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Transition-State Based Design and Synthesis of New Potential Inhibitors of DPP III

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To my family

„Die beste Bildung findet ein gescheiter Mensch auf Reisen.“

Johann Wolfgang von Goethe (1749 - 1832)

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Abstract

This thesis deals with the design and synthesis of new potential inhibitors of dipeptidyl peptidase III (DPP III). DPP III is a zinc dependent peptide cutting enzyme, which selectively cleaves peptide bonds at the *N*-terminus of physiologically important oligopeptides. Even though the exact role of DPP III is still unclear, it has been found that it might be involved in important physiological pathways, such as pain signaling, defense against oxidative stress, blood pressure regulation and inflammation. In order to understand the binding mechanism and to elucidate the exact role in physiological pathways, it is a desirable goal to synthesize a selective and potent inhibitor of DPP III. Already developed inhibitors are based on the structures of the neuropeptide enkephalin or synthetic pentapeptide tynorphin. Previous work on this project led to the synthesis of the transition-state mimicking pseudepeptide ***HER*** (*R*)-hydroxyethylene, which was used as lead structure in the course of this work. The newly synthesized inhibitors with different substitution patterns were tested in biological assays towards the inhibition of DPP III.

Kurzfassung

Diese Arbeit befasst sich mit der Entwicklung und Synthese von neuen potentiellen Inhibitoren von Dipeptidylpeptidase III (DPP III). DPP III ist ein Zink-abhängiges Peptid-spaltendes Enzym, welches Peptidbindungen selektiv am *N*-ständigen Ende von physiologisch wichtigen Oligopeptiden spaltet. Obwohl die genaue Rolle von DPP III noch immer nicht vollkommen verstanden ist, haben Untersuchungen gezeigt, dass es vermutlich an wichtigen physiologischen Vorgängen teilnimmt, wie zum Beispiel: Schmerzübertragung, Abwehr von oxidativem Stress, Blutdurckregulierung und Entzündungen. Um den Bindungsmechansimus und die genaue Rolle in physiologischen Vorgängen genauer erörtern zu können, ist es ein erstrebenswertes Ziel einen selektiven und aktiven Inhibitor von DPP III zu synthetisieren. Bereits entwickelte Inhibitoren basieren auf der chemischen Struktur des Neuropeptids Enkephalin oder dem synthetisch hergestellten Peptid Tynorphin. In früheren Arbeiten zu diesem Projekt haben zur Synthese des Pseudopeptids ***HER*** (*R*)-hydroxyethylene geführt, das den Übergangszustand nachahmt. Im Laufe dieser Arbeit wurde dieses als Grundstruktur für weitere Modifikationen verwendet. Alle neuen synthetisierten Inhibitoren, die unterschiedliche Substitutionsmuster aufweisen, wurden in biologischen Tests auf Aktivität für die Inhibierung von DPP III getestet.

1 Introduction

One major goal in chemistry and biochemistry is to understand the exact role of enzymes in the organism. Enzymes are significantly important in metabolic pathways, being ion pumps in active transport or for signal transduction and cell regulation. Due to this reason, it is a desirable goal to inhibit certain enzymes in order to prevent overactivity. Thus, modern drug design is targeting the inhibition of specific enzymes, rather than simultaneously inhibiting multiple enzymes.

Most inhibitors are related to the naturally occurring ligand of the enzyme in terms of structure and chemical behaviour. These inhibitors are analogues to transition states of the enzyme-substrate complex and may be synthetic peptides, natural peptides or other transition state mimetics. Therefore, inhibitors are small and stable molecules which form the substrate-enzyme complex quicker and bind more tightly to the enzyme than the natural substrate. Inhibitors may bind either reversible or irreversible, which means that the activity of the enzyme is shut down permanently.

Nowadays, a very efficient approach towards drug development is using the feature of structure-based design. This method is based on 3D data obtained via various methods in NMR-spectroscopy or X-ray crystallography.[1] Knowing the structure of a specific enzyme or biological substrate, the activity and selectivity of a potential drug candidate can be predicted to a certain extent. The knowledge of the structural feature of the binding site also gives rise to design a potential drug by assembling small molecule fragments and building up a competitive inhibitor. This de novo design of ligands is a very elegant approach in structure based drug design.[2,3] The use of this method provides access for the design and synthesis for a wide range of enzyme inhibitors. Major progress has not only been accomplished for therapeutical use, such as HIV-proteases or anticancer drugs, but also for herbicides and pesticides.[4-7]

A significant class of enzymes are metalloproteases. The catalytic mechanism requires the presence of a metal. The vast majority of these enzymes require zinc, one of the most abundant metals in nature. A lot of effort has been put into the investigation of these metalloproteases, in order to determine the exact structure and role in biological pathways. Dipeptidyl-peptidase III (DPP III) is a zinc-dependent metallopeptidase, which is able to cleave off dipeptides from oligopeptides at the *N*–terminus.[8] This enzyme can be found in multiple human cells such as liver brain, or muscle tissue but also in other mammals like rats.[9] While the exact biological role of DPP III is still unclear, various studies show indications in involvement of pain signalling, blood pressure control, inflammation, and defence against oxidative stress.[10,11]

In 2012 the group of Gruber reported that they obtained the crystal structure of human dipeptidyl-peptidase III (hDPP III).[11] These results provided a significant approach towards developing a potent

inhibitor of hDPP III, in order to get further insight into the exact biological role of this enzyme. Previously developed inhibitors were often non-selective, toxic or unstable under physiological conditions.[8] The most promising inhibitors so far are spinorphin, an endogenous inhibitor of enkephalin-degrading enzymes, and tynorphin, a chemically synthesized analogue to spinorphin. However, they are still both slowly degraded by DPP III.[10,12] These results suggest that the number of five amino acid residues seems to be crucial for effective inhibition. In order to increase knowledge and produce more specific data for hDPP III this work is focused on the design and synthesis of new hydroxy-ethylene bioisostere inhibitors.

2 Theoretical background

2.1 Metalloproteases – Metal dependent enzymes

Enzymes are involved in numerous biological pathways, playing an important role in catalysing a variety of different chemical reactions. Metalloproteases cover a wide range within the many different classes of enzymes. These enzymes require a metal for their catalytic activity. The vast majority of them use zinc in their catalytic mechanism. Most of these metalloproteases exhibit a very specific catalytic motif, which is the His-Glu-Xaa-Xaa-His (or HEXXH) motif. In further detail this motif comprises an uncharged residue, a hydrophobic residue and one which could be almost any amino acid. The exception is proline, because proline in this specific motif would break up the helix.[13]

Metallopeptidases are able to hydrolyse peptide bonds, which is mediated by the nucleophilic attack of a water molecule. In these enzymes a divalent metal cation, such as zinc, cobalt or manganese activates the water molecule. In the HEXXH motif only one amino acid is coordinating the metal ion. The most common known metal ligands in metallopeptidases are His, Glu, Asp or Lys residues.[14]

Since zinc is one of the most common metals utilized in the catalytic mechanism, the role of zinc in enzymes or gene expression has attracted a lot of interest in the past decades and this field has recently gained wide attention. Not only its role in catalysis and gene expression is of significant importance, but it also stabilizes the structure of proteins, participates in transport processes, plays a role in immune and viral phenomena and preserves the integrity of subcellular organelles.[15]

Metalloproteases can be divided into two subgroups: metalloendopeptidases and metalloexopeptidases. Very well known metalloendopeptidases would be matrix metalloproteases (MMPs) and membrane-anchored disintegrin metalloproteinases (ADAMs). The ADAMs are a family of transmembrane and secreted proteins of approximately 750 amino acids in length.[16] These membrane-anchored ADAM proteins play a major role in activating zymogens, including tumor necrosis factor-epidermal growth factor (EGF) and amyloid precursor protein (APP). Moreover, these proteins participate in a wide range of cellular processes, including cell adhesion and migration, ectodomain shedding, proteolysis, development, ovulation and angiogenesis.[17] Matrix metalloproteases are responsible for tissue remodelling and degradation of the extracellular matrix, including collagens, elastins and many more. Regulation of these enzymes is based on growth factors, hormones and cytokines and they are also involved in ovarian functions. Studies indicate that inhibition of MMP`s may be a powerful tool against the fight of malignant cancer.[18]

