Applications of the Ugi Reaction in the Synthesis of Cyclic and *N*-Alkylated Peptides

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"*The future is uncertain…but this uncertainty is at the very heart of human creativity.*"

Ilya Prigogine

(Nobel Prize in Chemistry 1977)

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List of abbreviations

[α]	specific rotation	*i*-	*Iso*-
Ac	acetyl	IC_{50}	median inhibitory concentration
Ala	alanine	*i.e.*	*id est* (that is)
atm	atmosphere	Ile	isoleucine
Aib	2-aminoisobutyric acid	IMCR	Isocyanide multicomponent reaction
Bn	benzyl	IPB	4-isocyanopermethylbutane-1,1,3-triol
Boc	*tert*-butoxycarbonyl	*J*	coupling constant (in NMR)
BOP	(benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate	M	molar
bs	broad singlet (in NMR)	m	milee
PyBOP	benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate	m	multiplet (in NMR)
°C	degrees Celcius (centigrade)	MCR	multicomponent reaction
calcd	calculated	Me	methyl
Cbz	benzyloxycarbonyl	h	hour (s)
4CR	four-component reaction	HATU	1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluoro phosphate
CSA	camphorsulfonic acid	HBTU	3-[bis(dimethylamino)methyliumyl]-3H-benzotriazol-1-oxide hexafluorophosphate
d	doublet in NMR	HOAt	1-hydroxy-7-azabenzotriazole
DCC	*N*,*N*-dicyclohexylcarbodiimide	HOBt	hydroxybenzotriazole
DMB	2,4-dimethoxybenzyl-	min	minutes
DMSO	dimethylsulfoxide	mp	melting point
d.r.	diastereomeric ratio	MS	mass spectrometry
EDCl	*N,N'*-1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride	*o*NB	*orto*-nitrobenzyl-

e.g.	*exempli gratia* (for example)	NMM	*N*-methylmorpholine
ESI	electron spray ionization	NMR	nuclear magnetic resonance
Et	ethyl	Nu	nucleophile
et al.	*et alia* (and others)	OBO	4-methyl-2,6,7-trioxabicyclo[2.2.2]octyl
equiv	equivalent	PFP	pentafluorophenol
Fmoc	9-fluorenylmethoxycarbonyl	Ph	phenyl
FT-ICR	Fourier transformation ion cyclotron resonance	ppm	parts per milion
HRMS	high resolution mass spectrum	*t*-	*tert*-
Hz	Hertz	TIPS	triisopropylsilane
PDA	photodiode array	TFA	triflouroacetic acid
q	quartet (in NMR)	THF	tetrahydrofuran
R_f	retention factor	TLC	thin layer chromatography
r.t.	room temperature	TMS	tetramethylsilane
s	singlet (in NMR)	UHPLC	ultra-high performance liquid chromatography
SPPS	solid-phase peptide synthesis	UV	ultraviolet
Sar	sarcosine		

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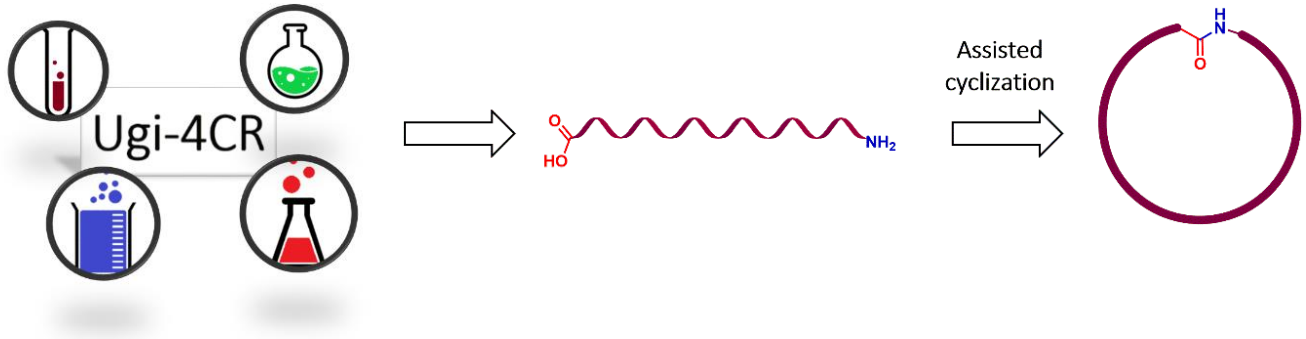
Chapter 1

Introduction

Abstract*



Macrocyclic peptides have found widespread applications across different disciplines such as medicinal chemistry, agriculture, nanoscience and material sciences. Conventional macrocyclization process comprises the activation of a peptide often in an entropically disfavored pre-cycling conformation prior to the desired product formation. Contemporary endeavors have focused on methods to improve the selectivity of macrocyclization. The chapter summarizes the main concepts of peptide macrocyclization and its applications in the preparation of cyclopeptides and cyclodepsipeptides.

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1.1 On the synthesis of macrocyclic peptides

Macrocycles are defined as molecules that bear at least one non-bridged ring larger than 12 members. Nevertheless, several authors include smaller ring sizes (8-, 9-, 10-, and 11-membered rings) for macrocycles or define the higher limit at 14 ring atoms.[1] In nature the most commonly occurring macrocycles are 14-, 16- and 18- membered.[2] Many natural compounds of saccharidic, peptidic and polyketide nature possess macrocyclic scaffolds, with a high incidence of remarkable biological activity.[1,3] In the bioactive compound space, macrocycles occupy a privileged intermediate position, displaying the binding power of biologics, while retaining at the same time the bioavailability of small molecules.[4] A classical example is the glycopeptide antibiotic vancomycin (**1**, **Figure 1.1**), which has been employed for many years as the last line resort against very resistant bacterial strains.[5]

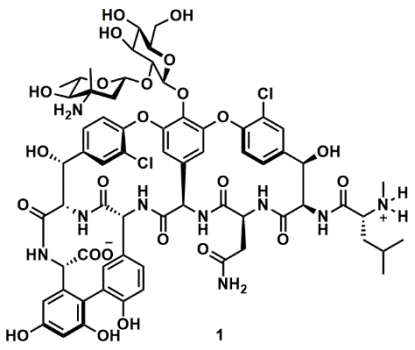
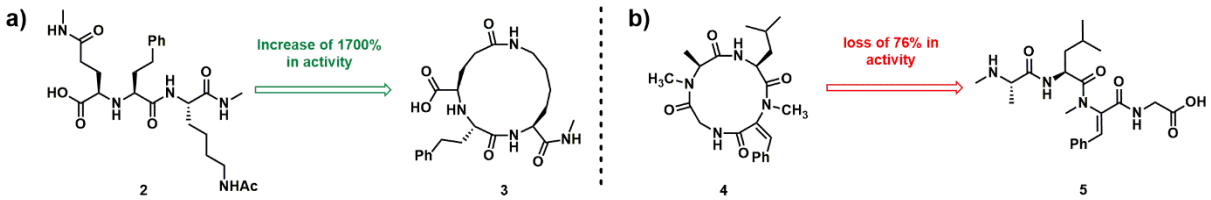


Figure 1.1 Vancomycin **1**.

The constrained structure inherent to macrocyclic compounds improves their ability to interact with protein receptors with a minimal entropic loss upon binding, which is also crucial for designing specific inhibitors of protein–protein interactions.[6–8] Moreover, macrocycles can display surface areas in a different way than their acyclic analogs, what makes them especially suitable for binding targets bearing flat surfaces.[8] A typical example that illustrates the positive effect of cyclization on the biological activity is the case of the synthetic matrix metalloproteinase MMP-8 inhibitor **3**, which displayed 17-fold higher potency compared with its linear precursor **2** (**Scheme 1.1a**).[8] By contrast, structure-activity studies have revealed that tentoxin acyclic precursor **5** loses 76% of the activity compared to the cyclic product (**4**) (**Scheme 1.1b**).[9] These results clearly highlight the influence of macrocyclization on the binding affinity of molecules to biological receptors.



Scheme 1.1 Effect of cyclization on biological activity. a) Synthetic matrix metalloproteinase MMP-8 inhibitor peptide **3** displays 17-fold higher potency compared with its linear precursor **2**. b) The linear precursor **5** of natural cyclotetrapeptide tentoxin **4** loses 76% of the toxic effect of the natural product.

Since the universe of macrocycle applications in medicinal and material chemistry seems to be infinite, an effective exploration of the macrocyclic chemotype space relies on the development of methods capable of

producing such compounds with relatively low synthetic costs and providing structural control and functional diversity at the cyclic scaffold.[10,11] The main problem related to macrocyclization, is how to face the drawbacks connected to the linear or cyclic oligomerization of the monomer to achieve the desired (mono-) cycle. In the case of peptides, it severely depends on the position of the ring-closing step. For example, in the synthesis of cyclo-[Pro–Ala–Ala–Phe–Leu] (**6**, **Figure 1.2**), the macrocyclization site influences on the reaction outcome.[12]

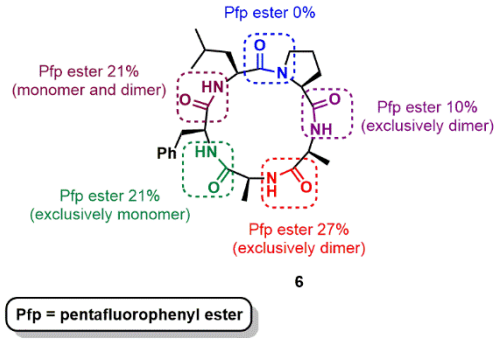


Figure 1.2 Site dependent macrocyclizations toward cyclo-[Pro–Ala–Ala–Phe–Leu] **6**.

The synthesis of natural macrocyclic peptides or peptidomimetics is very important to the development of the pharmaceutical industry. Different from linear peptides, the cyclic variants are usually more resistant to exo- and endoproteases,[13] what is one of the main reasons for the outstanding of these molecules in medicinal chemistry. The interest of the scientific community for macrocyclic peptides started during the Second Wold War, as the macrocyclic decapeptide Gramicidin S was extensively used in the treatment of septic gunshot wounds.[14] Since then, many naturally occurring macrocyclic peptides have been introduced as therapeutic drugs; and synthetic analogues in the lab for the same purpose.[15] One example is the widely employed drug cyclosporine A (**7**, **Figure 1.3**) an immunosuppressant agent that is frequently used to prevent rejection in organ transplant recipients.[16] Also, macrocyclic peptides are employed as chemotherapeutic drugs. Dactinomycin (**8**; **Figure 1.3**), is a cyclic polypeptide isolated from soil bacteria that is used as an intravenous antibiotic with anticancer activity.[17] It binds to DNA and subsequently inhibits RNA synthesis and is therefore used in the treatment of Wilm´s tumor, gestational trophoblastic neoplasia and rhabdomyosarcoma.[18]

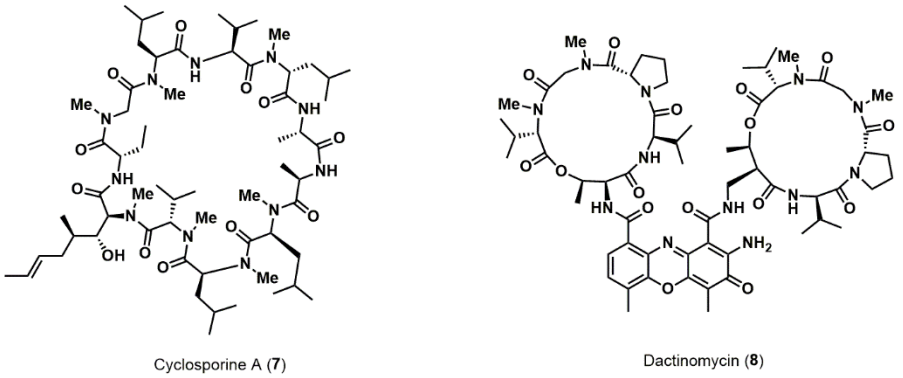


Figure 1.3 Macrocycle peptides used as chemotherapeutic and immunosuppressant agents.

Additionally, one of the strengths of total synthesis in drug discovery includes the generation of synthetic analogues of the natural product seeking to improve their biological activity. Somatostatin (**9**, **Figure 1.4**) is a natural macrocyclic peptide hormone that regulates the endocrine system and affects neurotransmission, cell proliferation and inhibits the production of various secondary hormones. The hormone also inhibits insulin and glucagon secretion.[19] Since native somatostatin is rapidly metabolized, it is not suitable for the treatment of juvenile diabetes, several metabolically stable analogues have been developed; for example, octreotide (Sandostatin®, Novartis Pharmaceuticals)[20] and lanreotide (Somatuline®, Ipsen Pharmaceuticals)[21] mimic the pharmacological activity of somatostatin (**10**, **11**, **Figure 1.4**).

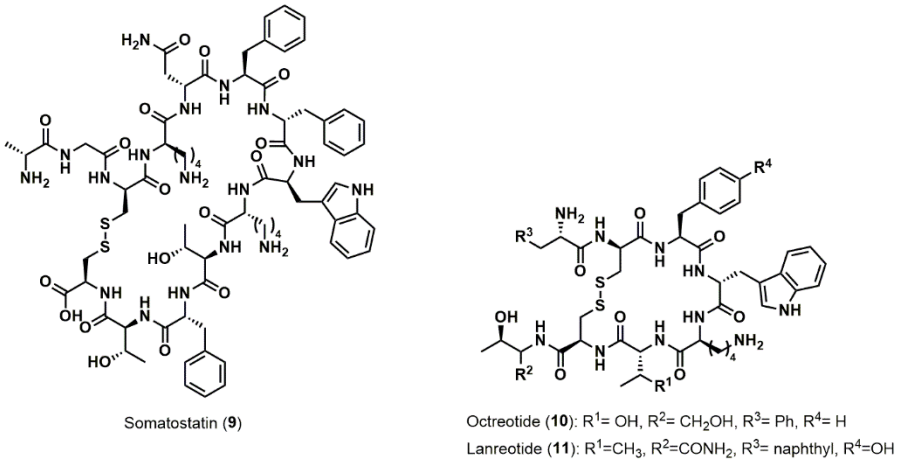


Figure 1.4 Macrocyclic peptide drug analogues of somatostatin.

Especially, octreotide exhibits a more potent inhibition of growth hormone, glucagon and insulin relative to somatostatin.[22] At the same time, lanreotide also shows an increased affinity for somatostatin receptors with a much longer half-life time than somatostatin.[23] Both peptide drugs are approved for the treatment of acromegaly and octreotide is prescribed to patients with metastasizing carcinoid and vasoactive tumors.[24]

1.2 Synthetic methodologies for macrocycles

Among macrocyclization methodologies, macrolactonization, macrolactamization, transition metal catalyzed coupling reaction, ring-closing metathesis, disulfide bridge formation, multicomponent reactions, nucleophilic aromatic substitution and click chemistry represent the most efficient and commonly used synthetic approaches to afford peptide macrocycles.[1,25–27]

1.2.1 General consideration of macrocyclization process

According to the functional groups present in the linear peptide backbone, the macrocyclization can be done with four different approaches: head-to-tail (*C*-terminal to *N*-terminally), head-to-side chain, side chain-to-tail, or side-chain-to-side-chain (**Figure 1.5**).[11,28] Macrocyclizations are best achieved under high dilution conditions to minimize undesired intermolecular processes such as oligo- and polymerization reactions.[29] In solutions, macrocyclizations are usually performed in submillimolar concentrations. By comparison, solid-phase macrocyclizations can have a pseudo-dilution phenomenon by themselves, since reactive functional groups attached to the polymeric support are less prone to encounter each other to react. In recent years, several new synthetic methodologies on solid phase have been established to perform peptide macrocyclizations.[30]

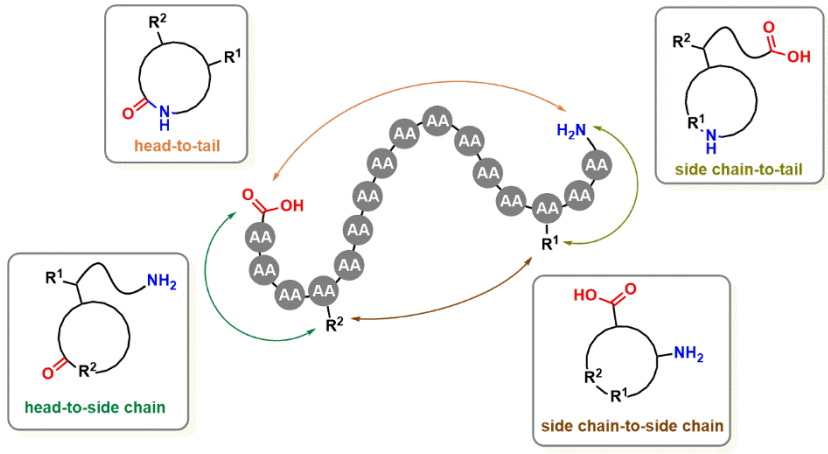


Figure 1.5 The four different sites in linear peptides to perform a macrocyclization.

Among the several factors that rule macrocyclization success, the ring size is one important parameter for the effectiveness of the process. In general, the closing of seven-amino-acid rings or higher is not problematic. Nevertheless, small peptide rings (three to five) are usually difficult to obtain without epimerization or oligomerization. Schmidt and Langner[12] studied small peptide cyclizations of tetra- and pentapeptides in the head-to-tail variant. In most of the cases, the researchers obtained cyclodimerization, and cyclotrimerization products. In addition, during the synthesis of the active cyclotetrapeptide cyclo-[Pro-Val-Pro-Tyr] almost 31% of the epimerization was found at the *C*-terminal. These facts indicate that

the cyclation of small peptides with the same configuration at the *N*- and *C*- termini are prone to invert the α-carbon chirality at the *C*-terminal during the cyclization process (**Figure 1.6**).

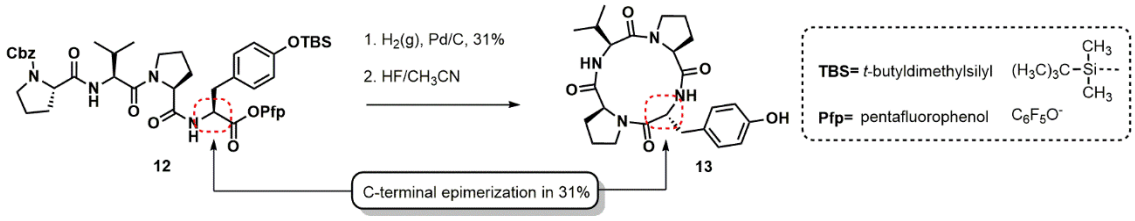


Figure 1.6 Inversion of configuration at the α-carbon of the activated *C*-terminus during the cyclization of the tetrapeptide Pro-Val-Pro-Tyr.

1.2.2 Parameters of efficiency of the macrocyclization reaction

The success of any cyclization is based on the control of the ring/chain equilibrium. Macrocyclization is an entropically disfavoured process, since the starting material is a highly flexible system and loses conformational freedom during formation of the more organized cyclic product. Hence, there is a high risk to obtain a dimeric side product. In this regard, Collins and James[25] established the Emac index that is defined as the log[yield^3×concentration] and is used to determine the efficiency of the macrocyclization process.

1.2.3 Conformational pre-organization

An arrangement of spatial proximity of the reacting atoms in the linear molecule are vital for successful macrocyclizations. Various strategies were developed in order to reach a pre-arrangement in the linear precursor for directing the macrocyclization reaction.[31] Some authors classified these strategies in two categories: (1) internal conformational element, which comprises structural moieties in the peptide backbone that helps the two reacting sides of the molecule to get closer; (2) external conformational element, which consists of scaffolds that are not connected covalently to the peptide backbone and helps to bring the molecule ends together.[11]

1.2.3.1 Internal elements for conformational pre-organization

A theoretical calculation performed by Cavelier-Frontin et al. for various cyclotetrapeptides found that the transition-state energy is the determining factor that rules the ring closing efficiency over the dimerization reaction.[32] This requirement is potentiated if an internal structural element assists the accommodation of a bonding conformation. On the other hand, in longer peptides the formation of internal hydrogen bonds can favour the formation of β-sheet structures, which allow distant reacting residues to get in contact easily if properly positioned.[33]

Secondary structural elements present in a protein thus can be useful to obtain a bended peptide backbone.[34] One approach to bring the two reacting sites to come closely together is introducing an s-*cis*-amide bond in the middle of the peptide chain. Accordingly, one of the most frequently encountered structural motifs found in proteins are reverse turns; especially the β-turn. In proteins, it is common to find these turns by the combination of proline with an L-α-amino-acid. This tertiary amide reduces the energy of the system equalizing or even favouring the conformational s-*cis* configuration over the s-*trans* one. Additionally, the presence of *N*-methylated amino acids in peptides also allows the s-*cis* conformation of an amide bond.[35] Hence, *N*-methylated peptides, similarly to proline-containing peptides are prone to have internal β-turns.[36,37]

The presence of D-amino acids in an otherwise all-L linear peptide backbone induces a turn in the molecule. Nowadays, this is a widely used strategy to improve macrocyclization efficiency.[38,39] Robison and co-workers combined this strategy with the previously commented L-proline bended approach; by the installation of a D-Pro-L-Pro template in a peptide chain. With this, they were able to induce a strong β-hairpin secondary structure in the molecule.[40] Besides, the cyclization reaction is favored also for a diastereomer possesses a D- and an L-amino acid at its termini.[41] Ehrlich et al. showed that the cyclation of an all-L peptide sequence usually afforded epimerization at the *C*-terminal alpha-carbon to D-configuration to accomplish or facilitate the cyclization.[42]

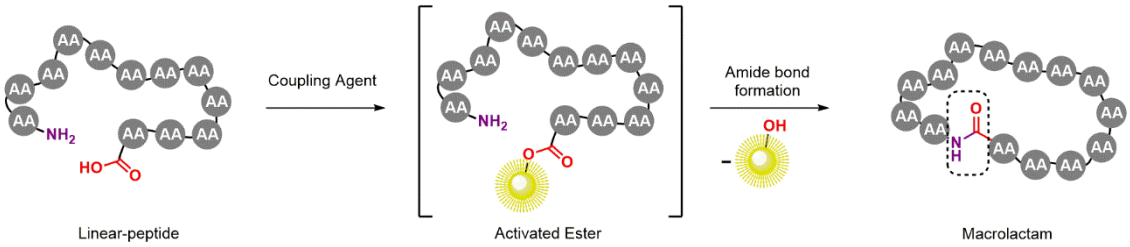
1.2.3.2 External control of conformational pre-organization

External influences to improve macrocyclization can include complexation or isolation of the molecule in a (polymeric) matrix to avoid the dimerization reactions. Tai and co-workers enhanced the difficult macrocyclizations of tetrapeptides by using molecularly imprinted cavities.[43] This strategy stabilized a turn structure with a *cis*-amide conformation imposed by a nano-sized cavity. This mechanism of action can be classified as a site-location mechanism and is enzyme mimetic.[11]

1.3 Macrolactamization and macrolactonization

Among all the cyclic compounds, macrolactams and macrolactones are the major part of the naturally occurring ones with broad and diverse biological activities.[44] For the synthesis of macrolactams, the most useful and efficient approach is to react the amino group with activated carboxylic acid (**Scheme 1.2**). As the typical amide bond formation in peptide chemistry, this process has to be assisted by a coupling reagent. In general, synthetic methodologies used to activate the carboxylic acid group in macrolactamizations include the use of uronium and phosphonium salts to form an active ester or a mixture of carbodiimide and an additive such as HOBt or HOAt. Similar to macrolactamization, the macrolactonization synthetic procedures have also been widely investigated. In general, the most attractive cyclic approach comes from

the direct lactonization of acids and alcohols employing diverse carboxylic acid activation protocols. Most of the linear intermediates used in macrolactonizations have a hydroxyl group on one side of the molecule, and a carboxylic acid group on the other side. Therefore, the most efficient synthetic method follows the three strategies: (1) increasing the electrophilicity of the carbonyl group (just as in macrolactamization); (2) interconverting the alcohol functionality into a better leaving group which could be easily attacked by the carboxylate anion; and (3) activating both functionalities at the same time.[45,46]



Scheme 1.2 Macrolactamization by peptide coupling.

Two representative reactions for the carboxylic acid activation strategy established for macrolactonization are: Yamaguchi and Keck-Boden lactonization. The Yamaguchi lactonization uses 2,4,6-trichlorobenzoyl chloride, combined with DMAP as basic catalyst, at high reaction temperatures.[47,48] In contrast, the Keck-Boden lactonization uses a carbodiimide for the same purpose. Usually, this protocol also uses a proton-supplying compound like DMAP-HCl, to increase the efficiency of the macrocyclation by eliminating the undesired *N*-acylurea.[49] Up to now, there are plenty applications of these two protocols for obtaining macrolactones. Yadav and co-workers[50] used the Yamaguchi lactonization in the synthesis of a novel macrolide antibiotic named FD-891.

Related to the carboxylic acid activation protocols, the activation of the alcohol at the opposite side of the molecular chain represents another choice to reach macrolactones. The Mitsunobu's lactonization combines diethylazodicarboxylate (DEAD) and triphenylphosphine (PPh_3) for turning the hydroxyl into a leaving group.[51] The Mitsunobu approach was used in the synthesis of the marine natural product (+)-tedanolide. In this case, the authors highlighted that Mitsunobu macrolactonization gave better yields than the Keck-Boden and Yamaguchi procedures.[52]

1.4 Amide bond formation and peptide coupling

The amide bond formation consists in the condensation of a carboxylic acid with an amine. In living organisms, long complex proteins are assembled by amino acids in a chemical factory; the ribosome.[53] Unfortunately, chemists do not have the privilege to work on a single-molecule scale; instead, they need to direct the collitions of the molecules in a specific trajectory to accomplish a single defined product-ideally.

In general, peptide coupling methods are based on the "activation" of the carboxylic acid by improving the leaving capacity of the hydroxyl group from the carboxylic acid. The substances used to "activate" the carboxylic acid are classified as a "coupling agent". In modern peptide synthesis, there are three main groups of coupling agents (**Figure 1.7**). The first are the carbodiimide coupling agents, such as EDC (**14**), DIC (**15**), DCC (**16**) (**Figure 1.7a**). The second ones are the phosphonium salts, e.g., BOP (**17**), PyBOP (**18**), AOP (**19**), and PyAOP (**20**) (**Figure 1.7b**). The third ones are the uronium salts reagents, e.g., HBTU (**21**), HATU, (**22**), and TBTU (**23**) (**Figure 1.7c**) among many others, like pentafluorophenols (**24**), T3P® (**25**) (**Figure 1.7d**), all those that have been developed to further increase the efficiency of amide coupling reactions.

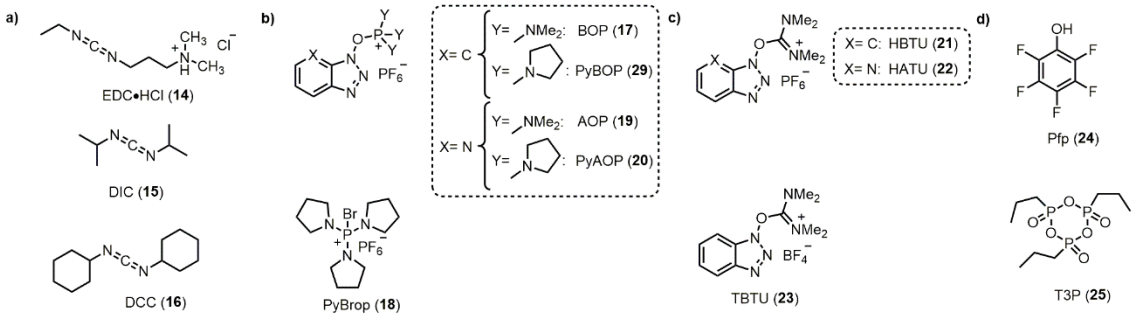
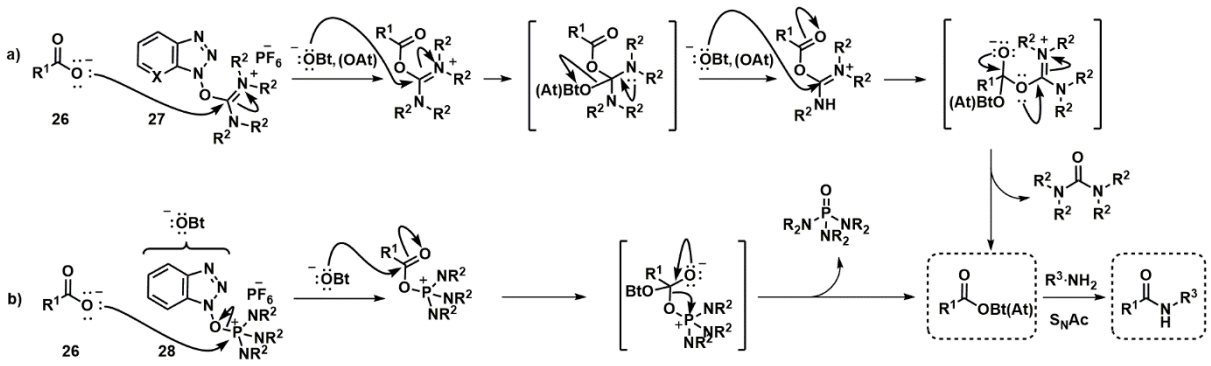


Figure 1.7 Different coupling agents a) carbodiimides; b) phosponium salt; c) uronium salt; d) pentafluorophenol and n-propane phosphonic anhydride.

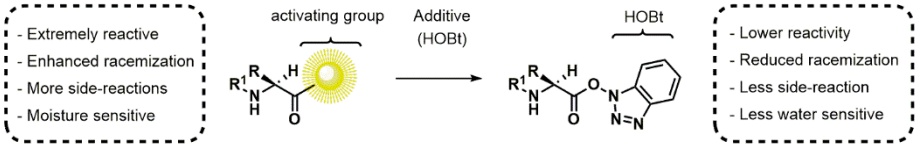
Some of the coupling reagents that hold a great utility in peptide chemistry by forming the active ester *in situ*. The main representatives are the uronium salt, and the phosphonium reagents, which find widespread applications in solution and solid-phase peptide chemistry. The uronium salt HATU (**22**) has been recognized as an excellent choice for performing sterically hindered amide bond formations, oftentimes with a negligible racemization at the α-carbon.[54,55] In many ways, the uronium salts show advantages over the carbodiimide-addition protocols. Nevertheless, high prices of these coupling reagents have limited their use in industry. Similar to uronium coupling reagents, phosphonium compounds like PyBrop and PyBOP are better to couple sterically hindered amino acids.[56,57] The reaction mechanisms of both reagents (uronium and phosphonium salts) work similar (**Scheme 1.3**). The carboxylate (**26**) anion performs a nucleophilic attack on the electrophilic position of the *O*-acyluronium (**27**) or acyloxy-phosphonium (**28**) salt that reacts with the benzotriazole oxyanion to produce the active ester, which reacts with the amine component at the end (**Scheme 1.3a,b**).



Scheme 1.3 Reaction mechanisms by onium-mediated peptide coupling: a) Uronium reagents: X = CH, HOBt (HBTU); X = N, HOAt (HATU), b) Phosphonium reagents: R^2 = Me (BOP); R^2 = (CH_2), (PyBOP).

n-Propanephosphonic acid anhydride (T3P®) is a versatile and cheap reagent used in many different reactions like: synthesis of heterocycles,[58–70] oxidation reactions,[71] dehydrating processes,[72,73] rearrangements,[74–77] esterification reactions,[78,79] carbon-carbon[80] and amide bond formations.[81–94] The major application of T3P® lies in coupling peptides since it has low toxicity, is easy to handle and the by-product stemming from the coupling is water-soluble. In addition, T3P® has become popular for its easy access to peptides with minimal racemization without the obligation to use an additive.[90,95–99]

While these coupling reagents are widely used and work for most simple amide couplings, they can show some inconvenient properties such as low conversion in the synthesis of large peptides or difficult couplings of disubstituted amines. An important issue is the racemization[1,100,101] at the α-carbon of the activated carboxylic acid.[102] Consequently, the optical purity and stereochemical integrity of the synthetic peptide is highly dependent on the degree of epimerization during the coupling steps. During peptide coupling the racemization of amino acid α-carbon follows two mechanisms: the direct enolization of the active ester or through a 5-(4*H*)-oxazolone intermediate.



Scheme 1.4 Main characteristic of the additive methods.

One of the synthetic strategies to diminish racemization and enhance the coupling rate is the "additive method" (**Scheme 1.4**). This method is based on the addition of a nucleophile that reacts fast to another active intermediate that eventually couples to the amino component and suppresses *N*-acyl urea formation

[1] While the expression racemization in organic chemistry is used for the complete conversion of a single enantiomer into the racemate, it is often used in peptide chemistry also for partial or total epimerization at one chiral center irrespective of whether a mixture of diastereomers or enantiomers is formed in the course of the process

and racemization. The addition of rate enhancers or racemization suppressants, such as dimethylaminopyridine DMAP (**29**) or HOBt (**30**), can help to lessen the degree of racemization observed; however, they cannot eliminate this undesired process totally (**Figure 1.8**).

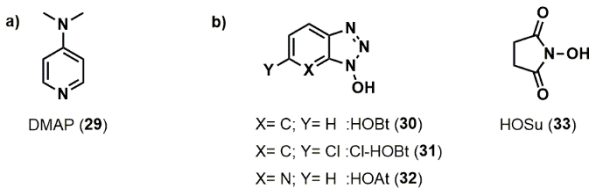
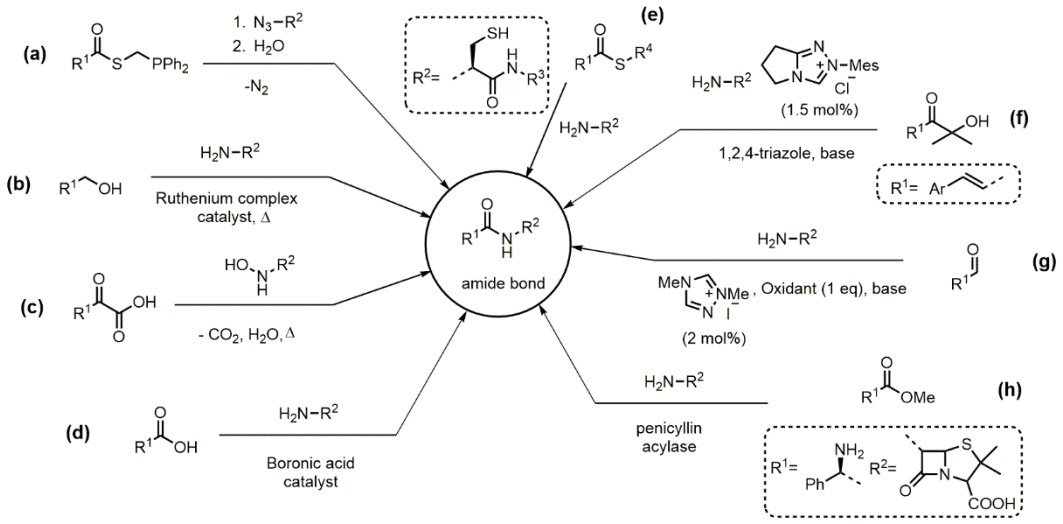


Figure 1.8 Common coupling additives. a) Rate enhancer additive; b) Racemization suppressant additive.

Regardless of these difficulties, the use of peptide coupling reagents has prevailed over the years as the favorite method for amide synthesis. The application of these reagents to solid phase synthesis, in which reagent excess can be used to drive the condensation to completion, has made the synthesis and purification of peptides much faster than in solution phase. Recently, alternatives to conventional amide synthesis have emerged since chemists try to address these practical challenges.[103] As seen in **Scheme 1.5**, these novel approaches include native chemical ligation,[104] Staudinger ligation,[105–111] oxidative amidation of alcohols,[112] ketoacid-hydroxylamine ligation,[113] using protease and amidase among others.



Scheme 1.5 Other approaches to amide synthesis: a) Staudinger ligation. b) Direct coupling of an alcohol with an amine mediated by a ruthenium catalyst. c) Ketoacid-hydroxylamine ligation. d) Direct condensation catalysed by boronic acid. e) Native chemical ligation. f) and g) Organocatalytic redox and oxidative amidation. h) Using enzymes (e.g., industrial ton-scale production of ampicillin).

1.5 Peptide macrocyclization on-resin

Modern applications of SPPS are in macrocyclization reactions, which are carried out while the peptide is still anchored to the resin. This approach takes benefits from the previously mentioned "pseudo-dilution" effect by favouring intramolecular reactions over the intermolecular ones.[29,114,115] On-resin cyclization methodologies possess the same attributes as the classical SPPS in which most by-products (except oligomers) can be easily removed by washing and filtration procedures. Moreover, on-resin cyclization can be used for new drug discovery approaches, since a combinatorial library can be readily constructed. The most used on-resin cyclization methodes include ring-closing metathesis (RCM),[116–124] intramolecular S_N2 or S_NAr,[125–133] transition metal catalyzed intramolecular coupling reactions,[134–140] Cu (I)-catalyzed "click chemistry",[141–155] thio-ene "click chemistry",[156–164] enzyme-mediated methods,[165–173] multicomponent reactions, and the classical coupling peptide reaction.[11,15]

Cyclic head-to-tail linked peptides can be synthesized in good purity using standard SPPS protocols. Nevertheless, the cyclization is a critical step and depends on the amino acid sequence, structural constraints and the desired ring size.

1.6 Multicomponent reactions

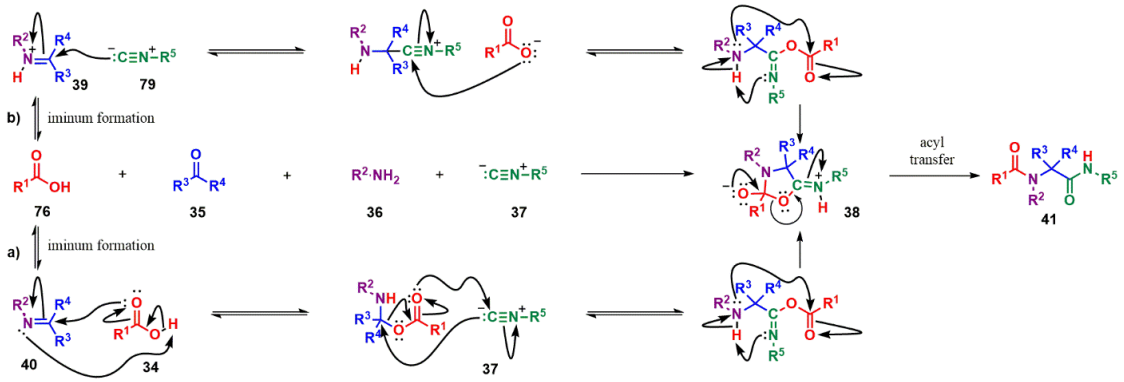
All reactions in which more than two reactants interact to form a product, ideally even in a way to keep all or most of the atoms of the starting material, are called multicomponent reactions (MCR).[174–178] Usually, the most important characteristic of MCRs are the high atom economy and the rapid generation of complicated structures in one-pot reactions. Therefore, MCRs are intensely used in drug discovery, compound library formation, and the synthesis of complex natural products.

The most useful MCRs are those which use isocyanides as one of the reactants. These reactions are classified as IMCRs (isocyanide-based multicomponent reactions), characterized by a final irreversible step. The most published IMCRs are the Passerini three component and Ugi four-component reaction. In 1921 Passerini discovered the condensation product α-acyloxycarboxamide by reacting a carbonyl, a carboxylic acid, and an isocyanide. This discovery allowed Ugi to find in 1959 a similar condensation product, this time to react a mixture of a carbonyl, a carboxylic acid, an isocyanide, and an amine component. Both reactions boosted the isonitrile chemistry development and enabled IMCRs to spread out into heterocyclic synthesis.[31,174,179–186]

1.6.1 Ugi four-component reaction (Ugi-4CR)

The reaction discovered by Ugi *et al*. in 1959 represents one of the most versatile multicomponent reactions (MCRs) of all times. This reaction is commonly classified as an isocyanide-based multicomponent reactions (IMCRs) and comprises the addition of four-component in one-pot: a carboxylic acid (**34)**, an oxo-

compound (aldehyde or ketone) (**35**), an amine (**36**), and an isocyanide (**37**) to give an *N*-acyl-α-aminoamide (**38**) (**Scheme 1.6**).[187]



Scheme 1.6 Proposed Ugi-4CR mechanism.

Two possible mechanism have been postulated to justify the Ugi product formation.[188] In both mechanisms, the first step comprises the condensation of the oxo and the amine component under the water loss to form an imine (**39** and **40**). In both mechanisms, the second step involves the protonation of the imine to form the electrophilic iminium ion (**39**). In the next step, the postulated mechanisms, differ. The first one (**Scheme 1.6a**), suggests the nucleophilic attack of the carboxylic acid component to the iminium ion followed by the reaction with the isocyanide via S_N2 mechanism. The second mechanism (**Scheme 1.6b**) postulates that isocyanide undergoes a nucleophilic addition to the iminium ion, followed by carboxylate addition to form the α-adduct. Moreover, in both mechanistic proposals, the final irreversible step comprises the intramolecular acyl rearrangement (Mumm rearrangement)[189] of the α-adduct affording the more stable Ugi product (**41**). The lack of mechanistic studies might be attributed to the occurrence of competing mechanisms.[188] In the course of this condensation, a new stereogenic center is formed when a prochiral oxo component is used. As a result, a mixture of both stereoisomers is usually obtained. The strength of the Ugi-4CR is settled over the high structural diversity generated by simple variation of the different four-components. This characteristic makes this reaction a very powerful synthetic tool for developing combinatorial chemistry approaches, drug discoveries, or in total synthesis.

1.6.2 Ugi four-component reaction in total synthesis

Structural design and biological properties of natural products are a source of inspiration for an organic chemist to get involved in a synthetic challenge. This motivation is the driving force for pushing forward the development of new protocols and synthetic methodologies for bond formation. In this endeavour, concepts such as: simplicity, resource effecacy, available starting materials, 100% yield, and environmentally-friendliness are relevant issues to be considered.[190] The Ugi-4CR is an excellent synthetic tool to obtain peptide-like structures, especially those related to natural products with a peptidic structure.

Even though this MCR lacks stereocontrol, enantiomers or diasteroisomers, they are usually separable and therefore quite valuable for biological screening (e.g., natural products and their analogues). As a result, many different natural products have been synthesized using the Ugi-4CR.[191–198] Among the natural products synthesized using Ugi-4CR there are several macrocyclic peptides (**Figure 1.9**). In 1999, Armstrong et al. synthesized Mutoporin, a natural marine macrocyclopeptide with a strong cytotoxicity against different cancer cell lines.[199] Armstrong showed the usefulness of Ugi-4CR to install *N*-methyl dihydroamino acid residues on a peptidic sequence. Similarly, the Kazmaier group synthesized the sterically high hindered endothiopeptides bottromycins A and B, using a thio-Ugi strategy.[195] The authors formed the amidine moiety by methylating the sulfur position in an inter- and in an intramolecular manner. Hutton et. al. also used Ugi-4CR to synthesize the highly, cyclic funtionalized natural product ustiloxin D.[200]

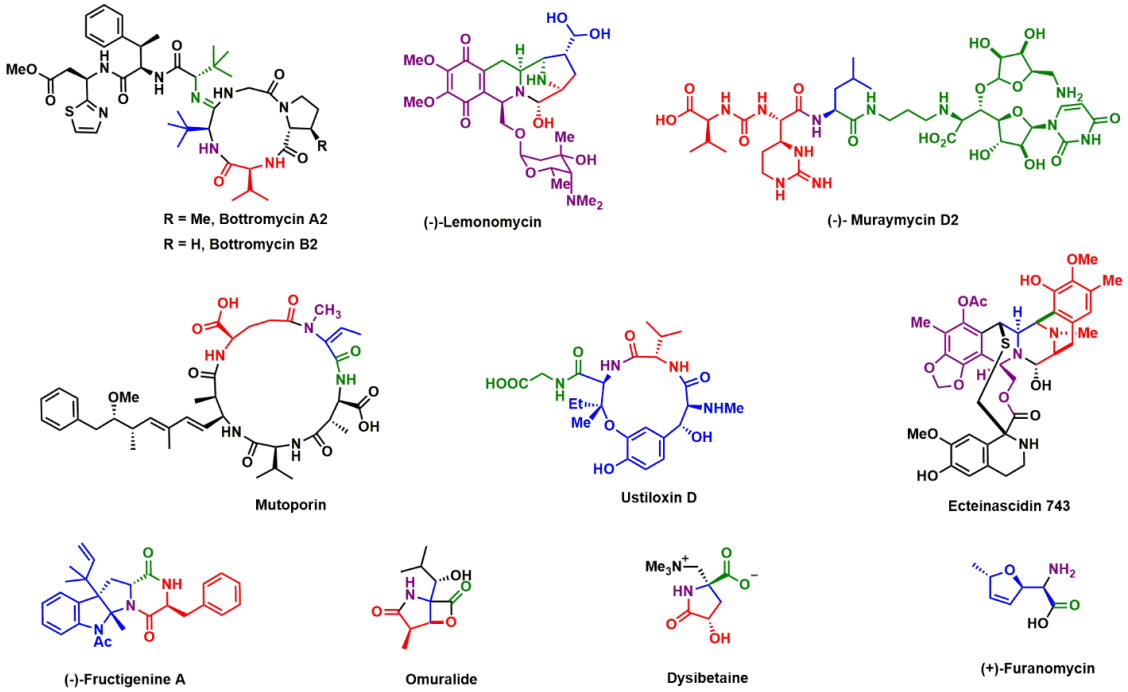


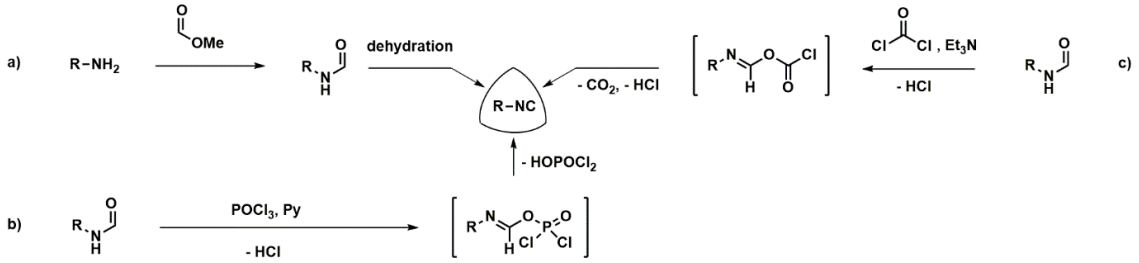
Figure 1.9 Natural products synthesized using Ugi-4CRs.

1.7 Isocyanide and convertible isocyanide

The isocyanide (also termed isonitrile) fuctional group was originally discovered in the late 1860´s,[201,202] as an isomer of cyanides. Ever since, it has turned into a versatile component of building blocks for organic synthesis.[203,204] It represents a particular kind of a stable organic compound with a formally divalent carbon atom[205], which allows these functionalities to react with electrophiles, nucleophiles and radicals.[206] The main organoleptic characteristic of isocyanides is an intense, mostly unpleasant odour of the volatile members, which is very often used to detect their presence in the reaction medium.

1.7.1 Synthesis of isocyanides

Until recently, isocyanides were prepared just before their use,[206–208] but now many are commercially offered. A straightforward synthetic method for their synythesis is the dehydration of *N*-monosubstituted formamides (**Scheme 1.7**).[209–212] Some of the most widely used reagents providing dehydration include: Burgess reagent,[213] Vilsmeier reagents,[214] cyanuric chloride,[215] trifluoromethanesulfonic anhydride,[216] tosyl chloride,[217,218] chlorodimethylformiminium chloride,[214] chlorophosphate derivatives,[219] CCl_4/PPh_3,[220] XtalFlour-E (X),[221] phosgene[222] and phosphoryl chloride.[223] The protocols with phosphorous oxychloride (**Scheme 1.7b**) and the phosgene derivatives (**Scheme 1.7c**) in basic media are the most widely used ones due to their high yield, low cost, and easy performance.[224,225]



Scheme 1.7 a) General scheme for amine formylation and dehydration to obtain isocyanides, b) Using $POCl_3$ as dehydrating agent, c) Using phosgene as a dehydrating agent.

1.7.2 Convertible isocyanides

Using an isocyanide in MCRs usually leads to the formation of a new secondary carboxamide group in the product. If *N*-substituents are used that allow a mild synthetic method for the amide convertion e.g., a carboxylic acid, ester or other functional group, the isocyanide is classified as "convertible isocyanide". This is one of the ways to increase the versatility of the IMCRs.

In 1986, Ivar Ugi reported the first reaction to an "Ugi-product".[226] Only in 1995, the formal term of a "convertible isocyanide" was introduced by Armstrong and Keating.[227] The well known "Armstrong isocyanide" (cyclohex-1-ene isonitrile) can be used in the Ugi-4CR to obtain a cyclohexenamide, that in acidic media and with a proper nucleophile can be turned into diverse functional groups like carboxylic acids, esters, thioesters, and primary amines. After the Armstrong isocyanide was discovered, newer convertable isocyanides have been found (**Figure 1.20**). Recently Zhu and colleagues reported a series of novel vinyl convertible isocyanides based on, the Wittig reaction.[228] Likewise, Orru and Ruijter published in 2016 a new convertible isocyanide named 2-bromo-6-isocyanopyridine, which was applied to the synthesis of a potent opioid carfentanil.[229] The authors also reported that contrary to most of the convertible isocyanides, the residual amide from the isocyanide conversion could be recovered and recycled.

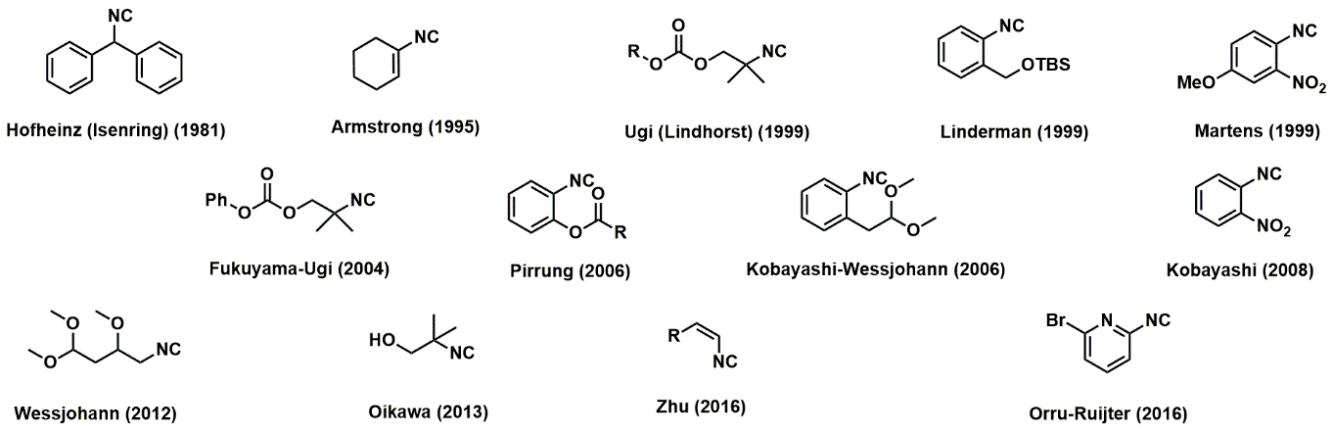
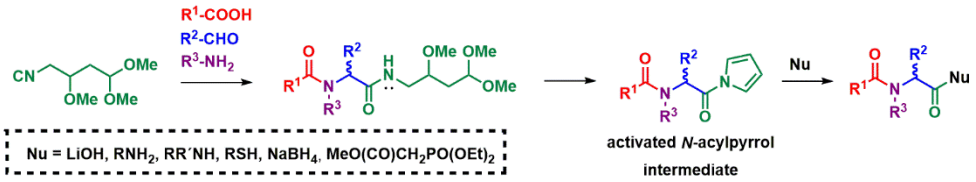


Figure 1.20 Convertible isocyanides.

In 2012 Wessjohann and co-workers published the synthesis and application of 4-isocyanopermethylbutane-1,1,3-triol (IPB) as a novel convertible isocyanide for IMCRs.[230] The convertible isocyanide obtained showed good to excellent reactivity in different IMCRs, great stability in storage and handling. IPB allows to perform a mild functional group interconversion via *N*-acylpyrrole intermediates (**Scheme 1.8**).



Scheme 1.8 Reactivity of IPB in Ugi-4CRs and conversions to different functional groups.

1.8 Turn-inducer and *N*-alkylated amides

The amide bond is the main architectural framework element of peptides. This functional group has been intensely studied, due to the property of forming proteins, which are the building blocks of life. Secondary amide bonds are the most abundant ones in nature, however, oftentimes *N*-methylated amino acids or proline are present in the peptidic backbone.[231,232] The existence of tertiary amide bonds in biomolecules increases their transport through the biological membranes by favouring the passive transcellular diffusion. The substitution of the NH functional group by *N*-Me decreases the hydrogen-bonding potential of *N*-alkylated peptides, while simultaneously increasing the hydrophobicity of the molecule.[233,234] Also, *N*-alkylated peptides show an increases resistance to proteolytic degradation and in drugs an enhancement of intestinal permeability.[102,235,236]

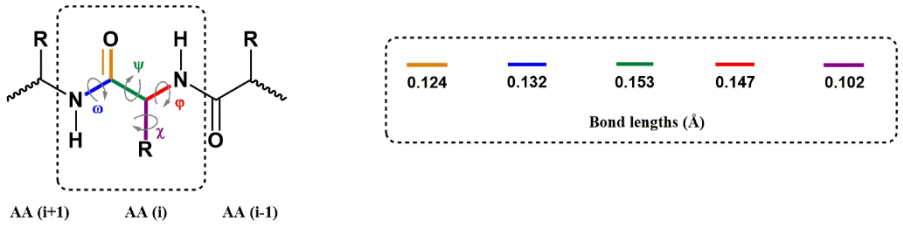


Figure 1.21 Depicted bond lengths and dihedral angles ω, ψ, φ and χ of amino acid AA (i) in a peptide.

A very special property of amide bonds is the presence of s-*cis* and s-*trans* equilibria around the carbonyl carbon and the nitrogen. The Nobel laureates Pauling[237] and Corey demonstrated by X-ray crystallography of several amino acid and simple linear peptides that the bond distance of the double bond of the amide carbonyl carbon (C=O) is longer than in aldehydes and ketones; also that the C-N distance in peptide bonds is shorter than typical C-N single bond (**Figure 1.21**). The electronic delocalization (tautomerism) over the peptide bond gives a partial double bond character to the C-N bond (**Scheme 1.9**). Therefore, a rotational barrier of 65-90 kJ/mol^{-1} restrains the free rotation around the C-N bond. Consequently, two rotamers of the peptide bond are distinguishable. The single-trans (s-*trans*) and the single-cis (s-*cis*) rotamers are established with an energetic difference of 8 kJ/mol^{-1}. The former is more stable and therefore the most abundant rotamer in polypeptide sequences. Nonetheless, the presence of *N*-alkyl ions like in *N*Me or proline amides in the molecule reduces the energy difference between s-*cis* and s-*trans*. One special *N*-variation moiety are the so-called peptoids, which are *N*-substituted oligomers of glycine monomers. These molecules are important for medical applications, since peptoids increase the hydrophobicity, enzymatic stability and intestinal permeability. Kessler and co-workers showed that *N*-Me increased the resistance to proteolytic degradation of the peptidic chain due to the lack of amide protons.[36]

Scheme 1.9 a) Resonance stabilization of peptide bond; b) s-*cis*/s-*trans* isomerization.

1.9 General goal of the work

The main goal of this work is: the development of synthetic methodologies based on the Ugi-4CR for the generation of *N*-alkylated peptide fragments either present in natural cyclopeptides or serving as turn inducers to facilitate peptide macrocyclization.

For accomplishing the general goal, this work has been divided into four parts, each one having a specific objective and corresponding to a chapter herein presented.

Chapter 3 describes the total synthesis of the *N*-alkylated cyclopeptide Cordyheptapeptide A. The objective of this chapter was *the development of an efficient synthetic methodology for the synthesis of this natural product employing the Ugi-4CR with convertible isocyanides.* An innovation of this part was the employment of a novel convertible isocyanide previously developed in the group for incorporating *N*-methylated peptide moieties present in the compound.

In **Chapter 4**, the aim was *to study the effect of the* N-*alkyl residue in the s*-cis/*s*-trans *isomerization process of the tertiary amide bond derived from the Ugi-4CR*. Consequently, a series of *N*-alkylated model peptides were prepared and studied by NMR to assess the influence of the *N*-alkyl residue on the population of the s-*cis* and s-*trans* isomers. The information provided by this study become useful for the design of the last two chapters of the thesis, which takes advantage of the turn inducing capacity of the *N*-alkylated fragment for favouring the cyclization of peptides.

In **Chapter 5** *a methodology for incorporating a traceless turn inducer capable of facilitating the head-to-tail macrocyclization of oligopeptides was developed*. In this chapter, the focus was posed on the implementation of solution protocols using the Ugi-4CR for installing the traceless *N*-alkylated peptide fragment serving as turn inducer. A crucial issue in this part was the assessment of the macrocyclization improvement of *N*-alkylated peptides as compared to the non-alkylated ones.

In the final **Chapter 6**, the goal was *to carry out the methodology of head-to-tail macrocyclization through the solid-phase synthesis*. In this chapter, the objective was *based on the application of the traceless turn inducer on solid-phase support*. Thus, the Ugi-4CR was used for the installation of the traceless *N*-alkyl peptide fragment on the solid-phase matrix, which assisted as turn inducer during the on-resin macrocyclization reaction.

Chapter 2

Experimental Section

2.1 General equipments, materials and methods

Solid-phase peptide synthesis was carried out either manually or in a ResPep SL peptide synthesizer (Intavis Bioanalytical Instruments, Germany). Fmoc amino acids and PyBOP were supplied from Novabiochem (Germany). Rink Amide MBHA and TentaGel-S-RAM resin were purchased from Iris Biotech GmbH (Germany). Piperidine, acetic acid, formic acid, DIPEA, TFA and DMF were bought from Sigma-Aldrich (Germany). Analytical thin layer chromatography (TLC) was performed on silica gel 60 F254 aluminum sheets and spots were visualized by UV (254 nm), or by staining with 5% phosphomolybdic acid hydrate in ethanol. Preparative RP18 HPLC was undertaken 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 μm, 300 Å, 250 x 9 mm internal diameter, VYDAC, 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). Flash column chromatography (FC) was accomplished by using silica gel (0.040-0.063 mm). Photochemical cleavage was carried out in a Rayonet RPR-200 photochemical chamber reactor (The Southern New England Ultraviolet Company, USA) equipped with 16 × 14 W, RPR-2537Å (low pressure Hg vapour lamp with > 80% emission at 254 nm) lamps. ^{1}H and ^{13}C NMR spectra were recorded at room temperature on a 400 or 600 MHz spectrometer. Chemical shifts are reported in ppm relative to TMS (^{1}H NMR) and to solvent signal (^{13}C NMR). High resolution ESI mass spectra were obtained either from an Apex III Fourier transform ion cyclotron resonance (FT-ICR), a mass spectrometer supplied with an Infinity™ cell, a 7.0 Tesla superconducting magnet, an RF-only hexapole ion guide and an external electrospray ion source (off axis spray) or 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). This latter MS system was coupled to an ultra-high performance liquid chromatography (UHPLC) system (Dionex UltiMate 3000, Thermo Fisher Scientific) provided with an RP18 column (Hypersil GOLD, 50 × 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 by 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 by using the software Xcalibur 2.7 SP1. Convertible isocyanide 4-isocyanopermethylbutane-1,1,3-triol (IPB) was prepared as previously reported.[230]

2.1.1 General solution-phase peptide synthesis

The peptide or amino acid methyl ester hydrochloride (1.0 mmol) and the Cbz-protected amino acid or peptide (1.1 mmol) are dissolved in dry DMF (10 mL) and cooled to 0 °C. HBTU (1.1 mmol) and DIPEA (0.52 mL, 3.0 mmol) are added, and the reaction mixture is allowed to warm up to room temperature and stirred for 12 h. The mixture is then poured into water (50 mL) and extracted with ethyl acetate (4 × 20 mL). The organic layer was washed with aqueous hydrochloric acid 10% *v*/*v* (2 × 20 mL), saturated aqueous $NaHCO_3$ (2 × 20 mL), brine (2 × 20 mL) and dried over Na_2SO_4. The organic phase was evaporated to dryness and the crude material purified by silica gel column chromatography.

2.1.2 General procedure for the Ugi-4CR

A suspension of the amine (1.5 mmol) and the aldehyde (1.5 mmol) in MeOH (5 mL) is stirred for 2 h at room temperature. DIPEA (1.5 mmol) is added when the amine is used as hydrochloride salt. Carboxylic acid (1.0 mmol) and isocyanide (1.0 mmol) components are added and the reaction mixture is stirred at room temperature for 24 h. The solvent is removed under reduce pressure and the crude product dissolved in EtOAc (30 mL). The solution is transferred to a separator funnel and washed with aqueous hydrochloric acid 10% *v*/*v* (2 × 50 mL), saturated aqueous $NaHCO_3$ (2 × 50 mL), brine (2 × 50 mL), dried over Na_2SO_4. The organic phase is evaporated to dryness and the crude material purified by silica gel column chromatography.

2.1.3 General Boc/DMB removal procedure

The peptide is dissolved in a trifluoroacetic acid (20% *v*/*v*) solution in CH_2Cl_2, and the mixture is stirred at room temperature for 2-4 h. As the material dissolved, gas evolution could be detected and the pressure that built up inside the reaction flask was released through a bubbler or in a very small scale was regularly relieved by opening the flask. The latter reaction is confirmed by ESI-MS analysis and TLC. The solvent is removed under reduced pressure and toluene (20 mL) is added; the contents are then concentrated under reduced pressure to dryness. This operation is repeated twice in order to remove the remaining TFA. In case of Boc removal, the resulting salt is used thereafter without further purification, assuming quantitative yield.

2.1.4 General methyl/ethyl ester removal procedure

The peptide (1.0 mmol) is dissolved in THF/H_2O (1:1, 6.0 mL) and LiOH.H_2O (105 mg, 2.5 mmol) is added at 0 °C. The mixture is stirred for 5 h and then acidified with aqueous 10% $NaHSO_4$ to pH 3.0. The resulting phases are separated and the aqueous phase is additionally extracted with ethyl acetate (2 × 20 mL), transferred to a separator funnel and washed with brine (2 × 20 mL). The combined organic phases are dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to yield the *C*-deprotected peptide.

2.1.5 General Cbz removal procedure

The Cbz-protected peptide is dissolved in MeOH (100 mL) and the palladium catalyst supported on charcoal (Pd/C, 10 wt%) is added. The reaction vessel is evacuated, purged and filled with hydrogen atmosphere and then stirred under hydrogen atmosphere (1 atm) for 12 h. After filtration through Celite™, the solvent is removed under reduced pressure, suspended in diethyl ether (40 mL) and evaporated to dryness again to afford a white solid.

2.1.6 General procedure for the synthesis of chiral isocyanopeptides

The peptide formamide (1.0 mmol) is dissolved in dichloromethane (10 mL) under argon, and cooled down to -60 °C. The resulting mixture was added 2,6-lutidine (175 μL, 1.5 mmol) and triphosgene (59 mg, 0.2 mmol), and stirred at that temperature for three hours. TLC checks are used to indicate whether the starting material has disappeared. The reaction mixture is diluted with dichloromethane (20 mL), and neutralized with saturated sodium bicarbonate solution (20 mL). The resulting organic phase is washed with brine, and dried over sodium sulfate. The solvent is evaporated at low pressure to yield the crude, and the residue purified by column chromatography to supply the pure product.

2.1.7 General solution-phase macrocyclization procedure with T3P®

To a solution of the fully deprotected *N*-alkylated peptide (0.2 mmol, 1.0 equiv) in dichloromethane (200 mL) are added DIPEA (3.0 equiv) and propylphosphonic anhydride (T3P®) (50% *w*/*w* solution in ethyl acetate, 1.5 equiv). After stirring the mixture for 12 h, the solvent is removed under reduced pressure. The crude residue is dissolved in ethyl acetate (50 mL) washed with water (2 × 30 mL), aqueous hydrochloric acid 10% *v*/*v* (2 × 30 mL), 10% *v*/*v* aqueous $NaHCO_3$ (2 × 30 mL), brine (2 × 30 mL), dried over Na_2SO_4 and evaporated to dryness. The crude product is purified by silica gel column chromatography.

2.1.8 General solution-phase macrocyclization procedure with PyBOP

To a solution of the fully deprotected *N*-alkylated peptide (0.2 mmol, 1.0 equiv) in DMF (200 mL) are added PyBOP (390 mg, 3 equiv.) and diisopropylethylamine (0.22 ml, 6 equiv.). The reaction mixture is stirred for 6 h at room temperature and then concentrated under vacuum. A mixture of water/acetonitrile

(1:1, 20 mL) is added and the suspension is sonicated and centrifuged. After removal of the supernatant, the resulting product is washed once using the same procedure, then suspended in water/acetonitrile (1:1, 5 mL) and lyophilized. The resulting product is purified by silica gel column chromatography.

2.1.9 General *N*-alkylation procedure

To a stirred solution of the amino acid (1.0 equiv) and methyl iodide (7.0 equiv) in anhydrous THF (150 mL) at 0 °C is added sodium hydride (60% dispersion in mineral oil; 3.0 g, 138 mmol) in portions of 1.0 g each ten minutes. The mixture was stirred at room temperature for 24 h under N_2 atmosphere. The reaction was cooled to 0 °C and carefully quenched by adding water (20 mL). The THF was evaporated under reduced pressure. To the remaining content were added water (100 mL) and ethyl acetate (200 mL). The organic layer was washed with water (1 × 50 mL) $Na_2S_2O_5$ aqueous solution (30% w/w, 1 × 50 mL), brine (1 × 50 mL) and was dried over Na_2SO_4. The solvent is evaporated under reduced pressure and the remaining crude residue is purified by column chromatography.

