 190-400 nm). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.65 – 8.55 (m, 1H), 8.41 (d, *J* = 4.4 Hz, 1H), 8.19 – 8.07 (m, 1H), 7.76 (d, *J* = 9.2 Hz, 1H), 7.54 (d, *J* = 7.8 Hz, 1H), 7.45 – 7.35 (m, 4H), 7.34 – 7.25 (m, 3H), 7.25 – 7.18 (m, 3H), 7.13 (d, *J* = 8.4 Hz, 2H), 6.91 – 6.84 (m, 2H), 5.05 (s, 2H), 4.46 (td, *J* = 8.3, 7.2, 3.7 Hz, 1H), 4.24 (td, *J* = 7.3, 6.8, 4.1 Hz, 1H), 4.12 – 4.00 (m, 2H), 3.93 – 3.81 (m, 2H), 3.49 – 3.35 (m, 2H), 3.06 – 2.89 (m, 3H), 2.69 – 2.60 (m, 1H), 2.03 – 1.85 (m, 1H), 1.83 – 1.72 (m, 1H), 1.53 – 1.42 (m, 1H), 1.21 – 1.08 (m, 1H), 0.88 – 0.79 (m, 6H), 0.66 (d, *J* = 6.8 Hz, 3H), 0.56 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.8, 171.3, 170.9, 170.4, 168.7, 168.3, 156.9, 137.2, 130.0, 129.4, 128.9, 128.4, 128.2, 127.7, 127.5, 126.5, 114.4, 69.8, 69.1, 59.2, 58.9, 55.8, 42.5, 42.2, 37.2, 35.9, 35.5, 29.9, 24.6, 19.3, 18.2, 15.5, 10.6. HRMS (ESI) *m/z*: 727.3812 $[M+H]^+$, calcd. for $C_{40}H_{51}O_7N_6$: 727.3814.

Cyclic peptide 3: The resin-bound peptide Fmoc-Ile-Phe-Gly-Gly-OEt (0.04 mmol) was subjected to one-step Fmoc/ethyl ester deprotection according to the general procedure above described. The resulting peptide was submitted to ring closure process by treatment with HATU (0.16 mmol, 4 equiv.) and DIPEA (0.48 mmol, 12 equiv.) in DMF (2 mL) according to the general procedure for the solid-phase macrocyclization. After cleavage from the resin, cyclic peptide **3** was obtained in 91% purity as determined by UHPLC (PDA range: 190-400 nm). Preparative RP-HPLC purification produced **3** (14 mg, 46%) in 99% purity, according to UHPLC (R_t = 13.92 min, PDA range: 190-400 nm). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.41 (t, *J* = 5.7 Hz, 2H), 8.08 (d, *J* = 6.9 Hz, 2H), 7.85 (d, *J* = 7.8 Hz, 2H), 7.69 (s, 2H), 7.28 – 7.15 (m, 10H), 4.44 – 4.36 (m, 2H), 3.93 (d, *J* = 6.7 Hz, 1H), 3.91 – 3.84 (m, 4H), 3.82 (d, *J* = 4.6 Hz, 1H), 3.60 (dd, *J* = 15.7, 6.5 Hz, 2H), 3.53 (d, *J* = 5.9 Hz, 2H), 3.12 (dd, *J* = 14.0,

4.6 Hz, 2H), 2.96 (dd, *J* = 14.1, 10.5 Hz, 2H), 1.71 – 1.59 (m, 2H), 1.07 – 0.97 (m, 3H), 0.89 – 0.80 (m, 1H), 0.75 – 0.60 (m, 12H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.8, 170.9, 170.5, 170.3, 137.9, 128.9, 128.1, 126.2, 58.5, 54.8, 42.6, 42.2, 36.4, 35.4, 23.9, 15.2, 11.2. HRMS (ESI) *m/z*: 749.3976 $[M+H]^+$, calcd. for $C_{38}H_{53}O_8N_8$: 749.3981.

Cyclic peptide 4: The resin-bound peptide Fmoc-Val-Phe-Gly-Gly-OEt (0.04 mmol) was subjected to one-step Fmoc/ethyl ester deprotection according to the general procedure above described. The resulting peptide was submitted to ring closure process by treatment with HATU (0.16 mmol, 4 equiv.) and DIPEA (0.48 mmol, 12 equiv.) in DMF (2 mL) according to the general procedure for the solid-phase macrocyclization. After cleavage from the resin, cyclic peptide **4** was obtained in 72% purity as determined by UHPLC (PDA range: 190-400 nm). Preparative RP-HPLC purification produced **4** (12 mg, 40%) in 97% purity, according to UHPLC (R_t = 13.22 min, PDA range: 190-400 nm). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.40 (t, *J* = 5.7 Hz, 2H), 8.00 (d, *J* = 7.1 Hz, 2H), 7.89 (d, *J* = 7.5 Hz, 2H), 7.83 – 7.77 (m, 2H), 7.28 – 7.16 (m, 10H), 4.44 – 4.36 (m, 2H), 3.92 (d, *J* = 6.7 Hz, 1H), 3.89 – 3.82 (m, 4H), 3.80 (d, *J* = 4.7 Hz, 1H), 3.67 (d, *J* = 6.7 Hz, 1H), 3.63 (d, *J* = 6.5 Hz, 1H), 3.48 – 3.43 (m, 2H), 3.10 (dd, *J* = 14.0, 4.8 Hz, 2H), 2.97 (dd, *J* = 14.1, 10.1 Hz, 2H), 1.92 (h, *J* = 6.8 Hz, 2H), 0.72 (d, *J* = 6.8 Hz, 6H), 0.65 (d, *J* = 6.8 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.8, 171.1, 170.4, 170.1, 137.8, 128.9, 128.2, 126.3, 59.3, 57.9, 54.8, 42.5, 42.3, 36.4, 29.1, 18.9, 17.6. HRMS (ESI) *m/z*: 721.3666 $[M+H]^+$, calcd. for $C_{36}H_{49}O_8N_8$: 721.3668.

Cyclic peptide 5: The resin-bound peptide Fmoc-Ile-Val-Phe-Gly-Gly-OEt (0.04 mmol) was subjected to one-step Fmoc/ethyl ester deprotection according to the general procedure above described. The resulting peptide was submitted to ring closure process by treatment with HATU (0.16 mmol, 4 equiv.) and DIPEA (0.48 mmol, 12 equiv.) in DMF (2 mL) according to the general procedure for the solid-phase macrocyclization. After cleavage from the resin, cyclic peptide **5** was obtained in 75% purity as determined by UHPLC (PDA range: 190-400 nm). Preparative RP-HPLC purification produced **5** (9 mg, 49%) in 99% purity, according to UHPLC (R_t = 13.14 min, PDA range: 190-400 nm). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (t, *J* = 5.5 Hz, 1H), 8.52 (d, *J* = 7.6 Hz, 1H), 8.24 (d, *J* = 8.9 Hz, 1H), 8.05 (t, *J* = 6.0 Hz, 1H), 7.68 (d, *J* = 9.0 Hz, 1H), 7.29 – 7.21 (m, 2H), 7.19 – 7.11 (m, 3H), 4.57 – 4.48 (m, 1H), 4.47 – 4.38 (m, 2H), 3.74 – 3.61 (m, 3H), 3.35 (d, *J* = 6.1 Hz, 1H), 2.97 (dd, *J* = 13.4, 8.3 Hz, 1H), 2.80 (dd, *J* = 13.4, 6.5 Hz, 1H), 1.95 – 1.81 (m, 2H), 1.54 – 1.43 (m, 1H), 1.19 – 1.07 (m, 1H), 0.84 – 0.73 (m, 12H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.5, 171.1, 170.7, 169.0, 168.2, 137.4, 128.9, 128.2, 126.3, 57.7, 56.2, 54.2, 43.6, 42.2, 37.6, 36.8,

30.2, 24.3, 19.1, 18.5, 15.4, 11.0. HRMS (ESI) *m/z*: 474.2708 $[M+H]^+$, calcd. for $C_{24}H_{36}O_5N_5$: 474.2711.

Cyclic peptide 6: The resin-bound peptide Fmoc-Ile-Val-Phe-Gly-Gly-OEt (0.04 mmol) was subjected to one-step Fmoc/ethyl ester deprotection according to the general procedure above described. The resulting peptide was submitted to ring closure process by treatment with HATU (0.16 mmol, 4 equiv.) and DIPEA (0.48 mmol, 12 equiv.) in DMF (2 mL) according to the general procedure for the solid-phase macrocyclization. After cleavage from the resin, cyclic peptide **6** was obtained in 76% purity as determined by UHPLC (PDA range: 190-400 nm). Preparative RP-HPLC purification produced **6** (14 mg, 36%) in 99% purity, according to UHPLC (R_t = 14.17 min, PDA range: 190-400 nm). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (t, *J* = 5.5 Hz, 2H), 8.52 (d, *J* = 7.6 Hz, 2H), 8.24 (d, *J* = 8.9 Hz, 2H), 8.05 (t, *J* = 6.0 Hz, 2H), 7.68 (d, *J* = 9.0 Hz, 2H), 7.29 – 7.21 (m, 4H), 7.19 – 7.11 (m, 6H), 4.57 – 4.48 (m, 2H), 4.47 – 4.38 (m, 4H), 3.74 – 3.61 (m, 6H), 3.35 (d, *J* = 6.1 Hz, 2H), 2.97 (dd, *J* = 13.4, 8.3 Hz, 2H), 2.80 (dd, *J* = 13.4, 6.5 Hz, 2H), 1.95 – 1.81 (m, 4H), 1.54 – 1.43 (m, 2H), 1.19 – 1.07 (m, 2H), 0.84 – 0.73 (m, 24H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.5, 171.1, 170.7, 169.0, 168.2, 137.4, 128.9, 128.2, 126.3, 57.7, 56.2, 54.2, 43.6, 42.2, 37.6, 36.8, 30.2, 24.3, 19.1, 18.5, 15.4, 11.0. HRMS (ESI) *m/z*: 947.5345 $[M+H]^+$, calcd. for $C_{48}H_{71}O_{10}N_{10}$: 947.5349.

Cyclic peptide 7: The resin-bound peptide Fmoc-Phe-Gly-Gly-OEt (0.04 mmol) was subjected to one-step Fmoc/ethyl ester deprotection according to the general procedure above described. The resulting peptide was submitted to transimination by treatment with paraformaldehyde (0.16 mmol, 4 equiv.) and piperidine (0.16 mmol) in THF/MeOH (1:1, *v*/*v*, 2 mL), and next reacted for 6 h with *n*-dodecylisocyanide (0.40 mmol) according to the general procedure for the solid-phase Ugi-4CR macrocyclization. After cleavage from the resin, cyclic peptide **7** was obtained in 92% purity as determined by RP-HPLC (215 nm). Preparative RP-HPLC purification produced **7** (6 mg, 32%) in 98% purity, according to RP-HPLC (R_t = 12.80 min, 215 nm). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.95 (t, *J* = 5.9 Hz, 1H), 7.65 (m, 1H), 7.60 (t, *J* = 5.8 Hz, 1H), 7.31 – 7.10 (m, 5H), 5.35 – 5.30 (m, 1H), 3.96 – 3.94 (m, 1H), 3.76 – 3.72 (m, 1H), 3.71 – 3.64 (m, 2H), 3.64 – 3.56 (m, 2H), 3.10 – 3.02 (m, 1H), 2.92 – 2.89 (m, 1H), 2.04 – 1.95 (m, 2H), 1.53 – 1.14 (m, 20H), 0.88 – 0.83 (m, 3H). ^{13}C NMR (151 MHz, DMSO-d_6, assigned through gHSQCAD) δ 129.7, 127.9, 127.5, 125.8, 57.5, 44.1, 42.0, 41.9, 38.5, 35.0, 31.2, 29.0, 28.9, 28.8, 26.3, 24.9, 21.9, 13.8. HRMS (ESI) *m/z*: 485.3130 $[M-H]^-$, calcd. for $C_{27}H_{41}O_4N_4$: 485.3133.

Cyclic peptide 8: The resin-bound peptide Fmoc-Gly-Phe-Gly-Gly-OEt (0.04 mmol) was subjected to one-step Fmoc/ethyl ester deprotection according to the general procedure above described. The resulting peptide was submitted to imination by treatment with paraformaldehyde (0.16 mmol, 4 equiv.) and piperidine (0.16 mmol) in THF/MeOH (1:1, *v*/*v*, 2 mL), and next reacted for 6 h with *n*-dodecylisocyanide (0.40 mmol) according to the general procedure for the solid-phase Ugi-4CR macrocyclization. After cleavage from the resin, cyclic peptide **8** was obtained in 87% purity as determined by RP-HPLC (215 nm). Preparative RP-HPLC purification produced **8** (9 mg, 41%) in 98% purity, according to RP-HPLC (R_t = 15.74 min, 215 nm). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.37 – 8.18 (m, 1H), 8.14 – 8.04 (m, 1H), 8.02 – 7.94 (m, 1H), 7.73 – 7.52 (m, 1H), 7.36 – 6.90 (m, 5H), 5.39 – 5.28 (m, 1H), 4.12 – 3.99 (m, 1H), 3.98 – 3.80 (m, 2H), 3.79 – 3.66 (m, 2H), 3.65 – 3.57 (m, 1H), 3.51 (s, 2H), 3.11 – 3.05 (m, 1H), 3.02 – 2.96 (m, 1H), 2.09 – 1.88 (m, 2H), 1.50 – 1.17 (m, 20H), 0.87 – 0.82 (m, 3H). ^{13}C NMR (151 MHz, DMSO-d_6, assigned through gHSQCAD) δ 128.8, 127.5, 127.4, 125.7, 125.5, 56.6, 50.8, 44.5, 41.6, 38.4, 38.0, 34.6, 30.8, 28.6, 28.5, 25.9, 24.6, 21.6, 13.5. HRMS (ESI) *m/z*: 542.3346 $[M-H]^-$, calcd. for $C_{29}H_{44}O_5N_5$: 542.3348.

Cyclic peptide 9: The resin-bound peptide Fmoc-Tyr(Bzl)-Gly-Phe-Gly-Gly-OEt (0.04 mmol) was subjected to one-step Fmoc/ethyl ester deprotection according to the general procedure above described. The resulting peptide was submitted to transimination by treatment with paraformaldehyde (0.16 mmol, 4 equiv.) and piperidine (0.16 mmol) in THF/MeOH (1:1, *v*/*v*, 2 mL), and next reacted for 6 h with *n*-dodecylisocyanide (0.40 mmol) according to the general procedure for the solid-phase Ugi-4CR macrocyclization. After cleavage from the resin, cyclic peptide **9** was obtained in 75% purity as determined by RP-HPLC (215 nm). Preparative RP-HPLC purification produced **9** (16 mg, 51%) in 98% purity, according to RP-HPLC (R_t = 16.19 min, 215 nm). In a parallel experiment under the same conditions, mini-cleavages and RP-HPLC analysis at 2 h, 4 h, 6 h and 8 h were made to assess the best reaction time. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.70 – 8.47 (m, 1H), 8.37 – 8.15 (m, 1H), 8.12 – 8.05 (m, 1H), 8.03 – 7.90 (m, 1H), 7.83 – 7.59 (m, 1H), 7.48 – 7.11 (m, 10H), 7.11 – 6.91 (m, 2H), 6.89 – 6.76 (m, 2H), 5.03 (s, 2H), 4.57 – 4.23 (m, 2H), 4.21 – 3.98 (m, 2H), 3.98 – 3.82 (m, 2H), 3.80 – 3.65 (m, 2H), 3.64 – 3.54 (m, 1H), 3.53 – 3.48 (m, 1H), 3.12 – 2.97 (m, 2H), 2.94 – 2.72 (m, 2H), 2.69 – 2.60 (m, 1H), 2.03 – 1.95 (m, 1H), 1.40 – 1.16 (m, 20H), 0.88 – 0.80 (m, 3H). ^{13}C NMR (151 MHz, DMSO-d_6, assigned through gHSQCAD) δ 129.6, 129.5, 129.4, 128.6, 127.9, 127.6, 127.2, 127.1, 125.7, 113.9,

69.4, 53.8, 53.6, 50.9, 41.9, 41.7, 38.2, 36.3, 35.3, 34.7, 30.8, 28.5, 28.4, 25.9, 24.7, 21.6, 13.5. HRMS (ESI) *m/z*: 795.4447 $[M-H]^-$, calcd. for $C_{45}H_{59}O_7N_6$: 795.4451.

Cyclic peptide 10: The resin-bound peptide Fmoc-Ile-Tyr(Bzl)-Gly-Phe-Gly-Gly-OEt (0.04 mmol) was subjected to one-step Fmoc/ethyl ester deprotection according to the general procedure above described. The resulting peptide was submitted to transimination by treatment with paraformaldehyde (0.16 mmol, 4 equiv.) and piperidine (0.16 mmol) in THF/MeOH (1:1, *v*/*v*, 2 mL), and next reacted for 6 h with *n*-dodecylisocyanide (0.40 mmol) according to the general procedure for the solid-phase Ugi-4CR macrocyclization. After cleavage from the resin, cyclic peptide 10 was obtained in 90% purity as determined by RP-HPLC (215 nm). Preparative RP-HPLC purification produced 10 (20 mg, 55%) in 98% purity, according to RP-HPLC (R_t = 18.39 min, 215 nm). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.63 (t, *J* = 5.7 Hz, 1H), 8.54 – 8.38 (m, 1H), 8.36 – 8.27 (m, 1H), 8.17 – 8.00 (m, 1H), 8.00 – 7.88 (m, 1H), 7.45 – 7.33 (m, 4H), 7.32 – 7.17 (m, 6H), 7.16 – 7.01 (m, 2H), 6.91 – 6.80 (m, 2H), 5.03 (s, 2H), 4.46 – 4.30 (m, 2H), 4.28 – 3.97 (m, 4H), 3.96 – 3.85 (m, 1H), 3.84 – 3.72 (m, 1H), 3.70 – 3.52 (m, 2H), 3.52 – 3.34 (m, 1H), 3.28 – 3.15 (m, 1H), 3.13 – 3.02 (m, 2H), 2.99 – 2.87 (m, 2H), 2.74 – 2.54 (m, 1H), 2.11 – 1.85 (m, 1H), 1.46 – 1.35 (m, 2H), 1.27 – 1.17 (m, 20H), 0.88 – 0.71 (m, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.2, 171.0, 170.2, 170.1, 169.3, 168.9, 168.8, 160.8, 156.9, 156.8, 137.1, 137.0, 130.0, 128.9, 128.4, 128.2, 127.7, 127.5, 126.5, 114.3, 69.8, 69.1, 65.1, 56.2, 55.7, 54.7, 54.0, 43.5, 40.7, 38.4, 37.0, 35.7, 31.3, 29.03, 29.01, 28.9, 28.9, 28.9, 28.8, 28.8, 28.7, 28.6, 26.3, 22.1, 15.4, 13.9, 11.7. HRMS (ESI) *m/z*: 908.5287 $[M-H]^-$, calcd. for $C_{51}H_{70}O_8N_7$: 908.5291.

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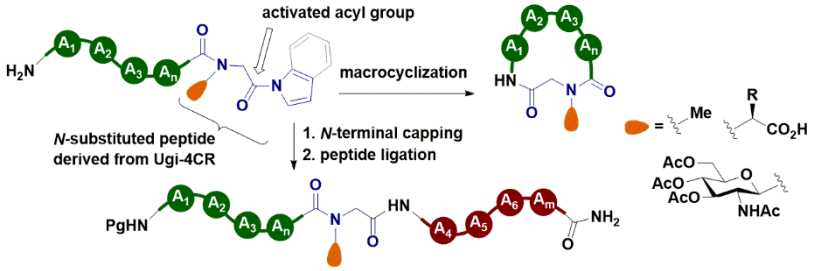
Chapter 5

Applications of Convertible Isonitriles in the Ligation and Macrocyclization of Multicomponent Reaction-Derived Peptides and Depsipeptides

*Abstract**



Peptide ligation and macrocyclization are among the most relevant approaches in the field of peptide chemistry. Whereas a variety of strategies relying on coupling reagents and native chemical ligation are available, there is a continuous need for efficient peptide ligation and cyclization methods. Herein we report on the utilization of convertible isonitriles as effective synthetic tools for the ligation and macrocyclization of peptides arising from isocyanide-based multicomponent reaction. The strategy relies on the use of convertible isonitriles – derived from Fukuyama amines – and peptide carboxylic acids in Ugi and Passerini reactions to afford *N*-alkylated peptides and depsipeptides, respectively, followed by conversion of the *C*-terminal amide onto either *N*-peptidoacyl indoles or pyrroles. Such activated peptides proved efficiency in the ligation to peptidic, lipidic and fluorescent-labeled amines and in macrocyclization protocols. As a result, a wide set of *N*-substituted peptides (with methyl, glycosyl and amino acids as *N*-substituents), cyclic *N*-methylated peptides and a depsipeptide were produced in good yields upon conditions involving either classical heating or microwave irradiation. This report improves the repertoire of peptide covalent modification methods by exploiting the synthetic potential of multicomponent reactions and convertible isonitriles.

* This Chapter was published: Wessjohann, L. A.; Morejón, M. C.**; Ojeda, G. M.; Rhoden, C. R. B.; Rivera, D. G. *J. Org. Chem.* **2016**, *81*, 6535–6545.

** Own contribution: Applications of IPB in the ligation and macrocyclization of MCR-derived peptides.

5.1 Introduction

Isonitrile-based multicomponent reactions (IMCRs) have proven to be powerful tools for the synthesis and derivatization of peptides and peptidomimetics.[1] Among the IMCRs, the Ugi four-component reaction[2] (Ugi-4CR) has the greatest applicability in this field,[1] not only because it utilizes amino and carboxylic groups but also due to its high chemical efficiency and diversity-generating character. A common application of the Ugi-4CR has been the ligation of – at least – two amino acids or peptide fragments to assemble *N*-substituted oligopeptidic skeletons. This Ugi-ligation strategy has enabled the preparation of naturally occurring peptides[3] as well as synthetic ones of medicinal,[4] catalytic[5] and biomimetic[6] importance. Another emerging application of this type of IMCR is its utilization in the synthesis of cyclic peptides[7] and peptidomimetics,[8] by means of approaches using the multicomponent process for assembling the acyclic scaffold, for the ring closure, or both.[9]

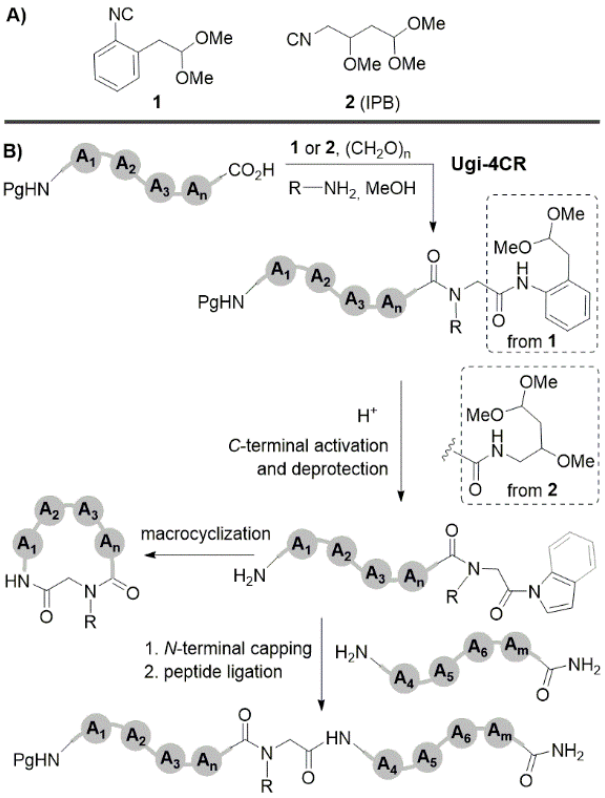
In peptide and medicinal chemistry, a strategy comprising an Ugi-4CR followed by either an efficient macrolactamization or peptide ligation can be a useful tool for the construction of *N*-functionalized (e.g., methyl, dye-labeled) (cyclo)peptides. However, a drawback limiting the implementation of this strategy is the poor reactivity in acylation processes of the *C*-terminal secondary amide. A solution for this can be found in the development of convertible isonitriles,[10,11] reagents that upon participation in the Ugi-4CR – and eventual activation – generate reactive amides suitable for follow-up derivatization. The utilization of these reagents has enabled effective applications of IMCRs in the synthesis of naturally occurring compounds and analogues.[3b,12]

The derivatizations reported so far for convertible isonitrile-derived amides include hydrolysis, methanolysis, intramolecular acylations to five and six-membered lactones and lactams, as well as acylation of aliphatic amines.[10,12] However, to our knowledge, applications in crucial approaches of peptide chemistry such as ligation of two peptide fragments and macrolactamization have remained elusive so far.

5.2 Synthetic Plan

Herein we report on the utilization of convertible isonitriles for the derivatization of peptides by IMCRs and their subsequent activation to enable either ligation to a second peptide or macrolactamization under dilution conditions. Of course, such a ligation is not limited to inter and intramolecular couplings, but can be extended to attaching lipids, labels and glycosidic moieties. To develop this strategy, we focused on the utilization of the structurally related and versatile convertible isocyanides **1** and **2**

(**Scheme 5.1 A**). 1-Isocyano-2-(2,2-dimethoxyethyl)benzene (**1**) was developed independently by the groups of Wessjohann[10e] and Kobayashi[10f] in 2007, while 4-isocyanopermethylbutane-1,1,3-triol (IPB, **2**) was reported by Wessjohann and co-workers in 2012.[10a] Both isonitriles show excellent reactivity in IMCRs and are available in multigram scale from amines previously introduced by Fukuyama *et. al.*[13]



Scheme 5.1 Strategy for the utilization of convertible isonitriles **1** and **2** in peptide ligation and macrocyclization.

As depicted in **Scheme 5.1**, the approach devised to exploit the potential of convertible isonitriles on peptide chemistry comprises their utilization in the Ugi-4CR by reaction with a peptidic carboxylic acid, a primary amine and an oxo-compound to produce a larger peptide incorporating an *N*-alkylated amino acid, in this case preferentially Gly by the use of paraformaldehyde. The internal *N*-alkylation of peptides, and specially the *N*-methylation,[14] has proven to be a successful way to improve pharmacological properties such as metabolic stability, membrane permeability and pharmacokinetics, as compared with their non-*N*-alkylated congeners.[14] As mentioned before, the reactivity of the *C*-terminal secondary amides derived from other convertible isocyanides has enabled their conversion to terminal carboxylic acids and esters, but not their utilization either in the direct ligation to another peptide fragment or in macrolactamization processes. On the other hand, the activation mode of terminal

amides derived from isonitriles **1** and **2** comprises the conversion – upon mild acidic treatment – to *N*-acyl indoles and pyrroles,[13] respectively. Both types of activated acyl groups are known to react readily with primary and secondary amines,[10a,e,13] thus paving the way for the development of both peptide ligation and intramolecular acylation of a peptidic amine, i.e., *N*-terminus or side chain.

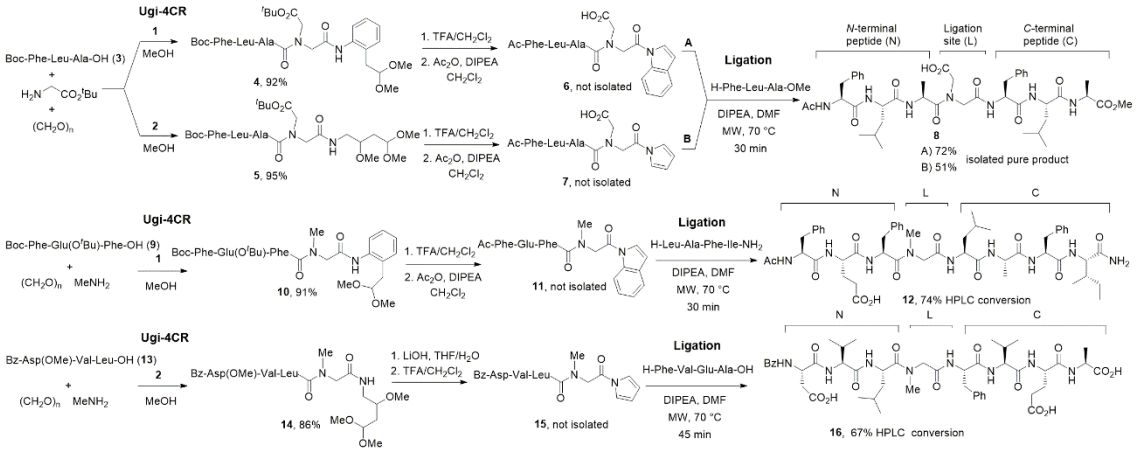
As shown in **Scheme 5.1 B**, our strategy encompasses the implementation of the Ugi-4CR with Boc-protected peptides as the carboxylic acid component and either isonitrile **1** or **2**, thus enabling the simultaneous activation of the *C*-terminus – by formation of either *N*-peptidoacyl indole or pyrrole – and the deprotection of the *N*-terminal residue upon mild acidic treatment (also known as UDAC, Ugi-deprotection-activation-cyclization/condensation). Consequently, head-to-tail macrocyclization can be straightforward by setting up dilution conditions typically required to cyclize oligopeptides, while ligation to another peptide fragment ideally requires previous capping of the *N*-terminus, e.g., by acetylation or a *N*-terminal protection not cleaved upon activation. Thus, the Fmoc protecting group may be installed at the *N*-terminus, while Lys side chains could be orthogonally protected with e.g., Cbz, if concomitant deprotection is not desired during acid-mediated *C*-terminal activation.

5.3 IMCR strategy towards peptide-peptide ligation of Ugi-modified peptides via *N*-peptidoacyl indoles and pyrroles

To prove the scope of this strategy, we decided to implement a ligation process wherein peptides having various carboxylic groups could be selectively ligated by the one taking part in the Ugi-4CR with a convertible isonitrile and being subsequently activated. Peptide synthesis was carried out either in solution using the Boc tactic or by a stepwise solid-phase Fmoc strategy on the Am-MBHA resin.[15] Oligopeptides used as substrates of the IMCRs and of the ligation processes are either known compounds reported by our group or were prepared as described in the **Experimental Part**.

As depicted in **Scheme 5.2**, we initially carry out a comparison of the ligation efficiency of an *N*-acyl indole and *N*-acyl pyrrole having identical peptide sequences and reacting them with the same nucleophilic aminopeptide. For this, tripeptide **3** was reacted in parallel with glycine *t*-butyl ester, paraformaldehyde and isonitriles **1** and **2** to furnish the branched *N*-substituted peptides **4** and **5**, respectively, in excellent yield after 24 h. Both intermediates were subjected to *C*-terminal activation and simultaneous Boc and *t*-butyl ester removal by treatment with 20% TFA in CH_2Cl_2, followed by acetylation of the *N*-terminus to render *N*-peptidoacyl indole **6** and pyrrole **7**, which were used without

further purification. A variety of protocols were studied to assess the best conditions to ligate the *C*-activated peptides to the model tripeptide Phe-Leu-Ala-OMe. Tested conditions include stirring a solution of both peptides in either DMF or THF and either at room temperature, 50 °C or 70 °C during several hours. The best conversion was found with DMF as solvent at 70 °C for 24 h. A solution to shorten the ligation time was the use of microwave irradiation at 70 °C for 30 min, which proved effective in the ligation of the two peptide fragments similar to the traditional heating during 24 h. Several parallel experiments demonstrated that peptides functionalized at the *C*-terminus as *N*-acyl indole are more activated than those having the *N*-acyl pyrrole. This was proved in the synthesis of peptide **8**, which was obtained as isolated pure product in 72% yield from **6** and only in 51% from **7**. HPLC monitoring of routes A and B shown in **Scheme 5.2** confirmed higher conversion into peptide **8** in the ligation process based on intermediate **6** than that involving **7** (see the **Attachments**).



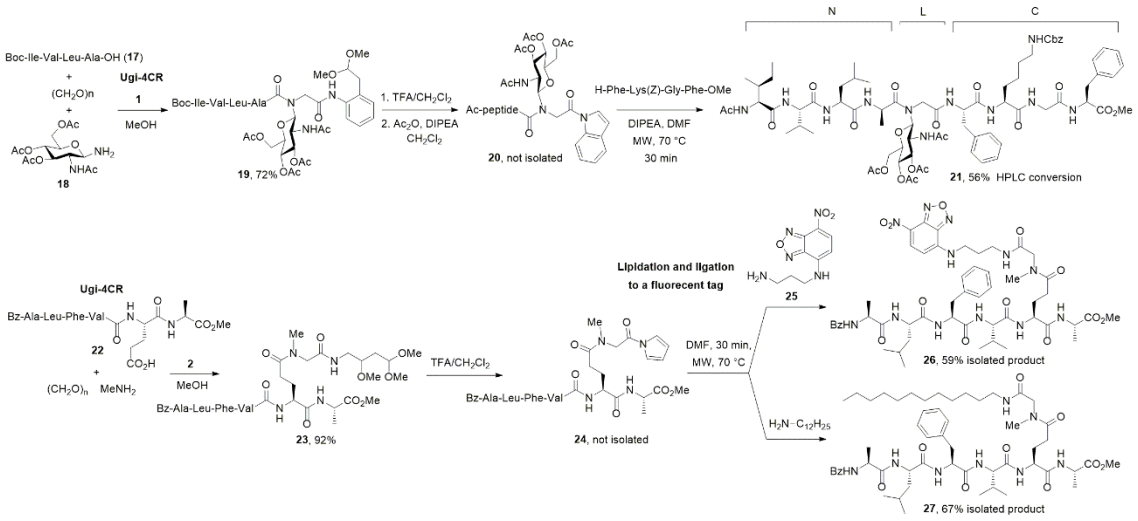
Scheme 5.2 Ligation of Ugi-modified peptides via *N*-peptidoacyl indoles and pyrroles.