2.2 Dipeptidyl-peptidase III

In 1967 *Ellis et al.* have reported the discovery of a new peptide cutting enzyme, which has been isolated from bovine pituitary.[19] Dipeptidyl arylamidase III and later renamed dipeptidyl-peptidase 3 (DPP III)[20] is a zinc dependent metallopeptidase that specifically cleaves the first two amino acids at the *N*-terminus from biologically important ogliopeptides, ranging from 3-10 amino acid residues, but more preferable 4-8 amino acids. It is a known member of the M49 peptidase family. DPP III harbours a very unique catalytic motif (HEXXGH) compared to the conventional HEXXH motif of common metallopeptidases. In 2008 *Baral et al.* were able to obtain the first crystal structure of yDPP III from *Saccharomyces cerevisiae* using X-ray crystallography. Figure 1 illustrates the crystal structure of DPP III, displaying two large domains which are separated through a large cleft.[21,22,8]

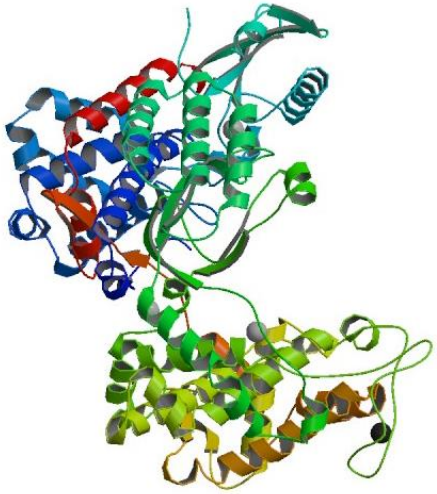


Figure 1: Crystal structure of human Dipeptidyl Peptidase III obtained by *Dong et al.*[23] Figure taken from *Dong et al.*[23]

Different synthetic dipeptidyl-β-naphthylamides (βNA) were tested towards hydrolysis, in order to get further insight into selectivity and activity. Results have shown that only Arg-Arg-βNA is readily hydrolysed, yielding Arg-Arg and β-naphthylamine, whereas other substrates such as Lys-Lys-βNA or Gly-Arg-βNA are not attacked. Suggesting that this Arg-Arg-βNA motif appears to be a specific substrate, it was also found that the optimum for hydrolysis lies at a pH 8.5 to 9. Furthermore, the cleavage of the dipeptidy1 residue is dependent on the presence of an unsubstituted amino terminus.[19]

In the past decades DPP III has turned out to be a ubiquitous peptidase. It has been isolated from a broad range from prokaryotic and eukaryotic species. In mammals, such as rats, DPP III is found in rat tissue, especially with elevated values in kidney, liver, heart, cerebrum and stomach.[24] In humans it has been found in erythrocytes, lymphocytes, neutrophils, skin, muscle and many more.[25-28]

Given the fact that DPP III can be found in a variety of prokaryotic and eukaryotic species, there is strong indication that this peptide cutting enzyme plays an important role in biological pathways in

mammals. However, the exact role in physiological pathways still remains unclear. Nevertheless, the ubiquitous distribution and broad specificity of action on oligopeptides of DPP III, contribute to the assumption that it is involved in intracellular catabolism.

In rat brains, DPP III was found to show a very high affinity towards angiotensins and enkephalins. In the metabolism of angiotensin, it is suggested that DPP III plays an important role due to a substantially higher affinity for both angiotensins II and III than any of the known angiotensin degrading enzymes. This leads to the assumption that DPP III plays a physiological role in regulating enkephalin and/or angiotensin disposition.[29] Another hint, that DPP III may be involved in biological pathways was reported within a study of *Sato et al.*[30] While elucidating the physiological role of spinorphin, it was found that DPP III was contained in human cerebrospinal fluid (CSF). With that information the activity of the enzyme was determined in CSF, derived from patients with pain. Results have shown that the activity of DPP III in CSF has been significantly lower for patients with pain compared to patients without pain.[30]

In 2010 *Chadwick et al.* reported the extraction of DPP III from cortical lipid rafts of 3xTgAD murine model of Alzheimer's disease. They identified it to be DPP III via mass spectroscopy, leading to the conclusion that this peptidase is located in membranes. However, the mechanism involved in targeting this cytosolic peptidase to the membrane is still unclear.[31] Even though there is a lack of any membrane targeting signal sequence, various studies suggest that posttranslational modifications of cytosolic proteins may be responsible for the localization in membranes of DPP III. Beyond that, even the ER-Golgi complex might not be involved in targeting this protein to the membrane.[32-34]

In recent years DPP III has attracted more attention due to its presumed involvement in other physiological pathways like the endogenous defense mechanisms against oxidative stress.[35] In 2003 *Šimaga et al.* reported that a significant rise in activity of DPP III has been observed in malignant ovarian tumors, compared to the activity in normal tissue.[36]

2.3 Structure and catalytic mechanism of DPP III

In 2008 *Baral et al.* reported the first crystal structure of yDPP III (Fig. 1).[21] Since the total understanding of the binding mechanism and the conformation during ligand binding was still unknown, it was a desirable goal to obtain an X-ray crystal structure of the enzyme in complex with a substrate. *Bezzera et al.* were the first one to report the crystal structure of hDPP III and its complex with the opioid peptide tynorphin.[11] ITC-measurements also revealed that the binding of the substrate is an entropy-driven process. This is very likely due to the release of ordered water molecules out the binding cleft.[11] Figure 2 illustrates the conformational change upon binding to the substrate.

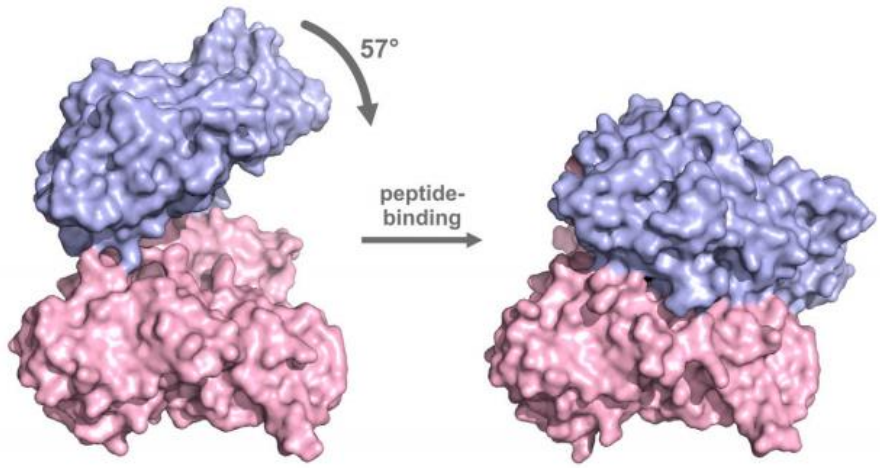


Figure 2: Domain movement upon tynorphin binding to hDPP III[11]

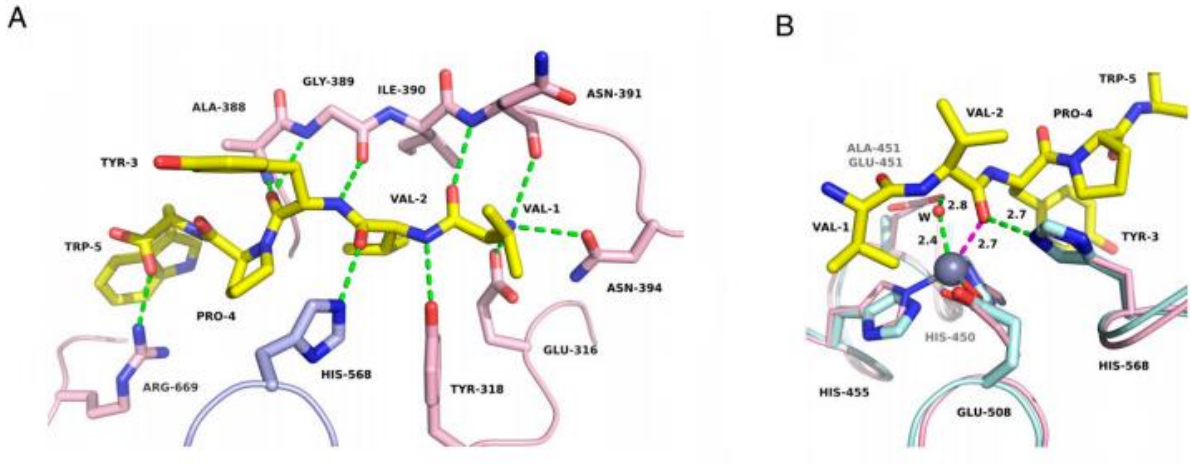


Figure 3: (A) Polar interactions of tynorphin with hDPP III (B) Superposition of the zinc-binding residues in the structures of the nonbound enzyme (light blue) and the peptide complex (pink). Pictures taken from *Bezzera et al.*[11]