2.1.10 General nucleophilic esterification procedure

To a suspension of *N*-terminally protected amino acid (1.0 equiv) and potassium carbonate (2.0 equiv) in DMF (30 mL) is added iodomethane (1.5 equiv), and the mixture is stirred at room temperature for 30 h. The mixture is filtered, and the filter cake was washed with ethyl acetate (50 mL), dissolved in water (100 mL), and extracted with ethyl acetate (2 × 100 mL). All the ethyl acetate solutions were combined with the filtrate, and the solution was evaporated under vacuum until most of the DMF has been removed. The residue was re-dissolved in ether (250 mL), washed with water (100 mL) and brine (50 mL), dried (Na_2SO_4), and evaporated to afford the desired product as a yellow oil. The resulting product is used forward without further purification.

2.1.11 General procedure for the multicomponent synthesis of backbone amide resin-linked peptides

The Fmoc-TentaGel S RAM resin (380 mg, loading 0.26 mmol/g) or Fmoc-Rink Amide MBHA resin (140 mg, loading 0.71 mmol/g) is treated with a 20% piperidine in DMF (*ca.* 4 mL) and swirled for 30 min to remove the Fmoc group. The resin is then washed successively with CH_2Cl_2, MeOH and THF prior to the on-resin Ugi reaction. A suspension of paraformaldehyde (0.4 mmol, 4 equiv.) and piperidine (0.4 mmol, 4 equiv.) in 2 mL of THF/MeOH (3:1, *v*/*v* for MBHA and 1:1, *v*/*v* for TentaGel) is added to the resin (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 × 2 mL). The isocyanopeptide allyl ester or ethyl isocyanoacetate (0.4 mmol, 4 equiv.) dissolved in THF/MeOH (1:1, *v*/*v*, 2 mL) and the Fmoc-amino acid (0.4 mmol, 4 equiv.) dissolved in MeOH (2 mL) are sequentially 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. The resin-bound peptide is dried and next the further peptide growing is carried out in an Automated Solid-Phase Peptide Synthesizer by stepwise Fmoc strategy in 0.1-0.3 mmol scale using the coupling cycle from the standard Intavis protocol.

2.1.12 General on-resin procedure for the simultaneous Fmoc and ethyl ester deprotection

The resin-bound peptide (arising from the automated solid-phase peptide synthesizer) is washed successively with DMF and CH_2Cl_2 and again with DMF. The resin is treated with a 0.25 M solution of KOH in EtOH/THF (1:1, *v*/*v*) 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 10% solution of $NaHSO_4$ during 15 minutes, washed with H_2O (2 × 2 mL), EtOH (2 × 2 mL), CH_2Cl_2 (2 × 2 mL) and dried.

2.1.13 General on-resin macrocyclization procedure

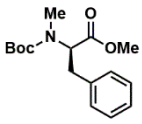
The deprotected resin-bound peptide is swelled in DMF (3 mL), washed and treated with in a solution of HATU (0.4 mmol, 4 equiv.) and DIPEA (1.2 mmol, 12 equiv.) in DMF (5 mL). The mixture is swirled for 4 h (a negative Kaiser ninhydrin test is required) and the resin is then washed sequentially with DMF and CH_2Cl_2. The resin-bound cyclic peptide is cleaved from the resin with the cocktail TFA/TIPS/H_2O (95:2.5:2.5, *v*/*v*, 5 mL), and the resulting mixture is concentrated to dryness. The crude cyclic peptide is taken up in 1:2 acetonitrile/water, lyophilized, analysed for purity by RP-UHPLC and purified by preparative RP-HPLC to > 95% purity.

2.1.14 General photochemical cleavage procedure

A solution of *o*NB-peptide in methanol (10 mL) is irradiated at 254 nm in a quartz flask inside a Rayonet photochemical reactor at room temperature. The reaction is monitored by ESI-MS and after 6 h the reaction is completed. The solvent is evaporated under reduced pressure and the remaining crude residue is purified by RP-HPLC.

2.2 Synthesized compounds in chapter 3

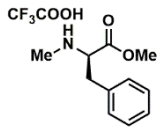
Boc-*N*Me-D-Phe-OMe (42)



The *N*-terminally protected amino acid Boc-D-Phe-OH (3.7 g, 14.0 mmol) was subjected to the general alkylation procedure of the *N*-amide group. The obtained material (3.63 g, 13.0 mmol) was subjected to the general esterification procedure of the free *C*-terminal. The pale yellow oil product **42** (3.8 g, quant) was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) 7.27 – 7.16 (m, 5H), 5.28 (s, 1H), 3.74 (s, 3H), 3.39 (m, 1H), 3.01 (m, 1H), 2.72 (s, 3H), 1.37

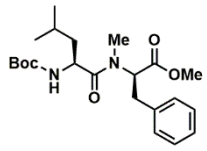
and 1.33 (2s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.7, 171.2, 155.6, 154.7 137.5, 137.2, 128.9, 128.8, 128.4, 128.2, 126.5, 126.3, 80.1, 79.8, 61.5, 59.4, 52.2, 35.4, 34.9, 32.4, 31.8, 28.1. HRMS (ESI+) *m*/*z* calcd for $C_{16}H_{24}NO_4$, $[M+H]^+$; 294.1705; found, 294.1124.

H-*N*Me-D-Phe-OMe·TFA (43)



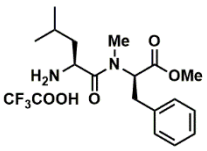
Peptide **42** (3.8 g, 12.9 mmol) was subjected to the general deprotection procedure of the *N*-terminally by Boc removal. The crude product **43** (3.9 g, quant) was used in the next step without further purification.

Boc-Leu-*N*Me-D-Phe-OMe (44)



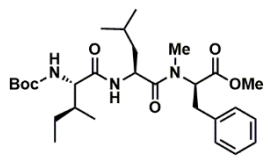
To a solution of Boc-Leu-OH (2.93 g, 12.7 mmol) in DMF (25 mL) at 0 °C were added **43** (3.9 g, 12.7 mmol), HBTU (4.81 g, 12.7 mmol) and DIPEA (4.92 g, 6.6 mL, 38.1 mmol). The reaction mixture was warmed up to room temperature and stirred for further 24 h. The mixture was poured in water (200 mL) and extracted with ethyl acetate (3 × 50 mL). The organic layer was washed with aqueous hydrochloric acid 10% v/v (2 × 50 mL), saturated aqueous $NaHCO_3$ (2 × 50 mL), brine (2 × 50 mL), dried over Na_2SO_4. The organic phase was evaporated to dryness and the crude material purified by silica gel column chromatography (ethyl acetate / hexane 1:4) as eluents to afford (3.93 g, 76%) of compound **44** as a colorless oil. R_f = 0.41 (hexane / ethyl acetate 1:1). $[\alpha]_D^{25}$ +37.1 (*c* 1.0, $CHCl_3$). $[\alpha]_D^{25}$ +37.1 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) 7.29 – 7.16 (m, 5H), 5.33 (dd, *J* = 11.6, 5.0 Hz, 1H), 5.16 (s, 1H), 4.49 (td, *J* = 9.6, 3.7 Hz, 1H), 3.74 (s, 3H), 3.42, 3.38 (2×d, *J* = 5.0 Hz, 1H), 3.03 (dd, *J* = 14.3, 5.0 Hz, 1H), 2.87 (s, 3H), 1.42 (s, 11H), 0.98 (m, 1H), 0.83 (d, *J* = 6.5 Hz, 3H), δ 0.74 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.8, 170.8, 155.4, 136.5, 128.7, 128.4, 126.7, 79.2, 58.1, 52.2, 48.6, 42.1, 34.7, 32.3, 28.2, 24.2, 23.1, 21.6. HRMS (ESI+) *m*/*z* calcd for $C_{22}H_{35}N_2O_5$, $[M+H]^+$; 407.2546; found, 407.2543.

H-Leu-*N*Me-D-Phe-OMe·TFA (45)



Peptide **44** (3.9 g, 9.60 mmol) was subjected to the general deprotection procedure of the *N*-terminally by Boc removal. The crude product **45** (4.06 g, quant) was used in the next step without further purification.

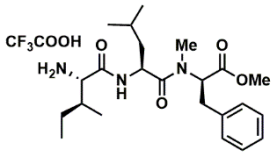
Boc-Ile-Leu-*N*Me-D-Phe-OMe (46)



To dipeptide **45** (4.06 g, 9.67 mmol) in DMF (20 mL) at 0 °C were added Boc-Ile-OH (2.68 g, 11.6 mmol), HATU (4.41 g, 11.6 mmol) and DIPEA (4.5 g, 6.06 mL, 34.8 mmol). The reaction mixture was warmed up to room temperature and stirred for further 24 h. The mixture was poured in water (200 mL) and extracted with ethyl acetate (3 × 50 mL).

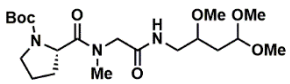
The organic layer was washed with aqueous hydrochloric acid 10% v/v (2 × 50 mL), saturated aqueous $NaHCO_3$ (2 × 50 mL), brine (2 × 50 mL), dried over Na_2SO_4. The organic phase was evaporated to dryness and the crude material purified by silica gel column chromatography (ethyl acetate / dichloromethane 7:3) as eluents to afford (5.0 g, 98%) of compound **46** as a colorless oil. R_f = 0.55 (hexane / ethyl acetate 1:1). $[\alpha]_D^{26}$ +4.45 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.14 (m, 5H), 6.60 (s, 1H), 5.38 (dd, *J* = 11.7, 4.8 Hz, 1H), 5.07 (d, *J* = 8.5 Hz, 1H), 4.84 (td, *J* = 9.6, 3.9 Hz, 1H), 4.01 – 3.93 (m, 1H), 3.73 (s, 3H), 3.41 (dd, *J* = 14.6, 5.0 Hz, 1H), 3.01 (dd, *J* = 14.5, 11.9 Hz, 1H), 2.88 (s, 3H), 1.79 (s, 1H), 1.53 – 1.45 (m, 1H), 1.42 (s, 9H), 1.37 – 1.25 (m, 1H), 1.17 – 1.00 (m, 2H), 0.96 – 0.85 (m, 7H), 0.82 (d, *J* = 6.5 Hz, 3H), 0.72 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9, 171.1, 170.7, 155.4, 136.5, 128.7, 128.4, 126.7, 79.5, 59.0, 58.1, 52.2, 47.1, 42.0, 37.5, 34.6, 32.4, 28.2, 28.1, 24.6, 24.2, 23.0, 21.6, 15.3, 11.3. HRMS (ESI+) *m*/*z* calcd for $C_{28}H_{45}N_3NaO_6$, $[M+Na]^+$; 542.3206 found, 542.3193.

H-Ile-Leu-*N*Me-D-Phe-OMe·TFA (47)



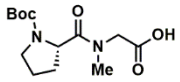
Peptide **46** (3.44 g, 7.0 mmol) was subjected to the general deprotection procedure of the *N*-terminally by Boc removal. The crude product **47** (3.76 g, quant) was used in the next step without further purification.

Boc-Pro-Sar-NHCH$_2$CH(OMe)CH$_2$CH(OMe)$_2$ (48)



To a solution of methylamine hydrochloride (2.16 g, 32.0 mmol) in methanol (320 ml) were added paraformaldehyde (0.9 g, 30.0 mmol) and triethylamine (3.2 g, 4.4 mL, 32.0 mmol). This suspension was stirred at room temperature for 24 h before Boc-Pro-OH (4.74 g, 22.0 mmol) and IPB (3.46 g, 20.0 mmol) were added subsequently. After stirring for 72 h the solvent was removed under reduced pressure in a rotavap. The crude residue was purified by column chromatography methanol / ethyl acetate 1:6) to give compound **48** (7.00 g, 80%) as a colorless oil. R_f = 0.43 (methanol / ethyl acetate 1:6). ^{1}H-NMR (400 MHz, CD_3OD): δ 4.61 – 4.53 (m, 2), 3.65 – 3.43 (m, 6H), 3.40 – 3.38 (m, 3H), 3.32 (s, 3H), 3.31 (s, 3H), 3.19 – 3.17 (2s, 3H), 2.17 – 1.70 (m, 8H), 1.45 (s, 9H). ^{13}C-NMR (100 MHz, CD_3OD): δ 173.8, 173.3, 172.6, 168.7, 168.6, 168.1, 154.8, 154.8, 153.6, 102.0, 101.9, 101.8, 80.1, 79.9, 79.5, 57.3, 55.6, 55.5, 53.1, 53.0, 52.9, 52.7, 52.5, 52.4, 46.9, 41.9, 41.7, 36.6, 35.9, 35.7, 29.3, 28.42, 28.3, 28.2, 24.7, 24.6. HRMS (ESI+) *m*/*z* calcd for, $C_{20}H_{37}N_3NaO_7$ $[M+Na]^+$; 454.2529 found, 454.2515.

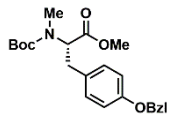
Boc-Pro-Sar-OH (49)



To a solution of compound **48** (1.2 g, 2.78 mmol) in toluene (20 mL) were added 10-camphorsulfonic acid (0.06 g, 0.28 mmol) and quinoline (0.04 g, 0.28 mmol). The contents were stirred for 1 min at room temperature and were then refluxed for 30 min. The contents were cooled,

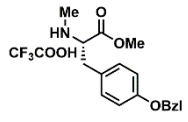
transferred to a separatory funnel and washed with 1M aqueous HCl (2 × 30 mL). The acidic aqueous phase was further extracted with ethyl acetate (1 × 20 mL). The organic layers were combined, washed with brine (2 × 20 mL), dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure in a rotavap. The obtained material (0.77 g, 2.3 mmol) was dissolved in a mixture of THF (8 mL) and water (8 mL) at 0 °C after $LiOH.H_2O$ (0.1 g, 2.4 mmol) was added in one portion. After stirring for 10 h, the mixture was transferred to a separatory funnel and water (10 mL) was added. The solution was washed with diethyl ether (2 × 20 mL). The aqueous layer was acidified to pH 3.0 using a saturated $NaHSO_4$ solution and brine (20 mL) was added. The contents were extracted with EtOAc (3 × 40 mL). The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure after filtration to afford (0.63 g, Overall yield: 77%) of product **49** as a colorless oil that was used in the next step without further purification. R_f = 0.31 (ethyl acetate / methanol / acetic acid 94:4:2). $[\alpha]_D^{24}$= -60.67 (*c* 1.0, MeOH). ^{1}H-NMR (400 MHz, CD_3OD): δ 5.93 (bs, 1H), 4.73 – 4.57 (m, 1H), 4.39 – 4.22 (m, 1H), 4.04 – 3.91 (m, 1H), 3.50 (m, 2H), 3.16 (2s, 3H), 2.24 – 1.78 (m, 4H), 1.52 – 1.34 (m, 9H). ^{13}C-NMR (100 MHz, CD_3OD): δ 173.6, 171.1, 80.3, 56.25, 50.5, 46.9, 46.5, 36.6, 30.1, 29.1, 28.4, 28.2, 24.2, 23.4. HRMS (ESI-) *m*/*z*: calcd for $C_{13}H_{21}N_2O_5$, $[M-H]^-$; 285.1456; found, 285.1456.

Boc-*N*Me-Tyr(Bzl)-OMe (50)



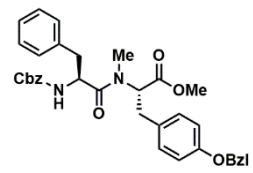
The *N*-terminally protected amino acid Boc-*N*Me-Tyr(Bzl)-OH (5,6 g, 15.0 mmol) was subjected to the general alkylation procedure of the *N*-amide group. The obtained material (5.4 g, 14.0 mmol) was subjected to the general esterification procedure of the free *C*-terminal. The pale yellow oil product **50** (5.5 g, quant) was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) 7.42 – 6.88 (m, 9H), 5.02 (s, 1H), 3.73 and 3.71 (2s, 3H), 3.22 (m, 1H), 2.95 (m, 1H), 2.72 (s, 3H), 1.37 and 1.33 (2s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8, 171.5, 157.5, 157.4, 155.7, 136.9, 114.8, 114.7, 80.1, 79.8, 69.9, 61.7, 50.5, 53.3, 52.0, 34.5, 34.0, 32.5, 31.7, 28.1. HRMS (ESI+) *m*/*z*: calcd for $C_{23}H_{30}NO_5$, $[M+H]^+$; 400.2124; found, 400.1571.

H-*N*Me-Tyr(Bzl)-OMe·TFA (51)



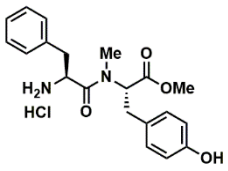
Peptide **50** (2.5 g, 6.2 mmol) was subjected to the general deprotection procedure of the *N*-terminally by Boc removal. The crude product **51** (2.7 g, quant) was used in the next step without further purification.

Cbz-Phe-*N*Me-Tyr(Bzl)-OMe (52)



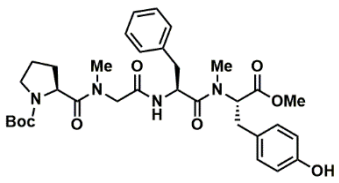
To Cbz-Phe-OH (2.4 g, 8.04 mmol), **51** (2.7 g, 6.7 mmol) and PyBrop (3.7 g, 8.04 mmol) in CH_2Cl_2 (150 mL) was added DIPEA (3.46 g, 4.66 mL, 26.8 mmol) at 0 °C under stirring. The ice bath was removed and stirring was continued for 18 h. The solvent was removed under reduced pressure in a rotavap and the residue dissolved in EtOAc (120 mL). The organic phase was washed with water (1 × 50 mL), HCl 5% v/v (2 × 50 mL), saturated $NaHCO_3$ (1 × 50 mL), brine (1 × 50 mL) and dried over Na_2SO_4. After filtration the solvent was evaporated under reduced pressure in a rotavap and the residue was purified by silica gel column chromatography (gradient 1:2, hexane / ethyl acetate) to afford compound **52** (3.7 g, 94%) as a colorless oil. R_f = 0.38 (hexane / ethyl acetate 1:1). $[\alpha]_D^{26}$ -69.41 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$). δ 7.41 – 7.14 (m, 15H), 7.01 (d, *J* = 8.5 Hz, 2H), 6.83 (d, *J* = 8.5 Hz, 2H), 5.35 (d, *J* = 8.9 Hz, 1H), 5.22 (dd, *J* = 9.8, 6.0 Hz, 1H), 5.02 (dd, *J* = 49.6, 12.3 Hz, 5H), 4.85 – 4.77 (m, 1H), 3.67 (s, 3H), 2.93 – 2.80 (m, 3H), 2.73 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.7, 170.7, 157.6, 155.4, 136.9, 136.3, 136.0, 129.8, 129.5, 128.8, 128.4, 128.4, 128.3, 128.0, 127.8, 127.8, 127.4, 126.8, 114.7, 69.9, 66.7, 58.5, 52.1, 51.9, 39.1, 33.7, 32.4. HRMS (ESI+) *m*/*z* calcd for $C_{35}H_{36}N_2NaO_6$, $[M+Na]^+$; 603.2573 found, 603.2461.

H-Phe-*N*Me-Tyr-OMe·HCl (53)



To a stirred solution of dipeptide **52** (1.0 g, 1.7 mmol) in MeOH (10 mL) were added $Pd(OH)_2$ (0.1 g, 10% w/w) and HCl 4M solution in 1,4-dioxane (0.3 mL). The reaction vessel was evacuated, purged with hydrogen and kept under H_2 atmosphere (1 atm.). The suspension was stirred for 2 h at room temperature. After filtration through Celite^{TM}, the solvent was removed under reduced pressure. The crude material was suspended in toluene (10 mL), evaporated under reduced pressure to dryness, suspended in diethyl ether (20 mL) and evaporated to dryness again to afford (0.82 g, quant) of product **53**, as a white solid which was used in the next step without further purification.

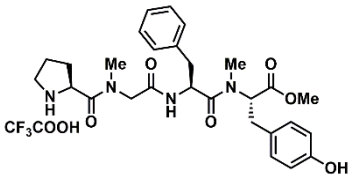
Boc-Pro-Sar-Phe-*N*Me-Tyr-OMe (54)



To dipeptide **53** (0.41 g, 1.4 mmol) in DMF (25 mL) at 0 °C were added dipeptide **49** (0.56 g, 1.96 mmol), HATU (0.75 g, 1.96 mmol) and DIPEA (0.72 g, 1.0 mL, 5.6 mmol). The contents were warmed up to room temperature and the mixture stirred for further 24 h. The mixture was poured in water (200 mL) and extracted with ethyl acetate (3 × 50 mL). The organic layer was washed with aqueous hydrochloric acid 10% v/v (2 × 50 mL), saturated aqueous $NaHCO_3$ (2 × 50 mL), brine (2 × 50 mL), dried over Na_2SO_4.

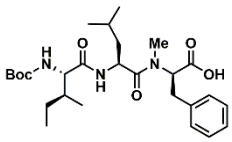
The organic phase was evaporated to dryness and the crude material purified by silica gel column chromatography (ethyl acetate / hexane / methanol 75:20:5) as eluents to afford (0.79 g, 82%) of compound **54** as a colorless oil. R_f = 0.24 (hexane / ethyl acetate / methanol 20:75:5). $[\alpha]_D^{23}$ -81.98 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (bs, 1H), 7.62 (bs, 1H), 7.37 – 7.06 (m, 5H), 6.99 – 6.76 (m, 2H), 6.75 – 6.45 (m, 2H), 5.46 (dd, *J* = 30.5, 10.8 Hz, 1H), 5.21 – 4.90 (m, 1H), 4.63 (m, 2H), 4.04 (m, 1H), 3.69 (3s, 3H), 3.50 (m, 2H), 3.28 – 2.50 (m, 9H), 2.42 – 1.69 (m, 5H), 1.60 – 1.32 (m, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7, 170.9, 170.8, 167.5, 167.2, 155.8, 155.6, 155.1, 154.8, 136.3, 136.2, 129.7, 129.5, 129.4, 128.4, 128.3, 127.4, 127.2, 126.9, 116.0, 115.6, 115.1, 80.6, 60.4, 58.2, 58.0, 56.5, 52.3, 50.6, 50.3, 49.9, 47.0, 38.4, 37.7, 36.3, 35.7, 33.2, 31.7, 31.4, 30.0, 29.6, 28.5, 24.2, 21.0, 14.2. HRMS (ESI+) *m*/*z* calcd for $C_{33}H_{44}N_4NaO_8$, $[M+Na]^+$; 647.7246 found, 647.3042.

H-Pro-Sar-Phe-*N*Me-Tyr-OMe·TFA (55)



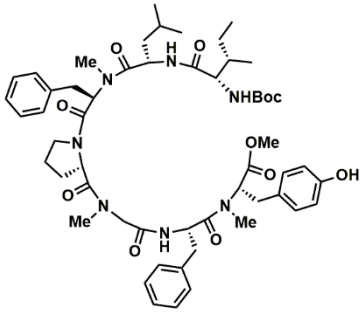
Peptide **54** (0.5 g, 0.8 mmol) was subjected to the general deprotection procedure of the *N*-terminally by Boc removal. The crude product **55** (0.51 g, quant) was used in the next step without further purification.

Boc-Ile-Leu-*N*Me-D-Phe-OH (56)



Peptide **46** (1.32 g, 2.5 mmol) was subjected to the general saponification procedure of the *C*-terminal methyl ester. The colorless oil product **56** (1.01 g, 92%) was used in the next step without further purification. R_f = 0.52 (ethyl acetate / methanol / acetic acid 94:4:2). $[\alpha]_D^{25}$ -9.31 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.20 (bs, 1H), 7.56 – 6.86 (m, 5H), 5.56 – 5.13 (m, 2H), 4.78 (m, 1H), 4.00 (m, 1H), 3.58 – 3.29 (m, 1H), 3.19 – 2.97 (m, 1H), 2.94 (s, 3H), 1.90 – 1.03 (m, 15H), 1.03 – 0.46 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.5, 173.1, 172.0, 155.9, 136.7, 129.9, 128.8, 128.6, 127.9, 126.9, 79.9, 59.0, 47.6, 41.4, 37.6, 34.7, 32.9, 28.3, 24.7, 24.3, 23.0, 21.8, 15.3, 11.2. HRMS (ESI-) *m*/*z* calcd for $C_{27}H_{42}N_3O_6$, $[M-H]^-$; 504.3074 found, 504.3079.

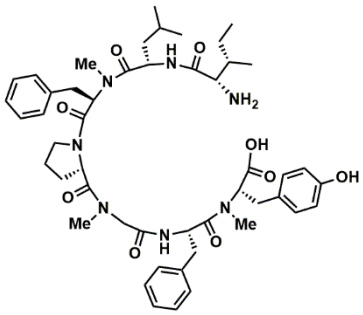
Boc-Ile-Leu-*N*Me-D-Phe-Pro-Sar-Phe-*N*Me-Tyr-OMe (57)



To tetrapeptide **55** (0.51 g, 0.8 mmol) in DMF (10 mL) at 0 °C were added tripeptide **56** (0.4 g, 0.8 mmol), HATU (0.3 g, 0.8 mmol) and DIPEA (0.41 g; 0.56 mL, 3.2 mmol). The contents were warmed up to room temperature and the mixture stirred for further 24 h. The mixture was poured in water (100 mL) and extracted with ethyl acetate (3 × 30 mL). The organic layer was washed with aqueous hydrochloric acid 10% v/v (2 × 30 mL), saturated

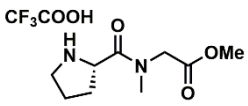
aqueous $NaHCO_3$ (2 × 20 mL), brine (2 × 20 mL), dried over Na_2SO_4. The organic phase was evaporated to dryness and the crude material purified by silica gel column chromatography (ethyl acetate / hexane / methanol 75:20:5) as eluents to afford (0.8 g, quant) of **57** as a colorless oil. R_f = 0.5 (ethyl acetate / methanol 95:5). $[\alpha]_D^{24}$ +17.01 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (s, 1H), 7.32 – 7.04 (m, 10H), 6.98 – 6.79 (m, 2H), 6.79 – 6.51 (m, 2H), 6.30 (dd, *J* = 21.8, 8.1 Hz, 1H), 5.71 – 5.40 (m, 2H), 5.16 – 4.88 (m, 2H), 4.83 – 4.55 (m, 2H), 4.14 – 3.37 (m, 8H), 3.33 – 2.35 (m, 16H), 2.14 – 1.60 (m, 7H), 1.33 (m, 9H), 1.14 – 0.37 (m, 13H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2, 171.9, 170.9, 168.3, 167.2, 155.6, 136.7, 135.9, 130.1, 129.6, 129.5, 128.8, 128.3, 128.2, 126.9, 126.6, 115.6, 79.9, 59.3, 57.3, 55.7, 52.3, 51.5, 50.0, 47.5, 47.3, 40.8, 38.6, 37.6, 36.9, 36.2, 34.6, 33.2, 31.2, 30.5, 28.3, 28.2, 25.5, 24.7, 24.4, 23.3, 21.1, 15.3, 11.1. HRMS (ESI+) *m*/*z* calcd for $C_{55}H_{77}N_7NaO_{11}$, $[M+Na]^+$; 1034.5681 found, 1034.5528.

H-Ile-Leu-*N*Me-D-Phe-Pro-Sar-Phe-*N*Me-Tyr-OH (59)



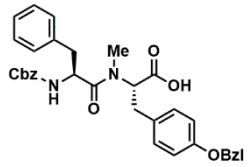
Peptide **57** (0.12 g, 0.11 mmol) was subjected to the general saponification procedure of the *C*-terminal methyl ester. The obtained material (0.1 g, 0.1 mmol) (**58**) was subjected to the general deprotection procedure of the *N*-terminally by Boc removal. The crude product **59** (0.121 g, 0.12 mmol) was used in the next step without further purification.

H-Pro-Sar-OMe·TFA (60)



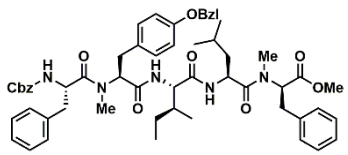
To a solution of compound **48** (4.31 g, 10.0 mmol) in toluene (200 mL) were added 10-camphorsulfonic acid (0.23 g, 1.0 mmol) and quinoline (0.2 g, 1.0 mmol). The contents were stirred for 1 min at room temperature and were then refluxed for 30 min. The contents were cooled, transferred to a separatory funnel and washed with 1M aqueous HCl (2 × 80 mL). The acidic aqueous phase was further extracted with ethyl acetate (1 × 80 mL). The organic layers were combined, washed with brine (2 × 80 mL), dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure in a rotavap. The obtained material (2.18 g, 6.5 mmol) was dissolved in anhydrous methanol (50 mL) before sodium methoxide (0.43 g, 8.0 mmol) was added. The mixture was stirred for 16 h, quenched with acetic acid (10 mL) and evaporated. The contents were dissolved in ethyl acetate (100 mL) washed with saturated aqueous $NaHCO_3$ (2 × 40 mL), brine (2 × 40 mL) and dried over Na_2SO_4. The obtained material (1.87 g, overall yield 58%) was subjected to the general deprotection procedure of the *N*-terminally by Boc removal. The white solid product **60** (1.94 g, quant) was used in the next step without further purification.

Cbz-Phe-*N*Me-Tyr(Bzl)-OH (61)



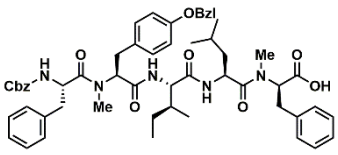
Peptide **52** (2.7 g, 4.65 mmol) was subjected to the general saponification procedure of the *C*-terminal methyl ester. Product **61** (2.58 g, 98%.) was a colorless oil used in the next step without further purification.

Cbz-Phe-*N*Me-Tyr(Bzl)-Ile-Leu-*N*Me-D-Phe-OMe (62)



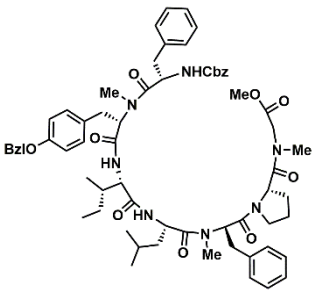
To tripeptide **47** (2.02 g, 3.79 mmol) in DMF (12 mL) at 0 °C were added peptide **61** (2.58 g, 4.55 mmol), HATU (1.44 g, 3.79 mmol) and DIPEA (1.47 g, 1.98 mL, 11.37 mmol). The contents were warmed up to room temperature and the mixture stirred for further 24 h. The mixture was poured in water (150 mL) and extracted with ethyl acetate (3 × 50 mL). The organic layer was washed with aqueous hydrochloric acid 10% v/v (2 × 50 mL), saturated aqueous $NaHCO_3$ (2 × 50 mL), brine (2 × 50 mL), dried over Na_2SO_4. The organic phase was evaporated to dryness and the crude material purified by silica gel column chromatography (ethyl acetate / dichloromethane 3:7) as eluents to afford (2.3 g, 63%) of compound **62** as an amorphous solid. R_f = 0.5 (ethyl acetate / methanol 95:5). $[\alpha]_D^{26}$ 24.27 (*c* 0.6, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, *J* = 8.3 Hz, 1H), 7.51 – 7.02 (m, 16H), 6.98 (d, *J* = 7.1 Hz, 2H), 6.79 (m, 4H), 6.39 (d, 9.3 Hz, 1H), 6.08 (bs, 1H), 5.34 (m, 1H), 5.20 – 5.05 (m, 1H), 5.04 – 4.90 (m, 2H), 4.87 – 4.57 (m, 4H), 4.40 – 4.18 (m, 1H), 3.71 (2s, 3H), 3.38 (dt, *J* = 16.8, 8.5 Hz, 1H), 3.23 (m, 2H), 3.18 – 2.66 (m, 9H), 2.40 (dd, *J* = 14.8, 11.0 Hz, 1H), 1.99 – 1.79 (m, 1H), 1.72 – 1.03 (m, 4H), 1.05 – 0.41 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 172.9, 172.8, 171.9, 170.9, 170.8, 170.0, 169.5, 169.2, 157.9, 157.6, 156.7, 155.7, 136.9, 136.7, 136.6, 136.5, 136.4, 135.9, 135.3, 130.3, 130.0, 129.8, 129.5, 128.9, 128.8, 128.5, 128.4, 128.2, 128.0, 127.9, 127.8, 127.4, 127.2, 126.9, 126.8, 115.4, 114.9, 69.9, 67.3, 66.8, 63.2, 59.2, 57.8, 52.7, 52.3, 50.0, 47.2, 41.8, 41.5, 38.9, 36.3, 34.7, 33.2, 33.0, 32.5, 32.2, 29.8, 25.0, 24.6, 24.3, 23.0, 21.8, 21.7, 15.6, 11.4, 10.8. HRMS (ESI+) *m*/*z* calcd for $C_{57}H_{69}N_5NaO_9$, $[M+Na]^+$; 990.4993 found, 990.4987.

Cbz-Phe-*N*Me-Tyr(Bzl)-Ile-Leu-*N*Me-D-Phe-OH (63)



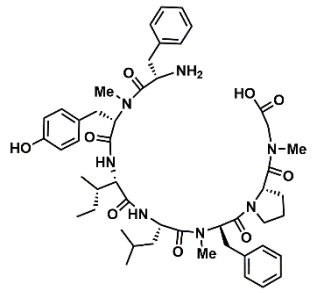
Peptide **62** (2.3 g, 2.4 mmol) was subjected to the general saponification procedure of the *C*-terminal methyl ester. Product **63** (2.19 g, 95%) was a colorless oil used in the next step without further purification.

Cbz-Phe-*N*Me-Tyr(Bzl)-Ile-Leu-*N*Me-D-Phe-Pro-Sar-OMe (64)



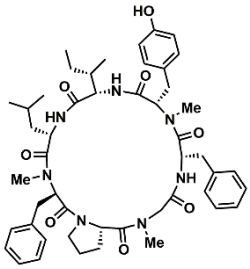
To dipeptide **60** (0.71 g, 2.25 mmol) in DMF (12 mL) at 0 °C were added the carboxylic acid **63** (2.19 g, 2.29 mmol), HATU (0.87 g, 2.29 mmol) and DIPEA (0.87 g; 1.2 mL, 6.75 mmol). The contents were warmed up to room temperature and the mixture stirred for further 24 h. The mixture was poured in water (200 mL) and extracted with ethyl acetate (3 × 50 mL). The organic layer was washed with aqueous hydrochloric acid 10% v/v (2 × 50 mL), saturated aqueous $NaHCO_3$ (2 × 50 mL), brine (2 × 50 mL), dried over Na_2SO_4. The organic phase was evaporated to dryness and the crude material purified by silica gel column chromatography (ethyl acetate) as eluents to afford (1.09 g, 43%) of **64** as a colorless oil. R_f = 0.33 (hexane / ethyl acetate 1:9). $[\alpha]_D^{24}$ -15.25 (*c* 0.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, *J* = 8.0 Hz, 1H), 7.49 – 6.67 (m, 24H), 6.55 (d, *J* = 8.7 Hz, 1H), 6.34 (d, *J* = 8.4 Hz, 1H), 5.74 – 5.52 (m, 2H), 5.19 – 4.36 (m, 8H), 4.26 – 4.08 (m, 1H), 3.93 – 3.66 (m, 4H), 3.50 (m, 2H), 3.35 – 2.72 (m, 15H), 2.43 – 1.82 (m, 10H), 1.65 – 0.50 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7, 170.2, 169.0, 164.7, 157.9, 138.6, 136.7, 130.6, 130.3, 129.8, 129.7, 129.6, 128.8, 128.5, 128.5, 128.3, 128.2, 128.0, 127.9, 127.5, 127.2, 126.5, 123.3, 115.5, 114.9, 71.9, 69.9, 69.4, 67.4, 67.2, 66.8, 64.1, 62.9, 60.2, 57.3, 56.2, 55.8, 55.7, 55.7, 53.2, 52.1, 51.8, 49.7, 48.8, 47.1, 44.9, 36.2, 33.6, 33.4, 31.5, 30.5, 29.7, 29.1, 27.9, 27.0, 25.0, 24.9, 24.5, 24.1, 23.4, 22.2, 21.4, 19.8, 15.4, 12.6, 10.8. HRMS (ESI+) *m*/*z* calcd for $C_{65}H_{81}N_7NaO_{11}$, $[M+Na]^+$; 1158.5892 found, 1158.5886.

H-Phe-*N*Me-Tyr(Bzl)-Ile-Leu-*N*Me-D-Phe-Pro-Sar-OH (65)



Peptide **64** (1.08 g, 0.95 mmol) was subjected to the general saponification procedure of the *C*-terminal methyl ester. The obtained material (1.01 g, 95%) was subjected to the general hydrogenolysis procedure of the free *N*-terminally. The product **65** (98 mg, quant) was a white solid used in the next step without further purification.

Cordyheptapeptide A (66). Cyclization strategy A:



To heptapeptide **65** (60 mg, 0.07 mmol) in MeCN (120 mL) at 0 °C were added HOAt (10 mg, 0.07 mmol), HATU (26 mg, 0.07 mmol) and DIPEA (0.026 g, 35 µL, 0.2 mmol). The contents were warmed up to room temperature and the mixture stirred for further 72 h. The solvent was evaporated and the residue dissolved in ethyl acetate (100 mL). The organic layer was washed with aqueous hydrochloric acid 10% v/v (2 × 20 mL), saturated aqueous $NaHCO_3$ (2 × 20 mL), brine (2 × 20 mL), dried over Na_2SO_4.

The organic phase was evaporated to dryness and the crude material purified by silica gel column chromatography (dichloromethane / methanol 9:1) as eluents to afford (1.1 mg, 4%) of cordyheptapeptide A as a white solid. Data see below.

Cordyheptapeptide A (66). Cyclization strategy B:

To heptapeptide **59** (0.12 g, 0.12 mmol) in MeCN (120 mL) at 0 °C were added HOAt (0.015 g, 0.11 mmol), HATU (0.041 g, 0.11 mmol) and DIPEA (0.09 g, 0.12 mL, 0.7 mmol). The contents were warmed up to room temperature and the mixture stirred for further 72 h. The solvent was evaporated and the residue dissolved in ethyl acetate (150 mL). The organic layer was washed with aqueous hydrochloric acid 10% v/v (2 × 50 mL), saturated aqueous $NaHCO_3$ (2 × 50 mL), brine (2 × 50 mL), dried over Na_2SO_4. The organic phase was evaporated to dryness and the crude material purified by silica gel column chromatography (dichloromethane / methanol 9:1) as eluents to afford 0.105 g of a mixture of cordyheptapeptide A (88 mg, 82%) and its epimer (8.4 mg, 8.3%) as a white solid. R_f = 0.54 (dichloromethane / methanol 9:1). $[\alpha]_D^{24}$ -49.77 (*c* 1.5, $CHCl_3$). $[\alpha]_D^{26}$ -68.50 (*c* 0.56, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.60 (d, *J* = 9.5 Hz, 1H), 8.20 (d, *J* = 9.6 Hz, 1H), 7.35 (m, 4H), 7.15 (m, 6H), 6.53 (d, *J* = 8.1 Hz, 2H), 6.22 (d, *J* = 8.1 Hz, 2H), 5.88 (d, *J* = 9.6 Hz, 1H), 5.56 (dd, *J* = 11.7, 4.5 Hz, 1H), 5.42 (m, 1H), 5.36 (m, 1H), 4.93 (bt, *J* = 10.8 Hz, 1H), 4.45 (dd, *J* = 9.6, 2.7 Hz, 1H), 4.39 (dd, *J* = 9.0, 2.4 Hz, 1H), 3.77 (m, 1H), 3.60 (m, 1H), 3.40 (m, 1H), 3.34 (m, 1H), 3.33 (dd, *J* = 12.5, 11.7 Hz, 1H), 3.14 (m, 1H), 3.05 (dd, *J* = 12.3, 11.7 Hz, 1H), 3.04 (s, 3H), 2.91 (s, 3H), 2.82 (dd, *J* = 12.3, 3.0 Hz, 1H), 2.74 (m, 1H), 2.72 (m, 1H), 2.61 (s, 3H), 2.42 (m, 2H), 2.33 (m, 1H), 2.03 (m, 1H), 1.85 (m, 1H), 1.55 (m, 1H), 1.39 (br t, *J* = 12.0 Hz, 1H), 1.35 (m, 1H), 1.00 (m, 1H), 0.93 (t, *J* = 6.6 Hz, 3H), 0.91 (d, *J* = 6.3 Hz, 3H), 0.89 (d, *J* = 6.9 Hz, 3H), 0.84 (d, *J* = 6.9 Hz, 3H), 0.12 (br t, *J* = 12.0 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.2, 172.2, 170.7, 170.4, 170.2, 168.3, 167.8, 154.5, 137.4, 136.9, 130.1, 129.9, 129.7, 128.6, 128.5, 127.9, 126.7, 115.6, 69.1, 58.2, 57.7, 54.4, 50.7, 50.0, 48.4, 47.4, 40.5, 39.9, 38.2, 35.5, 35.4, 35.2, 32.3, 31.8, 31.4, 30.0, 29.7, 29.4, 29.2, 29.1, 28.8, 26.7, 26.6, 24.7, 24.2, 23.7, 22.6, 22.0, 20.8, 16.3, 12.1. HRMS (ESI+) *m*/*z* calcd for $C_{49}H_{65}N_7NaO_8$, $[M+Na]^+$; 902.4895 found, 902.4786.

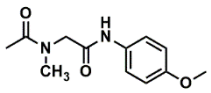
Epi cordyheptapeptide A

Yield: (8.4 mg, 8.3%). R_f = 0.54 (dichloromethane / methanol 9:1). $[\alpha]_D^{24}$ +19.54 (*c* 4.1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.61 (d, *J* = 9.5 Hz, 1H), 7.55 (d, *J* = 9.6 Hz, 1H), 7.33 (m, 4H), 7.12 (m, 6H), 6.99 (d, *J* = 8.1 Hz, 2H), 6.72 (d, *J* = 8.1 Hz, 2H), 5.56 (dd, *J* = 11.7, 4.5 Hz, 1H), 5.47 (d, *J* = 9.6 Hz, 1H), 5.42 (m, 2H), 4.78 (bt, *J* = 10.8 Hz, 1H), 4.48 (dd, *J* = 9.6, 2.7 Hz, 1H), 4.28 (dd, *J* = 9.0, 2.4 Hz, 1H), 3.75 (m, 1H), 3.59 (m, 1H), 3.34 (m, 3H), 3.21 (s, 3H), 3.14 (m, 1H), 3.05 (m, 2H), 3.00 (s, 3H), 2.90 (dd, *J* = 12.3, 3.0 Hz, 1H), 2.81 (s, 3H), 2.74 (m, 1H), 2.44 (m, 2H), 2.32 (m, 1H), 2.01 (m, 1H), 1.88 (m, 1H), 1.55 (m,

1H), 1.39 (br t, *J* = 12.0 Hz, 1H), 1.35 (m, 1H), 1.00 (m, 1H), 0.93 (t, *J* = 6.6 Hz, 3H), 0.91 (d, *J* = 6.3 Hz, 3H), 0.89 (d, *J* = 6.9 Hz, 3H), 0.84 (d, *J* = 6.9 Hz, 3H), 0.12 (br t, *J* = 12.0 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.4, 172.1, 171.8, 171.4, 171.1, 168.3, 167.5, 155.0, 137.6, 136.8, 130.3, 129.6, 129.5, 128.5, 128.4, 127.4, 126.7, 126.5, 115.7, 61.4, 57.6, 57.5, 54.6, 50.6, 50.2, 48.4, 47.4, 39.5, 37.9, 35.5, 35.3, 35.0, 34.2, 31.9, 31.3, 30.0, 24.5, 23.7, 23.6, 21.9, 20.5, 15.6, 11.1. HRMS (ESI+) *m*/*z* calcd for $C_{49}H_{65}N_7NaO_8$, $[M+Na]^+$; 902.4895 found, 902.4786.

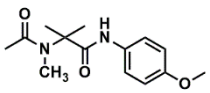
2.3 Synthesized compounds in chapter 4

Ac-Sar-*p*-NH-Ph-OMe (67)



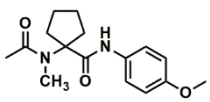
To a solution of methylamine hydrochloride (0.4 g, 6.0 mmol) in methanol (40.0 ml) were added paraformaldehyde (0.18 g, 6.0 mmol) and DIPEA (0.77 g, 1.04 mL, 6.0 mmol). This suspension was stirred at room temperature for 48 h before acetic acid (3.6 g, 3.43 mL, 4.0 mmol) and 4-methoxyphenylisocyanide (0.53 g, 4.0 mmol) were added subsequently. After stirring for 72 h the solvent was removed under reduced pressure in a rotavap. The crude residue was purified by column chromatography (methanol / ethyl acetate 2:8) to give compound **67** (0.57 g, 60%) as a white solid. R_f = 0.13 (methanol / ethyl acetate 2:8). Mixture of conformers. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.96 (s, 1H), 9.78 (s, 1H), 7.53 – 7.40 (m, 4H), 6.93 – 6.82 (m, 4H), 4.12 (s, 1H), 4.05 (s, 1H), 3.72 (s, 3H), 3.71 (s, 3H), 3.04 (s, 1H), 2.81 (s, 1H), 2.04 (s, 3H), 1.96 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.6, 170.3, 166.7, 166.4, 155.2, 155.1, 132.0, 131.8, 120.7, 120.6, 113.8, 113.7, 55.1, 55.1, 53.2, 50.4, 37.2, 36.6, 34.0, 21.3, 21.2. HRMS (ESI+) *m*/*z* calcd for $C_{12}H_{17}N_2O_3$, $[M+H]^+$; 237.1234 found, 237.1240.

Ac-*N*Me-Aib-*p*-NH-Ph-OMe (68)



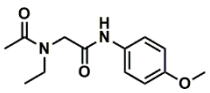
To a solution of methylamine hydrochloride (0.4 g, 6.0 mmol) in methanol (40.0 ml) were added acetone (2.3 g, 40.0 mmol) and DIPEA (0.77 g, 1.04 mL, 6.0 mmol). This suspension was stirred at room temperature for 48 h before acetic acid (3.6 g, 3.43 mL, 4.0 mmol) and 4-methoxyphenylisocyanide (0.54 g, 4.0 mmol) were added subsequently. After stirring for 72 h the solvent was removed under reduced pressure in a rotavap. The crude residue was purified by column chromatography (methanol / ethyl acetate 2:8) to give compound **68** (0.54 g, 50%) as a white solid. R_f = 0.11 (methanol / ethyl acetate 2:8). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.90 (s, 1H), 7.41 (d, *J* = 9.0 Hz, 2H), 6.83 (d, *J* = 9.0 Hz, 2H), 3.70 (s, 3H), 2.97 (s, 3H), 1.99 (s, 3H), 1.33 (s, 6H).^{13}C NMR (150 MHz, DMSO-d_6) δ 172.6, 170.0, 154.9, 132.7, 121.7, 113.3, 61.0, 55.1, 31.5, 23.3, 23.1. HRMS (ESI+) *m*/*z* calcd for $C_{14}H_{20}N_2NaO_3$, $[M+Na]^+$; 287.1366 found, 287.1358.

1-(Ac*N*Me)-1-(*p*-NH-Ph-OMe)-Cypen (69)



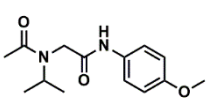
To a solution of methylamine hydrochloride (1.28 g, 19.0 mmol) in methanol (50.0 ml) were added cyclopentanone (10.6 g, 11.19 mL, 126.0 mmol) and DIPEA (2.46 g, 3.31 mL, 19.0 mmol). This suspension was stirred at room temperature for 2 weeks before acetic acid (0.75 g, 0.72 mL, 12.6 mmol) and 4-methoxyphenylisocyanide (1.67 g, 12.6 mmol) were added subsequently. After stirring for 72 h the solvent was removed under reduced pressure in a rotavap. The crude residue was purified by column chromatography (ethyl acetate) to give compound **69** (0.8 g, 21%) as a white solid. R_f = 0.36 (ethyl acetate). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.88 (s, 1H), 7.40 (d, J = 9.0 Hz, 2H), 6.84 (d, J = 9.0 Hz, 2H), 3.72 (s, 3H), 3.36 (s, 3H), 3.03 (s, 2H), 2.32 – 2.20 (m, 2H), 2.02 (s, 2H), 1.89 – 1.79 (m, 2H), 1.67 – 1.59 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.3, 170.8, 155.1, 132.5, 122.1, 113.3, 71.8, 55.1, 35.1, 33.0, 24.2, 23.4. HRMS (ESI+) *m*/*z* calcd for $C_{16}H_{23}N_2O_3$, $[M+H]^+$; 291.1703 found, 291.1701.

Ac-*N*Et-Gly-*p*-NH-Ph-OMe (70)



To a solution of methylamine hydrochloride (0.4 g, 6.0 mmol) in methanol (40.0 ml) were added paraformaldehyde (0.18 g, 6.0 mmol) and DIPEA (0.77 g, 1.04 mL, 6.0 mmol). This suspension was stirred at room temperature for 24 h before acetic acid (3.6 g, 3.43 mL, 4.0 mmol) and 4-methoxyphenylisocyanide (0.54 g, 4.0 mmol) were added subsequently. After stirring for 72 h the solvent was removed under reduced pressure in a rotavap. The crude residue was purified by column chromatography (ethyl acetate) to give compound **70** (1.45 g, 65%) as a white solid. R_f = 0.12 (ethyl acetate). Mixture of conformers. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.96 (s, 1H), 9.75 (s, 1H), 7.48 (d, J = 9.0 Hz, 2H), 6.88 (d, J = 9.0 Hz, 2H), 4.11 (s, 1H), 4.01 (s, 1H), 3.72 (s, 3H), 3.71 (s, 3H), 3.30 (q, J = 7.1 Hz, 2H), 2.05 (s, 3H), 1.95 (s, 3H), 1.12 (t, J = 7.1 Hz, 3H), 1.00 (t, J = 7.1 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.2, 169.8, 166.9, 166.9, 155.3, 155.1, 132.1, 131.8, 120.8, 120.7, 113.9, 113.8, 55.2, 55.1, 51.1, 48.4, 44.1, 41.5, 41.1, 40.6, 21.6, 20.9, 13.6, 12.7. HRMS (ESI+) *m*/*z* calcd for $C_{13}H_{18}N_2NaO_3$, $[M+Na]^+$; 273.1210 found, 273.1209.

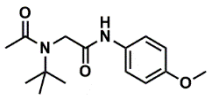
Ac-*N*(*i*Pr)-Gly-*p*-NH-Ph-OMe (71)



To a solution of methylamine hydrochloride (0.4 g, 6.0 mmol) in methanol (40.0 ml) were added paraformaldehyde (0.18 g, 6.0 mmol) and DIPEA (0.77 g, 1.04 mL, 6.0 mmol). This suspension was stirred at room temperature for 24 h before acetic acid (3.6 g, 3.43 mL, 4.0 mmol) and 4-methoxyphenylisocyanide (0.54 g, 4.0 mmol) were added subsequently. After stirring for 72 h the solvent was removed under reduced pressure in a rotavap. The crude residue was purified by recrystallization from ethyl acetate to give compound **71** (0.42 g, 40%) as a white solid. R_f = 0.11 (ethyl acetate). Mixture of conformers. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.95 (s, 1H), 9.64 (s, 1H), 7.52 – 7.44

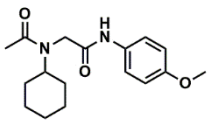
(m, *J* = 8.8 Hz, 4H), 6.92 – 6.83 (m, *J* = 9.5 Hz, 4H), 4.66 (dq, *J* = 13.6, 6.8 Hz, 1H), 4.10 (dq, *J* = 13.3, 6.6 Hz, 1H), 4.04 (s, 2H), 3.88 (s, 2H), 2.08 (s, 3H), 1.96 (s, 3H), 1.15 (d, *J* = 6.6 Hz, 6H), 1.01 (d, *J* = 6.8 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.2, 169.5, 167.6, 167.3, 155.3, 155.0, 132.2, 131.9, 120.8, 120.6, 113.9, 113.7, 55.1, 55.1, 48.5, 45.7, 43.7, 43.6, 22.1, 21.4, 20.6, 19.6. HRMS (ESI+) *m*/*z* calcd for $C_{14}H_{20}N_2NaO_3$, $[M+Na]^+$; 287.1366 found, 287.1371.

Ac-*N*(*t*Bu)-Gly-*p*-NH-Ph-OMe (72)



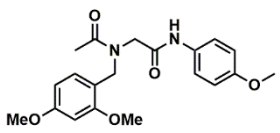
To a solution of *tert*-butylamine (1.4 g, 19.0 mmol) in methanol (50.0 ml) was added paraformaldehyde (0.57 g, 19.0 mmol). This suspension was stirred at room temperature for 24 h before acetic acid (0.75 g, 0.72 mL, 12.6 mmol) and 4-methoxyphenylisocyanide (1.7 g, 12.6 mmol) were added subsequently. After stirring for 72 h the solvent was removed under reduced pressure in a rotavap. The crude residue was purified by recrystalization from ethyl acetate to give compound **72** (3.4 g, 98%) as a yellow pale solid. R_f = 0.15 (ethyl acetate / hexane 6:4).^{1}H NMR (400 MHz, DMSO-d_6) δ 9.92 (s, 1H), 7.49 (d, *J* = 9.0 Hz, 2H), 6.89 (d, *J* = 9.0 Hz, 2H), 4.13 (s, 2H), 3.72 (s, 3H), 1.98 (s, 3H), 1.36 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.3, 167.9, 155.3, 131.9, 120.8, 113.9, 56.5, 55.2, 49.2, 28.3, 24.9. HRMS (ESI+) *m*/*z* calcd for $C_{15}H_{22}N_2NaO_3$, $[M+Na]^+$; 301.1528 found, 301.1526.

Ac-*N*(Cyhex)-Gly-*p*-NH-Ph-OMe (73)



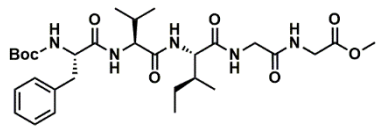
To a solution of cyclohexylamine (0.6 g, 0.7 mL, 6.0 mmol) in methanol (40.0 ml) was added paraformaldehyde (0.18 g, 6.0 mmol). This suspension was stirred at room temperature for 24 h before acetic acid (3.6 g, 3.43 mL, 4.0 mmol) and 4-methoxyphenylisocyanide (0.54 g, 4.0 mmol) were added subsequently. After stirring for 72 h the solvent was removed under reduced pressure in a rotavap. The crude residue was purified by column chromatography (ethyl acetate) to give compound **73** (0.85 g, 70%) as a white solid. R_f = 0.10 (ethyl acetate). Mixture of conformers. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.92 (s, 1H), 9.60 (s, 1H), 7.52 – 7.43 (m, *J* = 11.4, 9.0 Hz, 4H), 6.88 (t, *J* = 9.5 Hz, 4H), 4.33 – 4.24 (m, 1H), 4.06 (s, 2H), 3.92 (s, 2H), 3.72 (s, 2H), 3.72 (s, 3H), 3.66 – 3.56 (m, 1H), 2.09 (s, 3H), 1.97 (s, 3H), 1.81 – 1.65 (m, 7H), 1.63 – 1.51 (m, 4H), 1.45 – 1.19 (m, 9H), 1.13 – 0.99 (m, 3H).^{13}C NMR (100 MHz, DMSO-d_6) δ 170.2, 169.6, 167.5, 167.3, 155.3, 155.0, 132.2, 131.9, 120.7, 120.6, 113.8, 113.7, 56.8, 55.2, 55.1, 52.2, 46.5, 44.5, 30.8, 29.7, 25.4, 25.2, 25.0, 24.7, 22.1, 21.4. HRMS (ESI+) *m*/*z* calcd for $C_{17}H_{24}N_2NaO_3$, $[M+Na]^+$; 327.1679 found, 327.1674.

Ac-*N*(DMB)-Gly-*p*-NH-Ph-OMe (74)



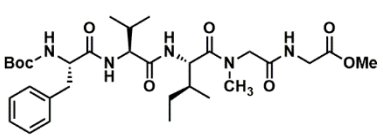
To a solution of 2,4-dimethoxybenzylamine (1.0 g, 0.9 mL, 6.0 mmol) in methanol (40.0 ml) was added paraformaldehyde (0.18 g, 6.0 mmol). This suspension was stirred at room temperature for 24 h before acetic acid (3.6 g, 3.43 mL, 4.0 mmol) and 4-methoxybenzylisocyanide (0.54 g, 4.0 mmol) were added subsequently. After stirring for 72 h the solvent was removed under reduced pressure in a rotavap. The crude residue was purified by column chromatography (ethyl acetate) to give compound **74** (1.11 g, 75%) as a white solid. R_f = 0.10 (ethyl acetate). Mixture of conformers. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.85 (s, 1H), 9.68 (s, 1H), 7.48 (t, *J* = 8.6 Hz, 6H), 7.06 (t, *J* = 8.7 Hz, 3H), 6.91 – 6.85 (m, 6H), 6.60 (d, *J* = 2.3 Hz, 1H), 6.55 (d, *J* = 2.3 Hz, 2H), 4.48 (s, 3H), 4.37 (s, 2H), 4.06 (s, 3H), 3.94 (s, 3H), 3.79 (s, 3H), 3.76 (s, 3H), 3.75 (s, 3H), 3.74 (s, 3H), 3.73 (s, 3H), 3.72 (s, 3H), 2.12 (s, 3H), 2.04 (s, 3H).^{13}C NMR (100 MHz, DMSO-d_6) δ 170.8, 170.4, 166.5, 166.5, 160.2, 159.8, 158.1, 158.1, 155.2, 155.1, 132.0, 131.8, 129.5, 129.1, 128.0, 125.4, 120.7, 120.6, 117.1, 116.6, 113.8, 113.7, 104.5, 104.5, 98.5, 98.1, 55.3, 55.3, 55.2, 55.1, 55.1, 55.1, 50.8, 47.8, 47.8, 43.4, 21.5, 21.1. HRMS (ESI+) *m*/*z* calcd for $C_{20}H_{25}N_2O_5$, $[M+H]^+$; 373.1758 found, 373.1756.

Boc-Phe-Val-Ile-Gly-Gly-OMe (75)



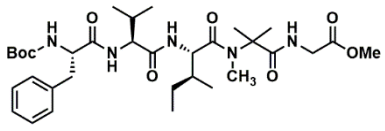
To a solution of Boc-Phe-Val-Ile-OH (0.23 g, 0.5 mmol) in DMF (15 mL) at 0 °C were added HCl·Gly-Gly-OMe (0.08 g, 0.45 mmol), HBTU (0.18 g, 0.5 mmol) and DIPEA (0.23 g, 0.31 mL, 1.8 mmol). The reaction mixture was warmed up to room temperature and stirred for further 24 h. The mixture was poured into water (20 mL) and extracted with ethyl acetate (3 × 25 mL). The organic layer was washed with aqueous hydrochloric acid 10% v/v (2 × 25 mL), saturated aqueous $NaHCO_3$ (2 × 25 mL), brine (2 × 25 mL), dried over Na_2SO_4. The organic phase was evaporated to dryness and the crude material purified by silica gel column chromatography (ethyl acetate) as eluents to afford (0.24 g, 90%) of compound **75** as white solid. R_f = 0.25 (ethyl acetate).^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 (m, 1H), 8.10 (m, 1H), 8.00 (m, 1H), 7.65 (m, 1H), 7.28 – 7.08 (m, 5H), 4.81 – 4.72 (m, 2H), 4.45 – 4.43 (m, 1H), 4.32 – 4.29 (m, 1H), 4.14 – 3.93 (m, 1H), 3.15 – 2.95 (m, 4H), 3.72 (s, 3H), 2.91 – 2.86 (m, 1H), 2.21 – 2.00 (m, 1H), 1.66 – 1.46 (m, 1H), 1.39 (s, 9H), 1.21 – 1.11 (m, 2H), 0.97 – 0.84 (m, 12H).^{13}C NMR (100 MHz, DMSO-d_6) δ 171.9, 171.7, 171.2, 170.3, 170.1, 169.9, 169.4, 169.0, 155.8, 142.5, 136.8, 135.1, 129.4, 128.2, 126.6, 79.7, 71.1, 71.0, 70.5, 60.2, 57.6, 57.2, 52.2, 52.1, 48.0, 42.8, 41.3, 41.1, 33.9, 31.6, 29.5, 28.2, 26.4, 24.9, 22.7, 22.0, 19.2, 19.1, 18.3, 18.0, 15.3, 14.7, 14.0, 11.8, 11.6. HRMS (ESI+) *m*/*z* calcd for $C_{30}H_{48}N_5O_8$, $[M+H]^+$; 606.3503 found, 606.3552.

Boc-Phe-Val-Ile-*N*Me-Gly-Gly-OMe (76)



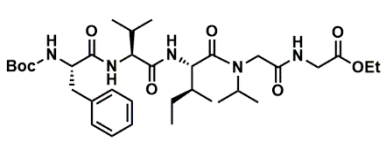
To a solution of methylamine hydrochloride (0.03 g, 0.45 mmol) in methanol (3.0 ml) were added paraformaldehyde (0.013 g, 0.45 mmol) and DIPEA (0.058 g, 0.078 mL, 0.45 mmol). This suspension was stirred at room temperature for 24 h before Boc-Phe-Val-Ile-OH (0.14 g, 0.3 mmol) and methyl isocyanoacetate (0.03 g, 0.027 mL, 0.3 mmol) were added subsequently. After stirring for 72 h the solvent was removed under reduced pressure in a rotavap. The crude residue was purified by column chromatography (ethyl acetate) to give compound **76** (0.03 g, 16%) as a white solid. R_f = 0.20 (ethyl acetate). Mixture of conformers. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.51 (t, *J* = 5.9 Hz, 1H), 8.21 (t, *J* = 5.9 Hz, 1H), 8.13, 8.09 (2×d, *J* = 8.0 Hz, 1H), 7.72 (d, *J* = 8.7 Hz, 1H), 7.27 – 7.24 (m, 2H), 7.21 – 7.16 (m, 1H), 7.02 – 6.98 (m, 2H), 4.55, 4.42 (2×t, *J* = 8.4 Hz, 1H), 4.50 (d, *J* = 17.2 Hz, 1H), 4.29 – 4.23 (m, 1H), 4.12 (d, *J* = 16.2 Hz, 1H), 3.91 – 3.79 (m, 4H), 3.62 (s, 3H), 3.10 (s, 3H), 2.98 – 2.93 (m, 1H), 2.76 – 2.70 (m, 1H), 1.54 – 1.47, 1.46 – 1.42 (2×m, 1H), 1.29, 1.23 (2×s, 9H), 1.12 – 1.06 (m, 2H), 0.87 – 0.76 (m, 12H).^{13}C NMR (100 MHz, DMSO-d_6) δ 171.6, 171.4, 170.9, 170.8, 170.0, 168.6, 168.5, 155.6, 138.2, 129.1, 128.0, 126.1, 78.1, 56.9, 55.7, 55.7, 52.4, 52.3, 51.7, 51.7, 51.6, 50.0, 40.4, 37.1, 36.3, 36.0, 34.3, 31.0, 28.1, 27.8, 24.1, 23.8, 19.0, 17.9, 15.4, 15.0, 10.8, 10.8. HRMS (ESI+) *m*/*z* calcd for $C_{31}H_{50}N_5O_8$, $[M+H]^+$; 620.3654 found, 620.3651.

Boc-Phe-Val-Ile-*N*Me-Aib-Gly-OMe (77)



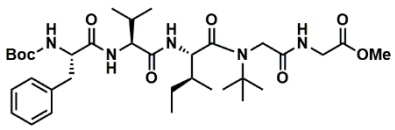
To a solution of methylamine hydrochloride (0.03 g, 0.45 mmol) in methanol (3.0 ml) were added acetone (0.17 g, 0.22 mL, 3.0 mmol) and DIPEA (0.058 g, 0.078 mL, 0.45 mmol). This suspension was stirred at room temperature for 24 h before Boc-Phe-Val-Ile-OH (0.14 g, 0.3 mmol) and methyl isocyanoacetate (0.03 g, 0.027 mL, 0.3 mmol) were added subsequently. After stirring for 72 h the solvent was removed under reduced pressure in a rotavap. The crude residue was purified by column chromatography (ethyl acetate) to give compound **77** (10 mg, 5%) as a white solid. R_f = 0.22 (ethyl acetate). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.31 (t, *J* = 7.5 Hz, 2H), 7.21 – 7.13 (m, 1H), 7.05 (m, 2H), 5.66 (s, 1H), 5.33 (t, *J* = 3.4 Hz, 1H), 5.22 (d, *J* = 2.2 Hz, 1H), 5.03 (s, 1H), 4.86 (d, *J* = 2.5 Hz, 1H), 4.51 (s, 1H), 4.30 (s, 1H), 4.12 (s, 1H), 3.88 (s, 1H), 3.61 (s, 3H), 3.30 (m, 1H), 3.11 (s, 3H), 2.93 (m, 1H), 2.48 – 2.36 (m, 1H), 2.26 (m, 1H), 1.61 (s, 6H), 1.29 (s, 9H), 1.33 (p, *J* = 6.8 Hz, 1H), 1.19 – 1.10 (m, 4H), 0.89 – 0.79 (m, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.0, 172. 1, 172. 3, 170.2, 164.6, 159.7, 137.5, 129.1, 127.9, 80.6, 69.5, 59.2, 54.0, 51.3, 40.1, 39.9, 39.7, 39.5, 39.2, 39.0, 38.8, 28.9, 28.0, 22.4, 22.0, 21.2, 21.0, 20.7, 20.3, 18.9, 14.0, 11.0. HRMS (ESI+) *m*/*z* calcd for $C_{33}H_{54}N_5O_8$, $[M+H]^+$; 647.3894 found, 647.3892.