In an endeavour to expand the scope of the peptide ligation, we turned to ligate peptides having unprotected Glu and Asp side chains. Peptide **9** was reacted with isonitrile **1**, methylamine and paraformaldehyde in the Ugi-4CR to produce *N*-methylated peptide **10** in excellent yield. The *C*-terminal activation/deprotection procedure followed by *N*-terminal capping rendered *N*-peptidoacyl indole **11** bearing an unprotected Glu side chain and activated *C*-terminus. As before, the MW-assisted ligation process proved success in the conjugation to tetrapeptide Leu-Ala-Phe-Ile-NH_2 yielding the *N*-methylated octapeptide **12** with 74% of conversion after 30 min, as indicated by analytical RP-HPLC analysis. As shown in **Scheme 5.2**, a third example of ligation was implemented with an *N*-peptidoacyl pyrrole as intermediate. For this, tripeptide **13** was combined with isonitrile **2** to render the *N*-methylated peptide **14**, which was subjected to deprotection of the Asp side chain by saponification followed by

C-terminal activation upon acidic treatment. Final ligation to tetrapeptide Phe-Val-Glu-Ala-OH was also undertaken under MW irradiation, albeit it required longer reaction time to achieve peptide **16** with a 67% conversion. With the last two examples, we demonstrated the success of the ligation of unprotected *N*-peptidoacyl indoles and pyrroles to aminopeptides bearing *C*-terminal methyl esters, carboxamides and free carboxylic groups. Finally, since peptide **13** was initially protected at the *N*-terminus with an acid stable benzoyl group, *N*-terminal capping between the Ugi-4CR and the *C*-terminal activation can be avoided, saving this extra step altogether.

5.4 Ligation strategy to glycosylated, lipidated and fluorescently tagged peptides

We next turned to assess the scope of the ligation process to produce glyco-, lipo- and labelled peptides. As depicted in **Scheme 5.3**, an initial strategy relied on employing glycosyl amine **18** – derived from the biologically relevant *N*-acetyl glucosamine – in the Ugi-4CR with peptide **17** and isonitrile **1** to afford *N*-glycosylated peptide **19** in good yield. After acidic treatment to enable the *C*-terminal activation and Boc removal, followed by *N*-terminal acetylation to yield *N*-acyl indole **20**, this latter intermediate was ligated to tetrapeptide H-Phe-Lys(Cbz)-Gly-Phe-OMe to afford the remarkably complex *N*-glycosylated nonapeptide **21**. HPLC monitoring of this reaction showed a moderate conversion of 56%, while a substantial amount of intermediate **20** remained unreacted after 45 min under MW irradiation. However, a longer reaction time was not considered, as decomposition of both compounds **20** and **21** becomes competitive after 1h.



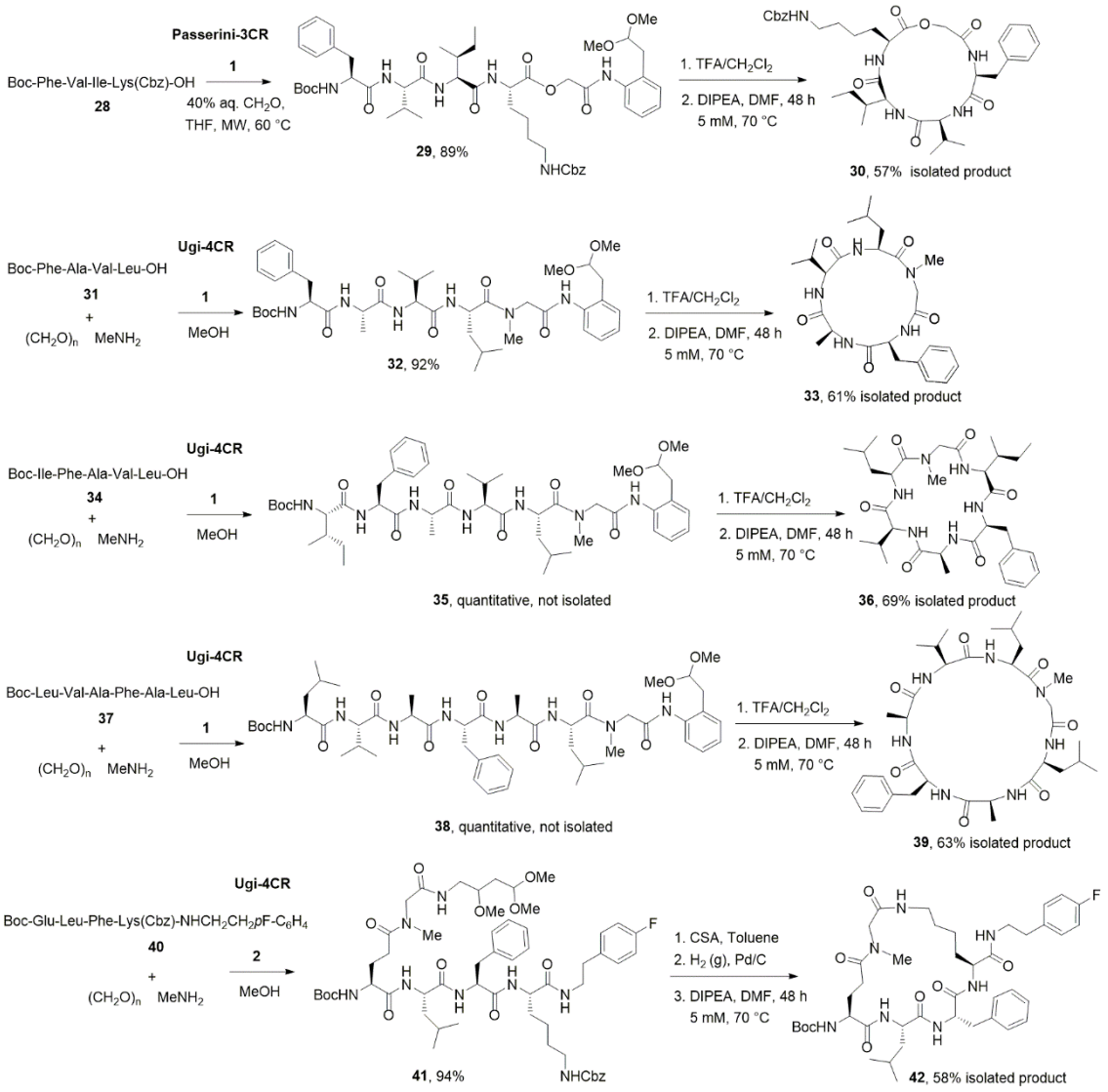
Scheme 5.3 Ligation strategy to produce glyco-, lipo- and labelled peptides.

Thus, far, ligation at the *C*-termini of peptides was the focus, while such a type of derivatization is also possible at the side chains of peptides endowed with Asp and Glu. **Scheme 5.3** illustrates the Ugi-4CRs of hexapeptide **22** – having an unprotected Asp side chain and protected *N*- and *C*-termini – with isonitrile **2** and the methylamine/paraformaldehyde combination to afford the side chain-functionalized peptide **23** in excellent yield. Classic activation by acidic treatment rendered *N*-peptidoacyl pyrrole **24** in quantitative yield, which was subsequently used in the ligation protocol without further purification. Thus, **24** was reacted with the 7-nitrobenz-2-oxa-1,3-diazol-4-yl (NBD)-derived fluorescent amine **25**[16] and *n*-docecylamine to furnish the side-chain fluorescently tagged peptide **26** and the lipidated version **27** in 59% and 67% yield, respectively, after column chromatography. It is worth-mentioning that first attempts to produce side-chain *N*-glycosides by reaction of activated peptide **24** with a glycosyl amine were thwarted by poor conversion and significant decomposition upon conjugation conditions with either MW irradiation or traditional heating.

5.5 Macrocyclization strategy

After demonstrating the scope of convertible isonitriles in Ugi-4CR/activation/ligation protocols, we turned to demonstrate applications in the synthesis of both cyclic depsipeptides and *N*-methylated peptides. Cyclic depsipeptides are natural products composed by amino acids (or bioisosters) and at least one hydroxy acid, thus forming a lactone bond in the cyclopeptidic skeleton. Previously, IMCRs have been employed to produce cyclic depsipeptide mimics,[17] though convertible isocyanides have not been used for such purposes yet. As shown in **Scheme 5.4**, our strategy to cyclic depsipeptides comprised the implementation of the Passerini 3-component reaction (Passerini-3CR) between *N*-protected peptide **28**, isonitrile **1** and aqueous formaldehyde in chloroform under MW irradiation to furnish depsipeptide **29** in excellent yield. Treatment of **29** with TFA in CH_2Cl_2 enabled the simultaneous *N*-terminal deprotection and the *C*-terminal activation (one spot Ugi-reaction/Deprotection/Activation/Cyclization, UDAC) by formation of the *N*-acyl indole, thus paving the way for the macrolactamization step. A variety of macrocyclization conditions were studied for this and other *C*-activated peptides (not including heating by MW irradiation), resulting in the selection of a 5 mM concentration in DMF under basic conditions at 70 °C as the most suitable one. The reaction time for such macrolactamizations was set to 48 h, but in some cases longer reaction times might be

required for higher yields. Under these conditions, the cyclic depsipeptide **30** was obtained in 57% yield of isolated pure product, while no byproducts derived from the feared cleavage of the lactone bond were detected by HPLC and ESI-MS analysis of the crude product.



Scheme 5.4 Macrocyclization strategy to cyclic depsipeptides and *N*-methylated peptides.

A similar approach was utilized for the synthesis of *N*-methylated peptides endowed with different sequences and macrocyclic ring sizes, but relying on the Ugi-4CR for the installation of the *N*-methylated amide bond. Initially, *N*-protected tetrapeptide **31** was reacted with isonitrile **1**, methylamine and paraformaldehyde in the Ugi-4CR to produce *N*-methylated peptide **32** in excellent yield. The *C*-terminal activation/deprotection procedure rendered the corresponding *N*-peptidoacyl indole, which was subjected to the macrocyclization protocol to furnish cyclic pentapeptide **33** in good yield over two steps.

The same UDAC sequence was employed for the synthesis of *N*-methylated cyclic hexapeptide **36** and heptapeptide **39** in 69% and 63% yield, respectively, over three steps. As shown in **Scheme 5.4**, the experience gained in the previous protocols led us to implement the synthesis of **36** in **39** without chromatographic purification of any intermediate, which was possible mainly due the high reactivity of isonitrile **1** in combination with methylamine, paraformaldehyde and peptide carboxylic acids, enabling the Ugi-4CRs to proceed quantitatively within 24 h.

Besides of the head-to-tail cyclization, the method should also enable the side chain-to-side chain cyclization of peptides. For this, peptide **40**, having both termini capped and an unprotected Glu side chain, was submitted to the Ugi-4CR with methylamine, paraformaldehyde and isonitrile **2** to produce the side chain-functionalized peptide **41** in excellent yield. As compound **41** is protected at the *N*-terminus with Boc, camphor sulphonic acid (CSA, 0.1 equiv) and quinoline (0.1 equiv) in toluene were chosen for the *C*-terminal activation, conditions upon which the Boc group remained unaffected.[13a] Subsequently, the Cbz group of the Lys side chain was orthogonally cleaved by hydrogenation, and the resulting intermediate was cyclized under previously described conditions to furnish the side chain cross-linked tetrapeptide **42** in 58% yield over three steps. With this final example, we have proven that convertible isonitriles **1** and **2** are suitable reagents for the incorporation of either *N*-alkylated or depsipeptide moieties by means of Ugi-4CR and Passerini-3CR, respectively, while enabling the mild activation of either the peptide *C*-terminus or side chain for ligation and macrocyclization purposes.

5.6 Conclusions

We have demonstrated the feasibility of using convertible isonitriles – derived from Fukuyama amines – as synthetic means to enable peptide ligation and macrocyclization. The strategy comprises their utilization in IMCRs such as the Ugi-4CR and the Passerini-3CR for the assembly of *N*-alkylated peptides and depsipeptides, respectively, followed by either side chain or *C*-terminal activation by acidic treatment to afford *N*-peptidoacyl indoles or pyrroles. The latter intermediates proved to be properly activated to enable either the ligation to nucleophilic aminopeptides or macrolactamization under diluted conditions. Both ligation and macrocyclization protocols required either microwave irradiation or heating to 70 °C to proceed in reasonable time, thus providing a variety of *N*-substituted (cyclo)peptides

and a cyclic depsipetide. These results provide further evidence of the potential of convertible isonitriles and IMCRs as powerful synthetic tools in peptide chemistry.

5.7 Experimental Part

General remarks

Convertible isonitriles **1** and **2** were synthesized according to references [11a] and [11b]. Peptides **3**, **9**, **17**, **28**, **31**, **34**, and **37** are known compounds and were produced according to references [6b] and [18]. Peptide synthesis grade DMF, CH_2Cl_2, DIPEA, TFA, and HPLC-grade acetonitrile were used. HPLC analysis was performed in a reverse-phase (RP) C18 column (4.6 × 150 mm, 5 *μ*m). A linear gradient from 5% to 60% of solvent B in solvent A over 35 min at a flow rate of 0.8 mL/min was used (solvent A: 0.1% (*v*/*v*) TFA in water, solvent B: 0.05% (*v*/*v*) TFA in acetonitrile). Detection was accomplished at 226 or 254 nm. Flash column chromatography was performed on silica gel 60 (>230 mesh) and analytical thin layer chromatography (TLC) was performed using silica gel on aluminum sheets. For peptides that were not purified to >95% by column chromatography on silica, purity was assessed by RP-HPLC and characterization of an analytical sample was made by electrospray ionization mass spectrometry (ESI-MS). The high resolution ESI mass spectra were obtained either from a 70e Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer equipped with an Infinity™ cell, a 7.0 Tesla superconducting magnet or from an Orbitrap Elite mass spectrometer equipped with an HESI electrospray ion source. Reactions involving microwave irradiation were performed in a Robotic Microwave Synthesizer (Biotage Emrys Personal Chemistry Optimizer Microwave Synthesizer). ^{1}H NMR and ^{13}C NMR spectra were recorded on a 400 spectrometer at 399.94 MHz and 100.57 MHz, respectively. Chemical shifts (δ) are reported in ppm relative to TMS (^{1}H NMR) and to the solvent signal (^{13}C NMR).

General procedure for the Ugi-4CR: A solution of the amine (0.6 mmol, 1.2 equiv.) and paraformaldehyde (0.6 mmol, 1.2 equiv.) in MeOH/CH_2Cl_2 (5 mL, 5:1, *v*/*v*) is stirred for 1 h at room temperature. Et_3N (0.6 mmol) is added when amine hydrochlorides were employed as amino components. The peptide carboxylic acid (0.5 mmol, 1 equiv.) and the convertible isocyanide (0.5 mmol, 1 equiv.) are then added and the reaction mixture is stirred at room temperature for 24 h. The volatiles are then concentrated under reduced pressure in a rotary evaporator and the resulting crude product is dissolved in 50 mL of $CHCl_3$. The organic phase is washed sequentially with an aqueous saturated solution of citric acid (30 mL), aqueous 10% $NaHCO_3$ (30 mL), and

brine (30 mL), and then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure in a rotary evaporator. The crude product is purified by flash column chromatography (CH_2Cl_2/MeOH) on silica to afford the corresponding *N*-alkylated peptide.

General procedure for the simultaneous Boc/*t*Bu removal and *C*-terminal activation by conversion of Ugi-4CR-derived amides into *N*-peptidoacyl indoles and pyrroles: The Ugi-4CR-derived peptide (0.5 mmol) is dissolved in CH_2Cl_2 (5 mL) and treated with trifluoroacetic acid (1 mL) at 0 °C. The reaction mixture is allowed to reach room temperature and stirred for 2 h, then concentrated under reduced pressure in a rotary evaporator. TFA is removed completely by repetitive addition and evaporation of CH_2Cl_2 to furnish the TFA salt of the *C*-activated peptide, which is used without further purification.

General acetylation procedure of the free *N*-terminal peptide: The peptide (0.5 mmol) is dissolved in 5 mL of CH_2Cl_2 and treated with DIPEA (0.52 mL, 3 mmol) and Ac_2O (0.28 μL, 3 mmol). The reaction mixture is stirred at room temperature for 2 h and then the volatiles are evaporated under reduced pressure in a rotary evaporator. The product is dissolved in EtOAc (25 mL) and washed vigorously with aq. 10% HCl (2×15 mL) and brine (15 mL). The organic phase is dried over anhydrous Na_2SO_4 and concentrated under reduced pressure in a rotary evaporator to afford the *N*-acetyl peptide.

General peptide ligation procedure under MW irradiation: The peptidyl or alkyl amine (0.25 mmol, 1 equiv.) and the *N*-peptidoacyl indole or pyrrole (0.25 mmol, 1 equiv.) are dissolved in 5 mL of DMF in a 10 mL glass tube. DIPEA (0.17 mL, 1 mmol, 4 equiv.) is added and the glass tube is sealed and introduced in the microwave reactor. The flask is irradiated for 30 min (150 W) under high-speed magnetic stirring at 70 °C, while the reaction course is monitored by TLC. Additional cycles of 15 min are applied in cases of poor consumption of the starting material. The volatiles are concentrated under reduced pressure in a rotary evaporator and the reaction product is washed several times with cold diethyl ether, then taken up in *ca.* 20-30 mL of MeOH and filtered through a pad of silica gel C18 to partially remove the indole or pyrrole derivatives. The resulting solution is concentrated to dryness and the crude product is purified either by flash column chromatography or analyzed by RP-HPLC and ESI-MS. In the latter case, the crude peptide is taken up in 2:1 acetonitrile/water and lyophilized prior to HPLC analysis and purification.

Peptide 4: HCl·Gly-O^{t}Bu (100 mg, 0.6 mmol), Et_3N (83μL, 0.6 mmol), paraformaldehyde (18 mg, 0.6 mmol), peptide **3** (225 mg, 0.5 mmol) and isonitrile **1** (95.5 mg, 0.5 mmol) were reacted in MeOH/CH_2Cl_2 (5 mL, 5:1, *v*/*v*) for 24 h according to the general Ugi-4CR procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 12:1) afforded peptide **4** (360 mg, 92%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.88 (s, 1H), 7.33 (m, 1H), 7.27 (m, 1H), 7.26 – 7.05 (m, 9H), 6.97 (m, 1H), 5.22 (m, 1H), 4.62 (t, *J* = 5.7 Hz, 1H), 4.57 (m, 1H), 4.54 – 4.50 (m, 2H), 4.38 – 4.31 (m, 2H), 4.17 – 4.06 (m, 2H), 3.38 (s, 3H), 3.37 (s, 3H), 3.06 (dd, *J* = 13.8, 5.2 Hz, 1H), 3.01 (m, 1H), 2.91 (m, 2H), 1.80 – 1.76 (m, 1H), 1.67 – 1.61 (m, 2H), 1.40 (s, 9H), 1.38 (s, 9H), 1.36 (d, *J* = 7.0 Hz, 3H), 0.89 (d, *J* = 6.4 Hz, 3H), 0.87 (d, *J* = 6.4 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 17.7, 22.6, 22.9 (*C*H_3), 25.1 (*C*H), 28.2, 28.4 (*C*H_3), 38.3, 40.8, 41.1 (*C*H_2), 47.7 (*C*H), 49.9, 51.6 (*C*H_2), 52.6 (*C*H), 55.3 (*C*H_3), 55.5 (*C*H), 80.5, 81.4 (*C*), 104.5, 122.3, 126.6, 126.8, 128.6, 129.1 (*C*H), 129.3 (*C*), 130.5, 131.2 (*C*H), 136.3, 136.5 (*C*), 155.9, 166.7, 167.3, 171.5, 171.6, 171.9, 173.0 (*C*O). HRMS (ESI-FT-ICR) *m/z*: 806.4322 $[M+Na]^+$, calcd. for $C_{41}H_{61}O_{10}NaN_5$: 806.4316.

Peptide 5: HCl·Glu-O^{t}Bu (100 mg, 0.6 mmol), Et_3N (83μL, 0.6 mmol), paraformaldehyde (18 mg, 0.6 mmol), peptide **3** (225 mg, 0.5 mmol) and isonitrile **2** (87 mg, 0.5 mmol) were reacted in MeOH/CH_2Cl_2 (5 mL, 5:1, *v*/*v*) for 24 h according to the general Ugi-4CR procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 10:1) afforded peptide **5** (364 mg, 95%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.36 (d, *J* = 9.2 Hz, 1H), 7.26 – 7.21 (m, 3H), 7.15 (m, 2H), 7.11 (m, 1H), 7.07 (m, 1H), 6.99 (m, 1H), 5.24 (m, 1H), 4.66 (m, 1H), 4.60 – 4.51 (m, 3H), 4.32 – 4.27 (m, 3H), 4.25 (dd, *J* = 17.2, 4.5 Hz, 1H), 4.12 (dd, *J* = 17.1, 4.6 Hz, 1H), 3.53 (m, 1H), 3.38 (s, 3H), 3.32 (s, 6H), 3.27 (m, 1H), 3.06 (dd, *J* = 13.8, 5.2 Hz, 1H), 2.96 (dd, *J* = 13.9, 7.9 Hz, 1H), 1.81 – 1.72 (m, 3H), 1.66 – 1.60 (m, 2H), 1.41 (s, 9H), 1.30 (s, 9H), 1.35 (d, *J* = 7.2 Hz, 3H), 0.88 (d, *J* = 6.4 Hz, 3H), 0.87 (d, *J* = 6.4 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 17.9, 22.0, 22.3 (*C*H_3), 25.1 (*C*H), 28.2 (*C*H_3), 38.2, 40.7, 42.6, 41.3 (*C*H_2), 47.6 (*C*H), 49.1, 49.9 (*C*H_2), 51.4 (*C*H), 55.3 (*C*H_3), 55.4 (*C*H), 56.2 (*C*H_3), 76.2 (*C*H), 80.3, 81.0 (*C*), 102.5, 126.9, 128.8, 129.2 (*C*H), 136.3 (*C*), 155.9, 166.2, 170.0, 171.6, 171.9, 172.6, 173.3 (*C*O). HRMS (ESI-FT-ICR) *m/z*: 788.4427 $[M+Na]^+$, calcd. for $C_{38}H_{63}O_{11}NaN_5$: 788.4422.

Peptide 8: Peptide **4** (235 mg, 0.3 mmol) was subjected to the general procedure for the simultaneous Boc/*t*Bu removal and *C*-terminal activation, followed by the general acetylation procedure to yield quantitatively the *N*-peptidoacyl indole **6**, which was used forward without previous purification. *C*-activated peptide **6** (151 mg, 0.25 mmol) was

reacted with the hydrochloride salt of tripeptide H-Phe-Leu-Ala-OMe (100 mg, 0.25 mmol) for 30 minutes in the presence of DIPEA according to the general ligation procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 10:1) afforded peptide **8** (153 mg, 72%) as a white amorphous solid. In parallel, peptide **5** was subjected to the general procedure for the simultaneous Boc/*t*Bu removal and *C*-terminal activation, followed by acetylation to give **7**, which was equally submitted to the ligation procedures to furnish **8** (108 mg, 51%) after column chromatography. Mixture of two conformers. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.27 – 7.16 (m, 10H), 5.13, 4.97 (2×m, 1H), 4.67 – 4.48 (m, 3H), 4.30 – 4.24 (m, 2H), 4.19 (d, *J* = 18.8 Hz), 4.17 (d, *J* = 17.2 Hz), 3.93 (d, *J* = 17.2 Hz), 3.91 (d, *J* = 17.2 Hz), 3.79 (d, *J* = 17.6 Hz), 3.74 (s, 3H), 3.19 – 3.09 (m, 2H), 2.95 – 2.85 (m, 2H), 1.90 (s, 3H), 1.75 – 1.42 (m, 6H), 1.36 (d, *J* = 6.8 Hz, 3H), 1.31 (d, *J* = 6.6 Hz, 3H), 0.99 (d, *J* = 6.6 Hz, 3H), 0.94 (d, *J* = 6.2 Hz, 3H), 0.90 (d, *J* = 6.4 Hz, 6H), 0.86 (d, *J* = 6.4 Hz, 3H), 0.85 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD): δ = 17.7, 17.8, 22.0, 22.4, 22.8, 22.9, 23.0, 23.2, 23.3, 23.4 (*C*H$_3$), 25.6, 5.7, 25.8 (*C*H), 38.5, 39.0, 39.7, 41.6, 41.8, 42.2, 46.1, 46.2 (*C*H$_2$), 46.8, 47.0 (*C*H), 50.6 (*C*H), 52.7 (*C*H$_3$), 53.0, 53.4, 53.5, 55.1, 55.3, 56.0, 56.5, 59.3 (*C*H), 127.6, 127.8, 129.4, 129.5, 130.2, 130.4, 130.6 (*C*H), 138.2, 138.4 (*C*), 171.0, 171.4, 172.0, 172.1, 172.6, 172.9, 173.1, 173.4, 173.5, 173.8, 174.4, 174.7, 174.8, 175.4 (*C*O). HRMS (ESI-FT-ICR) *m/z*: 874.4331 $[M+Na]^+$, calcd. for $C_{43}H_{61}O_{11}NaN_7$: 874.4327.

Peptide 10: HCl·$MeNH_2$ (40 mg, 0.6 mmol), Et_3N (83 μL, 0.6 mmol), paraformaldehyde (18 mg, 0.6 mmol), peptide **9** (334 mg, 0.5 mmol) and isonitrile **1** (95.5 mg, 0.5 mmol) were reacted in MeOH/CH_2Cl_2 (5 mL, 5:1, *v*/*v*) for 24 h according to the general Ugi-4CR procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 15:1) afforded peptide **10** (378 mg, 91%) as a pale yellow amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.07 (s, 1H), 7.30 – 7.07 (m, 14H), 6.79 (d, *J* = 8.6 Hz, 1H), 6.76 (d, *J* = 8.8 Hz, 1H), 5.73 (m, 1H), 5.07 (m, 1H), 4.94 (m, 1H), 4.69 (t, *J* = 5.8 Hz, 1H), 4.42 (m, 1H), 4.37 (d, *J* = 15.8 Hz, 1H), 4.08 (d, *J* = 15.8 Hz, 1H), 3.38 (s, 3H), 3.37 (s, 3H), 3.24 (s, 3H), 3.21 – 3.19 (m, 2H), 3.03 (m, 1H), 3.01 (m, 1H), 2.90 (m, 2H), 2.38 (m, 2H), 2.19 (m, 2H), 1.41 (s, 9H), 1.39 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 26.9 (*C*H$_2$), 28.2, 28.4 (*C*H$_3$), 30.6 (*C*H$_2$), 35.3 (*C*H$_3$), 38.3, 38.5, 40.8, 51.6 (*C*H$_2$), 53.2, 53.6 (*C*H), 55.2 (*C*H$_3$), 55.9 (*C*H), 80.8, 81.3 (*C*), 104.5, 122.3, 126.6, 127.7, 126.8, 127.0, 128.5, 128.6, 129.0, 129.1 (*C*H), 129.3 (*C*), 130.8, 131.3, 132.8 (*C*H), 136.1, 136.3, 136.4 (*C*), 157.9, 167.0, 170.7, 171.9, 172.1, 173.0 (*C*O). HRMS (ESI-FT-ICR) *m/z*: 854.4321 $[M+Na]^+$, calcd. for $C_{45}H_{61}O_{10}NaN_5$: 854.4316.

Peptide 12: Peptide **10** (249 mg, 0.3 mmol) was subjected to the general procedure for the simultaneous Boc/*t*Bu removal and *C*-terminal activation, followed by the acetylation procedure to yield quantitatively the *N*-peptidoacyl indole **11**, which was used forward without previous purification. *C*-activated peptide **11** (163 mg, 0.25 mmol) was reacted with the trifluoroacetate salt of tetrapeptide H-Leu-Ala-Phe-Ile-NH_2 (144 mg, 0.25 mmol) for 30 minutes in the presence of DIPEA according to the general ligation procedure to afford peptide **12** (239 mg) as a pale yellow amorphous solid. RP-HPLC analysis of the crude product showed 74% of conversion. An analytical sample was purified by RP-HPLC to >95% purity for ESI-MS characterization. R_t = 20.7 min. HRMS (ESI-FT-ICR) *m/z*: 996.5190 $[M-H]^-$, calcd. for $C_{52}H_{70}O_{11}N_9$: 996.5200.

General peptide coupling procedure. The Boc-protected L-amino acid (1.0 mmol, 1.0 equiv.), HOBt (168 mg, 1.1 mmol, 1.1 equiv.), EDC (210 mg, 1.1 mmol, 1.1 equiv.) and the L-amino acid methyl ester hydrochloride are suspended in dry CH_2Cl_2 (15 mL). Et_3N (0.15 mL, 1.1 mmol, 1.1 equiv.) is added via syringe in one portion and the resulting solution is stirred at room temperature overnight (~12 h). The reaction mixture is then diluted with 100 mL EtOAc, transferred to a separatory funnel and sequentially washed with 0.5 M aqueous solution of citric acid (2×50 mL) and saturated aqueous suspension $NaHCO_3$ (2×50 mL). The organic phase is dried over $MgSO_4$, filtered and concentrated under reduced pressure in a rotary evaporator.

General Boc removal procedure: The peptide is dissolved in a 4 M HCl solution in dioxane (2 mL) and the solution is stirred at room temperature. As the material dissolved, gas evolution could be detected and the pressure that built up inside the reaction flask is regularly relieved by a needle adapted in the lid of the reaction flask. After 30 min, usually no starting material is detected by thin layer chromatography and the reaction is concentrated under a stream of dry N_2. The volatiles are then fully removed by concentrating the resulting thick oily residue under reduced pressure in the rotary evaporator and then placing the flask under high vacuum for 2 h. The resulting salt was used forward assuming quantitative yield.

Peptide 13: *N*-Boc-Val-OH (217 mg, 1.0 mmol) was coupled to HCl·Leu-OBlz (257 mg, 1.0 mmol) according to the peptide coupling procedure, following by deprotection of the *N*-terminus by the Boc removal. The same protocol was employed for the coupling of *N*-Boc-Asp(OMe)-OH (247 mg, 1.0 mmol). Flash column chromatography purification (CH_2Cl_2/MeOH 15:1) furnished peptide Boc-Asp(OMe)-Val-Leu-OBzl (433 mg, 79%) as a white amorphous solid. This latter tripeptide (412 mg, 0.75 mmol) was subjected to deprotection of the *N*-terminus by the Boc removal procedure. The resulting crude peptide is dissolved in 10 mL of CH_2Cl_2 and treated with DIPEA (0.52 mL, 3 mmol) and

BzCl (0.28 μL, 3 mmol). The reaction mixture was stirred at room temperature for 8 h and then the volatiles evaporated under reduced pressure in a rotary evaporator. The crude product was dissolved in EtOAc (25 mL) and washed with aq. 10% HCl (2×10 mL) and brine (2×10 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure in a rotary evaporator to afford the N-benzoyl peptide. The resulting product was dissolved in MeOH (30 mL) and 10% Pd/C (80 mg) was added. The mixture was subjected successively to hydrogen atmosphere and vacuum and finally stirred under hydrogen atmosphere for 24 h. The catalyst was removed by filtration over a pad of Celite and the filtrate was evaporated under reduced pressure in a rotary evaporator. Flash column chromatography purification (CH_2Cl_2/MeOH 15:1) furnished peptide **13** (268 mg, 77%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 (d, *J* = 8.1 Hz, 1H), 7.82 (m, 2H), 7.56 (m, 1H), 7.53 – 7.47 (m, 1H), 7.41 (m, 2H), 7.17 (d, *J* = 8.1 Hz, 1H), 5.09 (m, 1H), 4.61 – 4.50 (m, 1H), 4.31 (m, 1H), 3.66 (s, 3H), 2.94 (d, *J* = 6.1 Hz, 2H), 2.24 – 2.11 (m, 1H), 1.66 (m, 3H), 0.90 (m, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 17.9, 19.3, 21.8, 23.0 (*CH*$_3$), 25.0, 30.5 (*CH*), 35.6, 40.8 (*CH*$_2$), 50.2, 51.2 (*CH*), 52.3 (*CH*$_3$), 59.3 (*CH*), 125.7, 127.5, 128.8, 132.2 (*CH*), 133.3 (*C*), 167.8, 171.3, 171.6, 172.5, 175.6 (*CO*). HRMS (ESI-FT-ICR) *m/z*: 462.2245 $[M-H]^-$, calcd. for $C_{23}H_{32}O_7N_3$: 462.2246.

Peptide 14: $HCl{\cdot}MeNH_2$ (40 mg, 0.6 mmol), Et_3N (83μL, 0.6 mmol), paraformaldehyde (18 mg, 0.6 mmol), peptide **13** (232 mg, 0.5 mmol) and isonitrile **2** (87 mg, 0.5 mmol) were reacted in MeOH/CH_2Cl_2 (5 mL, 5:1, *v*/*v*) for 24 h according to the general Ugi-4CR procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 18:1) afforded peptide **14** (292 mg, 86%) as a white amorphous solid. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.83 (m, 2H), 7.58 – 7.51 (m, 1H), 7.46 (m, 2H), 5.04 (t, *J* = 7.1 Hz, 1H), 4.87 (m, 1H), 4.52 (m, 1H), 4.28 (m, 1H), 4.11 – 3.97 (m, 2H), 3.69 (s, 3H), 3.45 – 3.39 (m, 1H), 3.37 (s, 3H), 3.34 – 3.30 (m, 6H), 3.27 – 3.14 (m, 3H), 3.02 (m, 1H), 2.92 (s, 1H), 2.86 (dd, *J* = 16.6, 7.7 Hz, 1H), 2.11 (m, 1H), 1.79 – 1.62 (m, 4H), 1.55 (m, 1H), 1.00 – 0.86 (m, 12H). ^{13}C NMR (100 MHz, CD_3OD): δ = 18.3, 19.8, 22.1, 23.7 (*C*H_3), 25.8, 32.1 (*C*H), 36.3 (*C*H_2), 36.7 (*C*H_3), 37.3, 41.3, 42.8 (*C*H_2), 51.7, 52.3, 52.5 (*C*H), 53.5, 53.7, 57.6, 57.7 (*C*H_3), 59.7, 77.6, 103.5, 128.5, 129.6, 133.0 (*C*H), 135.0 (*C*), 170.2, 170.8, 172.8, 173.1, 173.4, 174.7, (*C*O). HRMS (ESI) *m/z*: 678.3715 $[M-H]^-$, calcd. for $C_{33}H_{52}O_{10}N_5$: 678.3720.