Since first attempts of crystallising hDPP III failed, a truncated form of DPP III was synthesized, lacking 11 amino acid residues at the *C*-terminus. The overall fold of the modified hDPP III is very similar to the yeast homolog, even though there is only a 36% sequence identity. The enzyme consists of two domains, separated by a large cleft. The upper α-helical lobe contains the binding site of the zinc ion and also other catalytically important residues. One glutamate (Glu-451) and two histidine residues (His-450 and His-455) are tetrahedrally coordinating the zinc ion. These amino acid residues are also part of the catalytic motif, distinctive for the M49 metallopeptidase family, 450-HELLGH-455 and 507-EECRAE-512. An additional glutamate (Glu-451) is proposed to be responsible for the amide cleavage of the substrate. Similar to the mechanism of already known metallopeptidases like neprilysin[37] and thermolysin,[37] glutamate is responsible for the deprotonation of the water molecule and the subsequent nucleophilic attack of the amide bond. The amino acid residue histidine (His-568)

most likely provides stabilization of the tetrahedral transition state via hydrogen bonding. Among known DPP IIIs, both His-568 and Glu-451 are completely conserved.[11,38]

In addition to their work, where they investigated the binding mode and catalytic mechanism of hDPP III in complex with the synthetic opioid VVYPW, the group of Gruber[38] proposed another mechanism upon switching to other substrates. Endogenous opioid neurotransmitters Met-enkephalin (YGGFM) and Leu-enkephalin (YGGFL) can be found in spinal cords and brains of several animals, including humans. Mentioned neurotransmitters were already successfully tested towards peptide hydrolysis by hDPP III.[8] While in complex with Met-enkephalin (YGGFM) and Leu-enkephalin (YGGFL), a water molecule could be found near the P1 carbonyl of the peptide. Contrary to that result, X-ray crystallography revealed that while in complex with synthetic peptide IVYPW, no water molecule is found to be in close proximity to the scissile peptide bond. Instead, Glu-451 shows a close proximity, leading to the assumption that hydrolysis of the peptide bond is rather executed via an anhydride mechanism than a water-promoted mechanism, which is assumed to occur with substrates like Met/Leu-enkephalin (Fig. 4). Taking these findings into account, these could be an important approach towards the understanding which oligopeptides are rather a substrate than a competitive inhibitor.[38]

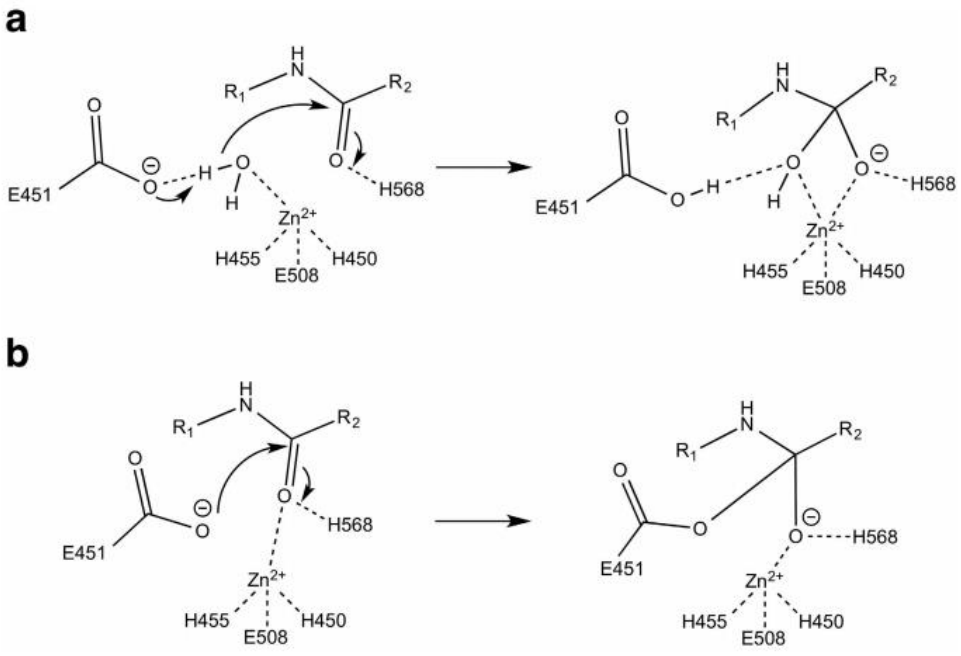


Figure 4: Possible mechanisms of peptide hydrolysis catalysed by hDPP III. (a) Promoted-water mechanism. (b) Anhydride mechanism. Picture taken from *Kumar et al.*[38]

2.4 Potential physiological role of hDPP III

Dipeptidyl peptidase 3 (DPP III) has been a subject of intense investigation for years. Yet, it is still not possible to assign a specific role and its involvement in physiological pathways for this peptide cutting enzyme. However, there are several indications that DPP III is involved in several catabolic mechanisms, ranging from protein turnover, and blood pressure regulation, even up to the correlation between overactivity of the enzyme and malignant tumor cells.

2.4.1 Protein turnover

Peptides, which are meant to be degraded by the ubiquitin proteosome system, are released into the cytosol.[39] These peptides exhibit a length of 3-24 amino acids, which are further hydrolysed into their constituent amino acids. Peptides with a chain length longer than 16 are degraded by tripeptidylpeptidase II (TPP II), while peptides with 6-17 amino acid residues are hydrolysed by thimet oligopeptidase (TOP).[8] The resulting shorter peptides are further degraded by various hydrolyases like leukotriene A_4 hydrolase or aminopeptidase B.[40] Given the fact that DPP III is a peptide cutting enzyme which preferable degrades ogliopeptides with a chain length of 4-8 amino acid residues an involvement in this process is assumed. The affinity towards such physiologically relevant peptides of DPP III at cellular pH is another indication of the involvement in protein turnover (Fig. 5).[8] Most peptides, which contain proline in their protein sequence, are resistant to hydrolysis. *Baršun et al.* reported that DPP III can act as a post-proline-cleaving enzyme,[41] which would be another strong recommendation of its inclusion in this process. Figure 5 roughly illustrates the possible involvement in this catabolic process.[8]

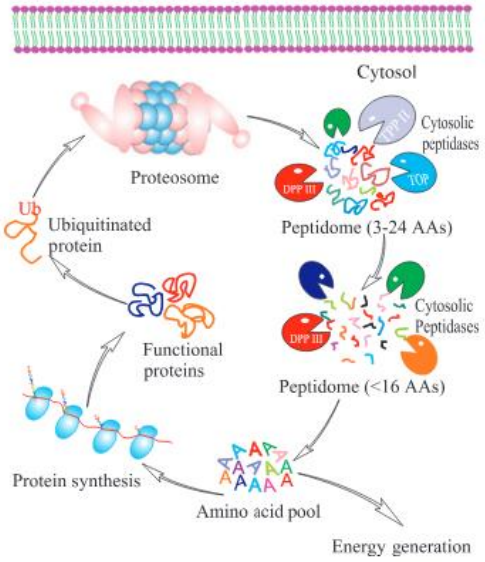


Figure 5: Potential role of DPP III in protein turnover. Figure taken from *Prajapati et al.*[8]