Boc-Phe-Val-Ile-*N*(*i*Pr)-Gly-Gly-OMe (78)



To a solution of isopropylamine (0.03 g, 0.45 mmol) in methanol (3.0 ml) was added paraformaldehyde (0.013 g, 0.45 mmol). This suspension was stirred at room temperature for 24 h before Boc-Phe-Val-Ile-OH (0.14 g, 0.3 mmol) and methyl isocyanoacetate (0.03 g, 0.027 mL, 0.3 mmol) were added subsequently. After stirring for 72 h the solvent was removed under reduced pressure in a rotavap. The crude residue was purified by column chromatography (ethyl acetate) to give compound **78** (17 mg, 8%) as a white solid. R_f = 0.31 (ethyl acetate). Mixture of conformers. ^{1}H NMR (400 MHz, DMSO-d_6) 8.45 (t, *J* = 5.8 Hz, 1H), 8.19 (d, *J* = 9.1 Hz, 1H), 8.12 (d, *J* = 8.3 Hz, 1H), 8.02 (t, *J* = 5.9 Hz, 1H), 7.71 (t, *J* = 8.2 Hz, 1H), 7.33 – 7.14 (m, 5H), 6.99 (t, *J* = 8.1 Hz, 1H), 4.63 (t, *J* = 8.6 Hz, 1H) ,4.59 – 4.52 (m, 1H), 4.48 (d, *J* = 17.8 Hz, 1H), , 4.63 (t, *J* = 8.6 Hz, 1H), 4.43 – 4.35 (m, 1H) 4.33 – 4.21 (m, 2H),4.23 – 4.15 (m, 1H) 4.08 (q, *J* = 7.1 Hz, 2H), 3.94 (d, *J* = 16.2 Hz, 1H), 3.71 (d, *J* = 16.1 Hz, 1H), 2.99 – 2.91 (m, 1H), 2.79 – 2.69 (m, 1H), 1.30 (s, 9H), 1.19, 1.18 (2×t, *J* = 6.0 Hz, 1H), 1.13 (d, *J* = 6.5 Hz, 6H), 0.98 (dd, *J* = 10.4, 6.8 Hz, 2H), 0.90 – 0.76 (m, 12H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.4, 171.3, 171.2, 171.0, 170.7, 170.4, 169.7, 169.7, 169.5, 169.0, 155.1, 138.2, 129.1, 127.9, 126.1, 78.1, 78.0, 60.3, 60.3, 56.8, 55.8, 55.6, 53.1, 52.3, 47.6, 44.7, 44.1, 42.9, 40.8, 40.6, 37.0, 36.3, 35.8, 31.0, 28.0, 27.8, 24.1, 24.0, 20.7, 20.5, 19.2, 19.1, 19.0, 18.8, 18.0, 17.8, 15.3, 15.1, 14.0, 10.9, 10.8. HRMS (ESI+) *m*/*z* calcd for $C_{34}H_{55}N_5NaO_8$, $[M+Na]^+$; 684.3943 found, 684.3935.

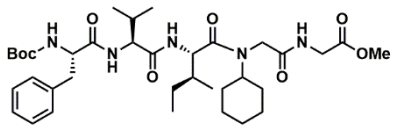
Boc-Phe-Val-Ile-*N*(*t*Bu)-Gly-Gly-OMe (79)



To a solution of *tert*-butylamine (0.032 g, 0.047 mL, 0.45 mmol) in methanol (3.0 ml) was added paraformaldehyde (0.013 g, 0.45 mmol). This suspension was stirred at room temperature for 24 h before Boc-Phe-Val-Ile-OH (0.14 g, 0.3 mmol) and methyl isocyanoacetate (0.03 g, 0.027 mL, 0.3 mmol) were added subsequently. After stirring for 72 h the solvent was removed under reduced pressure in a rotavap. The crude residue was purified by column chromatography (ethyl acetate / hexane (1:1) → ethyl acetate) to give compound **79** (60 mg, 30%) as a white solid. R_f = 0.33 (ethyl acetate / hexane 1:1). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.42 (bs, 1H), 8.15 (d, *J* = 6.4 Hz, 1H), 7.69 (d, *J* = 8.8 Hz, 1H), 7.31 – 7.22 (m, 3H), 7.21 – 7.13 (m, 2H), 6.99 (d, *J* = 8.6 Hz, 1H), 4.29 – 4.16 (m, 2H), 4.00 – 3.93 (m, 2H), 3.91 (d, *J* = 6.0 Hz, 1H), 3.86 (d, *J* = 5.8 Hz, 1H), 3.83 (d, *J* = 5.8 Hz, 1H), 3.62 (s, 3H), 2.95 (dd, *J* = 13.9, 3.8 Hz, 1H), 2.72 (dd, *J* = 13.8, 10.6 Hz, 1H), 1.92 (td, *J* = 13.3, 6.6 Hz, 1H), 1.84 – 1.77 (m, 1H), 1.48 – 1.41 (m, 1H), 1.32 (s, 9H), 1.29 (s, 9H), 1.02 – 0.95 (m, 1H), 0.84 (d, *J* = 2.9 Hz, 6H), 0.83 (d, *J* = 3.0 Hz, 3H), 0.79 (t, *J* = 7.4 Hz, 3H).^{13}C NMR (150 MHz, DMSO-d_6) δ 172.7, 171.4, 170.6, 170.3, 170.1, 155.5, 138.2, 129.1, 127.9, 126.1, 78.0, 57.3, 56.9, 55.7, 54.7, 51.6, 47.3, 47.1, 41.2, 40.6, 40.4, 38.4, 37.7, 37.1, 36.8, 36.1, 31.1, 28.1,

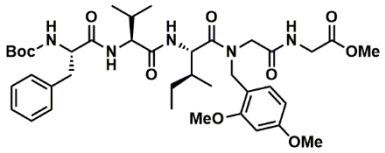
27.8, 27.6, 24.1, 18.8, 18.0, 15.2, 10.8. HRMS (ESI+) *m*/*z* calcd for $C_{34}H_{55}N_5NaO_8$, $[M+Na]^+$; 684.3943 found, 684.3917.

Boc-Phe-Val-Ile-*N*(Cyhex)-Gly-Gly-OMe (80)



To a solution of cyclohexylamine (0.044 g, 0.051 mL, 0.45 mmol) in methanol (3.0 ml) was added paraformaldehyde (0.013 g, 0.45 mmol). The suspension was stirred at room temperature for 24 h before Boc-Phe-Val-Ile-OH (0.14 g, 0.3 mmol) and methyl isocyanoacetate (0.03 g, 0.027 mL, 0.3 mmol) were added subsequently. After stirring for 72 h the solvent was removed under reduced pressure in a rotavap. The crude residue was purified by column chromatography (ethyl acetate) to give compound **80** (50 mg, 24%) as a white solid. R_f = 0.34 (ethyl acetate). Mixture of conformers.^{1}H NMR (600 MHz, DMSO-d_6) δ 8.43 (t, *J* = 5.8 Hz, 1H), 8.17 (d, *J* = 8.6 Hz, 1H), 8.01 (t, *J* = 5.9 Hz, 1H), 7.70 (d, *J* = 8.8 Hz, 1H), 7.31 – 7.16 (m, 5H), 7.02 (d, *J* = 8.6 Hz, 1H), 4.60 (t, *J* = 8.6 Hz, 1H), 4.47 (d, *J* = 17.8 Hz, 1H), 4.32 – 4.27 (m, 2H), 4.26 – 4.22 (m, 1H), 4.21 – 4.13 (m, 2H), 3.97 (d, *J* = 16.2 Hz, 1H), 3.75 (d, *J* = 16.3 Hz, 1H), 3.62 (s, 3H), 2.97 – 2.89 (m, 1H), 2.77 – 2.69 (m, 1H), 1.94 – 1.88 (m, 2H), 1.79 – 1.67 (m, 2H), 1.60 – 1.49 (m, 2H), 1.30 (s, 9H), 1.26 – 1.21 (m, 2H), 1.11 – 1.02 (m, 2H), 1.00 – 0.94 (m, 2H), 0.86 (d, *J* = 6.8 Hz, 6H), 0.85 – 0.76 (m, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.4, 171.2, 171.1, 170.6, 170.5, 170.3, 170.2, 170.1, 170.0, 169.8, 169.7, 169.1, 169.0, 155.1, 155.1, 138.2, 129.1, 128.0, 127.9, 126.1, 78.1, 78.0, 57.0, 56.8, 56.0, 55.8, 53.1, 53.0, 52.3, 51.6, 44.9, 44.1, 43.9, 40.6, 40.5, 40.4, 36.7, 36.1, 35.7, 31.2, 31.1, 30.8, 30.7, 29.3, 29.1, 28.0, 27.8, 25.4, 25.3, 25.3, 25.3, 25.2, 25.0, 24.7, 24.1, 24.0, 19.0, 18.8, 18.0, 17.8, 15.4, 15.2, 10.8, 10.8. HRMS (ESI+) *m*/*z* calcd for $C_{36}H_{57}N_5NaO_8$, $[M+Na]^+$; 710.4099 found, 710.4071.

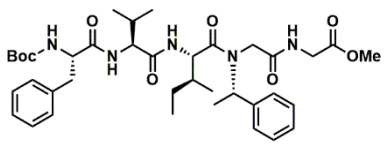
Boc-Phe-Val-Ile-*N*(DMB)-Gly-Gly-OMe (81)



To a solution of 2,4-dimethylbenzylamine (0.075 g, 0.068 mL, 0.45 mmol) in methanol (3.0 ml) was added paraformaldehyde (0.013 g, 0.45 mmol). This suspension was stirred at room temperature for 24 h before Boc-Phe-Val-Ile-OH (0.14 g, 0.3 mmol) and methyl isocyanoacetate (0.03 g, 0.027 mL, 0.3 mmol) were added subsequently. After stirring for 72 h the solvent was removed under reduced pressure in a rotavap. The crude residue was purified by column chromatography (ethyl acetate / hexane 6:4) to give compound **81** (40 mg, 20%) as a white solid. R_f = 0.12 (ethyl acetate / hexane 6:4). Mixture of conformers. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.45 (t, *J* = 5.9 Hz, 1H), 8.27, 8.05 (2×d, *J* = 8.8 Hz, 1H), 8.15 (t, *J* = 5.9 Hz, 1H), 7.78, 7.71 (2×d, *J* = 8.9 Hz, 1H), 6.90 (d, *J* = 8.4 Hz, 1H), 6.56 (dd, *J* = 18.6, 2.3 Hz, 2H), 6.48 (dd, *J* = 8.4, 2.3 Hz, 1H), 6.37 (dd, *J* = 8.4, 2.3 Hz, 1H), 4.81 (t, *J* = 8.3 Hz, 1H), 4.66 (d, *J* = 15.4 Hz, 1H), 4.57 (d, *J* = 16.5 Hz, 1H), 4.46 (d, *J* = 15.9 Hz, 1H), 4.41 (t, *J* = 8.9 Hz, 1H), 4.31 (m, 1H), 4.24 – 4.17 (m, 1H), 4.05 (d, *J* =

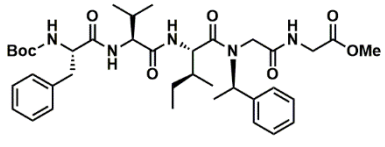
15.3 Hz, 1H), 3.95 (d, *J* = 16.2 Hz, 1H), 3.90 – 3.80 (m, 2H), 3.77, 3.76 (2×s, 3H), 3.75, 3.74 (2×s, 3H) 3.63 (s, 6H), 2.96 (m, 1H), 2.74 (m, 1H), 2.02 – 1.90 (m, 1H), 1.87 – 1.75 (m, 1H), 1.51 – 1.43 (m, 1H), 1.29 (s, 9H), 1.07 – 0.96 (m, 2H), 0.87 – 0.83 (m, 3H), 0.83 – 0.77 (m, 9H).^{13}C NMR (150 MHz, DMSO-d_6) δ 171.7, 171.6, 171.5, 171.4, 170.6, 170.5, 170.2, 170.0, 168.6, 168.3, 160.3, 159.7, 158.2, 158.0, 155.2, 155.2, 138.2, 129.6, 129.2, 129.1, 128.7, 127.9, 126.1, 116.5, 116.3, 104.5, 104.3, 98.4, 98.1, 78.0, 57.0, 56.9, 55.8, 55.7, 55.3, 55.2, 55.1, 52.5, 52.4, 51.7, 51.7, 48.9, 47.0, 46.7, 46.4, 46.2, 43.6, 40.6, 40.5, 40.4, 40.3, 37.1, 36.9, 36.7, 35.7, 35.5, 31.0, 30.9, 28.0, 27.8, 23.9, 23.7, 19.1, 19.0, 17.8, 17.8, 15.3, 15.2, 11.0, 10.8. HRMS (ESI+) *m*/*z* calcd for $C_{39}H_{57}N_5NaO_{10}$, $[M+Na]^+$; 778.3998 found, 778.3994.

Boc-Phe-Val-Ile-*N*(-L-PhEt)-Gly-Gly-OMe (82)



To a solution of (S)-1-phenylethyl-1-amine (0.15 g, 0.16 mL, 1.28 mmol) in methanol (8.0 ml) was added paraformaldehyde (0.038 g, 1.28 mmol). The suspension was stirred at room temperature for 24 h before Boc-Phe-Val-Ile-OH (0.4 g, 0.85 mmol) and methyl isocyanoacetate (0.082 g, 0.077 mL, 0.85 mmol) were added subsequently. After stirring for 72 h the solvent was removed under reduced pressure in a rotavap. The crude residue was purified by column chromatography (ethyl acetate) to give compound **82** (120 mg, 20%) as a white solid. R_f = 0.15 (ethyl acetate / hexane 1:1). Mixture of conformers.^{1}H NMR (600 MHz, DMSO-d_6) 8.24 (t, *J* = 5.7 Hz, 1H), 8.13 (d, *J* = 8.8 Hz, 1H), 8.10 (t, *J* = 5.9 Hz, 1H), 8.05 (d, *J* = 8.6 Hz, 1H), 7.36 – 7.15 (m, 10H), 7.00 (d, *J* = 8.6 Hz, 1H), 5.59 (dd, *J* = 14.0, 7.0 Hz, 1H), 5.53 (dd, *J* = 13.5, 6.6 Hz, 1H), 4.91 (t, *J* = 8.6 Hz, 1H), 4.43 (t, *J* = 8.3 Hz, 1H), 4.36, 4.30 (2×dd, *J* = 8.8, 6.5 Hz, 1H), 4.23 – 4.13 (m, 1H), 3.97 (d, *J* = 16.2 Hz, 1H), 3.89 (d, *J* = 6.2 Hz, 1H), 3.86 (d, *J* = 6.0 Hz, 1H), 3.61, 3.59 (2×s, 3H), 2.98 – 2.92 (m, 1H), 2.76 – 2.71 (m, 1H), 2.00 – 1.94 (m, 1H), 1.86 – 1.76 (m, 1H), 1.54, 1.40 (2×d, *J* = 6.9 Hz, 3H), 1.29 (d, *J* = 2.0 Hz, 9H), 0.97 (d, *J* = 6.7 Hz, 3H), 0.88 – 0.81 (m, 6H), 0.79 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.7, 171.5, 171.4, 170.6, 170.5, 170.2, 169.9, 168.7, 168.5, 157.6, 155.1, 153.2, 140.4, 140.3, 139.8, 138.1, 130.7, 129.1, 128.3, 128.0, 127.9, 127.7, 127.3, 127.2, 127.0, 126.6, 126.1, 78.7, 78.0, 57.7, 56.9, 56.8, 55.7, 54.7, 54.2, 53.8, 53.4, 52.6, 52.3, 52.2, 51.9, 51.6, 51.6, 50.1, 46.0, 44.9, 44.6, 42.6, 40.5, 40.4, 37.1, 36.8, 36.7, 31.0, 30.2, 28.0, 23.7, 19.3, 19.0, 17.8, 17.3, 16.1, 15.4, 15.2, 11.1, 11.0. HRMS (ESI+) *m*/*z* calcd for $C_{38}H_{56}N_5O_8$, $[M+H]^+$; 710.4123 found, 710.4126.

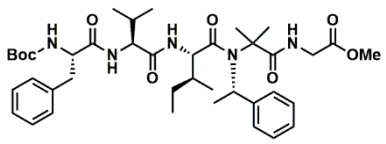
Boc-Phe-Val-Ile-*N*(-D-PhEt)-Gly-Gly-OMe (83)



To a solution of (R)-1-phenylethyl-1-amine (0.15 g, 0.16 mL, 1.28 mmol) in methanol (8.0 ml) was added paraformaldehyde (0.038 g, 1.28 mmol). This suspension was stirred at room temperature for 24 h before Boc-Phe-Val-Ile-OH (0.4 g, 0.85 mmol) and methyl isocyanoacetate (0.082 g, 0.077 mL, 0.85 mmol) were added

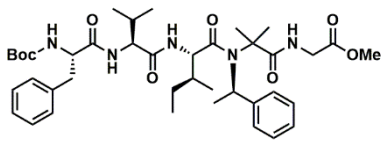
subsequently. After stirring for 72 h the solvent was removed under reduced pressure in a rotavap. The crude residue was purified by column chromatography (ethyl acetate) to give compound **83** (0.15 g, 25%) as a white solid. R_f = 0.15 (ethyl acetate / hexane 1:1). Mixture of conformers. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.48 – 8.39 (m, 2H), 8.30 (d, *J* = 8.6 Hz, 1H), 7.97 (t, *J* = 5.8 Hz, 1H), 7.75, 7.67 (2×d, *J* = 8.8 Hz, 1H), 7.43 – 7.15 (m, 10H), 7.04, 6.98 (2×d, *J* = 8.6 Hz, 1H), 6.98 (d, *J* = 8.6 Hz, 1H), 5.82 (q, *J* = 7.1 Hz, 1H), 5.53 (dd, *J* = 13.1, 6.3 Hz, 1H), 4.57 (d, *J* = 17.8 Hz, 1H), 4.33 (m, 1H), 4.20 (td, *J* = 10.2, 4.0 Hz, 1H), 4.06 (d, *J* = 16.2 Hz, 1H), 3.95, 3.91 (2×d, *J* = 6.2 Hz, 1H), 3.84 (d, *J* = 5.7 Hz, 1H), 3.63 (s, 3H), 2.94 (dd, *J* = 13.7, 3.7 Hz, 1H), 2.78 – 2.66 (m, 1H), 1.36 (d, *J* = 7.2 Hz, 3H), 1.29 (s, 9H), 1.04 – 0.93 (m, 1H), 0.90 (d, *J* = 6.7 Hz, 3H), 0.83 – 0.71 (m, 9H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.9, 171.7, 171.3, 170.9, 170.6, 170.1, 170.0, 169.9, 169.7, 169.1, 168.5, 155.1, 142.0, 141.1, 138.1, 129.1, 128.5, 128.2, 127.9, 127.2, 126.9, 126.6, 126.4, 126.1, 78.0, 60.5, 56.8, 55.7, 54.6, 53.0, 52.7, 51.6, 50.3, 45.0, 40.6, 40.4, 37.0, 35.8, 31.1, 28.0, 27.8, 24.0, 23.7, 20.3, 19.4, 19.2, 17.8, 17.7, 16.1, 15.4, 15.3, 10.7, 10.7. HRMS (ESI+) *m*/*z* calcd for $C_{38}H_{55}N_5NaO_8$, $[M+Na]^+$; 732.3943 found, 732.3936.

Boc-Phe-Val-Ile-*N*(-L-PhEt)-Aib-Gly-OMe (84)



To a solution of (R)-1-phenylethyl-1-amine (0.15 g, 0.16 mL, 1.28 mmol) in methanol (8.0 ml) was added acetone (0.5 g, 0.62 mL, 8.5 mmol). This suspension was stirred at room temperature for 24 h before Boc-Phe-Val-Ile-OH (0.4 g, 0.85 mmol) and methyl isocyanoacetate (0.082 g, 0.077 mL, 0.85 mmol) were added subsequently. After stirring for 72 h the solvent was removed under reduced pressure in a rotavap. The crude residue was purified by column chromatography (ethyl acetate) to give compound **84** (65 mg, 10%) as a white solid. R_f = 0.41 (ethyl acetate / hexane 7:3). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.21 – 7.89 (m, 2H), 7.69 (d, *J* = 8.6 Hz, 1H), 7.54 (s, 1H), 7.38 – 7.14 (m, 10H), 6.91 (d, *J* = 8.3 Hz, 1H), 6.43 (bs, 1H), 5.71 – 5.51 (m, 1H), 4.60 – 4.44 (m, 1H), 4.32 – 4.22 (m, 1H), 4.22 – 4.14 (m, 1H), 3.81 (dd, *J* = 11.7, 5.2 Hz, 1H), 3.60 (s, 3H), 2.99 – 2.90 (m, 1H), 2.77 – 2.71 (m, 1H), 1.99 – 1.87 (m, 1H), 1.78 (d, *J* = 6.1 Hz, 3H), 1.40 (s, 6H), 1.29 (s, 9H), 1.12 – 1.05 (m, 1H), 1.02 – 0.96 (m, 1H), 0.90 – 0.81 (m, 9H), 0.81 – 0.74 (m, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 174.5, 172.7, 171.6, 171.3, 170.6, 168.4, 155.3, 146.0, 142.7, 141.6, 138.2, 134.2, 129.2, 128.6, 128.3, 128.1, 127.6, 126.7, 126.5, 126.2, 117.5, 78.2, 70.7, 68.7, 68.5, 63.8, 58.9, 57.4, 56.9, 55.8, 55.0, 51.6, 42.1, 41.1, 40.7, 38.4, 37.1, 35.5, 32.2, 29.9, 29.6, 29.5, 28.1, 26.2, 25.5, 2.8, 19.3, 19.0, 18.0, 15.4, 11.1. HRMS (ESI+) *m*/*z* calcd for $C_{40}H_{60}N_5O_8$, $[M+H]^+$; 738.4436 found, 738.4442.

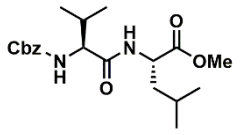
Boc-Phe-Val-Ile-*N*(-D-PhEt)-Aib-Gly-OMe (85)



To a solution of (S)-1-phenylethyl-1-amine (0.15 g, 0.16 mL, 1.28 mmol) in methanol (8.0 ml) was added acetone (0.5 g, 0.62 mL, 8.5 mmol). This suspension was stirred at room temperature for 24 h before Boc-Phe-Val-Ile-OH (0.4 g, 0.85 mmol) and methyl isocyanoacetate (0.082 g, 0.077 mL, 0.85 mmol) were added subsequently. After stirring for 72 h the solvent was removed under reduced pressure in a rotavap. The crude residue was purified by column chromatography (ethyl acetate / hexane 7:3) to give compound **85** (50 mg, 8%) as a white solid. R_f = 0.41 (ethyl acetate / hexane 7:3).^{1}H NMR (600 MHz, DMSO-d_6) 7.86 (d, *J* = 8.0 Hz, 1H), 7.73 (d, *J* = 8.8 Hz, 1H), 7.66 (bs, 1H), 7.52 (bs, 1H), 7.41 – 7.33 (m, 1H), 7.37 (t, *J* = 6.0 Hz, 1H), 7.33 – 7.15 (m, 10H), 5.34 – 5.30 (m, 1H), 4.98 (bs, 1H), 4.30 (s, 1H), 4.16 (dd, *J* = 12.6, 5.8 Hz, 1H), 3.89 – 3.77 (m, 1H), 3.76 – 3.67 (m, 1H), 3.63 (s, 3H), 2.92 (d, *J* = 12.4 Hz, 1H), 2.76 – 2.67 (m, 1H), 2.01 – 1.90 (m, 1H), 1.72 (s, 3H), 1.54 (s, 6H), 1.29 (s, 9H), 1.20 – 1.14 (m, 1H), , 0.89 – 0.82 (m, 12H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 174.6, 171.4, 170.6, 155.2, 143.0, 138.2, 129.1, 128.1, 128.0, 127.6, 126.9, 126.2, 78.1, 69.8, 56.9, 55.9, 54.1, 51.6, 28.1, 27.9, 25.9, 23.4, 22.9, 22.1, 19.0, 18.8, 18.4, 17.8, 16.4, 14.3, 13.9, 10.8. HRMS (ESI+) *m*/*z* calcd for $C_{40}H_{59}N_5NaO_8$, $[M+Na]^+$; 760.4256 found, 760.4260.

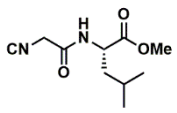
2.4 Synthesized compounds in chapter 5

Cbz-Val-Leu-OMe (86)



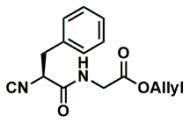
Cbz-Val-OH (527 mg, 2.1 mmol) was coupled to HCl·Leu-OMe (362 mg, 2.0 mmol) according to the general solution-phase peptide synthesis procedure. Flash column chromatography purification (hexane / ethyl acetate 1:1) furnished the title dipeptide (749 mg, >99%) as a white amorphous solid. R_f = 0.48 (hexane / ethyl acetate 1:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39 – 7.25 (m, 5H), 6.65 (d, *J* = 7.8 Hz, 1H), 5.56 (d, *J* = 9.0 Hz, 1H), 5.17 – 5.03 (m, 2H), 4.65 – 4.57 (m, 1H), 4.10 (dd, *J* = 9.1, 6.5 Hz, 1H), 3.71 (s, 3H), 2.09 (dq, *J* = 13.2, 6.5 Hz, 1H), 1.68 – 1.59 (m, 2H), 1.58 – 1.49 (m, 1H), 1.02 – 0.84 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.3, 171.4, 156.5, 136.4, 128.5, 128.2, 128.1, 127.9, 77.2, 67.0, 60.2, 52.2, 50.8, 41.3, 31.5, 24.9, 22.8, 21.9, 19.1, 17.9. HRMS (ESI+) *m*/*z* calcd for $C_{20}H_{30}N_2NaO_5$, $[M+Na]^+$; 401.2048 found, 401.2052. This product was subjected to methyl ester removal according to the general procedure to afford peptide Cbz-Val-Leu-OH (**87**) (1.02 g, 93%), which was used forward without further purification.

(CN)Gly-Leu-OMe (88)



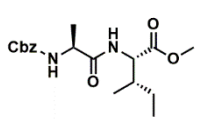
A mixture of potassium isocyanoacetate (320 mg, 2.60 mmol) and HCl·Leu-OMe (363 mg, 2.0 mmol) is stirred in DMF (15 mL) at room temperature. Triethylamine (0.6 mL, 4.2 mmol) was slowly added dropwise and the solution was stirred for 20 min and cooled to -10 °C (ice-salt bath; internal thermometer). TBTU (963 mg, 3.0 mmol) is added and the mixture is stirred for 12 h until completion of the reaction (monitored by TLC). The reaction mixture is then diluted with 100 mL EtOAc, transferred to a separatory funnel and sequentially washed with saturated suspension of $NaHCO_3$ (2×50 mL) and with 10% aqueous solution of HCl (2×30 mL). The organic phase is dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to dryness. The crude product is purified by flash column chromatography (n-Hex/EtOAc 1:1) to afford the title isocyanopeptide (233 mg, 55%) as an odorless, white amorphous solid. IR (KBr, cm^{-1}) ν_{max} 2160, 1740, 1656, 1454. R_f = 0.41 (hexane / ethyl acetate 1:1). ^{1}H NMR ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.59 (d, *J*=7.4 Hz, 1H), 4.46 (m, 1H), 4.12 (m, 2H), 3.71 (s, 3H), 1.71 – 1.57 (m, 2H), 1.36 (m, 1H), 0.80 (d, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.1, 162.4, 162.1, 52.7, 51.7, 45.0, 40.2, 25.4, 22.8, 21.3. HRMS (ESI+) *m*/*z* calcd for $C_{10}H_{16}N_2NaO_3$, $[M+Na]^+$; 235.1054 found, 235.1059.

(CN)Phe-Gly-OAll (89)



N-Formyl-Phe-Gly-OAll (290.12 mg, 1.0 mmol), triphosgene (47 mg, 0.4 mmol), and 2.6-lutidine (1.0 mL, 3.0 mmol), were reacted in dry dichloromethane (10 mL) for 3 h according to the general isocyanide synthesis protocol. Flash column chromatography purification (ethyl acetate / hexane 1:2) afforded the pure product (170 mg, 62%). IR (KBr, cm^{-1}) ν_{max} 3320, 3291, 2982, 2945, 2161, 1755, 1650, 1275, 1094 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 7.25 – 7.21 (m, 5H), 6.50 (br. s., 1H), 5.93 (m, 1H), 5.39 (dd, *J* = 17.2, 1.5 Hz, 1H), 4.45 (m, 1H), 4.27 (s, 2H), 4.14 – 4.10 (m, 2H), 3.20 – 3.14 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 171.3, 169.2, 163.6, 135.0, 129.1, 128.2, 128.3, 127.1, 119.8, 67.0, 60.0, 43.1, 38.4. HRMS (ESI+) *m*/*z* calcd for $C_{15}H_{16}N_3NaO_2$, $[M+Na]^+$; 294.1214 found, 294.1218.

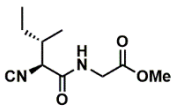
Cbz-Ala-Ile-OMe (90)



Cbz-Ala-OH (468 mg, 2.1 mmol) was coupled to HCl·Ile-OMe (363 mg, 2.0 mmol) according to the general solution-phase peptide synthesis procedure. Flash column chromatography purification (ethyl acetate / hexane 2:8) furnished the title dipeptide (588 mg, 84%) as a white amorphous solid. R_f = 0.35 (ethyl acetate / hexane 2:8). ^{1}H NMR (400 MHz, CD_3OD): *δ* 7.37 – 7.20 (m, 5H), 6.66 (br. s, 1H), 5.53 (br. s, 1H), 5.10 – 5.06 (m, 2H), 4.41 (d, *J* = 5.2 Hz, 1H), 4.26 (q, *J* = 7.1 Hz, 1H), 3.76 (s, 3H), 1.95 – 1.83 (m, 1H), 1.33 (d, *J* = 7.2 Hz, 3H), 1.31–1.16 (m, 2H), 0.98 –

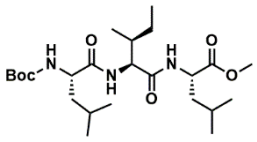
0.80 (m, 6H). ^{13}C NMR (100 MHz, CD_3OD): δ 175.4, 174.5, 158.1, 138.0, 129.4, 128.9, 128.7, 67.6, 57.9, 51.6, 52.2, 49.0, 38.4, 26.1, 18.2, 15.9, 11.9. HRMS (ESI+) *m/z* calcd for $C_{18}H_{27}N_2O_5$, $[M+H]^+$; 351.1917 found, 351.1920. This product was subjected to methyl ester removal according to the general procedure to afford peptide Cbz-Ala-Ile-OH (**91**) (527 mg, 94%), which was used forward without further purification.

(CN)Ile-Gly-OMe (92)



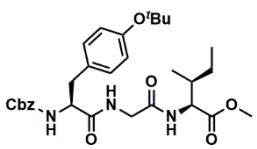
N-Formyl-Ile-Gly-OMe (230 mg, 1.0 mmol), triphosgene (118 mg, 0.4 mmol), and 2.6-lutidine (0.34 mL, 3.0 mmol), were reacted in dry dichloromethane (10 mL) for 3 h according to the general isocyanide synthesis protocol. Flash column chromatography purification (ethyl acetate / hexane 8:2) afforded the pure product (144 mg, 68%). IR (KBr, cm^{-1}) ν_{max} 2160, 1740, 1656, 1450. ^{1}H NMR ($CDCl_3$): δ = 6.39 (d, *J* = 7.1 Hz, 1H), 4.27 (m, 1H), 4.17 – 4.12 (m, 2H), 3.77 (s, 3H), 1.79 (m, 1H), 1.44 (m, 2H), 0.87 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.9, 164.8, 161.8, 53.8, 51.8, 42.0, 35.0, 24.9, 15.6, 11.6. HRMS (ESI+) *m/z* calcd for $C_{10}H_{16}N_2NaO_3$, $[M+Na]^+$; 235.1059 found, 235.1054.

Boc-Leu-Ile-Leu-OMe (93)



N-Boc-Leu-OH (508 mg, 2.2 mmol) was coupled to HCl·Ile-OMe (363 mg, 2.0 mmol) according to the peptide coupling procedure, following by deprotection of the *N*-terminally by Boc removal. The same protocol was employed for the coupling of *N*-Boc-Leu-OH (231 mg, 1.0 mmol). Flash column chromatography purification (hexane / ethyl acetate 1:1) furnished the title peptide (334 mg, 71%) as a white amorphous solid. R_f = 0.32 (hexane / ethyl acetate 1:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.65 (br. s. 1H), 6.4 (br. s, 1H), 5.8 (br. s, 2H), 4.44 – 4.3 (m, 1H), 4.2 – 4.1 (m, 1H), 3.71 (s, 3H), 2.0 – 1.7 (m, 5H), 1.40 (s, 9H), 1.3 – 1.1 (m, 4H), 1.06 – 0.95 (m, 18H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.9, 172.9, 171.1, 155.3, 79.7, 58.7, 52.7, 52.3, 50.9, 40.9, 40.1, 36.5, 28.3, 25.3, 24.8, 23.8, 22.2, 15.3, 11.9. HRMS (ESI+) *m/z* calcd for $C_{24}H_{46}N_3O_6$, $[M+H]^+$; 472.3387 found, 472.3382. This product was subjected to methyl ester removal according to the general procedure to afford peptide Boc-Leu-Ile-Leu-OH (**94**) (306 mg, 95%), which was used forward without further purification.

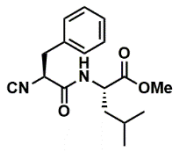
Cbz-Tyr(*t*Bu)-Gly-Ile-OMe (95)



Boc-Gly-OH (385 mg, 2.2 mmol) was coupled to HCl·Ile-OMe (363 mg, 2.0 mmol) according to the general solution-phase peptide synthesis procedure, following by deprotection of the *N*-terminally by Boc removal. The same protocol was employed for the coupling of Cbz-Tyr(*t*Bu)-OH (408 mg, 1.1 mmol). Flash column chromatography purification (hexane / ethyl acetate 1:1) furnished the title peptide (550 mg, 90%) as a white amorphous solid. R_f = 0.28

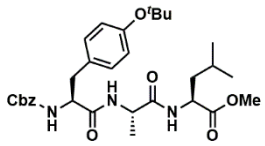
(hexane / ethyl acetate 1:1). ^{1}H NMR (400 MHz, CD_3OD): δ 7.38 – 7.11 (m, 5H), 7.01 (d, *J* = 8.4 Hz, 2H), 6.66 (d, *J* = 8.5 Hz, 2H), 5.07 (q, *J* = 12.5 Hz, 2H), 4.65 – 4.54 (m, 1H), 3.91 (d, *J* = 7.4 Hz, 1H), 3.86 (d, *J* = 5.8 Hz, 2H), 3.62 (s, 3H), 3.08 (dd, *J* = 13.8, 6.2 Hz, 1H), 2.83 – 2.72 (m, 1H), 1.74 – 1.62 (m, 1H), 1.40 – 1.27 (m, 2H), 1.33 (s, 9H), 0.82 (t, *J* = 7.4 Hz, 3H), 0.75 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD): δ 173.8, 173.1, 172.9, 158.6, 157.3, 138.1, 131.4, 129.0, 128.9, 128.7, 127.9, 116.2, 78.4, 67.8, 55.8, 55.3, 52.7, 41.0, 38.5, 38.1, 28.8, 28.5, 27.9, 25.7, 15.9, 11.4. HRMS (ESI+) *m/z* calcd for $C_{30}H_{42}N_3O_7$, $[M+H]^+$; 556.3023 found, 556.3017.

(CN)Phe-Leu-OMe (96)



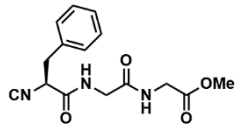
N-Formyl-Phe-Leu-OMe (320 mg, 1.0 mmol), triphosgene (118 mg, 0.4 mmol), and 2.6-lutidine (0.34 mL, 3.0 mmol), were reacted in dry dichloromethane (10 mL) for 3 h according to the general isocyanide synthesis protocol. Flash column chromatography purification (ethyl acetate / hexane 8:2) afforded the pure product (181 mg, 60%). IR (KBr, cm^{-1}) ν_{max} 3325, 3289, 2956, 2140, 1274, 1030 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29 – 7.20 (m, 5H), 6.53 (br. s. 1H), 4.51 (m, 1H), 4.41 (m, 1H), 3.67 (s, 3H), 3.22 – 3.11 (m, 2H), 1.55 – 1.29 (m, 3H), 0.84 (d, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.4, 164.5, 134.4, 129.7, 128.7, 127.7, 52.5, 51.1, 41.1, 38.3, 24.6, 22.7, 21.8. HRMS (ESI+) *m/z* calcd for $C_{17}H_{22}N_2NaO_3$, $[M+Na]^+$, 325.1528 found, 325.1524.

Cbz-Tyr(*t*Bu)-Ala-Leu-OMe (97)



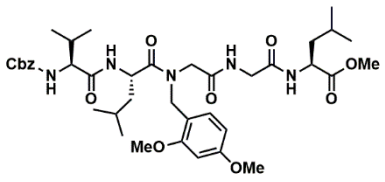
Boc-Ala-OH (416 mg, 2.2 mmol) was coupled to HCl·Leu-OMe (363 mg, 2.0 mmol) according to the general solution-phase peptide synthesis procedure, following by deprotection of the *N*-terminally by Boc removal. The same protocol was employed for the coupling of Cbz-Tyr-OH (408 mg, 1.0 mmol). Flash column chromatography purification (ethyl acetate / hexane 2:1) furnished the title peptide (495 mg, 87%) as a white amorphous solid. R_f = 0.32 (ethyl acetate / hexane 2:1). ^{1}H NMR (400 MHz, CD_3OD): *δ* 7.35 –7.26 (m, 5H), 7.05 (d, *J* = 8.2 Hz, 2H), 6.70 (d, *J* = 8.5 Hz, 2H), 5.02 (q, *J* = 12.5 Hz, 2H), 4.43 (dd, *J* = 9.3, 5.5 Hz, 1H), 4.33 (q, *J* = 6.9 Hz, 1H), 4.24 (dd, *J* = 8.8, 5.0 Hz, 1H), 3.71 (s, 3H), 3.01 (dd, *J* = 14.0, 4.9 Hz, 1H), 2.77 – 2.69 (m, 1H), 1.72 – 1.59 (m, 1H), 1.40 – 1.33 (m, 5H), 1.31 (s, 9H), 0.96 (d, *J* = 6.2 Hz, 3H), 0.92 (d, *J* = 6.1 Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD): δ 175.1, 174.7, 171.5, 157.8, 157.3, 138.1, 131.3, 129.1, 129.0, 128.8, 127.8, 116.2, 78.9, 67.8, 57.6, 52.6, 50.7, 41.8, 38.2, 28.9, 28.5, 28.4, 28.7, 25.8, 23.4, 17.9. HRMS (ESI+) *m/z* calcd for $C_{31}H_{44}N_3O_7$, $[M+H]^+$; 570.3179 found, 570.3174. This product was subjected to methyl ester removal according to the general procedure to afford peptide Cbz-Tyr(*t*Bu)-Ala-Leu-OH (**98**) (459 mg, 95%), which was used forward without further purification.

(CN)Phe-Gly-Gly-OMe (99)



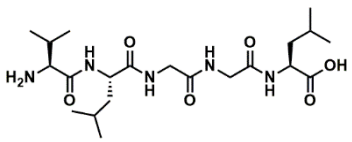
N-Formyl-Phe-Gly-Gly-OMe (321 mg, 1.0 mmol), triphosgene (118 mg, 0.4 mmol), and 2.6-lutidine (0.34 mL, 3.0 mmol), were reacted in dry dichloromethane (10 mL) for 3 h according to the general isocyanide synthesis protocol. Flash column chromatography purification (ethyl acetate / hexane 6:4) afforded the pure product (121 mg, 40%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.30 (m, 3H), 7.13 (m, 2H), 5.5 (br. s, 2H), 4.83 (dt, *J* = 7.8, 6.1 Hz, 1H), 4.12 (s, 2H), 3.75 (s, 3H,), 3.9 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.1, 170.2, 161.7, 162.0, 135.1, 129.1, 128.9, 127.5, 53.4, 52.6, 45.4, 43.4, 37.5. HRMS (ESI+) *m/z* calcd for $C_{15}H_{17}N_3NaO_4$, $[M+Na]^+$; 326.1117 found, 326.1112.

Cbz-Val-Leu-*N*(DMB)-Gly-Gly-Leu-OMe (100)



2,4-Dimethoxybenzylamine (**87**, 0.22 mL, 1.5 mmol), paraformaldehyde (45 mg, 1.5 mmol), Cbz-Val-Ile-OH (364 mg, 1.0 mmol) and CN-Gly-Leu-OMe (212 mg, 1.0 mmol) were reacted in MeOH (50.0 mL) for 24 h according to the general Ugi-4CR procedure. Flash column chromatography purification (ethyl acetate / hexane 9:1) afforded the Ugi-product **100** (527 mg, 82%) as a white amorphous solid. R_f = 0.70 (ethyl acetate / hexane 9:1). Mixture of conformers. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62 (d, *J* = 7.5 Hz, 1H), 7.37 – 7.26 (m, 5H), 7.17 – 7.07 (m, 2H), 6.45 – 6.41 (m, 2H) 5.14 – 5.05 (m, 3H), 4.76 – 4.50 (m, 6H), 4.15 (d, *J* = 15.9 Hz, 1H), 4.10 – 4.00 (m, 4H), 3.85 (d, *J* = 15.9 Hz, 1H), 3.80 (s, 3H), 3.77 (s, 3H), 3.70 (s, 3H), 2.12 – 1.99 (m, 2H), 1.67 – 1.43 (m, 4H), 0.93 – 0.80 (m, 18H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.4, 171.8, 170.3, 169.4, 161.3, 159.0, 136.4, 130.9, 128.6, 128.2, 128.1, 115.8, 104.0, 98.8, 77.1, 67.1, 60.5, 55.5, 55.3, 52.3, 51.6, 48.1, 42.4, 41.8, 41.1, 31.4, 24.8, 23.6, 23.2, 22.1, 21.7, 19.2, 17.9. HRMS (ESI+) *m/z* calcd for $C_{39}H_{58}N_5O_{10}$, $[M+H]^+$, 756.4184 found, 756.4181.

H-Val-Leu-Gly-Gly-Leu-OH (101)



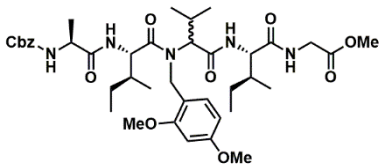
The parallel cyclization study of the model and *N*-alkylated peptides was accomplished under the same macrocyclization conditions (1.0 mM and T3P$^{@}$/DMAP as coupling system). The peptide **101** was synthesized on Wang resin by a standard protocol: Wang resin (300 mg, 1.11 mmol g^{-1}, 0.33 mmol) was swollen in CH_2Cl_2/DMF (8:2) for 1.5 h, and the first amino acid was coupled to the resin by use of Fmoc-Leu (2.0 equiv), diisopropylcarbodiimide (DIC, 2.0 equiv), and dimethylaminopyridine (DMAP, 0.1 equiv) in CH_2Cl_2/MeOH (8:2), with a reaction time of 20 h. The coupling step was repeated twice. The Fmoc group was cleaved with piperidine in DMF (20 %). The other four amino acids (Gly, Gly, Leu and Val) were sequencially coupled by use of the following method: 3.0 equiv of amino acid, 3.0 equiv of HBTU, 3.0

equiv of HOBt, 5.0 equiv of DIPEA in DMF for 6 h. Cleavage of the product from the resin was achieved by treatment with 50 % TFA in dichloromethane and precipitation by adding dry ether to the solution. The white solids were lyophilized with water containing 2 mL of 0.1 N HCl to guarantee the presence of a hydrochloric salt. The peptide **101** was obtained analytically pure as a white solid. The non-*N*-alkylated peptide (**101**) was cyclized under the same conditions as in the preparation of cyclo peptide **103**. The cyclation rendered a mixture of cyclic monomer (**104**) and dimer in 2.2:1 ratio and 21% yield of isolated cyclic peptide **104**.

Macrocycle 103: Peptide **100** (286 mg, 0.44 mmol) was deprotected at both termini according to the general Cbz and methy ester removal procedures. The resulting crude peptide (**102**) was then cyclized at 1.0 mM with T3P®/DMAP in dichloromethane (440 mL) according to the general macrocyclization procedure. The crude material was purified by silica gel column chromatography using (dichloromethane / methanol 9:1) to afford **103** (140 mg, 54%) as a white amorphous solid. R_f = 0.30 (dichloromethane / methanol 9:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.15 (d, *J* = 8.3 Hz, 1H), 6.58 (d, *J* = 2.3 Hz, 1H), 6.54 (dd, *J* = 8.3, 2.3 Hz, 1H), 4.88 (m, 1H), 4.79 (d, *J* = 15.3 Hz, 1H), 4.45 (d, *J* = 15.3 Hz, 1H), 4.39 (d, *J* = 15.3 Hz, 1H), 4.27 (dd, *J* = 9.9, 5.3 Hz, 1H), 3.98 (d, *J* = 9.0 Hz, 1H), 3.92 (m, 2H), 3.82 (s, 3H), 3.79 (s, 3H), 3.36 (d, *J* = 15.7 Hz, 1H), 3.31 (m, 2H), 2.10 (m, 1H), 1.86 – 1.77 (m, 1H), 1.70 – 1.56 (m, 4H), 1.05 – 0.88 (m, 18H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.7, 173.9, 171.9, 163.0, 160.2, 132.4, 116.7, 105.7, 99.8, 62.9, 56.1, 55.9, 43.7, 41.8, 30.5, 26.4, 26.0, 23.8, 23.3, 22.1, 22.0, 19.8, 19.5. HRMS (ESI+) *m*/*z* calcd for $C_{30}H_{48}N_5O_7$, $[M+H]^+$; 590.3554 found, 590.3551.

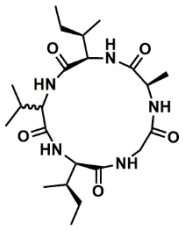
Macrocycle 104: The cyclopeptide **104** was subjected to DMB cleavage with 20% TFA in dichloromethane and purified by preparative RP-HPLC to afford **104** (95 mg, 49% from **100**) in 92% purity, according to UHPLC (*R*t = 13.38 min, PDA range: 190 – 400 nm). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.64 (t, *J* = 6.0 Hz, 1H), 8.24 (d, *J* = 7.6 Hz, 1H), 7.95 (d, *J* = 7.6 Hz, 1H), 7.84 (d, *J* = 9.2 Hz, 1H), 7.49 (br. s, 1H), 4.30 – 4.21 (m, 1H), 4.17 – 4.07 (m, 2H), 3.88 – 3.64 (m, 4H), 2.00 – 1.90 (m, 3H), 1.58 – 1.38 (m, 4H), 0.95 – 0.77 (m, 18H).^{13}C NMR (100 MHz, DMSO-d_6): δ 171.6, 171.6, 170.2, 169.3, 169.0, 60.9, 52.8, 51.9, 42.8, 41.2, 29.3, 24.5, 22.9, 22.6, 22.2, 21.6, 19.2, 18.6. HRMS (ESI+) *m*/*z* calcd for $C_{21}H_{38}N_5O_5$, $[M+H]^+$, 440.2873 found, 440.2870.

Cbz-Ala-Ile-*N*(DMB)-D,L-Val-Ile-Gly-OMe (105)

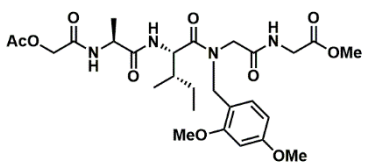


2,4-Dimethoxybenzylamine (0.15 mL, 1.02 mmol), isobutylaldehyde (93 µL, 1.02 mmol), Cbz-Ala-Ile-OH (228 mg, 0.68 mmol) and CN-Ile-Gly-OMe (144 mg, 0.68 mmol) were reacted in MeOH (15 mL) for 24 h according to the general Ugi-4CR procedure. Flash column chromatography purification (ethyl acetate / hexane 7:3) afforded the Ugi-product **105** (413 mg, 79%) as a white amorphous solid. R_f = 0.33 (ethyl acetate / hexane 7:3). Mixture of diasteromers, d.r. 1.4:1. ^{1}H NMR (400 MHz, CD_3OD): δ 7.39 – 7.24 (m, 7H), 7.03 (d, *J* = 8.4 Hz, 1H), 6.96 (d, *J* = 8.4 Hz, 1H), 6.51 (d, *J* = 2.2 Hz, 1H), 6.45, 6.39 (2×m, 1H), 6.42, 6.41 (2×d, *J* = 8.0 Hz, 1H), 5.12-5.07 (m, 3H), 4.91 (m, 1H), 4.79-4.75 (m, 1H), 4.64-4.57 (m, 2H), 4.49 (m, 1H), 4.30-4.06 (m, 6H), 3.82, 3.80 (2×s, 3H), 3.78, 3.77 (2×s, 3H), 3.75 (s, 3H), 3.74 – 3.68 (m, 2H), 2.51 (m, 1H), 2.43 (m, 1H), 1.83 (m, 3H), 1.54 (m, 3H), 1.36 – 1.21 (m, 3H), 0.93 – 0.77 (m, 18H). ^{13}C NMR (100 MHz, CD_3OD): δ 175.1, 175.0, 172.9, 172.8, 172.7, 172.3, 170.9, 170.8, 162.2, 159.6, 130.5, 129.5, 129.0, 128.9, 128.8, 118.4, 117.8, 105.4, 105.10, 99.3, 67.7, 62.3, 59.4, 59.2, 55.9, 55.9, 55.9, 55.8, 55.8, 55.5, 51.9, 42.0, 41.9, 39.9, 38.7, 37.9, 37.8, 28.8, 28.3, 25.9, 25.7, 24.9, 24.7, 20.5, 20.2, 19.5, 19.4, 18.3, 16.6, 16.5, 16.1, 14.6, 14.5, 11.7, 11.4, 11.3. HRMS (ESI+) *m*/*z* calcd for $C_{40}H_{60}N_5O_{10}$, $[M+H]^+$, 770.4340 found, 770.4336.

Macrocycle 108: Peptide **105** (381 mg, 0.59 mmol) was deprotected at both termini according to the general Cbz and methy ester removal procedures. The resulting product was cyclized with PyBOP/DIPEA according to the general macrocyclization procedure and the crude *N*-alkylated cyclopeptide **106** was subjected to DMB cleavage (**107**) with 20% TFA in dichloromethane and purified by preparative RP-HPLC to afford **108** (149 mg, 56%) in 91% purity, according to UHPLC (mixture of diastereomers, *R*t = 14.07 min and *R*t = 14.32, PDA range: 190 – 400 nm). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.64, 8.41 (2×d, *J* = 6.8 Hz, 1H), 8.27 – 8.22 (m, 2H), 7.88, 7.79 (2×m, 1H), 7.64, 7.25 (2×d, *J* = 8.6 Hz, 1H), 4.41 – 4.34 (m, 1H), 4.32 – 4.23 (m, 2H), 4.19 – 4.02 (m, 3H), 3.87 (dd, *J* = 16.4, 7.3 Hz, 1H), 3.74 – 3.66 (m, 2H), 2.25 (m, 1H), 2.05, 1.94 (2×m, 1H), 1.77 (m, 2H), 1.63 (m, 1H), 1.44 (m, 2H), 1.25 – 1.16 (m, 3H), 1.09 (m, 1H), 0.93 – 0.80 (m, 18H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.3, 171.7, 171.5, 171.4, 168.3, 79.2, 62.6, 60.8, 55.9, 49.1, 42.6, 39.5, 37.5, 33.5, 27.9, 25.3, 24.7, 24.0, 19.3, 19.3, 17.1, 15.5, 15.4, 11.7, 10.9, 10.1. HRMS (ESI+) *m*/*z* calcd for $C_{22}H_{40}N_5O_5$, $[M+H]^+$, 454.3029 found, 454.3023.

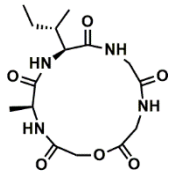


AcO-Glc-Ala-Ile-*N*(DMB)-Gly-Gly-OMe (110)



2,4-Dimethoxybenzylamine (220 μL, 1.5 mmol), paraformaldehyde (45 mg, 1.5 mmol), AcO-Glc-Ala-Ile-OH (**109**) (302 mg, 1.0 mmol) and methyl isocyanoacetate (90 μL, 1.0 mmol) are reacted in MeOH (15 mL) for 24 h according to the general Ugi-4CR procedure. Flash column chromatography purification (ethyl acetate / methanol 96:4) afforded the Ugi-product **110** (447 mg, 77%) as a white amorphous solid. R_f = 0.19 (ethyl acetate / methanol 96:4). Mixture of conformers, 1.5:1. ^{1}H NMR (400 MHz, CD_3OD): δ 7.33 (m, 1H), 7.17, 7.07 (2×d, *J* = 8.3 Hz, 1H), 6.55 (d, *J* = 2.4 Hz, 1H), 6.50 (m, 1H), 6.45 (dd, *J* = 8.3, 2.4 Hz, 1H), 5.00 (d, *J* = 7.6 Hz, 1H), 4.69 – 4.50 (m, 4H), 4.44 (m, 1H), 4.35 (d, *J* = 17.7 Hz, 1H), 4.07 (d, *J* = 17.7 Hz, 1H), 4.01 (m, 1H), 3.92 (m, 1H), 3.81, 3.79 (2×s, 3H), 3.79, 3.77 (2×s, 3H), 3.73, 3.72 (2×s, 3H), 1.93 – 1.82 (m, 1H), 1.61 – 1.55 (m, 1H), 1.37, 1.32 (2×d, *J* = 7.2 Hz, 3H), 1.16 – 1.09 (m, 1H), 0.91 – 0.84 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.5, 174.4, 174.4, 174.0, 171.9, 171.6, 171.5, 171.3, 171.2, 169.9, 169.8, 162.8, 162.3, 160.4, 160.2, 132.0, 131.9, 117.6, 117.3, 105.6, 105.6, 99.6, 99.2, 63.4, 63.4, 55.9, 55.9, 55.9, 55.8, 55.1, 55.1, 52.6, 52.6, 50.6, 50.3, 50.0, 49.0, 45.7, 41.9, 41.8, 38.6, 38.2, 25.5, 25.3, 20.5, 18.1, 17.9, 15.9, 15.8, 11.5, 11.4. HRMS (ESI+) *m/z* calcd for $C_{27}H_{41}N_4O_{10}$, $[M+H]^+$, 581.2823 found, 581.2819.

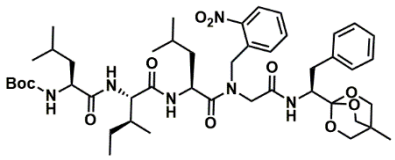
Macrocycle 113: Peptide **110** (424 mg, 0.74 mmol) is dissolved in THF/H_2O (1:1, 6.0 mL) and $LiOH.H_2O$



(105 mg, 2.5 mmol) is added at 0 °C. The mixture is stirred for 5 h and then acidified with aqueous 10% $NaHSO_4$ to pH 3.0. The resulting phases are separated and the aqueous phase is additionally extracted with EtOAc (2 × 20 mL), transferred to a separatory funnel and washed with brine (2×20 mL). The combined organic phases are dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to yield the *C*-deprotected peptide (**111**). The crude *N*-alkylated peptide (376 mg, 0.71 mmol) is dissolved in CH_2Cl_2 (710 mL) and is added EDC (136 mg, 0.71 mmol), and DMAP (171 mg, 1.4 mmol). After stirring the mixture for 12 h, the solvent is removed under reduced pressure. The crude residue is dissolved in ethyl acetate (50 mL) washed with water (2 × 30 mL), aqueous hydrochloric acid 10% v/v (2 × 30 mL), 10% v/v aqueous $NaHCO_3$ (2 × 30 mL), brine (2 × 30 mL), dried over Na_2SO_4 and evaporated to dryness to afford *N*-alkylated cyclopeptide **112**. This crude compound was subjected to DMB cleavage with 20% TFA in CH_2Cl_2 and purified by silica gel column chromatography using (dichloromethane / methanol 96:4) to afford **113** (179 mg, 68%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.24 (t, *J* = 5.4 Hz, 1H), 8.04 (d, *J* = 8.5 Hz, 1H), 7.72 (d, *J* = 7.8 Hz, 1H), 5.61 (t, *J* = 5.8 Hz, 1H), 4.18 (t, *J* = 7.9 Hz, 1H), 4.08 (q, *J* = 7.1 Hz, 2H), 3.84 (d, *J* = 5.8 Hz, 1H), 3.80 (d, *J* = 4.9 Hz, 2H), 3.75 (d, *J* = 5.7 Hz, 2H), 1.77 – 1.66 (m, 1H), 1.51 – 1.38 (m, 1H), 1.24 – 1.16 (m, 3H), 1.11 – 1.02 (m, 1H), 0.87 – 0.76 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.1, 171.2, 171.3, 169.8, 169.2, 61.3, 57.0, 47.5, 41.6, 40.7,

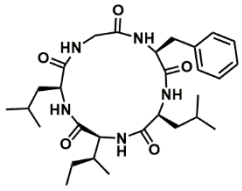
39.5, 36.6, 24.3, 18.9, 14.1, 11.1. HRMS (ESI+) *m*/*z* calcd for $C_{15}H_{25}N_4O_6$, $[M+H]^+$; 357.1774 found, 357.1770.

Boc-Leu-Ile-Leu-*N*(ONB)-Gly-Phe-OBO (114)



2-Nitrobenzylamide hydrochloride (188 mg, 1.0 mmol), paraformaldehyde (30 mg, 1.0 mmol), DIPEA (0.17 mL, 1.0 mmol), Boc-Leu-Ile-Leu-OH (329 mg, 1.0 mmol), and CN-Phe-OBO[238] (259 mg, 1.0 mmol) were reacted in MeOH (15.0 mL) for 24 h according to the general Ugi-4CR procedure. Flash column chromatography purification (ethyl acetate / methanol 96:4) furnished the title peptide (493 mg, 76%) as a white amorphous solid. Major conformer. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14 – 7.48 (m, 4H), 7.58 (dd, *J* = 7.5, 1H), 7.30 – 7.26 (m, 5H), 7.15 – 7.11 (m, 2H), 6.32 (d, *J* = 6.5, 1H), 5.90 (dd, *J* = 9.1), 5.51 (d, *J* = 6.9, 2H), 4.02 (s, 6H), 4.57 (m, 1H), 4.40 (m, 2H), 4.68 – 4.62 (m, 1H), 4.72 (m, 1H), 4.17 (t, *J* = 8.2, 1H), 3.01(m, 1H), 4.69 (d, *J* = 15.4, 1H), 4.52 (d, *J* = 15.4, 1H), 2.01(m, 1H), 1.89 – 1.75 (d, 1H), 1.74 – 1.48 (m, 4H), 1.44 (s, 9H), 1.19 – 1.04 (m, 2H), 0.97 – 0.80 (m, 21H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.1, 171,9, 170.3, 162.5, 158.3, 137.2, 128.8, 128.2, 126.7, 145.0, 147.9, 125.1, 127.0, 133.3, 133.8, 129.8, 106.7, 80.3, 73.1, 60.8, 48.5, 47.3, 35.9, 30.8, 14.2. 59.7, 50.9, 41.0, 37.2, 28.4, 24.8, 24.7, 22.8, 21.8, 15.3, 11.1. HRMS (ESI+) *m*/*z* calcd for $C_{46}H_{68}N_6NaO_{11}$, $[M+Na]^+$, 903.4844 found, 903.4840.

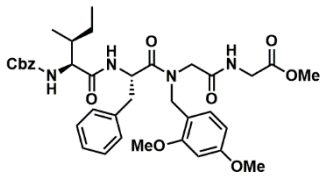
Macrocycle 117: To a solution of the peptide OBO-ester **114** (304 mg, 0.34 mmol) in 6 mL of CH_2Cl_2 is



added 0.025 mL of TFA and 0.1 mL of H_2O. The reaction mixture is stirred at room temperature for 30 min and then evaporated. The oily product is dissolved in 1 mL of THF, and then 3 mL of H_2O and 80 mg (2.0 mmol) of NaOH are added. The two-phase mixture was stirred at room temperature for 1 h for complete hydrolysis, while the mixture became homogeneous (TLC control: dichloromethane-methanol 10:1) The solution was then diluted with 15 mL of H_2O, acidified with concd. HCl to pH ~1, and extracted with EtOAc (2 × 15 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, and evaporated to afford the pure acid as a white solid. The crude peptide is cyclized with cyclized T3P®/DMAP according to the general macrocyclization procedure. The crude *N*-alkylated cyclopeptide **116** is subjected to *o*NB removal according to the general photo-cleavage procedure. The final product is purified by preparative RP-HPLC to afford **117** (89 mg, 48%) in 98% purity, according to UHPLC (*R*t = 13.37 min, PDA range: 190 – 400 nm). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.64 (t, *J* = 5.9 Hz, 1H), 8.38 (dd, *J* = 7.6 Hz, 1H), 8.06 (d, *J* = 7.9 Hz, 1H), 7.98 (d, *J* = 9.1 Hz, 1H), 7.59 (br. s, 1H), 7.29 – 7.19 (m, 5H), 4.42 (m, 1H), 4.29 (m, 1H), 4.15 (m, 1H), 3.91 (t, *J* = 8.1 Hz, 1H), 3.83 – 3.65 (m, 2H), 3.01 (dd, *J* = 13.6, 8.0 Hz, 1H), 2.89 (dd, *J* = 13.6, 7.0

Hz, 1H), 1.71 (m, 1H), 1.7 (m, 1H), 1.55 – 1.42 (m, 3H), 1.23 (m, 1H), 1.15 (m, 1H), 0.90 – 0.84 (m, 15H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.2, 172.0, 170.3, 169.5, 168.7, 137.3, 128.9, 128.2, 126.4, 57.9, 57.1, 55.6, 55.4, 43.6, 36.8, 24.5, 24.3, 22.8, 22.7, 21.3, 15.4, 11.0. HRMS (ESI+) *m/z* calcd for $C_{29}H_{46}N_5O_5$, $[M+H]^+$, 544.3499 found, 544.3495.

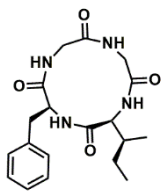
Cbz-Ile-Phe-*N*(DMB)-Gly-Gly-OMe (120)



2,4-Dimethoxybenzylamine (0.11 mL, 0.75 mmol), paraformaldehyde (22.5 mg, 0.75 mmol), Cbz-Ile-Phe-OH (206 mg, 0.5 mmol) and methyl isocyanoacetate (68 μL, 0.75 mmol) were reacted in MeOH (15 mL) for 24 h according to the general Ugi-4CR procedure. Flash column chromatography purification (ethyl acetate) afforded the Ugi-product **120** (290 mg, 84%) as a white amorphous solid. R_f = 0.37 (ethyl acetate). Mixture of conformers. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71 (d, *J* = 5.0 Hz, 1H), 7.35 – 7.02 (m, 14H), 6.50 – 6.38 (m, 3H), 5.61 (d, *J* = 8.4 Hz, 1H), 5.29 (t, *J* = 8.0 Hz, 1H), 4.98 (s, 2H), 4.81 (d, *J* = 15.0 Hz, 1H), 4.53 (d, *J* = 15.1 Hz, 1H), 4.17 (d, *J* = 15.8 Hz, 1H), 3.89 (d, *J* = 5.4 Hz, 1H), 3.81 (s, 3H), 3.76 (s, 3H), 3.64 (s, 3H), 3.13 – 3.04 (m, 1H), 3.00 – 2.83 (m, 1H), 1.92 – 1.81 (m, 1H), 1.52 – 1.40 (m, 1H), 1.08 – 0.97 (m, 1H), 0.92 (d, *J* = 6.8 Hz, 3H), 0.84 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.9, 170.9, 170.1, 169.1, 161.3, 159.2, 158.9, 156.2, 136.5, 131.3, 129.53, 129.4, 128.6, 128.6, 128.5, 128.5, 128.1, 127.9, 126.8, 116.0, 104.1, 98.9, 77.2, 66.9, 55.7, 55.5, 55.4, 53.8, 52.2, 48.6, 48.5, 41.0, 39.3, 38.5, 24.1, 15.7, 11.4. HRMS (ESI+) *m/z* calcd for $C_{37}H_{47}N_4O_9$, $[M+H]^+$; 691.3343 found, 691.3340.

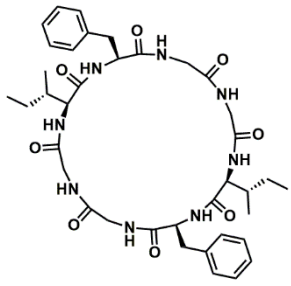
Macrocyclization of the *N*-alkylated tetrapeptide: Peptide **120** (282 mg, 0.4 mmol) was deprotected at both termini according to the general Cbz and methyl ester removal procedures (**121**). The resulting peptide is cyclized with PyBOP/DIPEA according to the general macrocyclation procedure to afford a mixture of *N*-alkylated cyclopeptides **122** and the corresponding cyclodimer **124**. The mixture of the *N*-alkylated cyclopeptides was subjected to DMB cleavage with 20% TFA in CH_2Cl_2 and purified by preparative RP-HPLC to afford cyclic tetrapeptide **123** (59 mg, 39%) and the corresponding cyclodimer **125** (25 mg, 17%).

Macrocycle 123: UHPLC (*R*t = 12.01 min, PDA range: 190 – 400 nm). ^{1}H NMR (400 MHz, DMSO-d_6):



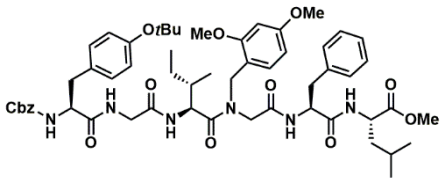
8.14 (br. s, 1H), 7.91 (br. s, 1H), 7.28 – 7.04 (m, 5H), 6.60 (br. s, 1H), 6.47 (br. s, 1H), 4.36 – 4.19 (m, 2H), 4.16 – 3.99 (m, 2H), 3.85 – 3.74 (m, 4H), 1.45 – 1.38 (m, 1H), 0.93 – 0.82 (m, 2H), 0.82 – 0.64 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.5, 170.9, 169.7, 169.2, 136., 127.7, 127.1, 125.3, 58.2, 56.2, 44.0, 42.3, 35.1, 34.5, 33.3, 23.5, 14.3, 10.1. HRMS (ESI+) *m/z* calcd for $C_{19}H_{27}N_4O_4$, $[M+H]^+$; 375.2032 found, 375.2037.