Peptide 16: Peptide **14** (204 mg, 0.3 mmol) was dissolved in THF/H_2O (2:1, 15 mL) and LiOH (50 mg, 1.2 mmol) is added at 0 °C. The mixture was stirred at 0 °C for 3 h and then acidified with aquouse 10% $NaHSO_4$ to pH 3. The resulting phases were separated and the aqueous phase was additionally extracted with EtOAc (2×20 mL).

The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure in a rotary evaporator. The resulting crude was subjected to the general procedure for the *C*-terminal activation to yield quantitatively the *N*-peptidoacyl pyrrole **15**, which was used forward without previous purification. *C*-activated peptide **15** (146 mg, 0.25 mmol) was reacted with the trifluoroacetate salt of tetrapeptide H-Phe-Val-Glu-Ala-OH (145 mg, 0.25 mmol) for 45 minutes in the presence of DIPEA according to the general ligation procedure to afford peptide **16** (212 mg) as a white amorphous solid. RP-HPLC analysis of the crude product showed 67% purity. A sample was purified by RP-HPLC to >95% purity for characterization. R_t = 9.72 min. ^{1}H NMR (600 MHz, CD_3OD) δ 7.89 – 7.80 (m, 2H), 7.52 (m, 1H), 7.48 – 7.40 (m, 2H), 7.30 – 7.14 (m, 5H), 5.00 (t, *J* = 7.3 Hz, 1H), 4.77 – 4.69 (m, 1H), 4.65 (m, 1H), 4.49 – 4.39 (m, 1H), 4.32 (m, 1H), 4.27 (m, 1H), 4.24 – 4.15 (m, 1H), 4.08 (m, 1H), 4.04 – 3.89 (m, 2H), 3.21 – 3.15 (m, 1H), 3.11 – 3.03 (m, 2H), 3.01 – 2.88 (m, 3H), 2.86 – 2.79 (m, 1H), 2.58 – 2.47 (m, 1H), 2.46 – 2.36 (m, 2H), 2.15 – 2.02 (m, 3H), 1.98 – 1.89 (m, 1H), 1.71 – 1.59 (m, 2H), 1.59 – 1.48 (m, 1H), 1.45 – 1.40 (m, 1H), 1.40 – 1.34 (m, 3H), 1.30 (dd, *J* = 14.1, 6.6 Hz, 1H), 1.02 – 0.75 (m, 18H). ^{13}C NMR (151 MHz, CD_3OD) δ 18.9, 19.8, 19.9, 22.1 (*CH*$_3$), 23.6 (*CH*), 25.8 (*CH*$_2$), 28.5, 30.2 (*CH*), 31.2 (*CH*$_2$), 36.3 (*CH*$_3$), 37.3, 38.7, 41.5 (*CH*$_2$), 52.1 (*CH*), 53.8 (*CH*$_2$), 56.1, 57.5, 57.6, 59.2, 60.2, 61.0, 127.7, 128.4, 128.5, 128.6, 129.3, 129.5, 129.6, 130.3, 130.4, 131.2 (*CH*), 134.1, 138.5 (*C*), 166.7, 168.4, 171.4, 172.0, 172.2, 172.6, 173.2, 173.5, 174.5, 175.3, 180.4 (*CO*). HRMS (ESI) *m/z*: 965.4619 $[M-H]^-$, calcd. for $C_{47}H_{65}O_{14}N_8$: 965.4626.

Peptide 19: Glucosyl amine **18** (208 mg, 0.6 mmol), paraformaldehyde (18 mg, 0.6 mmol), peptide **17** (257 mg, 0.5 mmol) and isonitrile **1** (95.5 mg, 0.5 mmol) were reacted in MeOH/CH_2Cl_2 (10 mL, 5:1, *v*/*v*) for 36 h according to the general Ugi-4CR procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 12:1) afforded glycopeptide **19** (383 mg, 72%) as a pale yellow amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ **=** 9.12 (s, 1H), 7.62 (d, *J* = 7.2 Hz, 1H,), 7.48 (m, 1H), 7.24 – 7.08 (m, 4H), 5.62 (d, *J* = 8.2 Hz, 1H), 5.50 (d, *J* = 8.2 Hz, 1H), 5.39 (t, *J* = 9.4 Hz, 1H), 5.00 (m, 1H), 4.87 (m, 1H), 4.75 – 4.64 (m, 3H), 4.58 (t, *J* = 5.6 Hz, 1H), 4.44 – 4.40 (m, 1H), 4.36 – 4.30 (m, 3H), 4.14 (m, 1H), 4.06 (d, *J* = 15.8 Hz, 1H), 3.87 (m, 1H), 3.39 (s, 3H), 3.37 (s, 3H), 2.90 (m, 2H), 2.01, 2.04, 2.05, 2.08 (4×s, 4×3H), 1.81 (m, 1H), 1.73 – 1.62 (m, 2H), 1.56 – 1.46 (m, 2H), 1.43 (s, 9H), 1.36 (d, *J* = 7.0 Hz, 3H), 0.96 (d, *J* = 6.0 Hz, 3H), 0.93 – 0.86 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ **=** 15.9, 17.4, 18.9, 20.7, 21.3, 21.4, 22.2, 22.7, 22.8 (*C*H$_3$), 24.6 (*C*H), 24.9 (*C*H$_2$), 28.3, 28.4 (*C*H$_3$), 30.5, 37.5 (*C*H), 40.0, 41.2, 43.5 (*C*H$_2$), 48.6, 51.4, 51.9, 52.5 (*C*H), 53.6 (*C*H$_3$), 57.2, 59.3 (*C*H), 62.8 (*C*H$_2$), 68.6, 69.7, 73.2 (*C*H), 79.7 (*C*), 84.4 (CH), 104.5 (*C*H), 122.3, 126.9 (*C*H), 129.3

(*C*), 130.5, 131.1 (*C*H), 136.1 (*C*), 158.3, 1710.2, 171.4, 171.7, 171.9, 172.3, 172.5, 172.8 (*C*O). HRMS (ESI-FT-ICR) *m/z*: 1086.5590 $[M+Na]^+$, calcd. for $C_{51}H_{81}O_{17}NaN_7$: 1086.5587.

Peptide 21: Peptide **19** (320 mg, 0.3 mmol) was subjected to the general procedure for the simultaneous Boc/*t*Bu removal and *C*-terminal activation, followed by the acetylation procedure to yield quantitatively the *N*-peptidoacyl indole **20**, which was used forward without previous purification. *C*-activated peptide **20** (235 mg, 0.25 mmol) was reacted with the hydrochloride salt of tetrapeptide H-Phe-Lys(Cbz)-Gly-Phe-OMe (170 mg, 0.25 mmol) for 30 minutes in the presence of DIPEA according to the general ligation procedure to afford peptide **21** (257 mg) as a pale yellow amorphous solid. RP-HPLC analysis of the crude product showed 56% purity. An analytical sample was purified by RP-HPLC to >95% purity for ESI-MS characterization. R_t = 17.3 min. HRMS (ESI-FT-ICR) *m/z*: 1492.7234 $[M+Na]^+$, calcd. for $C_{73}H_{103}O_{21}N_{11}Na$: 1492.7228.

Bz-Ala-Leu-Phe-Val-Glu-Ala-OMe (22): *N*-Boc-Glu(OBzl)-OH (337 mg, 1.0 mmol) was coupled to HCl·Ala-OMe (139 mg, 1.0 mmol) according to the peptide coupling procedure, following by deprotection of the *N*-terminus by Boc removal. The same protocol was employed for the sequential coupling of *N*-Boc-Val-OH (217 mg, 1.0 mmol) and *N*-Boc-Phe-OH (265 mg, 1.0 mmol). Flash column chromatography purification (CH_2Cl_2/MeOH 15:1) furnished peptide *N*-Boc-Phe-Val-Glu(OBzl)-Ala-OMe (411 mg, 72%) as a white amorphous solid. This latter tetrapeptide (400 mg, 0.6 mmol) was subjected to deprotection of the *N*-terminus by the Boc removal procedure, followed by sequential coupling of *N*-Boc-Leu-OH (139 mg, 0.6 mmol) and *N*-Boc-Ala-OH (113 mg, 0.6 mmol). Flash column chromatography purification (CH_2Cl_2/MeOH 12:1) furnished hexapeptide Boc-*N*-Ala-Leu-Phe-Val-Glu(OBzl)-Ala-OMe (414 mg). This latter peptide was subjected to *N*-terminal deprotection by the Boc removal procedure, followed by benzoylation of the *N*-terminus and removal of the benzyl protecting group of the Glu side chain according to the procedures described for peptide **13**. Flash column chromatography purification (CH_2Cl_2/MeOH 12:1) furnished the hexapeptide **22** (264 mg, 71%) as a white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.54 – 8.40 (m, 2H), 8.35 – 8.21 (m, 1H), 8.08 – 7.99 (m, 1H), 7.98 – 7.90 (m, 1H), 7.90 – 7.82 (m, 2H), 7.52 (m, 1H), 7.45 (m, 2H), 7.26 – 7.07 (m, 5H), 6.39 (m, 2H), 4.61 – 4.51 (m, 1H), 4.45 (m, 1H), 4.36 – 4.29 (m, 1H), 4.25 (m, 2H), 4.15 (m, 1H), 3.96 – 3.84 (m, 2H), 3.63 – 3.56 (m, 3H), 3.45 (s, 3H), 3.26 (s, 1H), 3.19 (m, 2H), 3.04 (dd, *J* = 14.0, 4.0 Hz, 1H), 2.95 (m, 2H), 2.83 – 2.74 (m, 2H), 2.45 – 2.40 (m, 2H), 2.39 – 2.32 (m, 1H), 2.01 – 1.91 (m, 1H), 1.84 (m, 4H), 1.66 (p, *J* = 5.4 Hz, 1H), 1.55 (m, 3H), 1.37 (t, *J* = 7.3 Hz, 1H), 1.27 (m, 6H), 1.21 – 1.13 (m, 1H), 0.76 (m, 9H), 0.71 (dd,

J = 6.5, 3.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 16.7, 17.6, 18.1, 19.1, 21.5, 21.7 (*CH*$_3$), 22.9 (*CH*), 23.0, 24.1, 28.4 (*CH*$_2$), 29.2 (*CH*), 36.1 (*CH*$_2$), 36.2 (*CH*$_3$), 36.5, 37.1, 41.2 (*CH*$_2$), 45.0, 47.5, 48.5 (*CH*), 48.9 (*CH*$_2$), 51.9 (*CH*$_3$), 52.8, 53.3, 57.8, 99.2, 102.0, 126.1, 127.4, 127.5, 127.9, 128.2, 129.1, 129.3, 131.3 (*CH*), 134.0, 137.6, 137.7, 137.9, 144.6, 145.2 (*C*), 166.0, 166.2, 168.4, 171.0, 171.1, 172.1, 172.2, 172.9, 174.9 (*CO*). HRMS (ESI-FT-ICR) *m/z*: 765.3823 [M-H]$^-$, calcd. for $C_{39}H_{53}O_{10}N_6$: 765.3829.

Peptide 23: HCl·MeNH$_2$ (40 mg, 0.6 mmol), Et$_3$N (83μL, 0.6 mmol), paraformaldehyde (18 mg, 0.6 mmol), peptide **22** (383 mg, 0.5 mmol) and isonitrile **2** (87 mg, 0.5 mmol) were reacted in MeOH/CH_2Cl_2 (8 mL, 5:1, *v*/*v*) for 24 h according to the general Ugi-4CR procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 15:1) afforded peptide **23** (452 mg, 92%) as a white amorphous solid. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.88 (d, *J* = 7.5 Hz, 2H), 7.59 – 7.50 (m, 1H), 7.46 (m, 2H), 7.24 – 7.10 (m, 5H), 6.92 (s, 1H), 4.68 (dd, *J* = 9.9, 4.8 Hz, 1H), 4.55 – 4.46 (m, 2H), 4.45 – 4.35 (m, 2H), 4.34 – 4.26 (m, 1H), 4.20 (dd, *J* = 11.7, 7.6 Hz, 1H), 4.16 – 4.06 (m, 1H), 4.02 (m, 1H), 3.70 (s, 3H), 3.39 (m, 1H), 3.38 – 3.34 (m, 3H), 3.31 (m, 9H), 3.20 (dd, *J* = 13.4, 4.7 Hz, 1H), 3.05 (m, 2H), 3.02 – 2.94 (m, 1H), 2.93 – 2.89 (m, 1H), 2.58 (m, 1H), 2.48 (m, 1H), 2.15 – 2.07 (m, 2H), 2.01 – 1.92 (m, 1H), 1.72 (m, 2H), 1.60 (m, 1H), 1.45 (m, 4H), 1.40 (m, 6H), 0.98 – 0.79 (m, 12H). ^{13}C NMR (101 MHz, CD_3OD): δ = 17.3, 17.8, 19.8, 22.1, 23.4, 25.8 (*C*H$_3$), 28.7 (*C*H), 30.0, 30.9 (*C*H$_2$), 31.9 (*C*H), 35.4 (*C*H$_3$), 36.8, 37.4, 38.4, 42.8 (*C*H$_2$), 51.8, 52.2, 52.7, 53.1, 53.6, 53.7 (*C*H), 55.9, 56.5, 57.5, 57.7 (*C*H$_3$), 60.4, 77.7, 103.6, 126.1, 127.7, 128.7, 129.4, 129.5, 130.2, 130.3, 132.9, 135.0, 138.4 (*C*H), 138.6, 139.2 (*C*), 170.1, 170.4, 171.3, 173.3, 173.7, 174.5, 175.2, 175.3, 175.5 (*C*O). HRMS (ESI) *m/z*: 981.5287 [M-H]$^-$, calcd. for $C_{49}H_{73}O_{13}N_8$: 981.5303.

Peptide 26: Peptide **23** (392 mg, 0.4 mmol) was subjected to the general procedure for the *C*-terminal activation to yield quantitatively the *N*-peptidoacyl pyrrole **24**, which was used forward without previous purification. *C*-activated peptide **24** (133 mg, 0.15 mmol) was reacted with the NBD-derived fluorescent amine **25** (48 mg, 0.20 mmol) for 30 minutes in the presence of DIPEA according to the general ligation procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 15:1) afforded pure peptide **26** (94 mg, 59%) as a white amorphous solid. R_t = 11.7 min. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.54 – 8.40 (m, 2H), 8.35 – 8.21 (m, 1H), 8.08 – 7.99 (m, 1H), 7.98 – 7.90 (m, 1H), 7.90 – 7.82 (m, 2H), 7.52 (m, 1H), 7.45 (m, 2H), 7.26 – 7.07 (m, 5H), 6.39 (m, 2H), 4.61 – 4.51 (m, 1H), 4.45 (m, 1H), 4.36 – 4.29 (m, 1H), 4.25 (m, 2H), 4.15 (m, 1H), 3.96 – 3.84 (m, 2H), 3.63 – 3.56 (m, 3H), 3.45 (s, 3H), 3.26 (s, 1H), 3.19 (m, 2H), 3.04 (dd, *J* = 14.0, 4.0 Hz, 1H), 2.95 (m, 2H), 2.83 – 2.74 (m, 2H), 2.45 – 2.40 (m, 2H), 2.39 – 2.32

(m, 1H), 2.01 – 1.91 (m, 1H), 1.84 (m, 4H), 1.66 (d, *J* = 5.4 Hz, 1H), 1.55 (m, 3H), 1.37 (t, *J* = 7.3 Hz, 1H), 1.27 (m, 6H), 1.21 – 1.13 (m, 1H), 0.76 (m, 9H), 0.71 (m, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 16.7, 17.6, 18.1, 19.1, 21.5, 21.7 (*C*H$_3$), 22.9 (*C*H), 23.0, 24.1, 28.4 (*C*H$_2$), 29.2 (*C*H), 36.1 (*C*H$_2$), 36.2 (*C*H$_3$), 36.5, 37.1, 41.2 (*C*H$_2$), 45.0, 47.5, 48.5, (*C*H), 48.9 (*C*H$_2$), 51.9 (*C*H$_3$), 52.8, 53.3, 57.8, 99.2, 102.0, 126.1, 127.4, 127.5, 127.9, 128.2, 129.1, 129.3, 131.3 (*C*H), 134.0, 137.6, 137.7, 137.9, 144.6, 145.2 (*C*), 166.0, 166.2, 168.4, 170.9, 171.03, 172.1, 172.2, 172.9, 174.9 (*C*O). HRMS (ESI) *m/z*: 1055.4952 [M-H]$^-$, calcd. for $C_{51}H_{67}O_{13}N_{12}$: 1055.4956.

Peptide 27: *N*-peptidoacyl pyrrole **24** (133 mg, 0.15 mmol), obtained as describe above, was reacted with *n*-dodecylamine (100 mg, 0.20 mmol) for 30 minutes in the presence of DIPEA according to the general ligation procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 20:1) afforded pure peptide **27** (102 mg, 67%) as a white amorphous solid. R_t = 16.0 min. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.07 – 7.94 (m, 1H), 7.93 – 7.84 (m, 2H), 7.56 – 7.50 (m, 1H), 7.49 – 7.40 (m, 2H), 7.29 – 7.09 (m, 5H), 4.57 (m, 1H), 4.47 (m, 1H), 4.31 (m, 1H), 4.25 (m, 2H), 4.20 – 4.08 (m, 1H), 3.93 – 3.80 (m, 2H), 3.63 – 3.56 (m, 3H), 3.35 (m, 6H), 3.07 – 2.99 (m, 3H), 2.95 – 2.90 (m, 2H), 2.79 – 2.72 (m, 2H), 2.42 – 2.32 (m, 1H), 2.07 (s, 1H), 2.03 – 1.92 (m, 1H), 1.88 (m, 1H), 1.78 (m, 1H), 1.60 – 1.49 (m, 1H), 1.41 – 1.33 (m, 2H), 1.30 (m, 2H), 1.28 (m, 2H), 1.27 – 1.25 (m, 2H), 1.25 – 1.20 (m, 18H), 0.87 – 0.69 (m, 15H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 14.0, 16.8, 17.0, 17.7, 18.1, 18.2, 19.2 (*C*H$_3$), 21.6, 21.7, 22.1, 22.9, 23.0 (*C*H$_2$), 24.1 (*C*H), 26.4, 27.7, 28.7, 29.0 (*C*H$_2$), 30.4 (*C*H), 31.3, 34.0, 34.1, 36.1 (*C*H$_2$), 37.1 (*C*H$_3$), 38.4, 41.0 (*C*H$_2$), 47.5, 49.1, 50.2, 50.8 (*C*H), 51.1 (*C*H$_3$), 51.9, 53.7, 57.8, 126.1, 126.2, 127.4, 127.5, 127.9, 128.0, 128.2, 129.2, 129.3, 131.3 (*C*H), 134.0, 138.0 (*C*), 166.0, 167.6, 167.9, 170.6, 171.0, 171.9, 172.1, 172.8, 173.0, (*C*O). HRMS (ESI) *m/z*: 1003.6232 [M-H]$^-$, calcd. for $C_{54}H_{83}O_{10}N_8$: 1003.6238.

General macrocyclization procedure: The *C*-activated peptide (0.25 mmol, 1 equiv.) is dissolved in DMF (50 mL) and treated with DIPEA (0.17 mL, 1 mmol, 4 equiv.). The reaction mixture is stirred for 48 h at 70 °C and then concentrated under reduced pressure in a rotary evaporator. The reaction product is taken up in *ca.* 20-30 mL of MeOH and filtered through a pad of silica gel C18 to partially remove the indole or pyrrole derivatives and the resulting solution is concentrated to dryness. The resulting crude cyclic peptide is purified by flash column chromatography (CH_2Cl_2/ MeOH).

Depsipeptide 29: Peptide **28** (222 mg, 0.3 mmol) was dissolved in 5 mL of THF a 10 mL glass tube and treated with 0.1 mL of 40% aqueous formaldehyde and isonitrile **1** (57 mg, 0.3 mmol). The mixture was irradiated for 30 min (300 W) under high-speed magnetic stirring to 60 °C. After completion of the reaction, volatiles were evaporated

under reduced pressure in a rotary evaporator. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH 20:1) to furnish depsipeptide **29** (256 mg, 89%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ **=** 9.18 (s, 1H), 7.74 (d, *J* = 7.8 Hz, 1H), 7.29 (d, *J* = 7.2 Hz, 1H), 7.34 – 7.18 (m, 10H), 7.25 – 7.20 (m, 2H), 7.14 – 7.08 (m, 2H), 6.84 (d, *J* = 8.2 Hz, 1H), 5.60 (m, 1H), 5.15 (m, 1H), 5.08 (s, 2H), 4.85 (d, *J* = 15.4 Hz, 1H), 4.67 (d, *J* = 15.4 Hz, 1H), 4.61 (m, 1H), 4.43 (m, 2H), 4.22 (m, 1H), 3.40 (s, 3H), 3.38 (s, 3H, C*H*$_3$O), 3.24 – 3.14 (m, 2H), 3.04 (dd, *J* = 14.0, 5.2 Hz, 1H), 2.96 – 2.88 (m, 3H), 2.14 (m, 1H), 1.96 (m, 2H), 1.85 (m, 1H), 1.56 – 1.44 (m, 4H), 1.37 (s, 9H), 0.93 – 0.81 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.3, 15.4, 17.4, 19.2, 23.4 (*C*H$_3$), 22.7, 24.6 (*C*H$_2$), 28.1 (*C*H$_3$), 29.2 (*C*H$_2$), 30.2 (*C*H), 30.9 (*C*H$_2$), 36.2 (*C*H), 36.7, 37.9, 40.4 (*C*H$_2$), 42.1 (*C*H), 52.3 (*C*H$_3$), 54.8, 55.3, 56.0, 58.0, 59.0 (*C*H), 63.6, 66.4 (*C*H$_2$), 80.7, 107.0 (*C*), 124.8, 125.6, 127.0, 127.5, 127.9, 128.4, 128.7, 129.1, 131.0 (*C*H), 135.5, 135.9, 136.6 (*C*), 155.9, 156.9 165.4, 170.4, 170.9, 171.5, 172.1 (*C*O). HRMS (ESI-FT-ICR) *m/z*: 983.5105 [M+Na]$^+$, calcd. for $C_{51}H_{72}O_{12}NaN_6$: 983.5106.

Cyclic Depsipeptide 30: Depsipeptide **29** (240 mg, 0.25 mmol) was subjected to the procedure for the simultaneous Boc/*t*Bu removal and *C*-terminal activation to yield quantitatively the intermediate *N*-peptidoacyl indole, which was next submitted to the general macrocyclization procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 18:1) afforded the pure cyclic depsipeptide **30** (98 mg, 57%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.21 (d, *J* = 7.8 Hz, 1H), 7.64 (d, *J* = 7.4 Hz, 1H), 7.59 (d, *J* = 7.0 Hz, 1H), 7.51 (d, *J* = 7.1 Hz, 1H), 7.35 – 7.31 (m, 5H), 5.11 (s, 2H), 4.92 (d, *J* = 15.8 Hz, 1H), 4.72 (d, *J* = 15.7 Hz, 1H), 4.50 (m, 1H), 4.37 (m, 1H), 4.28 (m, 2H), 3.18 – 3.13 (m, 3H), 3.08 (dd, *J* = 14.0, 5.2 Hz, 1H), 2.09 (m, 1H), 1.86 (m, 2H), 1.72 (m, 1H), 1.52 – 1.49 (m, 4H), 0.93 – 0.84 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ **=** 11.1, 15.3, 17.6, 19.0 (*C*H$_3$), 24.2 (*C*H$_2$), 29.4, 30.3 (*C*H$_2$), 31.4 (*C*H), 36.5 (*C*H$_2$), 36.7 (*C*H), 39.6, 40.8 (*C*H$_2$), 53.4, 56.6, 58.7, 59.9 (*C*H), 63.4, 66.5 (*C*H$_2$), 126.9, 127.7, 127.9, 128.1, 128.5, 128.8, 129.1, 129.3 (*C*H), 136.3, 136.7 (*C*), 157.8, 169.8, 170.9, 171.5, 172.2, 172.8 (*C*O). HRMS (ESI-FT-ICR) *m/z*: 702.3477 [M+Na]$^+$, calcd. for $C_{36}H_{49}O_8NaN_5$: 702.3479.

Peptide 32: HCl·MeNH$_2$ (20 mg, 0.3 mmol), Et$_3$N (44 μL, 0.3 mmol), paraformaldehyde (9 mg, 0.3 mmol), peptide **31** (137 mg, 0.25 mmol) and isonitrile **1** (48 mg, 0.25 mmol) were reacted in MeOH/CH_2Cl_2 (5 mL, 5:1, *v*/*v*) for 24 h according to the general Ugi-4CR procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 15:1) afforded peptide **32** (180 mg, 92%) as a pale yellow amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.91 (s, 1H), 7.31 (m, 1H), 7.27 (m, 1H), 7.26 – 7.07 (m, 9H), 6.96

(m, 1H), 5.78 (m, 1H), 5.24 (m, 1H), 4.63 (t, *J* = 5.6 Hz, 1H), 4.59 – 4.52 (m, 4H), 4.36 (m, 1H), 4.08 (m, 1H), 3.39 (s, 3H), 3.37 (s, 3H), 3.25 (s, 3H), 3.08 (dd, *J* = 13.9, 5.0 Hz, 1H), 3.08 (dd, *J* = 13.9, 5.0 Hz, 1H), 2.96 (dd, *J* = 13.9, 8.0 Hz, 1H), 2.90 (m, 2H), 1.81 – 1.74 (m, 1H), 1.66 – 1.60 (m, 1H), 1.55 – 1.42 (m, 2H), 1.38 (s, 9H), 1.37 (d, *J* = 7.1 Hz, 3H), 0.96 (d, *J* = 6.4 Hz, 3H), 0.94 (d, *J* = 6.4 Hz, 3H), 0.87 (d, *J* = 6.4 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 17.9, 18.8, 19.0, 21.9, 22.1 (*C*H_3), 25.1 (*C*H), 28.2, 38.3 (*C*H_3), 40.4, 40.7, 41.3 (*C*H_2), 47.6 (*C*H), 51.4 (*C*H_2), 51.7, 51.9 (*C*H), 55.3 (*C*H_3), 55.7 (*C*H), 80.4 (*C*), 104.5 (*C*H), 122.3, 126.6, 126.9, 128.6, 129.2 (*C*H), 129.3 (*C*), 130.5, 131.2 (*C*H), 136.2, 136.6 (*C*), 155.8, 166.7, 171.5, 170.8, 171.9, 172.9 (*C*O). HRMS (ESI-FT-ICR) *m/z*: 805.4472 $[M+Na]^+$, calcd. for $C_{41}H_{62}O_9NaN_6$: 805.4476.

Cyclic peptide 33: Peptide **32** (156 mg, 0.2 mmol) was subjected to the procedure for the simultaneous Boc/*t*Bu removal and *C*-terminal activation to yield quantitatively the intermediate *N*-peptidoacyl indole, which was next submitted to the general macrocyclization procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 16:1) afforded the pure cyclic peptide **33** (101 mg, 61%) as a white amorphous solid. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.28 – 7.21 (m, 5H), 4.84 (dd, *J* = 9.9, 4.6 Hz, 1H), 4.55 (dd, *J* = 11.0, 3.8 Hz, 1H), 4.45 (q, *J* = 6.9 Hz, 1H), 4.38 (d, *J* = 14.1 Hz, 1H), 3.44 (d, *J* = 10.1 Hz, 1H), 3.34 (m, 1H), 3.31 (m, 1H), 3.24 (d, *J* = 14.1 Hz, 1H), 3.17 (s, 3H), 2.82 (dd, *J* = 11.0, 14.1 Hz, 1H), 2.51 – 2.43 (m, 1H), 1.73 – 1.62 (m, 1H), 1.53 – 1.46 (m, 2H), 1.39 (d, *J* = 6.9 Hz, 3H), 1.01 – 0.95 (m, 12H). ^{13}C NMR (100 MHz, CD_3OD): δ = 17.0, 20.0, 21.7, 23.6 (*C*H_3), 26.2, 29.7 (*C*H), 37.8 (*C*H_2), 38.3 (*C*H_3), 41.9 (*C*H_2), 49.3, 51.0 (*C*H), 54.6 (*C*H_2), 56.6, 66.2 (*C*H), 127.8, 129.5, 129.9 (*C*H), 138.9 (*C*), 172.1, 173.1, 174.5, 174.5, 175.2 (*C*O). HRMS (ESI-FT-ICR) *m/z*: 783.5354 $[M+H]^+$, calcd. for $C_{40}H_{73}O_{10}N_5$: 783.5357.

Cyclic peptide 36: $HCl{\cdot}MeNH_2$ (20 mg, 0.3 mmol), Et_3N (44 μL, 0.3 mmol), paraformaldehyde (9 mg, 0.3 mmol), peptide **34** (165 mg, 0.25 mmol) and isonitrile **1** (48 mg, 0.25 mmol) were reacted in MeOH/CH_2Cl_2 (5 mL, 5:1, *v*/*v*) for 24 h according to the general Ugi-4CR procedure to afford quantitatively peptide **35**. This latter peptide was subjected without further purification to the procedure for the simultaneous Boc removal and *C*-terminal activation to yield quantitatively the intermediate *N*-peptidoacyl indole, which was next submitted to the general macrocyclization procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 18:1) afforded the pure cyclic peptide **36** (106 mg, 69%) as a white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ **=** 7.54 (d, *J* = 7.2 Hz, 1H), 7.27 (br. m, 5H), 4.84 – 4.74 (m, 4H), 4.62 – 4.58 (m, 2H), 4.46 (m, 1H), 4.14 (m, 1H), 4.04 (d, *J* = 5.7 Hz, 1H), 3.97 (d, *J* = 7.2 Hz, 1H), 3.42 (d, *J* = 15.2 Hz, 1H), 3.40 (dd, *J* = 14.0, 4.4 Hz, 1H), 3.18 (s, 3H, C*H*$_3$N), 3.13 (m, 1H),

2.27 (m, 1H), 1.85 – 1.79 (m, 2H), 1.63 (m, 4H), 1.48 (d, *J* = 7.2, 3H,), 1.36 – 1.28 (m, 4H), 1.01 – 0.89 (m, 12H), 0.74 (d, *J* = 8.4 Hz, 3H), 0.72 (d, *J* = 9.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ **=** 11.0, 15.4, 15.9, 18.3, 19.6, 22.4, 22.9 (*C*H_3), 24.6 (*C*H), 25.0 (*C*H_2), 28.5, 29.7 (*C*H), 34.0 (*C*H), 36.3 (*C*H_3), 38.5, 40.7 (*C*H_2), 48.3 (*C*H), 54.6 (*C*H_2), 55.8, 55.9, 56.0, 56.1 (*C*H), 126.9, 128.6, 129.0 (*C*H), 137.0 (*C*), 170.3, 172.0, 172.8, 173.0, 174.1, 174.3 (*C*O). HRMS (ESI-FT-ICR) *m/z*: 637.3685 $[M+Na]^+$, calcd. for $C_{32}H_{50}O_6NaN_6$: 637.3689.

Cyclic peptide 39: $HCl{\cdot}MeNH_2$ (20 mg, 0.3 mmol), Et_3N (44 μL, 0.3 mmol), paraformaldehyde (9 mg, 0.3 mmol), peptide **37** (183 mg, 0.25 mmol) and isonitrile **1** (48 mg, 0.25 mmol) were reacted in MeOH/CH_2Cl_2 (5 mL, 5:1, *v*/*v*) for 24 h according to the general Ugi-4CR procedure to afford quantitatively peptide **38**. This latter peptide was subjected without further purification to the procedure for the simultaneous Boc removal and *C*-terminal activation to yield quantitatively the intermediate *N*-peptidoacyl indole, which was next submitted to the general macrocyclization procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 16:1) afforded the pure cyclic peptide **39** (108 mg, 63%) as a white amorphous solid. Mixture of the *S-cis* and *S-trans* isomers of the *N*-methylated amide bond. ^{1}H NMR (400 MHz, $CDCl_3$): δ **=** 8.81, 8.45 (2×d, *J* = 8.8 Hz, 1H), 8.41, 8.34 (2×d, *J* = 6.2 Hz, 1H), 7.71 (d, *J* = 9.5 Hz, 1H), 7.67, 7.47 (2×d, *J* = 5.4 Hz, 1H), 7.55, 7.41 (2×br. s, 1H), 7.27 (br. m, 5H), 6.73, 6.60 (2×d, *J* = 7.8 Hz, 1H), 4.70 (td, *J* = 10.2, 4.5 Hz, 1H), 4.60 (m, 1H), 4.54 – 4.45 (m, 2H), 4.27 – 4.12 (m, 4H), 3.30 (m, 1H), 3.29 (s, 3H, C*H*$_3$N), 3.20 (dd, *J* = 14.0, 4.4 Hz, 1H), 3.03 (dd, *J* = 14.1, 10.0 Hz, 1H), 1.91 (m, 2H), 1.69 (m, 2H), 1.62 (m, 1H), 1.42 (d, *J* = 7.3, 3H), 1.23 (d, *J* = 7.0, 3H), 1.01 – 0.84 (m, 18H, 6×C*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 18.1, 18.9, 20.9, 21.4, 22.3, 22.9, 23.1, 23.4 (*C*H_3), 24.6, 24.8, 32.4 (*C*H), 33.9 (*C*H_2), 36.8 (*C*H_3), 40.2, 40.2 (*C*H_2), 49.1, 49.7, 51.4, 52.2 (*C*H), 52.6 (*C*H_2), 55.8, 57.7 (*C*H), 127.0, 128.5, 128.6, 128.7, 129.0 (*C*H), 137.0 (*C*), 169.3, 171.3, 171.8, 172.3, 172.6, 173.0, 173.2 (*C*O). HRMS (ESI-FT-ICR) *m/z*: 708.4063 $[M+Na]^+$, calcd. for $C_{35}H_{55}O_7NaN_7$: 708.4060.