2.4.2 Defence against oxidative stress

The activity of DPP III has been found to exhibit significantly high levels in malignant primary ovarian carcinomas. Interestingly, only a higher activity in malignant ovarian tumors could be observed, but not in benign ovarian tumors, compared to the activity in normal tissue.[36] In 2007 *Liu et al.* reported activation of the antioxidant response element (ARE) in primary mouse derived cortical neurons by a subset of cDNAs, which also encodes DPP III. The overexpression of DPP III stimulated NF-E2-related factor 2 (NRF2) nuclear translocation and led to increased levels of NAD(P)H:quinone oxidoreductase 1, a protein which is transcriptionally regulated by the ARE. Overexpression of DPP III also contributes to the resistance against H_2O_2 or rotenone-induced toxicity.[35] *Wilson et al.*[42] suggested a linkage between H_2O_2, a tumor-derived factor, and increased Ets-1 expression, which leads to the proposal that this reactive oxygen species (ROS) may be an important factor in directly regulating the expression of Ets-1 in tumor cells. Hence, elevated expression of DPP III in cancer cells may be a result of increased Ets-1 levels, which would mean that overexpressed DPP III may promote tumor growth.[8,42]

2.4.3 Blood pressure regulation and inflammation

Peptides with a chain length of <10 amino acid residues are bioactive peptides. They participate in various physiological pathways, such as signal transduction, blood pressure regulation or reproduction.[8] The high affinity of DPP III to neurotransmitters such as enkepahlins and angiotensins[29] and the colocalisation of DPP III in several body fluids, leads to the assumption that it might contribute to the dislocalisation of the these small peptides, further leading to homeostasis.[8] An increase in blood pressure may be caused by the hydrolysis of hemopressin by DPP III.[43]

2.4.4 Nociception

Small peptides, such as enkephalins, angiotensins, endorphins and endomorphins, play an important role in nociception. Figure 6[44] displays the involvement of these opioids within this process. After an injury or chronic inflammation, immune cells release pro-inflammatory cytokines and chemokines which initiate and maintain the noxious message. Subsequently, opioid peptides that are secreted by activated immune cells promote an antinociceptive effect. This effect is initiated through the activation of their peripheral receptor. The ascending noxious message is subsequently sent into upper brain areas.[44]

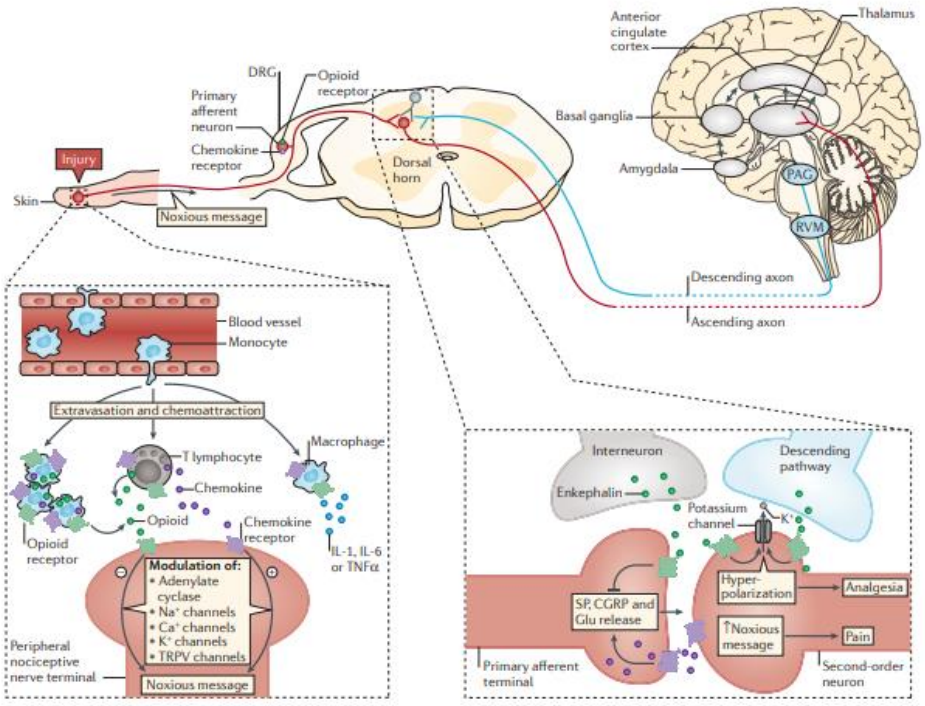


Figure 6: Involvment of chemokine and opioids in nocicepticve pathways. Figure taken from *Mélik et al.*[44]

DPP III is already known for its high affinity to small physiologically important oligopeptides such as enkepahlins and angiotensins,[29] *Hambrook et al.* reported the rapid degradation of small peptides in human plasma and rat brain homogenates via peptide bond hydrolysis. Even though the nature and specificity of the enzymes involved is still unclear,[45] DPP III with its high affinity for these compounds might be a strong indication for its involvement.

2.5 Known inhibitors of DPP III

The exact structure and binding conformation of DPP III remained unknown for a long time. Recent work on crystallisation attempts of hDPP III in complex with their substrates and inhibitor respectively, provided further insight into the ongoing peptide hydrolysis.[11,21,38] Since DPP III is assumed to be included in many important physiological pathways, like blood pressure regulation,[43] pain signalling[45] and protein turnover,[8] inhibition of this enzyme was always a desirable goal, in order to prevent overactivity.

Contrary to expectations that EDTA would shut down or at least lower the activity of zinc-dependent peptidase DPP III from bull reproductive tissues and rat skin, no effective inhibition could be achieved.[46] Mammalian DPP III is significantly inhibited by different small molecules which are potent cysteine peptidase inhibitors such as pCMB, *p*-chloromercuriphenylsulfate (pCMS), *p*-hydroxy-mercuribenzoate (pHMB) or *p*-chloromercuribenzene sulfonic acid (*p*CMBS).[47,48] In the same fashion DPP III is also inhibited by multiple serine peptidase inhibitors like phenylmethanesulfonylfluoride

(PMSF), diisopropylfluorophosphate (DFP), 3,4-dichloroisocoumarin (DCI) or tosyl-phenylalanyl chloromethyl ketone (TPCK).[48-50]

The addition of metal ions also seems to effectively lower the activity of DPP III. Addition of metal ions like Cd^{2+}, Cu^{2+}, Mn^{2+} or Zn^{2+} has been reported to inhibit DPP III, probably due to interaction of the metal with cysteine residues or alcohol and carboxylic groups of other amino acids, leading to a significant change in overall conformation.[8,21,48,51] Especially, the addition of Hg^{2+} shuts down the activity of DPP III entirely, even at very low concentrations.[52] Most of these inhibitors were still either non-selective, toxic or both. The discovery and isolation of fluostatins A and B out of *Streptomyces sp*. TA-3391 was a significant progress in competitive inhibition. They turned out to be very potent and almost exclusively selective for placental DPP III. With arginyl-arginine-2-naphthylamide as a synthetic substrate, fluostatins A and B exhibited IC_{50} values of 0.44 and 24.0 µg/mL, respectively.[53]

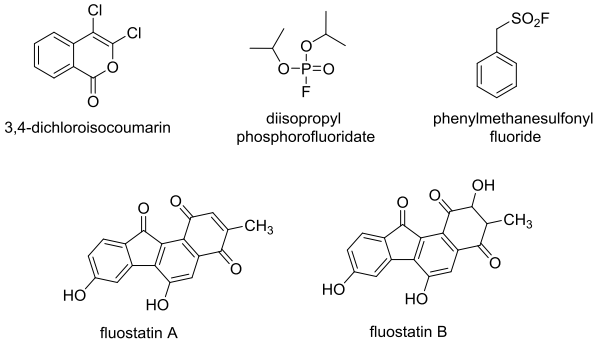


Figure 7: Selected inhibitors for DPP III.[48,49,53]

The most potent inhibitors so far are actually oligopeptides. The distinction between an inhibitor and a substrate is pretty blurry. In fact, these oligopeptides act as inhibitor as well as a substrate, meaning that it is well designed to fit into the active site of DPP III, but being very slowly degraded. This very slow degradation leads to competitive inhibition. *Yamamoto et al*.[12] and *Nishimura et al*.[54] reported the finding and isolation of very potent and highly selective inhibitors, tynorphin (VVYPW) (Fig. 8) and spinorphin (LVVYPWT), respectively. With tynorphin in particular, a very selective inhibitor for enkephalin-degrading enzymes was found. Unfortunately, experiments revealed that it slowly gets hydrolysed by the enzyme. Complete degradation of tynorphin in human serum at 37 °C could be observed already after 4 h.[12] However, this promotes the search for new inhibitors which are stable under physiological conditions.