Macrocycle 125: UHPLC (*R*t = 13.92 min, PDA range: 190 – 400 nm). ^{1}H NMR (400 MHz, DMSO-d_6):



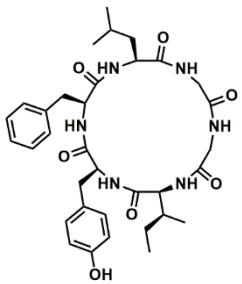
δ 8.21 (br. s, 2H), 7.92 (br. s, 2H), 7.25 – 7.07 (m, 10H), 6.61 (br. s, 2H), 6.44 (br. s, 2H), 4.41 – 4.22 (m, 4H), 4.15 – 4.02 (m, 4H), 3.86 – 3.73 (m, 8H), 1.44 – 1.37 (m, 2H), 0.94 – 0.84 (m, 4H), 0.83 – 0.67 (m, 12H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.4, 170.9, 169.8, 169.2, 136.2, 127.8, 127.1, 125.4, 58.2, 56.4, 44.0, 42.4, 35.2, 34.5, 33.3, 23.5, 14.3, 10.3. HRMS (ESI+) *m/z* calcd for $C_{38}H_{53}N_8O_8$, $[M+H]^+$; 749.3986 found, 749.3982.

Cbz-Tyr(*t*Bu)-Gly-Ile-*N*(DMB)-Gly-Phe-Leu-OMe (126)



2,4-Dimethoxybenzylamine (0.11 mL, 0.75 mmol), paraformaldehyde (22 mg, 0.75 mmol), Cbz-Tyr(*t*Bu)-Gly-Ile-OH (270 mg, 0.5 mmol) and CN-Phe-Leu-OMe (151 mg, 0.5 mmol) are reacted in MeOH (15 mL) for 24 h according to the general Ugi-4CR procedure. Flash column chromatography purification (dichloromethane / methanol 98:2) afforded the Ugi-product **126** (409 mg, 81%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38 – 7.07 (m, 10H), 7.04 – 6.89 (m, 2H), 6.72 – 6.58 (m, 3H), 6.56 – 6.41 (m, 3H), 5.18 – 4.99 (m, 1H), 4.75 – 4.56 (m, 3H), 4.50 – 4.28 (m, 3H), 4.09 – 3.83 (m, 4H), 3.85 – 3.72 (m, 7H), 3.20 – 2.51 (m, 7H), 1.95 – 1.86 (m, 1H), 1.85 – 1.75 (m, 1H), 1.73 – 1.38 (m, 5H), 1.37 – 1.09 (m, 3H), 3.20 – 2.51 (m, 7H), 1.95 – 1.86 (m, 1H), 1.85 – 1.75 (m, 1H), 1.73 – 1.38 (m, 5H), 1.37 – 1.09 (m, 3H), 1.40 (s, 9H), 0.97 – 0.69 (m, 14H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.4, 175.9, 174.7, 172.7, 171.4, 162.7, 160.2, 159.9, 157.2, 138.4, 138.2, 131.5, 131.3, 130.5, 130.4, 129.5, 129.4, 129.3, 128.9, 128.2, 127.9, 127.7, 127.7, 116.3, 116.2, 105.6, 99.6, 79.9, 65.2, 58.6, 57.0, 55.9, 55.8, 55.9, 50.8, 41.8, 38.8, 38.6, 38.5, 38.4, 28.9, 28.7, 28.5, 28.2, 27.4, 26.1, 25.8, 25.3, 23.8, 23.4, 22.0, 21.9, 16.3, 15.9, 14.9, 12.2, 12.1. HRMS (ESI+) *m/z* calcd for $C_{56}H_{75}N_6O_{12}$, $[M+H]^+$; 1023.5443 found, 1023.5439.

Macrocycle 129: Peptide **126** (409 mg, 0.4 mmol) was deprotected at both termini according to the general

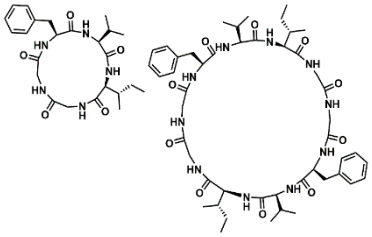


Cbz and methy esther removal procedures (**127**) and then cyclized with T3P®/DMAP according to the general macrocyclation procedure. The crude *N*-alkylated cyclopeptide **128** was subjected to DMB/*t*Bu cleavage with 20% TFA in CH_2Cl_2 and purified by preparative RP-HPLC to afford to afford **129** (149 mg, 61%) in 99% purity, according to UHPLC (*R*t = 13.22 min, PDA range: 190 – 400 nm). ^{1}H NMR (400 MHz, DMSO-d_6): 9.17 (s, 1H), 8.61 (dd, *J* = 7.2, 3.9 Hz, 1H), 8.40 (d, *J* = 4.0 Hz, 1H), 8.35 (br. s, 1H), 8.18 (br. s, 1H), 8.16 (br. s, 1H), 7.72 (d, *J* = 6.5 Hz, 1H), 7.35 – 7.08 (m, 5H), 6.85 (d, *J* = 8.3 Hz, 2H), 6.57 (d, *J* = 8.2 Hz, 2H), 4.50 – 4.42 (m, 1H), 4.09 – 3.90 (m, 4H), 3.86 (t, *J* = 8.6 Hz, 1H), 2.85 – 2.73 (m,

4H), 1.66 – 1.40 (m, 3H), 1.28 – 1.12 (m, 2H), 1.03 – 0.95 (m, 1H), 0.92 (d, *J* = 6.4 Hz, 3H), 0.85 (d, *J* = 6.4 Hz, 3H), 0.81 – 0.76 (m, 2H), 0.71 (t, *J* = 7.4 Hz, 3H), 0.45 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 172.3, 172.1, 171.6, 170.8, 168.9, 168.5, 155.8, 129.8, 129.4, 127.9, 114.9, 81.1, 78.7, 59.1, 39.5, 35.4, 24.1, 22.7, 22.0, 15.0, 10.8, 10.0. HRMS (ESI+) *m/z* calcd for $C_{34}H_{47}N_6O_7$, $[M+H]^+$; 651.3506 found, 651.3502.

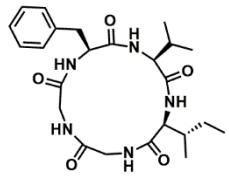
2.5 Synthesized compounds in chapter 6

Macrocycles 130 and 131: The resin Rink-Amide MBHA (140 mg, loading 0.71 mmol/g resin) was



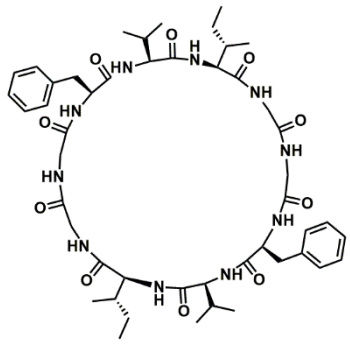
subjected to the on-resin Ugi reaction with Fmoc-Phe-OH, paraformaldehyde and ethyl isocyanoacetate, followed by sequential couplings of Fmoc-Val-OH and Fmoc-Ile-OH according the general procedure for the multicomponent synthesis of resin-bound peptides. The resulting resin-bound peptide was then deprotected at both termini and subjected to the on-resin macrocyclization procedure with HATU/DIPEA. After cleavage from the resin, a mixture of cyclic peptides **130** and dimer **131** was obtained. Preparative RP-HPLC purification produced **130** (6.6 mg, 14%) and the cyclodimer **131** (34 mg, 36%) in 99% purity.

Macrocycle 130: 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), 7.26 (t, *J* = 7.5 Hz, 2H), 7.19 – 7.11 (m, 3H), 4.53 (q, *J* = 7.4 Hz, 1H), 4.43 (q, *J* = 7.9 Hz, 2H), 3.67 (dd, *J* = 20.2, 5.4 Hz, 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), 1.89 (dq, *J* = 14.9, 7.1 Hz, 2H), 1.54 – 1.43 (m, 1H), 1.19 – 1.07 (m, 1H), 0.84 – 0.73 (m, 12H). ^{13}C NMR (100 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* calcd for $C_{24}H_{36}O_5N_5$, $[M+H]^+$; 474.2711 found, 474.2708.

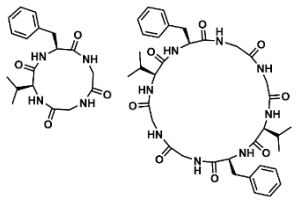
Macrocycle 131: UHPLC (*R*t = 14.17 min, PDA range: 190-400 nm). ^{1}H NMR (400 MHz, DMSO-d_6) δ



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), 7.26 (t, *J* = 7.5 Hz, 4H), 7.19 – 7.11 (m, 6H), 4.53 (q, *J* = 7.4 Hz, 2H), 4.43 (q, *J* = 7.9 Hz, 4H), 3.67 (dd, *J* = 20.2, 5.4 Hz, 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), 1.89 (dq, *J* = 14.9, 7.1 Hz, 4H), 1.54 – 1.43 (m, 2H), 1.19 – 1.07 (m, 2H), 0.84 – 0.73 (m, 24H). ^{13}C NMR (100 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,

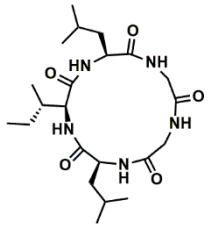
37.6, 36.8, 30.2, 24.3, 19.1, 18.5, 15.4, 11.0. HRMS (ESI+) *m/z* calcd for $C_{48}H_{71}O_{10}N_{10}$, $[M+H]^+$; 947.5355 found, 947.5345.

Macrocycles 132 and 133: TentaGel S RAM resin (380 mg, loading 0.26 mmol/g) was subjected to the



on-resin Ugi reaction with Fmoc-Phe-OH, paraformaldehyde and ethyl isocyanoacetate, followed by N-terminal deprotection and coupling of Fmoc-Val-OH according the general procedure for the multicomponent synthesis of resin bound peptides. The resulting resin-bound peptide was then deprotected at both termini and subjected to the on-resin macrocyclization procedure with HATU/DIPEA. After cleavage from the resin, a mixture of cyclic peptide **132** and the dimer **133** was obtained. Preparative RP-HPLC purification produced **132** (6.5 mg, 18%, *R*t = 11.72 min.) and **133** (28 mg, 39%, *R*t = 13.22 min). ^{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), 7.81 (q, *J* = 6.2, 4.7 Hz, 2H), 7.28 – 7.16 (m, 10H), 4.40 (ddd, *J* = 10.2, 7.5, 4.8 Hz, 2H), 3.92 (d, *J* = 6.7 Hz, 1H), 3.86 (ddd, *J* = 10.0, 5.9, 3.1 Hz, 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 (100 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* calcd for $C_{36}H_{49}O_8N_8$, $[M+H]^+$; 721.3668 found, 721.3662.

Macrocycle 134: TentaGel S RAM resin (380 mg, loading 0.26 mmol/g) was subjected to the on-resin Ugi



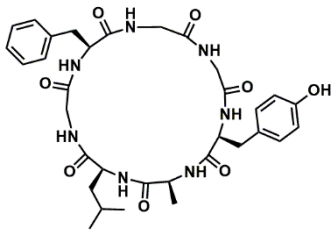
reaction with Fmoc-Leu-OH, paraformaldehyde and ethyl isocyanoacetate, followed by sequential couplings of Fmoc-Ile-OH and Fmoc-Leu-OH according the general procedure for the multicomponent synthesis of resin-bound peptides. The resulting resin-bound peptide was then deprotected at both termini and subjected to the on-resin macrocyclization procedure with HATU/DIPEA. After cleavage from the resin, cyclic peptide **134** was obtained in 78% purity as determined by UHPLC (PDA range: 190 – 400 nm). No dimer cyclopeptide was detected by UHPLC/ESI-MS. Preparative RP-HPLC purification rendered cyclic peptide **134** (21 mg, 47%) 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 8.64 (t, *J* = 6.0 Hz, 1H), 8.37 (d, *J* = 7.8 Hz, 1H), 8.06 (d, *J* = 8.0 Hz, 1H), 7.98 (d, *J* = 9.3 Hz, 1H), 7.59 (s, 1H), 6.64 (s, 1H), 4.33 – 4.24 (m, 1H), 4.15 (q, *J* = 7.3 Hz, 1H), 3.90 (t, *J* = 8.1 Hz, 1H), 3.84 – 3.63 (m, 4H), 3.57 – 3.49 (m, 2H), 1.76 – 1.65 (m, 2H), 1.57 – 1.38 (m, 6H), 1.16 – 1.06 (m, 2H), 0.92 – 0.78 (m, 12H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.6, 170.2, 169.2, 168.9, 59.6, 52.7, 51.9, 43.1, 42.9, 41.3, 39.5, 35.7, 24.9, 24.6, 24.6, 22.9, 22.6, 22.2, 21.7, 15.5, 10.9. HRMS (ESI+) *m/z* calcd for $C_{22}H_{40}N_5O_5$, $[M+H]^+$; 454.3029 found, 454.3024.

Macrocycle 135: TentaGel S RAM resin (380 mg, loading 0.26 mmol/g) was subjected to the on-resin Ugi reaction with Fmoc-Ile-OH, paraformaldehyde and CN-Phe-Gly-OAll, followed by sequential couplings of Fmoc-Val-OH and Fmoc-Tyr(Bzl)-OH according the general procedure. The resin-bound peptide is washed with DMF (4 × 2 mL) and the *C*-terminal allyl ester is cleaved by treatment with $Pd(PPh_3)_4$ (0.5 mmol) and $PhSiH_3$ (5.0 mmol) in dry CH_2Cl_2 under argon atmosphere at 25 °C. The peptide-bound resin is then washed with THF (3 × 2 mL), DMF (3 × 2 min), CH_2Cl_2 (3 × 2 min), and the Fmoc group is removed by treatment with a 20% piperidine in DMF (*ca.* 5 mL) during 30 min, followed by washings with DMF (3 × 1 min) and CH_2Cl_2 (3 × 1 min). The deprotected resin-bound peptide was then subjected to the on-resin macrocyclization procedure with HATU/DIPEA. After cleavage from the resin, cyclic peptide **135** was obtained in 78% purity as determined by UHPLC (PDA range: 190-400 nm). Preparative RP-HPLC purification produced **135** (37 mg, 51%) 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 (d, *J* = 1.4 Hz, 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), 1.95 (dh, *J* = 27.3, 7.1 Hz, 1H), 1.78 (m, 1H), 1.48 (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 (100 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* calcd for $C_{40}H_{51}O_7N_6$, $[M+H]^+$; 727.3814 found, 727.3810.

Macrocycle 136: TentaGel S RAM resin (380 mg, loading 0.26 mmol/g) was subjected to the on-resin Ugi reaction with Fmoc-Phe-OH, paraformaldehyde and ethyl isocyanoacetate, followed by sequential couplings of Fmoc-Gly-OH, Fmoc-Val-OH and Fmoc-Ile-OH according the general procedure for the multicomponent synthesis of resin-bound peptides. The resulting resin-bound peptide was then deprotected at both termini and subjected to the on-resin macrocyclization procedure with HATU/DIPEA. After cleavage from the resin, cyclic peptide **136** was obtained in 80% purity as determined by UHPLC (PDA range: 190-400 nm). Preparative RP-HPLC purification produced **136** (28 mg, 61%) 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), 4.47 (m, 1H), 4.13 – 4.07 (m, 1H), 3.99 – 3.92 (m, 1H), 3.84 (dt, *J* = 15.8,

3.8 Hz, 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 (m, 1H), 1.80 – 1.71 (m, 1H), 1.32 (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 (100 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* calcd for $C_{26}H_{39}O_6N_6$, $[M+H]^+$; 531.2926 found, 531.2922.

Macrocycle 141: TentaGel S RAM resin (380 mg, loading 0.26 mmol/g) was subjected to the on-resin Ugi

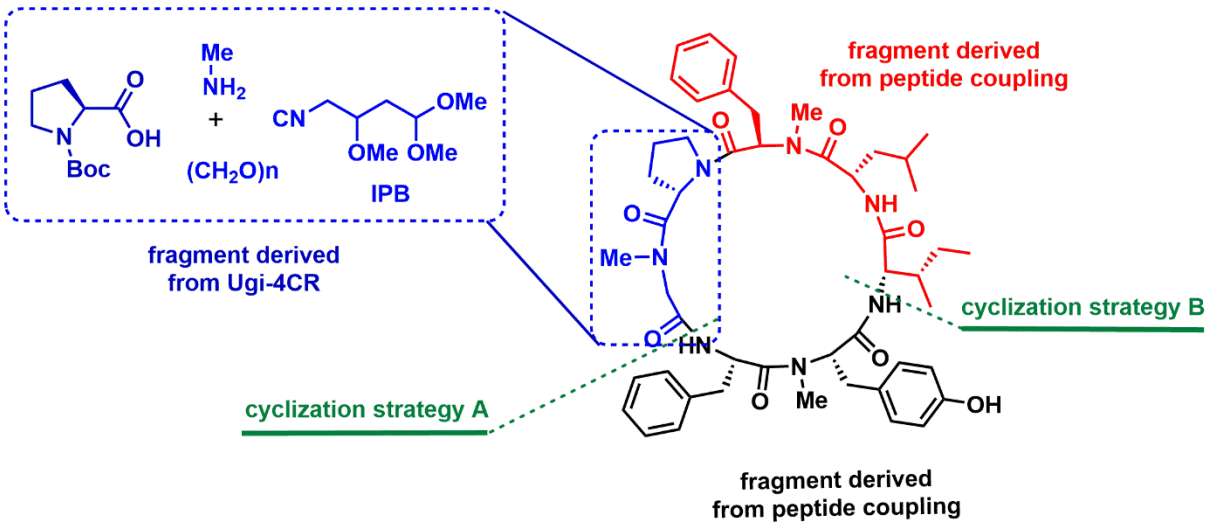


reaction with Fmoc-Phe-OH, paraformaldehyde and ethyl isocyanoacetate, followed by sequential couplings of Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Ala-OH and Fmoc-Tyr(*t*Bu)-OH according the general procedure for the multicomponent synthesis of resin-bound peptides. The resulting resin-bound peptide was then deprotected at both termini and subjected to the on-resin macrocyclization procedure with HATU/DIPEA. After cleavage from the resin, cyclic peptide **141** was obtained in 82% purity as determined by UHPLC (PDA range: 190-400 nm). Preparative RP-HPLC purification the produced natural product Crassipin B (**141**, 35 mg, 53%) in 99% purity, according to UHPLC (*R*t = 12.48 min, PDA range: 190-400 nm). ^{1}H NMR (400 MHz, DMSO-d_6): 10.05 (s, 1H), 9.45-9.38 (t, *J* = 4.0 Hz, 1H), 9.32 (t, *J* = 4.0 Hz, 1H), 9.27 (d, *J* = 5.1 Hz, 1H), 9.05 (br. s, 1H), 8.14 – 7.97 (m, 5H), 7.86 (d, *J* = 8.5, 2H), 7.47 (d, *J* = 8.5 Hz, 2H), 5.31 – 5.21 (m, 2H), 5.09 – 5.01 (m, 2H), 4.56 – 4.48 (m, 1H), 4.36 – 4.32 (m, 1H), 3.88 – 3.78 (m, 1H), 3.83 (m, 1H), 3.78 – 3.62 (m, 2H), 3.61 – 3.52 (m, 1H), 2.96 (m, 3H), 2.48 – 2.22 (m, 5H), 2.14 (d, *J* = 6.9 Hz, 3H), 2.01 (m, 1H), 1.74 (d, *J* = 6.1 Hz, 3H),1.68 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): 172.8, 172.6, 171.0, 170.7, 169.6, 169.5, 168.9, 155.8, 137.4, 129.9, 129.12, 128.2, 126.5, 115.0, 55.5, 55.4, 52.9, 48.1, 43.0, 42.2, 24.2, 22.7, 22.1, 17.8. HRMS (ESI+) *m/z* calcd for $C_{35}H_{47}N_8O_9$, $[M+H]^+$; 723.3251 found, 723.3247.

Chapter 3

Total Synthesis of Cordyheptapeptide A

Abstract*



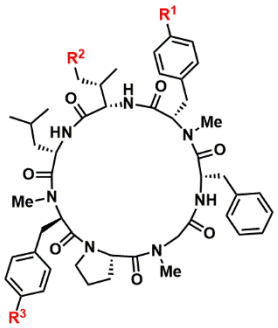
The first convergent total synthesis of Cordyheptapeptide A is reported. The strategy included the rational combination of peptide coupling and the Ugi-4CR for the assembly of the linear peptide and a cyclization study to assess the most suitable peptide bond for undertaking the macrocyclic-ring-closing step. The use of Ugi-4CR involved the special convertible isocyanide IPB for surrogating two peptide couplings in a single step. This synthetic strategy allowed to prepare the main molecular building blocks and the final natural product on a multi-gram scale.

* This chapter was published: Alfredo R. Puentes**, Ricardo N. Filho, Daniel G. Rivera, and Ludger A. Wessjohann. *Synlett.* **2017**; *28* (15), 1971-1974

** Own contribution: Total synthesis of Cordyheptapeptide A.

3.1 Introduction

The cordyheptapeptides are members of a macrocyclicpeptide family, classified from A to E, which were isolated from different fungi strains of both terrestrial and marine origin (**Figure 3.1**).[239,240]



Cordyhept	R^1	R^2	R^3
A	OH	Me	H
B	H	Me	H
C	OH	H	H
D	OH	H	OH
E	OH	Me	OH

Figure 3.1 Cordyheptapeptides family A-E.

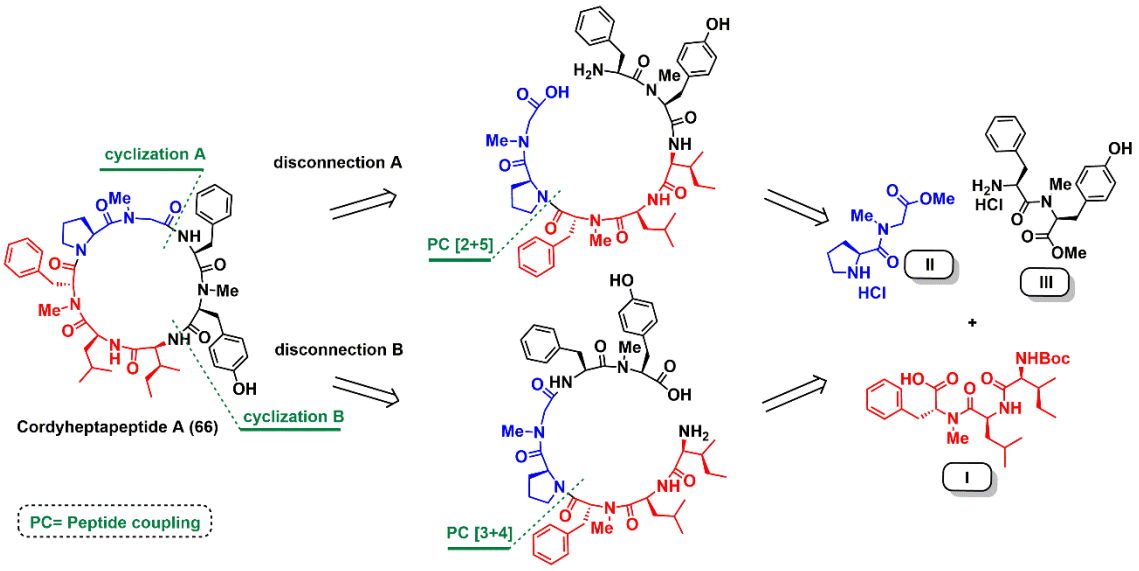
The cordyheptapeptides C to E were isolated from the marine fungus *Acremonium persicinum* and were tested in cytotoxicity assays against various tumor cell lines such as human glioblastoma, breast cancer and lung cancer. Cordyheptapeptides C and E showed significant cytotoxicity against the indicated tumour cell lines, whereas the more polar D macrocycle showed no activity at all.

Cordyheptapeptides A and B, on the other hand, were isolated from fungi of the genus *Cordyceps*, which are pathogens of different insects. In particular, the cordyheptapeptide A exhibits a weak toxicity against healthy Vero cells (IC_{50} > 56.88 μM) and high cytotoxicity against several cancer cell lines, including oral human epidermoid carcinoma (KB, IC_{50} = 0.78 μM), breast carcinoma (BC, IC_{50} = 0.20 μM), and human small cell lung cancer (NCI-H187, IC_{50} = 0.20 μM).[239,241] The potent and selective cytotoxicity profile of cordyheptapeptide A makes it an interesting target for the development of a total synthesis methodology capable of providing not only the natural compound but also varied analogues. The lack of appropriate amounts of isolated materials from natural sources to permit the enhancement of anticancer assays, makes it necessary to develop an efficient and milder synthesis of cordyheptapeptide A.

3.2 Synthesis design

As the **Scheme 3.1** depicted, the retrosynthetic strategy includes disconnection A and B, which comprise the cyclization between Sar and Phe – to avoid epimerization issues – and between Tyr and Ile, respectively. Whereas the latter conveys the risk of Cα-racemization at Tyr, it is opposed to an internal β-turn centered at the *N*-Me-D-Phe-Pro moiety, a fact that should facilitate the macrolactamization. Further disconnections of the two acyclic precursors lead to the key intermediates **I** – **III**. All the key intermediates were planned to be assembled via peptide couplings comprising smaller building blocks, of which the fragment II was to be obtained by Ugi-4CR. We devised the utilization of the Ugi-4CR to construct the fragment II, thus aimin

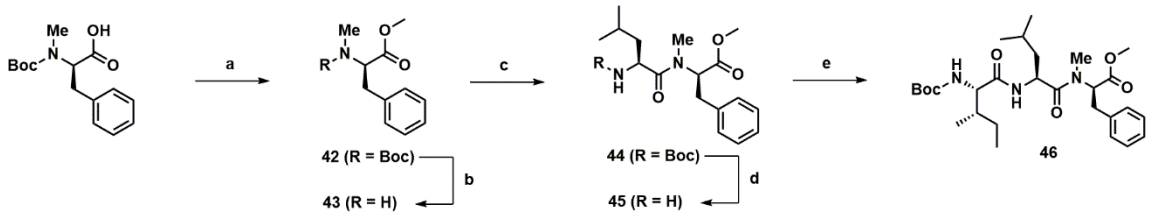
to seeking a more versatile approach to target *N*-alkylated peptide backbones. The interest on utilizing this approach lies in the possibility of introducing not only an *N*-methyl substituent[194,242–244] at a selected amide bond, but also varied *N*-substituents aiming to produce natural product analogues.



Scheme 3.1 Retrosynthetic approach of cordyheptapeptide A.

3.3 Synthesis of building block I

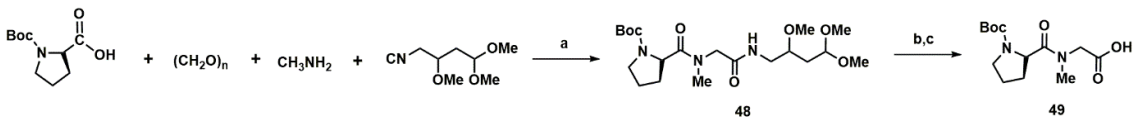
Synthesis of building block I started with the coupling of Boc-Leu-OH and H-D-*N*Me-Phe-OMe by treatment with HATU and DIPEA in DMF to afford **42** in 91% yield (**Scheme 3.2**). Removal of the Boc group from dipeptide **44** with TFA in dichloromethane, followed by treatment with Boc-Ile-OH, coupling agent HBTU, DIPEA in DMF, allowed to afford tripeptide **46** in 88% yield. It should be noted that no significant differences were detected during the optimization process of the coupling reaction in which HBTU and HATU were used, since they belong to the same series of coupling reagents (uronium salt).



Scheme 3.2 Reagents and conditions: a) MeI, K_2CO_3, DMF, r.t., 24 h, quant. b) 30% TFA, CH_2Cl_2, r.t., 4 h, quant. c) Boc-Leu-OH, HATU, DIPEA, DMF, r.t., 24 h, 76%. d) 30% TFA, CH_2Cl_2, r.t., 4 h, quant. e) **45**, Boc-Ile-OH, HBTU, DIPEA, DMF, r.t., 24 h, 98%.

3.4 Synthesis of building block II

Synthesis of dipeptide **49** started with the Ugi-4CR of methylamine, paraformaldehyde, Boc-Pro-OH, and the convertible isonitrile IPB (**Scheme 3.3**) to afford the intermediate **48** in 80% yield. Using a convertible isocyanide in this protocol was necessary in order to generate a reactive secondary amide, which may be transformed into an ester moiety at the *C*-terminal.

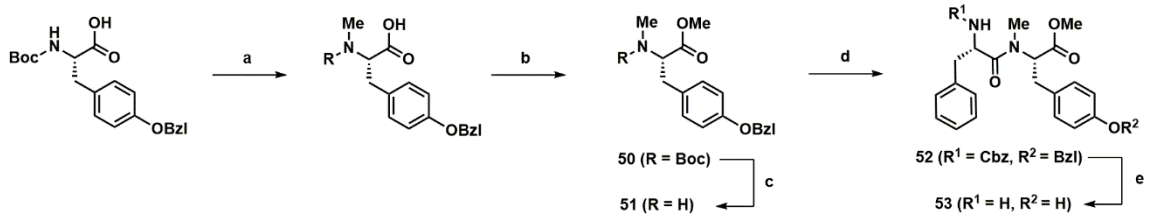


Scheme 3.3 Reagents and conditions: a) MeOH, r.t., 80%. b) 10-camphorsulfonic acid, quinoline, toluene, reflux, 30 min, 80%. c) $LiOH.H_2O$, THF/H_2O (1:1), 77% (overall yield).

Although other convertible isocyanides might have been employed in this step,[191,212,227,229,245–250] isocyanide IPB[230] was selected because of its easy preparation and mild conversion conditions. Ugi product **48** was treated under mild acid conditions to obtain the *N*-acylpyrrole, which was subsequently transformed into the carboxy group of *C*-terminal dipeptide **49**, and proceeded in a good 49% overall yield over three steps.

3.5 Synthesis of building block III

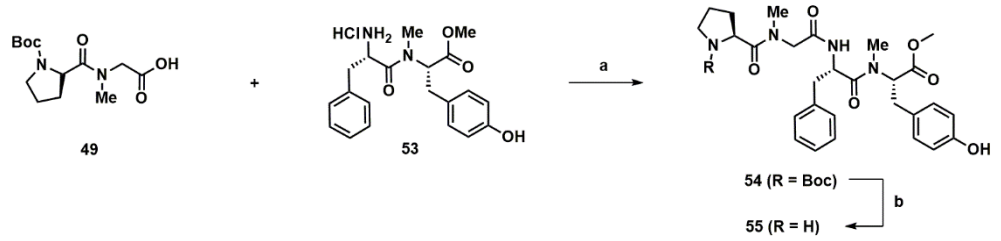
Synthesis of fragment **III** started with the exhaustive methylation of protected amino acid Boc-Tyr-OH through protocols MeI, NaH, in THF and later under MeI, K_2CO_3 in DMF condition to afford completely protected amino acid **50**, in almost quantitative yield (**Scheme 3.4**). Deprotection of Boc-group under mild acidic conditions gave the salt **51** in quantitative yield. Convenctional peptide coupling of **51** and Cbz-Phe-OH using PyBroP as coupling reagent with DIPEA in dichloromethane produces dipeptide **52** in 78% yield. Once the *N*-methylated dipeptide **52** was obtained, deprotection of the benzyl (Bzl) and benzyloxycarbonyl (Cbz) groups were carried out by hydrogenolysis in acidic media, in order to avoid any formation of unwanted 1,5-diketopiperazine (DKP) by-product.



Scheme 3.4 Reagents and conditions: a) MeI, NaH, THF, 0 °C, quant. b) MeI, K_2CO_3, DMF, 0 °C, quant. c) 30% TFA, CH_2Cl_2, r.t., 4 h, quant. d) Cbz-Phe-OH, PyBroP, DIPEA, CH_2Cl_2, r.t., 12 h, 94%. e) H_2, Pd / C (10% by weight), MeOH, 4M HCl in 1,4-dioxane, r.t., 30 min, quant.

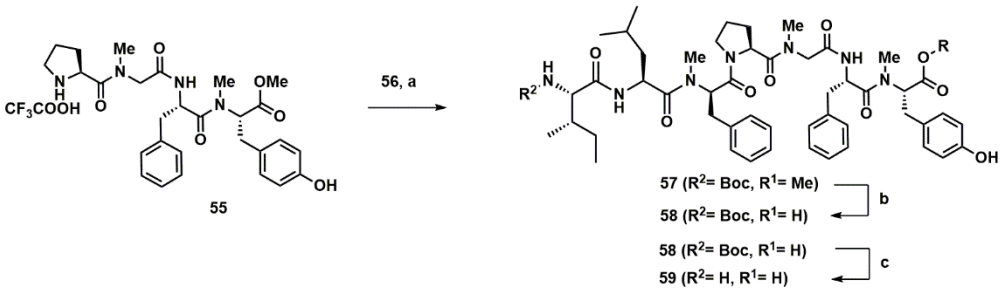
3.6 Completion of total synthesis of Cordyheptapeptide A

Completion of the synthesis started by merging fragments **I** and **II**, employing the HATU coupling protocol in 70% yield. During the coupling reaction, no by-products were detected in the reaction between the free tyrosine hydroxyl group with the HATU-activated ester of intermediate **49**. This result can be explained by the presence of the electronic withdrawing effect of the aromatic ring over the phenolic hydroxyl group reducing its nucleophilic character. Therefore, the selected protecting group combination for dipeptide **53** did not need to be orthogonal respect to the Cbz-Phe- and the hydroxyl group (**Scheme 3.5**). In order to improve the preparation of intermediate **54** the highly activated *N*-acyl pyrrole derived from **48** was directly coupled with **53**. To our disappointment, the reaction was completely unsuccessful.



Scheme 3.5 Reagents and conditions: a) HATU, DIPEA, DMF, r.t., 24 h, 82%. b) 30% TFA, CH_2Cl_2, r.t, 4 h, quant.

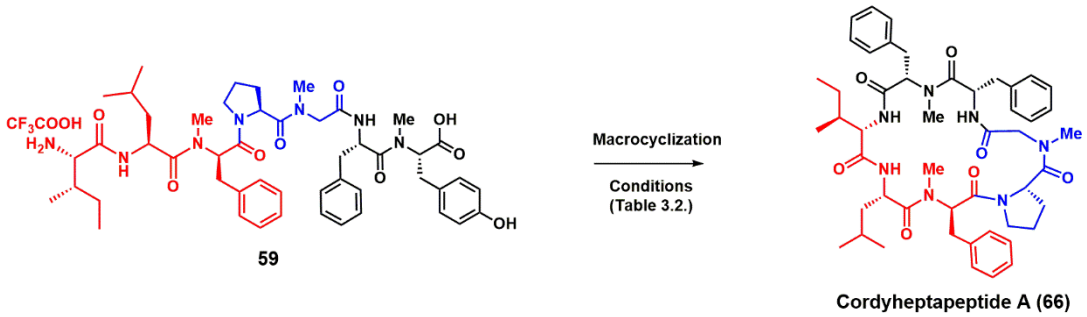
The synthesis of heptapeptide **57** *en route* to the advanced intermediate **59**, begins with the coupling of Boc-deprotected tetrapeptide **55** to **56** by treatment with HATU and DIPEA in DMF to afford **57** in quasi-quantitative yield (**Scheme 3.6**). The acyclic peptide **57** was subjected to the sequential deprotection of the *C*- and *N*- termini to afford the crude product **59** in 86% yield.



Scheme 3.6 a) HATU, DIPEA, DMF, r.t., 24 h, quant. b) $LiOH.H_2O$, THF/H_2O (1:1). c) 30% TFA, CH_2Cl_2, r.t, 4 h, quant.

Prior completing the final cyclization step (**Scheme 3.7**), the ring closure conditions were investigated. The results of this study is presented in **table 3.1**. The HPLC analysis of the crude reaction exhibit that only the conditions derived from protocols EDCl / HOBt / CH_2Cl_2 (reaction A), HATU / HOAt / DIPEA / MeCN (reaction C) and DEPBT / DIPEA / THF (reaction D) generate the desired macrocycle. Of all reaction

conditions, the mixture of HATU / HOAt / DIPEA produced the best results, yielding the cyclopeptide in 97% conversion with less than 2% epimerization (**Table 3.1**).



Scheme 3.7 Macrocyclization optimization study in the total synthesis of **66.**

Table 3.1 Reaction conditions studied for the cyclization of peptide **59**.

Reaction label	Reagents / Solvent[a]	Conversion rate (%)	Epimerization[b] (%)
A	EDCI/HOBt/CH_2Cl_2	73	17
B	PyBroP/DIPEA/CH_2Cl_2	no reaction	-
C	HATU/HOAt/DIPEA/MeCN	97	2
D	DEPBT/DIPEA/THF	1	-
E	T3P®/DMAP/DIPEA/CH_2Cl_2	no reaction	-
F	Cyclohexyisocyanide/HOBt/CH_2Cl_2	no reaction	-

a) Reaction conditions: 0,2 mM (**59**), 72 h and room temperature. b) Determined by HPLC/NMR.

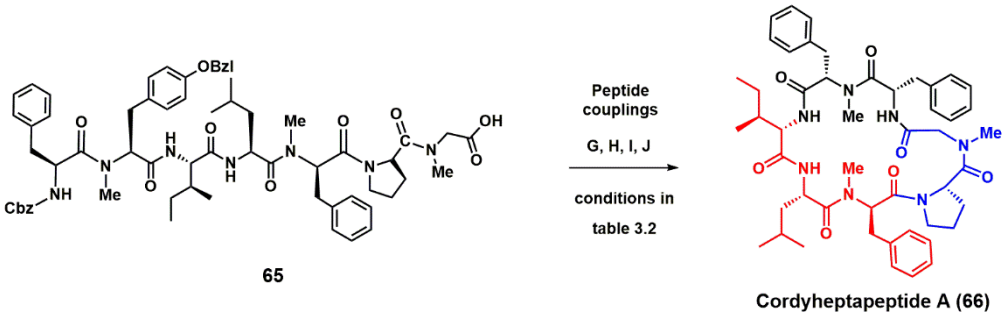
In 1992, Kopple and co-workers[251] demonstrated that the sequence D-Pro-L-Pro induce a strong preference for a type-II' β-turn formation, which can be used to generate a more rigid conformation in cyclopeptides. Therefore, the D-Phe-Pro residue was strategically locaded in the acyclic intermediate **59** halfway between amino and carboxylic acid termini. This position allows the two reacting sites, to stay most of the time in a bent conformation bringing the ends in close proximity. Consequently, cyclization processes are favoured over dimerization. From all the coupling reagents studied, only the HATU protocol consumed all the acyclic starting material, therefore it was the selected protocol to perform the final cyclation. In this way the protocol of HATU, HOAt and DIPEA in MeCN, was used at 1 mM, room temperature, over 3 days to afford cordyheptapeptide A in 86% isolated yield, without dimerization. In order to increase the amount of the natural product the former reaction was up scaled. Surprisingly, the up scaled reaction was completed

in 15 minutes, without dimerization. Additionally, the ESI-MS analysis of the cyclization step evidenced the gradual disappearance of the starting material and the appearance of the $[M+Na]^+$ fragment peak of the cyclo peptide product.

3.7 Study of the cyclization position

In order to improve the macrocyclic-ring-closing step a different cyclization position was studied. The position comprises the cyclation between Sar and Phe, - to avoid the risk of the epimerization at the *C*-terminus-. In contrast to the previous section, the positions for the cyclization are not equidistant from the proline inducer. Therefore, the results presented in **table 3.2** indicate that all the evaluated protocols generate very low yield of macrocycle. These results are probably due to the lack of turn inducer assistance of the Pro in the linear peptide, which means that the two reacting sites are not close enough to interact effectively (**Scheme 3.8**). The cyclization experiments reinforce the initial idea that the best position to cyclize cordyheptapeptide A is having reactive ends equidistant to the D-Phe-Pro moiety.

Finally, the ^{1}H-NMR and ^{13}C spectroscopic information of cordyheptapeptide A (**66**) are compatible to the isolated natural product reported by Rukachaisirikul and co-workers.[239]



Scheme 3.8 Optimization experiment of peptide **65** cyclation.

Table 3.2 Reaction conditions for the study of the step of macrocyclation of the peptide **65**.

rute	condition	obtained mass (mg)	yield (%)
G	1.DMAP/EDC/PFP/DIPEA/ CH_2Cl_2 2. H_2 Pd/C (20% w/w)	3	7
H	1. H_2 Pd/C (20% w/w) 2. HBTU/DIPEA/DMF	1	2
I	DMAP/T3P®/ CH_2Cl_2	Non-isolable quantity	-
J	EDCI/HOBt/DIPEA/ CH_2Cl_2	Non-isolable quantity	-

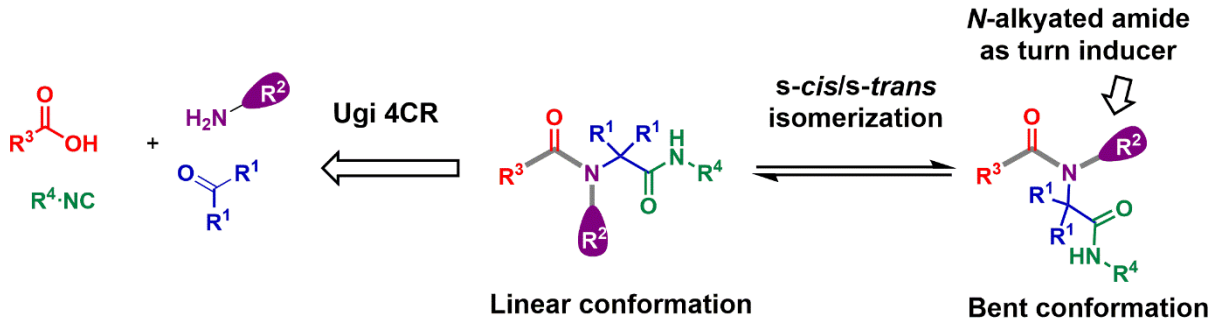
3.8 Summary

In summary, the first total synthesis to cordyheptapeptide A was successfully achieved in 30% overall yield. The convergent approach featured a rational combination of peptide coupling and the Ugi-4CR employing a convertible isonitrile for the gram-scale preparation of the main building blocks **I**, **II** and **III**. Using such multicomponent processes seems to be promising for the future creation of a library of cordyheptapeptide analogues by a variation of the Ugi components, thus enabling a structure-activity relationship analysis. The conformation of the acyclic precursor turned out to be crucial to the efficiency of the macrocyclization, thus if correctly positioned opposite and equidistant to the internal β-turn.

Chapter 4

Studies on the s-*cis*/s-*trans* Isomerism for Peptidic Model Compounds Featuring *N*-Alkylated Ugi Reaction Fragments in Solution-Phase

Abstract*



The existence of similar energy between *Z* and *E* isomers around the amide bond has been intensively investigated in order to reach a better understanding of the molecular mechanism of their isomerization in chemistry and biology. Through ^{1}H and ^{13}C NMR spectroscopy, the s-*cis*/s-*trans* isomerization (CTI) with two different *N*-alkylated tertiary amide models were investigated. The *N*-alkylated peptides were prepared through the Ugi four-component reaction as a general one-pot multicomponent approach by connecting peptide carboxylic acids and peptide isocyanides in the presence of different amides and oxo- components to form a new linked *N*-alkyl-amino acid moiety.

* This chapter will be published: Alfredo R. Puentes**, Aldrin V. Vidal, Celia G. Moya, Carlos S. Perez. Martinez, Daniel G. Rivera, and Ludger A. Wessjohann. *Manuscript in preparation.*

** Own contribution: Installation of *N*-alkyl moieties in peptides and study of their effect in the s-*cis*/s-*trans* isomerization.

4.1 Introduction

The architecture of the tertiary amide skeleton in peptoids makes them incapable of stabilizing putative folded structures by the formation of intramolecular hydrogen bonds. Several authors had thoroughly studied the impact of different functionalities of the *N*-alkyl side chain on this s-*cis*/s-*trans* equilibrium in peptoids.[252–255] Although the inherent flexibility of the peptoids, the secondary structures of the linear and cyclic peptoids have been studied in detail by NMR and X-ray crystallography,[256–259] and some requirements for the formation have been identified of secondary peptoid structure.[258,260,261] To acquire experimental information about the folding propensity of *N*-substituted peptides we decided to prepare a serie of peptidic models and evaluate the influence of s-*cis*/s-*trans* amide bond isomerization by NMR.

There are several methods to prepare *N*-alkylated compounds but the most widely used are the direct *N*-alkylation of *N*α-protected amino acids or α-amino esters[262–273] and the 5-oxazolidinone formation with reduction.[274–282] Also, exist other methods that involve reductive amination,[283–286] Mitsunobu reaction,[287,288] Diels-Alder/retro-Diels-Alder sequences[289] and unconventional procedures.[290–295] Unfortunately, most of these methods are limited to aliphatic amino acids and are characterized by harsh reaction conditions or long synthetic sequences, causing an unpleasant partial racemization of the substrate.[269,284,296,297] Conversely, the Ugi-4CR is an amenable multicomponent process to assemble peptidic backbones with at least one *N*-alkyl or *N*-aryl peptide bond in only one single operation. Therefore, the Ugi-4CR was used in this work to synthesize the differents *N*-alkylated peptides required to study the amide s-*cis*/s-*trans* isomerization (CTI).

4.2 Synthetic design

In order to simplify the CTI exploration we limited the study to two series of *N*-alkylated model compounds. As shown in **figure 4.1** the two constructed models differ in size and in the chemical environment around the amide bond studied. On the other hand, the formation of the stereoisomer during the Ugi-4CR was evaded by employing acetone or paraformaldehyde as the oxo- component.

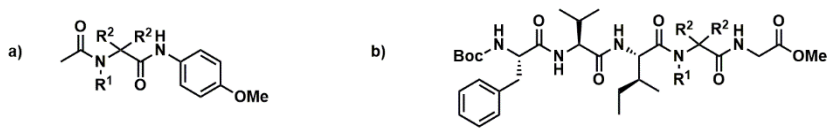
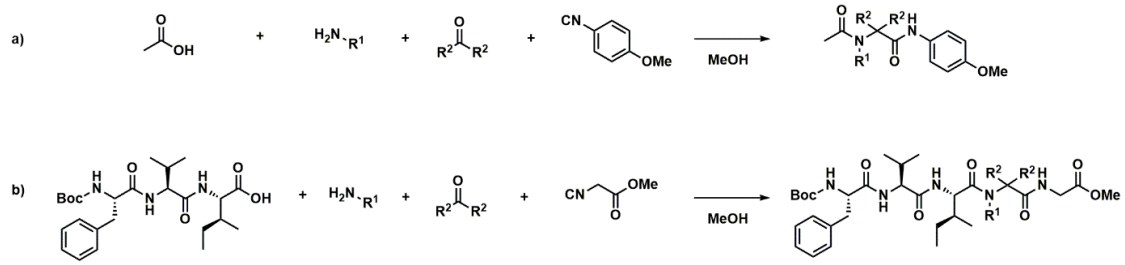


Figure 4.1 Generic structures of model compounds for the study of the s-*cis*/s-*trans* amide isomerization of the terciary amides. a) small constrained model dipeptide (I); b) model pentapeptide (II).

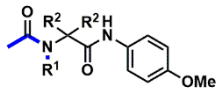
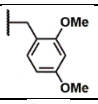
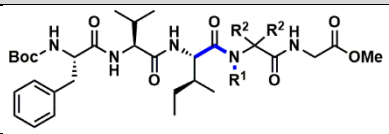
Scheme 4.1 indicate the synthetic approach to obtain the two series for structural model compounds mentioned. Model I series was synthesized by mixing acetic acid, *p*-methoxyphenylisocyanide and different congested amines; also combined with acetone or paraformaldehyde as the oxo- component of the Ugi-

4CR. Similarly, the structural model II series was built using the same amine and oxo- components as in model I, but varying the acidic and isocyanide components using larger units. The model I series was designed for NMR signal simplification, in order to clearly measure and evaluate the CTI process. Therefore, compounds members of Model I have an unambiguous aromatic pattern, acetate and hydrogen amide signal, which appear in their characteristic NMR regions. On the other hand, model II was planned to evaluate the role of the side chain steric hindrance in promoting the cis conformer in a pentapeptide backbone. In that sense **table 4.1** depicts the different substituents on the α carbon and the nitrogen of the amide bond, studied in both structural models.



Scheme 4.1 General Ugi-4CR scheme for the synthesis of the different *N*-alkylated compounds. a) synthesis of small *N*-alkylated compounds; b) synthesis of large *N*-alkylated peptidic compounds.

Table 4.1 Structure of the peptidic model system and the structure of the amine and carbonyl component examined in this study.

Model I								
R^1								
R^2								
	(**67**)	(**68**)	(**69**)	(**70**)	(**71**)	(**72**)	(**73**)	(**74**)
Yield	60%	50%	21%	40%	65%	98%	70%	75%
Model II								
R^1											
R^2											
	(**75**)	(**76**)	(**77**)	(**78**)	(**79**)	(**80**)	(**81**)	(**82**)	(**83**)	(**84**)	(**85**)
Yield	16%	5%	90%	8%	30%	24%	20%	20%	25%	10%	8%

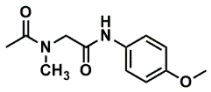
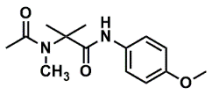
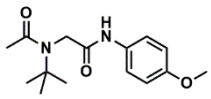
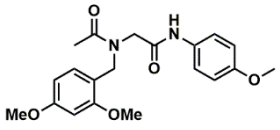
4.3 NMR assignment and kinetics of the s-*cis* / s-*trans* isomerization

As the previous **scheme 4.1a** depicted the expected model I series was synthetized using the Ugi-4CR. in technical grade methanol at room temperature. Imine preformation was performed overnight, and the acid and isocyanide component were added on the next day. All dipeptides (**67**-**74**) were obtained in yields ranging from 40% to 98% (**Table 4.1**).

The peptide analysis by NMR were used to determine the s-*cis*/s-*trans* distribution present in solution phase, since the CTI is usually rather slow on the NMR time scale. As mentioned before, one of the consequences of the *N*-substitution of the amide bond is that the population of the s-*cis* conformation increases compared to non alkylated peptides. Therefore, the NMR analysis allowed to observe the presence of both conformers in most of the samples.

The distribution of s-*cis* and s-*trans* conformers of model I compounds is shown in **table 4.2**. As expected the, 1D NMR analysis indicated that the highly congested Aib-derivate **68** only display the presence of the s-*trans* conformer, while the *N*-*tert*-butylated glycine derivative **72** produced the opposite result, generating only the s-*cis* conformer. On the other hand, the less bulky *N*-Me derivative **67** generated lower amount of s-*cis* conformer. Special interest evoked compound **74** since the ratio of the conformers was almost 1:1, i.e. the *N*-benzylated sample induced a higher proportion of s-*cis* conformer in comparison to *N*-methylated sample **67**. This result is probably related to the significant steric bulkiness but also because of the presence of the aromatic ring. Blackwell and co-workers[253] established that the presence of an aromatic ring in the peptidic chain can stabilize the s-*cis* configuration through carbonyl-aromatic orbital n→π* interactions.

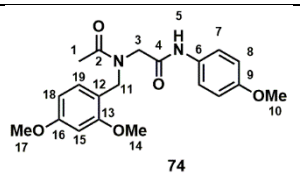
Table 4.2 Percentage of conformers s-*cis* and s-*trans* of compounds series I.

	Compound	% *s-cis*	% *s-trans*
	**67**	38.8	61.2
	**68**	-	100
	**72**	100	-
	**74**	42.5	54.5

The procedure used for the assignment of the ^{1}H and ^{13}C-NMR spectra of each of the confomers was based on the identification of the signal sets taking into account the integration and the peaks in the TOCSY, COSY, HSQC and HMBC spectra. The ROESY spectrum allowed to assign the sets of values

corresponding to each conformer. As shown in **figure 4.2**, the ROESY spectrum display a cross peak between the backbone of the peptoid and the CH_3 of the acetyl group. According to the **figure 4.3**, this result can be firmly assigned to the s-*cis* isomer. To better illustrate the procedure followed, the assignment of the ^{1}H and ^{13}C spectra is shown for compound **74** (**Table 4.3**).

Table 4.3 NMR assignments for the s-*cis* and s-*trans* conformer of compound **74.**

Atoms label	 74			
	s-*cis*		**s-*trans***	
	^{1}H (ppm)	**^{13}C (ppm)**	**^{1}H (ppm)**	**^{13}C (ppm)**
CH_3 (1)	2.02	21.3	2.11	20.9
CO (2)	-	170.8	-	170.5
CH_2 (3)	4.05	50.6	3.92	47.6
CO (4)	-	166.0	-	166.0
NH (5)	9.85	-	9.68	-
C (6)	-	131.0	-	132.0
CH (7)	7.47	120.0	7.45	120.0
CH (8)	6.88	113.8	6.87	113.7
C (9)	-	155.2	-	155.1
CH_3 (10)	3.71	55.1	3.71	55.1
CH_2 (11)	4.36	43.2	4.47	47.6
C (12)	-	117.1	-	116.7
C (13)	-	158.1	-	158.1
CH_3 (14)	3.73	55.4	3.77	55.4
CH (15)	6.53	97.7	6.59	98.3
C (16)	-	159.8	-	160.3
CH_3 (17)	3.73	55.2	3.75	55.2
CH (18)	6.48	104.3	6.52	104.2
CH (19)	7.04	129.0	7.07	128.9

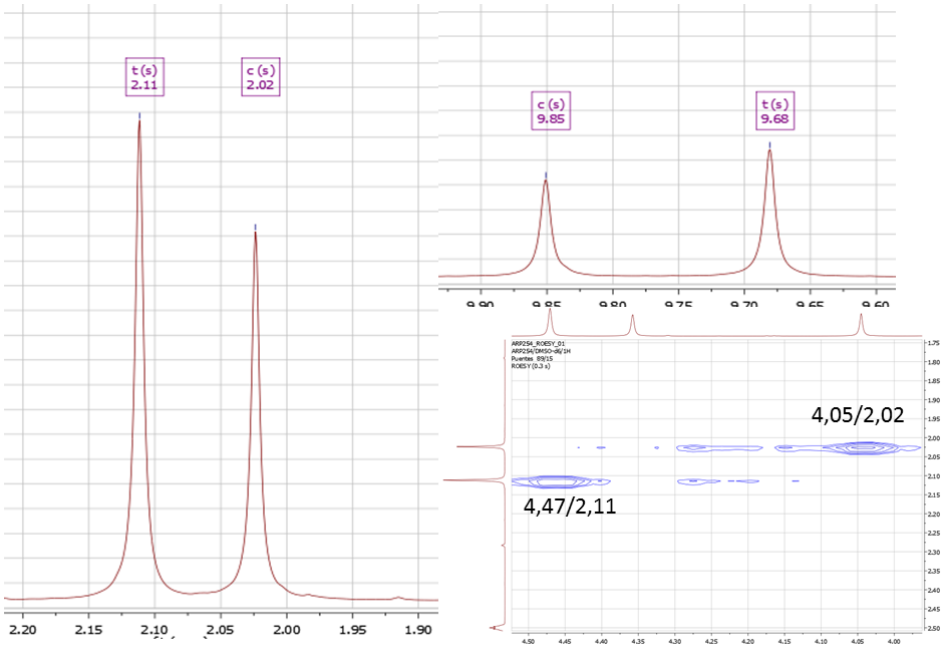


Figure 4.2 ^{1}H-NMR and ROESY region for compound **74**. Spectral regions from 1.90 to 2.20 and 9.60 to 9.90 related to H1 and H5 (NH) respectively.

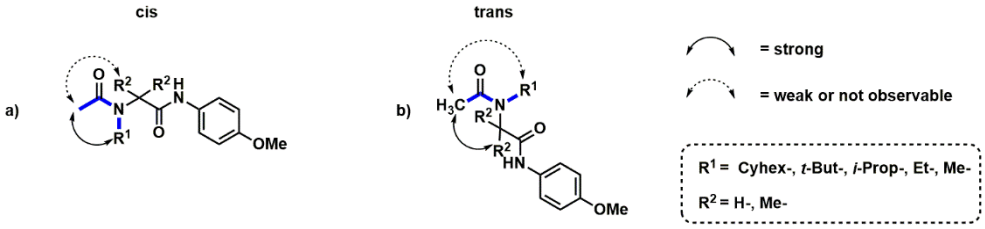


Figure 4.3 NOE contacts expected for the different conformation of the amide bond.

Since compound **74** was almost 50% of its time in s-*cis* conformation, it was studied in detail by 2D-NMR in order to quantify the CTI process. **Table 4.3** shows the chemical shifts of the two dominant conformational isomers of **74**, which were assigned by TOCSY, COSY, HSQC and HMBC spectral analyses. For example, the ROESY spectrum revealed a cross peak between CH_3 (1) and CH_2 (3), corresponding to the s-*cis* isomer, as well as a cross-point between CH_3 (1) and CH_2 (11) for the s-*trans* isomer.

The percentage of s-*cis* and s-*trans* conformers for the compounds of model series II are shown in **table 4.4**. Similar to the results obtained for model I, the 1D NMR analysis indicated that the highly congested compound **79** generated only the s-*cis* conformer, while the *N*-benzylated and *N*-methylated samples (**76** and **81**) produced a mix of conformers.

Table 4.4 Percentage of *s-cis* and s-*trans* conformers of model compounds of series II

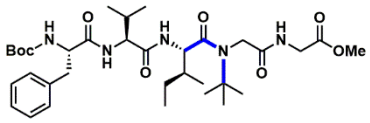
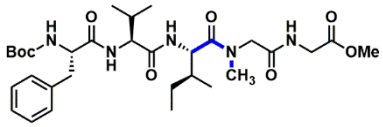
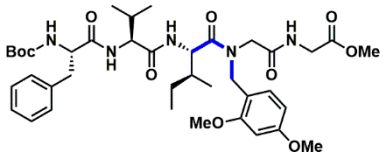
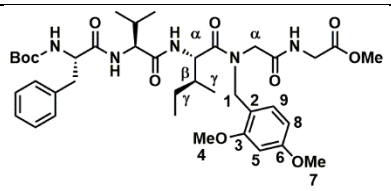
	Compound	% *s-cis*	% *s-trans*
	**79**	100	0
	**76**	30	70
	**81**	47	53

Table 4.5 shows the 2D NMR assignment of the spectral signals for the determination of the s-*cis* and s-*trans* isomer of pentapeptide **81**. The NOE contacts between the aromatic proton 9 at 7.09 ppm with the gamma proton of the isoleucine at 4.81 ppm endorses this isomer to the s-*trans*. On the other hand, the s-*cis* isomer did not display any spatial contact between the methoxylated aromatic ring with the side chain of isoleucine.

Table 4.5 NMR assignment for conformer s-*cis* and s-*trans* of **81** compound



s-*trans* conformer						
Group	***H*N signa**	**^{1}H/^{13}C NMR signal**				
		H_α/C_α	**H_β/C_β**	**H_γ/C_γ**	**C=O**	**Others**
Boc		1.28/27.7	-	-	159.7	
Phe	7.00	4.19/54.4	2.74/2.93/36.7	7.25, 7.26, 7.17/ 129.		
Val	7.77	4.31/56.63	1.98/30.7	0.85/18.8	170.5	
Ile	8.03	4.81/52.0	1.79/36.5	0.79/14.9(CH_3) 1.04/23.5 (CH_2)	171.6	
*N*Gly	-	3.73/4.57/48.6			168.6	
Gly	8.44	3.87/40.6			170.8	3.64/ 51.35 (OMe)
		H or C				
CH_2 (1)	-	4.57,4.46/ 35.9	-	-	-	-
C_{Ar} (2)	-	116.4	-	-	-	-
C_{Ar} (3)	-	158.2	-	-	-	-
MeO (4)	-	3.76/54.9	-	-	-	-
CH_{Ar} (5)	-	6.57/97.9	-	-	-	-
C_{Ar} (6)	-	160.3	-	-	-	-

MeO (7)	-	3.75/54.8	-	-	-	-
CH_{Ar} (8)	-	6.49/104.1	-	-	-	-
CH_{Ar} (9)	-	7.09/129.3	-	-	-	-
s-*cis* conformer						
Group	***H*N signa**	**$^{1}H/^{13}C$ NMR signal**				
		H_α/C_α	**H_β/C_β**	**H_γ/C_γ**	**C=O**	**Others**
Boc		1.28/27.7	-	-	159.7	
Phe	7.00	4.19/54.4	2.74/2.93/36.7	7.25, 7.26, 7.17/ 129.	-	
Val	7.68	4.29/56.6	1.93/30.6	0.81/18.8	170.7	
Ile	8.26	4.4/52.28	1.84/35.4	1.01(CH_2)/23.6 0.8/17.5(CH_3)	171.7	
*N*Gly	-	3.93/3.75/46.3			168.23	
Gly	8.14	3.85/40.5			170.8	3.64/ 51.35 (OMe)
		H or C				
CH_2 (1)	-	4.04, 4.66/43.2	-	-	-	-
C_{Ar} (2)	-	116.35	-	-	-	-
C_{Ar} (3)	-	158.1	-	-	-	-
MeO (4)	-	3.76/54.9	-	-	-	-
CH_{Ar} (5)	-	6.54/97.8	-	-	-	-
C_{Ar} (6)	-	159.7	-	-	-	-
MeO (7)	-	3.73/54.7	-	-	-	-
CH_{Ar} (8)	-	6.37/103.9	-	-	-	-
CH_{Ar} (9)	-	6.9/128.3	-	-	-	-

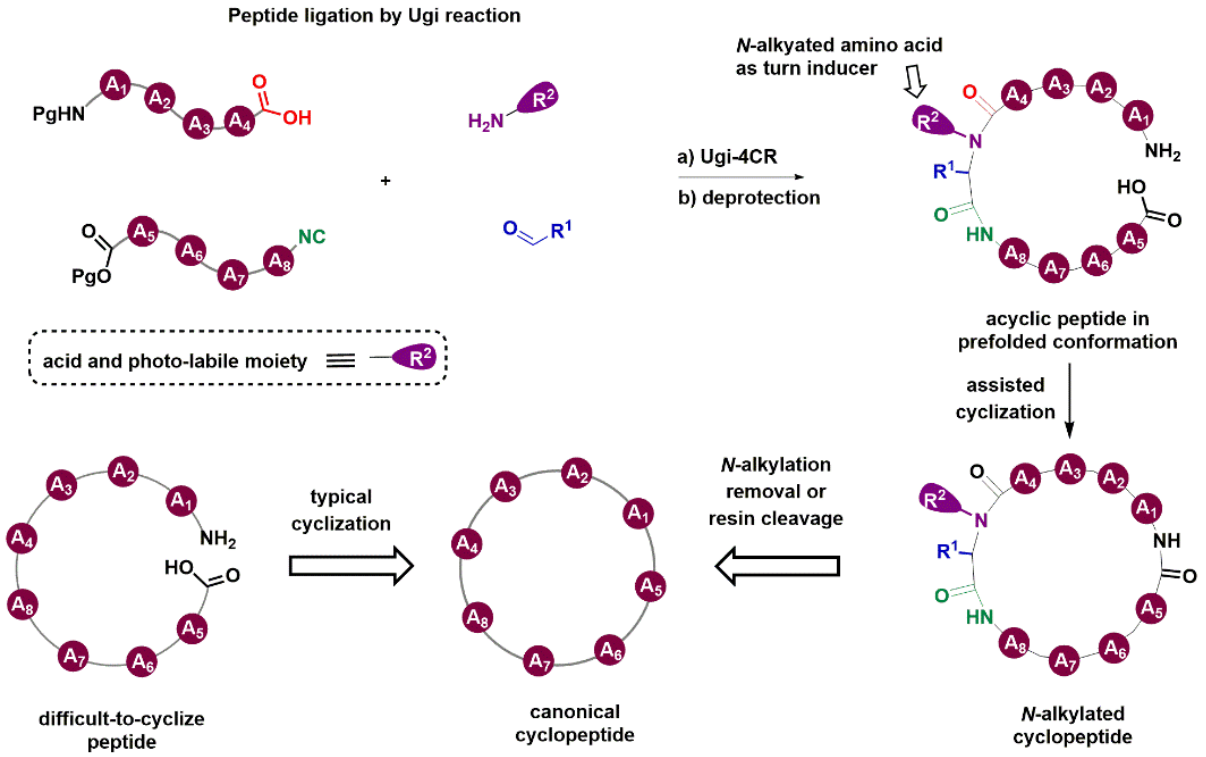
4.4 Summary

In this work a study of s-*cis*/s-*trans* forms of peptoids has been performed. The versatile Ugi-4CR was used for the incorporation of an *N*-alkylated moieties that serves as turn-inducer moiety and through facilitating the formation of the s-*cis* conformer. The short peptides sequences from model I afforded the expected products excellent yield (40–98%), while the pentapeptides sequences from model II generate moderated to low yields (5–90%). The highly congested and non-hindered at the substituted nitrogen peptides displayed only the s-*trans* isomer while highly hindered *N*-alkylated amides exist mainly as the s-*cis* conformer. In both model the s-cis/s-trans preference of 2,4-dimethoxybenzyl moiety produce a 1:1 s-*cis*/s-trans rate, independely of the size of the peptidic backbone. These result suggest that the presence of 2,4-dimethoxybenzyl group at the nitrogen amide position act as a turn inducer in the molecule. Therefore, the 2,4-dimethoxybenzyl moiety can be use as a turn inducer to assist a peptide macrocyclization.

Chapter 5

Peptide Macrocyclization Assisted by Traceless Turn-inducers Featuring *N*-Alkylated Ugi Reaction Fragments in Solution-Phase

Abstract*



A new type of traceless turn inducing moiety – featuring an *N*-alkylated peptide fragment – that facilitates the macrocyclization of short and medium size oligopeptides is described in this chapter. Installating the traceless turn inducer is accomplished through the Ugi ligation of peptide carboxylic acids and isocyanopeptides in the presence of acid and photo-labile amines and aldehydes. Such cleavable backbone *N*-substituents are quantitatively removed after cyclization to render canonical cyclopeptides. Consequently, diverse cyclopeptides were produced in good yields.

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* This chapter was published: Alfredo R. Puentes**, Micjel C. Morejón, Daniel G. Rivera, and Ludger A. Wessjohann. *Organic Letters* **2017** *19* (15), 4022-4025

** Own contribution: Development of a new traceless turn inducing moiety on solution phase for improving macrolactam and lactone cyclization.