Boc-Glu-Leu-Phe-Lys(Cbz)-NHCH$_2$CH$_2$*p*F-C$_6$H$_4$ (40): N^{α}-Boc-Lys(Cbz)-OH (380 mg, 1.0 mmol) was coupled to 2-(4-fluorophenyl)ethan-1-amine (139 mg, 1.0 mmol) according to the peptide coupling procedure, following by deprotection of the *N*-terminus by Boc removal subsequent coupling of *N*-Boc-Phe-OH (265 mg, 1.0 mmol). Flash column chromatography purification (CH_2Cl_2/MeOH 18:1) furnished peptide Boc-Phe-Lys(Cbz)-NHCH$_2$CH$_2$*p*F-C$_6$H$_4$ (544 mg, 84%) as a white amorphous solid. This latter peptide (389 mg, 0.6 mmol) was subjected to deprotection of the *N*-terminus by the Boc removal procedure, followed by sequential coupling of *N*-Boc-Leu-OH (139 mg,

0.6 mmol) and *N*-Boc-Glu(OMe)-OH (157 mg, 0.6 mmol). The resulting crude peptide was dissolved in THF/H_2O (2:1, 10 mL) and LiOH (105 mg, 2.5 mmol) was added at 0 °C. The mixture was stirred at 0 °C for 3 h and then acidified with aquouse 10% $NaHSO_4$ to pH 3. The resulting phases are separated and the aqueous phase is additionally extracted with EtOAc (2×30 mL). The combined organic phases are dried over anhydrous Na_2SO_4 and concentrated under reduced pressure in a rotary evaporator to yield the crude deprotected peptide. Flash column chromatography purification (CH_2Cl_2/MeOH 15:1) furnished the peptide **40** (368 mg, 69%) as a white amorphous solid. ^{1}H NMR (400 MHz, CD_3OD) δ = 7.32 (d, *J* = 4.4 Hz, 4H), 7.28 – 7.16 (m, 8H), 6.98 (t, *J* = 8.8 Hz, 2H), 5.05 (m, 2H), 4.58 (dt, *J* = 14.4, 7.1 Hz, 1H), 4.24 (m, 2H), 4.11 – 3.99 (m, 1H), 3.36 (m, 2H), 3.11 (m, 3H), 2.97 (dd, *J* = 14.1, 8.7 Hz, 1H), 2.75 (t, *J* = 7.3 Hz, 2H), 2.37 (t, *J* = 7.5 Hz, 2H), 1.99 (m, 1H), 1.85 (m, 1H), 1.73 (m, 1H), 1.66 – 1.56 (m, 2H), 1.54 – 1.46 (m, 3H), 1.43 (s, 9H), 1.35 – 1.21 (m, 3H), 0.89 (d, *J* = 6.6 Hz, 3H), 0.85 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ = 22.1, 23.4 (*CH*$_3$), 24.1 (*CH*$_2$), 25.7 (*CH*), 28.1 (*CH*$_3$), 28.7, 30.4, 31.2, 32.7, 35.6, 38.2, 41.6, 41.9 (*CH*$_2$), 53.8, 54.9, 55.5, 56.1 (*CH*), 67.3 (*CH*$_2$), 80.8 (*C*), 115.9, 116.2, 127.9, 128.8, 128.9, 129.4, 129.5, 130.3, 130.4, 131.5, 131.6 (*CH*), 136.3, 138.2, 138.4 (*C*), 158.0, 158.8, (*CO*), 163.0 (d, $^1J_{C\text{-}F}$ = 242.7 Hz), 173.3, 173.9, 174.7, 174.8, 176.5 (*CO*). HRMS (ESI) *m/z*: 889.4509 [M-H]$^-$, calcd. for $[C_{47}H_{62}FN_6O_{10}]^-$ 889.4511.

Peptide 41: HCl·$MeNH_2$ (20 mg, 0.3 mmol), Et_3N (44 μL, 0.3 mmol), paraformaldehyde (9 mg, 0.3 mmol), peptide **40** (223 mg, 0.25 mmol) and isonitrile **2** (43.5 mg, 0.25 mmol) were reacted in MeOH/CH_2Cl_2 (5 mL, 5:1, *v*/*v*) for 24 h according to the general Ugi-4CR procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 15:1) afforded peptide **41** (260 mg, 94%) as a pale yellow amorphous solid. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.32 (d, *J* = 4.2 Hz, 4H), 7.29 – 7.16 (m, 8H), 6.99 (m, 2H), 5.49 (m, 1H), 5.06 (m, 2H), 4.60–4.48 (m, 2H), 4.29 – 4.16 (m, 2H), 4.14 – 3.97 (m, 4H), 3.41 – 3.34 (m, 6H), 3.30 (m, 6H), 3.20 – 3.06 (m, 4H), 3.02 (m, 2H), 2.98 – 2.87 (m, 2H), 2.76 (t, *J* = 7.3 Hz, 2H), 2.55 – 2.33 (m, 2H), 2.01 (m, 2H), 1.87 (m, 2H), 1.76 – 1.69 (m, 2H), 1.61 (m, 2H), 1.54 – 1.46 (m, 3H), 1.42 (s, 9H), 1.25 (m, 3H), 0.90 (d, *J* = 6.4 Hz, 3H), 0.86 (m, 3H). ^{13}C NMR (101 MHz, CD_3OD): δ = 22.0 (*C*H_3), 23.4 (*C*H), 24.1, 25.7 (*C*H_2), 28.8 (*C*H_3), 30.5, 32.7 (*C*H_2), 35.6 (*C*H_3), 36.8, 37.3, 38.1, 41.6, 42.0, 42.8, 52.1 (*C*H_2), 53.5, 53.6, 53.7, 56.2 (*C*H), 57.7, 57.7, (*C*H_3), 67.3 (*C*H_2), 77.7 (*C*H), 80.8 (*C*), 103.6, 116.0, 116.2, 127.8, 128.8, 128.9, 129.5, 129.6, 130.3, 131.5, 131.6 (*C*H), 136.3, 138.4, 138.5 (*C*), 158.9 (*C*O), 163.0 (d, $^1J_{C\text{-}F}$ = 242.7 Hz), 170.8, 171.3, 173.3, 173.9, 174.8, 174.9, 175.2 (*C*O). HRMS (ESI) *m/z*: 1105.5985 [M-H]$^-$, calcd. for $C_{57}H_{82}O_{13}N_8F$: 1105.5991.

Cyclic peptide 42: Peptide **41** (221 mg, 0.2 mmol) was dissolved in toluene (10 mL) and 10-camphorsulfonic acid (4.6 mg, 0.02 mmol) and quinoline (2.6 mg, 0.02 mmol) were added. The reaction mixture was stirred at reflux for 30 min, then diluted with 30 mL of EtOAc and washed with aqueous 10% HCl (15 mL). The aqueous phase was additionally extracted with EtOAc (2 × 25 mL) and the combined organic phases were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure in a rotary evaporator to yield the *C*-activated peptide. This latter compound was dissolved in EtOH (25 mL) and 10% Pd/C (60 mg) was added. The mixture was subjected successively to hydrogen atmosphere and vacuum and finally stirred under hydrogen atmosphere for 24 h. The catalyst was removed by filtration over a pad of Celite and the filtrate was evaporated under reduced pressure in a rotary evaporator. The resulting *N*-deprotected and *C*-activated peptide was subjected to the general macrocyclization procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 18:1) afforded the pure cyclic peptide **42** (94 mg, 58%) as a white amorphous solid. R_t = 11.9 min. ^{1}H NMR (600 MHz, CD_3OD): δ = 8.02 (m, 1H), 7.90 – 7.74 (m, 1H), 7.32 – 7.16 (m, 7H), 7.00 (m, 2H), 4.61 – 4.46 (m, 1H), 4.38 (m, 1H), 4.29 – 4.16 (m, 2H), 4.12 – 4.04 (m, 1H), 4.04 – 3.95 (m, 1H), 3.94 – 3.80 (m, 1H), 3.71 – 3.60 (m, 1H), 3.56 – 3.40 (m, 1H), 3.40 – 3.32 (m, 2H), 3.24 – 3.13 (m, 1H), 3.08 (m, 2H), 3.01 (m, 1H), 2.96 – 2.89 (m, 1H), 2.83 – 2.69 (m, 2H), 2.55 – 2.27 (m, 2H), 2.11 – 1.94 (m, 2H), 1.91 – 1.79 (m, 1H), 1.76 – 1.61 (m, 2H), 1.52 (m, 2H), 1.47 – 1.37 (m, 11H), 1.34 – 1.26 (m, 2H), 0.96 – 0.70 (m, 6H). ^{13}C NMR (151 MHz, CD_3OD): δ = 21.7, 23.5 (*C*H_3), 25.7 (*C*H), 28.7 (*C*H_3), 29.6, 30.8, 31.8, 32.1 (*C*H_2), 35.6 (*C*H_3), 37.2, 37.7, 40.0, 41.4, 41.9 (*C*H_2), 52.7, 53.7, 54.8, 56.3, 57.5 (*C*H), 80.7 (*C*), 116.0, 116.1, 127.9, 129.6, 130.1, 130.3, 131.6, (*C*H), 136.3, 138.4, (*C*), 157.8 (*C*O), 163.0 (d, $^1J_{C\text{-}F}$ = 242.7 Hz), 171.2, 173.5, 174.0, 174.9, 175.2 (*C*O). HRMS (ESI) *m/z*: 808.4416 $[M\text{-}H]^-$, calcd. for $C_{42}H_{59}O_8N_7F$: 808.4415.

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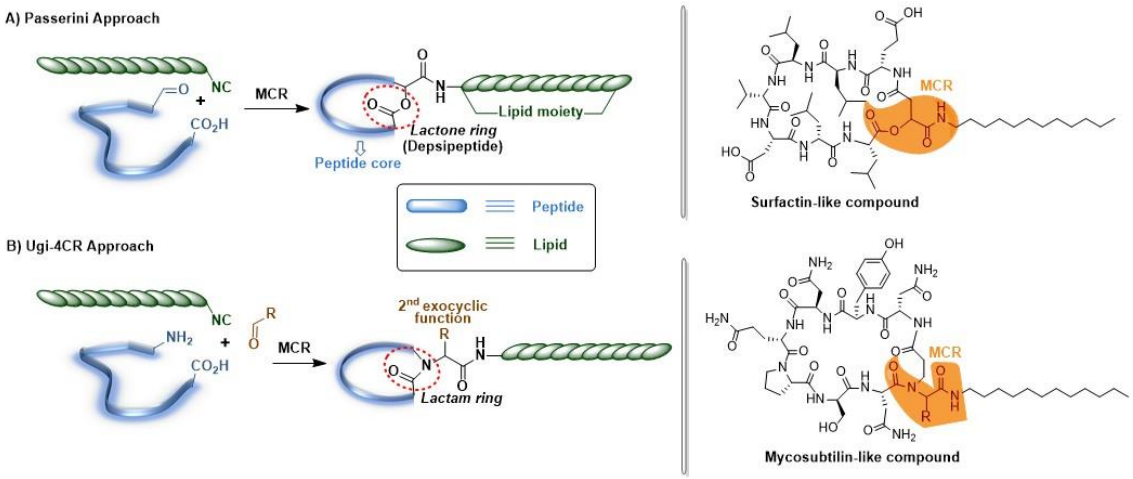
Summary and Outlook

Peptide functionalization and macrocyclization stands among the most challenging issues in peptide chemistry. While derivatization endows the peptide scaffold with some desired features, such as, reactive or biologically active moieties, cyclization is commonly used to anchor bioactive conformations as well as to study the structure - activity relationship of peptides. Combining both approaches in a single methodology is perfectly addressed by using multicomponent reactions (MCRs), which is able to accomplish ring closure and functionalization of peptides in one single step, providing an efficient and step-economical route of synthesis. The goal of this research project was to develop new strategies based on MCRs towards functionalization and macrocyclization of peptides as well as their applications in the synthesis of natural (-like) cyclic lipopeptides.

Chapter 1 highlights the current relevance of macrocycles and their potential applications in diverse ranging areas covering their scope in chemistry and biology. It also presents an overview about basic concepts of the IMCR-based macrocyclization, its uses in the synthesis of cyclopeptide scaffolds and its future perspectives.

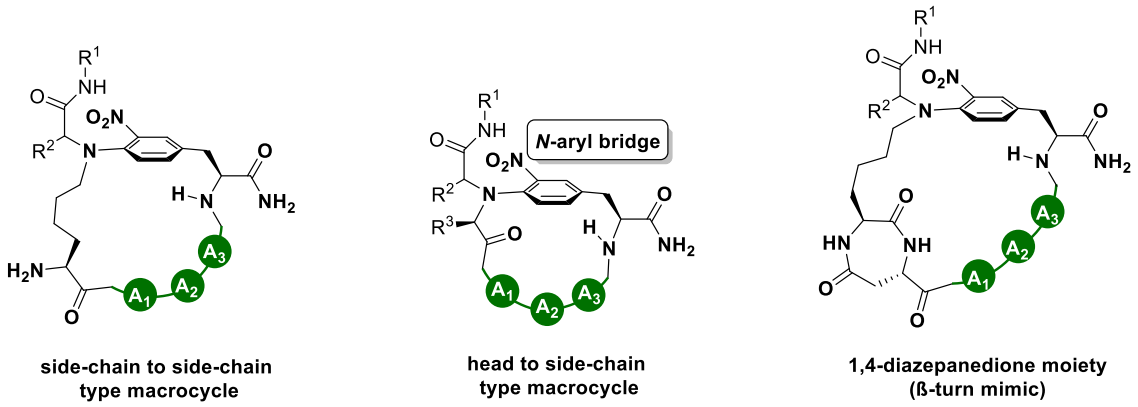
In Chapter 2, the synthesis and anticancer evaluation of surfactin and mycosubtilin analogues is described. The strategy makes use of the Ugi and Passerini reactions to carry on the peptides and depsipeptides macrocyclization and the construction of a small library of analogues of the above mentioned natural products (**Scheme 1**). The main feature of this method is the simultaneous incorporation of either one or two exocyclic lipid tails along with the macrocyclic ring closure, which is only possible due to the multicomponent nature of the macrocyclization step.



Scheme 1. Employment of a lipidic isocyanide in Passerini and Ugi reactions for the synthesis of surfactin and mycosubtilin analogues.

Interestingly, the installation of a second lipid moiety in the surfactin scaffold potentiate the cytotoxic activity up to five times in comparison to the natural product and changes the mode of action against the B16F10 cell line.

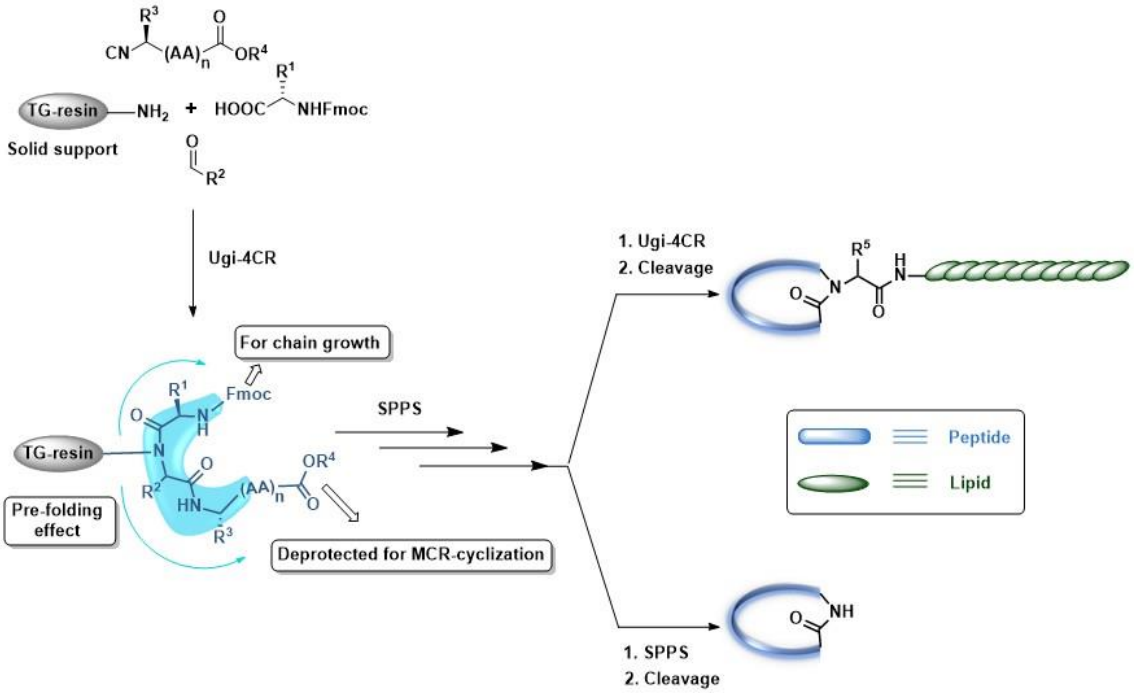
The focus of Chapter 3 lays on the development of a new multicomponent methodology for the solution and solid-phase macrocyclization of peptides via Ugi-Smiles reaction. This approach allows to create a structurally novel kind of cyclic peptides featuring an *N*-aryl bridge between the Tyr(NO_2) side chain and the *N*-terminus or a lysine side chain and containing an exocyclic moiety (lipidic or fluorescent) arising from the isocyanide component (**Scheme 2**). Both the on-resin and solution cyclizations took place with good to excellent efficiency in the presence of an aldehyde and a lipidic isocyanide, while the use of paraformaldehyde required an aminocatalysis-mediated imine formation prior to the on-resin Ugi-Smiles ring closure. Finally, the installation of a 1,4-diazepanedione moiety (ß-turn mimic) (**Scheme 2**) in the resin-bound peptide shortened the reaction time, likely by bringing closer the two reactive ends of the peptide precursor. HPLC and NMR evidence confirmed the presence of a single diastereomer of compounds produced with paraformaldehyde, i.e., either a single atropisomer is formed or atropisomers rapidly interconvert. This work constitutes the first application of the Ugi-Smiles reaction in the synthesis of cyclic peptides, and in macrocyclization approaches in general.



Scheme 2. Archetypal structures of the *N*-aryl bridged macrocycles obtained by the Ugi-Smiles reaction.

Chapter 4 describes the development of a new methodology to peptide head-to-tail cyclization on solid phase. The approach makes use of an Ugi-4CR to functionalize a commercial solid support endowing it with a bifunctional building block that acts as a loop-inducer mimic favoring the final on-resin cyclization (**Scheme 3**). The use of HATU as coupling reagent to trigger the cyclization showed a fast reaction (in 1 h all the starting material is consumed) and high cyclization efficiency in the synthesis of

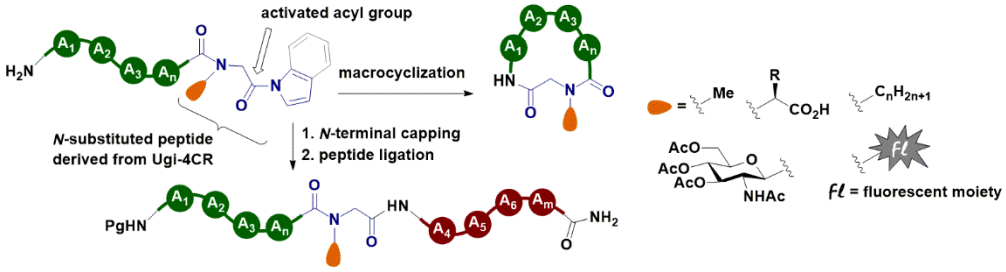
cyclic penta- and hexa- peptides. However, with classical amide formation reagents, the use of on-resin tetrapeptides yielded selectively the corresponding cyclic dimers. This outcome must be associated to intersite reactions on resin. On the other hand, the use of an Ugi reaction to perform the macrocyclizations on-resin allows ring closure and lipid moiety insertion in a single reaction step to produce cyclic lipopeptides of all sizes. An outstanding result of this cyclizing approach thus is the possibility to synthesize small cyclic peptide composed of three to six amino acids in excellent efficiency and without or at most minor formation of cyclic oligomers.



Scheme 3. An overview of the development of a turn-inducer in SPPS and its applications towards the synthesis of cyclic peptides.

In the Chapter 5 the applications of convertible isonitriles in the ligation and macrocyclization of multicomponent reaction-derived peptides and depsipeptides is described. The strategy relies on the use of convertible isonitriles – derived from Fukuyama amines – and peptide carboxylic acids in Ugi and Passerini reactions to afford *N*-alkylated peptides and depsipeptides, respectively, followed by conversion of the *C*-terminal amide onto either *N*-peptidoacyl indoles or pyrroles. Such activated peptides proved efficient in the ligation to peptidic, lipidic and fluorescent-labeled amines and in macrocyclization protocols (**Scheme 4**). Both ligation and macrocyclization procedures required either microwave irradiation or heating to 70 °C to proceed in reasonable time, thus providing a variety of *N*-substituted (cyclo)peptides

and a cyclic depsipetide. These results provide further evidence of the potential of convertible isonitriles and IMCRs as powerful synthetic tools in peptide chemistry.



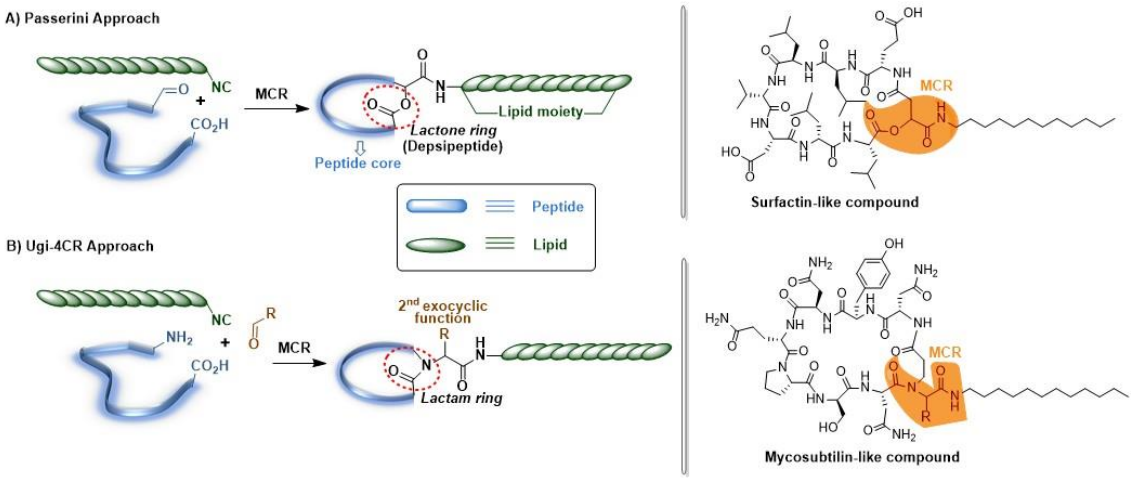
Scheme 4. Applications of convertible isonitriles in the ligation and macrocyclization of multicomponent reaction-derived peptides.

Zusammenfassung und Ausblick

Die Funktionalisierung und Makrozyklisierung von Peptiden stellt eine der größten Herausforderungen in der Peptidchemie dar. Während die Funktionalisierung der Peptidstruktur, wie zum Beispiel durch die Einführung von reaktiven oder biologisch aktiven Gruppen, zu erwünschten Eigenschaften führt, werden Zyklisierungen hingegen verwendet, um aktive Konformere zu stabilisieren und die Struktur-Wirkungsbeziehung besser zu bestimmen. Multikomponentenreaktionen (MCRs) sind bestens geeignet, um beide Modifizierungen in einem Reaktionsschritt durchzuführen. MCRs stellen nicht nur eine effiziente, sondern auch eine ökonomisch attraktive Syntheseroute dar. Das Ziel dieser Dissertation war die Entwicklung neuer Synthesestrategien unter Verwendung von Multikomponentenreaktionen, um die Funktionalisierung und Makrozyklisierung von Peptiden sowie die Synthese von naturähnlichen zyklischen Lipopeptiden zu realisieren.

Kapitel 1 stellt die gegenwärtige Bedeutung von Makrozyklen und deren potenzielle Anwendungen in verschiedenen Bereichen der Chemie und Biologie heraus. Weiterhin wird eine Einführung in die Grundkonzepte der Isonitril basierten Multikomponenten-Makrozyklisierungen gegeben und deren Anwendung in der Zyklopeptidsynthese sowie eine mögliche zukünftige Verwendung näher erläutert.

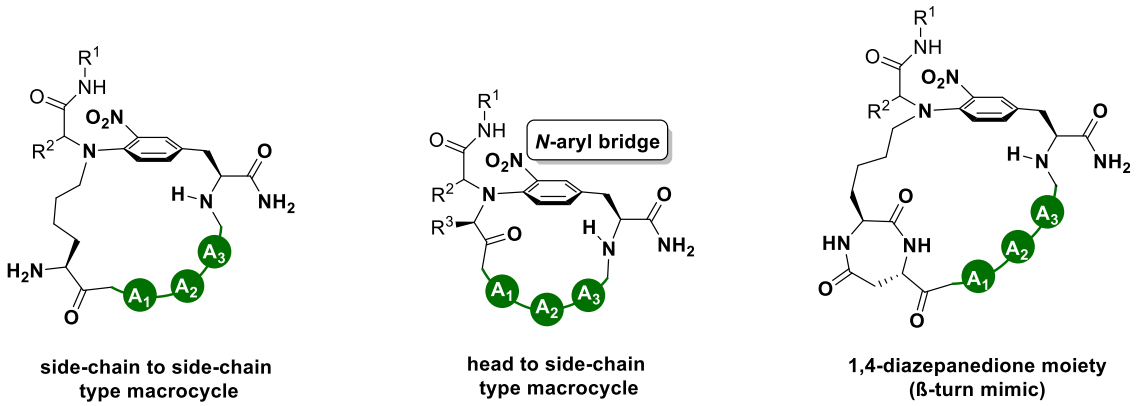
Im zweiten Kapitels wird die Synthese und biologische Aktivitätsuntersuchung bezüglich der Antitumorwirkung von Surfactin- und Mycosubtilin-Analoga beschrieben. Die angewendete Synthesestrategie beinhaltet Ugi- und Passerini-Reaktionen, um Peptid- und Depsipeptidmakrozyklisierungen durchzuführen und somit kleinere Bibliotheken von Analoga bereitzustellen (**Schema 1**).



Schema 1. Verwendung von langkettigen Alkylisocyaniden in Passerini- und Ugi-Reaktionen zur Synthese von Surfactin- und Mycosubtilin-Analoga.

Die Besonderheit dieser Methode besteht in der gleichzeitigen Makrozyklisierung und Einführung von ein oder zwei exozyklischen Alkylketten, welche effizient nur durch eine Multikomponentenreaktion ermöglicht werden kann. Interessantweise bewirkt die Einführung einer zweiten Alkylkette in das Surfactin-Grundgerüst eine Erhöhung der zytotoxischen Aktivität um das bis zu fünffache im Vergleich zum Naturstoff selbst und ändert die Wirkungsweise gegenüber der Zelllinie B16F10.

Fokus des dritten Kapitels ist die Entwicklung einer neuen Multikomponentenmethode, die Peptidmakrozyklisierungen via Ugi-Smiles-Reaktion in Lösung und an einer Festphase ermöglicht. Mit dieser Herangehensweise können neuartige Strukturen von zyklischen Peptiden hergestellt werden, die eine *N*-Aryl Brücke zwischen der Tyr(NO_2)-Seitenkette und dem *N*-Terminus oder einer Lysinseitenkette enthalten. Weiterhin kann eine exozyklische Gruppe, zum Beispiel Alkylketten oder fluoreszierende Sonden, mit Hilfe der Isonitrilkomponente eingeführt werden (**Schema 2**).

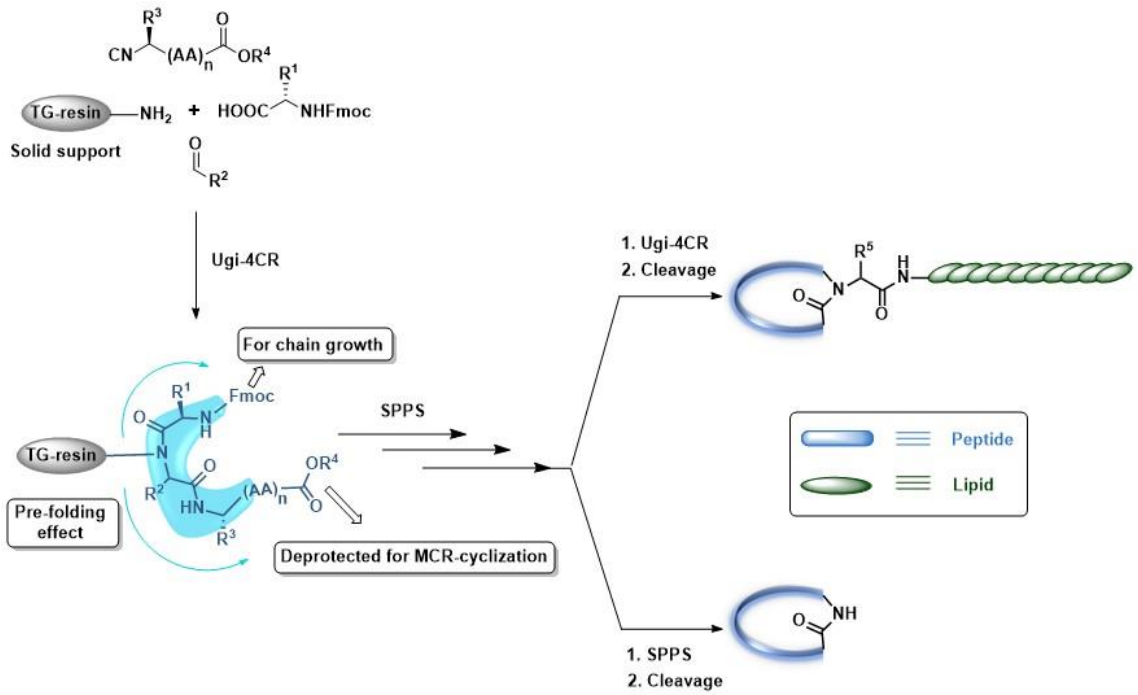


Schema 2. Grundstrukturen der *N*-Aryl verbrückten Makrozyklen, hergestellt mittels Ugi-Smiles-Reaktion.

Sowohl die an Festphase als auch die in Lösung durchgeführten Zyklisierungen wurden mit guter bis exzellenter Ausbeute in Gegenwart von Aldehyden und langkettigen Alkylisonitrilen durchgeführt. Bei der Verwendung von Paraformaldehyd war die Anwendung einer aminkatalysierten Transiminierung notwendig, woraufhin mittels Ugi-Smiles-Reaktion der Ringschluss am Harz vorgenommen werden konnte. Die Verwendung einer 2,5-Dioxohomopiperazinstruktur (ß-Schleifen Mimetika) (**Schema 2**) im harzgebundenen Peptid verkürzte die Reaktionszeit durch Zusammenführung der reaktiven Enden des Ausgangspeptides. Mit Hilfe von HPLC und NMR konnte nachgewiesen werden, dass nur ein Diastereomer mit Paraformaldehyd hergestellt wurde. Daher kann geschlussfolgert werden, dass nur ein Atropisomer gebildet wird oder mehrere Atropisomere, welche jedoch schnell

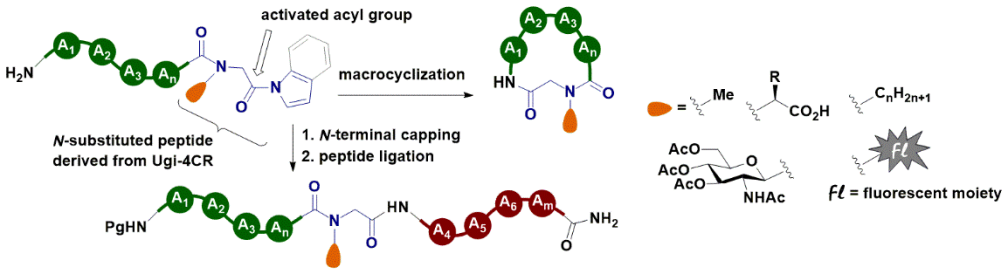
isomerisieren. Diese Arbeit stellt die erste Anwendung der Ugi-Smiles-Reaktion zur Synthese von zyklischen Peptiden und für Makrozyklisierungen dar.

In Kapitel 4 wird die Entwicklung einer neuen Festphasenmethode für die Kopf-Schwanz-Verknüpfung von Peptiden vorgestellt. Bei diesem Ansatz wird die Ugi-4CR für die Modifizierung einer kommerziell erhältlichen Festphase mit einem bifunktionellen Baustein genutzt, der Peptidschleifen induziert und so die Zyklisierung am Harz begünstigt (**Schema 3**). Die Verwendung von HATU als Kupplungsreagenz bewirkte schnelle Reaktionen, bei denen innerhalb einer Stunde das Startmaterial verbraucht war und zyklische Penta- sowie Hexapeptide mit hoher Effizienz hergestellt werden konnten. Jedoch entstehen bei Verwendung der klassischen Peptidknüpfungsreagenzien im Fall der harzgebundenen Tetrapeptide selektiv zyklische Dimere. Diese Ergebnisse stehen in Zusammenhang mit Nebenreaktionen zwischen den Peptidketten an der Festphase. So kann mit der Ugi-Reaktion sowohl der Ringschluss als auch die Einführung langkettiger Alkylketten in einem Reaktionsschritt erfolgen, wodurch zyklische Lipopeptide beliebiger Größe dargestellt werden können. Somit ist ein bedeutendes Ergebnis dieser Methode der neue Syntheseweg zu kleinen zyklischen Peptiden mit drei bis sechs Aminosäuren in exzellenter Ausbeute und sehr geringer Oligomerenbildung.



Schema 3. Übersicht über die Entwicklung eines Peptidschleifeninitiators in der Festphasensynthese und die Anwendung in der Synthese von zyklischen Peptiden.