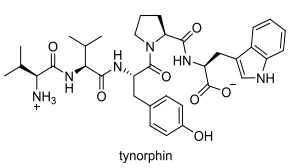


Figure 8: Tynorphin, one of the most promising oligopeptide-based inhibitors so far[12]

2.6 Transition state mimetics

In recent years drug design for the inhibition of specific enzymes has experienced a remarkable progress. For efficient drug development a lot of information about the enzyme, its substrates and most importantly its binding mode and catalytic mechanism is necessary. Major progress in structural biology, X-ray crystallography and NMR-spectroscopy are responsible for the better understanding of proteins.[1] These techniques have provided a significant approach to elucidate the ongoing mechanism of enzyme-substrate interactions. With the knowledge of the exact binding mode, the corresponding catalytic motif and the understanding of the ongoing chemical reaction, a transition state during substrate modification can be proposed. Modern drug design is targeting the synthesis of molecules, which mimic the transition state of the natural substrate under the premise that the transition state analogue binds way more tightly to the enzyme than the substrate.[55]

Several compounds, which are transition state analogues have been synthesized over the last couple of decades. Some prominent examples are: phosphonamidate transition state analogues, which inhibit the activity of thermolysin,[56] an enzyme which cleaves peptide bonds, similar to DPP III. Amino acid sulfonamide transition state analogue, which inhibits arginase, which is a binuclear manganese metalloenzyme that catalyses the hydrolysis of L-arginine to form L-ornithine plus urea.[57] A hydroxyethylene isostere has been used to inhibit human immunodeficiency virus-1 protease.[58] Oseltamivir, a neuroamidase inhibitor was designed for the treatment and prophylaxis of influenza.[59]

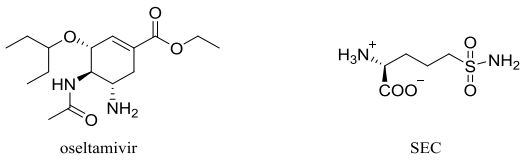
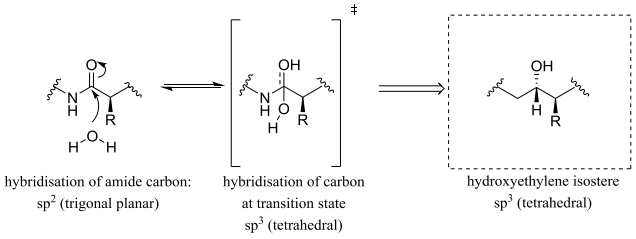


Figure 9: Selected inhibitors, which are transition state analogues[57,59]

Similar to these examples, one goal to potently and selectively inhibit hDPP III is to introduce a hydroxyethylene bioisostere. Since, tynorphin and spinorphin, one of the best oligopeptide-based inhibitors so far, get slowly hydrolysed by the enzyme under physiological conditions,[12,54] the introduction of this transition state mimicking moiety, should bring inhibition activity (nM scale) and selectivity towards a new level (Scheme 1).



Scheme 1: Exchange of the classic amide moiety to hydroxyethylene transition state analogue.

3 Aims of this thesis

In 2000 *Yamamoto et al.* reported the synthesis of tynorphin (VVYPW), an oligopeptide with 5 amino acid residues. This peptide significantly inhibits the activity of hDPP III.[12] X-ray crystal structure of hDPP III in complex with tynorphin, described by *Bezzera et al.*[11] provided further insight into the mechanism of the enzyme upon substrate binding. Unfortunately, synthetic opioid tynorphin gets slowly degraded by hDPP III and turned out to be not stable under physiological conditions.[12] However, these results strongly contributed to the development of new specific inhibitors of hDPP III.

Based on tynorphin as lead structure, Jakov Ivković developed a novel potent inhibitor for hDPP III.[60] While preserving the original peptide sequence Val-Val-Tyr-Pro-Trp,[12] the idea was to replace the amide bond of Val-Val by a hydroxyl-ethylene moiety. Since the peptide is supposed to be cleaved at this position,[19] the introduction of this bioisostere might lead to the conservation of the original structure of the peptide. ITC measurements of two novel inhibitors, ***SHE*** (*S*)-hydroxyethylene and ***HER*** (*R*)-hydroxyethylene, revealed an endothermal binding profile, similar to tynorphin. With IC_{50}-values of 91 µM and 8.5 µM respectively, a major improvement has been achieved. With a non-cleavable peptide bond and based on these knowledge, extensive work has also been carried out during the thesis of Bernhard Berg[61] and Christian Lembacher-Fadum.[62] Based upon these results and the lead structure of ***HER*** (*R*)-hydroxyethylene, this work shall target the synthesis and modifications of this potent inhibitor, in order to push activity towards the nM range.

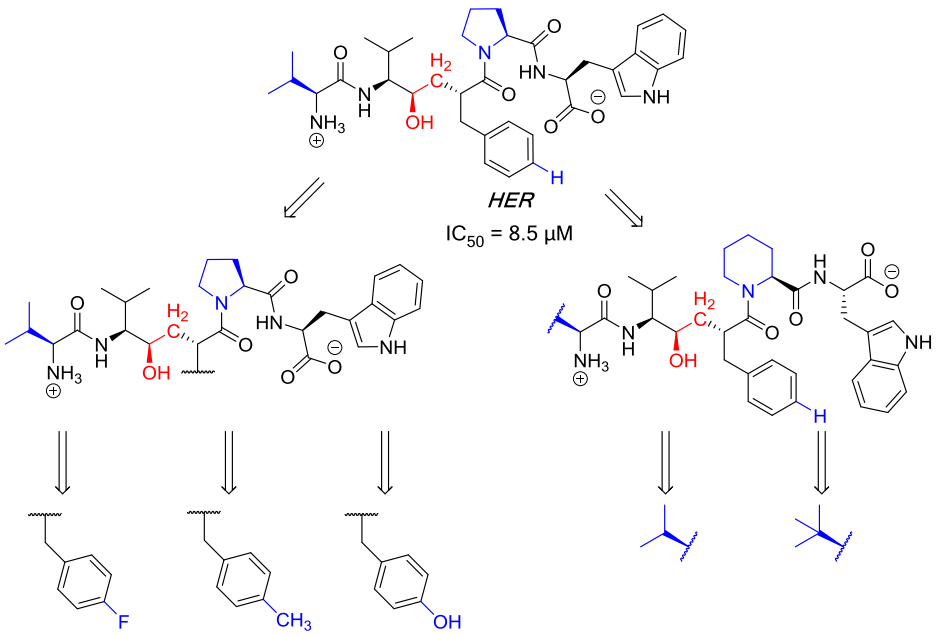


Figure 10: Design of new potential inhibtors for DPP III

Figure 10 illustrates, where modifications and changes within this molecule should be installed (highlighted in blue), while preserving the crucial hydroxyethylene moiety (highlighted in red). On the one hand, the first idea is to introduce new substituents on the aromatic moiety in the phenylalanine

residue, with a special focus on the introduction of the hydroxyl moiety, since this is almost as close as it gets to the original structure of tynorphin.[12] On the other hand, an exchange of the proline residue by a six-membered ring is desired, while simultaneously replacing the valine residue by a *tert*-leucine residue and preserving the original valine, respectively. A similar synthetic route, which was already established for the synthesis of ***HER*** (*R*)-hydroxyethylene, was applied. Inhibitors ***HER***, ***SHE***, and other synthesized compounds, have already proven to be very efficient inhibitors.[60,62] These new compounds should provide further insight and understanding of the inhibition of hDPP III and give a significant boost in activity.

4 Results and discussion

4.1 Synthesis and modification of hydroxyethylene inhibitors of hDPP3

The theses of J. Ivković, C. Lembacher-Fadum and B. Berg, have already provided a strong foundation for the successful synthesis of powerful and promising inhibitors of DPP III.[60–62] Within this work we wanted to synthesize even more inhibitors, using the already established synthesis route, in order to gain more knowledge about DPP III and improve activity towards the desired nM range. Via the introduction of new substituents and changing amino acid residues we wanted to achieve a boost in activity of already active inhibitors. Figure 11 compares the structures of synthetic oligopeptide tynorphin (VVYPW)[12] and ***HER*** (*R*)-hydroxyethylene.