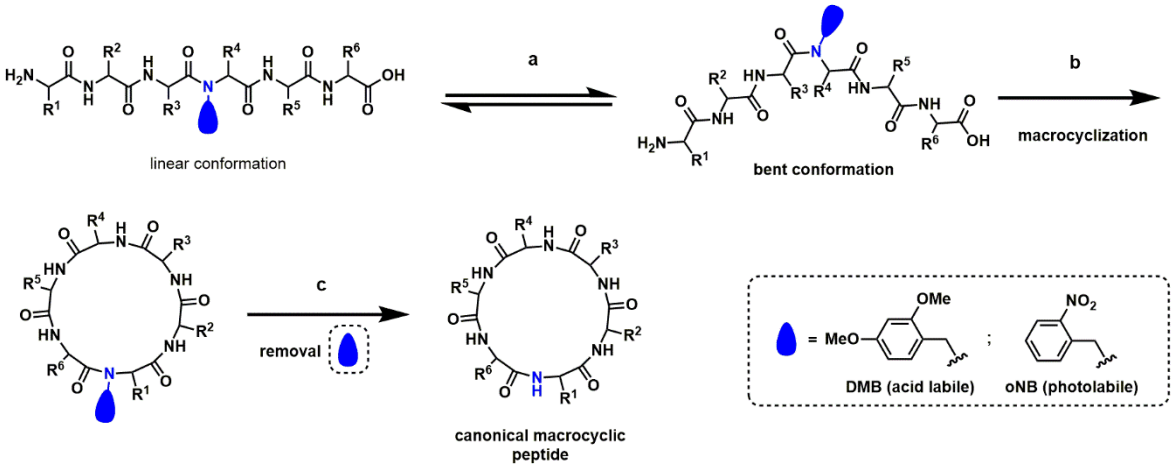
5.1 Introduction

The head-to-tail cyclization of short peptides remains as one of the most difficult procedures in synthetic chemistry.[11,298] The main problems in this process are the *C*-terminal epimerization and cyclooligomerization.[11] Whereas new coupling reagents[299,300] and synthetic tools[11,301,302] have been developed to partially solve these issues, they are intrinsically dependent on both the sequence and the ring size, and therefore difficult to generalize in short peptides. The selected strategy for increasing the macrocyclization efficiency was the incorporation of turn-inducing elements, since they are capable of facilitating the macrocyclic ring closure even at higher concentration by bringing both termini closer together.[11]

Due to their capacity to favor the *cis*-amide bond, proline and *N*-alkylated amino acids are well known turn-inducing elements that improve peptide cyclization efficiency when embedded midway in the linear precursor.[11,36,40,303,255] The inclusion of D-amino acids[38,304] and *pseudo*-prolines[305–308] – i.e., (4*S*)-oxazolidine-4-carboxylic acids derived from serine and threonine – or the analogous sulfur compound from cysteine into linear peptides also makes macroyclization much more efficient. Nonetheless, the use of these structural elements to assist peptide cyclization is limited to the presence of such amino acids in the target cyclic peptide. A more versatile strategy is using a traceless turn inducer, i.e., a moiety that can be installed in the middle of any peptide to bend its backbone and be subsequently cleaved or transformed into a native peptide structure after cyclization. Other research examples are using the photo-labile 2-hydroxy-6-nitrobenzyl *N*-substituent[309] and dehydrophenylalanine[310] as turn inducers capable of assisting the cyclization of short peptides. Herein, a novel strategy for the solution-phase macrocyclization of peptide assisted by traceless turn inducer is reported.

5.2 Synthetic design

The synthetic design proposed by us is shown in **Scheme 5.1**. This approach comprises the ligation of a peptide acid and an isocyanopeptide by the Ugi four-component reaction (Ugi-4CR), using a cleavable amine (or resin amine linker) and an aldehyde, thus leading to a peptide including an *N*-alkylated amino acid midway along the sequence. The 2,4-dimethoxybenzylamine (DMB) and *orto*-nitrobenzylamine (*o*NB) were selected as traceless turn inducer moieties in this research. Our endeavor was using the known turn inducing capability of the resulting *N*-benzylated peptide fragment to facilitate the macrocyclization in solution (see Chapter 4).

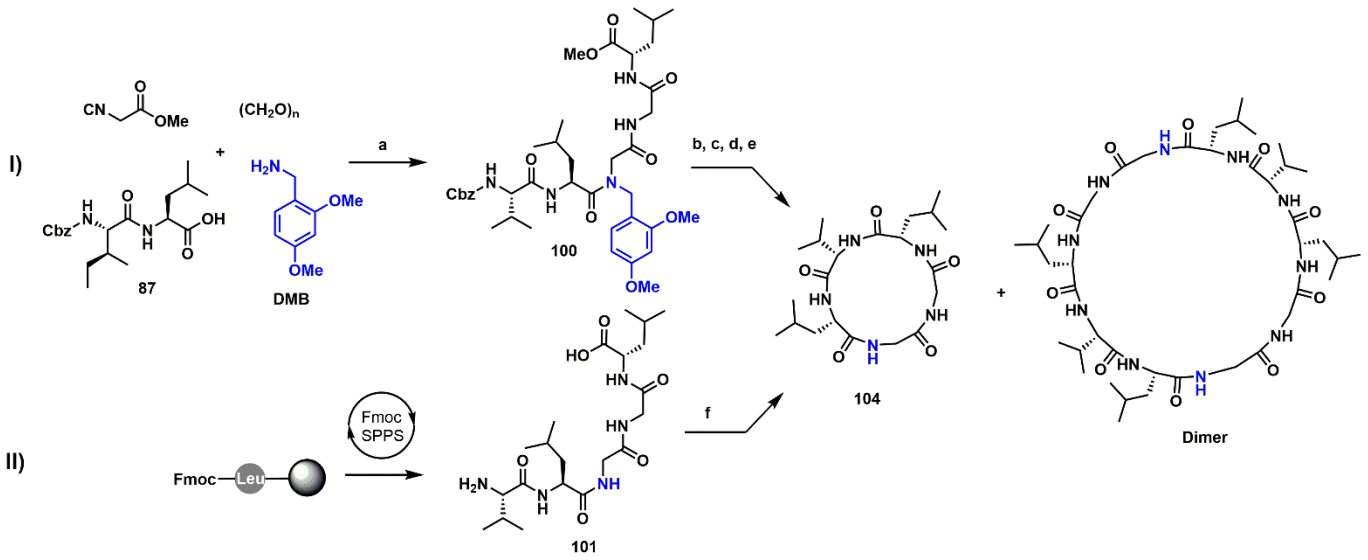


Scheme 5.1 General synthetic design: a) The turn inducer rules equilibria between straight and bent conformation. b) The turn inducer lowers the energy for bending of the linear molecule. c) Cleavage of a temporary turn inducer after macrocyclation to afford a canonical macrocyclic peptide or depsipeptides.

5.3 Macrocyclization in solution-phase assisted by turn inducer

5.3.1 Study on macrocyclization efficacy

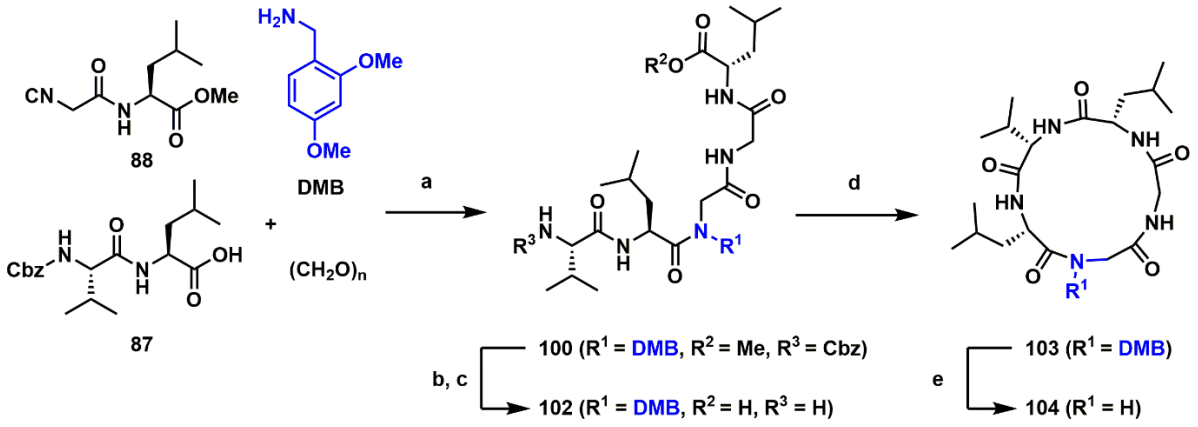
Model peptide sequence was established for a macrocyclization efficacy study. The model peptide sequences are H-Val-Leu-Gly-Gly-Leu-OH, and the H-Val-Leu-*N*(DMB)-Gly-Gly-Leu-OH, i.e., without and with turn inducer (**Scheme 5.2**). The pentapeptides were cyclized under the same reaction conditions and results were compared by HPLC/ESI-MS analysis. Both experiments were performed using syringe pump methodology, for reducing the dimerization. Since there is no risk of epimerization of the *C*-terminal, it was decided to use DMAP as base during the macrocyclization which resulted in a significant yield improvement. The study proved that even at 1 mM concentration, the non-*N*-alkylated peptide (**101**) rendered a mixture of cyclic monomer (**104**) and dimer in 2.2:1 ratio and only 21% yield of isolated cyclic peptide **104** (**Figure 5.2**). On the other hand, the deprotected peptide of **100** led to a monomer/dimer ratio of 21:1 and 53% yield of isolated **104** (see experimental section). The study showed that the *N*-alkylation strongly assisted the macrocyclic ring closure in peptide. Therefore, it was proceeded to demonstrate the applicability in the protocol for the synthesis of different size macrocyclopeptides. Also, the protocol was employed for the synthesis of macrocyclodepsipeptides.



Scheme 5.2 Reagents and conditions: I) a) MeOH, r.t, 24 h. 52% b) LiOH.H_2O, THF/H_2O (1:1), r.t. c) H_2, Pd/C (10% w/w), MeOH, r.t., 12 h d) 1mM, T3P®, DMAP, CH_2Cl_2, 3 h. e) 5% TFA, r.t., 30 min., 49% from **100**. II) f) 1mM, T3P®, DMAP, CH_2Cl_2, 3 h. 21%.

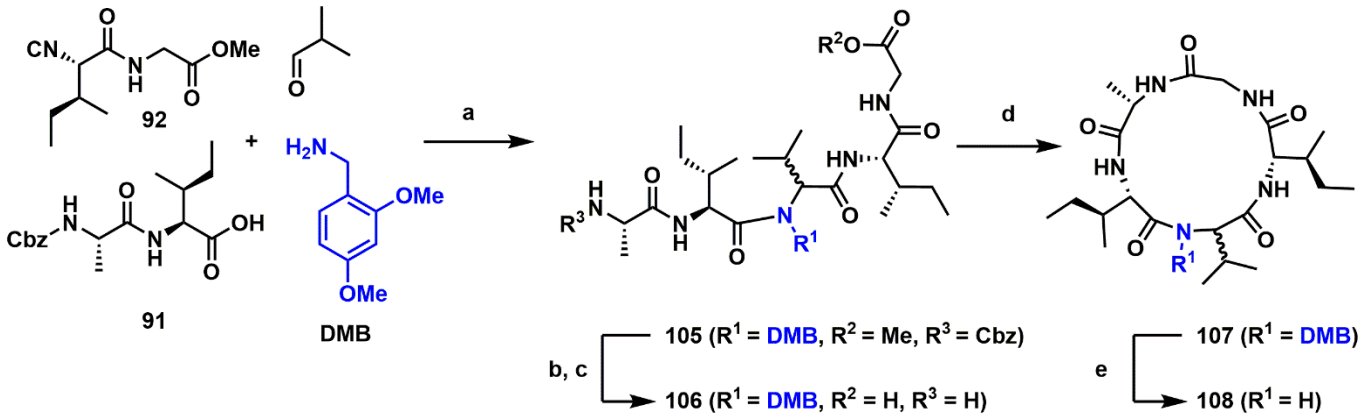
5.3.2 Synthesis of macrocyclopeptapeptides assisted by traceless acid-labile *N*-substituent Ugi reaction fragment

According to the positive results obtained from the previous macrocyclization study it was decided to synthesize two macrocyclic pentapeptides as an application of the macrocyclization methodology. The first synthesis afforded the same macrocycle taken as the model for the optimization cyclization study. Therefore, the synthesis of cyclopeptapeptide **103** started with the Ugi-4CR of Cbz-protected dipeptide **87**, paraformaldehyde, peptide-isocyanide **88**, and DMB amine to give the intermediate **100** in 82% yield. The Ugi-product **100** was saponified and the *N*-terminally deprotected by hydrogenolysis of the benzyl carbamate moiety. The final macrocyclization of deprotected acyclic *N*-alkyl peptide **102** with further acidic cleavage gave cyclopeptide **104** in 52% overall yield without epimerization (**Scheme 5.3**).



Scheme 5.3 Reagents and conditions: a) MeOH, r.t, 24 h., 82% b) LiOH.H_2O, THF/H_2O (1:1), r.t. c) H_2, Pd/C (10% w/w), MeOH, r.t., 12 h d) 1mM, T3P®, DMAP, CH_2Cl_2, r.t. e) 5% TFA, r.t., 30 min., 52%, from **100**.

The second macrocyclopeptide was obtained through the Ugi-4CR, using Cbz-protected dipeptide **91**, isobutyric aldehyde, peptide isocyanide **92** and the turn inducer DMB. Ugi-product **105** was saponified and the Cbz group removed from the *N*-terminally by hydrogenolysis. After optimizing the coupling condition, linear peptide **106** was macrocyclized using PyBOP and DIPEA to afford **107** in 56% yield. The use of a prochiral oxo- component leads to a *ca.* 1:1 mixture of diastereomers due to the poor stereoselectivity of the Ugi-4CR, which is perhaps the only drawback of this strategy. Next, to allow *N*-alkylated macrocyclopeptide **107** to be transformed into the canonical macrocycle, an acidic cleavage was performed to afford **108** in quantitative yield. The final product was obtained as a diasteromeric mixture (**Scheme 5.4**).

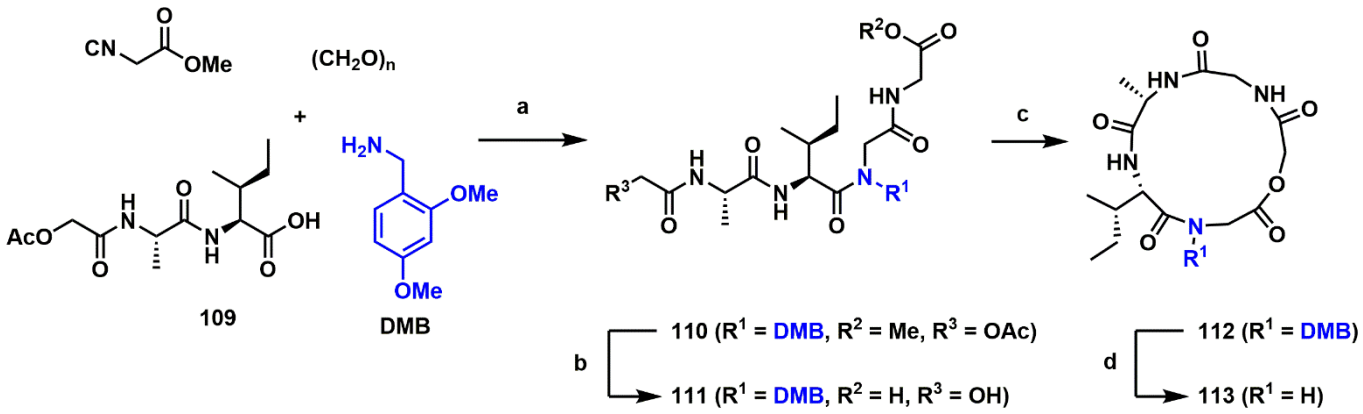


Scheme 5.4 Reagents and conditions: a) MeOH, r.t, 24 h. 79%. b) LiOH.H_2O, THF/H_2O (1:1), r.t. c) H_2, Pd/C (10% w/w), MeOH, r.t., 12 h. d) 1 mM, PyBOP, DIPEA, CH_2Cl_2, r.t. e) 5% TFA, r.t., 30 min., 56%.

5.3.3 Synthesis of macrocyclodepsipeptides assisted by traceless cleavable *N*-substituent introduced by Ugi-ligation

To study the potential of the acid-labile turn inducer DMB one example of macrolactone was synthesized. The synthesis of pentapeptide **111** *en route* to macrocyclolactone **113**, begins with the Ugi-4CR involving carboxylic acid **109**, paraformaldehyde, methylisocyanoacetate and the amine turn inducer DMB to afford *N*-alkylated peptapeptide **110** in 77% yield (**Scheme 5.5**). Intermediate **110** was treated under basic conditions to deprotect both termini of the molecule at the same time, to give **111**. Crude *N*-alkylated hydroxyl carboxylic acid **111** was treated under standard Keck macrolactonization conditions, with EDC, DMAP in dichloromethane to afford macrolactone **112** in 68% yield. It is important to mention that during the optimization experiments it was observed that intermediate **112** easily hydrolyzes during chromatography on the silica column. A possible explanation is that the acidic sites of the silica and activated water favor the hydrolysis of the lactone during the purification process. For that reason, advanced *N*-alkylated intermediate **112** was further on employed without purification. Finally, *N*-alkylated

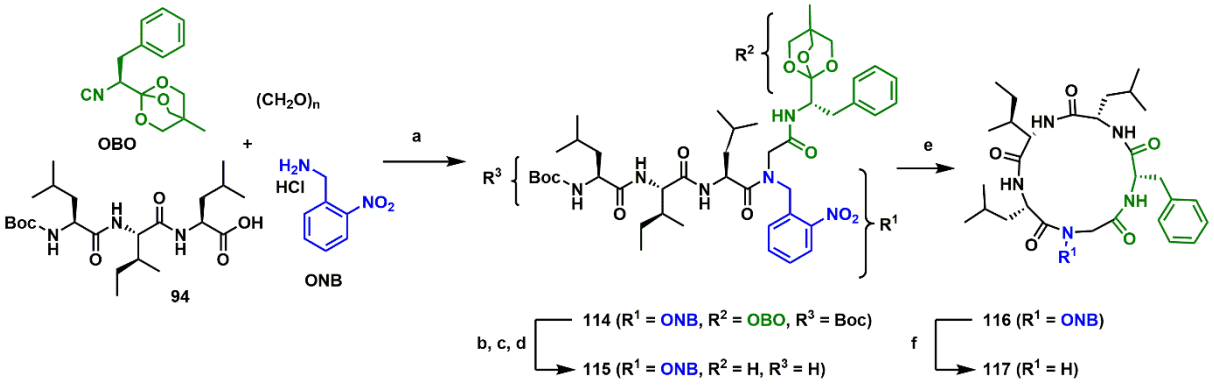
macrolactone **112** was treated in mild acidic media to give the desired macrolactone **113** in quantitative yield.



Scheme 5.5 Reagents and conditions: a) MeOH, r.t, 24 h. 77%. b) LiOH.H_2O, THF/H_2O (1:1), r.t. c) 1 mM, EDCI, DIPEA, CH_2Cl_2, r.t., d) 5% TFA, r.t., 30 min., 68%, from **110**.

5.3.4 Synthesis of a macrocyclic pentapeptide assisted by a traceless cleavable photo-labile *N*-substituent introduced by Ugi reaction fragment

The selected turn inducer was the photo-labile *o*-nitrobenzylamine, because it is sterically similar to DMB. The synthesis of the model macrocycle started with the Ugi-4CR of Boc-protected peptide **94**, paraformaldehyde, 4-methyl-2,6,7-trioxabicyclo[2.2.2]octyl (OBO) ester of phenyl isocyanide, and the turn inducer component *o*-nitrobenzylamine hydrochloride (*o*NB) in 76% yield. The OBO ester developed by Nenajdenko et al.[238] was used with the aim to vary the isocyanide α-carbon position. Also, the OBO ester strategy was selected to guarantee the stereochemical integrity of isocyanide OBO ester in Ugi-4CR under various reaction conditions. A decrease in the acidity of the α-hydrogen atom avoids epimerization at this center. The Ugi-product was treated under mild acidic conditions and saponified to afford pentapeptide **114** in 76% yield. One advantage of the *o*NB protocol is the deprotection of *N*- and *C*- termini at the same time under mild reaction conditions and without epimerization risk. Next, the advanced intermediate **115** was cyclized at the previously established 1 mM concentration using T3P® as the coupling agent and DMAP / dichloromethane. The macrocyclation optimization showed almost the same result when DIPEA instead of DMAP was used as the base, also with no detectable epimerization. Nevertheless, DMAP was selected since it is easier to remove from the reaction. Finally, macrocyclopentapeptide **117** was obtained by exposing **116** to light for 6 h (**Scheme 5.6**).

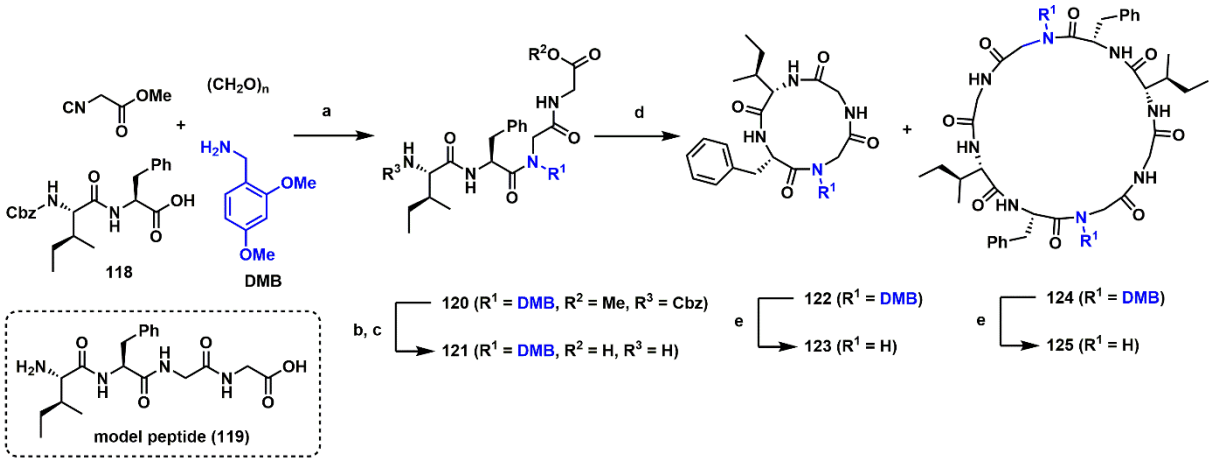


Scheme 5.6 Reagents and conditions: a) MeOH, r.t, 24 h. 76%. b) 5% TFA, r.t., 15 min., quant. c) $LiOH.H_2O$, THF/H_2O (1:1), r.t., 7 h d) 5% TFA, r.t., 1 h min., quant. e) 1 mM, T3P®, DMAP, CH_2Cl_2, r.t., f) sun light or irradiation lamp (λ = 253 nm), r.t., 6 h, 48%.

5.4.1 Macrocyclization of tetra- and hexapeptides assisted by traceless *N*-alkylated Ugi reaction fragment

5.4.1.1 Synthesis of macrocyclotetrapeptides

After proving the efficacy of this method with pentapeptides, we thought assessing the scope with tetra- and hexapeptides, as the cyclodimerization propensity of the former ones are known. Similary to the former macrocyclic synthesis with DMB as the turn inducer, cyclotetrapeptide starter was synthesized by an Ugi-4CR, followed by *C*-terminal saponification and *N*-terminally hydrogenation to obtain the pre-cyclic molecule **121** (**Scheme 5.7**).



Scheme 5.7 Reagents and conditions: a) MeOH, r.t, 24 h. 84% b) $LiOH.H_2O$, THF/H_2O (1:1), r.t., c) H_2, Pd/C (10% w/w), MeOH, r.t., 12 h, quant. d) 1mM, PyBOP, DIPEA, CH_2Cl_2, r.t. e) 5% TFA, r.t., 30 min., quant, monomer 39%, dimer 17%.

The final macrocyclization was performed at 1 mM concentration by using PyBOP as coupling agent, DIPEA and dichloromethane. It is important to highlight that peptide **121** has two Gly in its sequence. We

intentionally introduced the bulky Ile at the *N*-terminally, so that cyclization is not greatly facilitated by the sequence and an accurate assistant effect an be assessed better. Cyclization of **121** followed by DMB cleavage rendered cyclic tetrapeptide **123** in 39% yield and the cyclodimer **125** in 17% yield (**Scheme 5.7**) (**Figure 5.1**). Nonetheless, the proposed method did prove much more effective than the cyclization of the non-assisted model peptide H-Ile-Phe-Gly-Gly-OH (**119**, **Scheme 5.7**), which led almost exclusively to formation of cyclodimer **125** (see experimental section). HPLC/ESI-MS analysis of the crude cyclization reaction showed a monomer/dimer ratio of 2.6:1 for *N*-alkylated peptide **121** and a ratio of 1:19 for cyclization of non-*N*-alkylated one **119**.

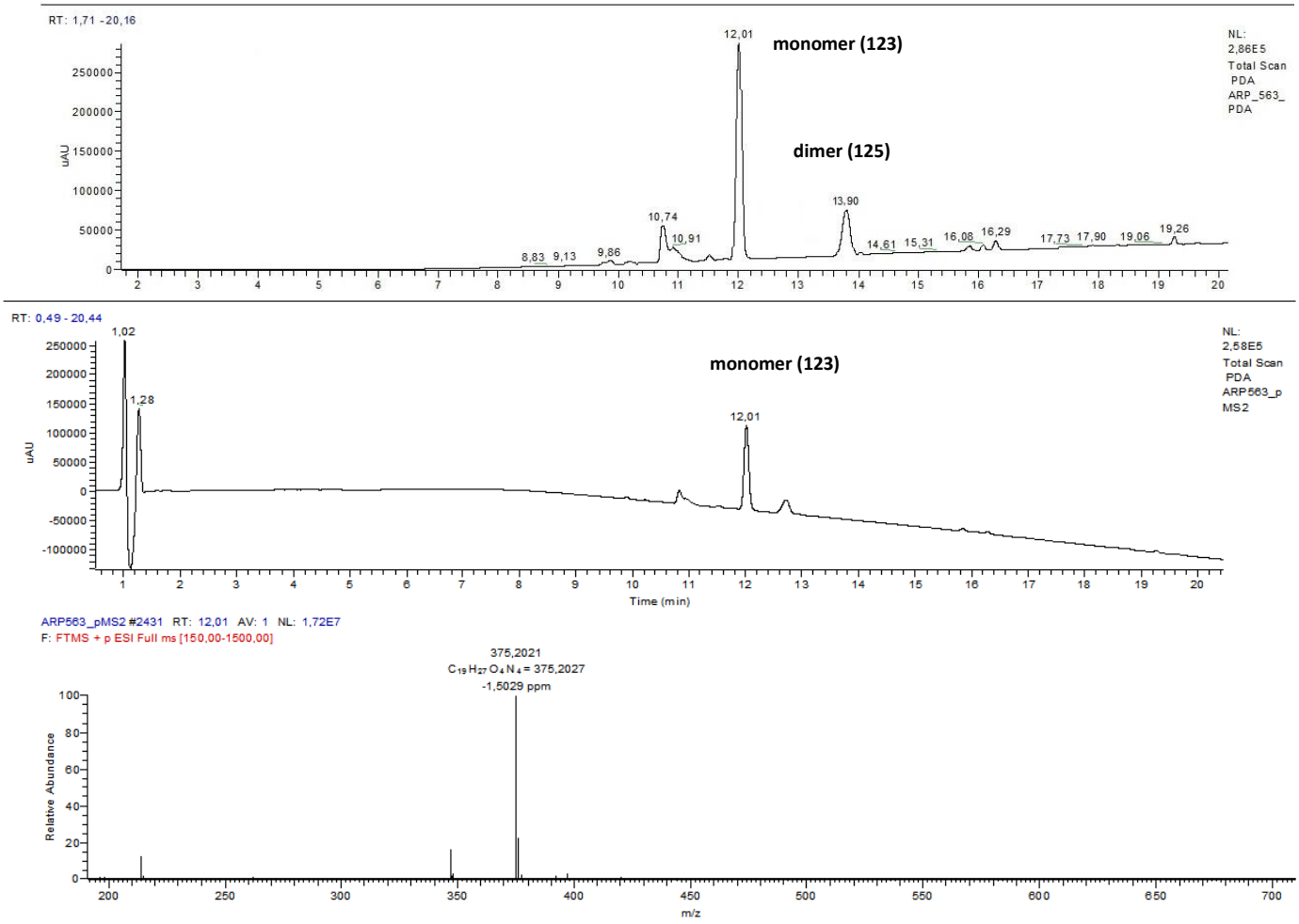
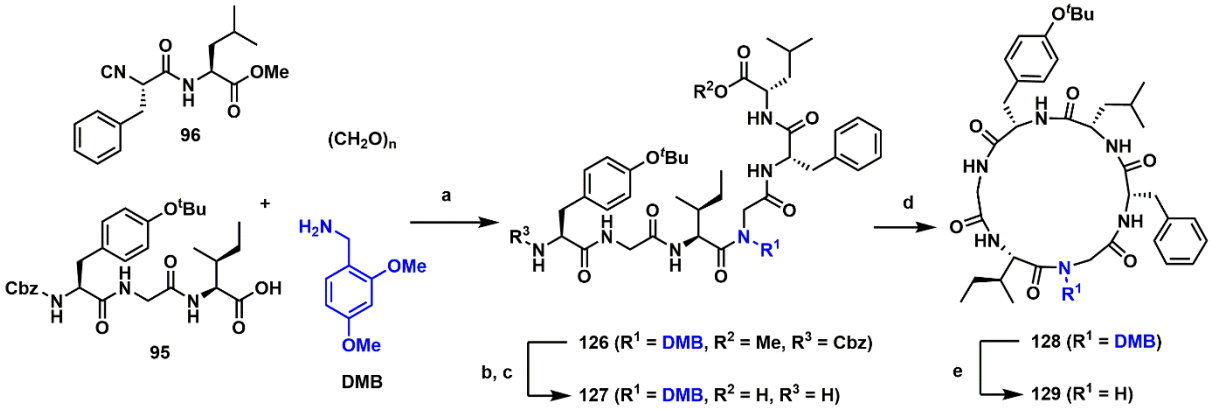


Figure 5.1 RP-UHPLC chromatograms and ESI-HRMS of crude and pure cyclic peptide **123**.

5.4.2 Synthesis of macrocyclohexapeptides

As known by DMB protocols, the macrocyclohexapeptide was synthesized using similar conditions. The first reaction was an Ugi-4CR of carboxylic acid **95**, paraformaldehyde, peptide isocyanide **96**, and the DMB amide turn inducer. As expected, cyclization of deprotected hexapeptide **127** proceeded smoothly to furnish cyclic peptide **129** in 61% yield after DMB/*t*Bu cleavage and purification. Only a minor amount of

a diastereomer (apparently *C*-terminal epimer) was detected by analytical HPLC, but it was not isolated by column chromatography (see experimental section).



Scheme 5.8 Reagents and conditions: a) MeOH, r.t, 24 h. 81% b) LiOH.H_2O, THF/H_2O (1:1), r.t., c) H_2, Pd/C (10% w/w), MeOH, r.t., 12 h, quant. d) 1mM, T3P®, DMAP, CH_2Cl_2, r.t. e) 5% TFA, r.t., 30 min., 61%.

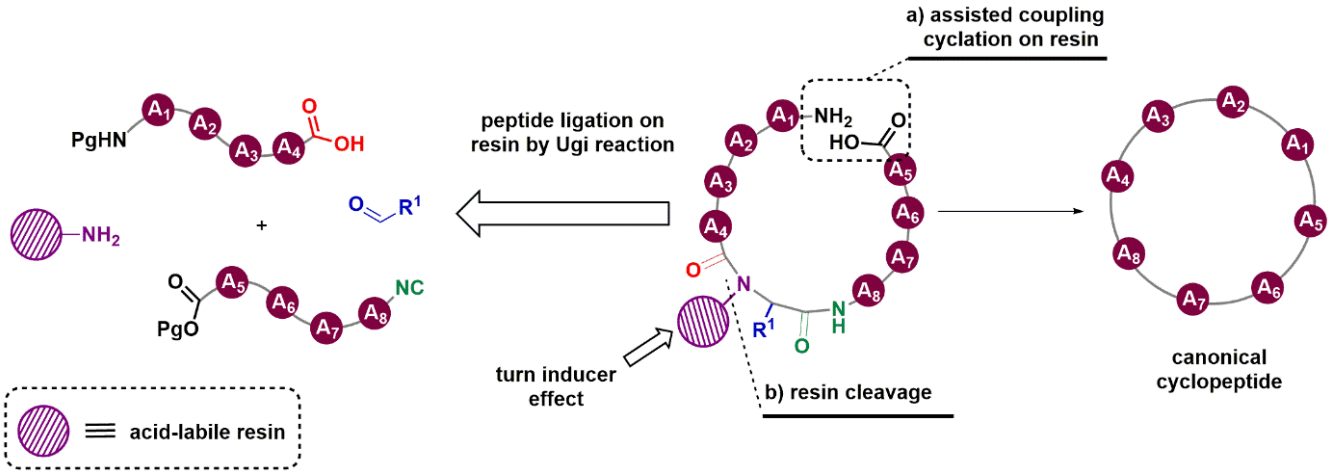
5.5 Summary

In summary, an efficient methodology to assist peptide macrocyclization in solution-phase has been developed in this work. The approach uses the versatile Ugi-4CR for simultaneous incorporation of an *N*-alkylated peptide fragment that serves as turn-inducing moiety and thus facilitates the macrocyclic ring closure. Assistance to macrocyclization was proved with a variety of penta- and tetrapeptides, in good yields (52–68%). The cyclizations were less effective without the presence of an internal *N*-alkylated moiety. Importantly, the Ugi-derived turn inducer is traceless due to the utilization of either acid- or photo-labile amines, which are quantitatively cleaved after cyclization to render the canonical cyclopeptides. Moreover, it is remarkable that preparative HPLC purifications were not required for most of the obtained cyclo peptides. Therefore, the multicomponent turn inducer strategy can be usefull for the upscaling preparation of higher amounts of cyclo peptides. Also, the developed strategy allows to prepare macrocyclodepsipeptides.

Chapter 6

Solid-phase Synthesis of Cyclic Peptides by a Multicomponent Backbone Amide Linker (BAL) Strategy

Abstract*



The transfer of the syntheses of chapter 5 to solid-phase is described in this chapter. The foundation of the solid-phase application lay in the structural similarities of the turn inducer DMB used in chapter 5 and Rink amide resin linker structure. The approach was successfully implemented in solid-phase by using resins functionalized with the Rink amide linker, which represents a new class of amine backbone linker strategy. The on-resin variant proved to be highly efficient, as it comprised the multicomponent incorporation into the resin of several amino acids in the first step, followed by peptide growing, cyclization and final cleavage. Herein, a novel strategy for the solid-phase macrocyclization of peptides assisted by traceless turn inducers is described. Consequently, diverse cyclopeptides were produced in solid phase in good yields, including a naturally occurring cyclic heptapeptide.

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** Own contribution: Development of a new multicomponent backbone amide-linked (BAL) peptides with the subsequent derivatization by peptide growth and final macrolactamization.

6.1 Introduction

Over the years, much effort has been devoted to developing effective protocols for the on-resin head-to-tail cyclization of peptides that do not have trifunctional amino acids such as Glu, Asp, Lys, His and Ser, whose side chains are commonly linked to the resin leaving the two termini available for cyclization.[311–314] Using resins of low loading, solid-phase peptide macrocyclizations usually reproduce the dilution conditions required to avoid cyclodimerization. A major advance in this field was the development of the so-called Backbone Amide Linker (BAL) strategy, introduced independently by Albericio and Barany[315–317] and Ellman.[318,319] This approach enables the on-resin *C*-terminal modification and cyclization of peptides anchored to the resin by an internal amide *N*-substituent. Several linkers have been introduced after those reports,[320–322] thus permitting a variety of *C*-terminal derivatizations. The strategy comprises the attachment of an acid-labile substituted benzaldehyde to the polymeric support, followed by reductive amination – generally with a *C*-protected amino acid – and acylation with another amino acid. Herein, we extend the BAL concept into a multicomponent strategy for the synthesis of backbone amide-linked peptides and their subsequent derivatization by peptide growth and final cyclization.

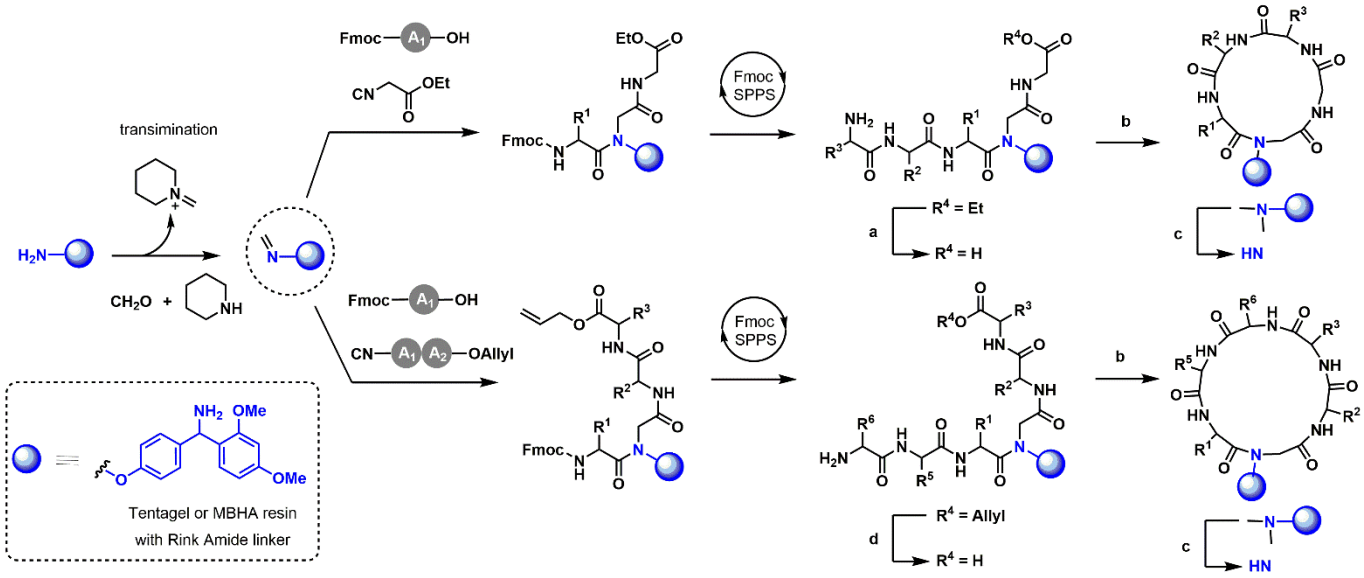
6.2 Synthetic design

The Ugi-4CR was the selected methodology to functionalize the resin, since it can install an *N*-subtituted peptide bond in only one single operation. Resins Tentagel S RAM and Rink MBHA were selected as the amino free components of the Ugi-4CR. Of the two resins the "tentacle polymer gel" (Tentagel) resin possesses PEG chains that allow good swelling and solvation in methanol, which is the most efficient solvent to perform an Ugi reaction. The rest of the selected components for the Ugi reaction are: paraformaldehyde, Fmoc-Phe-OH, and the ethyl isocyanoacetate. Paraformaldehyde (oxo- component) is chosen to guarantee the optical purity of the resin, because of to the intrinsic poor stereoselectivity of the Ugi reaction. Moreover, the insertion of paraformaldehyde in the resin is easy through an aminocatalysis-mediated transimination protocol developed in our group.[323] The transimination reaction is necessary, because both reacting sites (oxo- and the amino linked to the solid-phase) are solid and therefore, if the Ugi reaction is allowed to happen without the pre-transimination step, reactivity will be tremendously decreased. Once both resins are functionalized by using the Ugi-4CR, these would be the main building blocks to be used along all the macrocycle synthesis on solid phase.

6.3 Functionalization of the resin with Ugi-4CR

The functionalization of the Tentagel S RAM resin was achieved by an Ugi-4CR. Initially Tentagel S RAM resin was swollen in methanol and treated with a mixture of paraformaldehyde and piperidine.[323] Aminocatalytic transimination allows to form the methylene imine easily on the resin. Next, Fmoc-Phe-

OH was added, as well as ethyl isocyanoacetate to afford the functionalized resin. A similar functionalization process was performed on the MBHA resin. This functionalization approach does not require the initial incorporation of a cleavable aldehyde linker and subsequent reductive amination and acylation; instead, it comprises the direct incorporation (only one step) of an Fmoc-amino acid and an isocyanide peptide or amino acid to the polymer support functionalized with the Rink amide linker. Other aldehydes could also be employed without such transimination step, but resulting in a final mixture of diastereomers.



Scheme 6.1 Solid-phase synthesis of cyclic peptides by a multicomponent backbone amide linker (BAL) strategy. a) KOH in EtOH/THF (1:1), 0.25M. b) HATU, DIPEA, DMF, r.t. c) TFA/H_2O/TIS d) $Pd(PPh_3)_4/PhSiH_3/N_2$.

Once the resin was functionalized, the efficacy of the on-resin Ugi reaction was evaluated by chromathography and spectrometric techniques. The Ugi product linked on the resin was completely deprotected (*C*- and *N*-termini) and cleaved from the resin. The release H-Phe-Gly-Gly-OH peptide was analyzed by UHPLC/ESI-MS technique showing 82% purity from the crude reaction (see attachments). In addition, the multicomponent BAL strategy preserves a great flexibility for protecting groups, as either ethyl or allyl esters were used at the *C*-terminal and Boc/*t*Bu groups at the side chains, being orthogonal with the Fmoc tactic for peptide elongation.

6.4 Multicomponent BAL strategy for the synthesis of macrocyclopeptides

The scope of this multicomponent BAL strategy was assessed using oligopeptides ranging from four to seven amino acids. Typical Fmoc/tBu strategy was used for the peptide chain growing in an automated synthesis process. After removing the Fmoc group from the *N*-terminally and saponifying the *C*-terminal position, both reacting site were ready to be coupled. Before the cyclization step, it was necessary to study

the best coupling conditions for the on-resin cyclization. **Figure 6.1** depicts the cyclization of peptide **136** using PyBOP, HBTU, HATU and oxyma/DIC as coupling agents. The experiment performed with PyBOP, HBTU or HATU after 1 h of reaction showed no detectable linearpeptide once cleaved from the resin – as hoped. In contrast, the use of oxyma/DIC mixture was completely unsuccessful, since after 1 h reaction the desired cyclic peptide was not detected. Therefore, the standard protocol to carry out on-resin cyclization was established with HATU coupling reagent and 1 h reaction time.

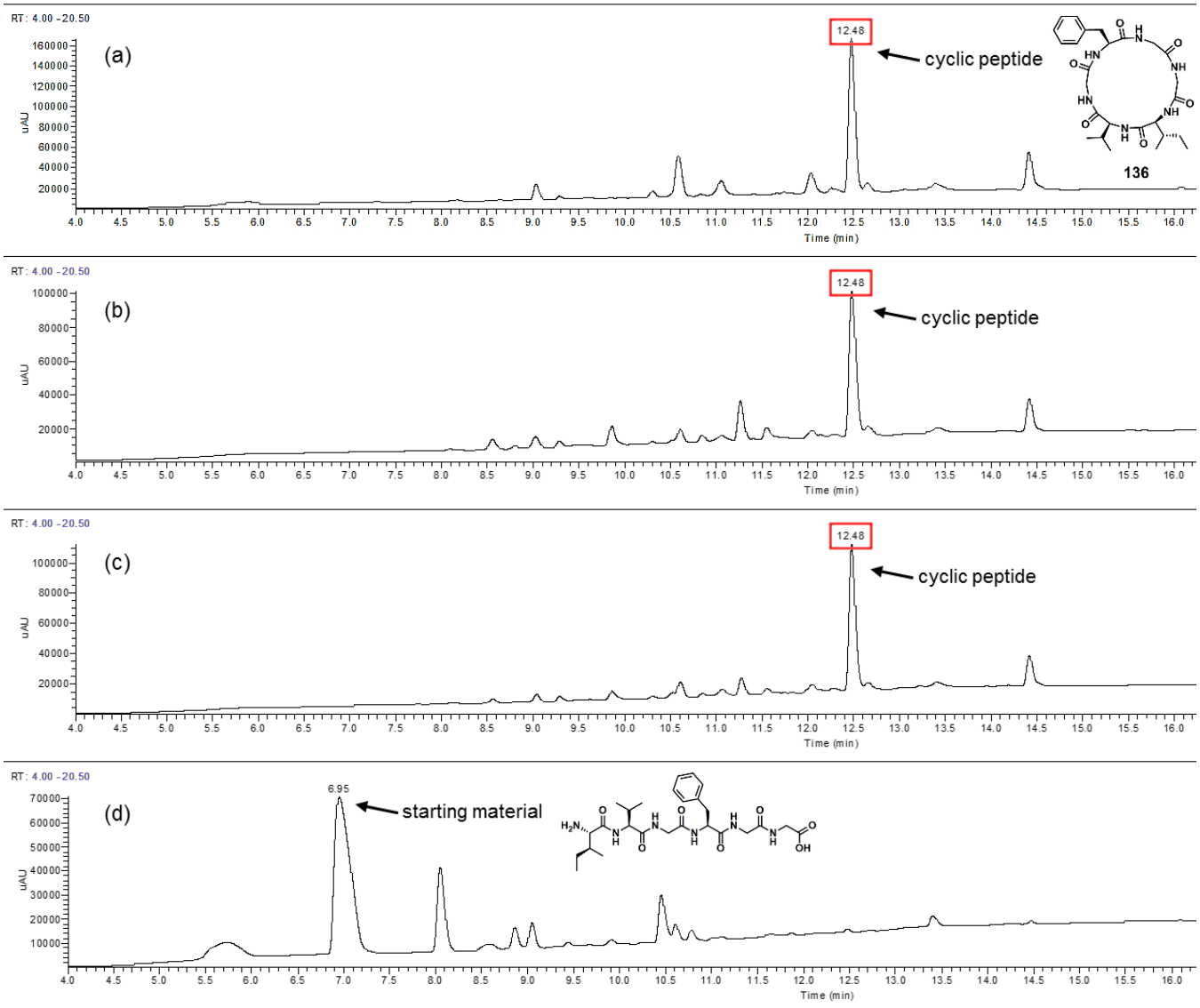
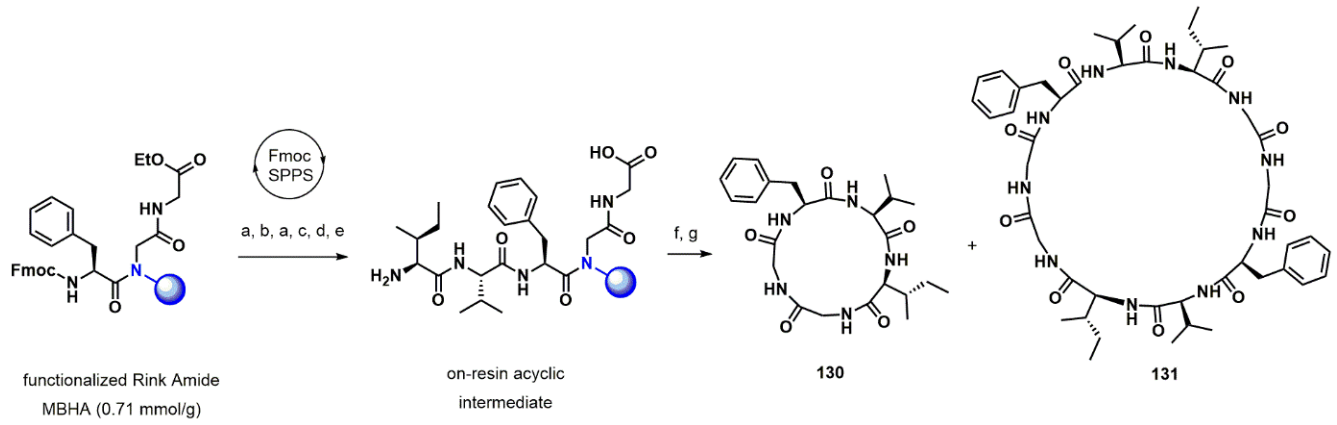
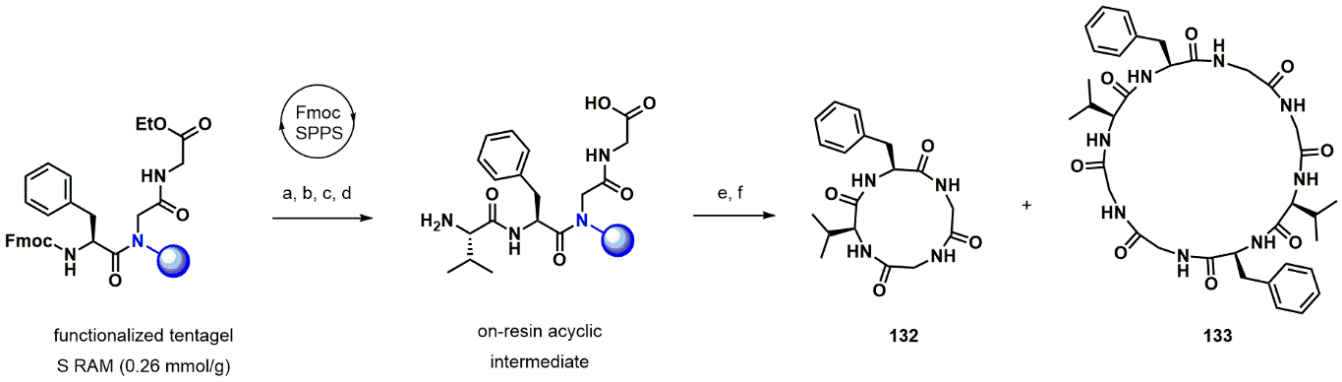


Figure 6.1 RP-UHPLC chromatogram of crude cyclic peptide **136** using different coupling reagents. a) PyBOP/DIPEA/DMF b) HBTU/HOAt/DMF c) HATU/DIPEA/DMF d) Oxyma/DIC/DMF.



Scheme 6.2 Reagents and conditions: a) Piperidine/DMF b) Fmoc-Val-OH, HBTU/HOBt, DIPEA in DMF, c) Fmoc-Ile-OH, HBTU/HOBt, DIPEA in DMF, d) KOH in EtOH/THF (1:1), 2 h, e) $NaHSO_4$(aq) (10%), 15 min, f) HATU, DIPEA, DMF, g) TFA/TIPS/H_2O (95:2.5:2.5), **130**, 14%, **131**, 36%.

As shown in **Scheme 6.2**, using the Rink amide polystyrene resin MBHA with a loading of 0.71 mmol/g led to cyclic pentapeptide **130** in only 14% yield, while dimer **131** was isolated in 36% yield. The ^{1}H and ^{13}C NMR spectra of cyclopeptides **130** and **131** are identical. Both compounds show the same chemical shift, number of signals, and signal patterns. In order to elucidate the correct structural assignment of cyclo peptide **130** and **131** tandem mass spectrometry experiments were performed. The mass spectra showed five fragment molecular ions due to the ring opening during collision. By sequential assignment of the fragments, it was possible to determine that **130** belonged to the monomeric cyclopeptide (see attachments).

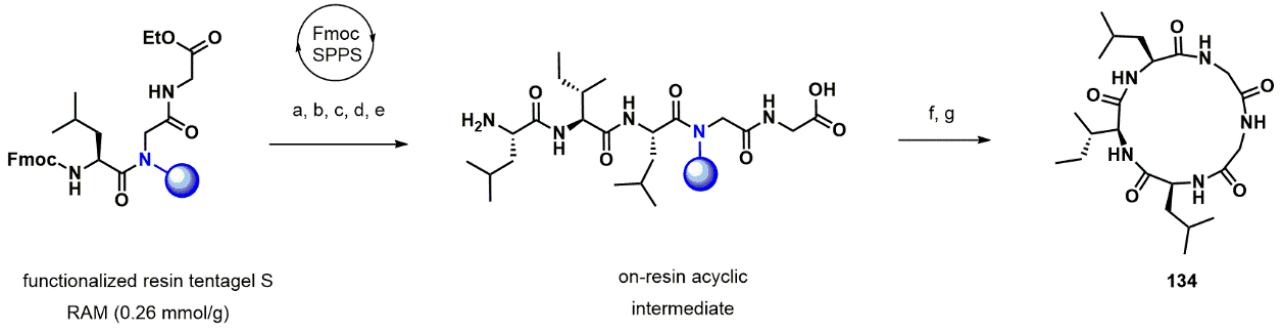


Scheme 6.3 a) Piperidine/DMF, b) Fmoc-Val-OH, HBTU/HOBt, DIPEA in DMF, c) KOH in EtOH/THF (1:1), 2 h, d) $NaHSO_4$(aq) (10%), 15 min, e) HATU, DIPEA, DMF, f) TFA/TIPS/H_2O (95:2.5:2.5), **132**, 18%, **133**, 39%.

A similar analysis was performed on cyclic peptides **132** and **133**. As expected, the NMR spectra of both compounds were identical. For that reason, a tandem mass spectrometry experiments were carried out on both compounds. As in the former case (**130** and **131**), the fragmentation pattern in mass spectra of **132** and **133** allowed to differentiate unequivocally the monomer from the dimer (**Scheme 6.3**). High dimerization rate is probably related to the high solvation rate of PEG chains present in the TentaGel resin, which confer

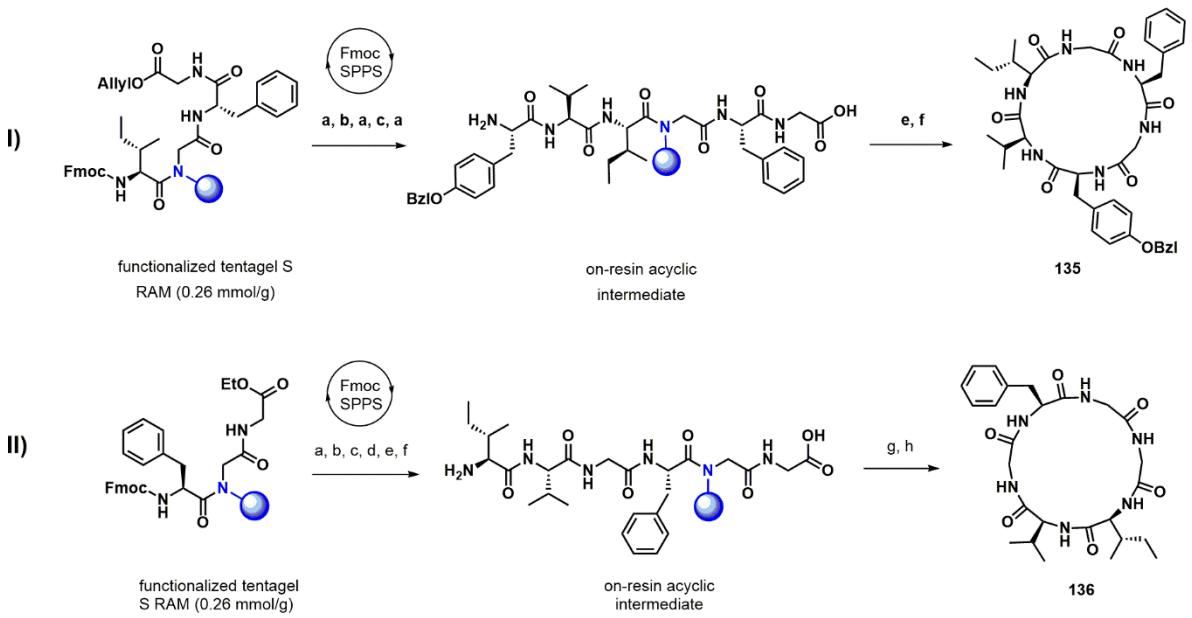
high mobility on the peptide bound to the copolymer. Phenomenal dimerization on-resin cyclation was previously reported.

Alternative, when using the Tentagel S RAM resin with a lower loading (0.26 mmol/g), only cyclic pentapeptide **134** (monomer) was isolated in very good overall yield (**Scheme 6.4**). However, using the same Tentagel S RAM resin, a mixture of cyclic tetrapeptide **132** (monomer) (18%) and dimer **133** (39%) was obtained, proving the difficulty of cyclizing tetrapeptides without cyclodimerization. For avoiding that, perhaps a more rigid resin with a much lower loading might be required, as it has been proven in previous works reports.[311–314]



Scheme 6.4 a) Piperidine/DMF, b) Fmoc-Ile-OH, HBTU/HOBt, DIPEA in DMF, c) Fmoc-Leu-OH, HBTU/HOBt, DIPEA in DMF d) KOH in EtOH/THF (1:1), 2 h, e) $NaHSO_4$(aq) (10%), 15 min, f) HATU, DIPEA, DMF, g) TFA/TIPS/H_2O (95:2.5:2.5), **134**, 47% (only monomer).

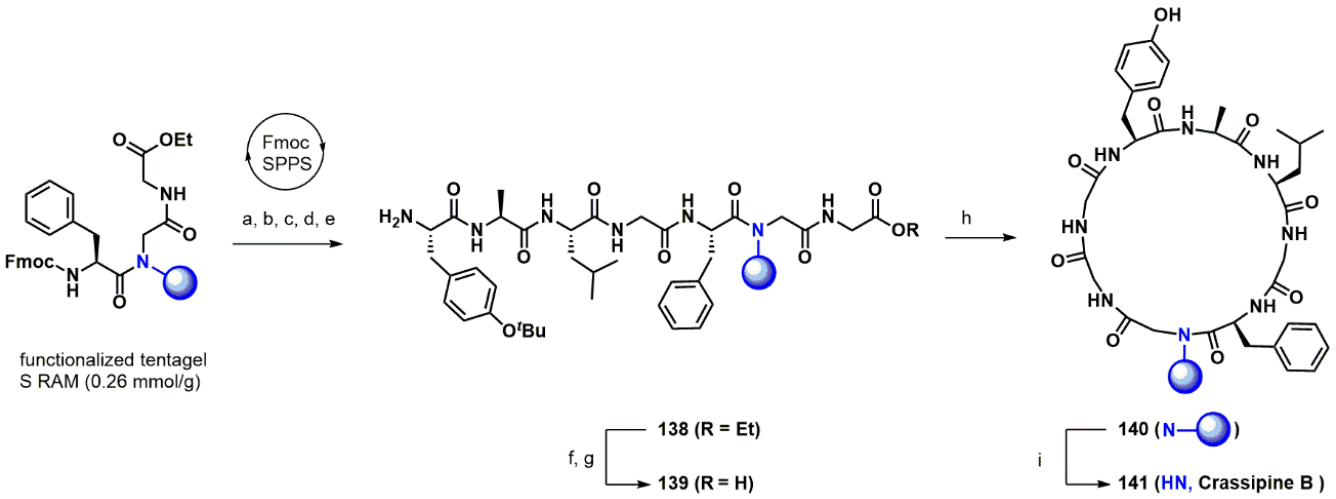
As expected, the on-resin macrocyclization of hexapeptides proceeded smoothly to furnish cyclic peptides **135** and **136** in very good overall yield and without cyclodimerization (**Scheme 6.5**). Peptide **135** derives from the initial incorporation of Fmoc-Phe-OH and isocyanide CN-Phe-Gly-OMe, while **136** comprises the use of ethyl isocyanoacetate. As a final look at this multicomponent BAL strategy, we consider it as a highly valuable and efficient method, in spite of the lack of diastereoselectivity of the Ugi-4CR that eventually constricts it to cyclic peptides having at least one Gly in their sequences. Several factors such as low synthetic cost for incorporating the first three or four amino acids into the resin, the great tolerance of protecting groups and the complete absence of DKP formation, among others, make it a plausible alternative to classic BAL approaches.



Scheme 6.5 I) a) Piperidine/DMF, b) Fmoc-Val-OH, HBTU/HOBt, DIPEA in DMF c) Fmoc-Tyr(Bzl)-OH, HBTU/HOBt, DIPEA in DMF d) $Pd(PPh_3)_4/PhSiH_3/N_2$ e) piperidine/DMF f) HATU, DIPEA, DMF, g) TFA/TIPS/H_2O (95:2.5:2.5), **135**, 51% (only monomer). II) a) Piperidine/DMF, b) Fmoc-Gly-OH, HBTU/HOBt, DIPEA in DMF c) Fmoc-Val-OH, HBTU/HOBt, DIPEA in DMF d) Fmoc-Ile-OH, HBTU/HOBt, DIPEA in DMF e) KOH in EtOH/THF (1:1), 2 h f) $NaHSO_4$(aq) (10%), 15 min g) HATU, DIPEA, DMF h) TFA/TIPS/H_2O (95:2.5:2.5) **136**, 54% (only monomer).

6.4.1 Total synthesis of macrocycloheptapeptide Crassipin B by multicomponent BAL strategy

Crassipin B is a cyclic heptapeptide isolated from an arctic tree from the Caryophyllacae family. Usually the members of the Caryophyllacae are used as medicinal herbs in China. To prove the scope of the multicomponent BAL method on solid-phase, we undertook the total synthesis of natural product Crassipin B,[324] that was produced in overall 53% yield by the initial multicomponent attachment of Fmoc-Phe-OH and ethyl isocyanoacetate to the resin, followed by peptide growth using the Fmoc strategy and consecutive macrocyclization and acidic cleavage from resins. Because the on-resin Ugi product had an isocyanide methyl ester, the approach was performed without epimerization risk on the macrocyclation closuring site (**Scheme 6.6**).



Scheme 6.6 Reagents and conditions: a) Piperidine/DMF, b) Fmoc-Gly-OH, HBTU/HOBt, DIPEA in DMF c) Fmoc-Leu-OH, HBTU/HOBt, DIPEA in DMF d) Fmoc-Ala-OH, HBTU/HOBt, DIPEA in DMF e) Fmoc-Tyr(*t*Bu)-OH, HBTU/HOBt, DIPEA in DMF f) KOH in EtOH/THF (1:1), 2 h g) $NaHSO_4$(aq) (10%), 15 min h) HATU, DIPEA, DMF i) TFA/TIPS/H_2O (95:2.5:2.5) **141**, 53% (only monomer).

6.5 Summary

In summary, a new multicomponent BAL strategy to improve peptide macrocyclization on solid-phase has been developed. The solid phase led to the development of a new backbone amide linker strategy, relying for the first time on the multicomponent incorporation of at least three amino acids in one step, instead of the three on-resin steps required in traditional BAL protocols for attaching a dipeptide fragment. Different from the classic BAL strategy, our protocol has no risk of diketopiperazine formation, which is common in BAL approaches if Fmoc deprotection is used at the dipeptide stage. In our method, diketopiperazines cannot be formed because the initial on-resin multicomponent step directly leads to a tripeptide, or an even longer peptide if isocyanopeptides are employed. Owing to its synthetic usefulness, this BAL approach was employed to perform the first total synthesis of the cycloheptapeptide Crassipine B in 53% yield. The high flexibility of the new multicomponent BAL strategy can be very useful for the combinatorial and medicinal chemistry development.

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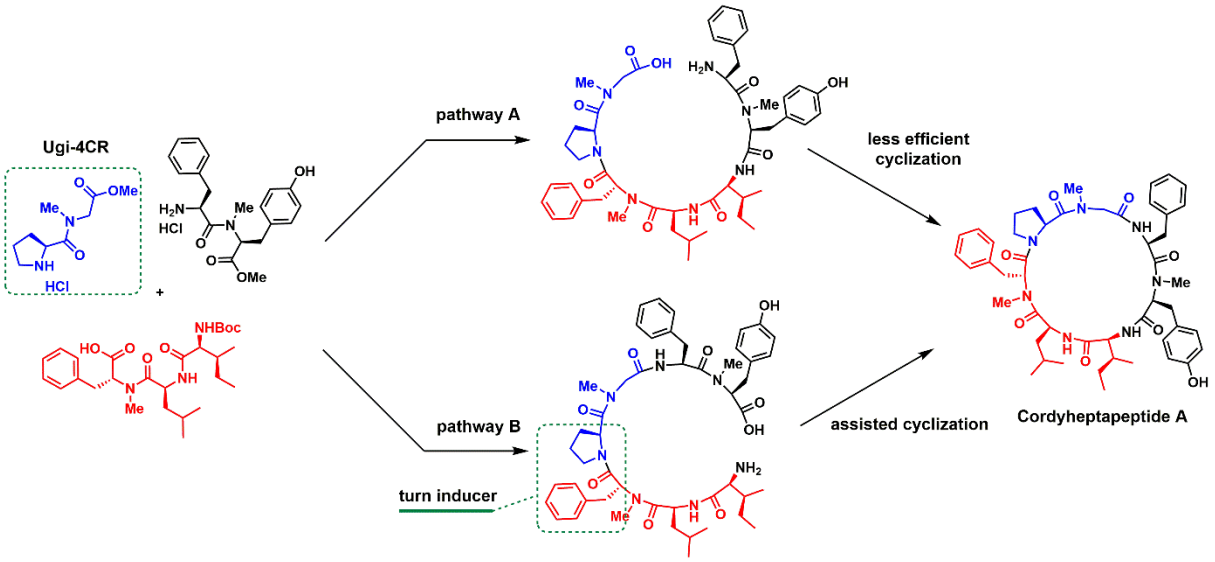
Summary and Outlook

Macrocyclopeptides have long been of interest to the scientific community. Also the pharmaceutical industry found in these molecules the opportunity to open new areas for medicinal treatments. The head-to-tail macrocyclization is one of the ways to prepare molecules of this class and is often a critical step during the synthetic. The installation of an intramolecular ring contraction element is an effective way of alleviating the entropic penalty of macrocyclization in order to yield the desired product. This thesis describes a strategy for increasing the macrocyclization efficacy by the incorporation of a turn-inducing element since it is capable to facilitate the macrocycling ring closure.

Chapter 1 outlines the most relevant aspects of macrocycles, and their applications in medicinal chemistry. It also presents an overview about basic concepts of the Ugi-4CR, including a compilation about the isocyanide and amide development.