Im fünften Kapitel wird die Anwendung von konvertierbaren Isonitrilen in der Ligation und Makrozyklisierung von Peptiden und Depsipeptiden beschrieben. Die Synthesestrategie basiert auf der Anwendung konvertierbarer Isonitrile, die aus dem Fukuyama-Amin hergestellt wurden, und Peptiden mit freiem *C*-Terminus, welche in Ugi- und Passerini-Reaktionen zu den gewünschten *N*-alkylierten Peptiden und Depsipeptiden umgesetzt werden. Nachfolgend wird das am *C*-Terminus vorkommende Amid entweder in *N*-Acyl-Indole oder -Pyrrole überführt. Diese aktivierten Amide zeigen effiziente Ligationen zu peptidischen, lipidischen und fluoreszierenden Aminen als auch in Makrozyklisierungsprotokollen (**Schema 4**). Beide Verfahren, Ligation und Makrozyklisierung, benötigen Energiezufuhr durch Mikrowellenstrahlung oder durch Erhitzen auf 70 °C, um in angemessener Zeit vollständig abzulaufen. Hierbei liefern diese Reaktionen verschiedene *N*-substituierte zyklische Peptide und Depsipeptide. Diese Ergebnisse zeigen das Potential von konvertierbaren Isonitrilen und der IMCR als leistungsstarke Synthesemethode in der Peptidchemie.



Schema 4. Anwendung von konvertierbaren Isonitrilen in Ligations- und Makrozyklisierungsreaktionen multikomponentbasierter Peptide.

Attachments

S1- RP-UHPLC chromatogram of crude cyclic peptide **1** (Chapter 4).

S2- RP-UHPLC chromatogram and ESI-HRMS of pure cyclic peptide **1** (Chapter 4).

S3- ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of cyclic peptide **1** (Chapter 4).

S4- ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of cyclic peptide **1** (Chapter 4).

S5- RP-UHPLC chromatogram and ESI-HRMS of cyclic peptide **2** (Chapter 4).

S6- ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of cyclic peptide **2** (Chapter 4)

S7- ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of cyclic peptide **2** (Chapter 4).

S8- RP-UHPLC chromatogram and ESI-HRMS of cyclic peptide **3** (Chapter 4).

S9- ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of cyclic peptide **3** (Chapter 4).

S10- ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of cyclic peptide **3** (Chapter 4).

S11- RP-UHPLC chromatogram and ESI-HRMS of cyclic peptide **4** (Chapter 4).

S12- ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of cyclic peptide **4** (Chapter 4).

S13- ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of cyclic peptide **4** (Chapter 4).

S14- RP-UHPLC chromatogram and ESI-HRMS of crude cyclic peptide **5** (monomer) and **6** (associated dimer) (Chapter 4).

S15- RP-UHPLC chromatogram and ESI-HRMS of pure cyclic peptide **5**. (Chapter 4).

S16- ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of cyclic peptide **5** (Chapter 4).

S17- ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of cyclic peptide **5** (Chapter 4).

S18- RP-UHPLC chromatogram and ESI-HRMS of pure cyclic peptide **6**. (Chapter 4).

S19- ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of cyclic peptide **6** (Chapter 4).

S20- ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of cyclic peptide **6** (Chapter 4).

S21- RP-HPLC chromatogram and ESI-HRMS of cyclic lipopeptide **7** (Chapter 4).

S22- ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of cyclic peptide **7** (Chapter 4).

S23- gHSQCAD (150 MHz, DMSO-d_6) spectrum of cyclic peptide **7** (Chapter 4).

S24- RP-HPLC chromatogram and ESI-HRMS of cyclic lipopeptide **8** (Chapter 4).

S25- ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of cyclic peptide **8** (Chapter 4).

S26- gHSQCAD (150 MHz, DMSO-d_6) spectrum of cyclic peptide **8** (Chapter 4).

S27- RP-HPLC chromatogram and ESI-HRMS of cyclic lipopeptide **9** (Chapter 4).

S28- ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of cyclic peptide **9** (Chapter 4).

S29- gHSQCAD (150 MHz, DMSO-d_6) spectrum of cyclic peptide **9** (Chapter 4).

S30- RP-HPLC chromatogram and ESI-HRMS of cyclic lipopeptide **10** (Chapter 4).

S31- ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of cyclic peptide **10** (Chapter 4).

S32- ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of cyclic peptide **10** (Chapter 4).

S33- Standard Intavis peptide synthesis protocol.

S35- Curriculum Vitae and List of Publications.

S39- Declaration (Erklärung).

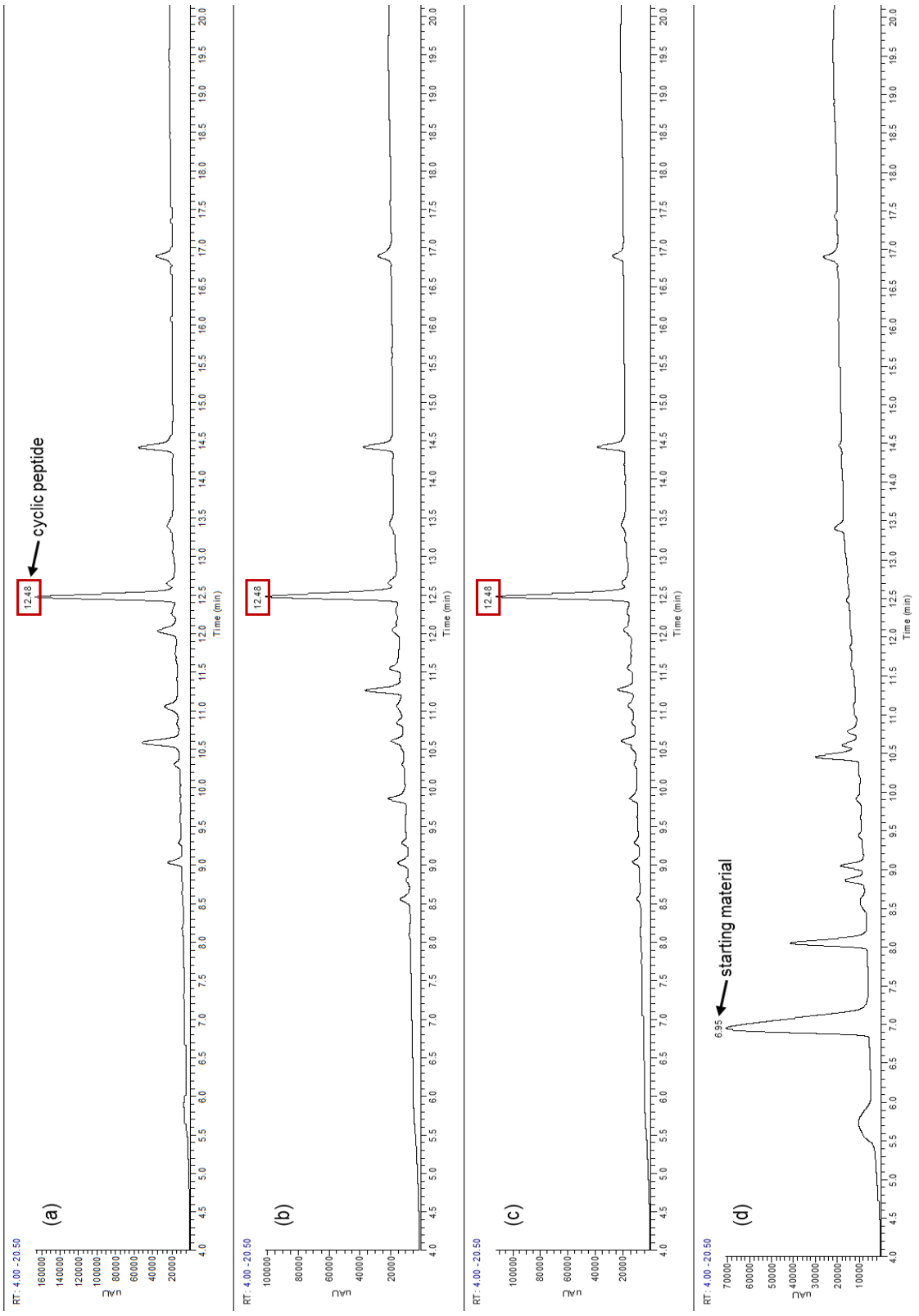


Figure S1 - RP-UHPLC chromatogram of crude cyclic peptide **1** (Chapter 4) under different coupling reagent. (a) PyBOP. (b) HBTU. (c) HATU. (d) Oxyma/DIC.

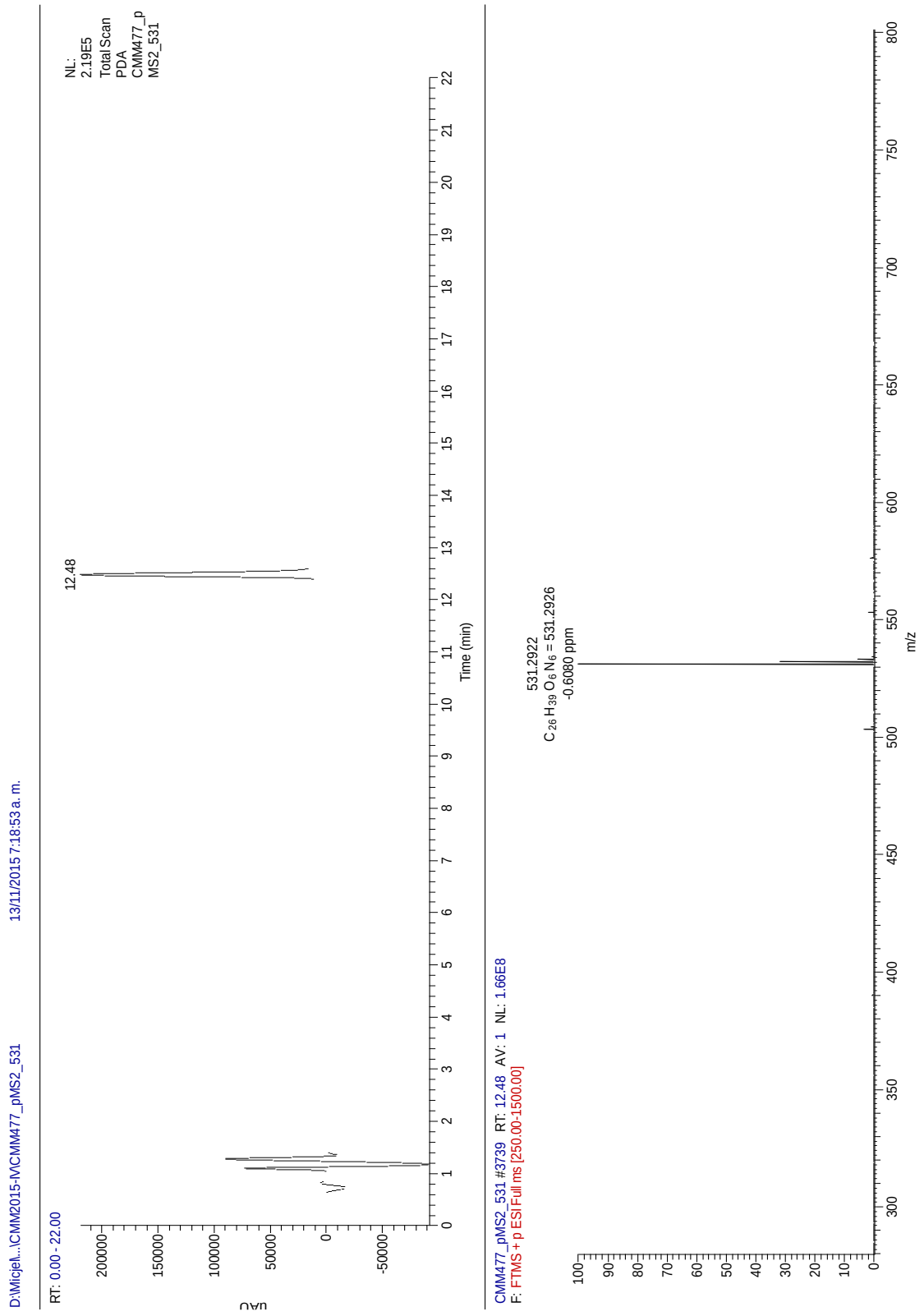


Figure S2 - RP-UHPLC chromatogram and ESI-HRMS of purified cyclic peptide **1** (Chapter 4).

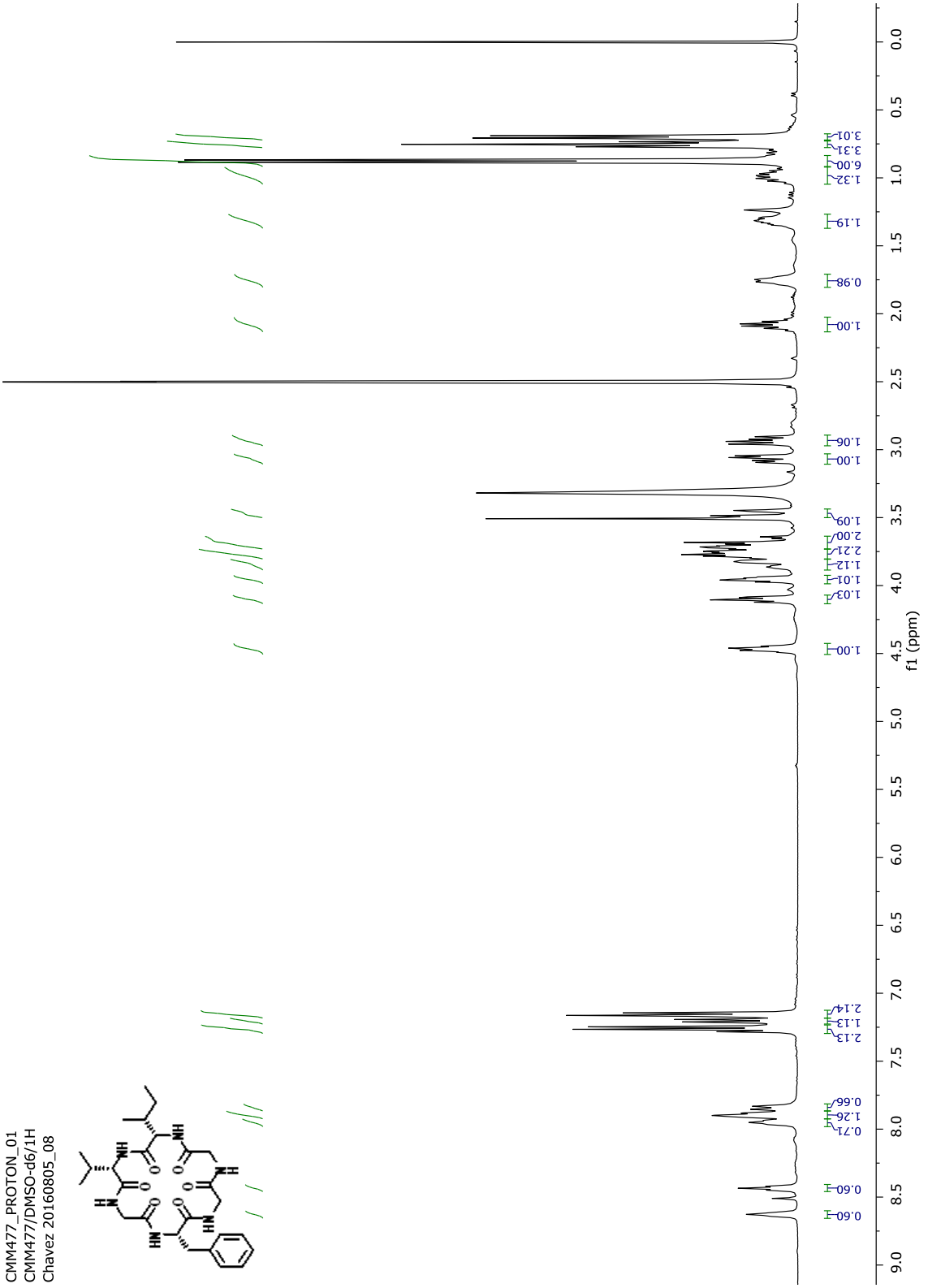


Figure S3 - ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of cyclic peptide **1** (Chapter 4).

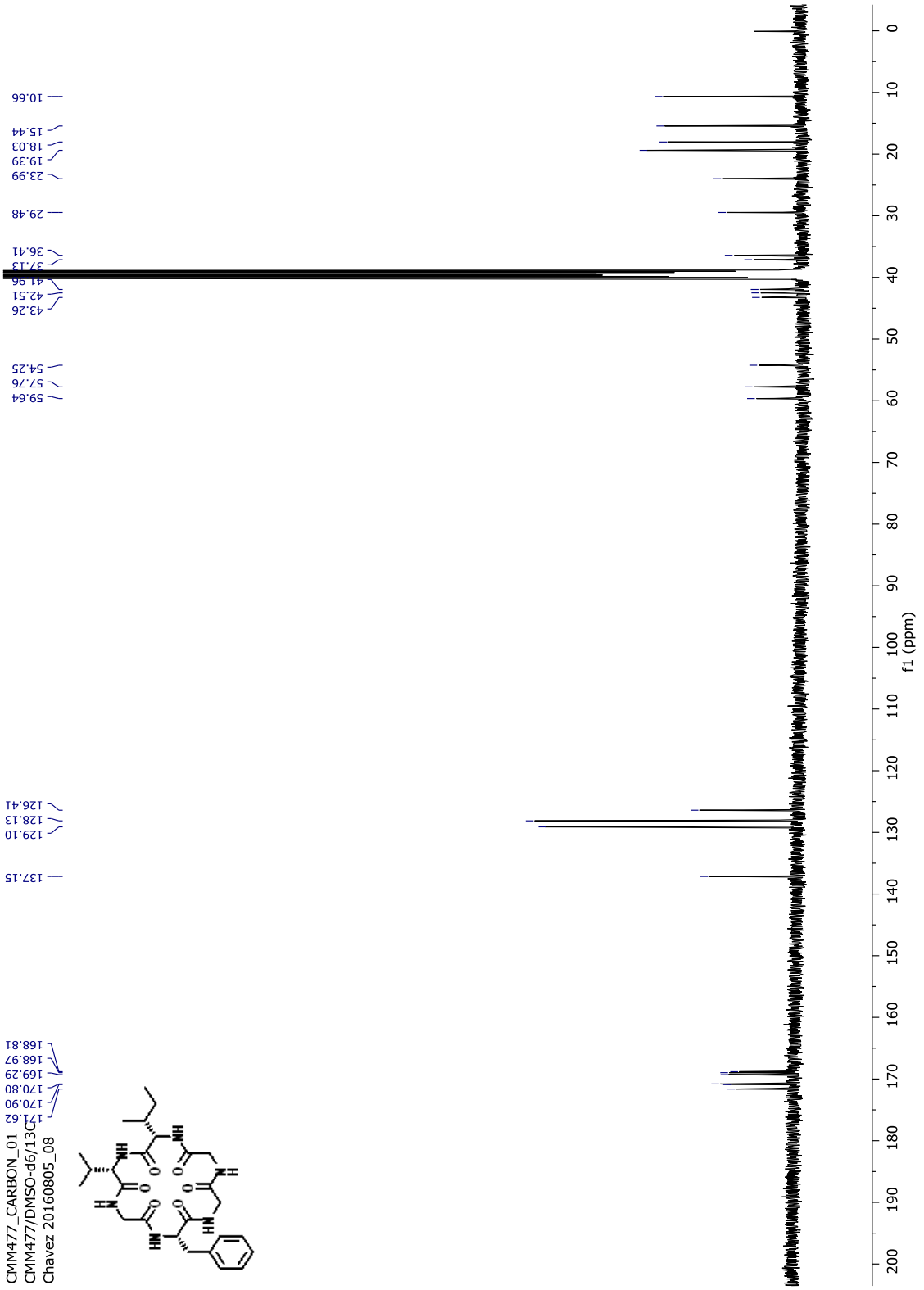


Figure S4 - ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of cyclic peptide **1** (Chapter 4).

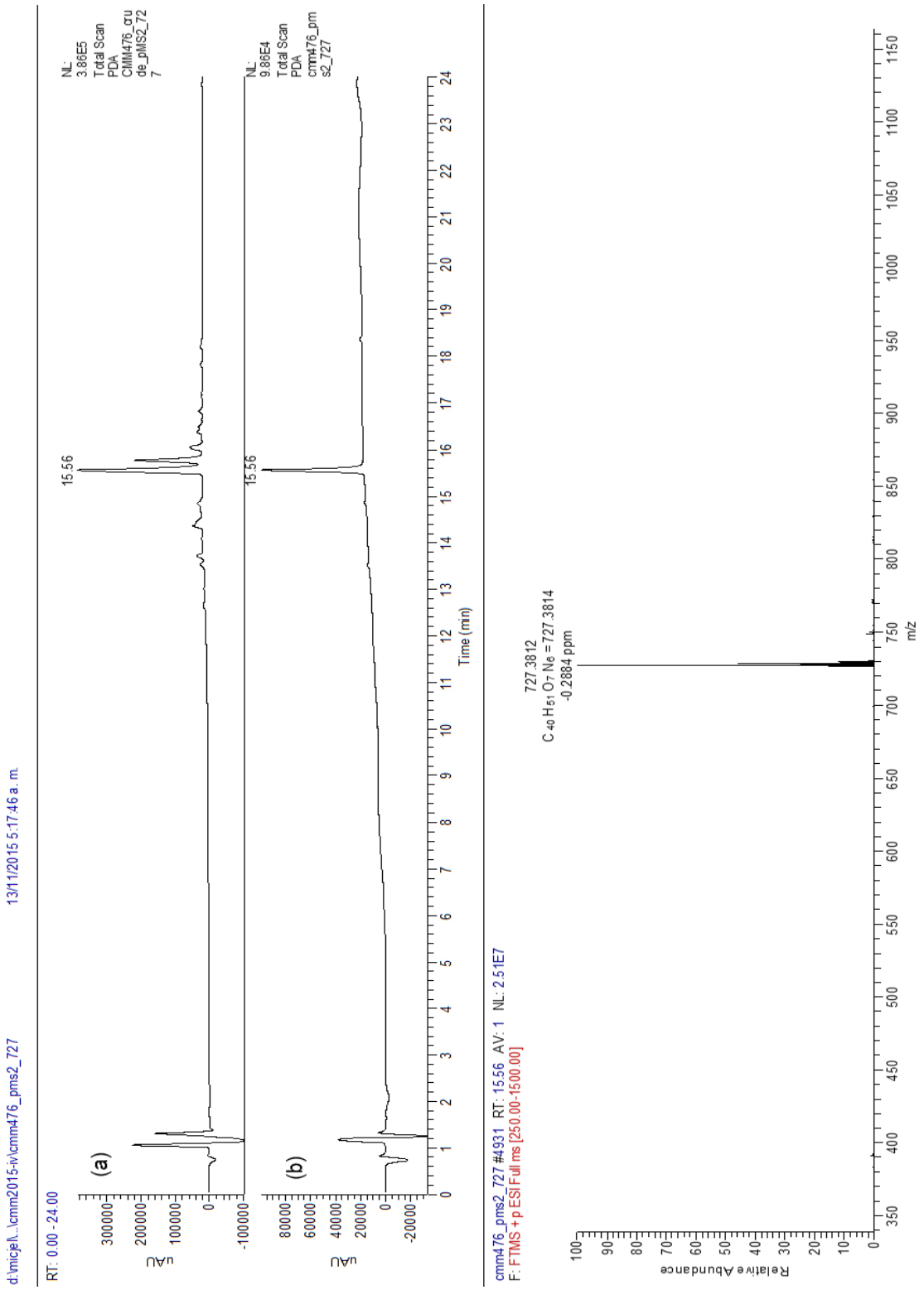


Figure S5 - RP-UHPLC chromatogram and ESI-HRMS of cyclic peptide **2** (Chapter 4). (a) Crude material after resin cleavage. (b) Pure compound after preparative HPLC purification.

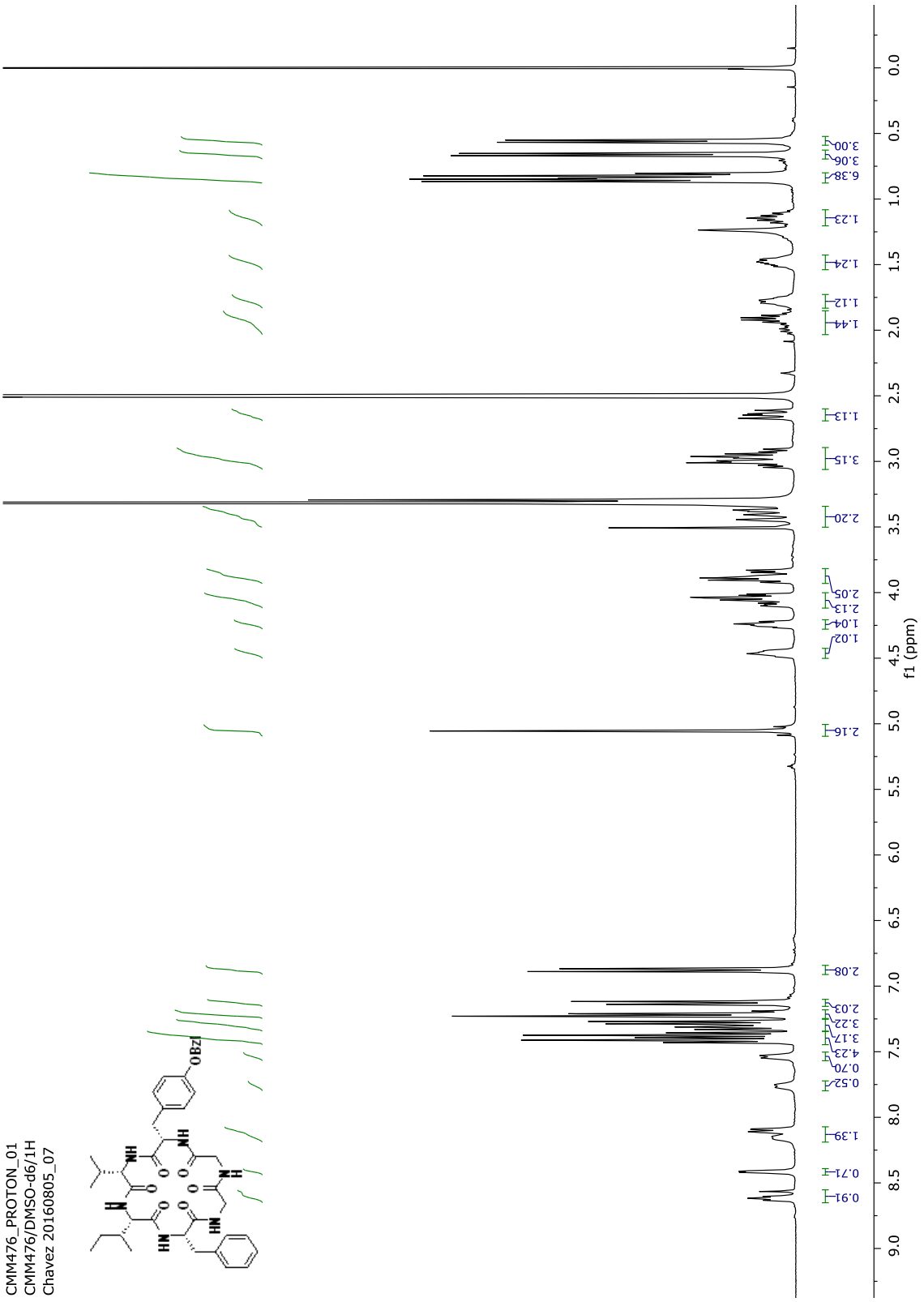


Figure S6 - ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of cyclic peptide **2** (Chapter 4).

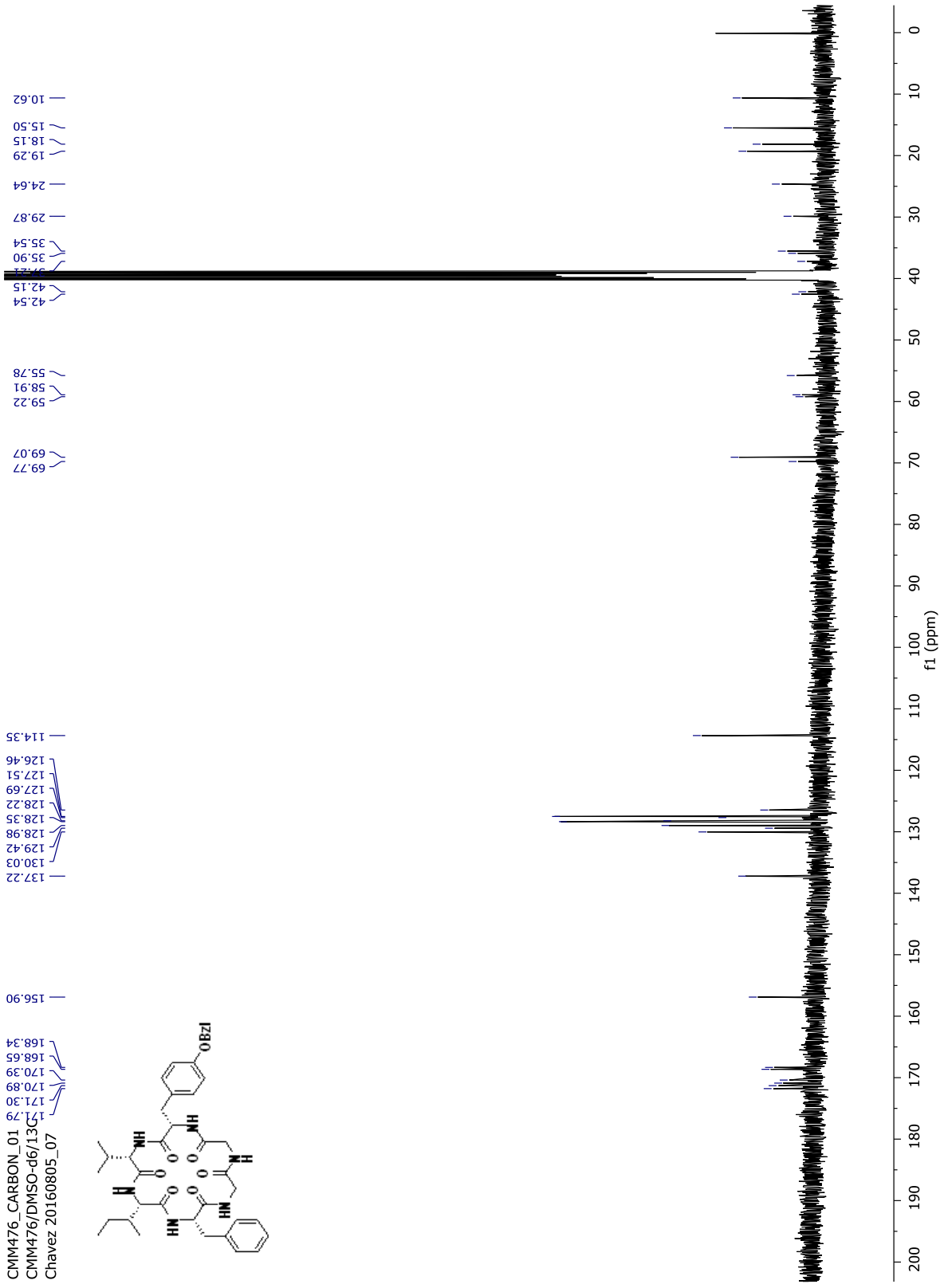


Figure S7 - ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of cyclic peptide **2** (Chapter 4).

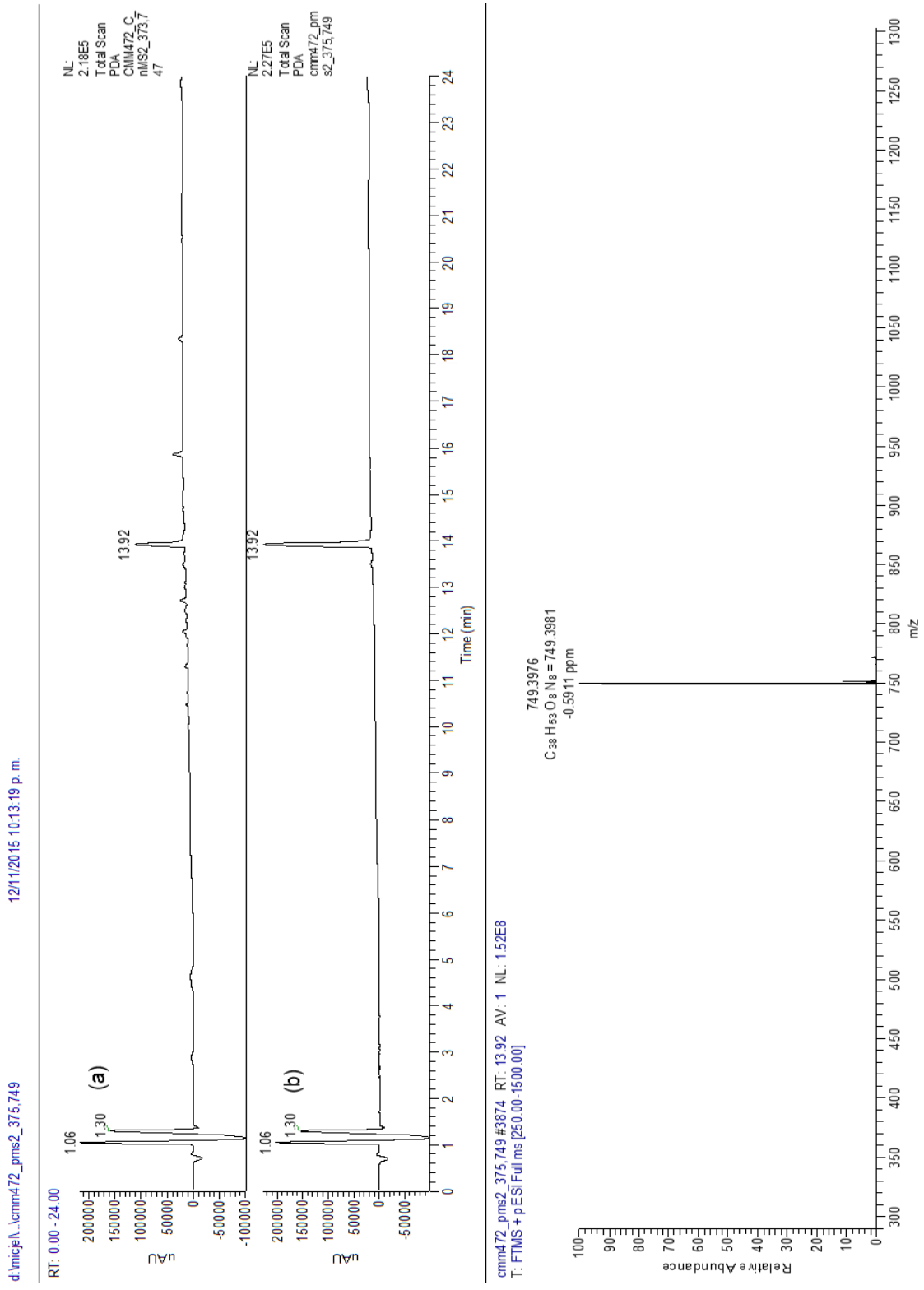


Figure S8 - RP-UHPLC chromatogram and ESI-HRMS of cyclic peptide **3** (Chapter 4). (a) Crude material after resin cleavage. (b) Pure compound after preparative HPLC purification.