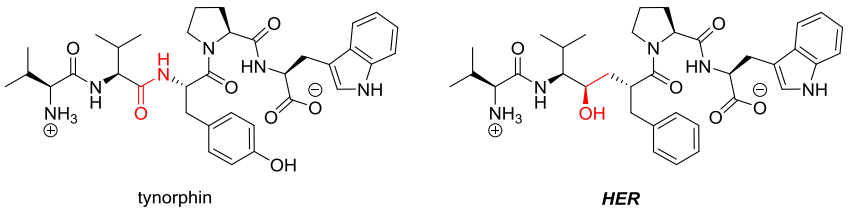


Figure 11: Comparison of the structures of tynorphin and *HER*.

The peptide bond (red), which is cleaved by the enzyme in tynorphin, was replaced by the hydroxyethylene moiety in novel compound ***HER***. With this non-cleavable bond, this compound is not supposed to be cleaved by the enzyme, contrary to the fact that tynorphin gets slowly degraded by the enzyme.[12] At first glance, the structures seem to be identical, apart from the replaced peptide bond. However, ***HER*** exhibits a structural difference, since the Tyr residue is replaced by the Phe residue, simply due to easier synthetic access.[60] This is exactly where we targeted our focus at – the introduction of a new substituent onto the aromatic ring. Furthermore, we wanted to exchange the proline residue by a pipecolic acid residue and simultaneously varying amino acid residue Val. Figure 12 displays all desired new inhibitors, which we wanted to synthesize in the course of this work.

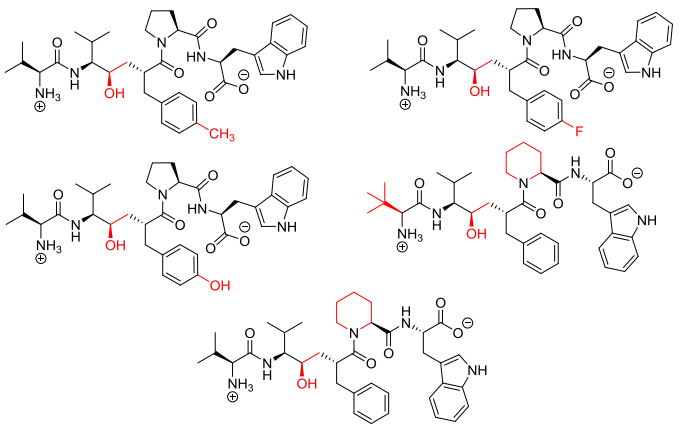
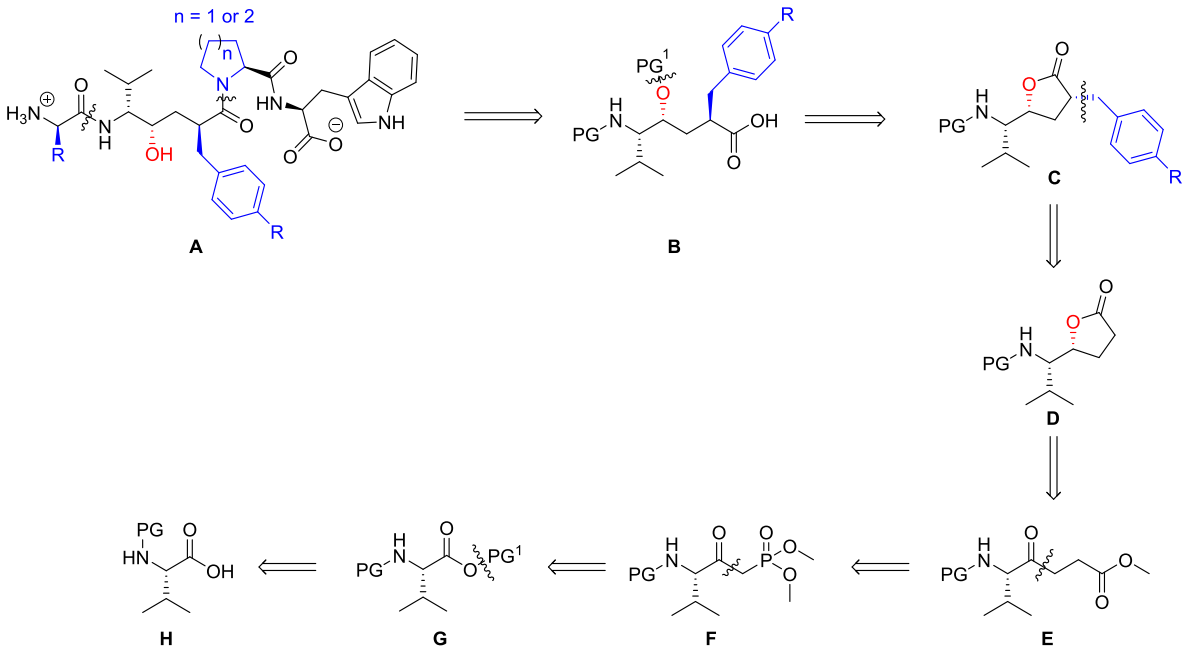


Figure 12: Desired inhibitors, which should be synthesized during this work.

The modifications we wanted to install are highlighted in red (Fig. 12). To access the desired molecules, an established synthesis route was chosen. Based on the work of *Haug et al.*[63] where a synthesis route of a Gln-Phe hydroxyethylene isostere is described, we wanted to access a major core fragment, in order to introduce our various new substituents. Scheme 2 displays the breakdown of this multiple step synthesis in a retrosynthetic manner.

4.1.1 Retrosynthetic approach



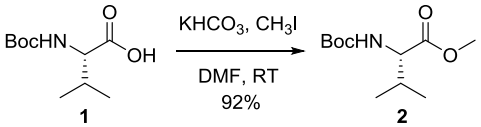
Scheme 2: Retrosynthetic analysis of the desired pseudopentapeptides.

Target molecule **A** can be obtained by a two-step standard coupling procedure and deprotection sequence. Different amino acids can be used in order to access our desired compounds. The *O*-protected acid **B** can be synthesized by ring opening and subsequent alcohol protection from alkylated lactone **C**. The latter compound exhibits different aromatic residues, hence the synthetic pathway from **D** to **C** allows the introduction of the respective different amino acid residues (e.g. Tyr). Compound **D** can be accessed via the stereoselective reduction and subsequent acid-catalysed lactonization from compound **E**. A *Horner-Wadsworth-Emmons* reaction of **F** with methyl glyoxalate allows the synthesis of **E.** Introduction of a phosphonate moiety into *O*-protected L-Val gives access to starting material **F** for the HWE reaction. Overall starting material is *N*-protected essential amino acid L-valine.

4.2 Synthesis of the pseudopeptide core fragment

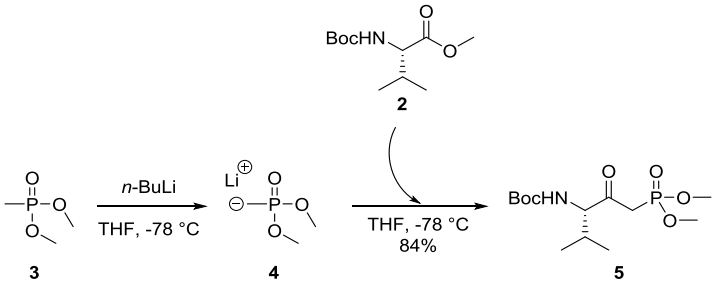
4.2.1 From L-valine to the ketophosphonate

The first step in the synthesis of the pseudopeptide core fragment was the protection of commercially available Boc-protected L-Val. Following a straightforward procedure of *Brenner et al.*[64] **2** could be methyl-protected using methyl iodide and potassium bicarbonate in 92% yield (Scheme 3). According to NMR, simple aqueous work up of the reaction provided the desired compound in excellent quality and **2** could be used in the next step without further purification.



Scheme 3: *O*-protection of Boc-Val-OH.[64]

For the introduction of the phosphonate group, dimethyl methylphosphonate was first lithiated with *n*-butyl lithium in THF at -78 °C. Deprotonated dimethyl methylphosphonate was then allowed to react with *O*- and *N*-protected compound **2** to yield keto phosphonate **5** in 84% yield. Yet again, only simple aqueous work was necessary to furnish the desired compound in satisfying purity. No further purification needed to be performed, since NMR indicated no significant impurities.