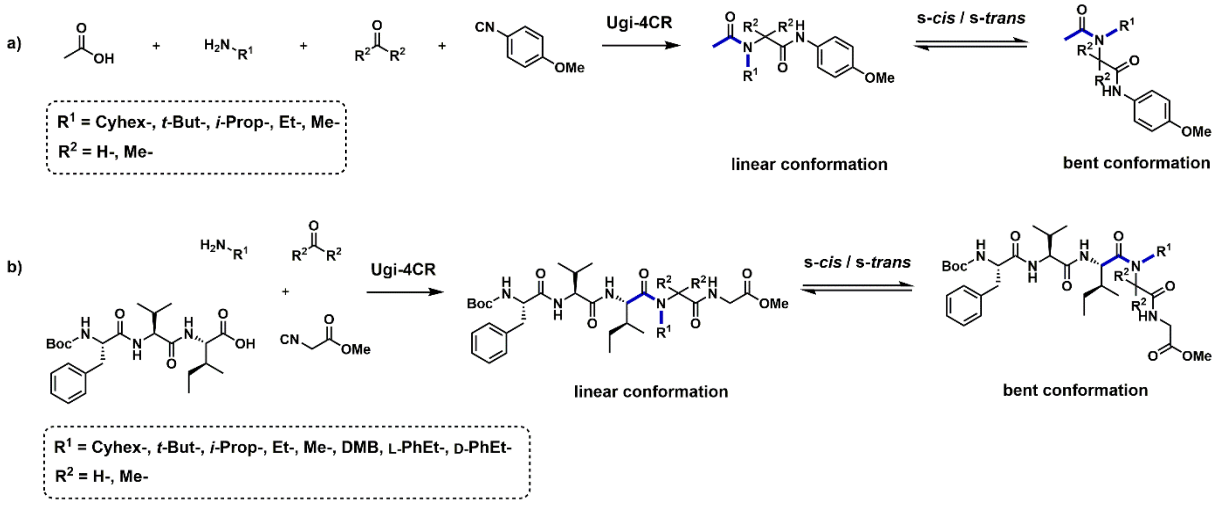
Chapter 2 includes the experimental part of the work.

In the Chapter 3 the first total synthesis of cordyheptapeptide A is described. The synthesis is achieved by a convergent approach featuring a combination of peptide coupling and the Ugi-4CR for the synthesis of the main building blocks and the acyclic precursors. The assembly of an *N*-methylated fragment by the Ugi-4CR included the utilization of a convertible isocyanide followed by the activation of the *C*-terminal amide. Two different macrocyclization positions were evaluated, providing greater efficacy the macrolactamization at the Ile-Tyr position, probable due of a convenient conformational bias of the acyclic precursor having an internal β-turn centered at the *N*-Me-D-Phe-Pro moiety (**Scheme 1**). Evidently, the acyclic precursor synthesized in pathway B was crucial for the efficacy of the macrocyclization, resulting in the selection of a macrolactamization site opposed to an internal β-turn (**pathway B**, **Scheme 1**). The approach enables the gram scale preparation of the main building blocks, which were joined in a rational way to afford the natural product in 30% overall yield. The approach might be used to prepare a library of cordyheptapeptide analogues by varying the carboxylic, amino, and oxo components, thus enabling a structure–activity relationship analysis.



Scheme 1 The two pathways to synthesize the cordyheptapeptide A.

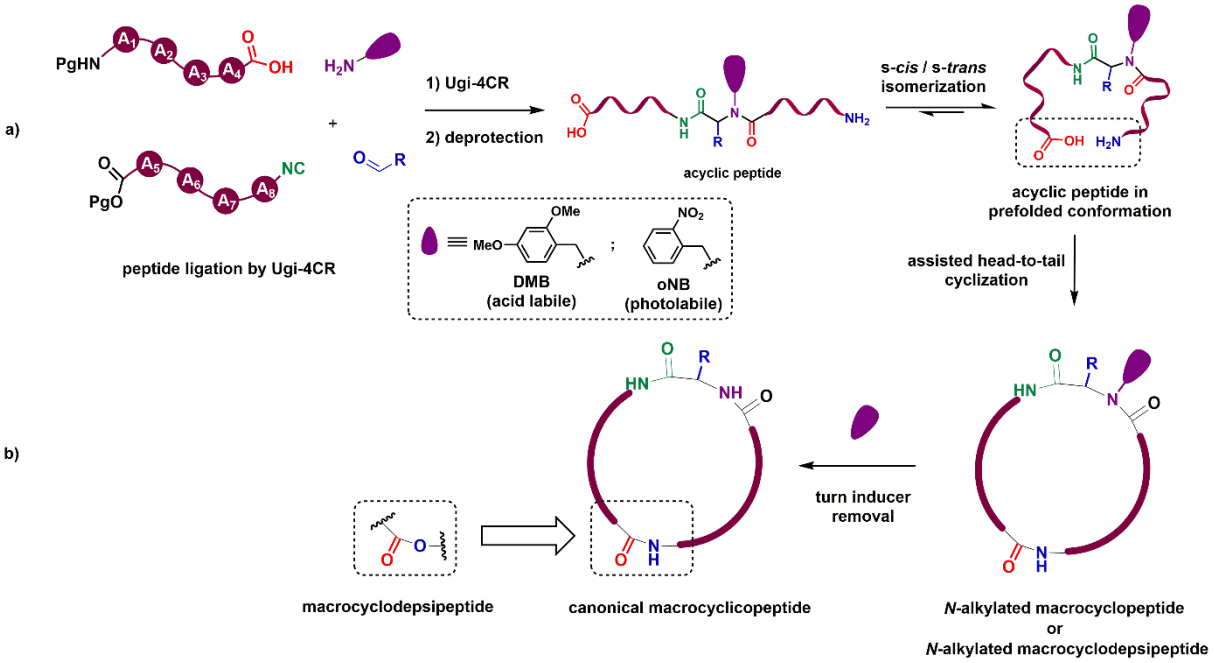
Chapter 4 describes a study of the conformational s-*cis* / s-*trans* equilibrium around the peptide bond close to the *N*-alkylated position in two peptidic models. The different modifications of the two *N*-alkylated model peptides were achieved by the Ugi-4CR. The NMR analysis of 2,4-dimethoxybenzyl derivatives showed a significant presence of the bent conformation in dipeptides and pentapeptides (**Scheme 2**). This result was taking into account for the design of the turn inducer strategy in chapter 5 and 6. The approach is flexible enough to install more complex *N*-substituents into peptidic backbone in order to study their influence in the s-*cis*/s-*trans* amide bond isomerization.



Scheme 2 Preparation of different *N*-substituted peptides by Ugi-4CR for studying the s-*cis*/s-*trans* isomerization of the amide bond. a) model dipeptide I, b) model pentapeptide II.

Chapter 5 presents a novel strategy for the solution-phase macrocyclization of peptides assisted by traceless turn inducers. The approach comprises the ligation of a peptide carboxylic acid and an isocyanopeptide by

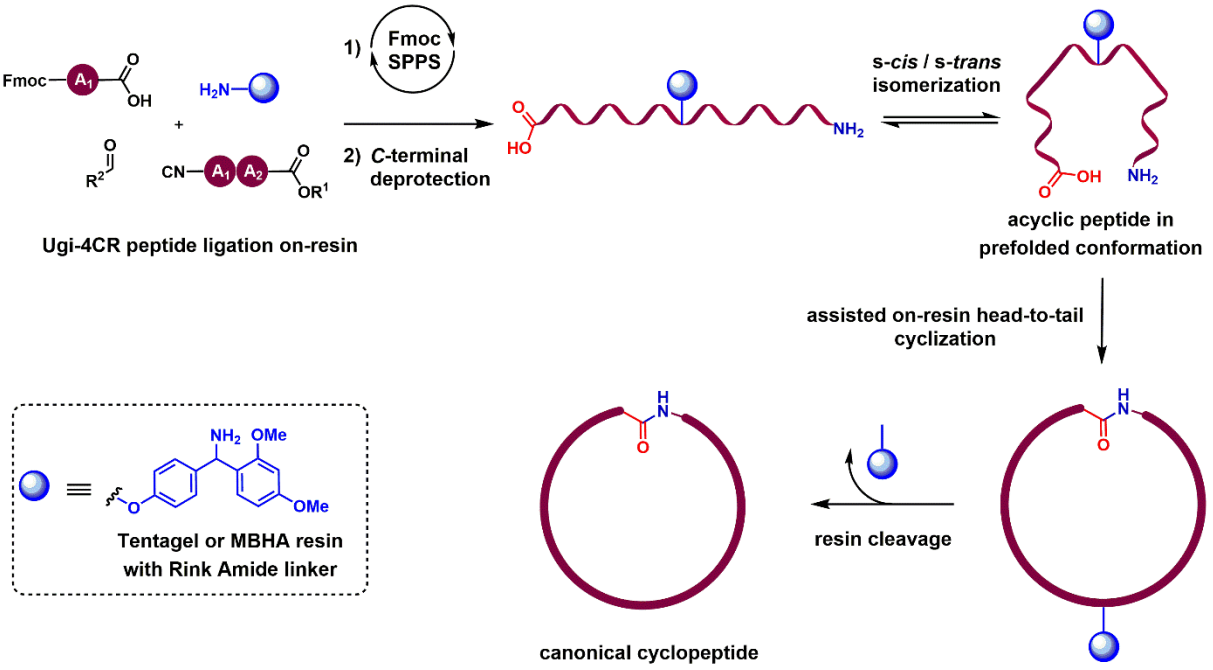
the Ugi-4CR using a cleavable amine, thus leading to a peptide including an *N*-alkylated amino acid equidistant along the sequence (**Scheme 3**). The incorporated removable *N*-alkyl substituent serves as a turn-inducing moiety and facilitates the macrocyclic ring closure. The facilitation of the macrocyclization by the turn inducing assistance was proved with a variety of tetra- to heptapeptides. The assisted macrocyclization was also used for the synthesis of cyclic depsipeptides. This novel auxiliary strategy for ring closure might be used for accessing difficult-to-cyclize peptides, towards the expansion of the repertoire of cyclic peptides and peptidomimetics that are available synthetically, as well as providing new biological probes and therapeutics.



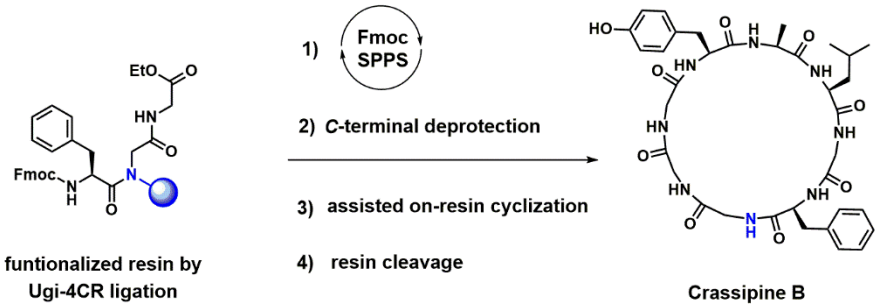
Scheme 3 Head-to-tail solution-phase macrocyclization of peptides assisted by traceless turn inducers. a) The turn inducer controls the equilibria between the linear acyclic and the prefolded conformation. b) cleavage of a traceless turn inducer after macrocyclation to afford a canonical macrocyclic peptide or depsipeptides.

The focus of Chapter 6 is the extention of the multicomponent strategy to the synthesis of backbone amide-linked peptides (BAL) and their subsequent derivatization by peptide growth and final cyclization. The new BAL strategy, relies for the first time on the multicomponent combination of at least three amino acids in one step instead of the three on-resin steps required in traditional BAL protocols for attaching a dipeptide fragment (**Scheme 4**). As at least a tripeptide is attached to the resin in the first step, this enables bypassing the difficult acylation step of the secondary amine and, even more important, avoids diketopiperazine (DKP) formation, which is common in BAL approaches if Fmoc deprotection is used at the dipeptide stage. The scope of this multicomponent BAL strategy was assessed using oligopeptides ranging from four to seven amino acids. To further prove the scope of this method, we undertook the total synthesis of the natural product crassipin B (**Scheme 5**). This cyclic heptapeptide was produced in overall 53% yield through the

initial multicomponent attachment of Fmoc-Phe-OH and ethyl isocyanoacetate to the resin, followed by peptide growing using the Fmoc strategy and consecutive macrocyclization and acidic cleavage from the resin. Due to its synthetic projections, this concept can be very useful for the peptide, combinatorial, and medicinal chemistry communities.



Scheme 4 Multicomponent strategy to the synthesis of backbone amide-linked peptides (BAL) and their successive derivatization by peptide growth and final cyclization.



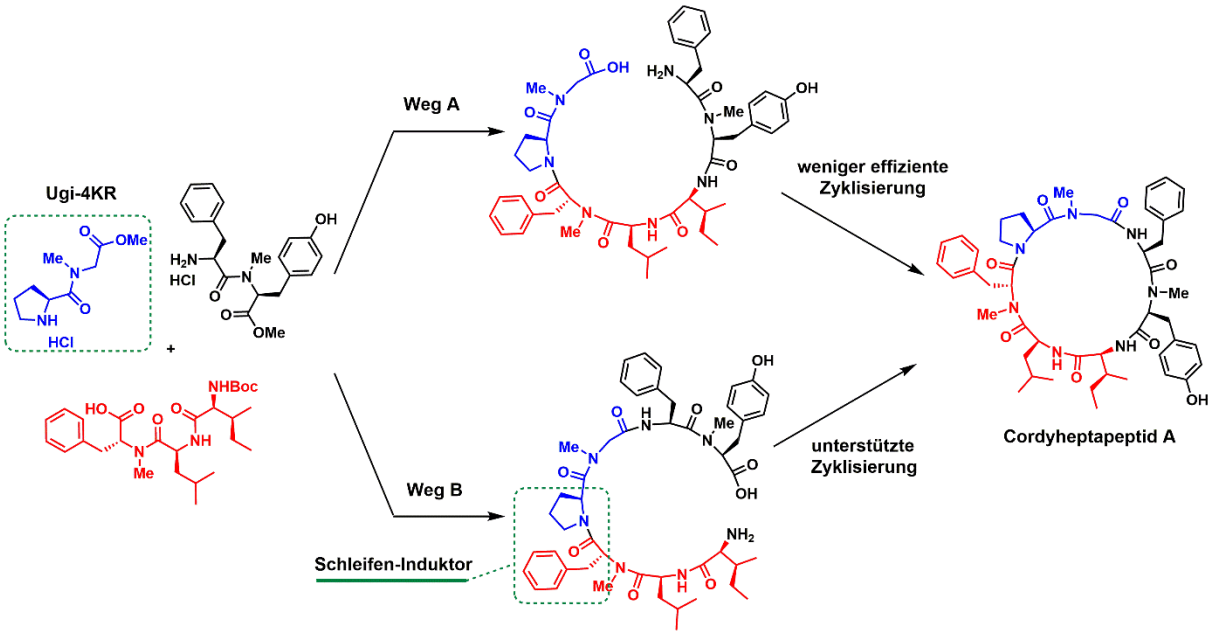
Scheme 5 Total synthesis of Crassipin B by the multicomponent Backbone Amide Linker (BAL) Strategy.

Zusammenfassung und Ausblick

Makrozyklische Peptide sind seit langem von Interesse für die Wissenschaftsgemeinschaft. Auch die pharmazeutische Industrie fand in diesen Molekülen eine Möglichkeit zur Eröffnung neuer Wege in der medizinischen Versorgung. Die Kopf-Schwanz-Makrozyklisierung stellt einen der Wege zur Herstellung von Molekülen dieser Klasse dar und ist oft ein kritischer Schritt während der Synthese. Der Einbau eines intramolekularen Ringkontraktionselementes ist ein effektiver Schritt um den entropischen Nachteil der Makrozyklisierung zu mindern und das gewünschte Produkt freizusetzen. Die vorliegende Arbeit beschreibt eine Strategie, die Wirksamkeit der Makrozyklisierung durch die Eingliederung eines Schleifen-induzierenden Elementes, welches in der Lage ist den Ringschluss zu erleichtern, zu erhöhen.
Kapitel 1 umreißt die relevantesten Aspekte der Makrozyklen und ihrer Anwendungen in der medizinischen Chemie. Außerdem präsentiert es einen Überblick über die grundlegenden Konzepte der Ugi-4KR, einschließlich einer Zusammenstellung über die Entwicklung von Isocyaniden und Amiden.

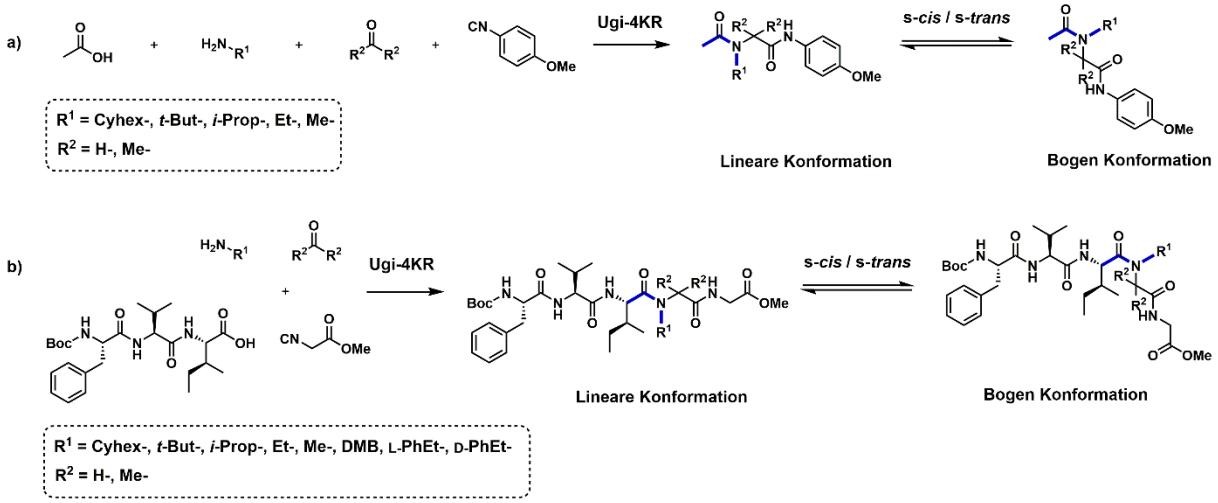
Kapitel 2 enthält den experimentellen Teil der Arbeit.

Im Kapitel 3 wird die erste Totalsynthese von Cordyheptapeptid A beschrieben. Die Synthese wird durch einen konvergenten Ansatz, der sich durch eine Kombination von Peptidkopplung und Ugi-4KR für die Synthese der Grundbausteine und azyklischen Vorstufen auszeichnet, erreicht. Die Herstellung eines *N*-methylierten Fragments durch die Ugi-4KR beinhaltete die Verwendung eines konvertierbaren Isocyanids, gefolgt von der Aktivierung des *C*-terminalen Amids. Es wurden zwei unterschiedliche Makrozyklisierungspositionen untersucht, wodurch die größere Wirksamkeit für die Makrolaktamisierung an der Ile-Tyr-Position deutlich wurde, die wahrscheinlich auf die günstige konformative Anordnung der azyklischen Vorstufe, die eine interne β-Schleife zentriert am *N*-Me-D-Phe-Pro-Rest besitzt, zurückzuführen war (**Schema 1**). Offenbar war das in Weg B synthetisierte, azyklische Vorläufermolekül entscheidend für die Wirksamkeit der Makrozyklisierung, was zur Wahl der Makrolaktamisierungsstelle gegenüberliegend einer internen β-Schleife führte (**Weg B**, **Schema 1**). Der Ansatz erlaubt die Herstellung der Grundbausteine, die in rationaler Art und Weise verbunden wurden um den Naturstoff in einer endgültigen Ausbeute von 30 % zu liefern, im Gramm-Maßstab. Der Ansatz könnte dafür genutzt werden durch die Variation der Carboxyl-, Amino- und Oxo-Komponenten eine Bibliothek aus Cordyheptapeptid-Analogen zu synthetisieren, was eine Analyse der Beziehung zwischen Struktur und Aktivität ermöglichen würde.



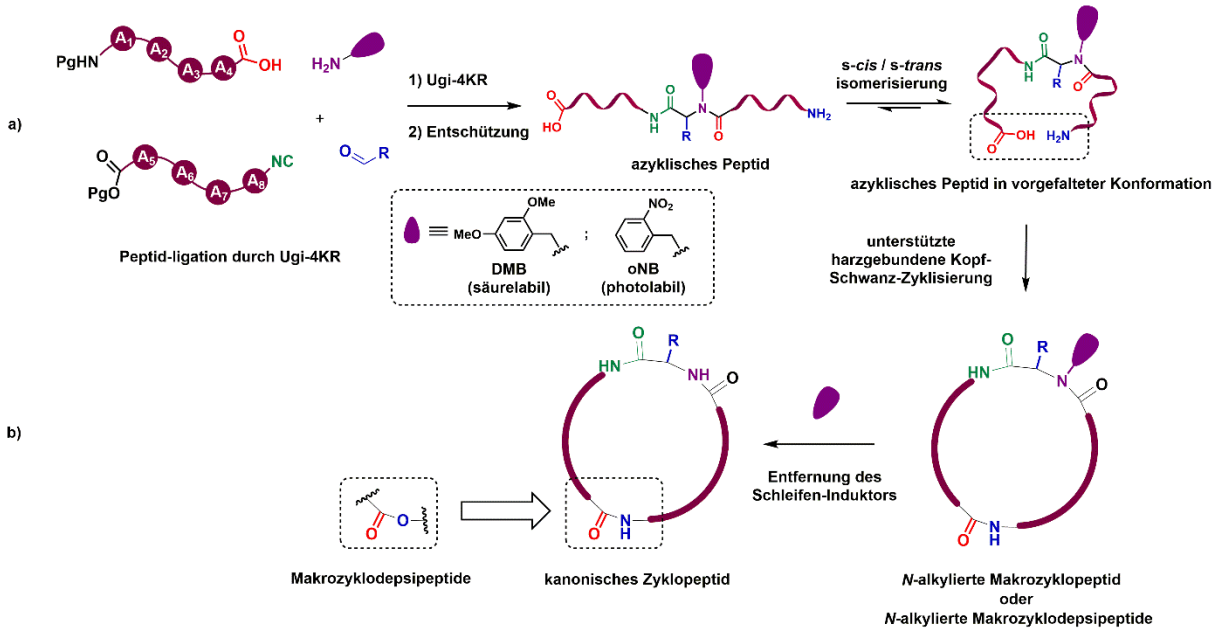
Schema 1 Die beiden Wege der Cordyheptapeptid A-Synthese.

Kapitel 4 beschreibt mittels zweier Modellpeptiden eine Untersuchung des konformativen s-*cis*/s-*trans* Equilibriums an der Peptidbindung nahe der *N*-alkylierten Position. Die unterschiedlichen Modifikationen der zwei *N*-alkylierten Modellpeptide wurden durch die Ugi-4KR erreicht. Die NMR-Analyse der 2,4-Dimethoxybenzylderivate zeigte ein signifikantes Vorhandensein der Bogen-Konformation in Di- und Pentapeptiden (**Schema 2**). Dieses Ergebnis wurde für das Design der Schleifen-Induktor-Strategie in Kapitel 5 und 6 in Betracht gezogen. Der Ansatz ist flexibel genug um mehrere *N*-Substituenten in das Peptidgrundgerüst einzubauen um deren Einfluss auf die s-*cis*/s-*trans* Isomerisierung der Amidbindung zu untersuchen.



Schema 2 Synthese verschiedener *N*-substituierter Peptide durch die Ugi-4KR um die s-*cis*/s-*trans* Isomerisierung der Amidbindung zu untersuchen.

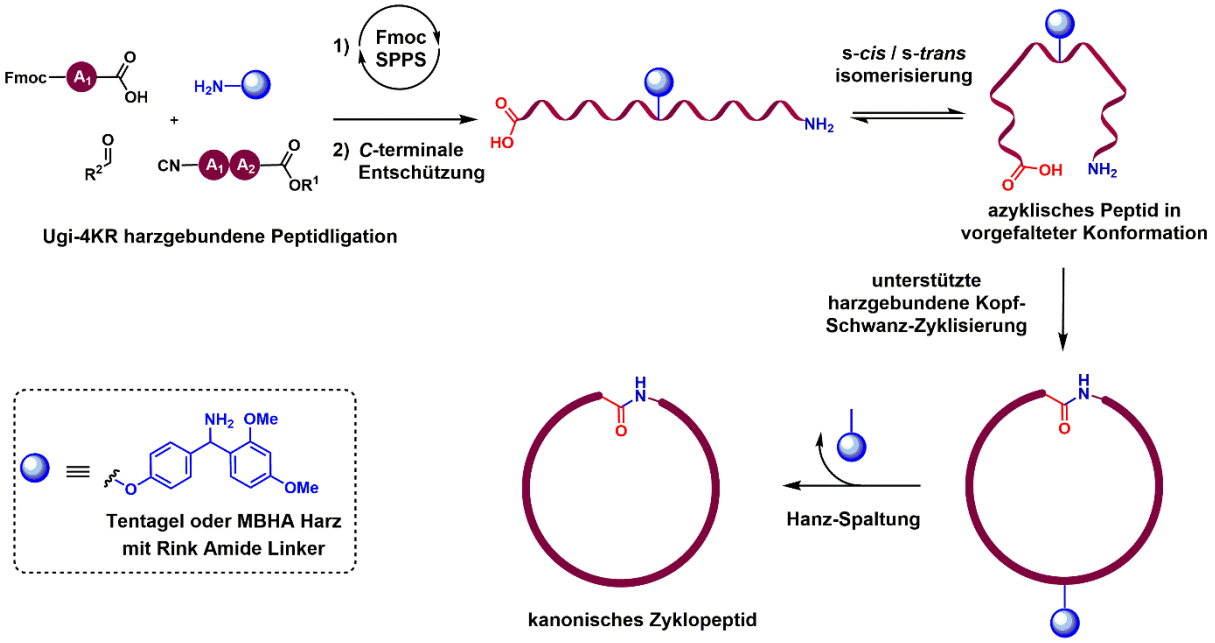
Kapitel 5 beschreibt eine neue Strategie für die Flüssigphasen-Makrozyklisierung von Peptiden, unterstützt durch „rückstandlose“ Schleifen-Induktoren. Der Ansatz umfasst die Ligation einer Peptidcarboxylsäure und eines Isocyanopeptids durch die Ugi-4KR unter Verwendung eines spaltbaren Amins, was ein Peptid entstehen lässt, welches eine *N*-alkylierte Aminosäure in der Sequenzmitte aufweist (**Schema 3**). Der eingebaute, ablösbare *N*-alkyl-Substituent dient als Schleifen-induzierender Baustein und erleichtert den Ringschluss. Die Erleichterung der Makrozyklisierung durch die Schleifen-Induktion wurde mittels einer Auswahl von Tetra- und Heptapeptiden belegt. Außerdem wurde die unterstützte Makrozyklisierung für die Synthese von zyklischen Depsipeptiden verwendet. Diese neue Hilfsstrategie für den Ringschluss könnte als Zugang für schwer zu zyklisierende Peptide dienen, hin zu einer Erweiterung des Repertoires an synthetisch verfügbaren, zyklischen Peptiden und Peptidomimetika, sowie zur Bereitstellung neuer biologischer Tester und Therapeutika.



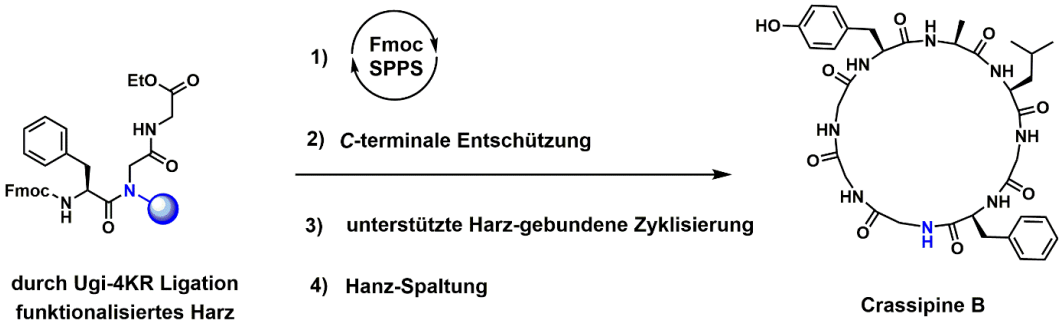
Schema 3 Kopf-Schwanz Festphasen-Makrozyklisierung von Peptiden unterstützt von „rückstandslosen“ Schleifen-Induktoren. a) Der Schleifen-Induktor kontrolliert das Equilibrium zwischen der linearen, azyklischen und der vorgefalteten Konformation. b) Spaltung des „rückstandslosen“ Schleifen-Induktors nach der Makrozyklisierung um ein kanonisches Makrozyklopeptid oder Depsipeptide zu generieren.

Der Fokus des Kapitels 6 liegt auf dem Ausbau der Multikomponenten-Strategie zur Synthese von Peptiden mit Amid-verlinktem Grundgerüst (BAL) und ihrer anschließenden Derivatisierung durch die Peptidkettenverlängerung und finaler Zyklisierung. Diese neue BAL-Strategie beruht erstmals auf einer Multikomponentenkombination von mindestens drei Aminosäuren in einem Schritt, anstelle der drei harzgebundenen Schritte, die in herkömmlichen BAL-Protokollen für den Einbau des Dipeptid-Fragments nötig sind (**Schema 4**). Solange wenigstens ein Tripeptid im ersten Schritt mit dem Harz verbunden ist,

kann der schwierige Acylierungsschritt des zweiten Amins umgangen und die Bildung von Diketopiperazin, welche häufig in BAL-Protokollen mit der Nutzung einer Fmoc-Entschützung im Dipeptid-Stadium auftritt, vermieden werden. Die Reichweite dieser Multikomponenten-BAL-Strategie wurde mittels Verwendung von Oligopeptiden aus vier bis zu sieben Aminosäuren beurteilt. Außerdem führten wir die Totalsynthese des Naturstoffs Crassipin B durch (**Schema 5**). Das zyklische Heptapeptid wurde durch den initialen Multikomponenten-Einbau von Fmoc-Phe-OH und Isocyanoacetat in das Harz, gefolgt von der Peptidverlängerung durch die Fmoc-Strategie und aufeinanderfolgender Makrozyklisierung und Säurespaltung vom Harz, mit einer Gesamtausbeute von 53 % produziert. Aufgrund seiner synthetischen Projektionen kann dieses Konzept für die Peptid-, die kombinatorische- und die Gemeinschaft der medizinischen Chemie von großem Nutzen sein.



Schema 4 Multikomponenten-Strategie zur Synthese von Peptiden mit Amid-verlinktem Grundgerüst (BAL) und ihre schrittweise Derivatisierung durch Peptidverlängerung und finaler Zyklisierung.



Schema 5 Totalsynthese von Crassipin B durch die Multikomponenten-Strategie mit Amid-verlinktem Peptid-Grundgerüst.

Attachments

S1 – ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of compound **66** (Chapter 3).

S2 – ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of compound **66** (Chapter 3).

S3 – gDQCOSY (600 MHz, $CDCl_3$) spectrum of compound **66** (Chapter 3).

S4 – gHSQCAD (150 MHz, $CDCl_3$) spectrum of compound **66** (Chapter 3).

S5 – HPLC chromatogram of compound **59** (Chapter 3).

S6 – HPLC chromatograms related to the table 3.2 (Chapter 3).

S7 – ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **74** (Chapter 4).

S8 – ^{13}CNMR (100 MHz, DMSO-d_6) spectrum of compound **74** (Chapter 4).

S9 – ROESY (600 MHz, DMSO-d_6) spectrum of compound **74** (Chapter 4).

S10 – ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of compound **81** (Chapter 4).

S11 – ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of compound **81** (Chapter 4).

S12 – ROESY (600 MHz, DMSO-d_6) spectrum of compound **81** (Chapter 4).

S13 – ^{1}H NMR (400 MHz, CD_3OD) spectrum of compound **103** (Chapter 5).

S14 – ^{13}C NMR (100 MHz, CD_3OD) spectrum of compound **103** (Chapter 5).

S15 – ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **104** (Chapter 5).

S16 – ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **104** (Chapter 5).

S17 – ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **108** (Chapter 5).

S18 – ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **108** (Chapter 5).

S19 – ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **113** (Chapter 5).

S20 – ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **113** (Chapter 5).

S21 –^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **123** (Chapter 5)

S22 – ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **123** (Chapter 5).

S23 – RP-UHPLC chromatogram and ESI-HRMS of H-Phe-Gly-Gly-OH after resin cleavage with 82% purity from the crude reaction (Chapter 6).

S24 – RP-UHPLC chromatogram after resin cleavage and ESI-HRMS of compound **130** and **131** (Chapter 6).

S25 – Positive ion HR-ESI-MS^2 spectrum of $[M+H]^+$ of cyclic peptide **130** (monomer) and **131** (dimer) (Chapter 6).

S26 – ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **130** (Chapter 6).

S27 –^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **130** (Chapter 6).

S28 –^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **131** (Chapter 6).

S29 – ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **131** (Chapter 6).

S30 – ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **133** (Chapter 6).

S31 – ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **133** (Chapter 6).

S32 – RP-UHPLC chromatogram after resin cleavage and ESI-HRMS of compound **134** (Chapter 6).

S33 – ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **134** (Chapter 6).

S34 – ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **134** (Chapter 6).

S35 – RP-UHPLC chromatogram and ESI-HRMS of compound **135** (Chapter 6).

S36 – ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **135** (Chapter 6).

S37 – ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **135** (Chapter 6).

S38 – RP-UHPLC chromatogram and ESI-HRMS of compound **136** (Chapter 6).

S39 – ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **136** (Chapter 6).

S40 – ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **136** (Chapter 6).

S41 – RP-UHPLC chromatogram and ESI-HRMS of compound **141** (Chapter 6).

S42 –^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **141** (Chapter 6).

S43 – ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **141** (Chapter 6).

S44 – Curriculum Vitae and list of publication.

S45 – Declaration (Erklärung).

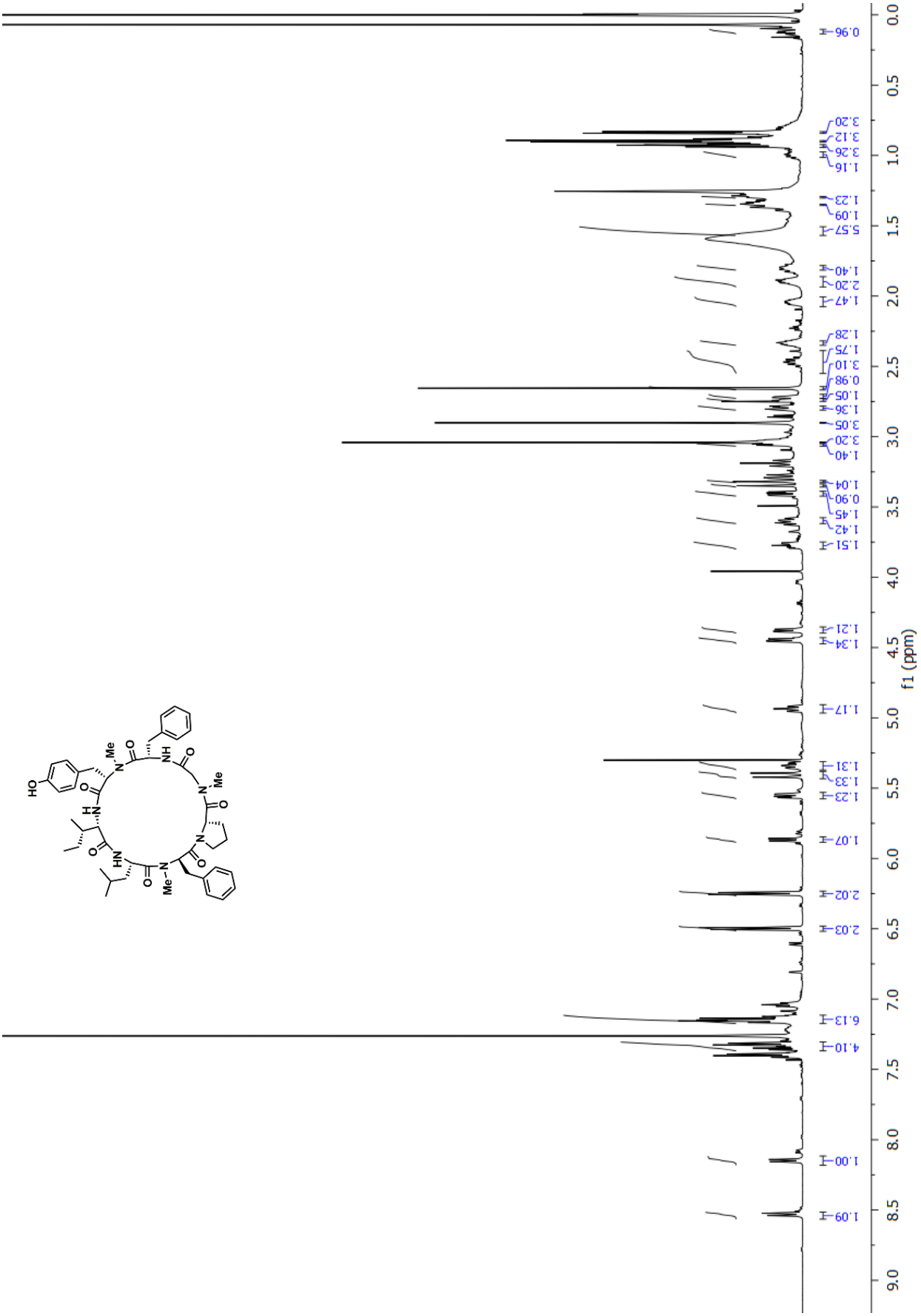


Figure S1 – ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of compound **66** (Chapter 3).

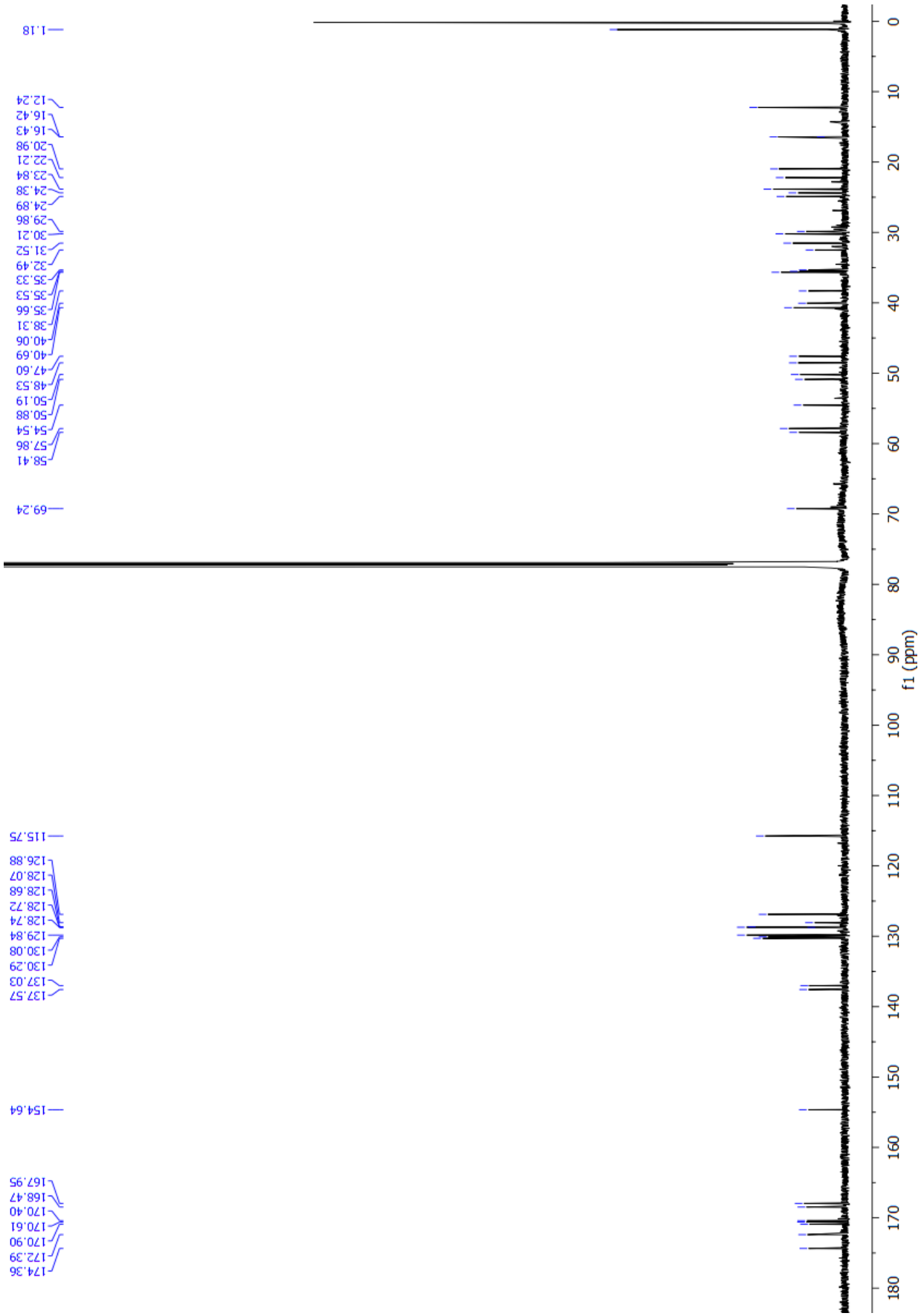


Figure S2 – ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of compound **66** (Chapter 3).

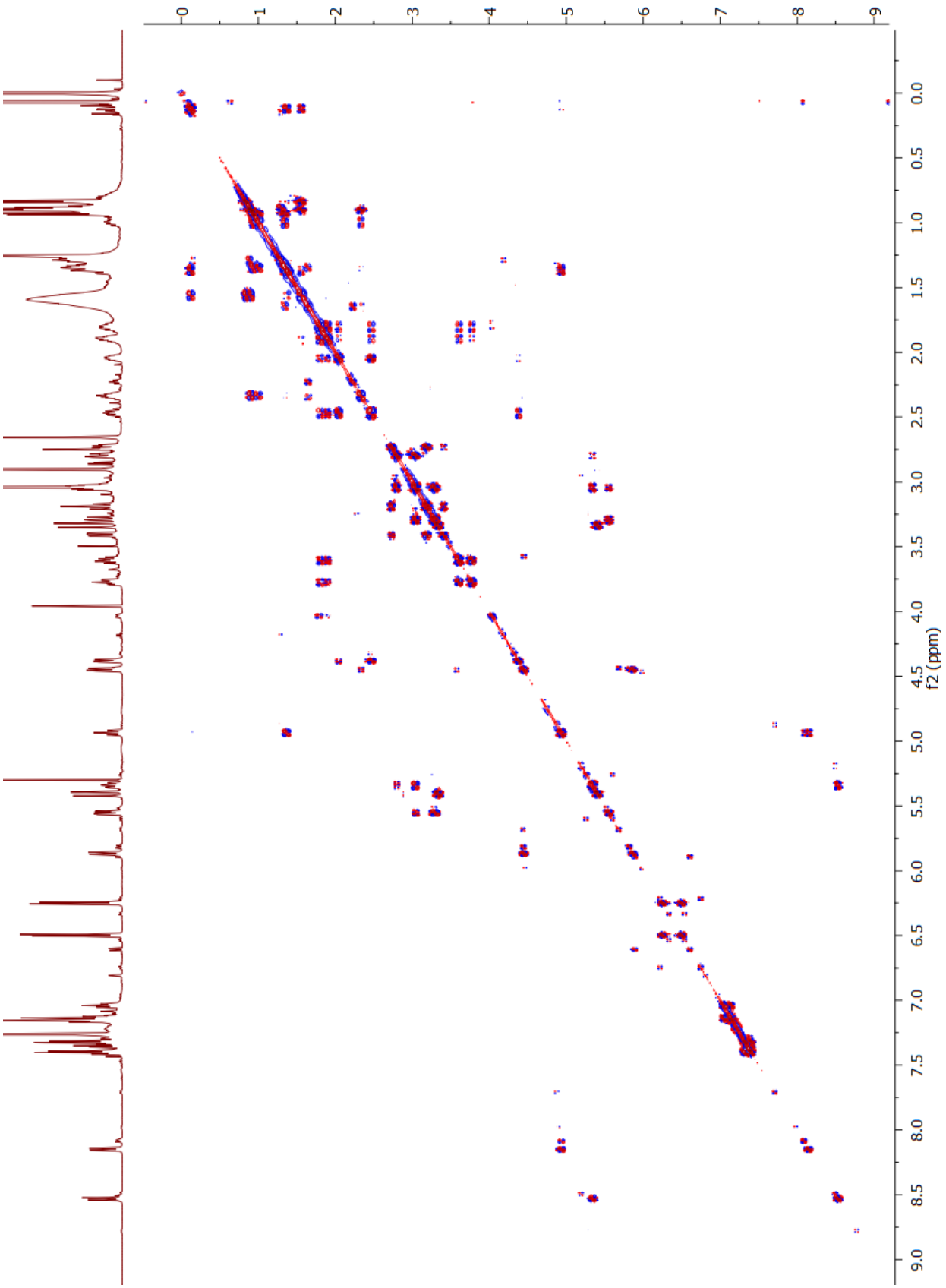


Figure S3 – gDQCOSY (600 MHz, $CDCl_3$) spectrum of compound **66** (Chapter 3).

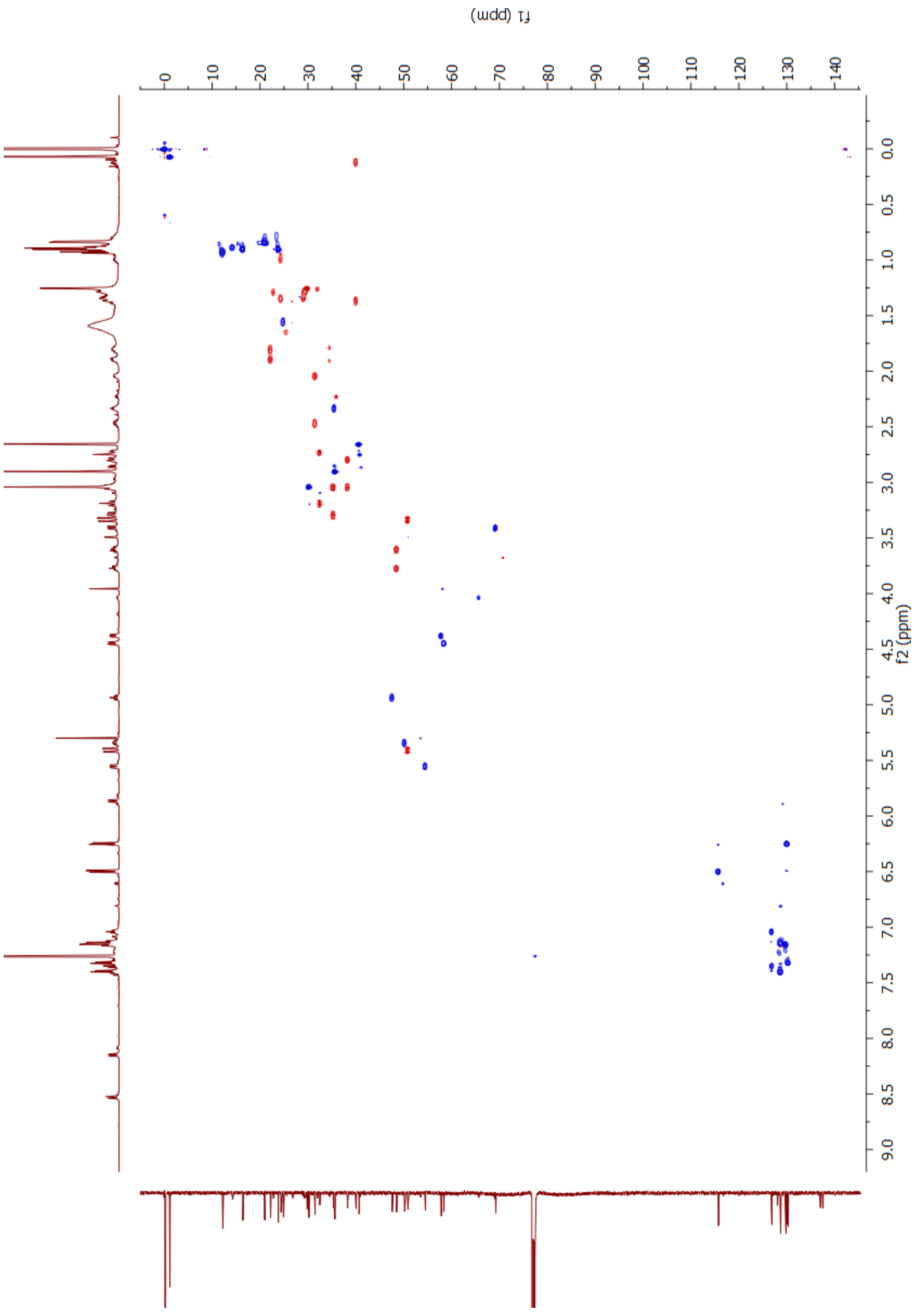


Figure S4 – gHSQCAD (150 MHz, $CDCl_3$) spectrum of compound **66** (Chapter 3).

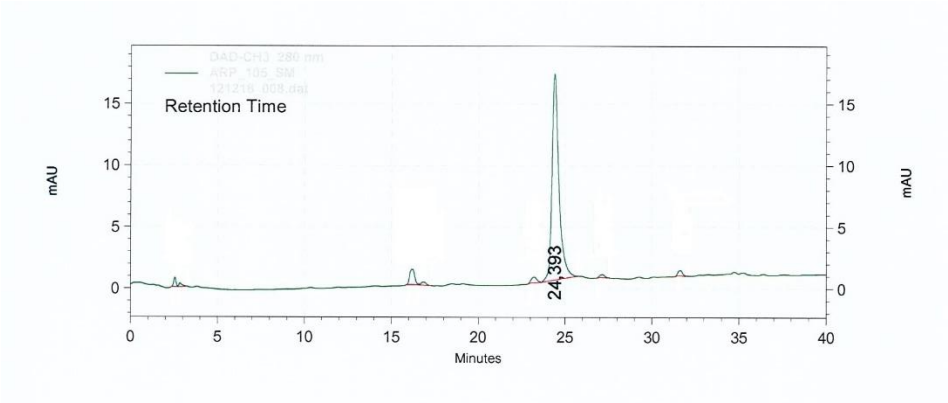


Figure S5 – HPLC chromatogram of compound **59** (Chapter 3).

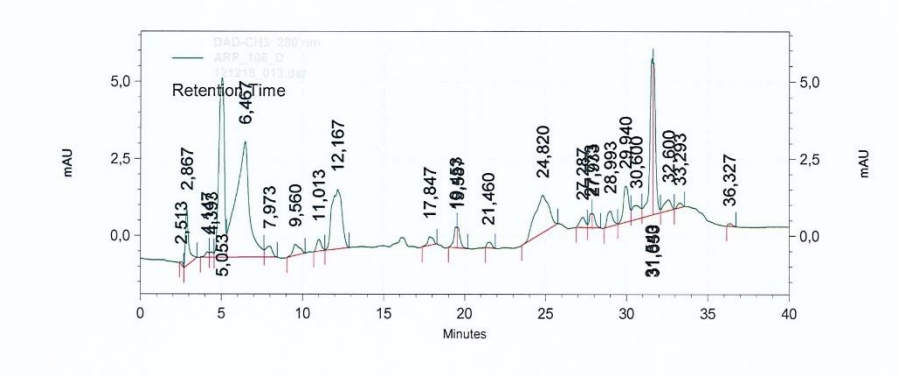
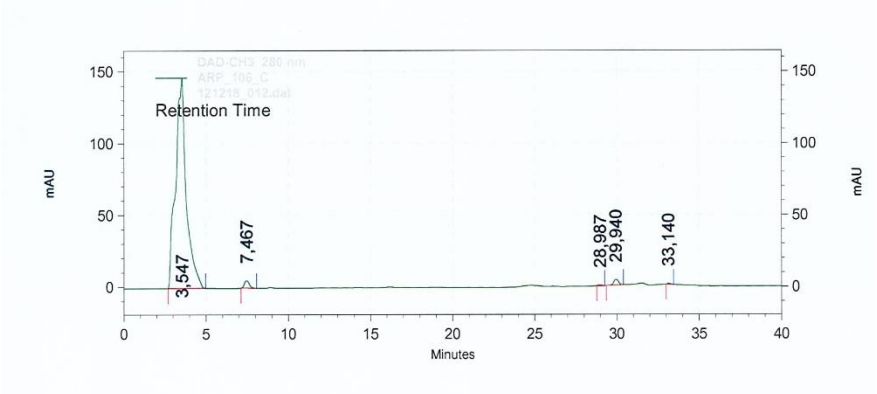
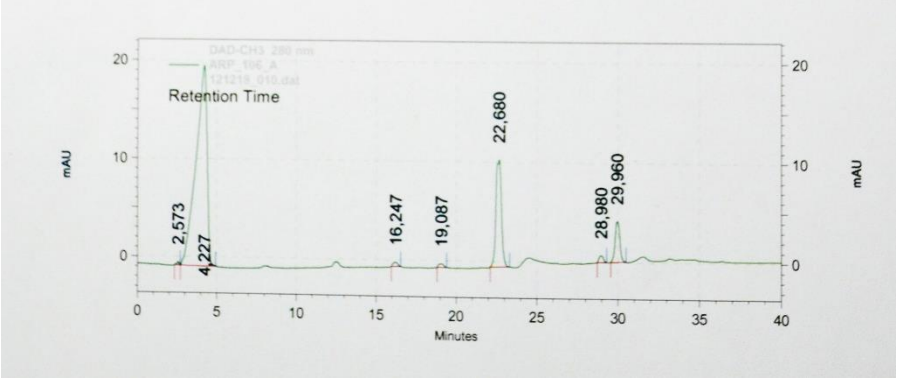
reaction D	
reaction C	
reaction A	

Figure S6 – HPLC chromatograms related to the table 3.2 (Chapter 3).

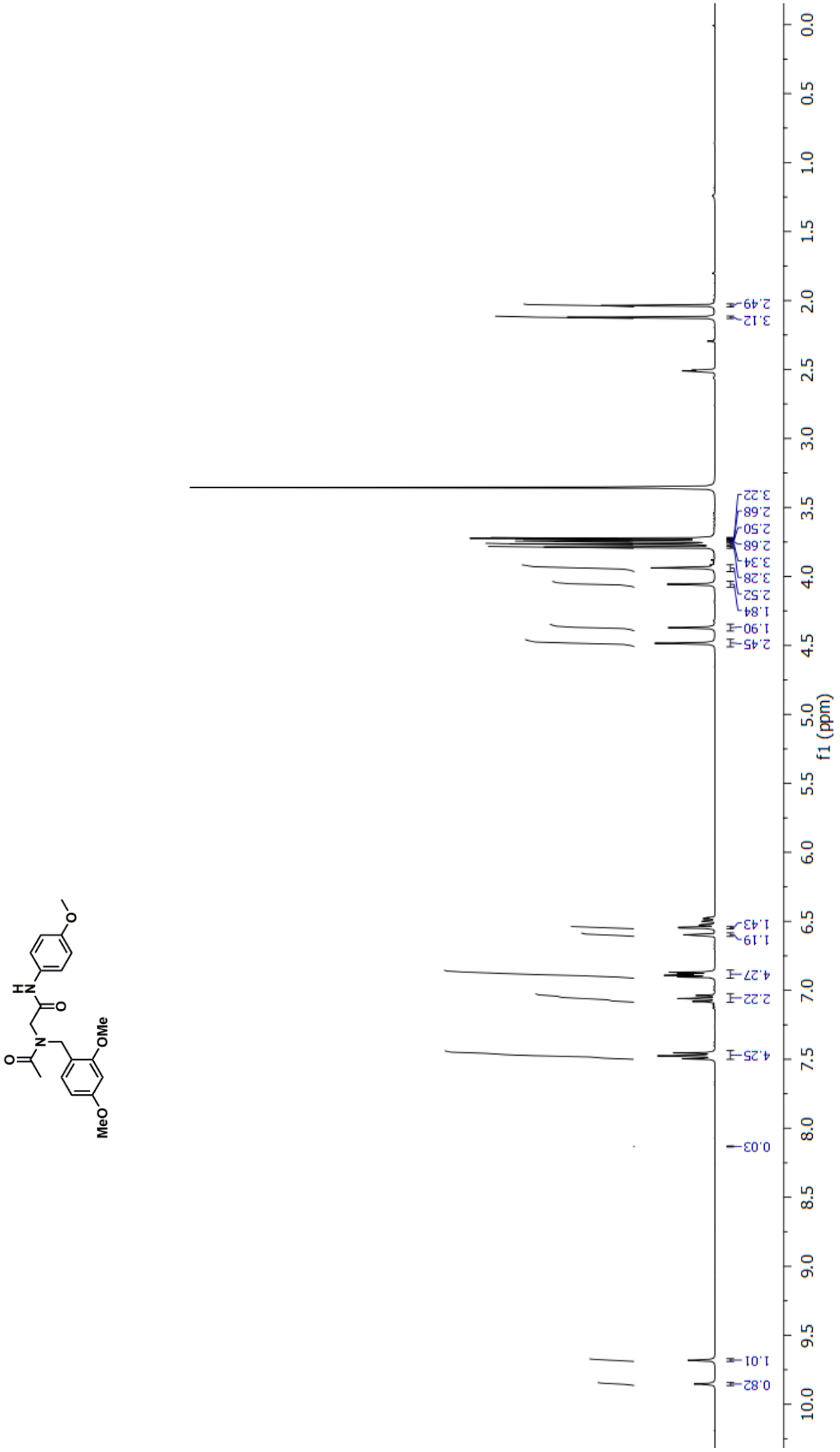


Figure S7 – ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **74** (Chapter 4).

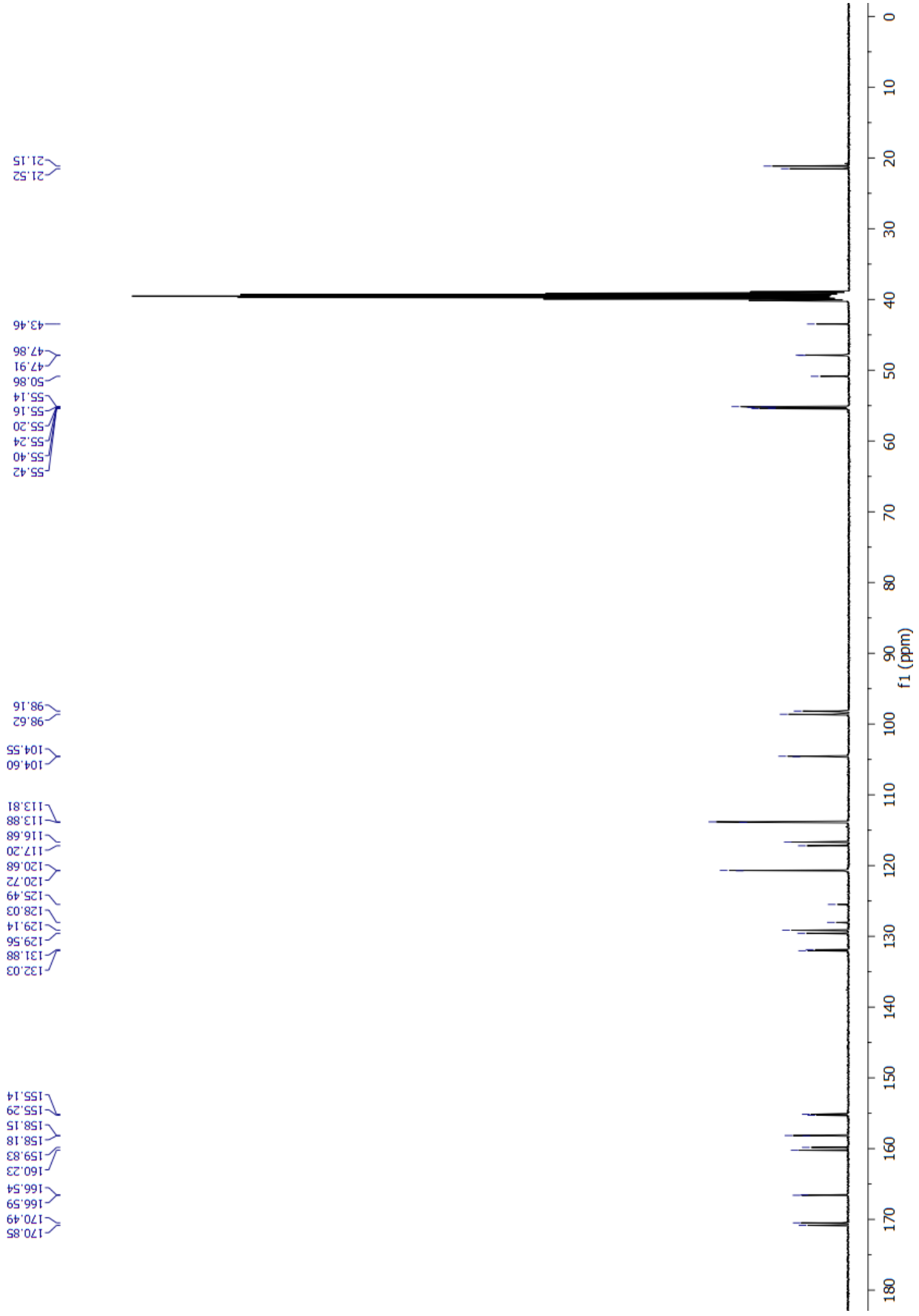


Figure S8 – ^{13}CNMR (100 MHz, DMSO-d_6) spectrum of compound **74** (Chapter 4).

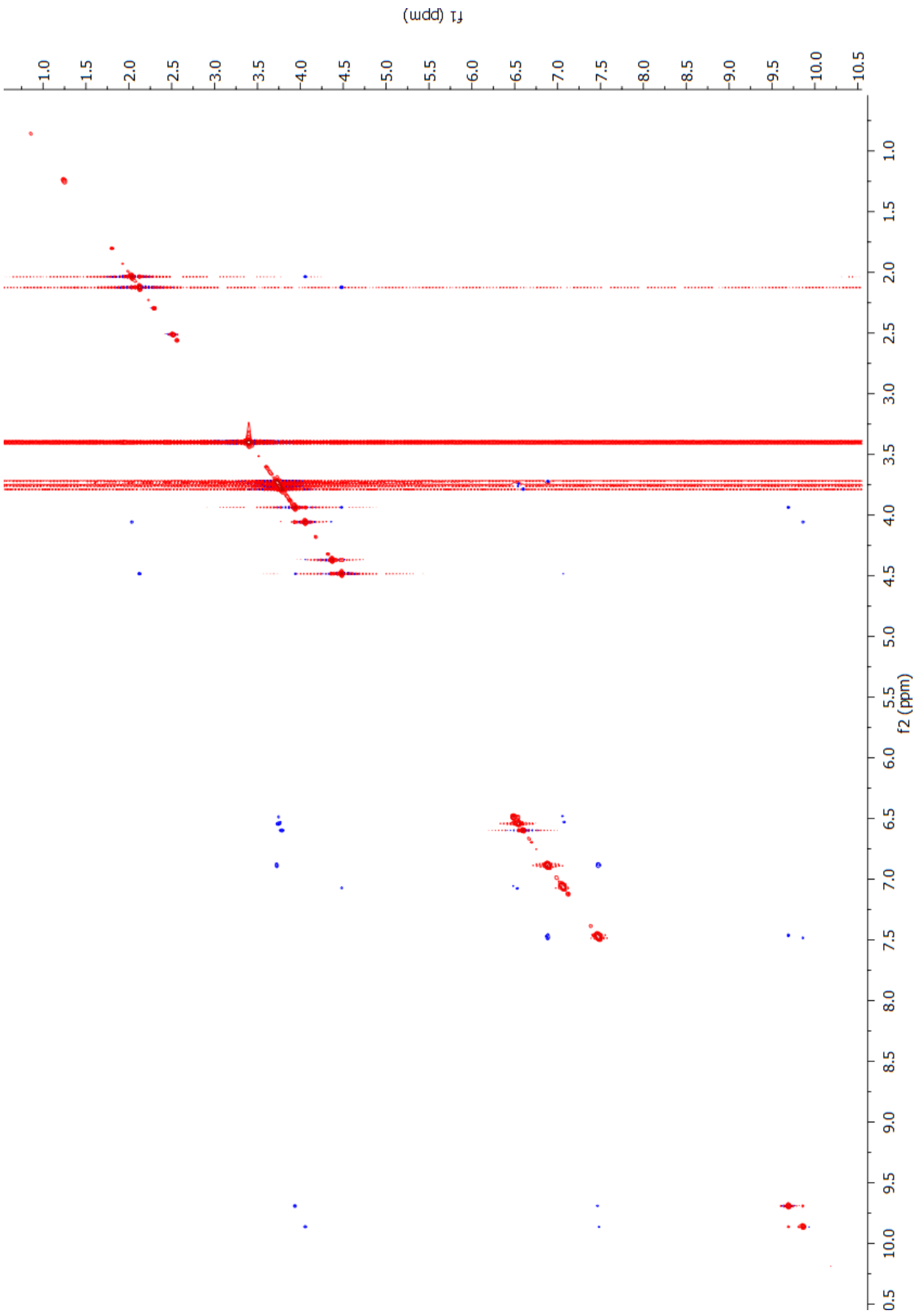


Figure S9 – ROESY (600 MHz, DMSO-d_6) spectrum of compound **74** (Chapter 4).