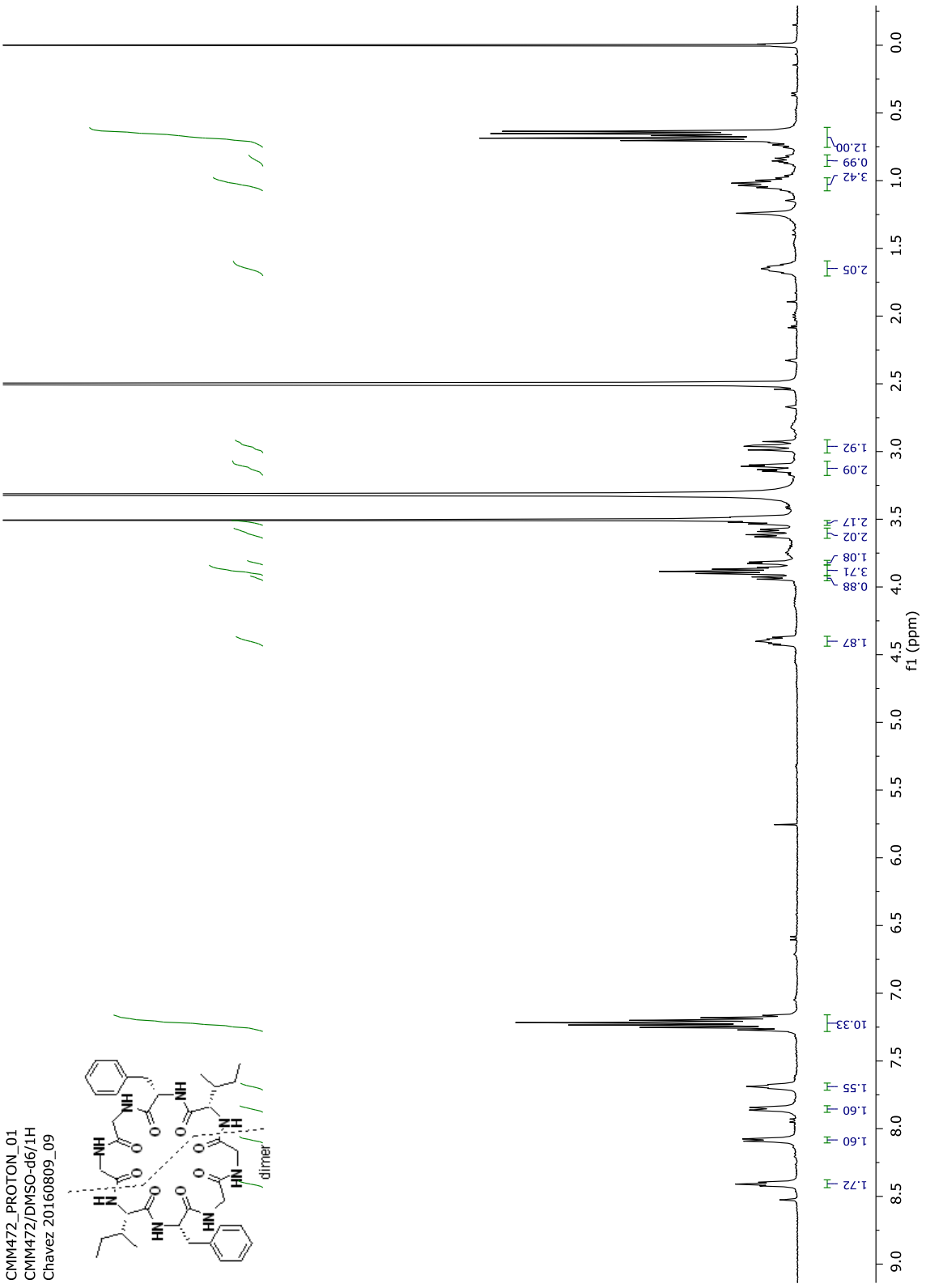


Figure S9 - ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of cyclic peptide **3** (Chapter 4).

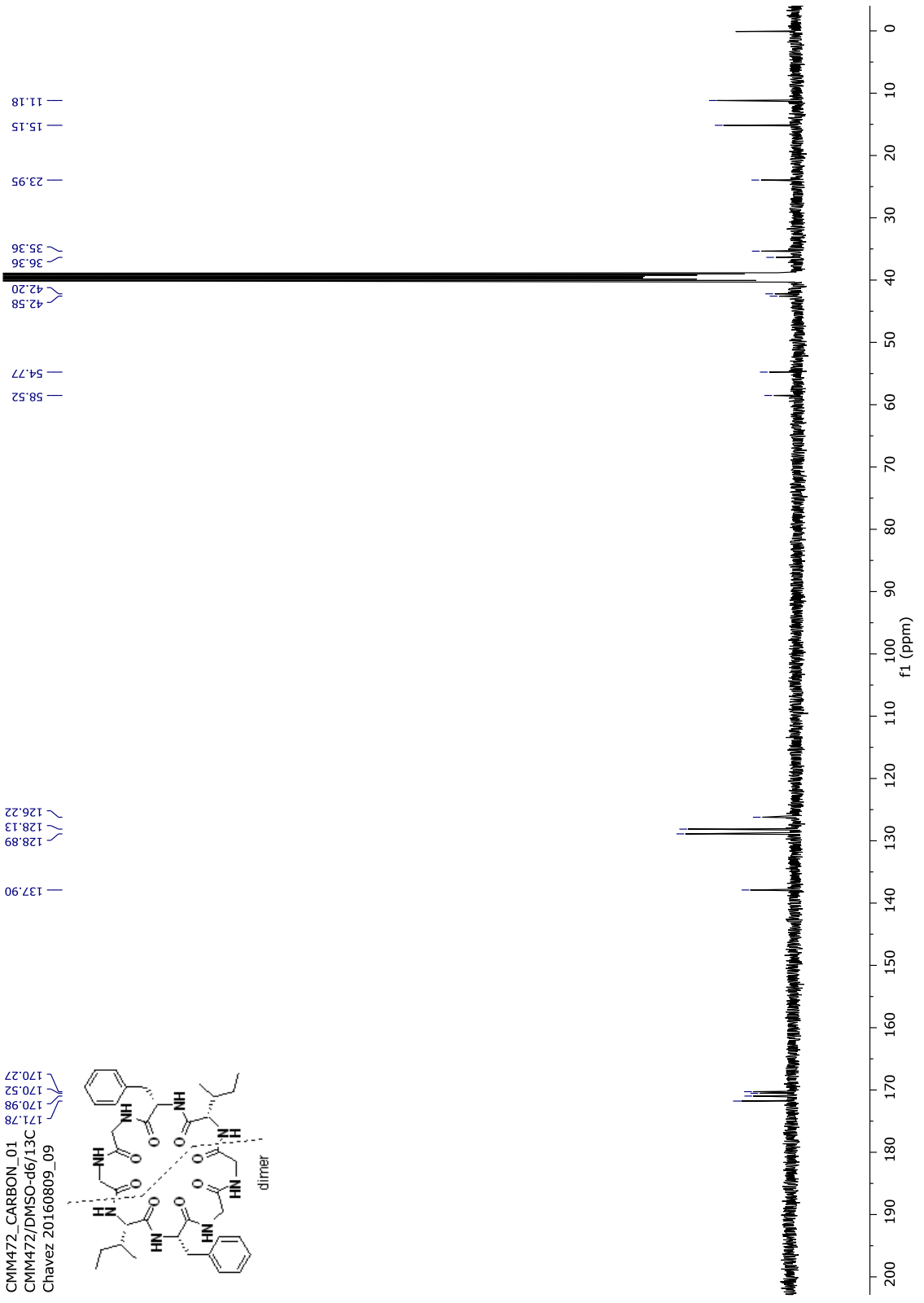


Figure S10 - ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of cyclic peptide **3** (Chapter 4).

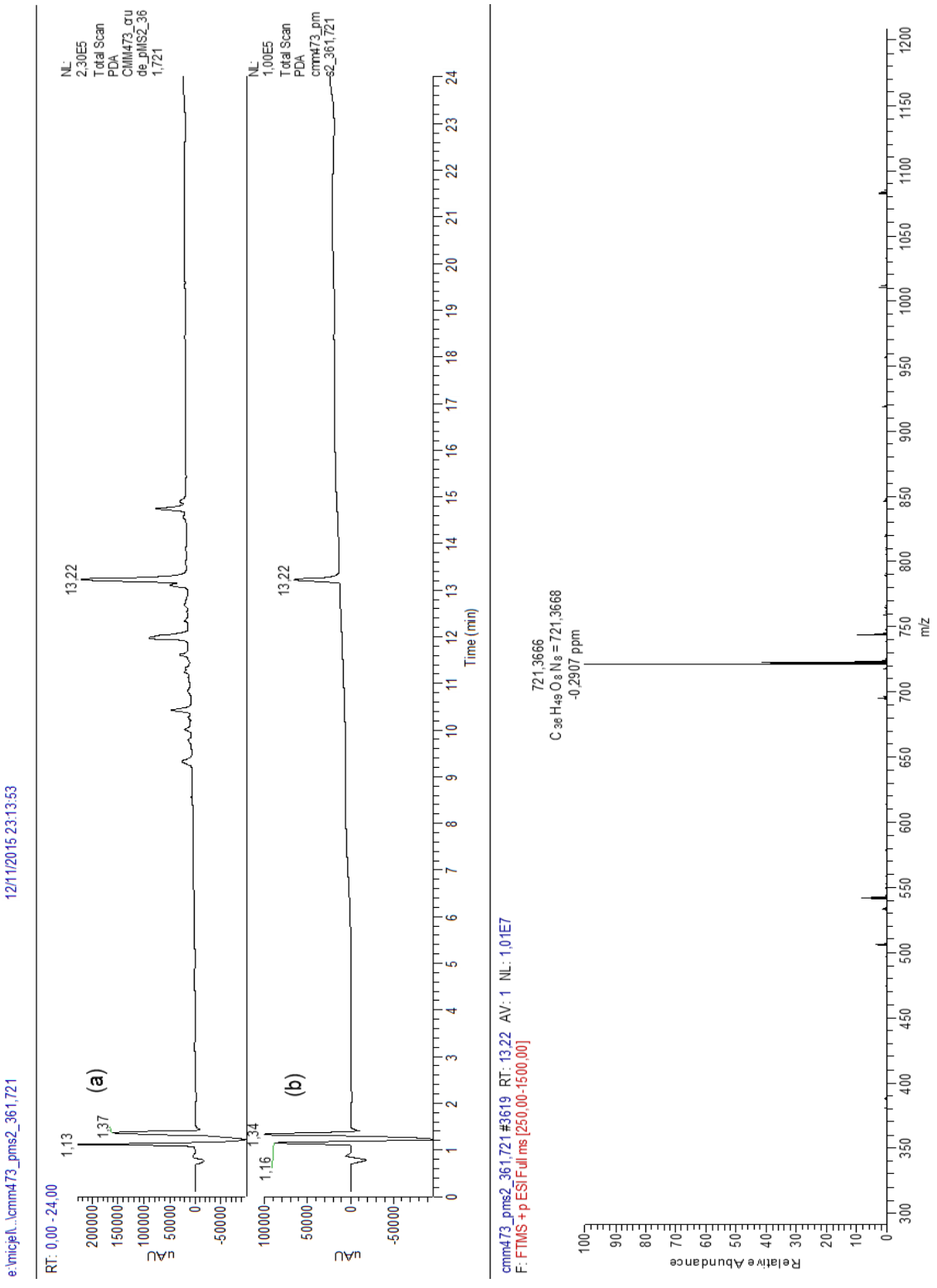


Figure S11 - RP-UHPLC chromatogram and ESI-HRMS of cyclic peptide **4** (Chapter 4). (a) Crude material after resin cleavage. (b) Pure compound after preparative HPLC purification.

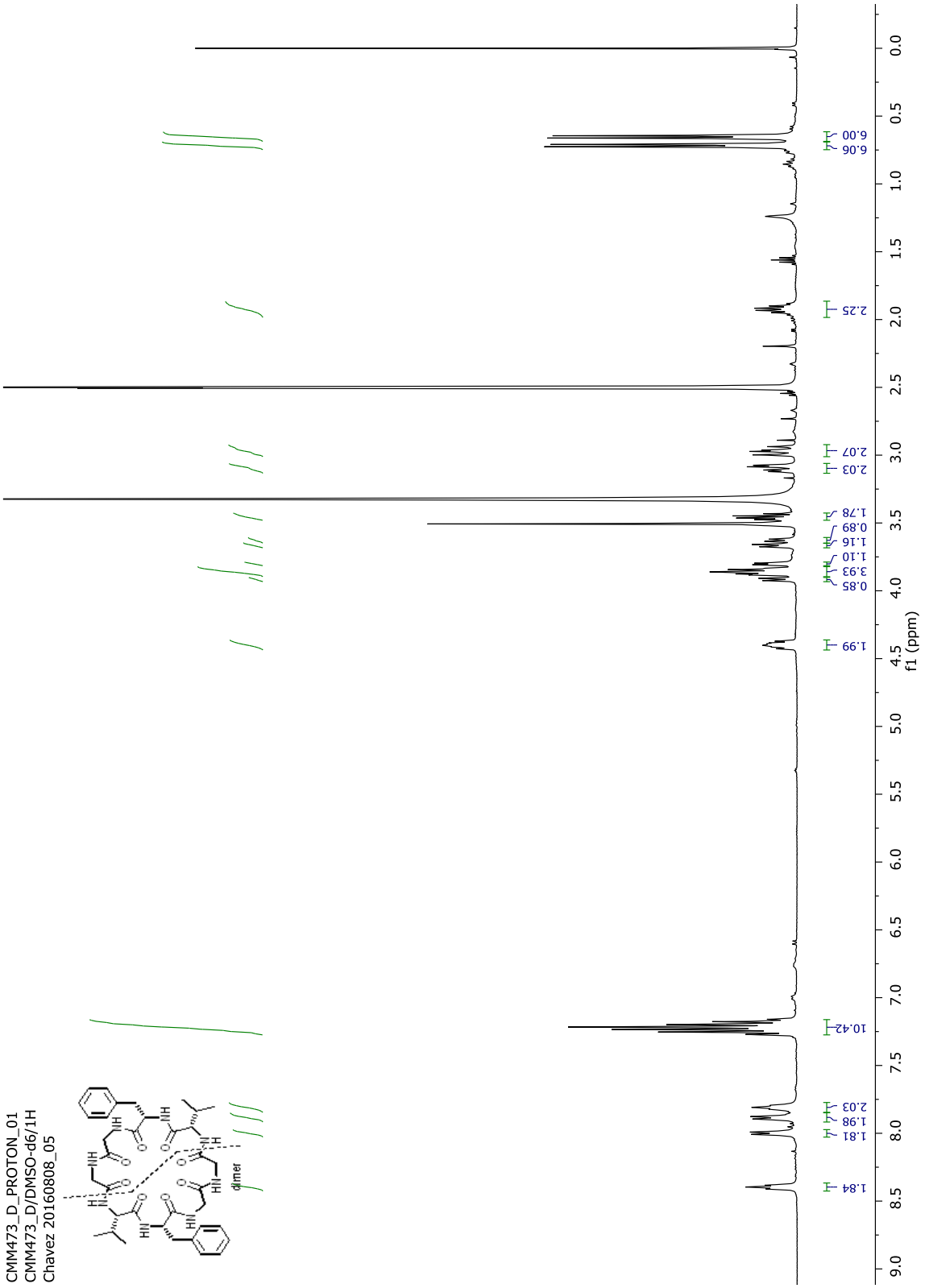


Figure S12 - ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of cyclic peptide **4** (Chapter 4).

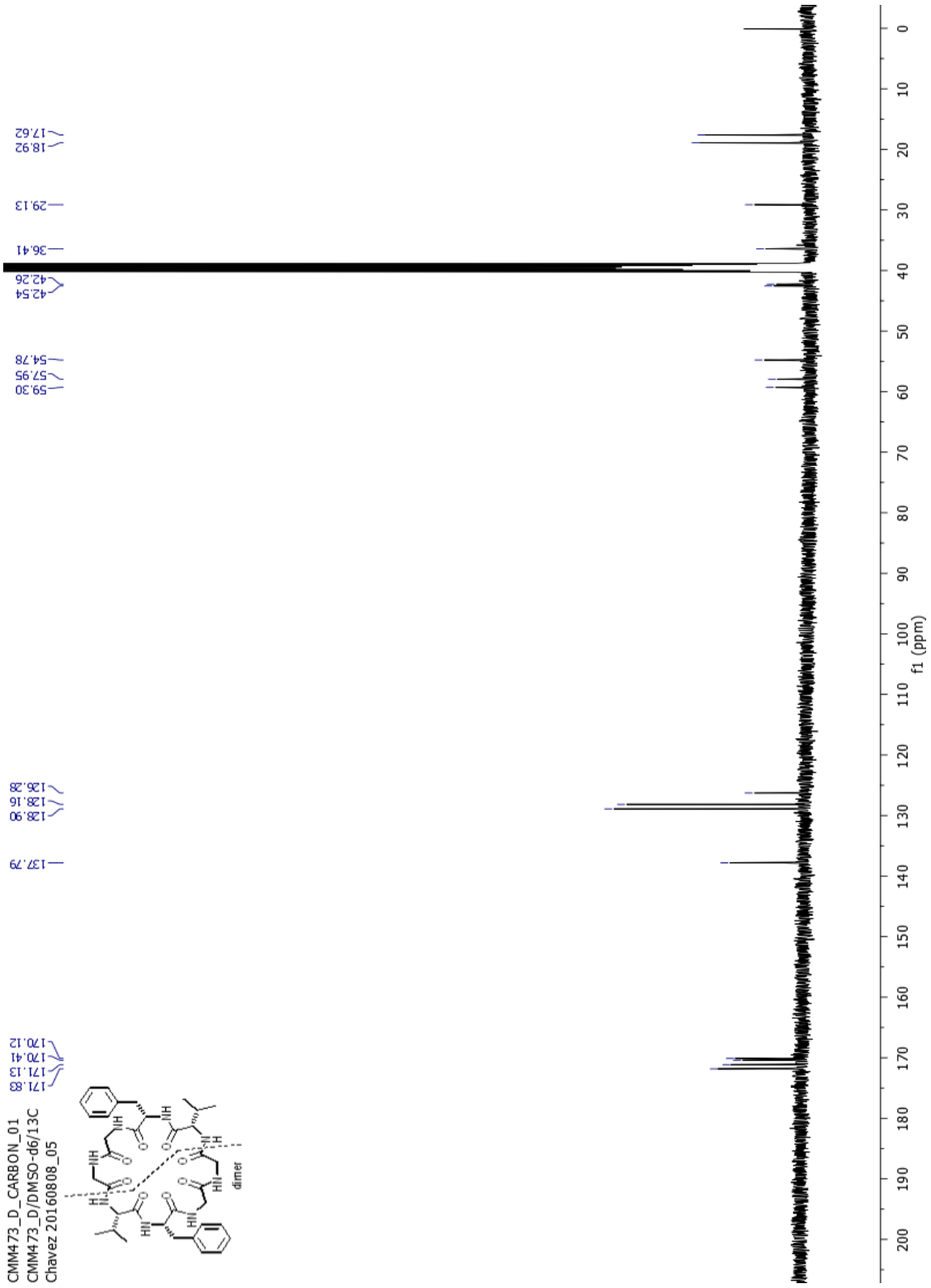


Figure S13 - ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of cyclic peptide **4** (Chapter 4).

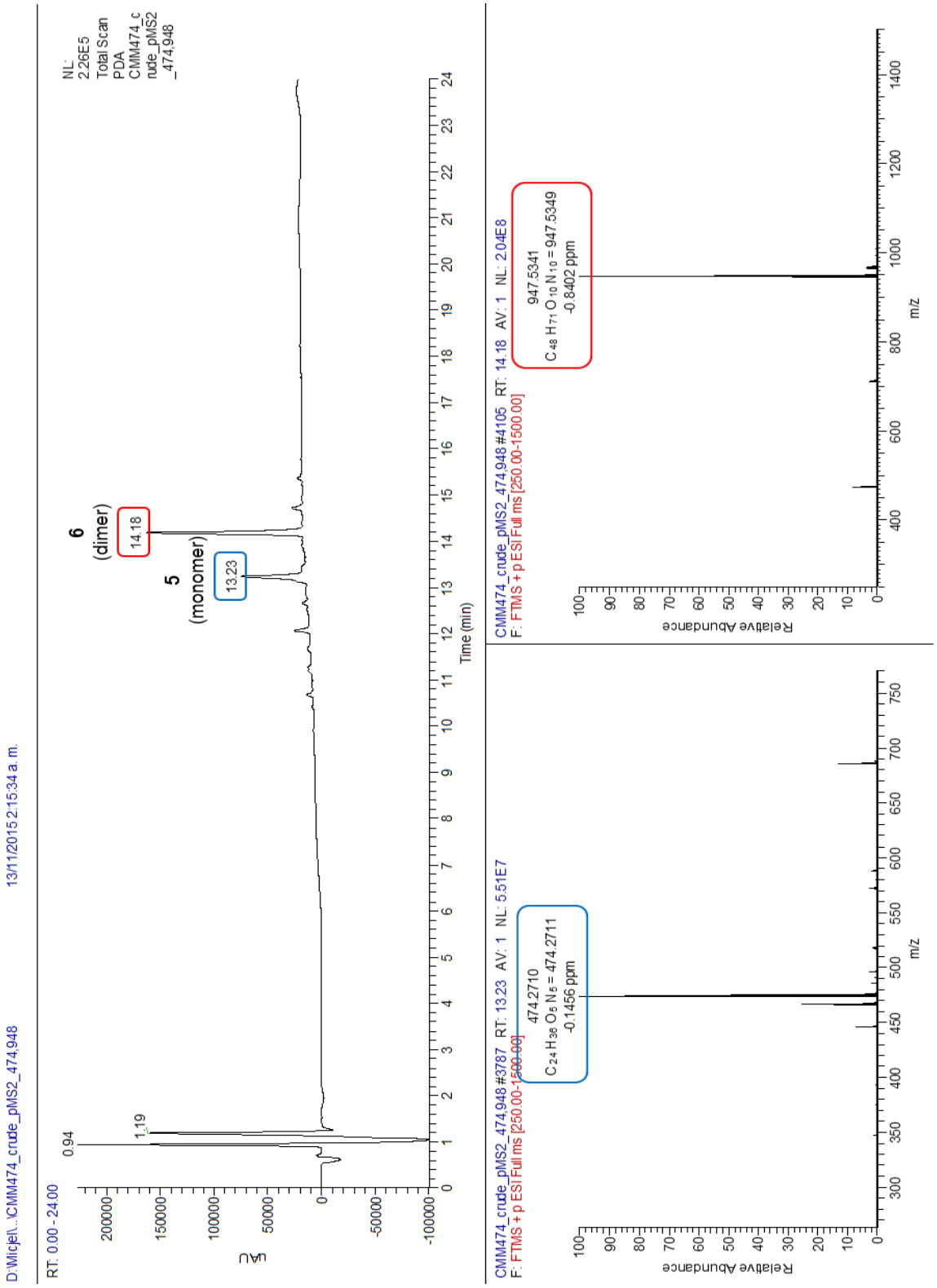


Figure S14 - RP-UHPLC chromatogram of crude material after resin cleavage and ESI-HRMS of cyclic peptide **5** (monomer) and **6** (associated dimer) (Chapter 4).

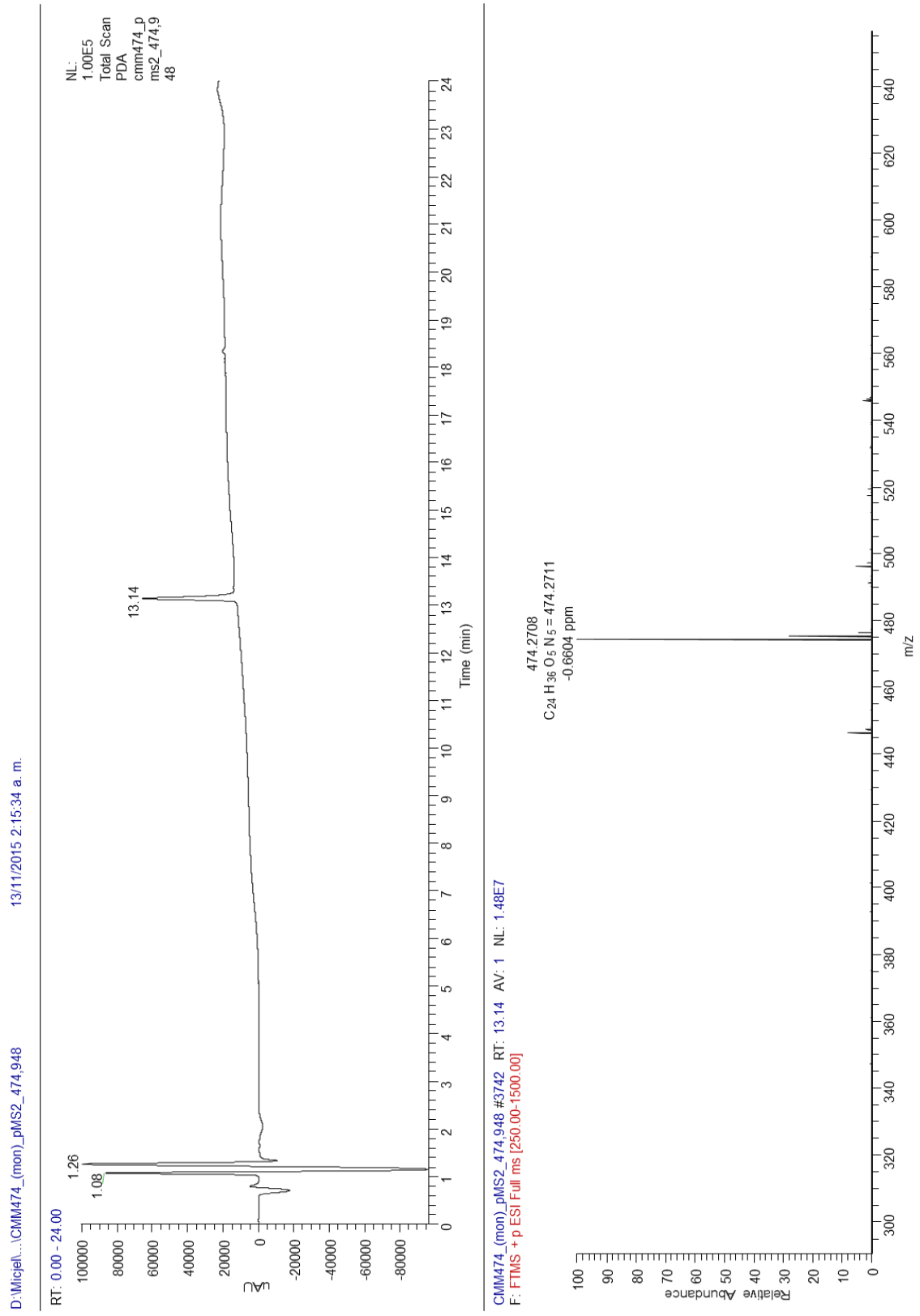


Figure S15 - RP-UHPLC chromatogram and ESI-HRMS of pure cyclic peptide **5**. (Chapter 4).

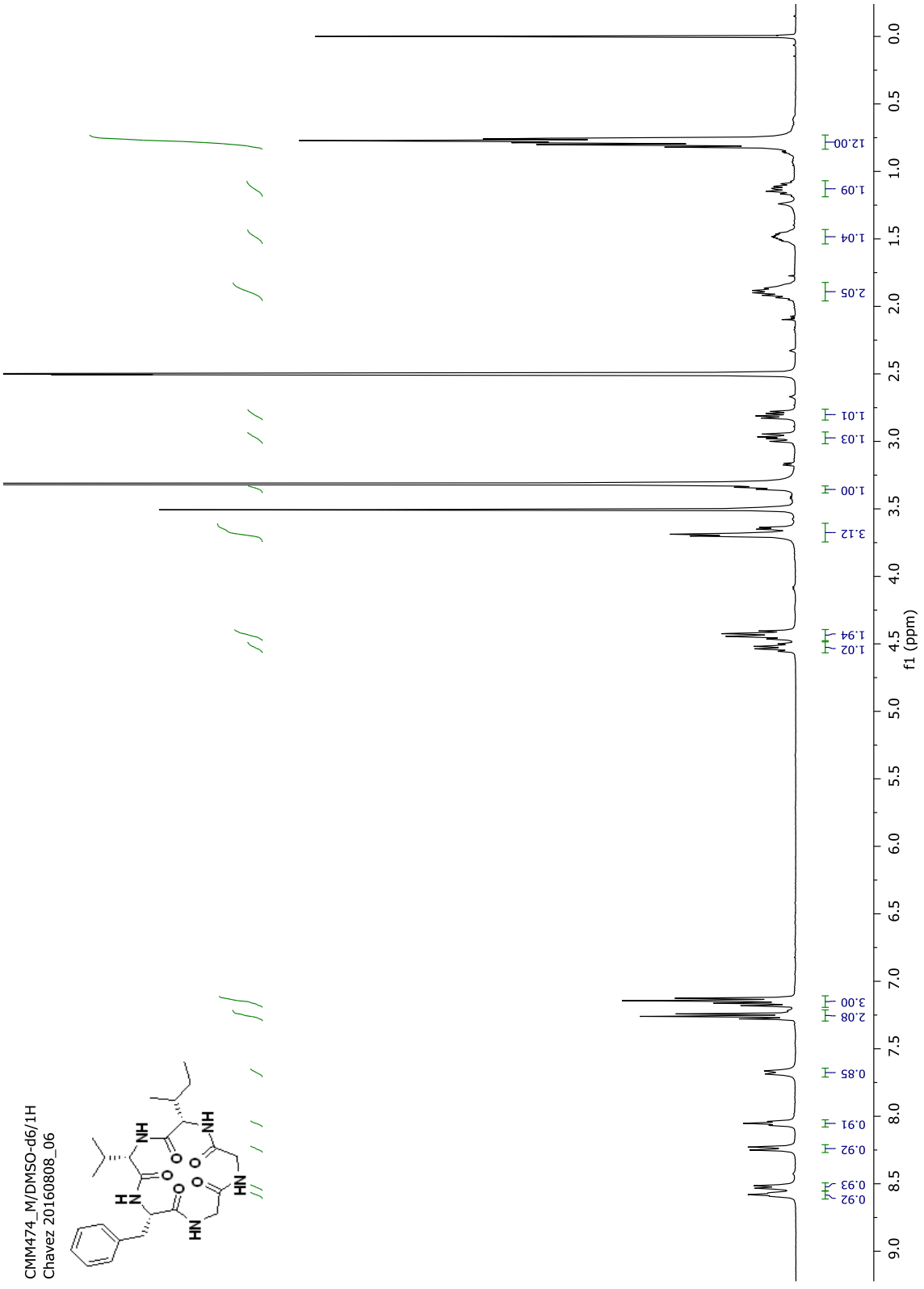


Figure S16 - ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of cyclic peptide **5** (Chapter 4).

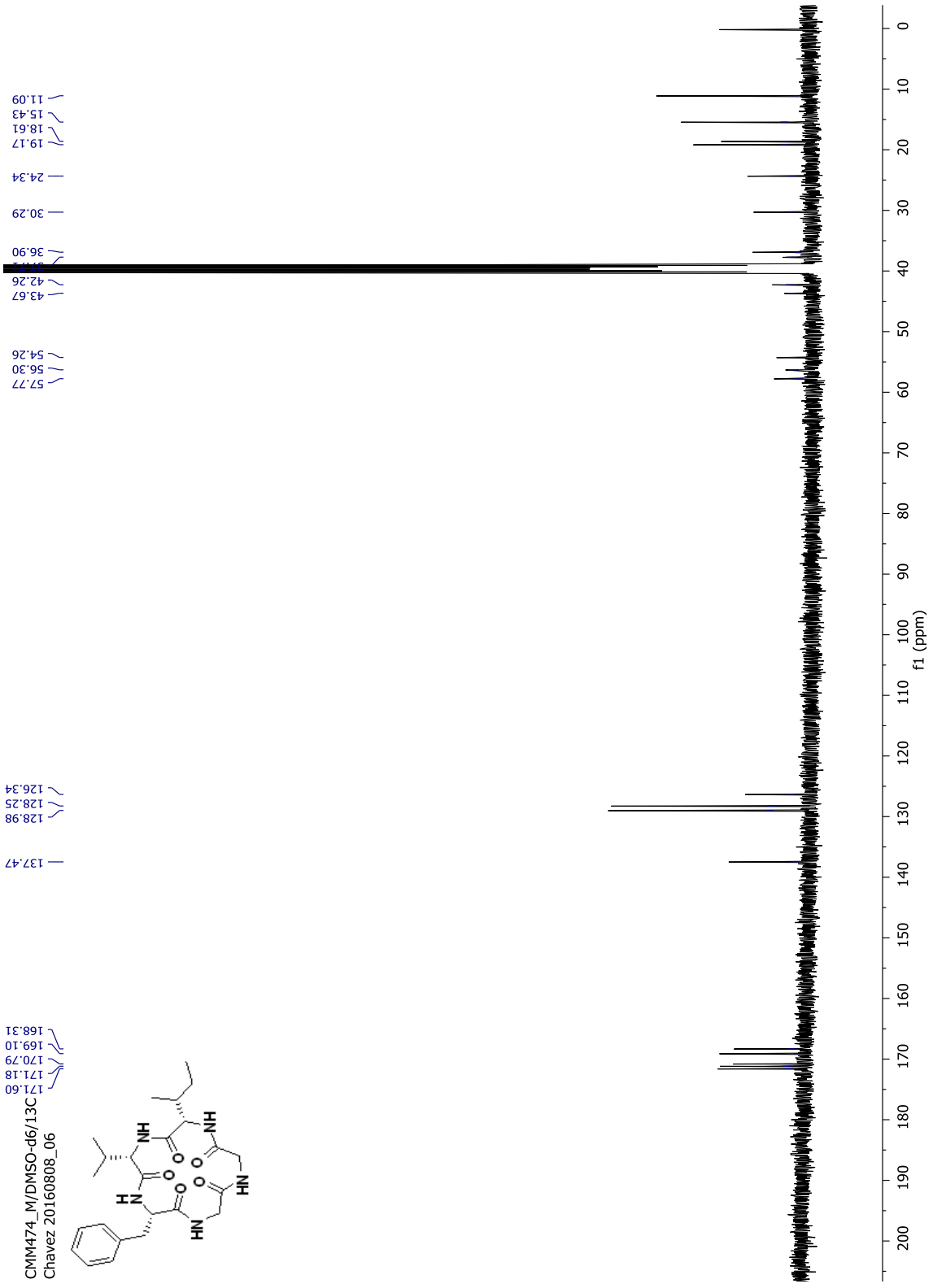


Figure S17 - ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of cyclic peptide **5** (Chapter 4).