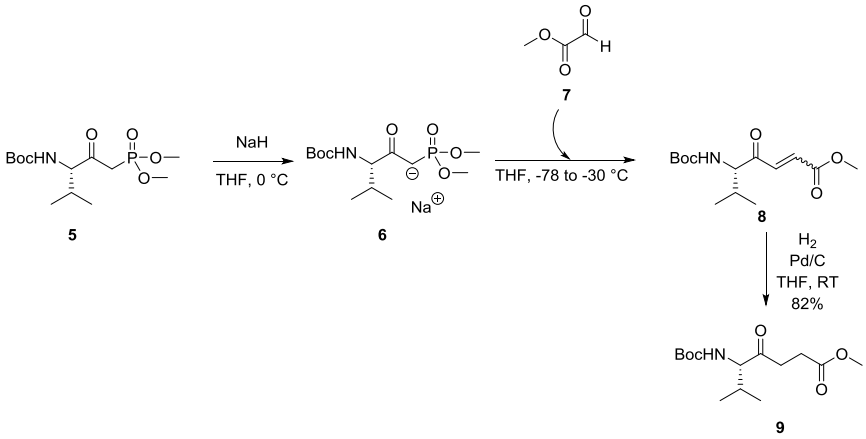


Scheme 4: Synthetic route towards the synthesis of the keto phosphonate.

4.2.2 Horner-Wadsworth-Emmons reaction

The synthesis of keto phosphonate **5** gave access to the following HWE reaction. The corresponding aldehyde had first to be synthesized. This was achieved by the oxidative cleavage of dimethyl tartrate using periodic acid. The procedure of *Schuda et al.*[65] was applied and no further purification of the aldehyde was performed, since this was not necessary for this purpose, even though there is a equilibrium of hydrated and non-hydrated form. However, since aldehyde **7** is not considered to be stable over a long time, it was freshly prepared and directly used in the HWE reaction. Scheme 5 illustrates the synthesis of olefinic compound **8** and subsequent catalytic reduction with hydrogen to

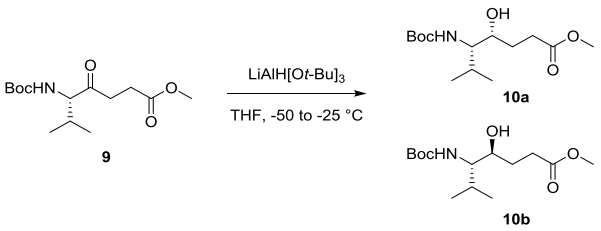
obtain keto ester **9**.[63] The ketophosphonate was first deprotonated at 0 °C with sodium hydride and was allowed to react with freshly prepared aldehyde **7** at -30 °C, to yield the *cis/trans* olefin **8** which was not purified and subsequently reduced with molecular hydrogen over palladium on activated charcoal. Keto ester **9** was obtained after flash chromatography in 82% yield, showing no other impurities (Scheme 5).



Scheme 5: HWE reaction and subsequent hydrogen mediated reduction for the synthesis of the keto ester.

4.2.3 Stereoselective reduction and lactonization

With the isolation of keto ester **9**, we have reached a crucial step in the total synthesis of our new inhibitors - the stereoselectively very important introduction of the hydroxyethylene moiety via reduction. Scheme 6 displays how this stereoselective reduction was performed. In order ensure that only one major isomer is formed, a very bulky metal hydride was used. Since bulkiness and temperature have a significant influence on the outcome of the reaction,[66] we tried to approach this reduction with both temperature and choice of reducing agent. Using LiAlH[O*t*-Bu]$_3$,[63] a bulky reducing agent was involved in the reaction in order to control stereoselectivity. To monitor the reaction progress as well as the distribution of compounds **10a** and **10b**, GC-MS was used. Monitored by continuous measurements, the temperature was gradually increased very slowly, starting at -50 °C to ensure that stereoselective control will take place.



Scheme 6: Stereoselective reduction with LiAlH[O*t*-Bu]$_3$.

Figure 13 displays the reaction progress and the consumption of the starting material respectively. After 50 h, conversion was still not complete and had been stagnant since approximately 30 h. At this point a temperature of -25 °C was reached, but reaction progress could not be pushed further towards

full completion. Since it was desirable not to increase temperature any further, the reaction was quenched after a conversion of 89% of the starting material and a ratio of desired compound **10a** to **10b** of 75:25.

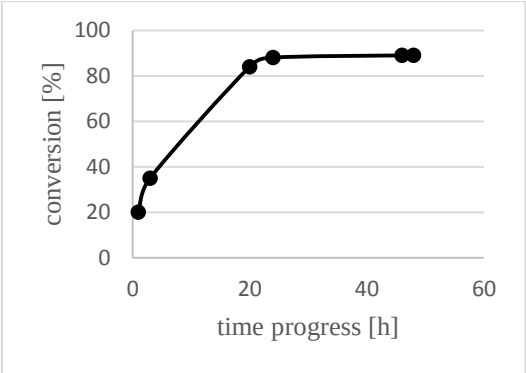
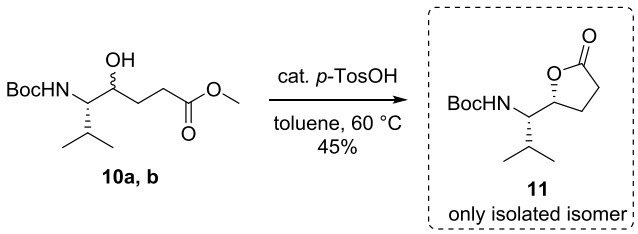


Figure 13: Reaction progress of the stereoselective reduction, determined with GC-MS measurements.

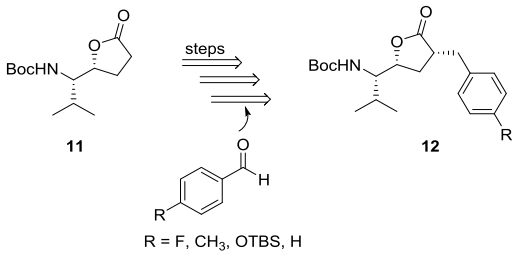
Although the ratio of compounds **10a:10b** was only 3:1, the crude mixture of **10a** and **10b** was directly used in the next step without further purification. Scheme 7 illustrates the acid-catalysed lactonization of the alcohol, leading to compound **11**. After 24 h the crude reaction mixture was concentrated and simply crystallised from hot *n*-pentane to furnish the pure desired (*R,S*)-γ-lactone as a white powder. NMR as well as HPLC analyses revealed that no further purification was needed, since no impurities could be detected. After two steps core fragment **11** could be isolated in acceptable 45% yield and provided the possibility to introduce different aromatic substituents.



Scheme 7: Acid-catalysed lactonization, in order to obtain pseudopeptide core fragment.

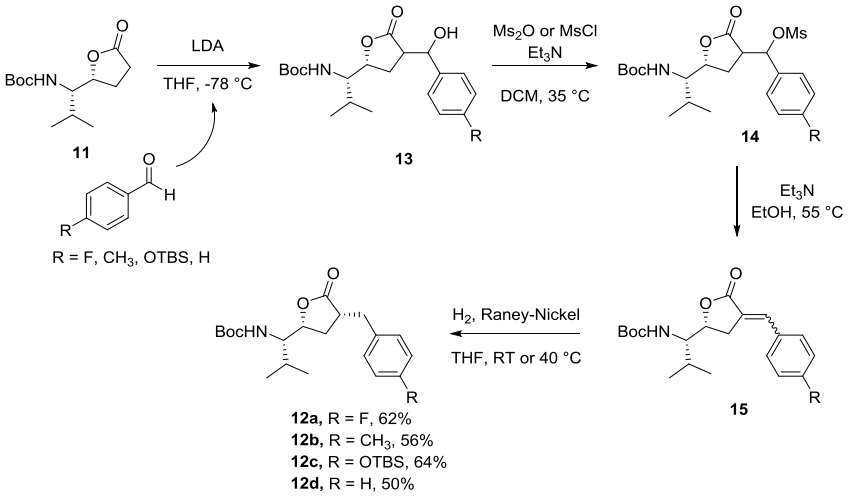
4.3 Introduction of the new aromatic moiety

Having γ-lactone **11** in hand it was now possible to introduce various aromatic substituents. Our synthetic approach is displayed in Scheme 8.