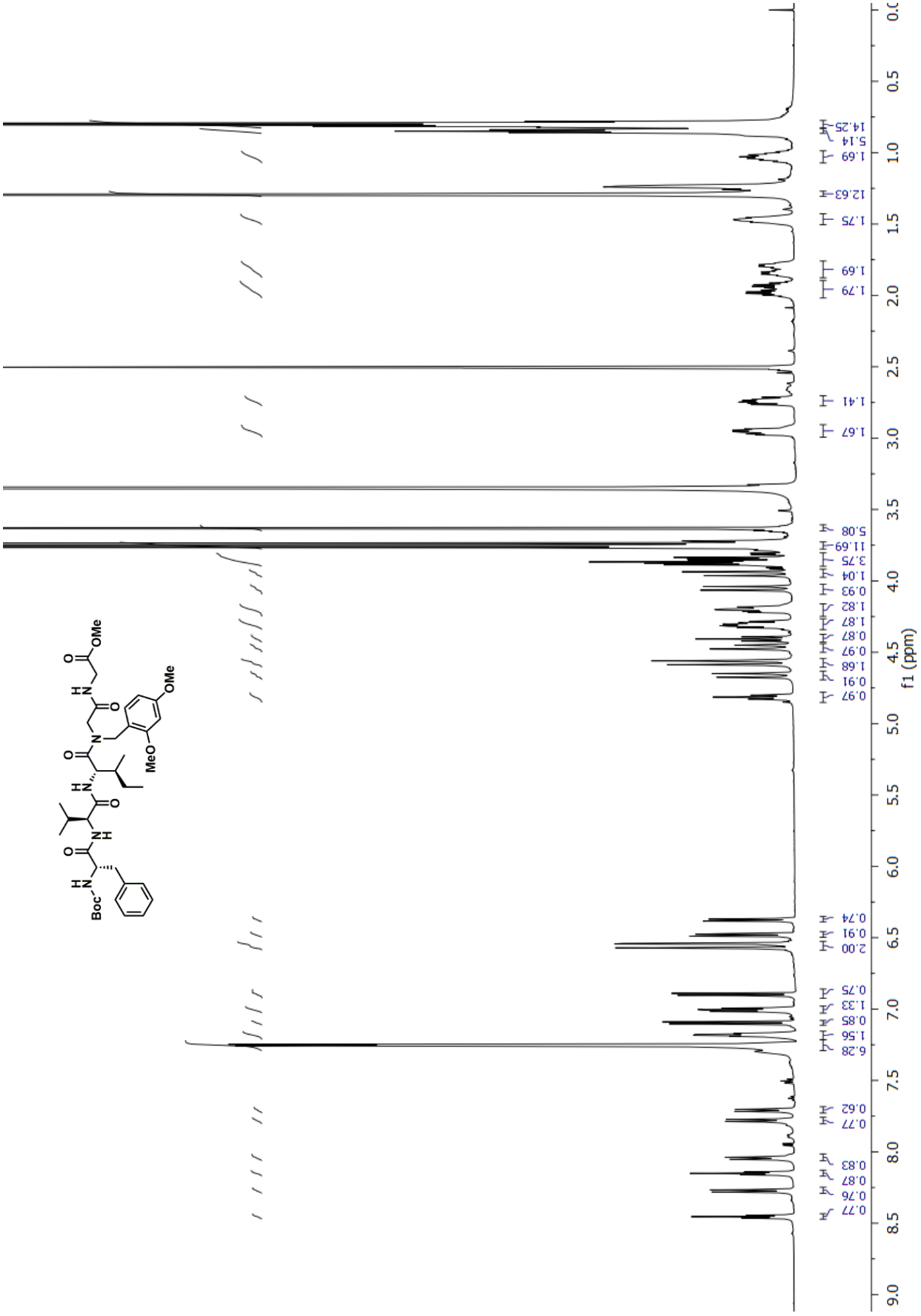


Figure S10 – ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of compound **81** (Chapter 4).

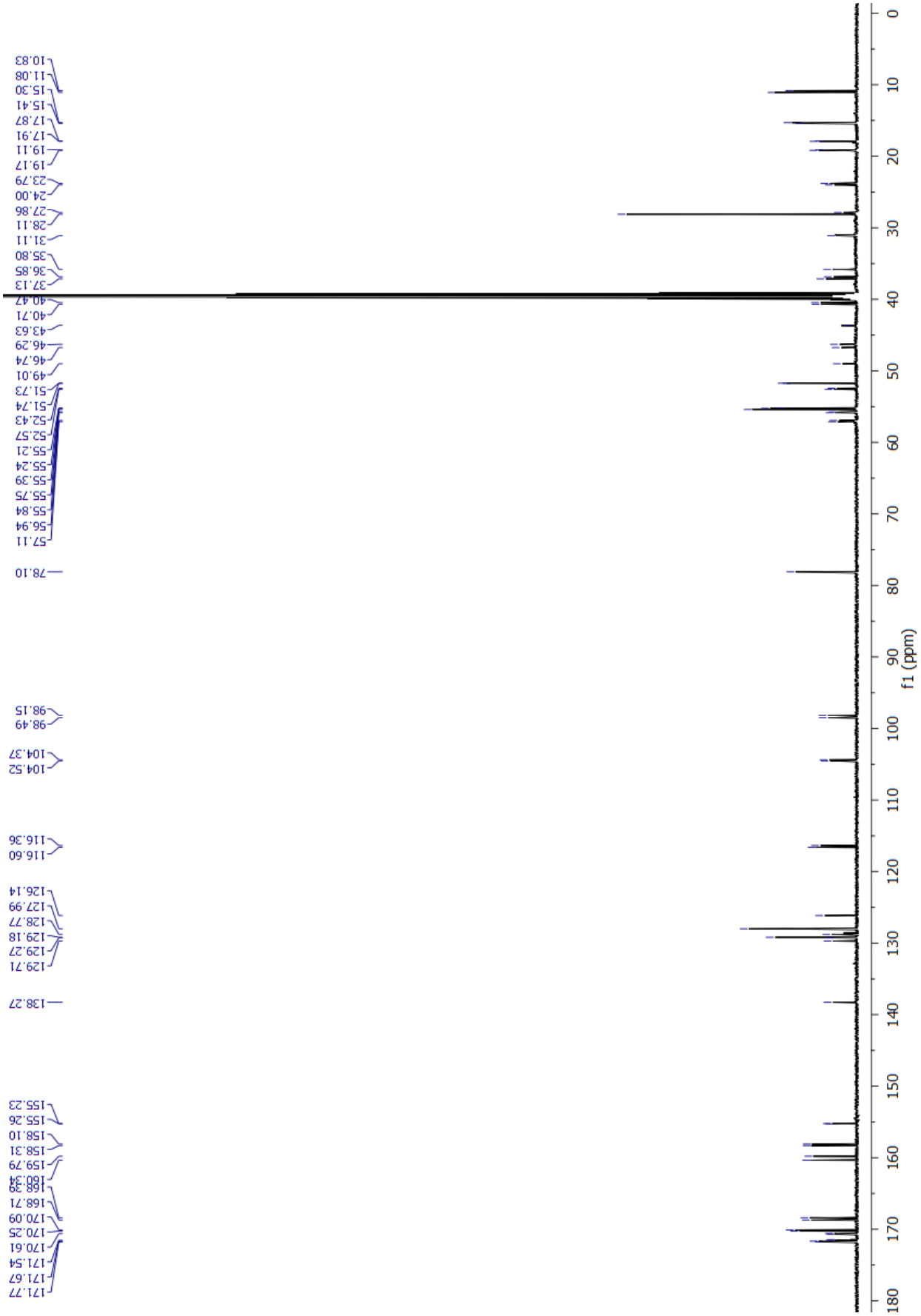


Figure S11 – ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of compound **81** (Chapter 4).

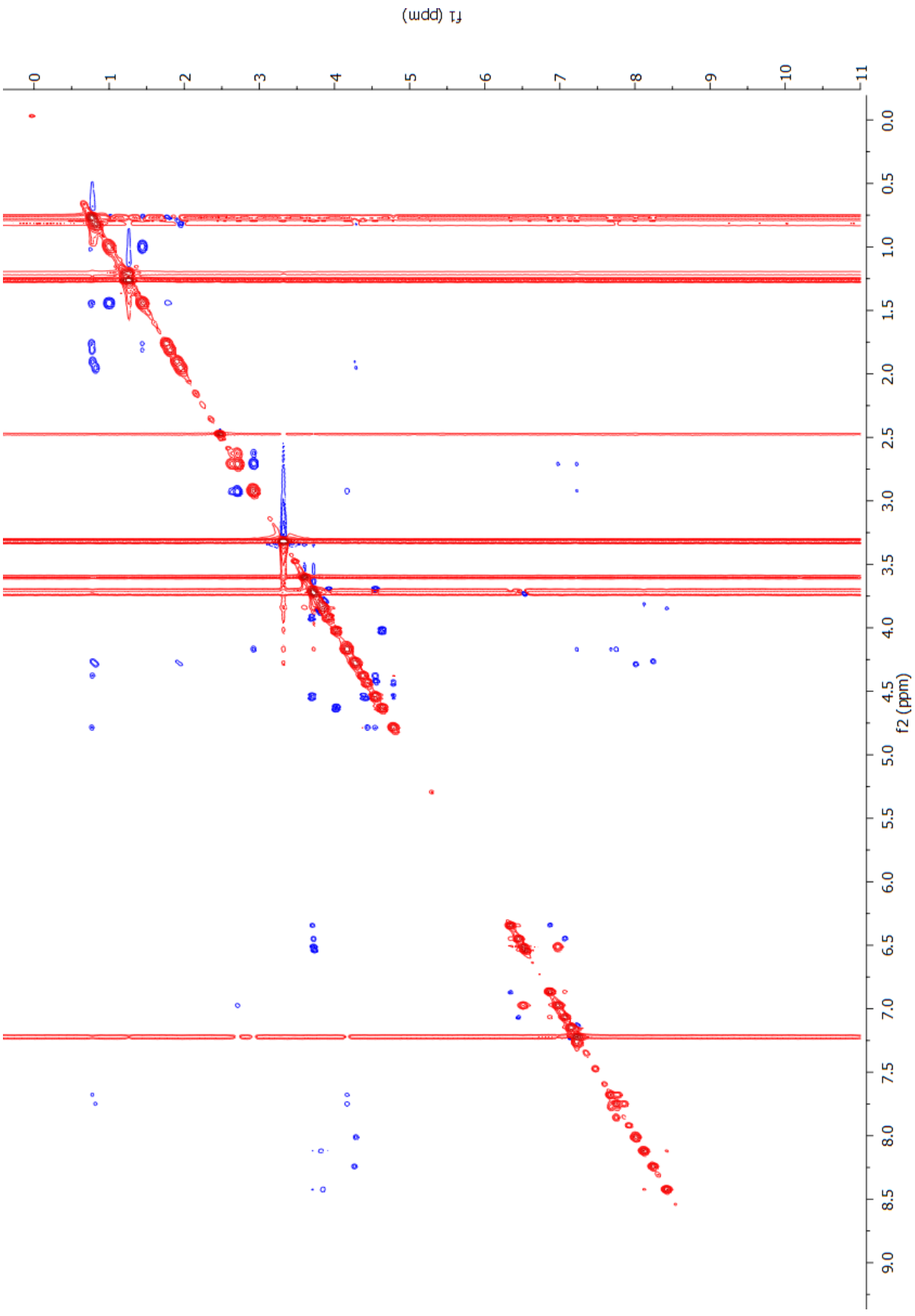


Figure S12 – ROESY (600 MHz, DMSO-d_6) spectrum of compound **81** (Chapter 4).

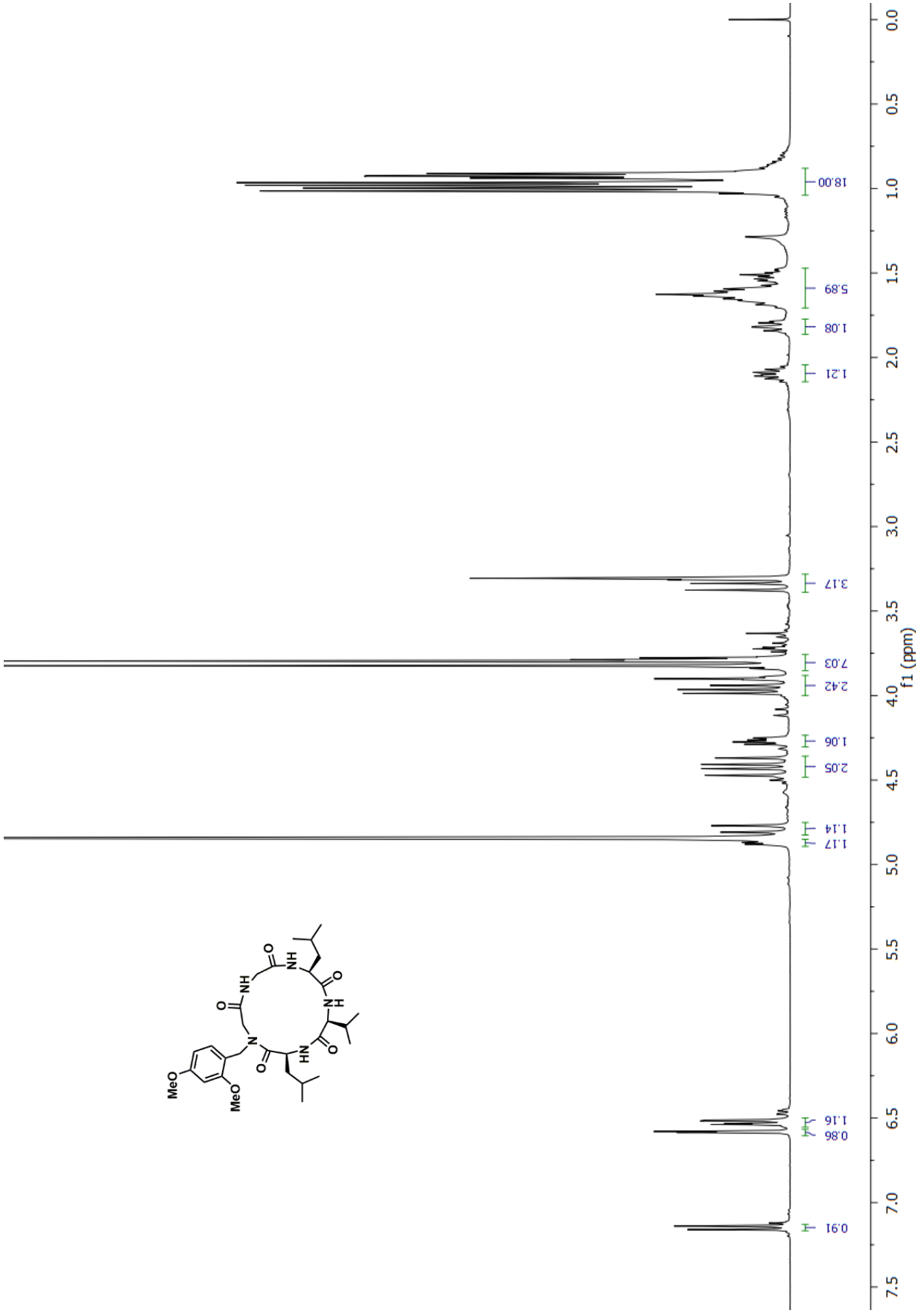


Figure S13 – ^{1}H NMR (400 MHz, CD_3OD) spectrum of compound **103** (Chapter 5).

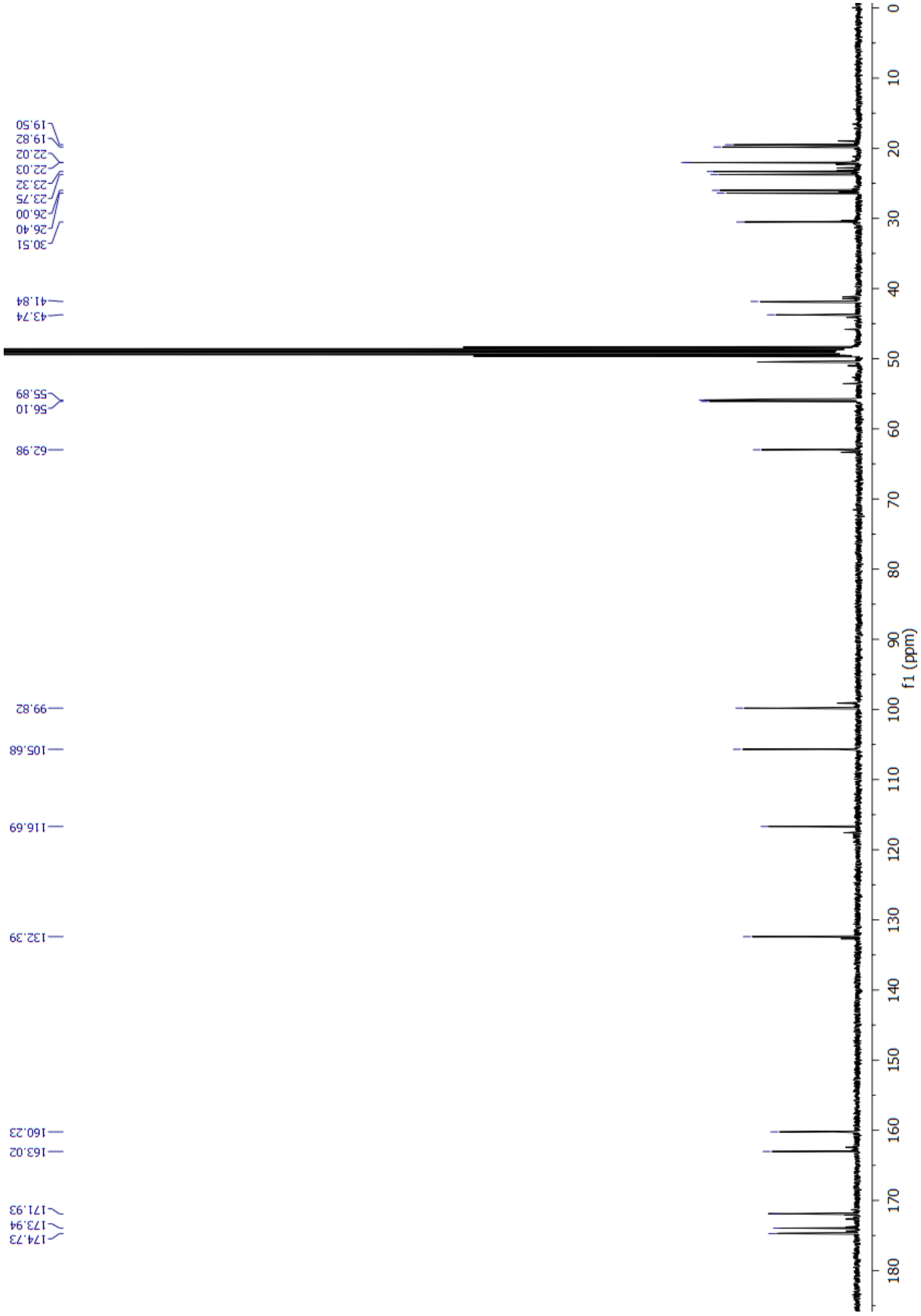


Figure S14 – ^{13}C NMR (100 MHz, CD_3OD) spectrum of compound **103** (Chapter 5).

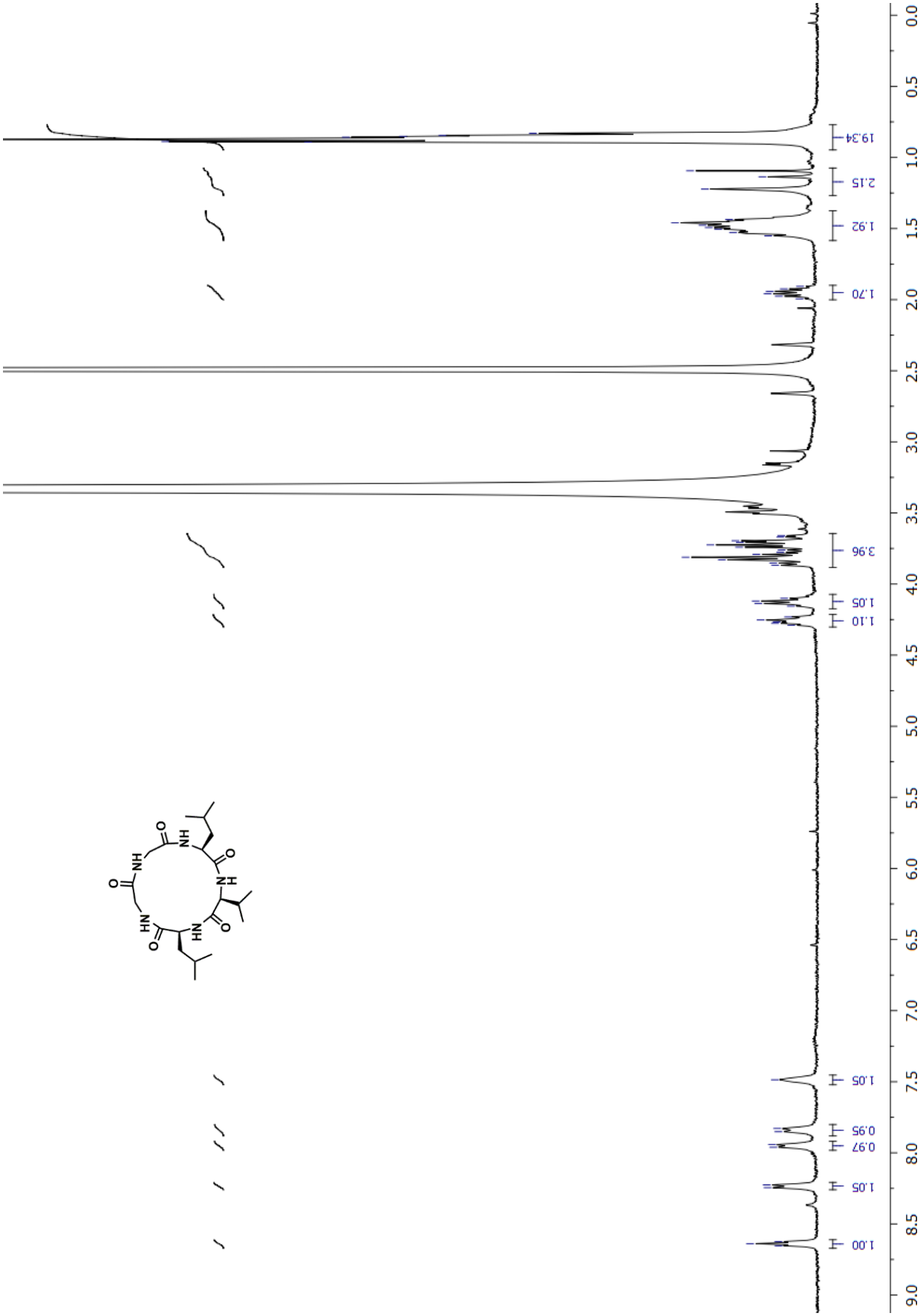


Figure S15 – ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **104** (Chapter 5).

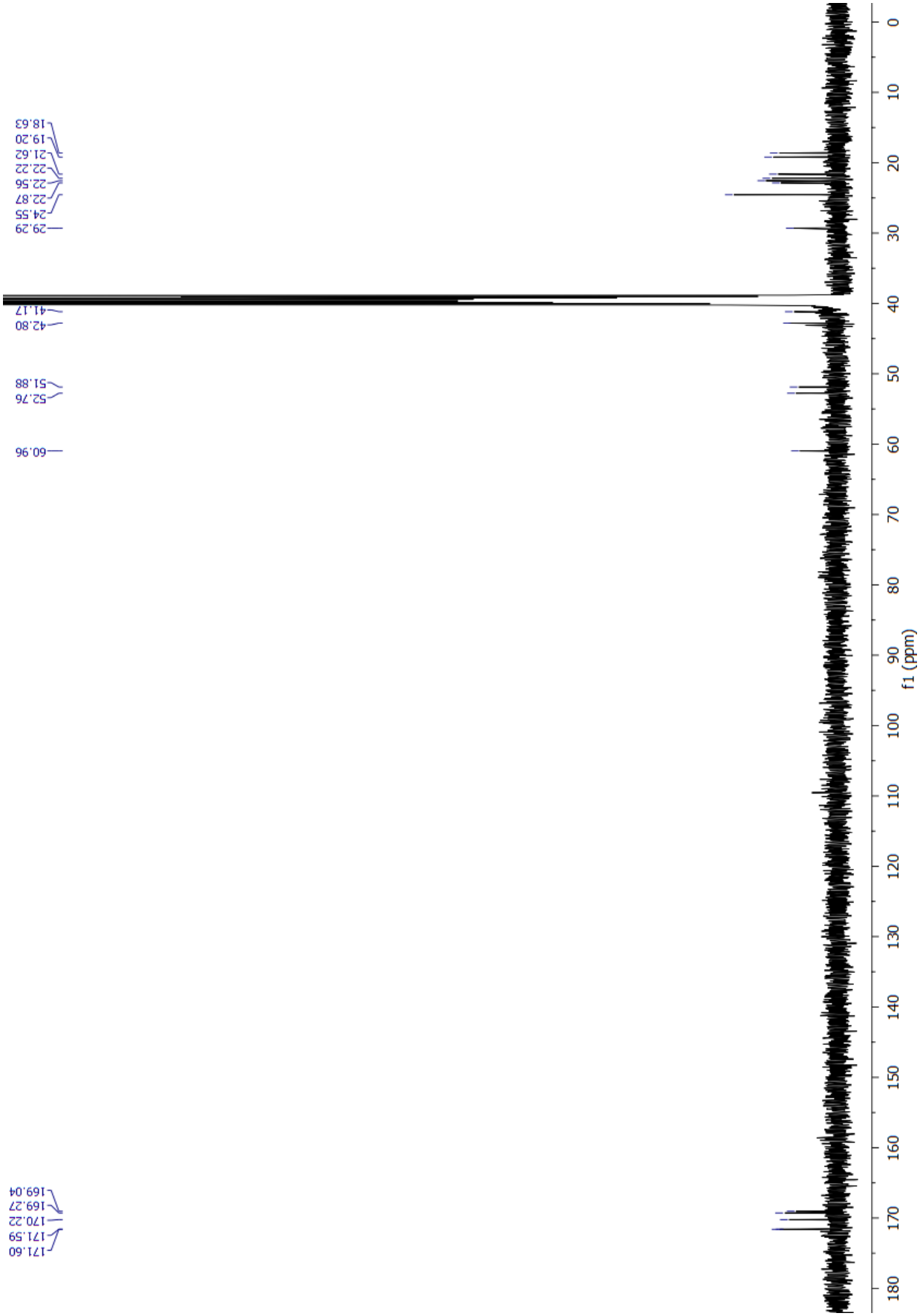


Figure S16– ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **104** (Chapter 5).

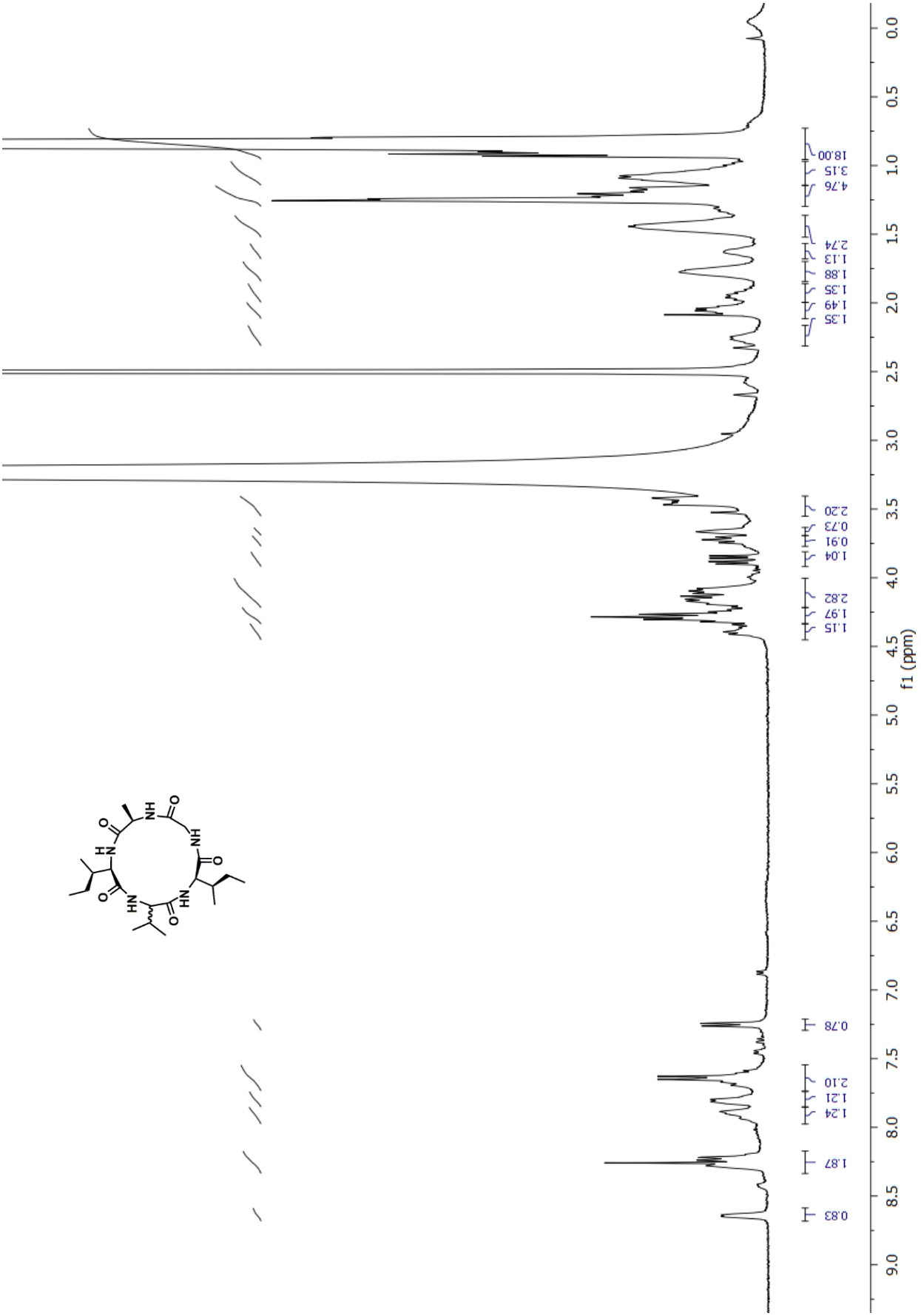


Figure S17 – ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **108** (Chapter 5).

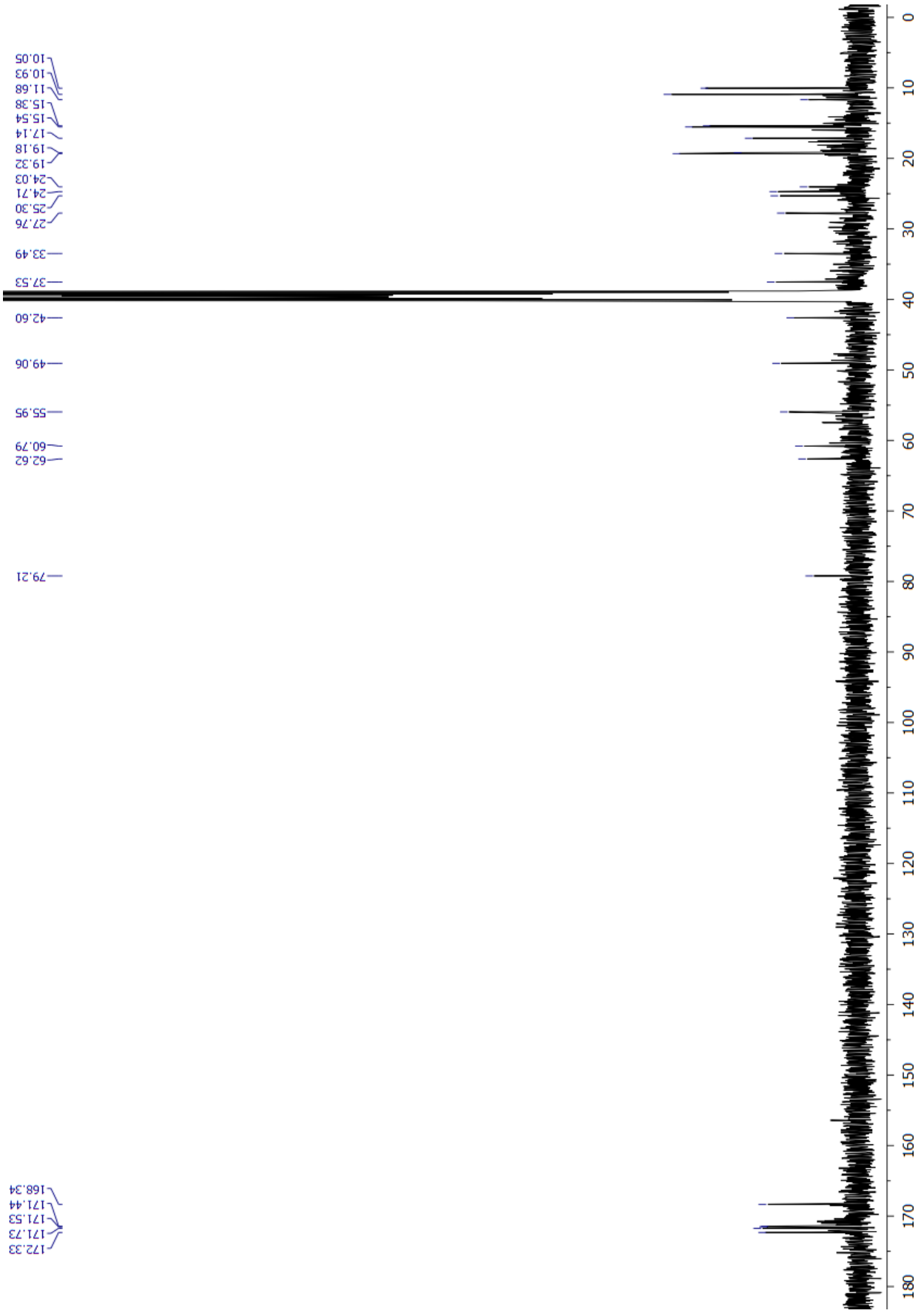


Figure S18 – ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **108** (Chapter 5).

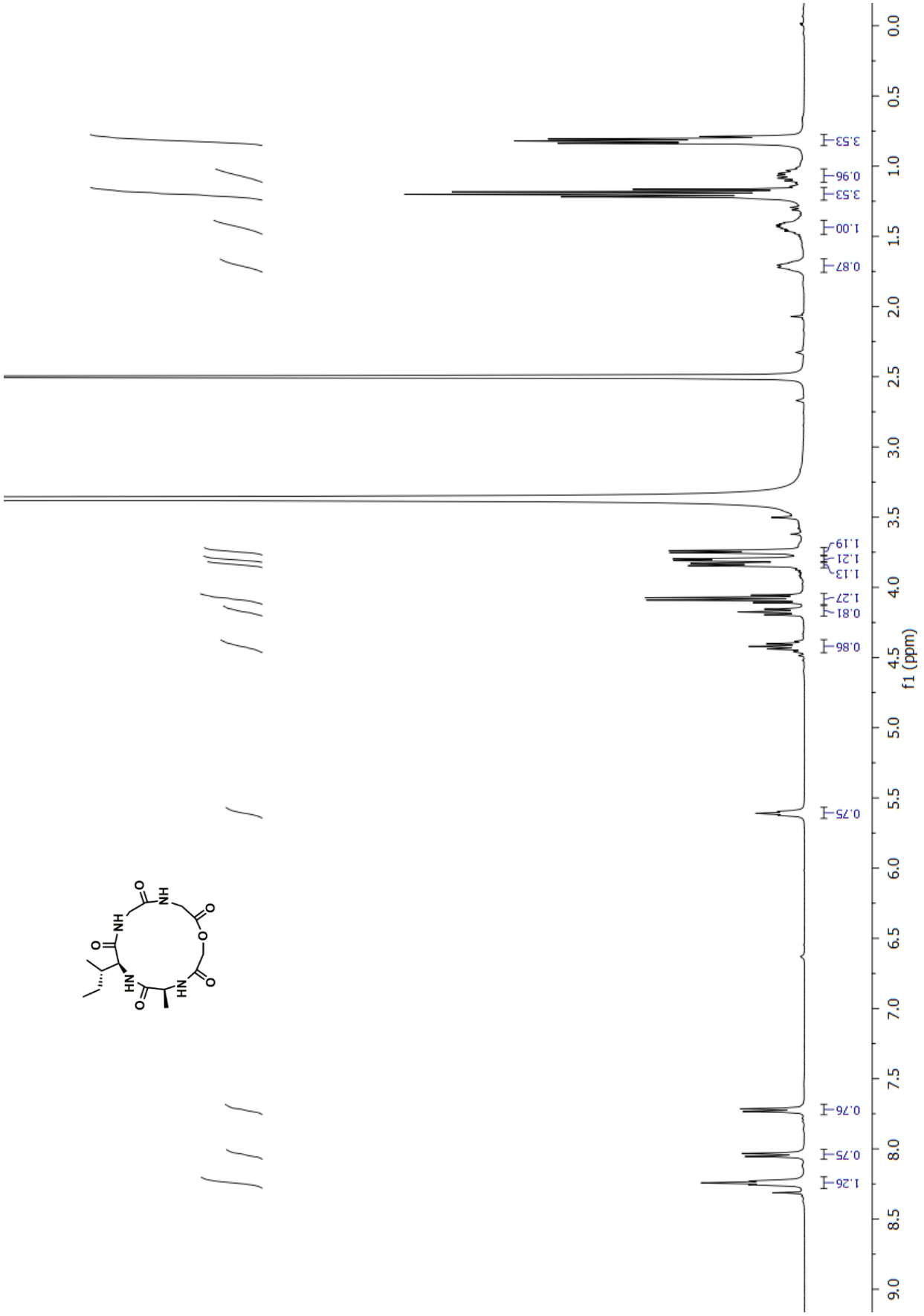


Figure S19 – ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **113** (Chapter 5).

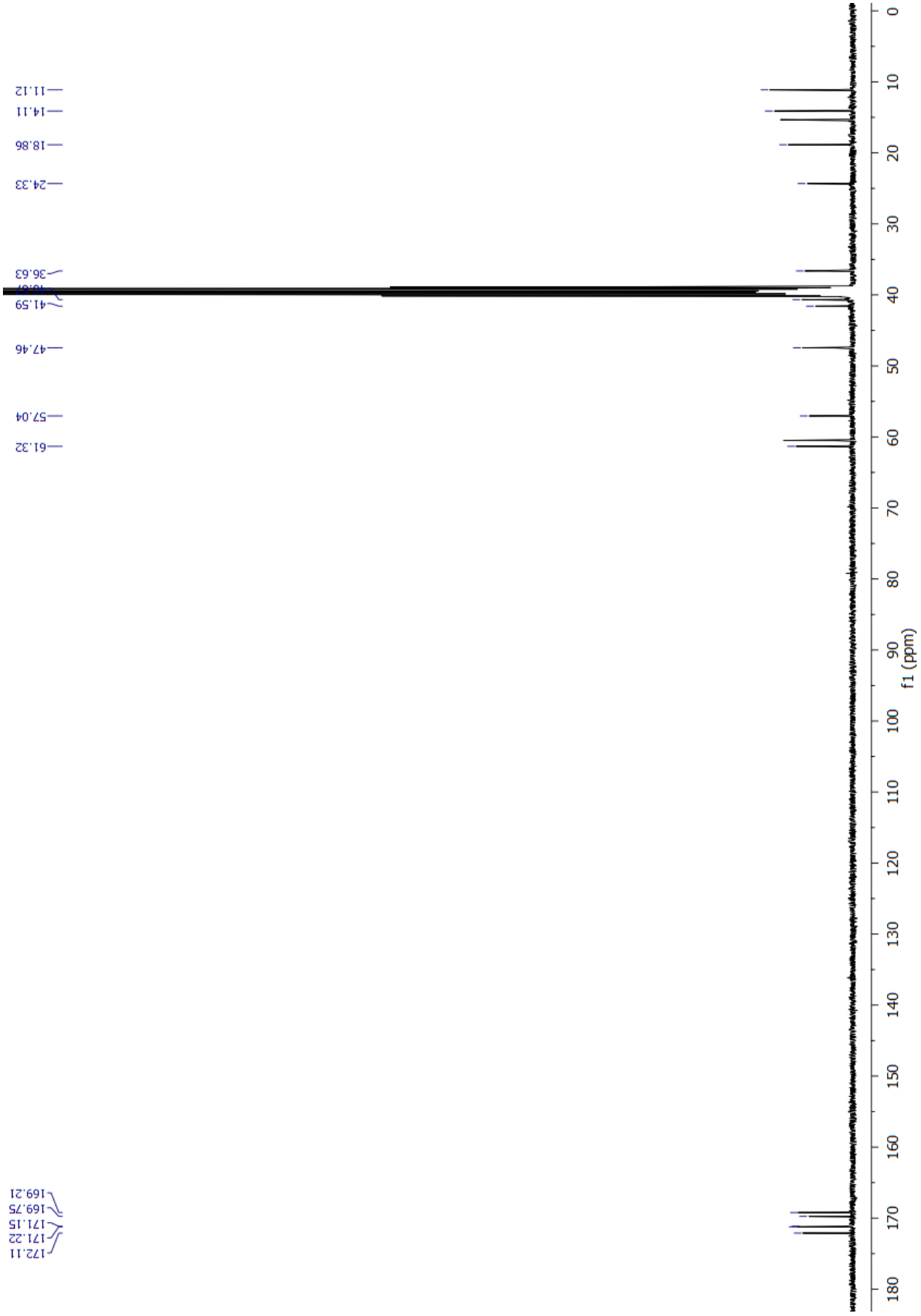


Figure S20 – ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **113** (Chapter 5).

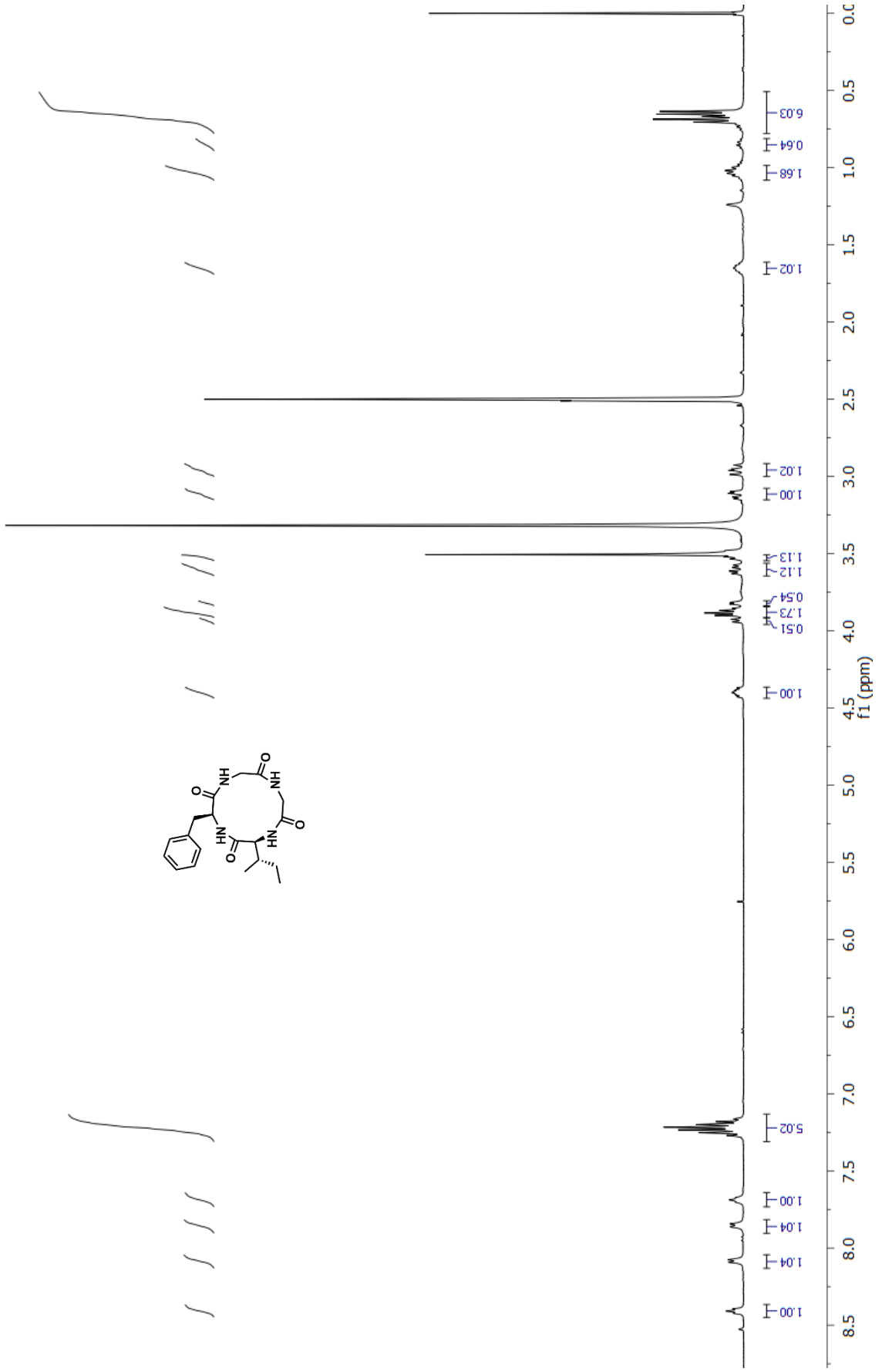


Figure S21 –^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **123** (Chapter 5).

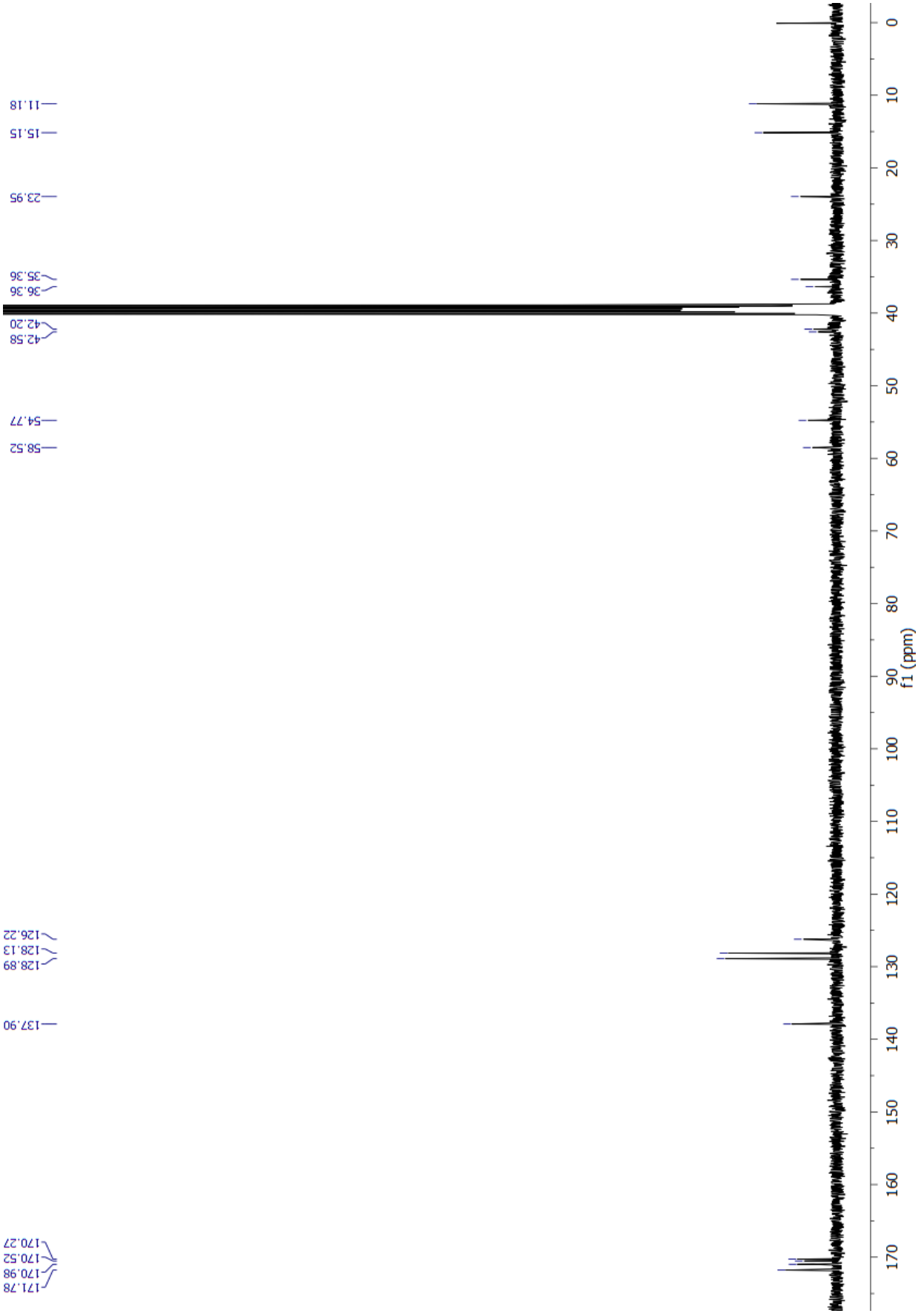


Figure S22 – ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **123** (Chapter 5).

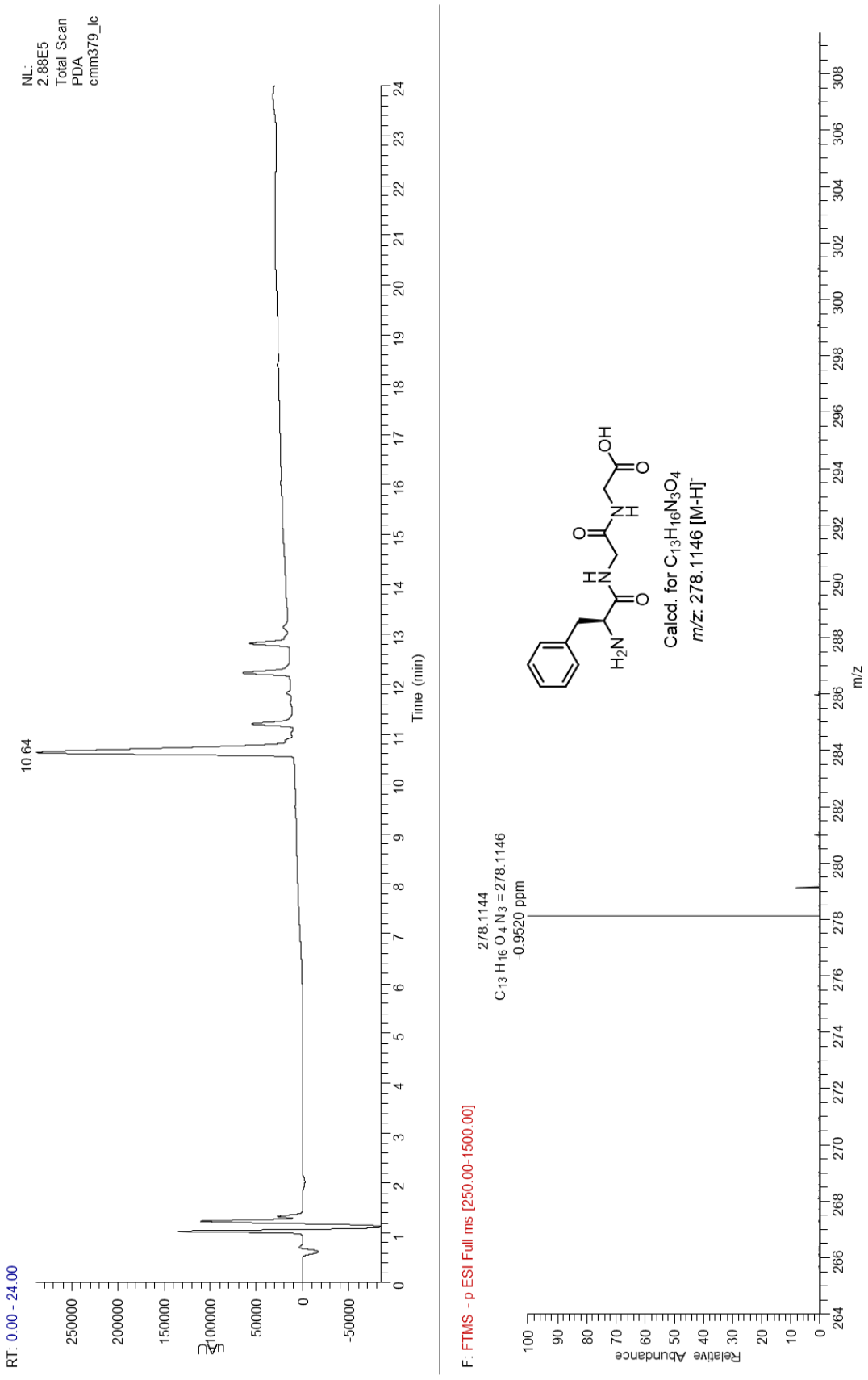


Figure S23 – RP-UHPLC chromatogram and ESI-HRMS of H-Phe-Gly-Gly-OH after resin cleavage with 82% purity from the crude reaction (Chapter 6).

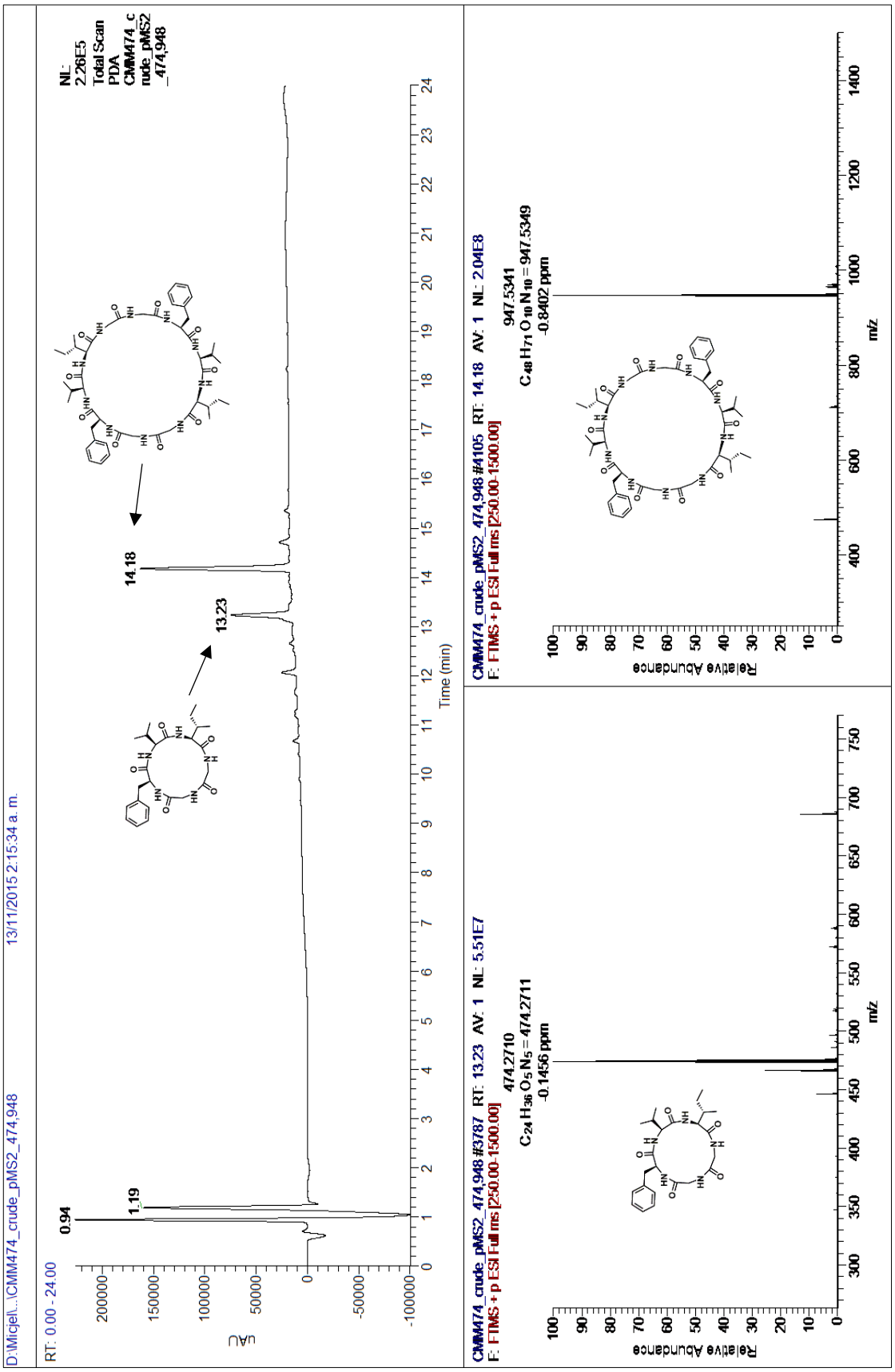


Figure S24 – RP-UHPLC chromatogram after resin cleavage and ESI-HRMS of compound **130** and **131** (Chapter 6).

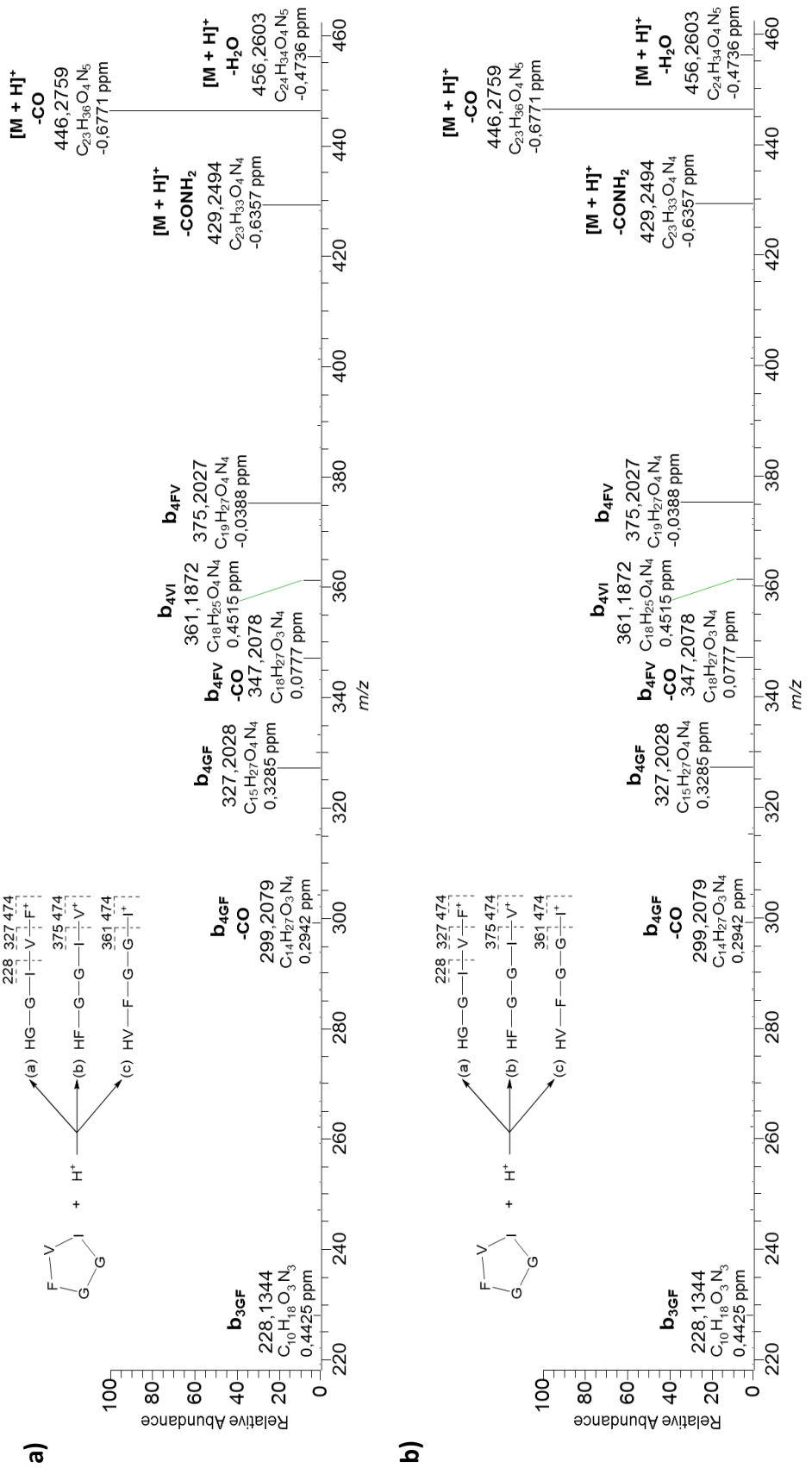


Figure S25 – Positive ion HR-ESI-MS^2 spectrum of $[M+H]^+$ of cyclic peptide **130** (monomer) and **131** (dimer) (Chapter 6).

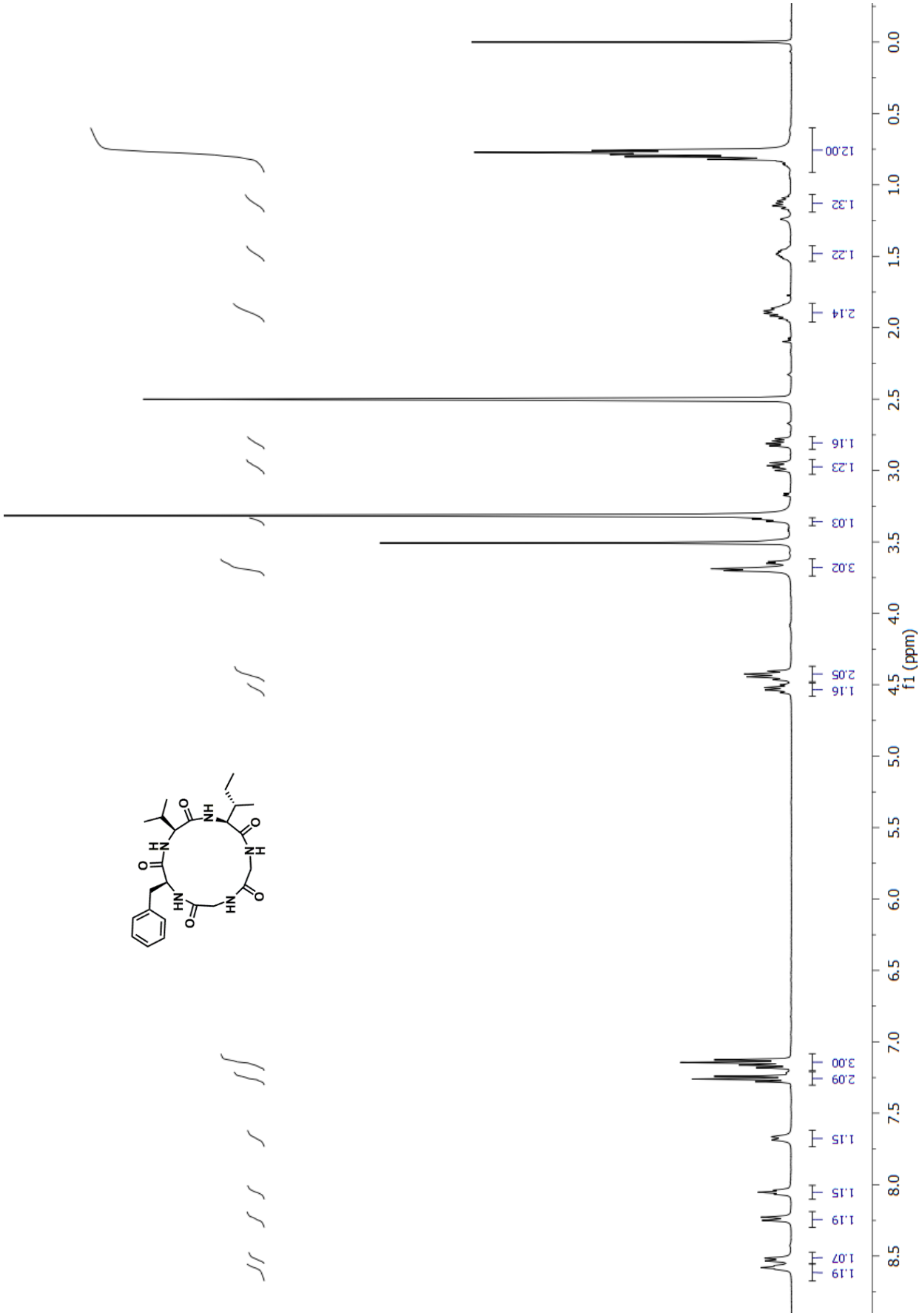


Figure S26 – ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **130** (Chapter 6).

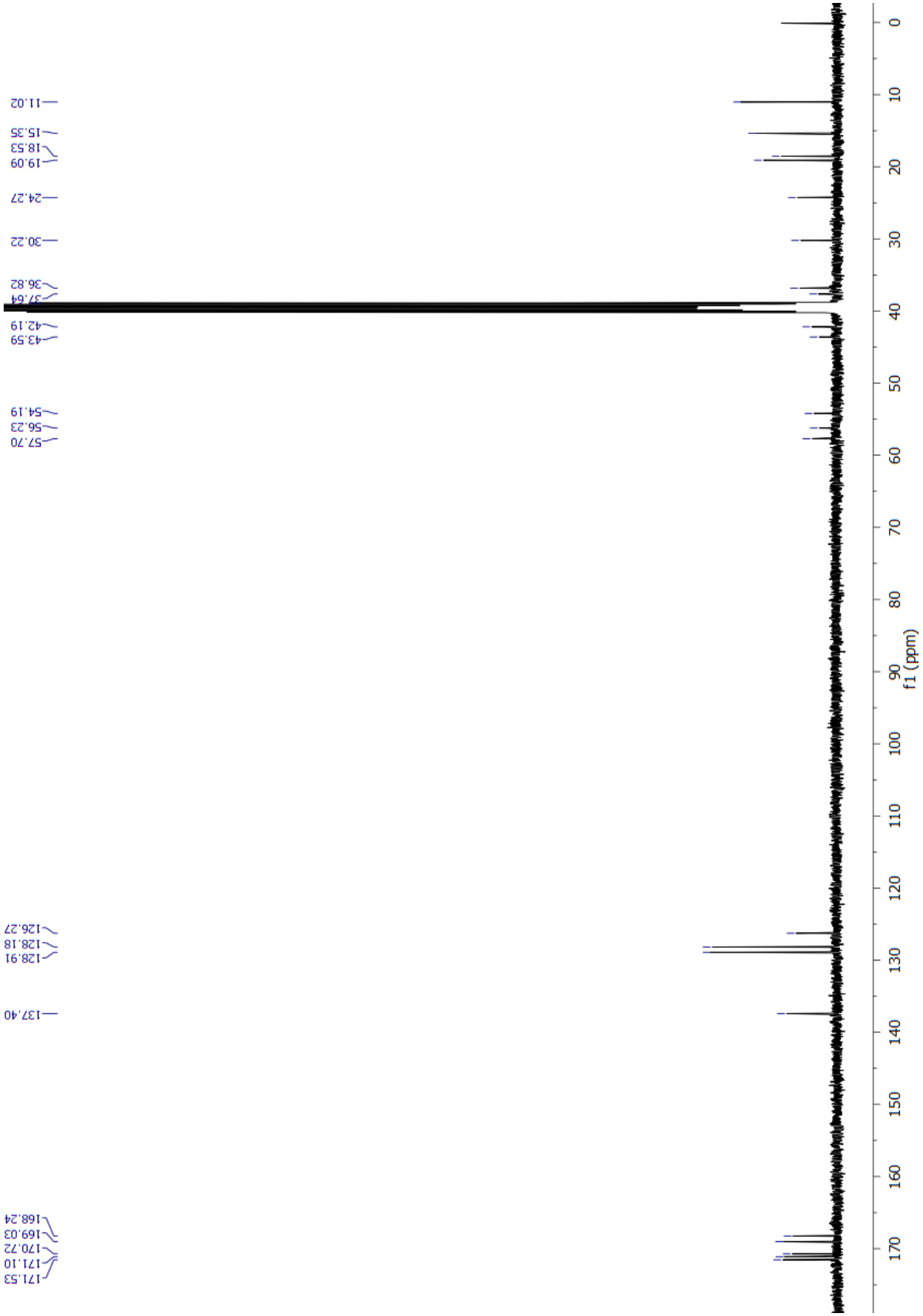


Figure S27 –^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **130** (Chapter 6).