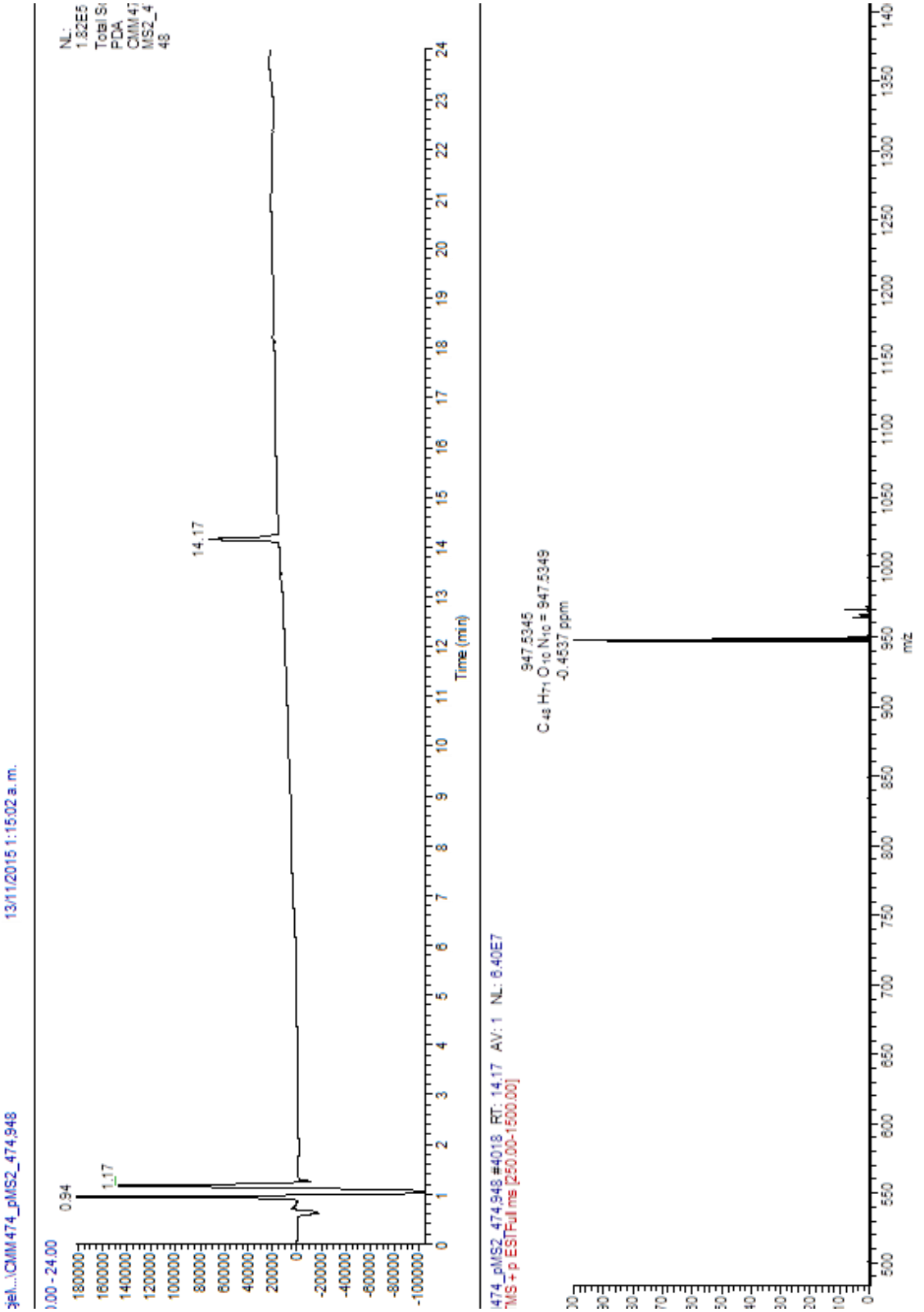


Figure S18 - RP-UHPLC chromatogram and ESI-HRMS of pure cyclic peptide **6**. (Chapter 4).

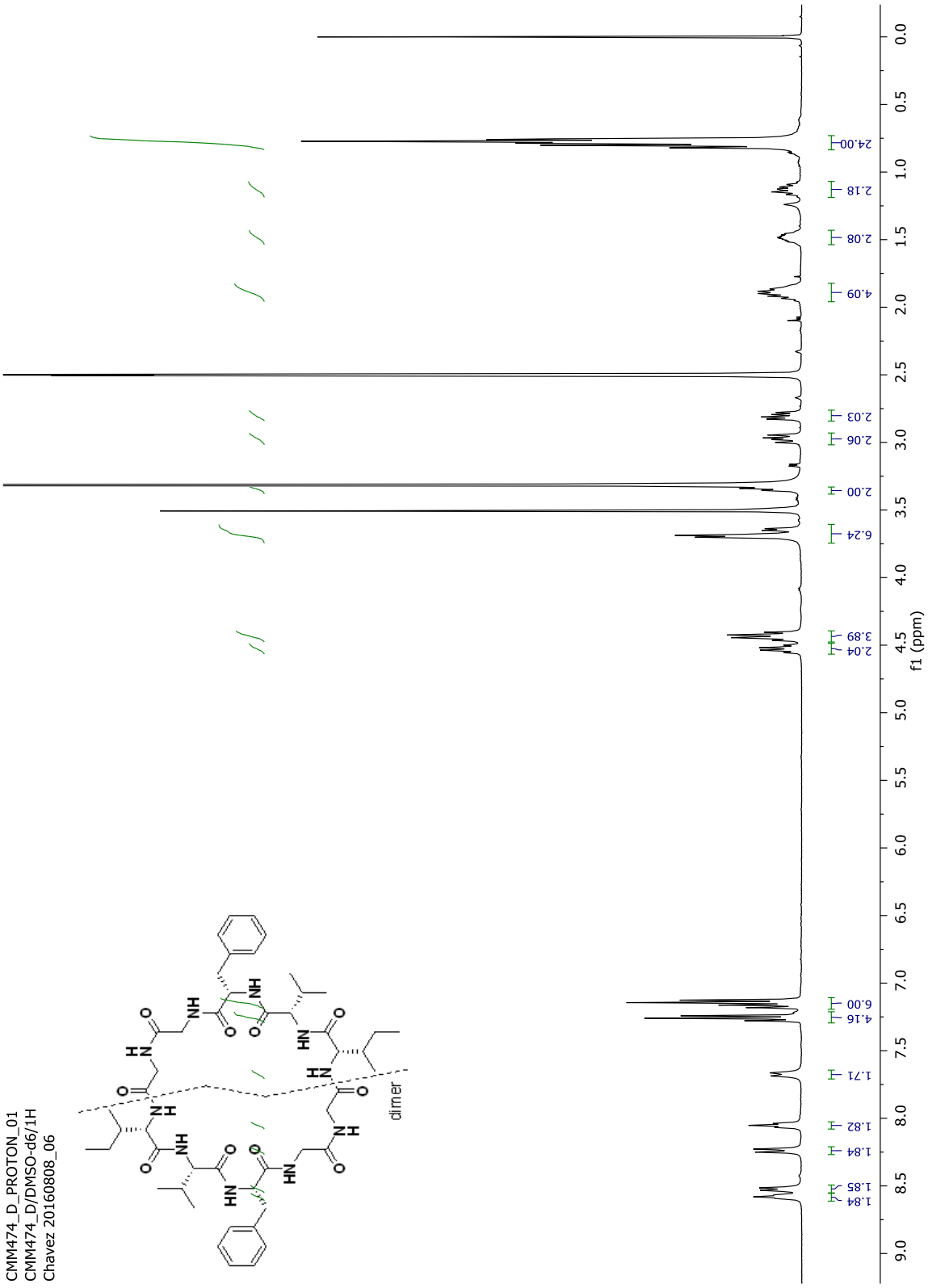


Figure S19 - ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of cyclic peptide **6** (Chapter 4).

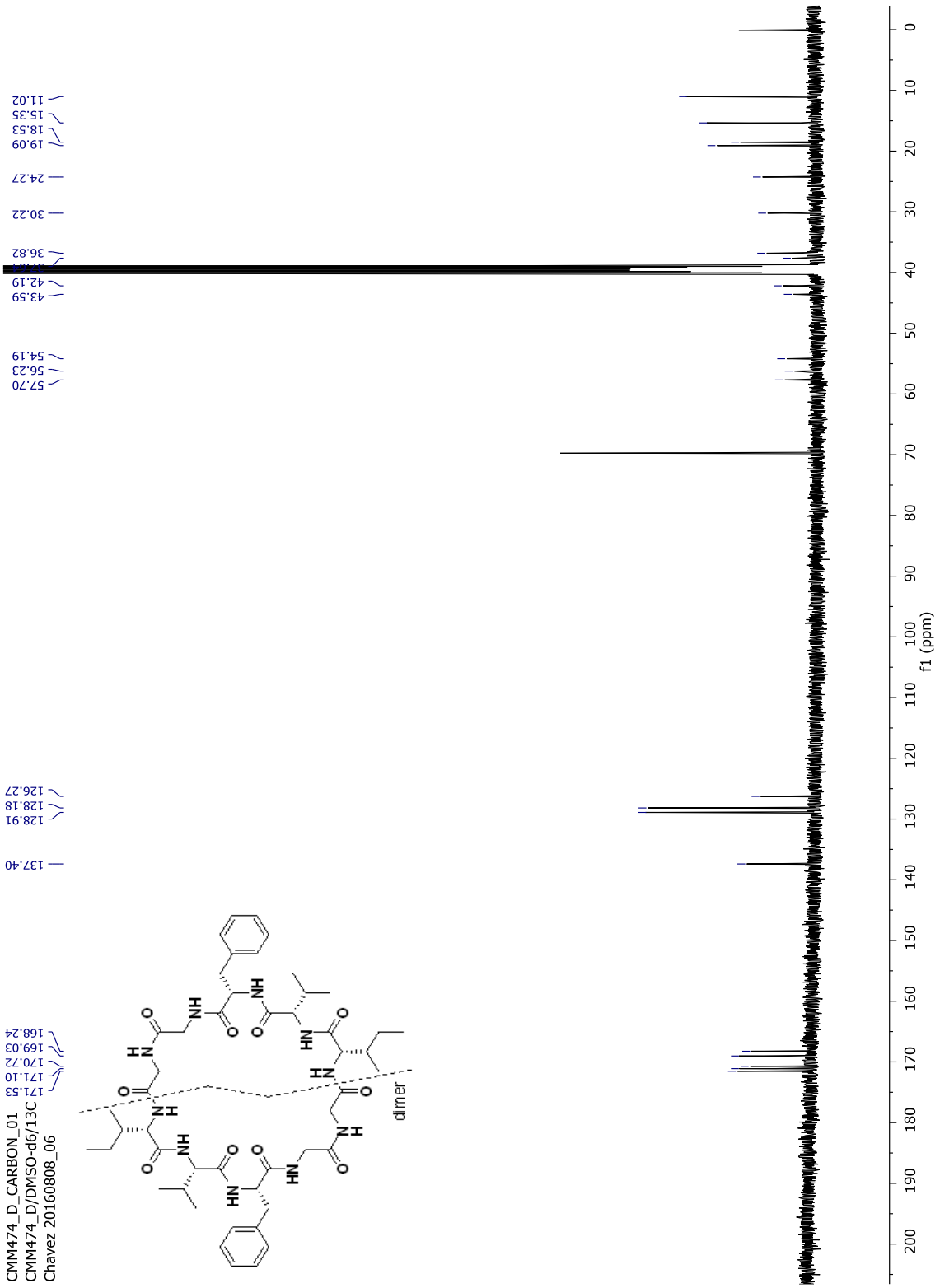


Figure S20 - ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of cyclic peptide **6** (Chapter 4).

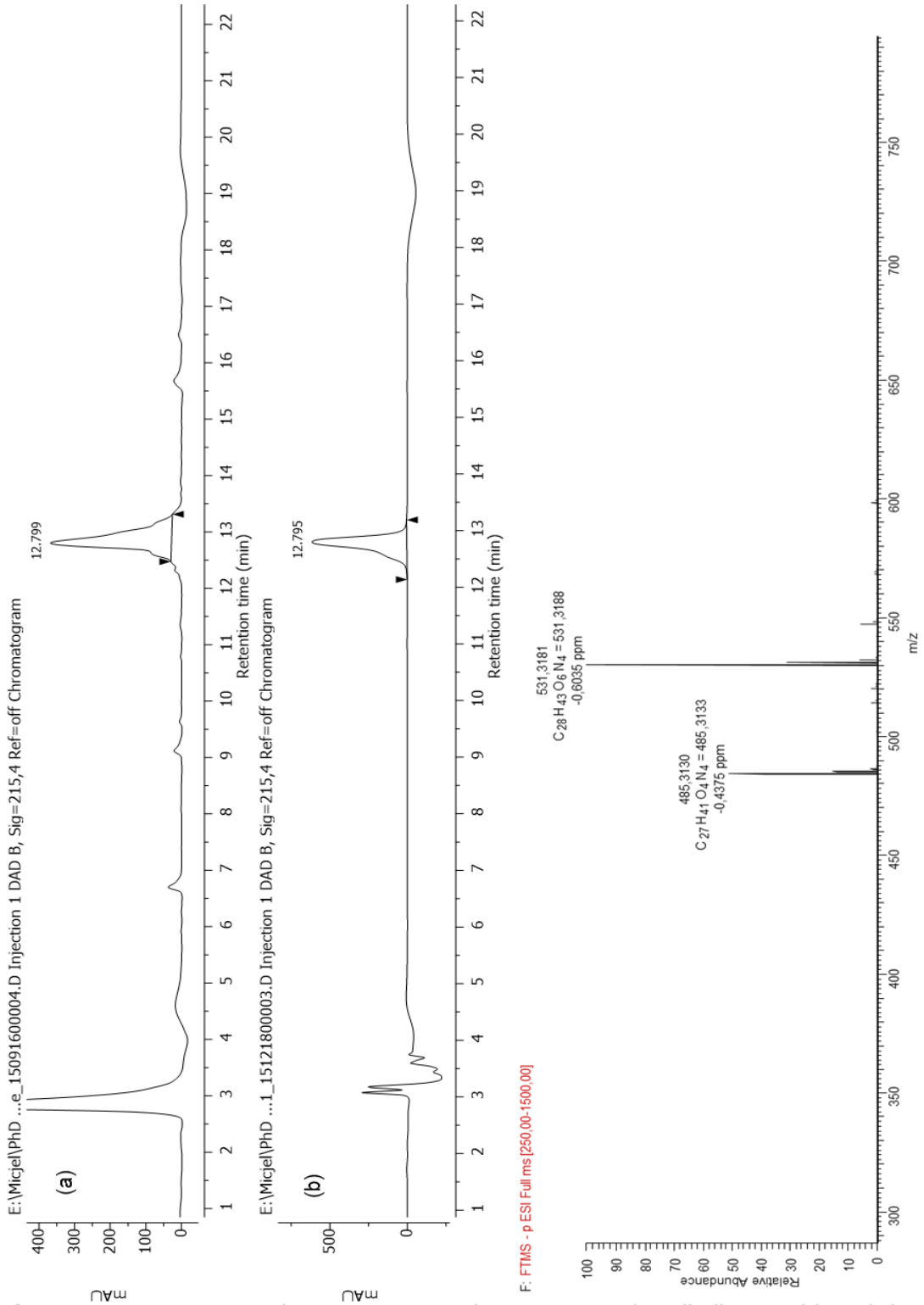


Figure S21 - RP-HPLC chromatogram and ESI-HRMS of cyclic lipopeptide **7** (Chapter 4). (a) Crude material after resin cleavage. (b) Pure compound after preparative HPLC purification.

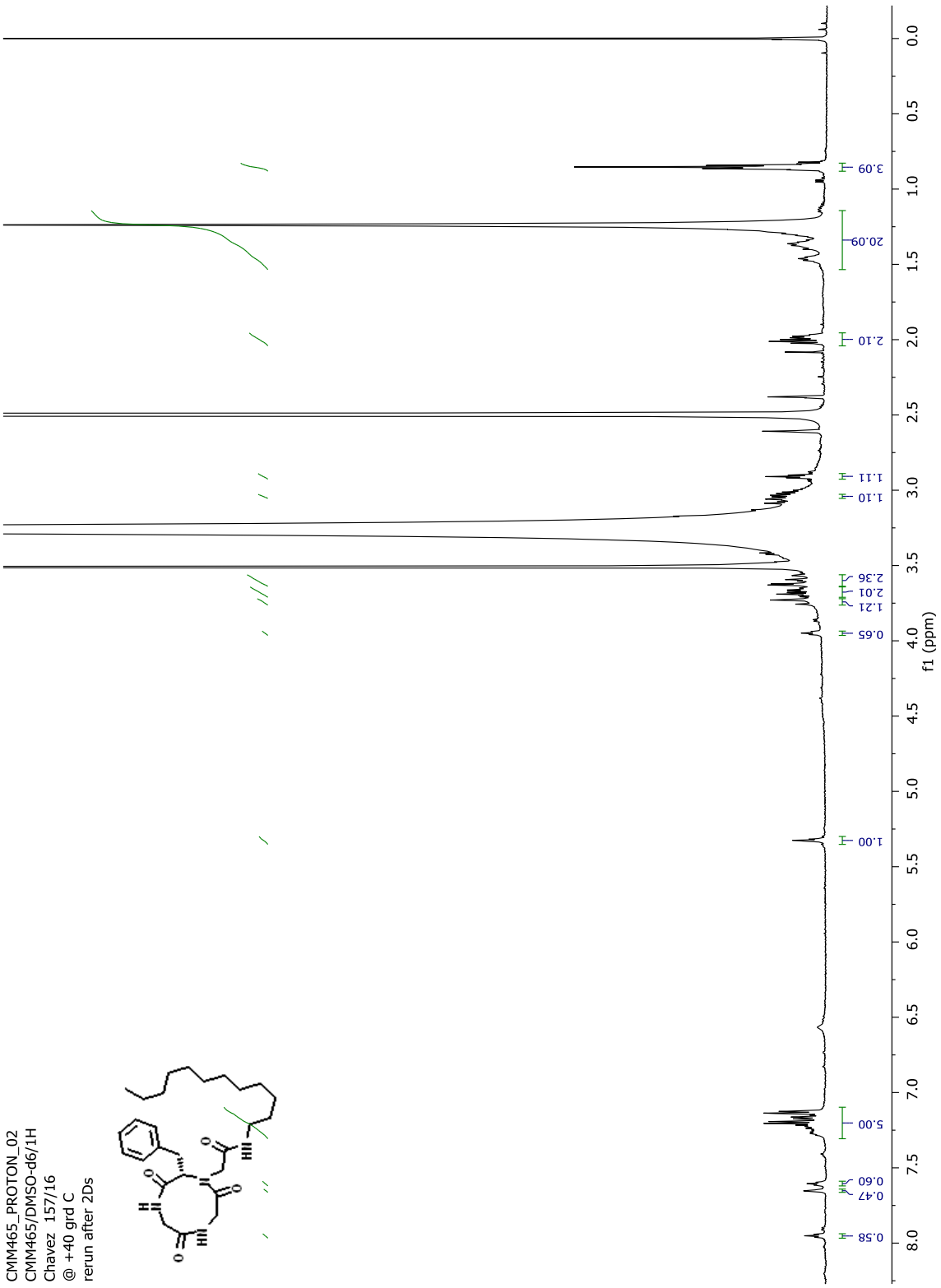


Figure S22 - ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of cyclic peptide **7** (Chapter 4).

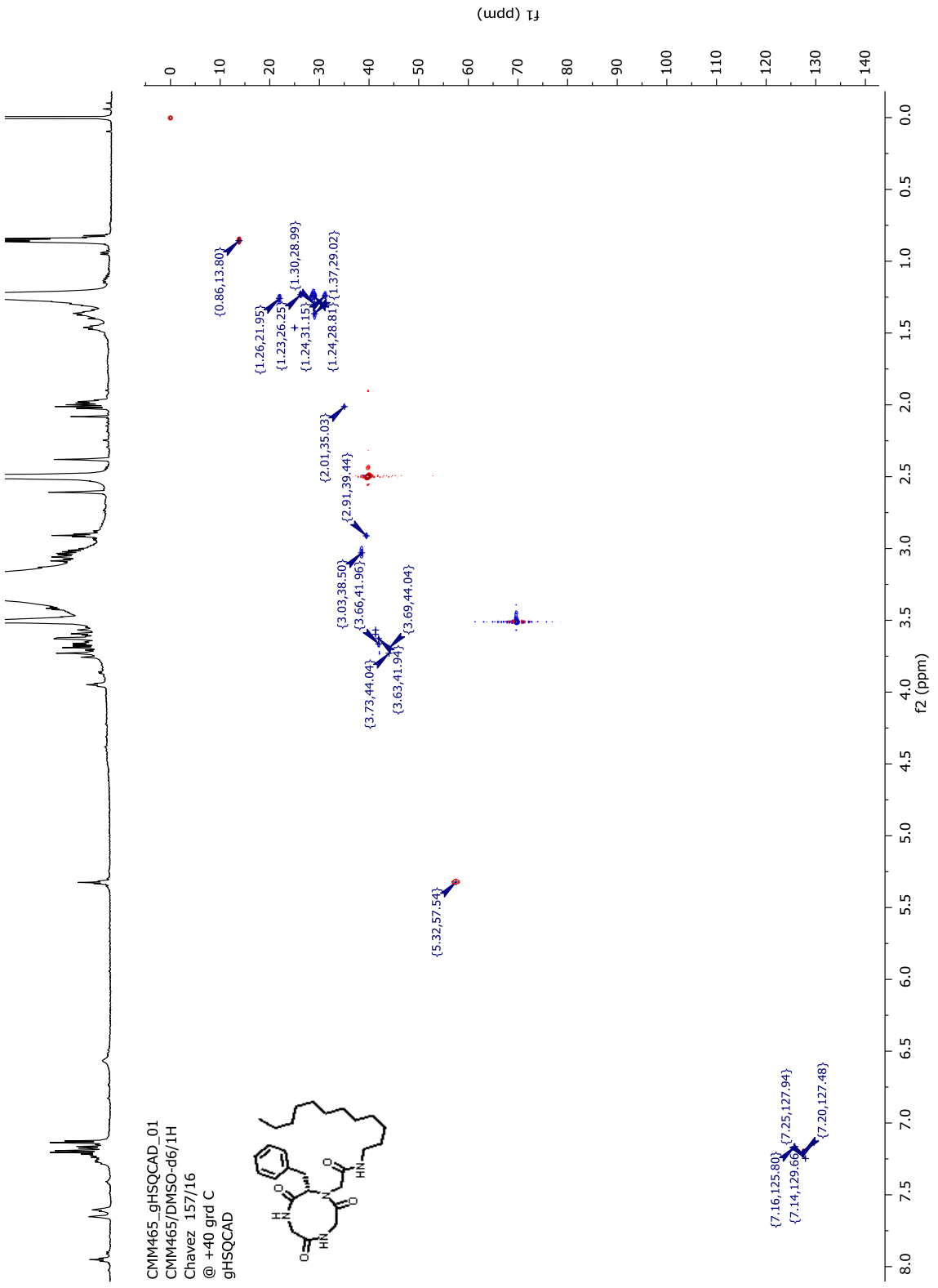


Figure S23 - gHSQCAD (150 MHz, DMSO-d_6) spectrum of cyclic peptide **7** (Chapter 4).

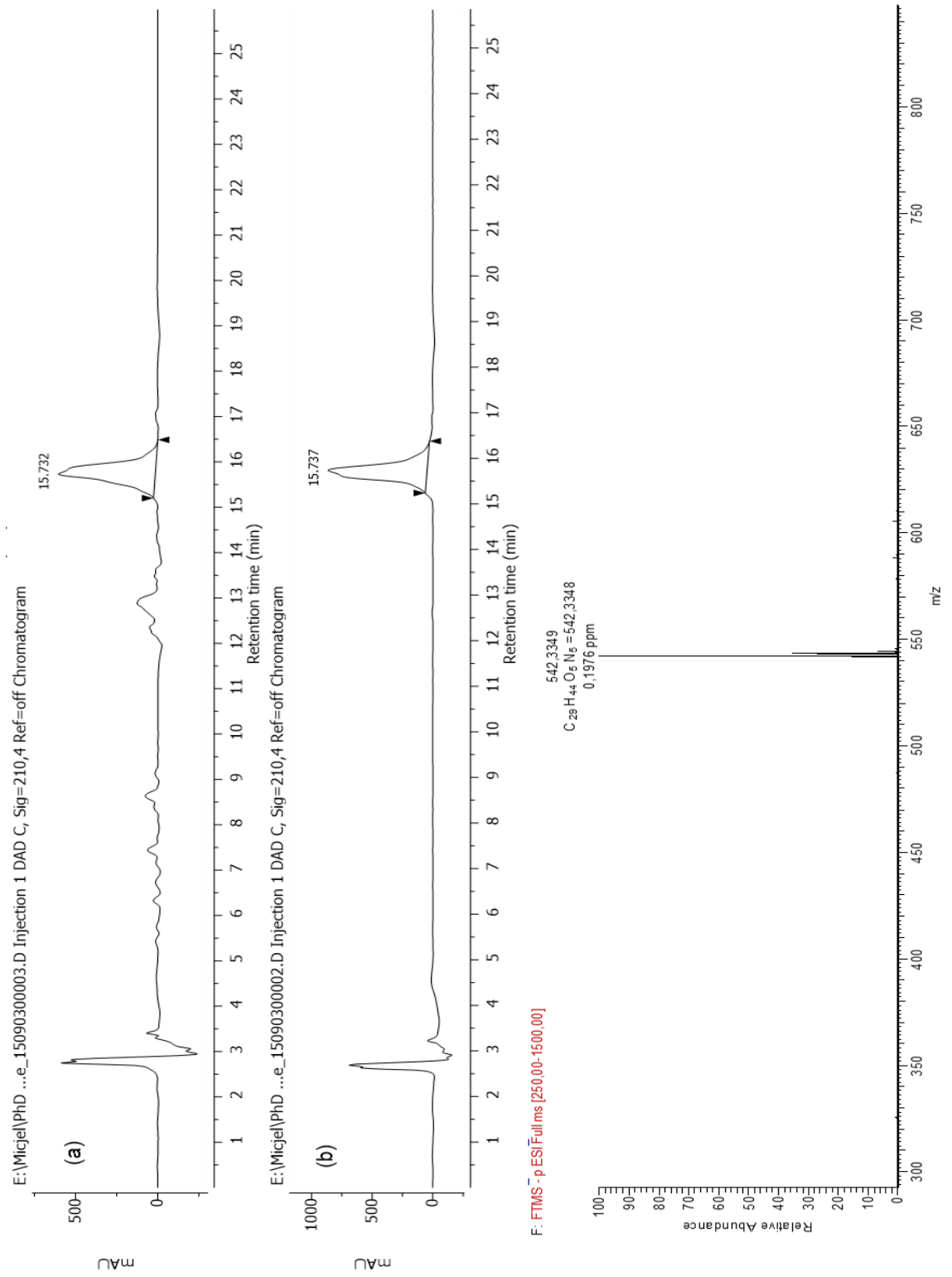


Figure S24 - RP-HPLC chromatogram and ESI-HRMS of cyclic lipopeptide **8** (Chapter 4). (a) Crude material after resin cleavage. (b) Pure compound after preparative HPLC purification.

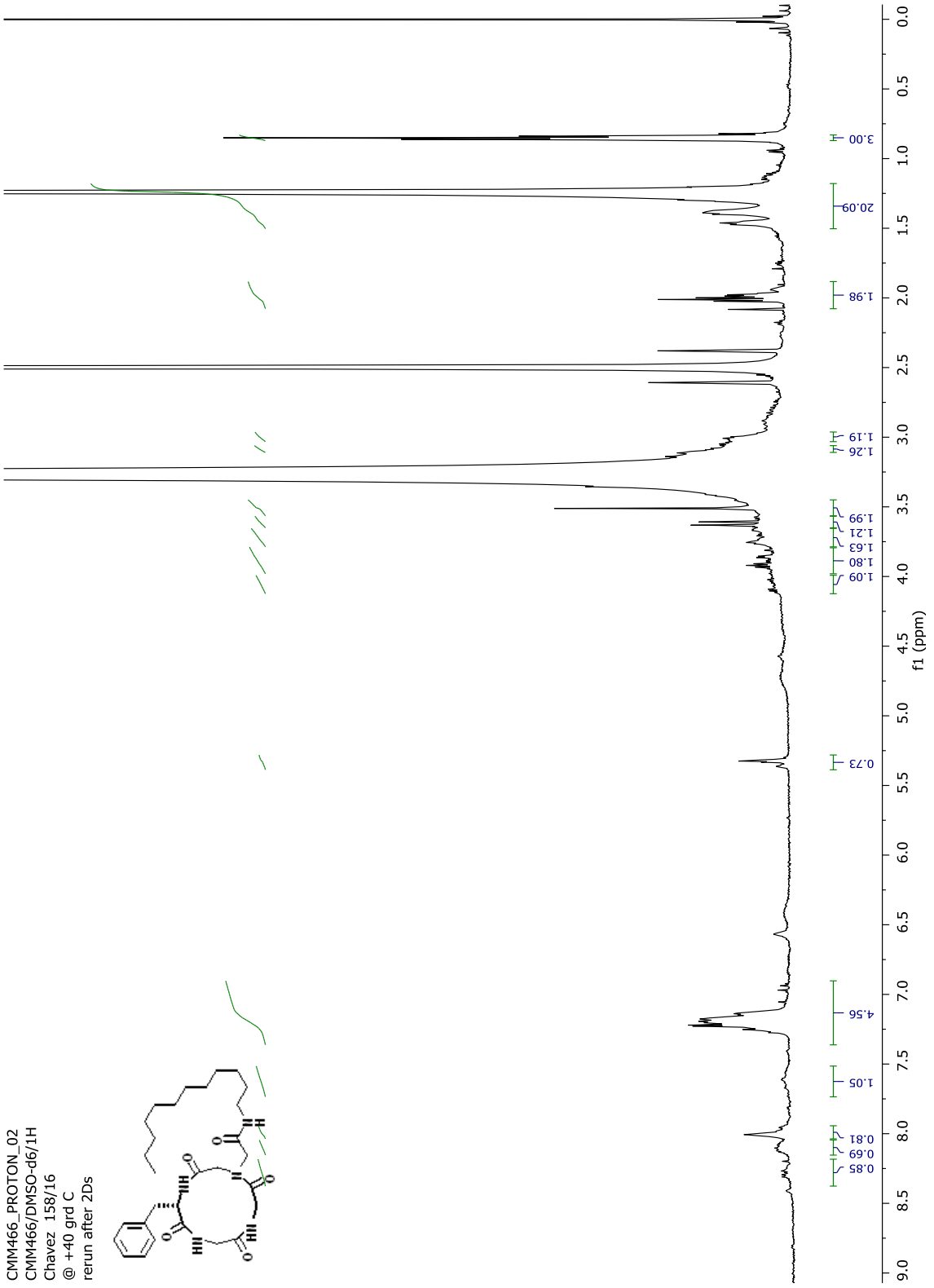


Figure S25 - ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of cyclic peptide **8** (Chapter 4).

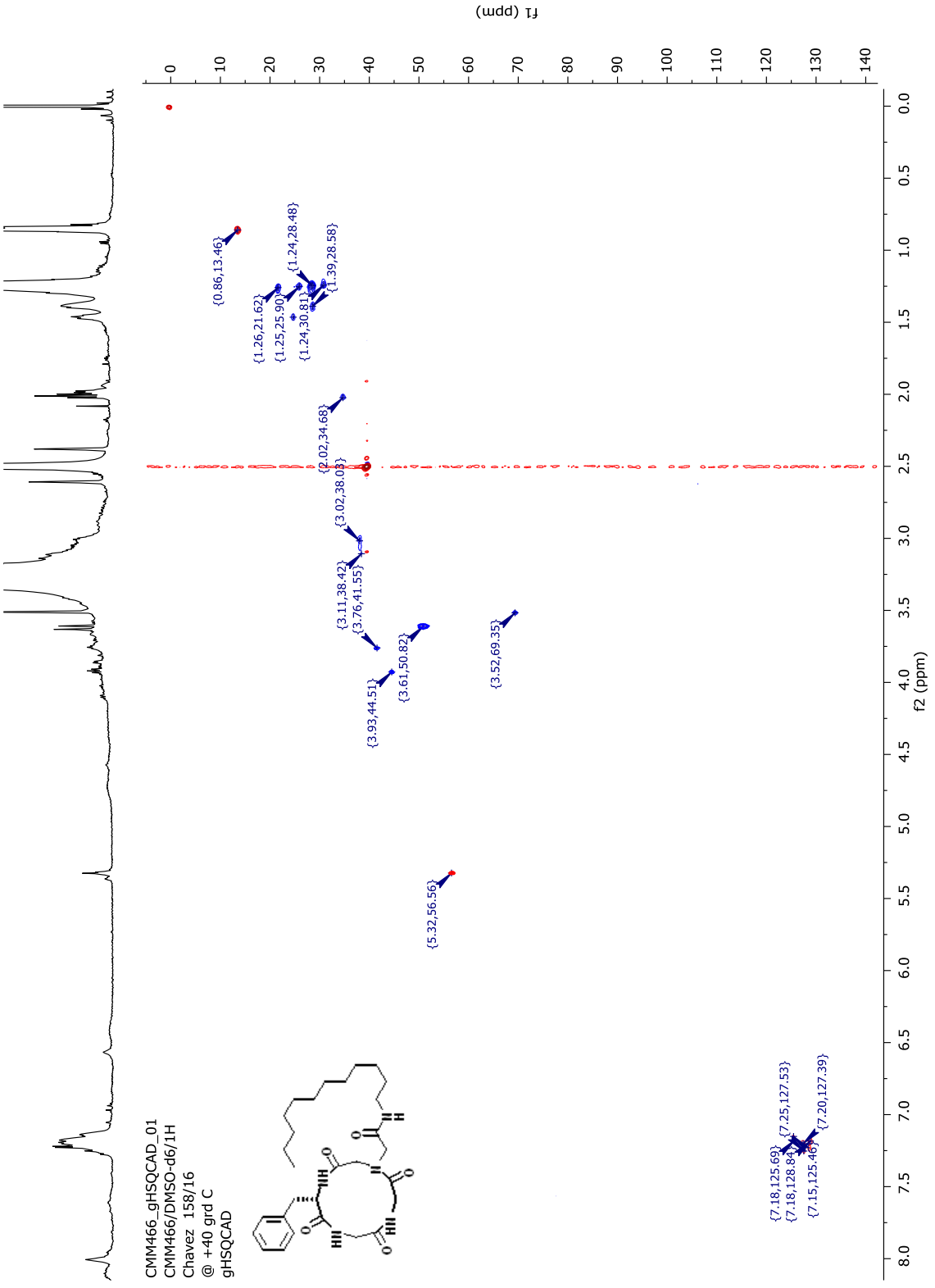


Figure S26 - gHSQCAD (150 MHz, DMSO-d_6) spectrum of cyclic peptide **8** (Chapter 4).

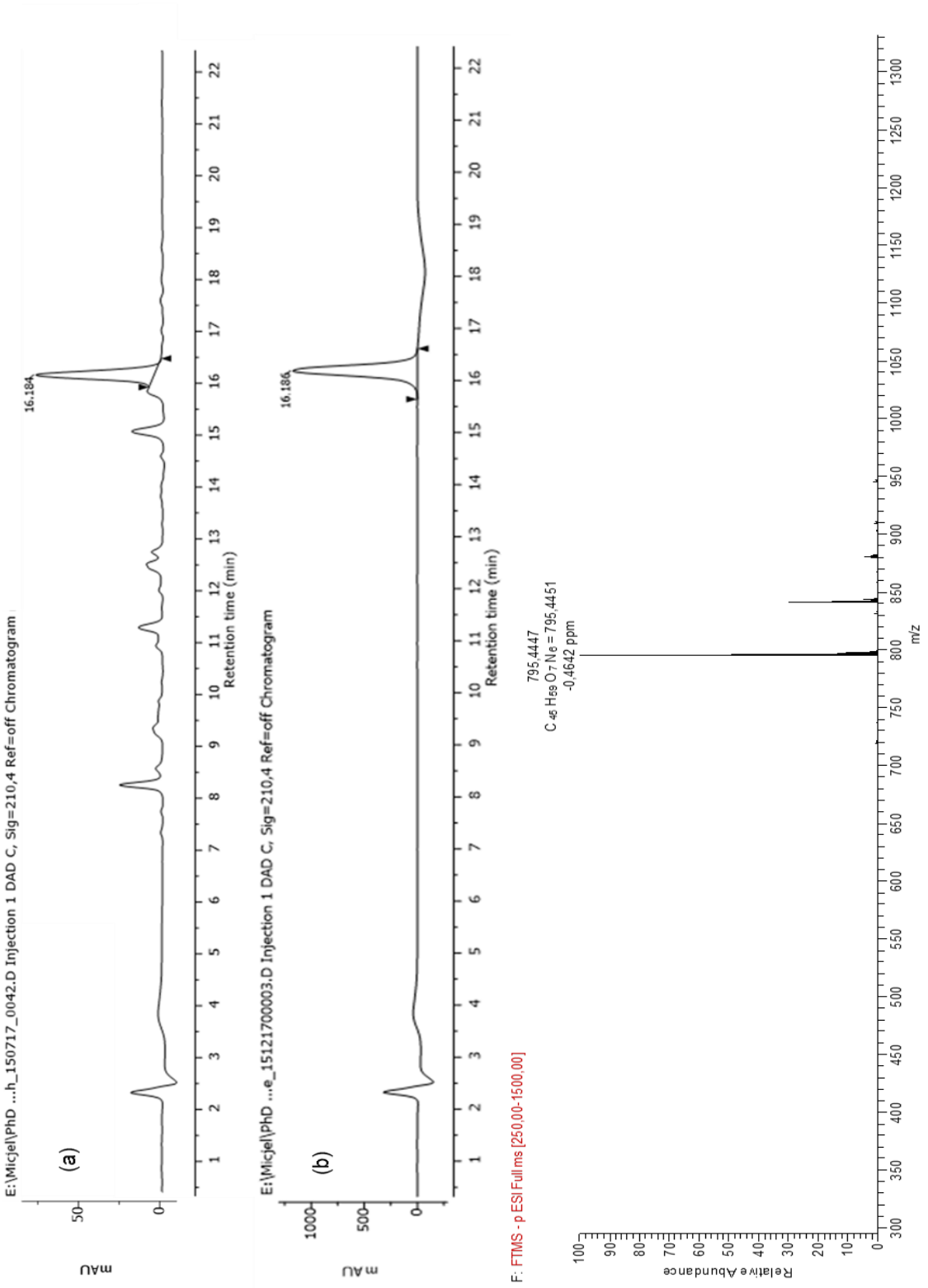


Figure S27 - RP-HPLC chromatogram and ESI-HRMS of cyclic lipopeptide **9** (Chapter 4). (a) Crude material after resin cleavage. (b) Pure compound after preparative HPLC purification.

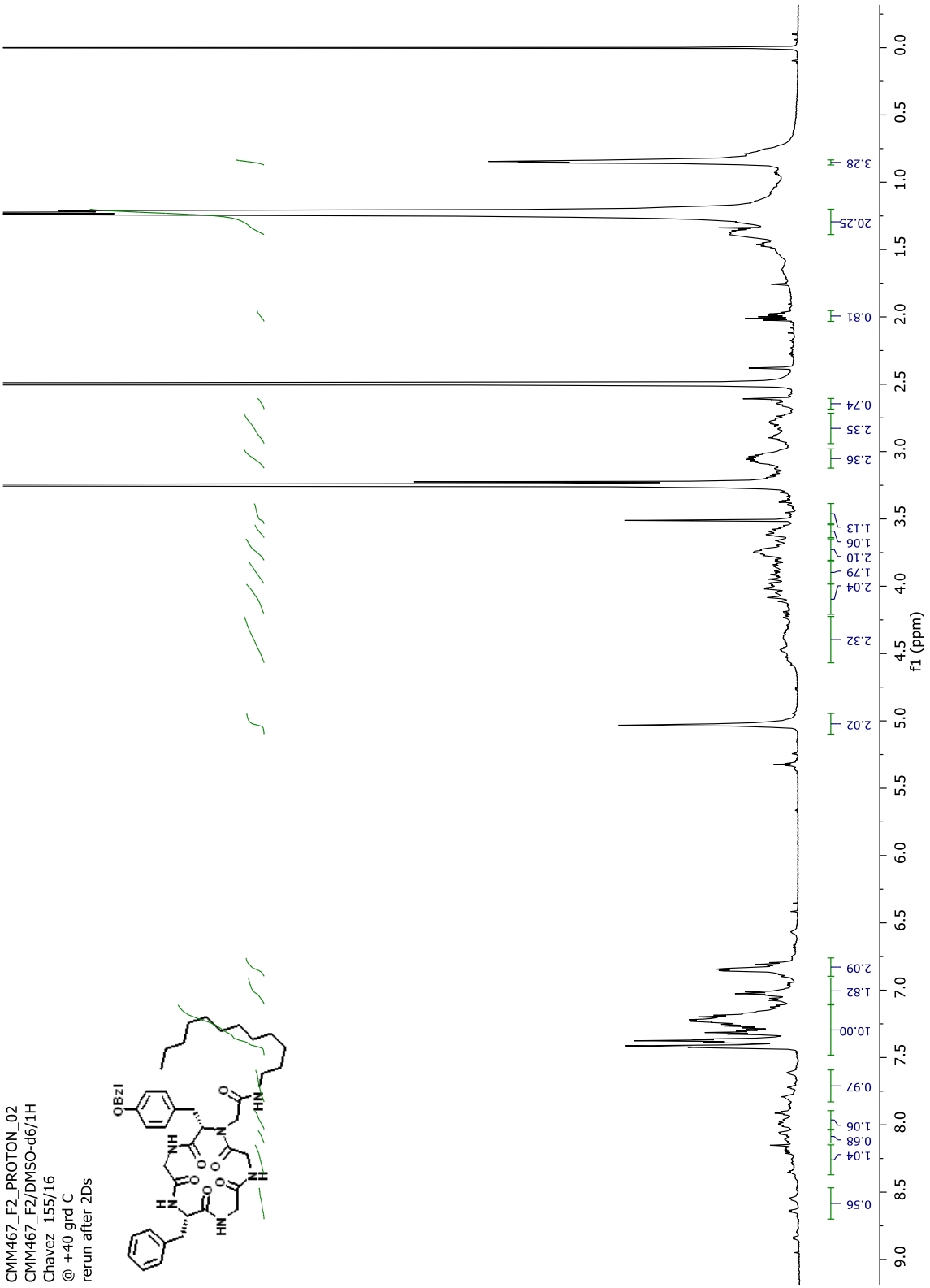


Figure S28 - ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of cyclic peptide **9** (Chapter 4).

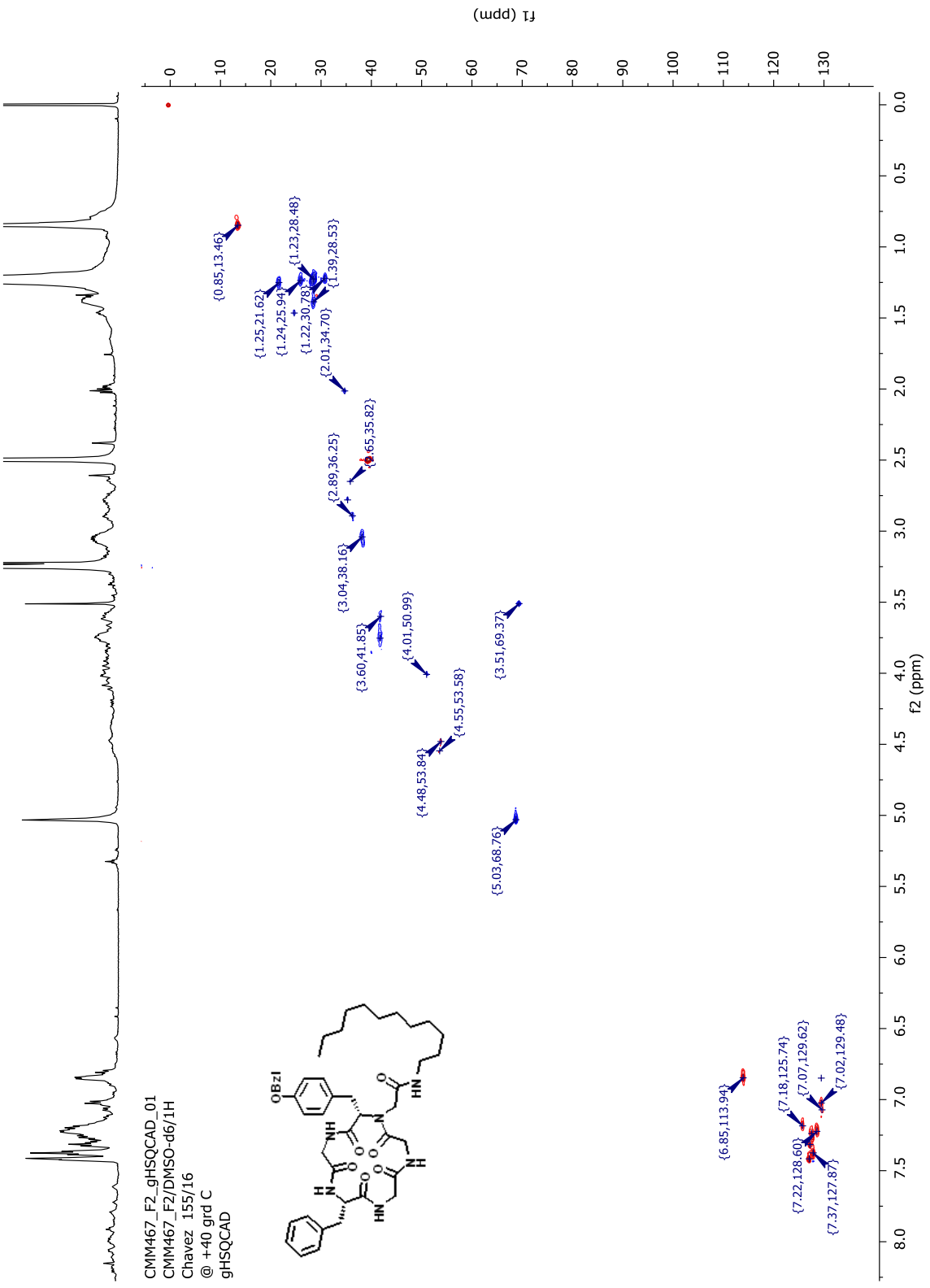


Figure S29 - gHSQCAD (150 MHz, DMSO-d_6) spectrum of cyclic peptide **9** (Chapter 4).

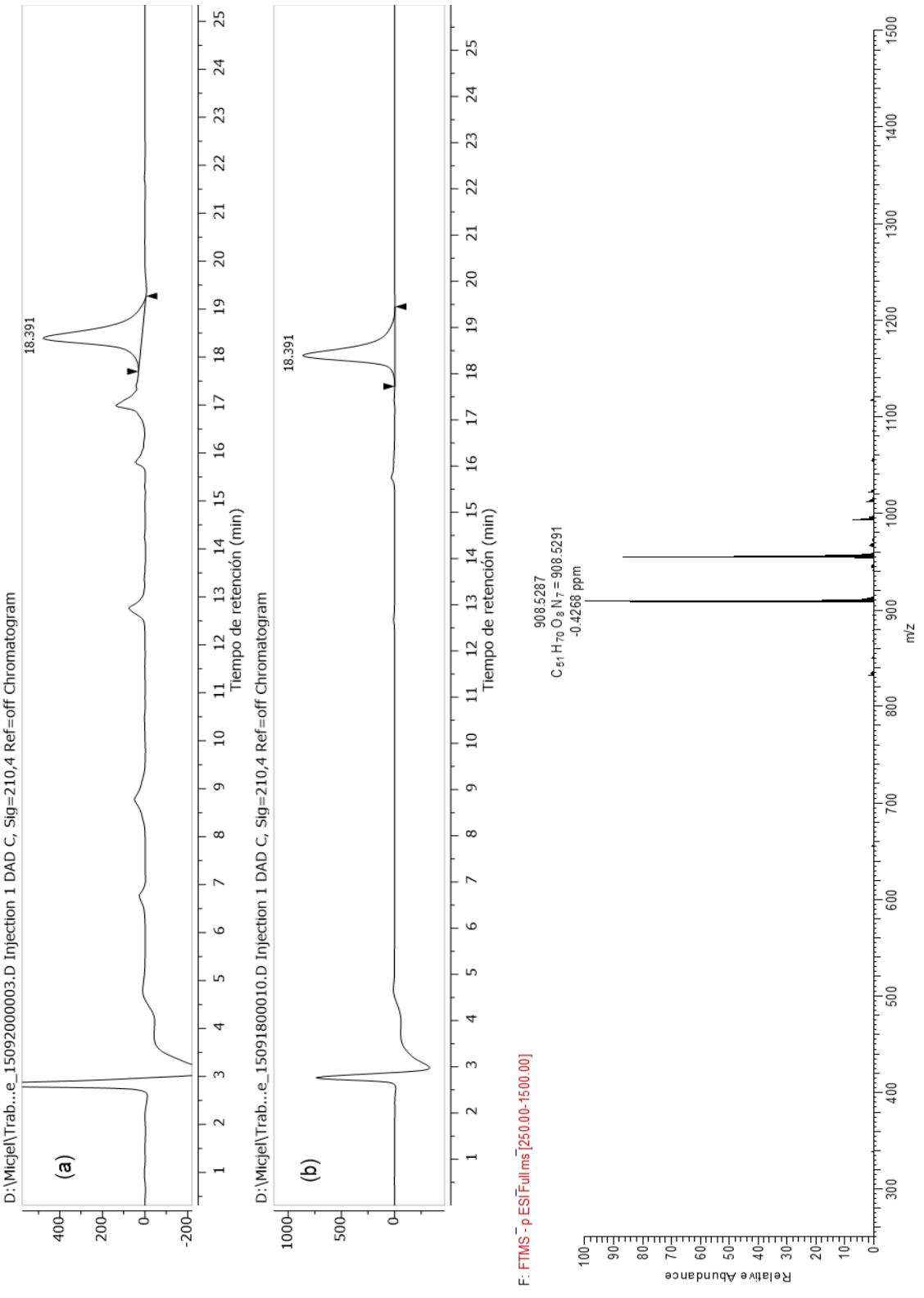


Figure S30 - RP-HPLC chromatogram and ESI-HRMS of cyclic lipopeptide **10** (Chapter 4). (a) Crude material after resin cleavage. (b) Pure compound after preparative HPLC purification.

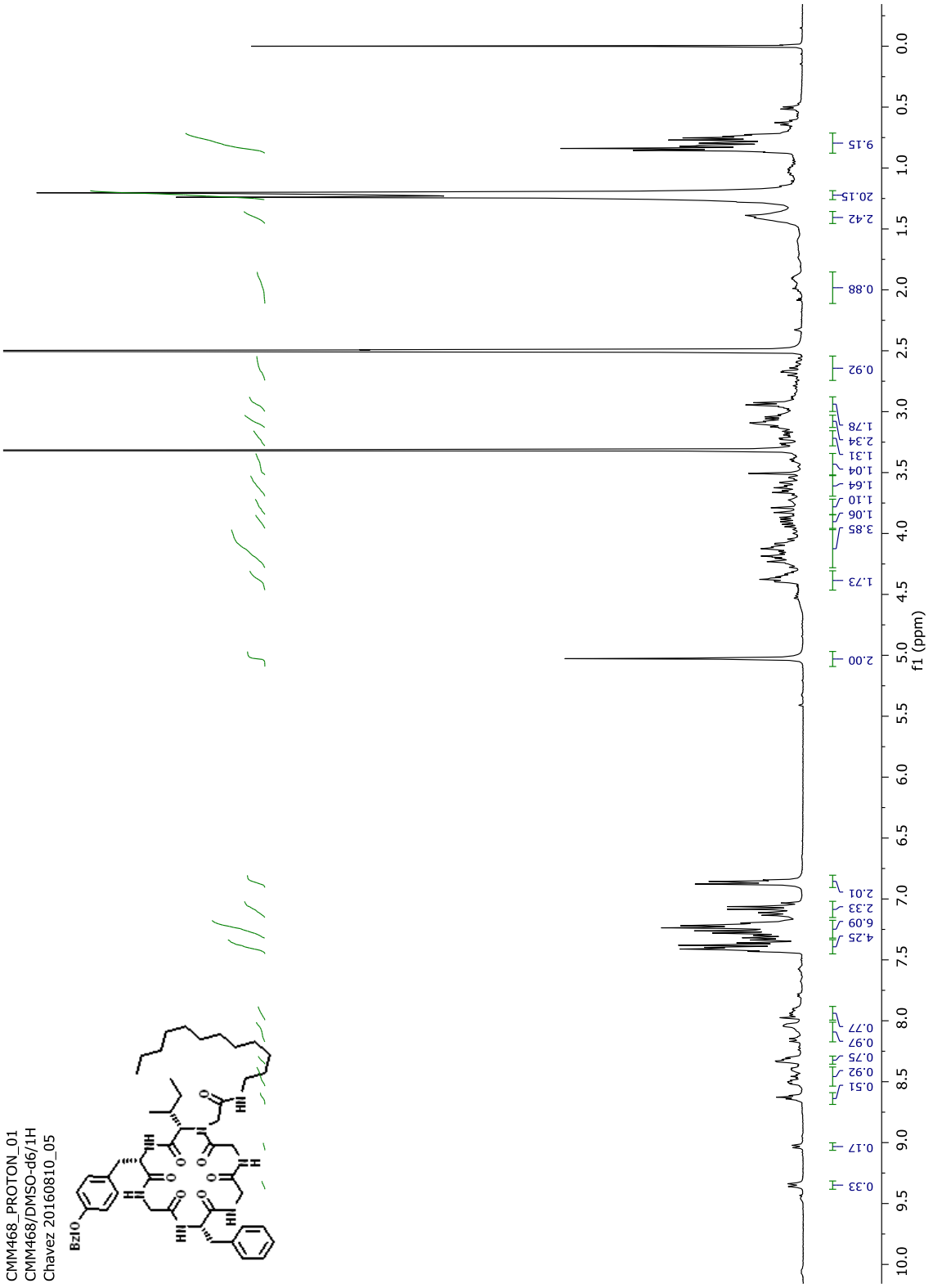


Figure S31 - ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of cyclic peptide **10** (Chapter 4).

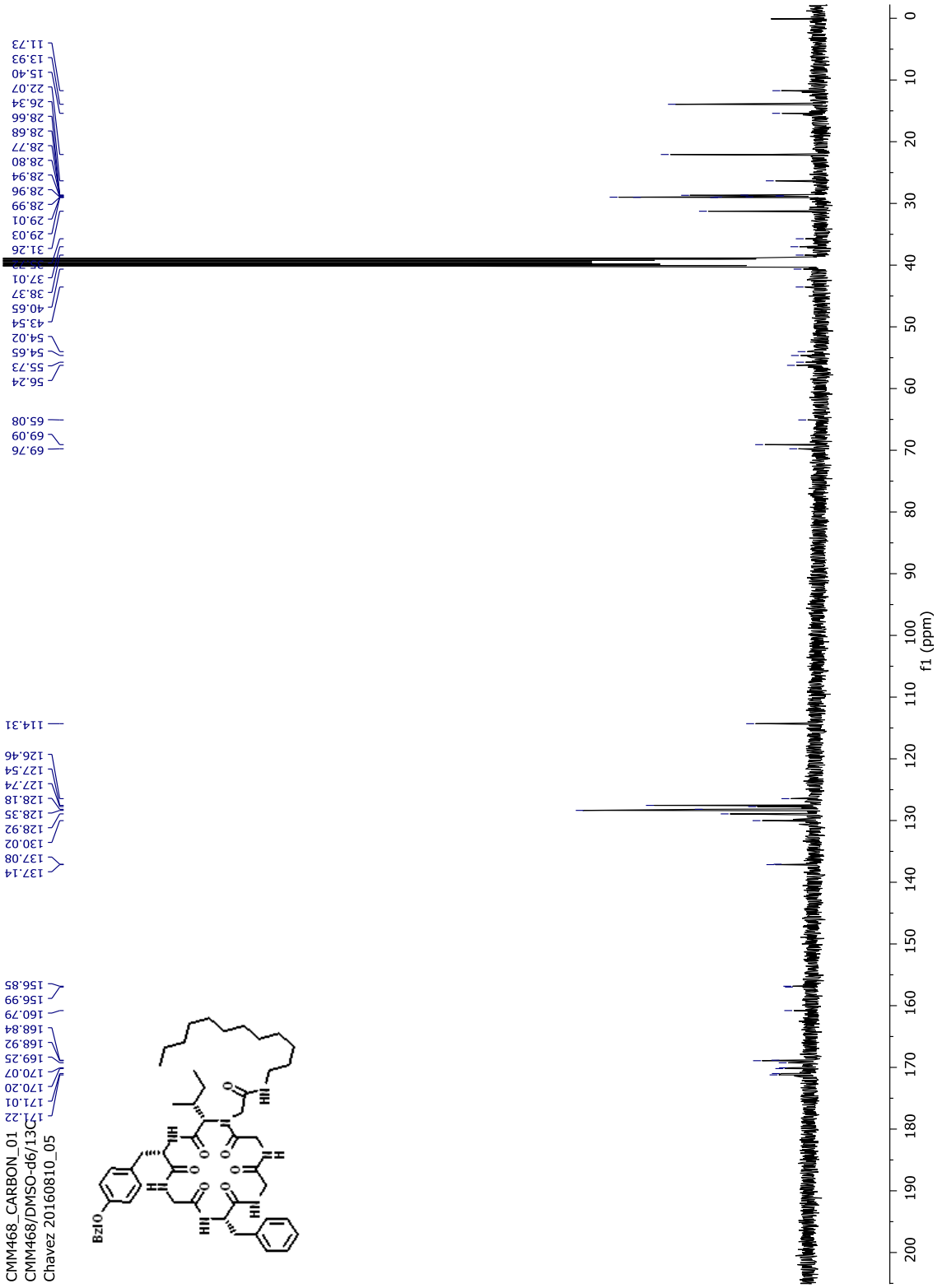


Figure S32 - ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of cyclic peptide **10** (Chapter 4).

Prepare	
1 Memo	ResPep SL version, 100 µmol peptide synthesis, 3-columns
2 RinseNeedle	2000 / 2500 µl
3 WashColumns	3200 µl, Reservoir ->Peptides
4 WashColumns	3200 µl, DCM ->Peptides, 2x
5 WashColumns	3200 µl, Reservoir ->Peptides, 2x
6 RinseNeedle	500 / 1500 µl
7 Extract	20 s
Cycle: 1 -> ...	
8 Deprotection	1800 µl, Piperidine ->Peptides
9 Deprotection	1800 µl, Piperidine ->Peptides
10 Agitate	700 / 300 µl, 00:08 hh:mm
11 RinseNeedle	1000 / 2000 µl
12 WashColumns	3200 µl, Reservoir ->Peptides, 2x
13 WashColumns	2500 µl, Reservoir ->Peptides
14 WashColumns	3200 µl, Reservoir ->Peptides, 3x
15 RinseNeedle	1000 / 1500 µl
16 Activation/Coupling	808+250+10+833 ->Peptides
17 Agitate	700 / 300 µl, 00:18 hh:mm
18 Capping	1800 µl, CapMixture ->Peptides
19 WashColumns	3200 µl, Reservoir ->Peptides
20 WashColumns	2500 µl, Reservoir ->Peptides
21 WashColumns	3200 µl, Reservoir ->Peptides, 3x
Final	
22 Deprotection	1800 µl, Piperidine ->Peptides
23 Deprotection	1800 µl, Piperidine ->Peptides
24 Agitate	700 / 300 µl, 00:10 hh:mm
25 Deprotection	2000 µl, Piperidine ->Peptides
26 Agitate	700 / 300 µl, 00:12 hh:mm
27 RinseNeedle	1000 / 2000 µl
28 WashColumns	3200 µl, Reservoir ->Peptides, 3x
29 WashColumns	2500 µl, Reservoir ->Peptides
28 WashColumns	3200 µl, Reservoir ->Peptides, 5x
29 WashColumns	4000 µl, DCM ->Peptides
30 WashColumns	3200 µl, DCM ->Peptides
31 RinseNeedle	1000 / 2500 µl
32 Extract	60 s

Table S33. Standard Intavis peptide synthesis protocol.

M.Sc. Micjel Chávez Morejón

1. General Information

Date and place of Birth: February, 6th 1985 – Pinar del Rio - Cuba

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Phone:+49(0)34555821347
e-mail: Micjel.ChavezMorejon@ipb-halle.de
Private e-mail: jesmac85@gmail.com

2. Education

2012-Currently	PhD Student at the Leibniz Institute of Plant Biochemistry – Department of Bioorganic Chemistry, IPB, Halle (Saale), Germany. Supervisors: Prof. Dr. L.A. Wessjohann Prof. Dr. D.G. Rivera Mentor: Prof. Dr. B. Westermann
2011-2016	Instructor of Organic and Biomolecular Chemistry at the Faculty of Chemistry, University of Havana, Cuba.
2009-2016	Adjunt Investigator at the Center for Natural Products Research and Department of Organic Chemistry, Faculty of Chemistry, University of Havana, Cuba.
2009-2011	*M. Sc.* in Organic Chemistry, Faculty of Chemistry, University of Havana. "*Multicomponent synthesis of policationic peptidomimetic hybrids of peptide-peptoids*". Mention: excellent. Supervisor: Dr. Daniel Garcia Rivera (dgr@fq.uh.cu).
2008-2009	*Diploma* thesis at the Center for Natural Products Research, Faculty of Chemistry, University of Havana. Project: "*Multicomponent Synthesis of peptidomimetics with potential antibacterial activity*". Mention: excellent. Supervisor: Dr. Daniel Garcia Rivera.
2004-2009	*B.Sc.* in Chemistry, Faculty of Chemistry, University of Havana.

3. Languages

Spanish, English, Portuguese, German.

4. Publications

Citation Report Author = (MOREJON MC or Morejon, Micjel C.)
Timespan=All Years. Databases = SCI-EXPANDED, SSCI, A&HCI.
Results found: 5
Sum of the Times Cited: 36
Average Citations per Item: 7.20
h-index: 3
Source: Web of Science (December, 28th 2017)

2017 Morejón, M. C.; Puentes, A. R.; Rivera, D. G.; Wessjohann, L. A. Peptide Macrocyclization Assisted by Traceless Turn Inducers Derived from Ugi Peptide Ligation with Cleavable and Resin-Linked Amines. *Org. Lett.* **2017**, *19*, 4022–4025.

Morejón, M. C.; Laub, A.; Kaluđerović, G.; Puentes, A. R.; Hmedat, A. N.; Otero-González, A. J.; Rivera, D. G.; Wessjohann, L. A. A Multicomponent Macrocyclization Strategy to Natural Product-Like Cyclic Lipopeptides: Synthesis and Anticancer Evaluation of Surfactin and Mycosubtilin Analogues. *Org. Biomol. Chem.* **2017**, *15*, 3628–3637.

2016 Morejón, M. C.; Laub, A.; Westermann, B.; Rivera, D. G.; Wessjohann, L. A. Solution and Solid-Phase Macrocyclization of Peptides by the Ugi-Smiles Multicomponent Reaction: Synthesis of *N*-Aryl-Bridged Cyclic Lipopeptides. *Org. Lett.* **2016**, *18* (16), 4096–4099.

Wessjohann, L. A.; Morejón, M. C.; Ojeda, G. M.; Rhoden, C. R. B.; Rivera, D. G. Applications of Convertible Isonitriles in the Ligation and Macrocyclization of Multicomponent Reaction-Derived Peptides and Depsipeptides. *J. Org. Chem.* **2016**, *81* (15), 6535–6545.

2012 Neves, R. A. W.; Stark, S.; Morejon, M. C.; Westermann, B.; Wessjohann, L. A. 4-Isocyanopermethylbutane-1,1,3-triol (IPB): a convertible isonitrile for multicomponent reactions. *Tetrahedron Letters* **2012,** *53* (40), 5360-5363. Highlighted in*: Chem Inform* **2013**, *44*, 0520.

5. Book Chapters

2015 Wessjohann, L. A., Neves Filho, R.A.W., Puentes, A.R.; Morejon, M.C. Macrocycles from Multicomponent Reactions, *In Multicomponent Reactions in Organic Synthesis*, 1st Edition. Zhu, J.; Wang, Q.; Wang, M. Eds. Wiley-VCH Verlag GmbH & Co. KGaA, **2015**, pp 231.

2013 Wessjohann, L. A., Kaluderovic, G., Neves Filho, R.A.W., Morejon, M.C., Lemanski, G., Ziegler, T. Ed. Müller T.J.J. Multicomponent reactions 1. *In Science of Synthesis*, **2013**, pp 415.

6. Selected conference presentations

2015 Morejón, M. C.; Laub, A.; Rivera, D. G.; Wessjohann, L. A.; Macrocyclic lipopeptides as potential antibiotics by MCR. At 50. Doktoranworkshop “Naturstoffe: Chemie, BIologie und Ökologie”, Würzburg, Germany, 2015. Oral presentation.

Neves Filho, R.A.W.; Morejon, M.C.; Puentes, A.R.; Westermann, B.; Wessjohann, L. A.; PS-IPB as a new resin-immobilized convertible isonitrile for multicomponent reactions. At 6th International Conference on MultiComponent Reactions and Related Chemistry (MCR 2015), Brasilia, Brazil. Poster presentation.

2013 Morejon, M.C.; Neves Filho, R.A.W.; Stark, S.; Rivera, D.G.; Westermann, B.; Wessjohann, L. A.; Total synthesis of Spiroidesin a homotyrosine-containing lipopeptide. At 18th European Symposium on Organic Chemistry (ESOC 2013), Marseille, France. Poster presentation.

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Declaration

“I declare that I have completed this dissertation without unauthorized help of a second party and only with the assistance acknowledged therein. I have appropriately acknowledged and referenced all text passages that are derived literally from or based on the content of published or unpublished work of others authors.”

Erklärung

Hiermit erkläre ich an Eides Statt, dass ich die vorliegende Arbeit selbständig und nur unter Verwendung der angegebenen Literatur und Hilfsmittel angefertigt habe.

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