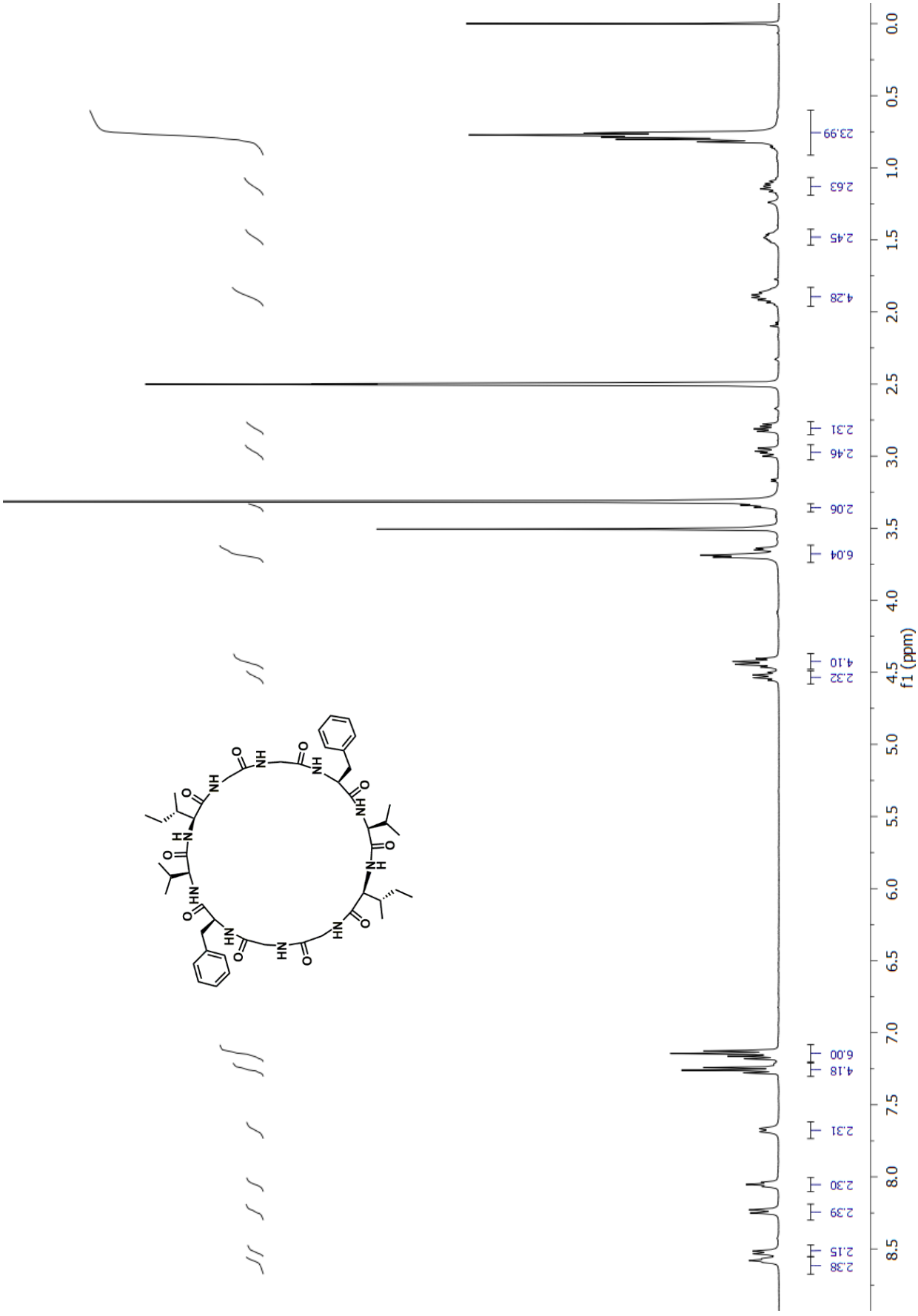


Figure S28 –^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **131** (Chapter 6).

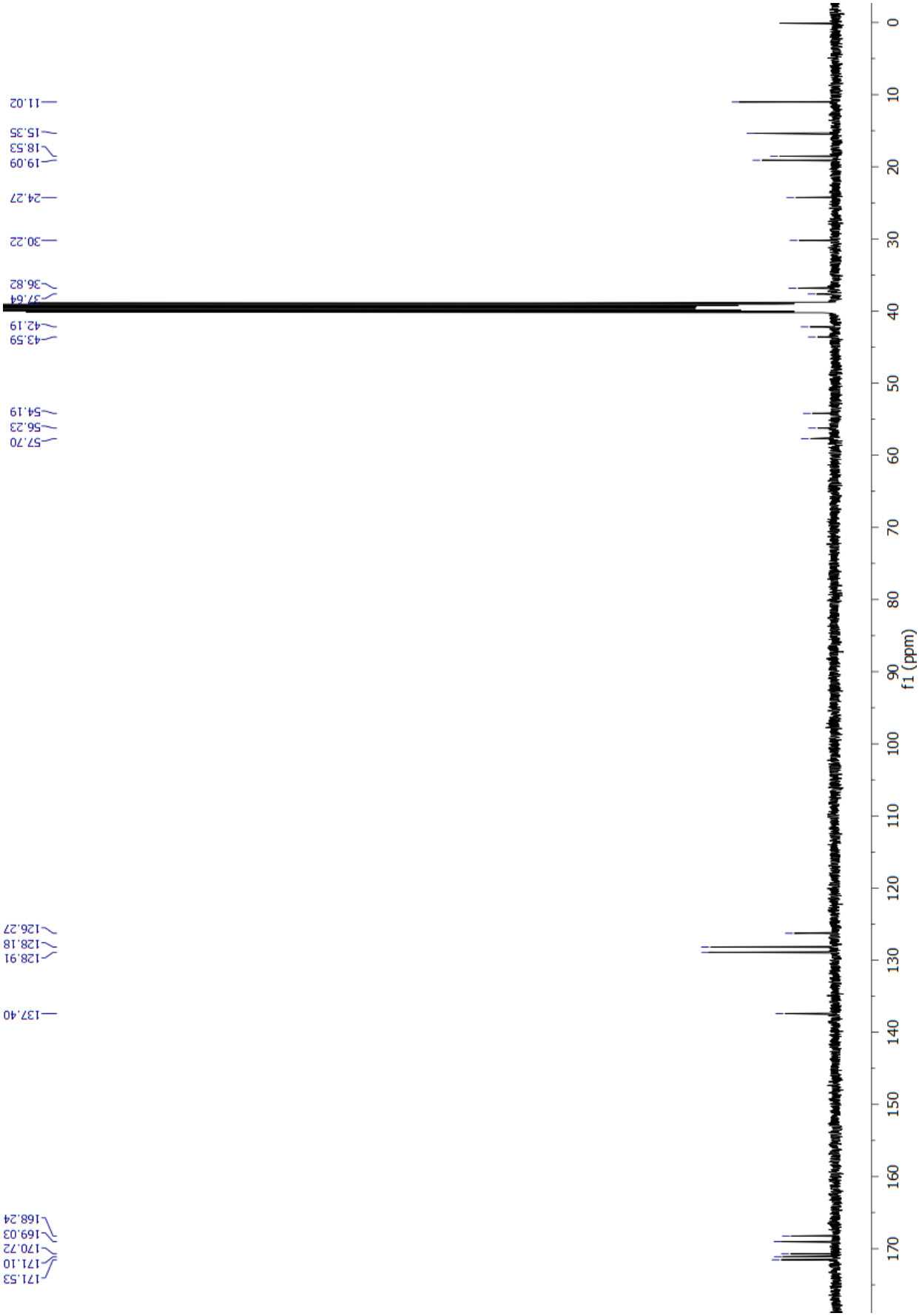


Figure S29 –^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **131** (Chapter 6).

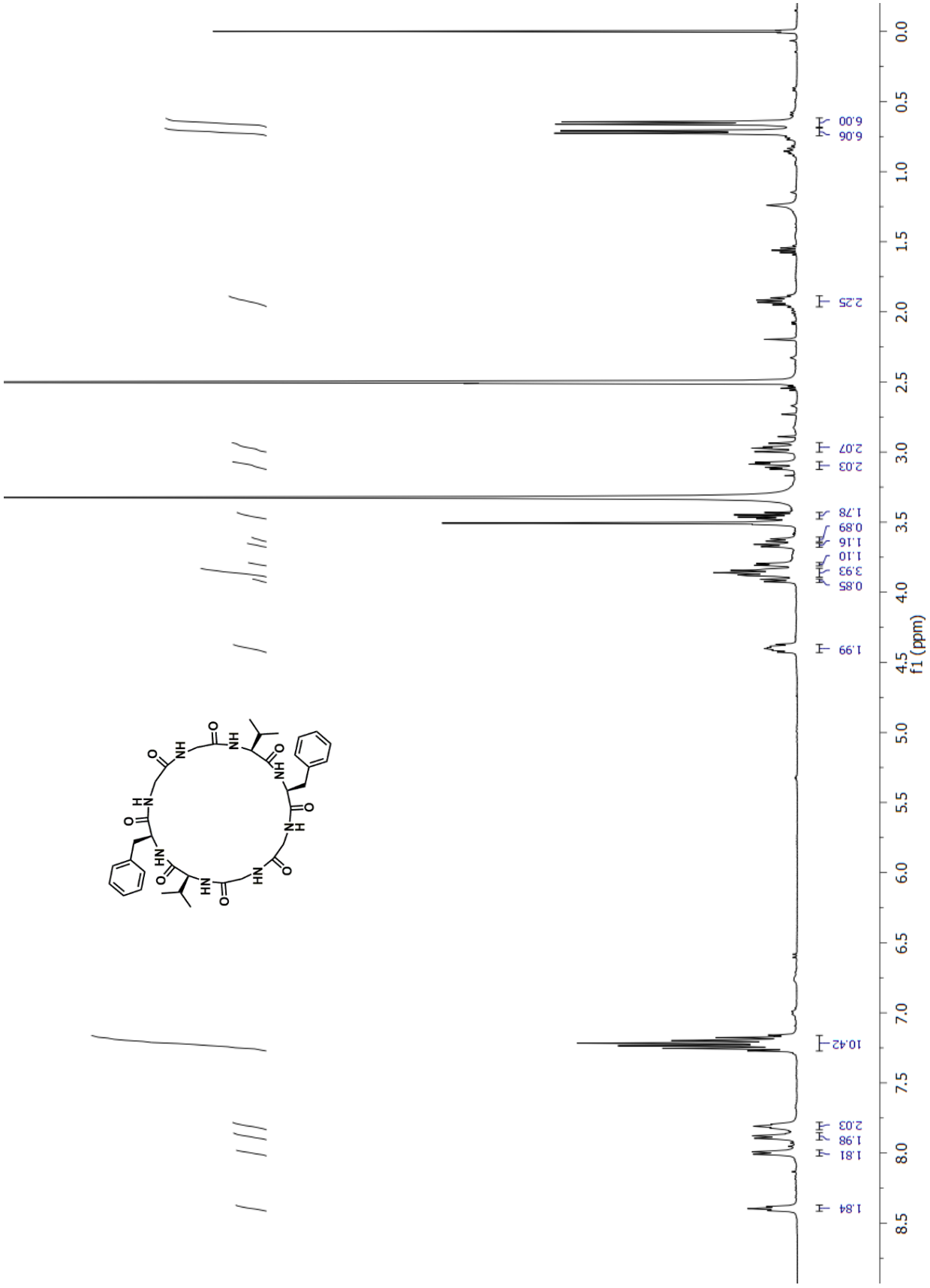


Figure S30 –^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **133** (Chapter 6).

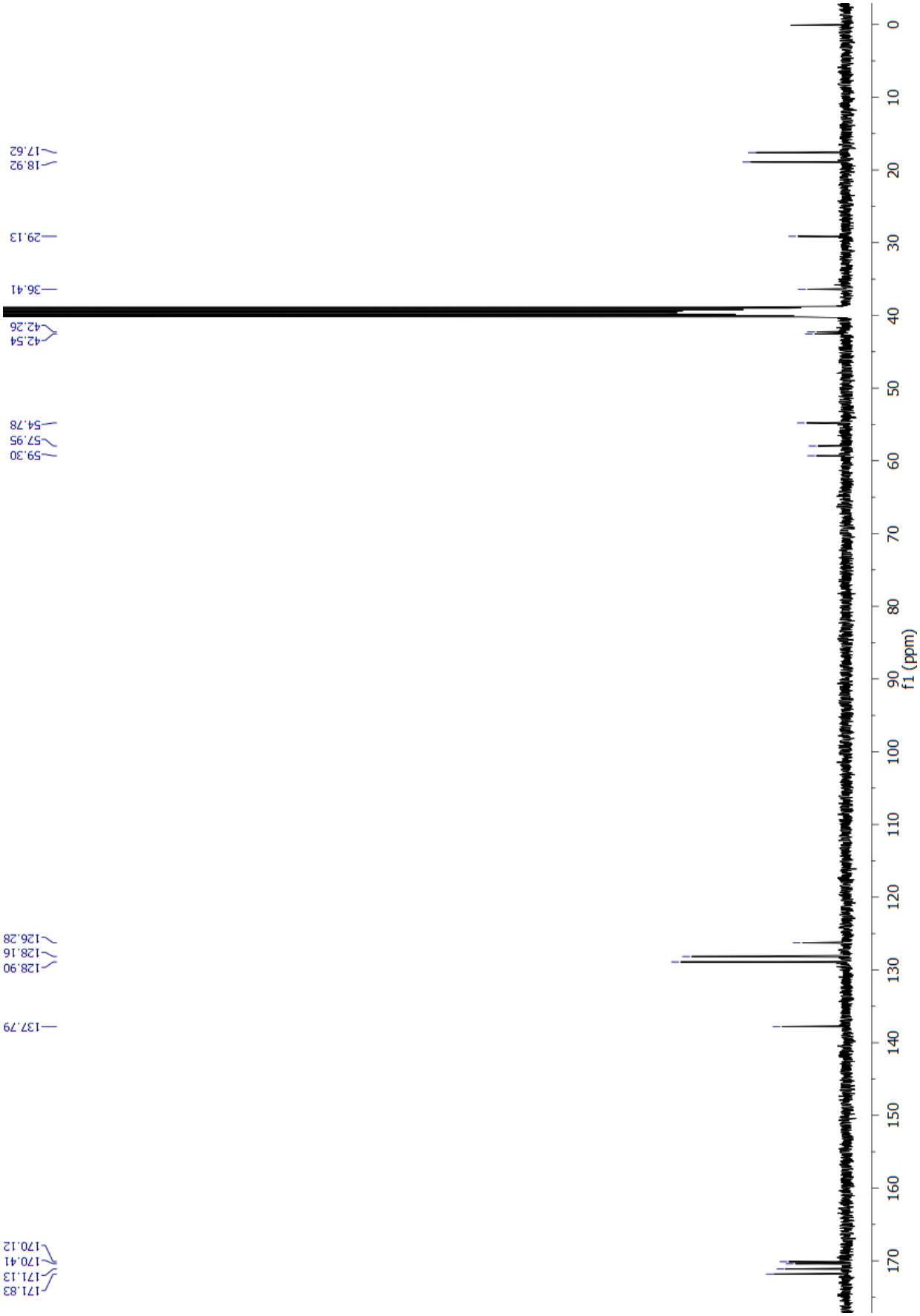


Figure S31 –^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **133** (Chapter 6).

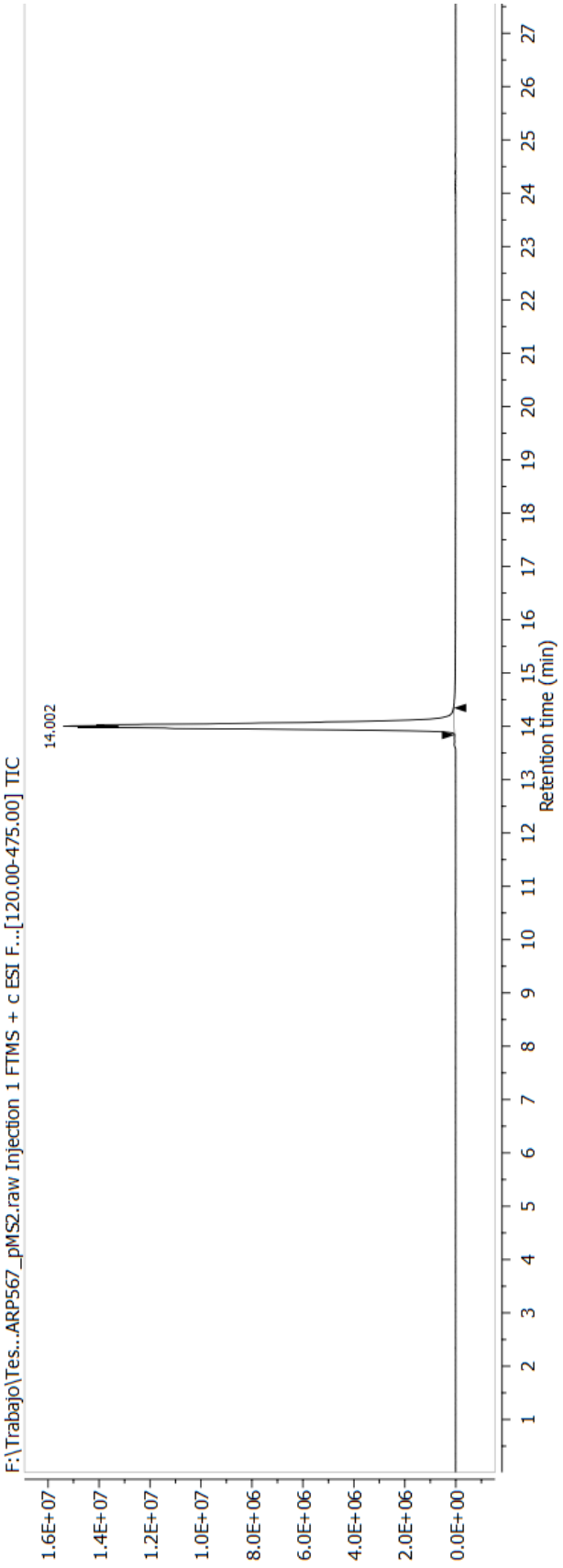


Figure S32 – RP-UHPLC chromatogram after resin cleavage and ESI-HRMS of compound **134** (Chapter 6).

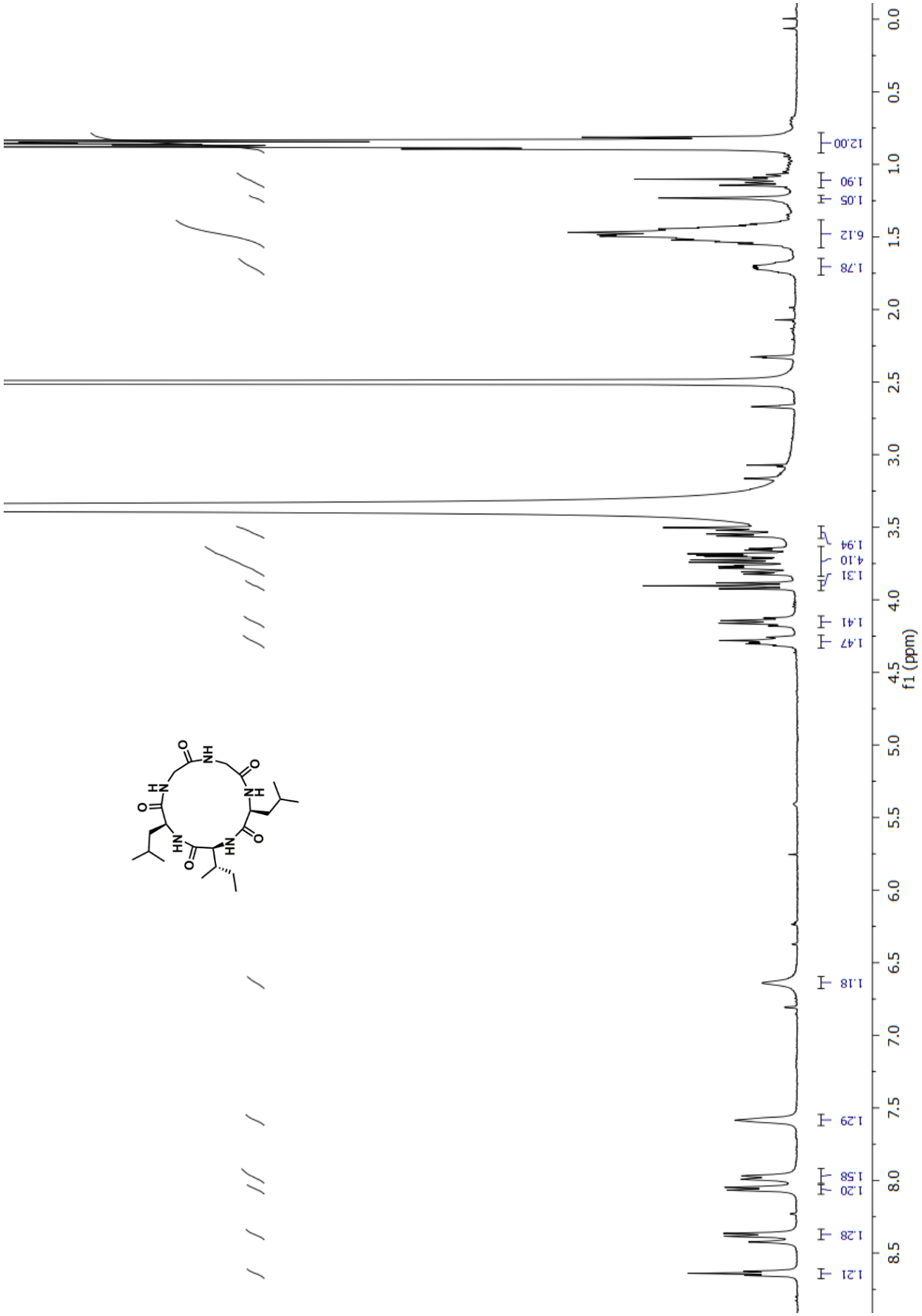


Figure S33 –^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **134** (Chapter 6).

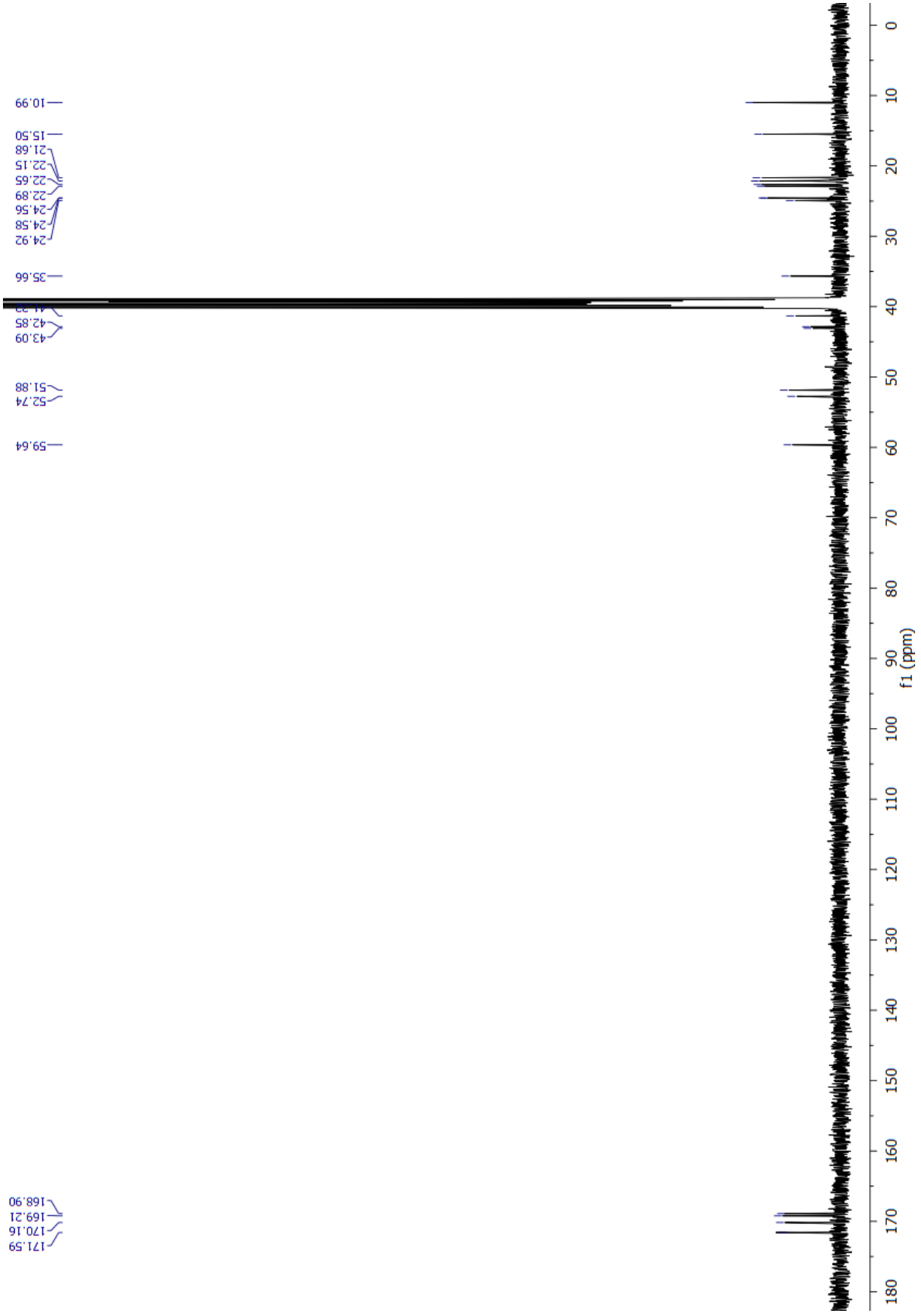


Figure S34 –^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **134** (Chapter 6).

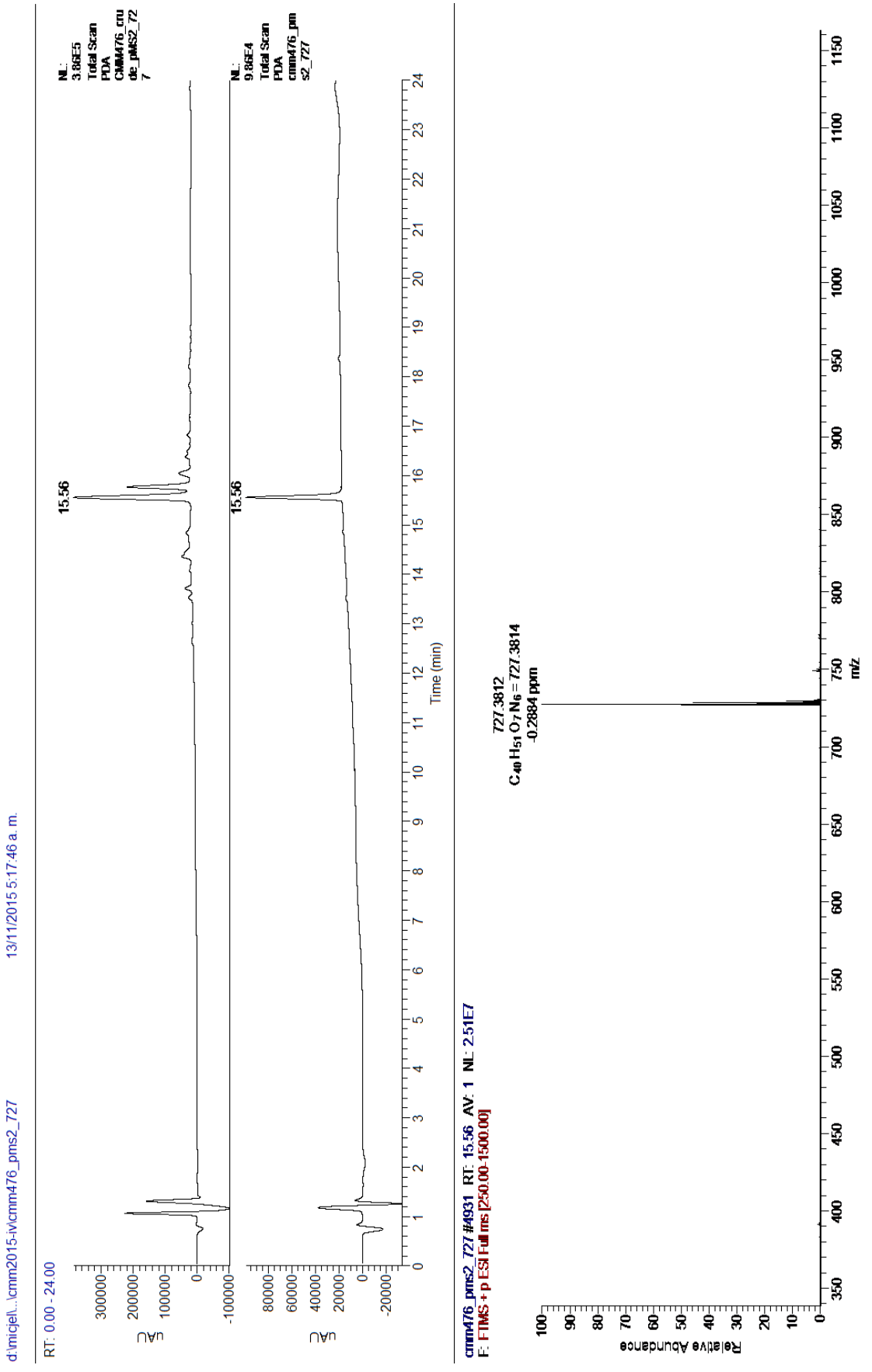


Figure S35 –RP-UHPLC chromatogram and ESI-HRMS of compound **135** (Chapter 6).

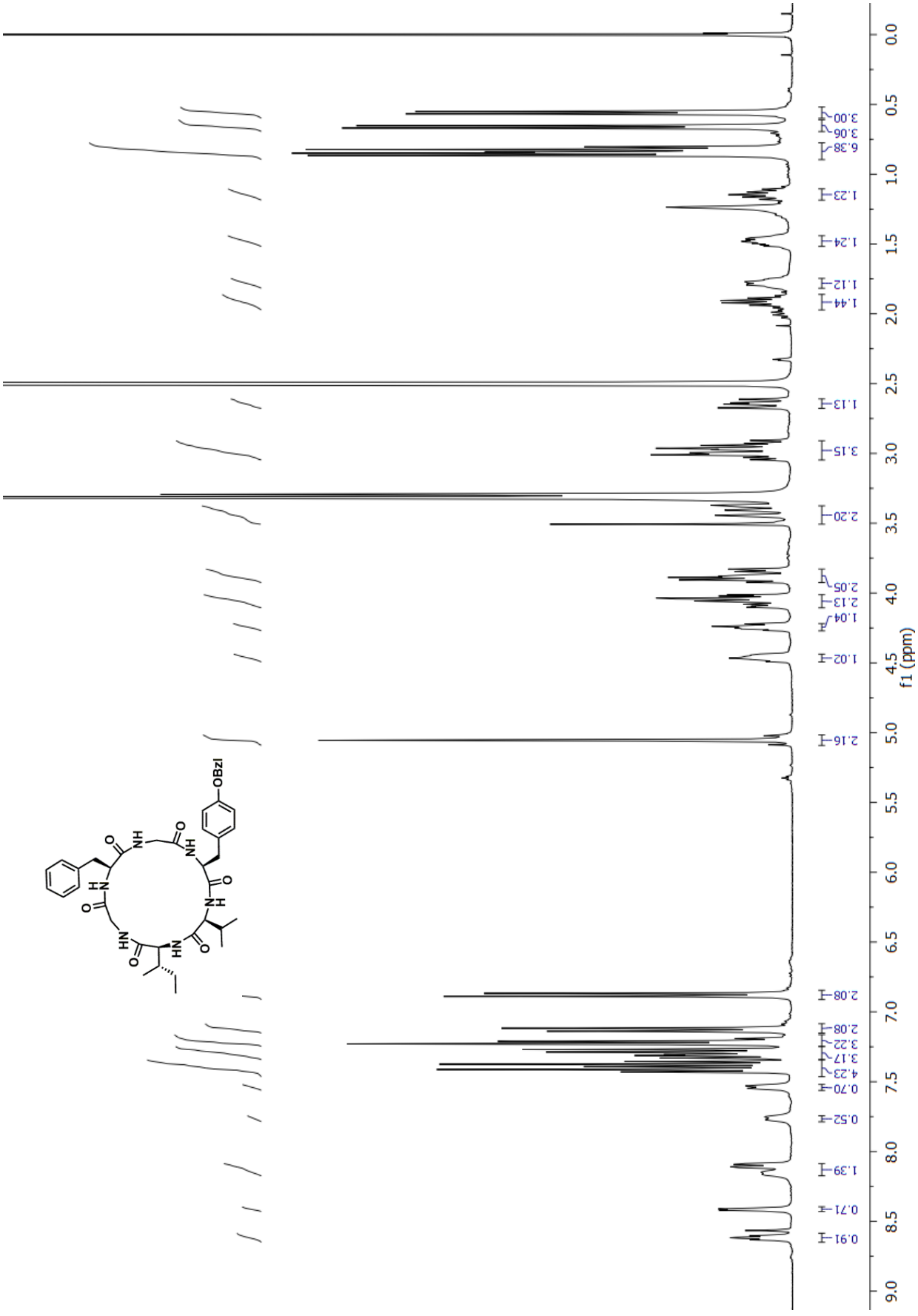


Figure S36 –^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **135** (Chapter 6).

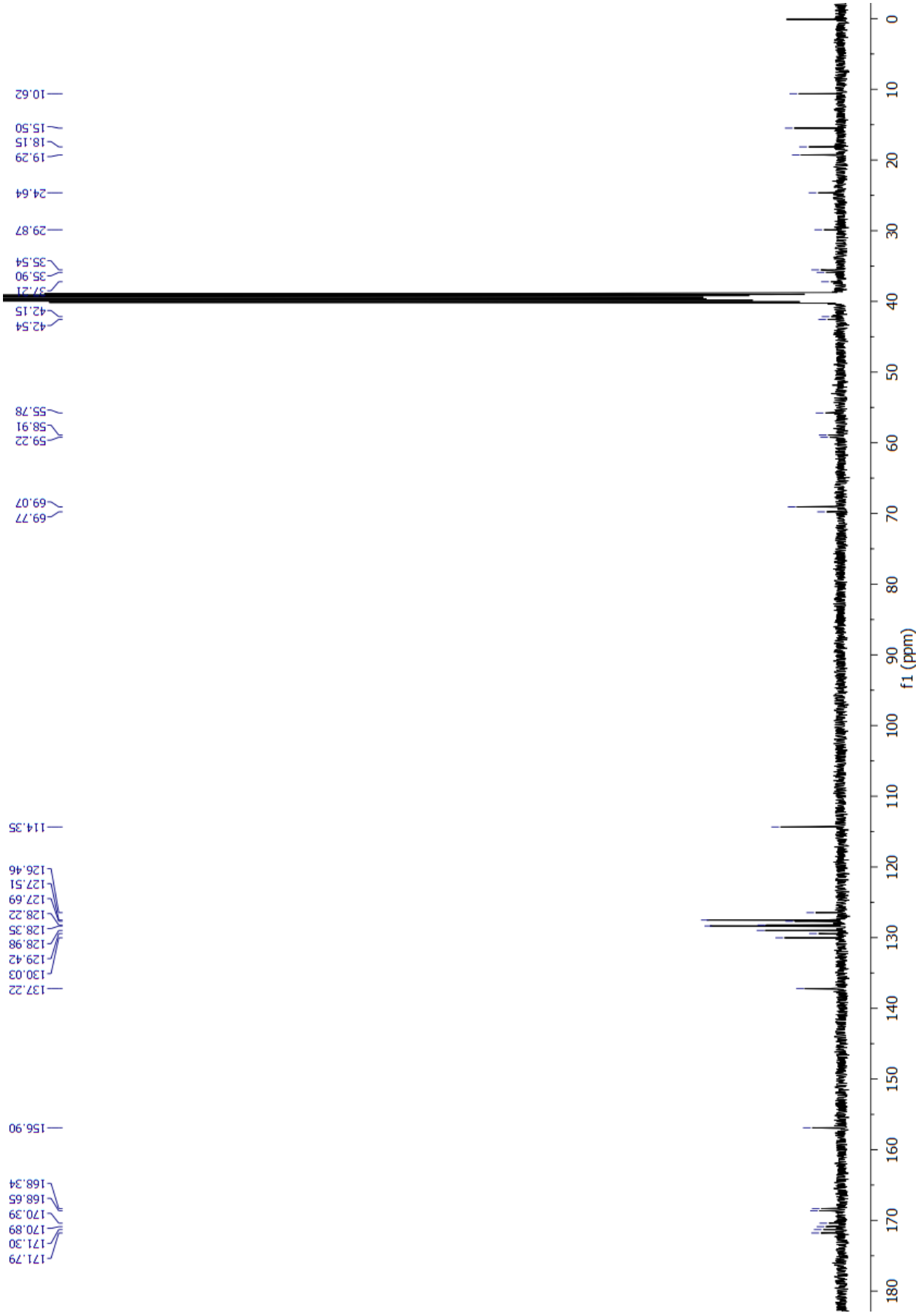


Figure S37 –^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **135** (Chapter 6).

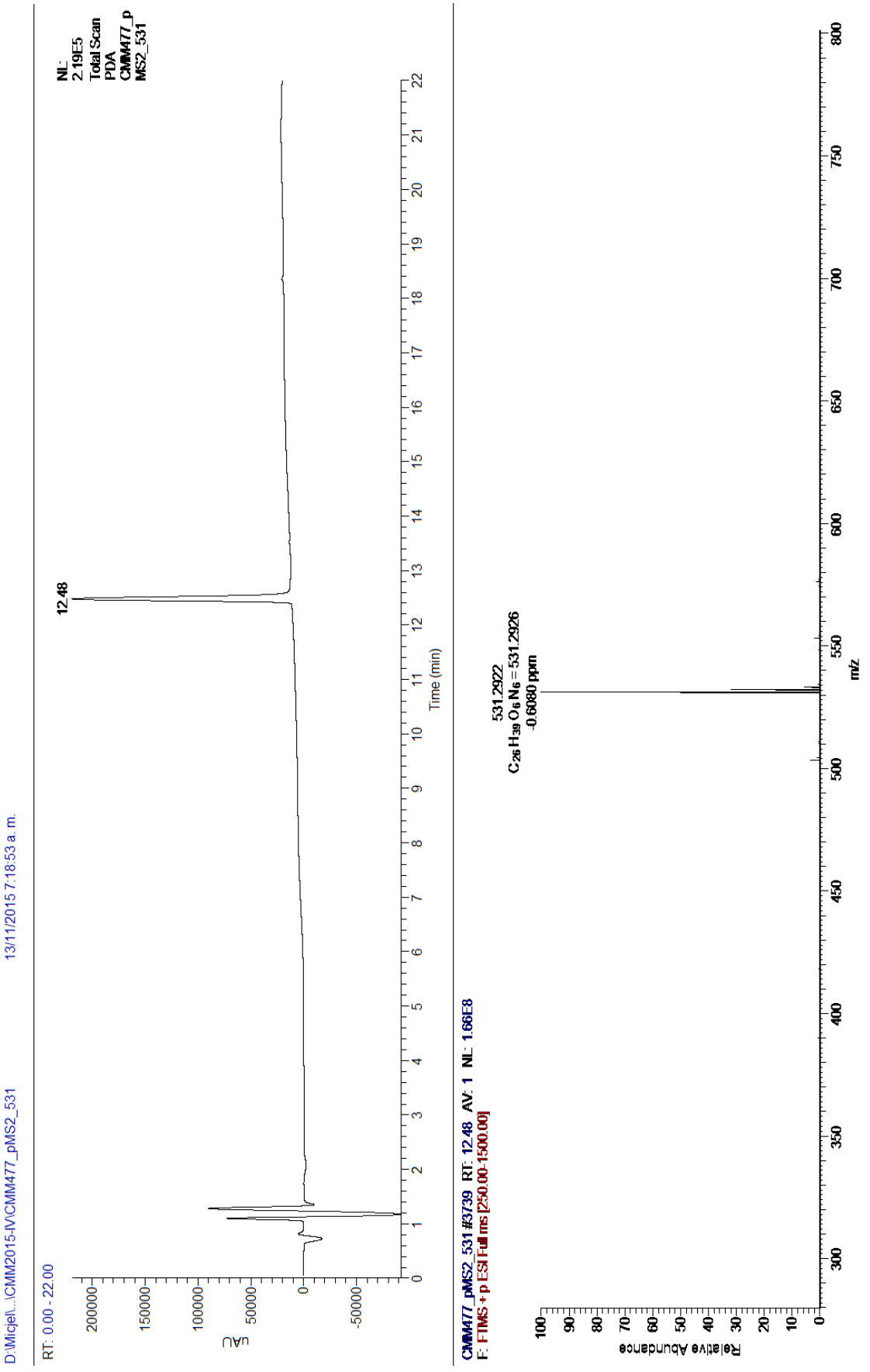


Figure S38 – RP-UHPLC chromatogram and ESI-HRMS of compound **136** (Chapter 6).

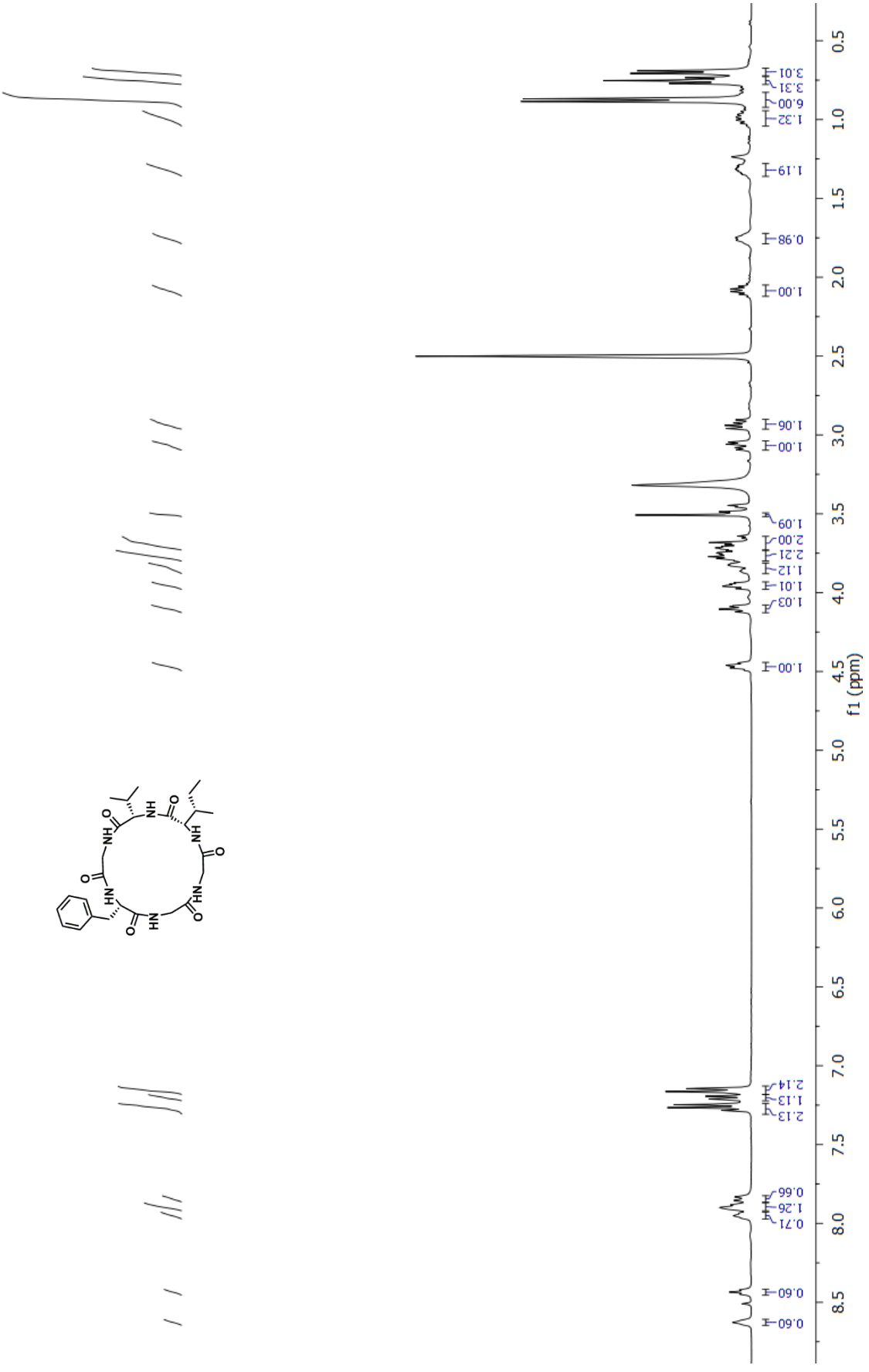


Figure S39 –^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **136** (Chapter 6).

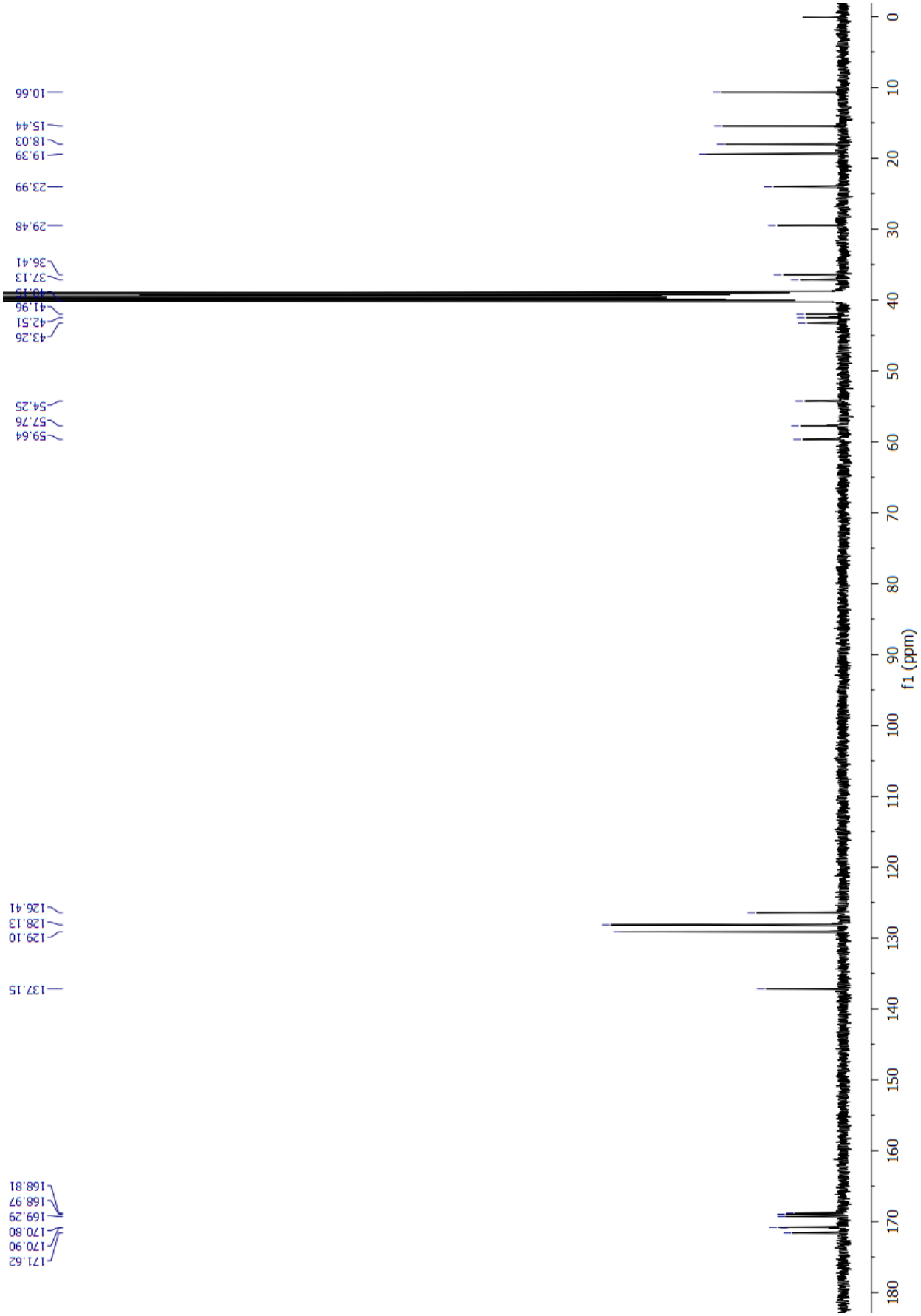


Figure S40 –^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **136** (Chapter 6).

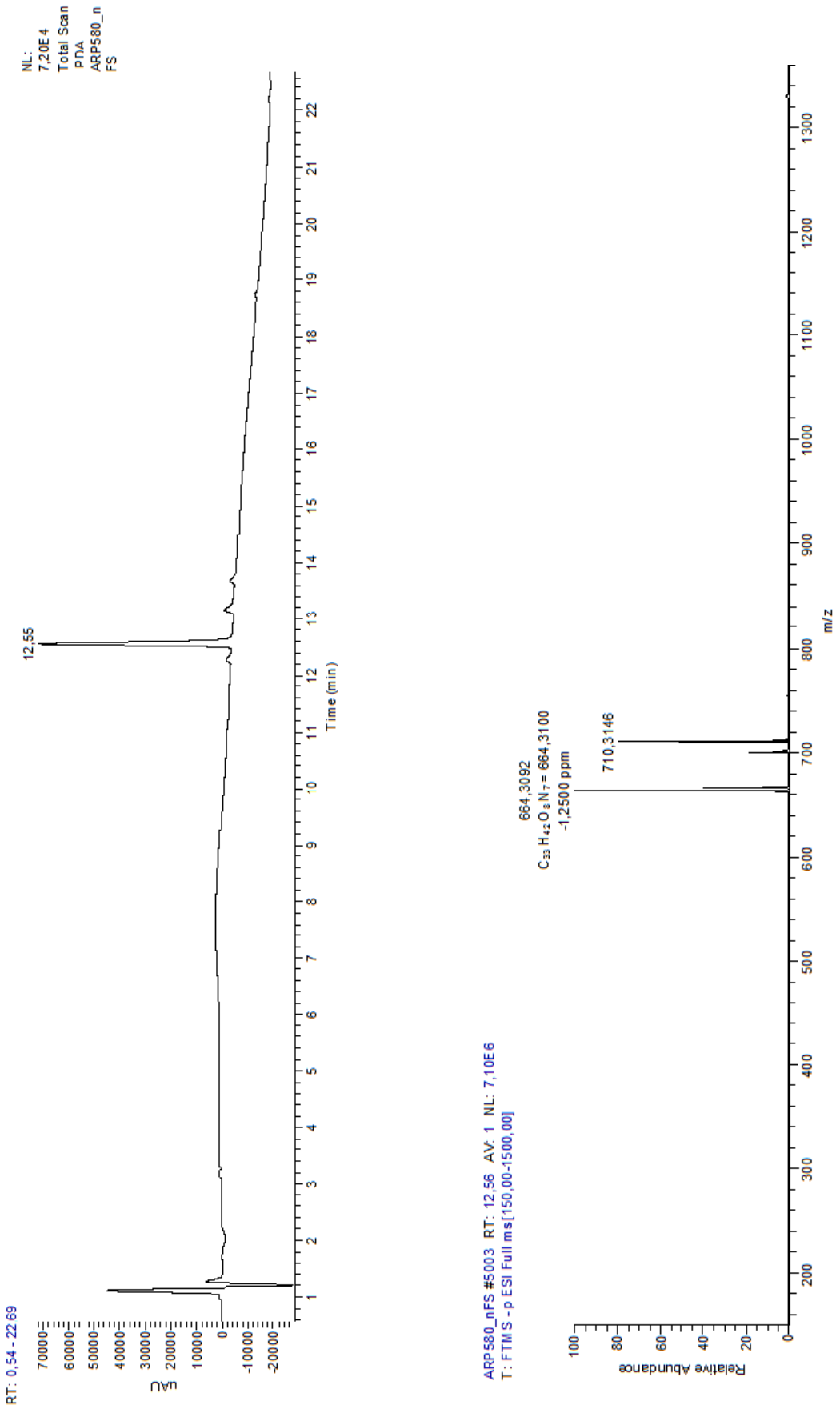


Figure S41 –RP-UHPLC chromatogram and ESI-HRMS of compound **141** (Chapter 6).

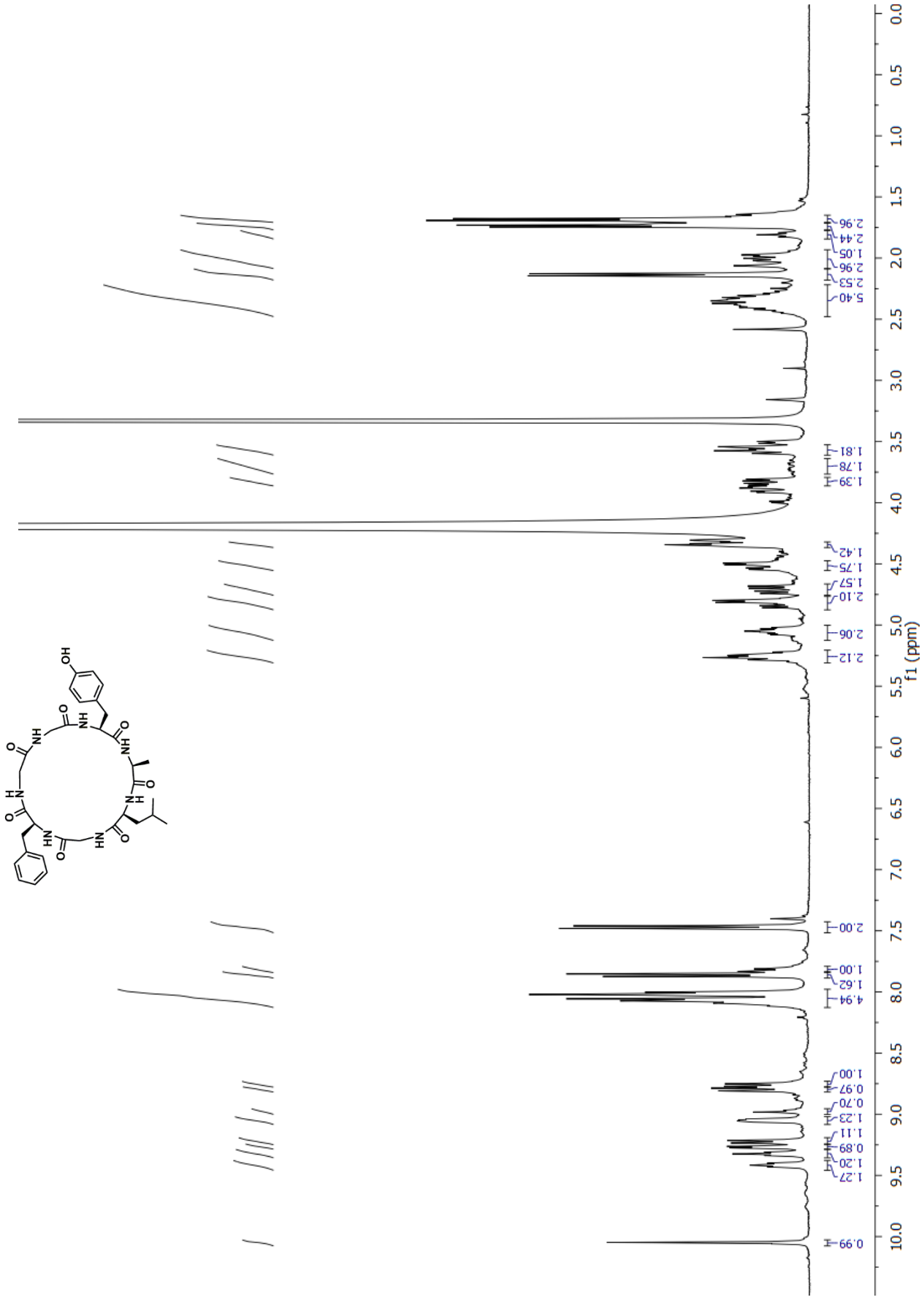


Figure S42 –^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **141** (Chapter 6).

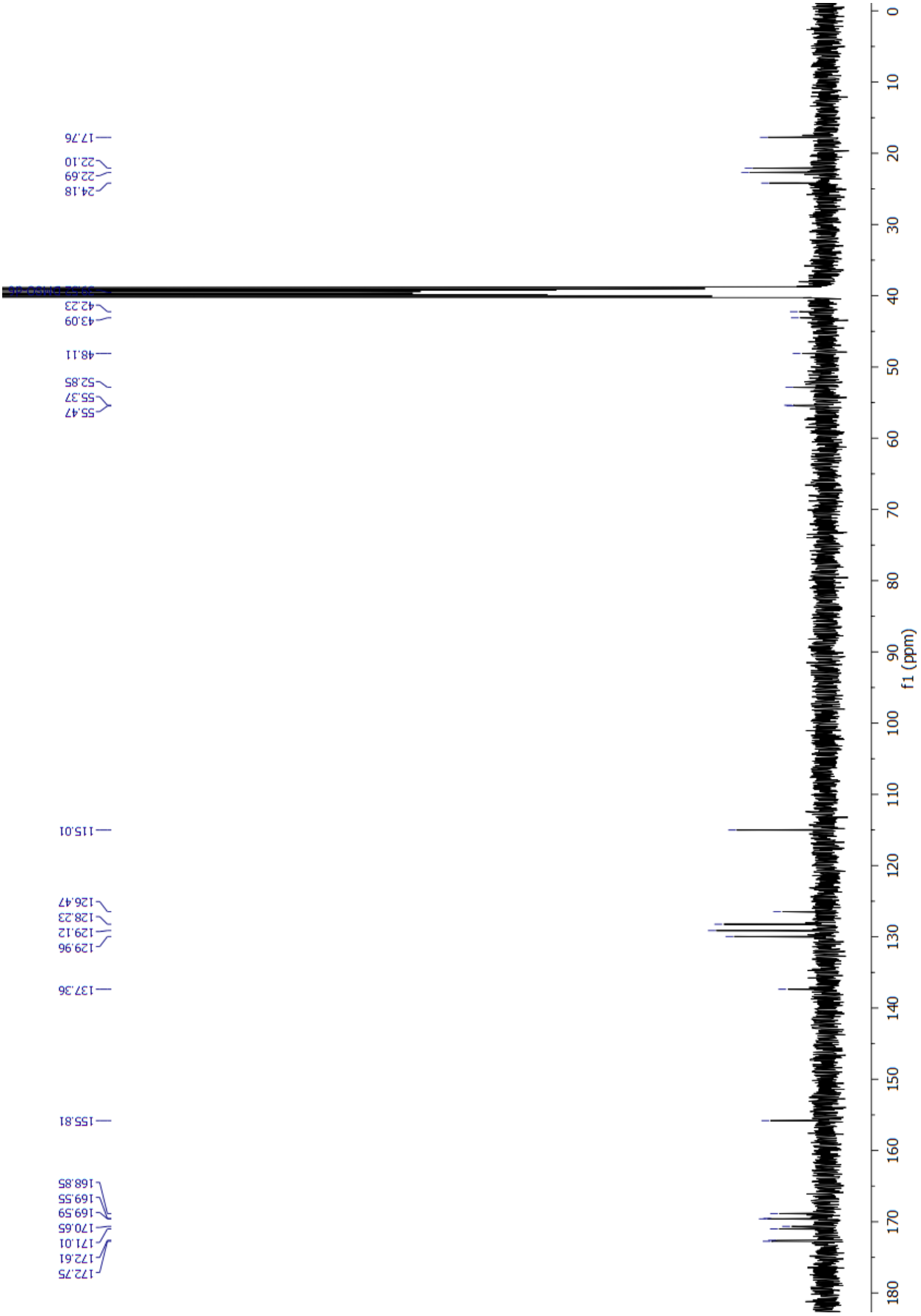


Figure S43 –^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **141** (Chapter 6).

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1. General informations

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2. Education

2016 – 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
2017 – onwards	Assistant professor, Department of organic chemistry, Faculty of Chemistry, University of Havana. Disciplines: Organic Chemistry II, Experimental Organic Chemistry.
2012 – 2015	Scientific assistance at the Leibniz Institute of Plant Biochemistry, Department of Bioorganic Chemistry, IPB, Halle (Saale). Supervisors: Prof. Dr. L.A. Wessjohann.
2012 – 2014	*M. Sc.* in Organic Chemistry. Faculty of Chemistry, University of Havana. *Total Synthesis of Cordyheptapeptide A*. Evaluation: Excellent. Supervisors: Prof. Dr. L.A. Wessjohann, Prof. Dr. D.G. Rivera
2009 – 2016	Instructor teacher and researcher, Faculty of Chemistry, Center for Natural Products Research. Department of Organic Chemistry. University of Havana. Graduate: *Diplom in Chemistry*, Summa cum Laude. Faculty of Chemistry, University of Havana. Diploma thesis at the Laboratoy of Organic Synthesis

2004 – 2009 (LSO). *Theoretical models on the structural and electronic characteristics of the bis-tiadiazine t1a and their reactivity with amino acids and proteins.* Evaluation: excellent. Supervisors: Prof. Dra. Edelsys Codorniu Hernández, Prof. Dra. Hortensia Rodríguez Cabrera

3. Languages

Spanish (Native), English, Portuguese, German (A1).

4. Publication

Plutín, A.M., Suárez, M., Machado, T., Álvarez, A., **Puentes, R, A.**, Martínez, R., Duque, J., Martínez-Álvarez, R. and Martín, N., **2010**. On the selective methylation of benzoyl and furoylthiocarbamates. *Arkivoc*, *10*, pp.276-290.

Ruiz, E., Rodríguez, H., Coro, J., Niebla, V., **Puentes, R, A.**, Martínez-Alvarez, R., de Armas, H.N., Suárez, M. and Martín, N., **2012**. Efficient sonochemical synthesis of alkyl 4-aryl-6-chloro-5-formyl-2-methyl-1, 4-dihydropyridine-3-carboxylate derivatives. *Ultrasonics sonochemistry*, *19*(2), pp.221-226.

Puentes, A.R., Neves Filho, R.A.W., Rivera, D.G., Wessjohann, L.A., **2017**. Total Synthesis of Cordyheptapeptide A. *Synlett* 28, 1971–1974.

Morejón, M.C., Laub, A., Kaluđerović, G.N., **Puentes, A.R.**, Hmedat, A.N., Otero-González, A.J., Rivera, D.G., Wessjohann, L.A., **2017**. A multicomponent macrocyclization strategy to natural product-like cyclic lipopeptides: synthesis and anticancer evaluation of surfactin and mycosubtilin analogues. *Org. Biomol. Chem*. 15, 3628–3637.

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

5. Book chapters

Wessjohann, L.A., Neves Filho, R.A.W., **Puentes, A.R.**, Morejon, M.C., **2015**. 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. 231–264.

Marsault, E., & Peterson, M. L. (**2017**). *Practical Medicinal Chemistry with Macrocycles: Design, Synthesis, and Case Studies*. John Wiley & Sons. In *Macrocycles from multicomponent reaction* (pp. 339–376). Ludger A. Wessjohann, Ricardo A.W. Neves Filho, **Alfredo R. Puentes** and Micjel C. Morejon.

6. Selected conference presentations

2018 7th International Conference on Multicomponent Reactions and Related Chemistry, Düsseldorf, Germany (Poster Presentation)

2018 Naturstoffe: Chemie, Biologie und Ökologie, Bayreuth Universität, Germany. (Oral presentation)

2016 Ernest Eliel Symposium: US and Cuba collaboration in chemistry education and neglected disease drug discovery. Havana. Cuba. Oral presentation).

2015 9th Congress of Chemical Science, Technology and Innovation. Quimicuba. (Poster Presentation) *Total Synthesis of Cordyheptapeptide A*. Havana. Cuba.

2011 VIII Seminars of Advanced Studies on Molecular Design and Bioinformatics. (Poster Presentation) *Theoretical studies on the structure of differen N,N-dialquil-N-aciltioureas derivatives and their cobalt complexes*. Havana. Cuba.

2009 VII International Congress of Chemistry and Chemical Engineering**.** *Molecular Modeling Workshop*. Havana. Cuba

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.

Alfredo Rodríguez Puentes