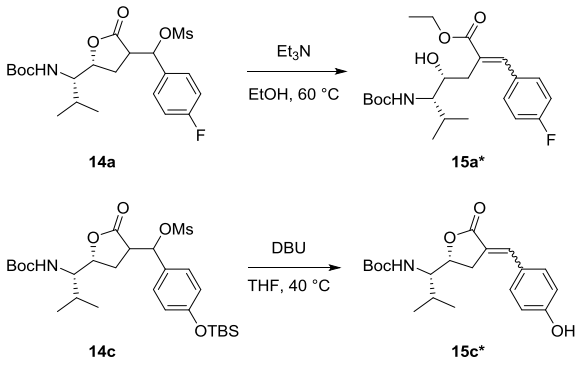
Scheme 8: Reaction scheme to furnish differently substituted alkylated lactones.

Following literature procedures from *Nadin et al.*[67] we started with a simple aldol condensation of the corresponding aromatic aldehydes. All of them were commercially available, except for the TBS-protected *p*-hydroxybenzaldehyde. For that purpose, the hydroxyl moiety of the benzaldehyde derivative was protected with TBSCl under standard protection conditions. The procedure of *Faler et al.*[68] allowed the isolation of TBS-protected *p*-hydroxybenzaldehyde in an acceptable 67% yield. With all aromatic aldehydes ready to use, it was possible to perform the first reaction in this four-step sequence. Scheme 9 displays the overall reaction scheme in order to obtain the desired alkylated lactones.



Scheme 9: Reaction cascade for the synthesis of different alklyated lactones.

The first step was the aldol condensation of γ-lactone **11.** Deprotonation of the α-hydrogen with LDA at -78 °C led to the deprotonated intermediate, which was allowed to react with the aromatic aldehydes, leading to the corresponding diastereomeric mixture of alcohols in yields between 63-86%. The alcohol moiety was subsequently converted into a leaving group for the following elimination step by using methanesulfonyl chloride and methanesulfonic anhydride respectively. Elimination under basic conditions with triethylamine gave the corresponding olefin which was subsequently stereoselectively reduced to the alkylated lactone. The reduction was performed with hydrogen in the presence of Raney®-Nickel. Stereoselective control was achieved due to the bulkiness of the substituents while the lactone ring is in one plane with the aromatic ring. A general *cis*-hydrogenation mechanism[69] allowed access to the proposed structure and subsequent NOE experiments confirmed the correct configuration. After a four-step sequence quite good yields for the alkylated lactones between 50-64% could be obtained.

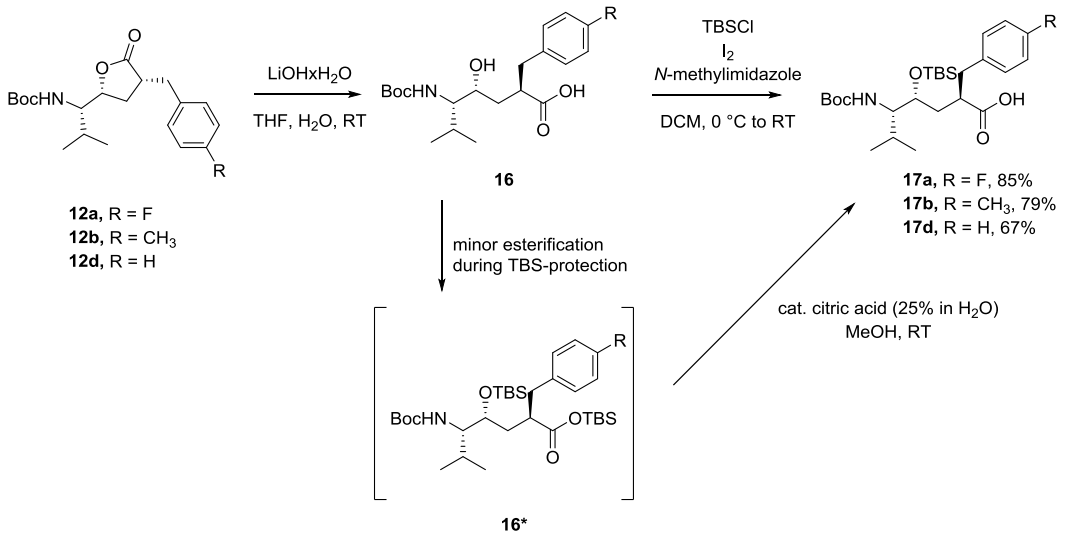


Scheme 10: Observed side reactions during the elimination step.

However, a few troubles while performing the elimination reactions of two compounds were faced. As shown in Scheme 10, side reactions for compounds **14a** and **14c** could be observed. Since for none of the mentioned reactions complete conversion of the elimination, according to TLC, could be observed, two new synthetic approaches were tried. On the one hand we switched to another base and on the other hand we tried to increase reaction temperature. Treatment with DBU instead of triethylamine as a base for compound **14c** led to full conversion after only 4 h. However, a simultaneous cleavage of the TBS moiety could be observed. Increasing the reaction temperature for compound **14a** led to the total decomposition of the starting material by transesterification with the solvent ethanol. Due to these results we stuck to the already established reaction conditions. In both cases, prolonged reaction times up 72 h finally led to full consumption of the staring material.

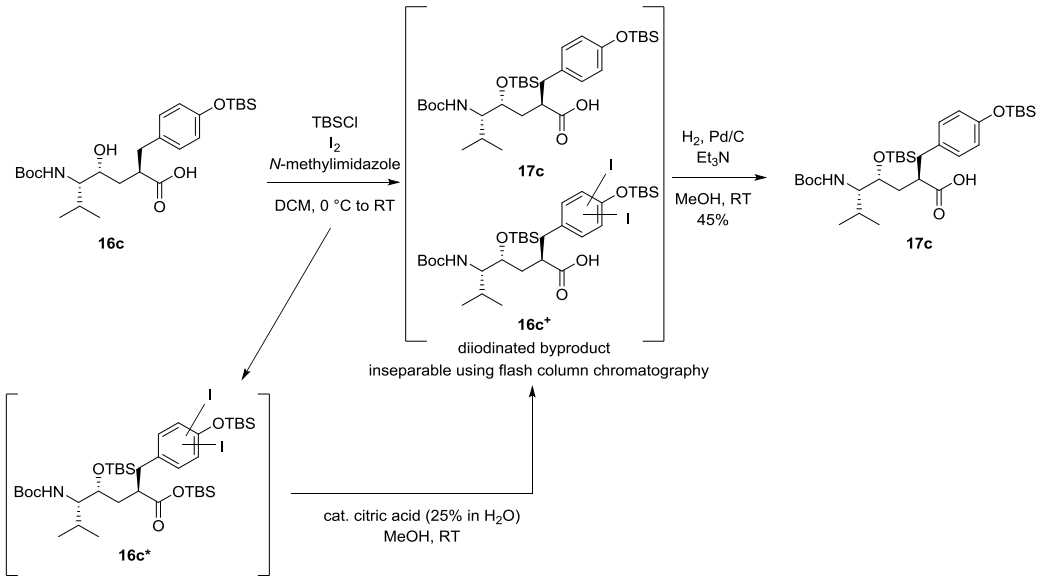
4.4 Ring opening and subsequent alcohol protection

The next step of the synthesis was the lactone ring opening and the subsequent protection of the hydroxyl moiety with TBSCl (Scheme 11). Opening the ring structure with a base in particular, was a very sensitive procedure, since the γ-hydroxy acid intermediate **16** is very prone to perform relactonization. The first step was the saponification with $LiOHxH_2O$ in THF/H_2O at room temperature. Subsequently, a pH of ~4 was adjusted with 25% citric acid at 0 °C. Performing this acidification at 0 °C was a crucial precaution in order to prevent the mentioned relactonization under acidic conditions. Crude intermediate **16** was then treated with TBSCl, in the presence of *N*-methylimidazole and iodine.[70] According to the procedure of *Bartoszewicz et al.*[70] the presence of iodine is a very important factor to accelerate the reaction. The formation of I_2Cl^- ensures a significant boost in reaction progress. Due to the reason that during TBS-protection partly conversion to the undesired TBS-ester **16*** could be observed, the final step included the treatment of the mixture of **16** and **16*** with 25% citric acid in methanol in order to remove byproduct **16***. After a three-step sequence the desired pseudodipeptide fragments could be isolated in yields between 67-85%.



Scheme 11: Three-step synthesis in order to obtain dirfferent γ-protected hydroxy acids.

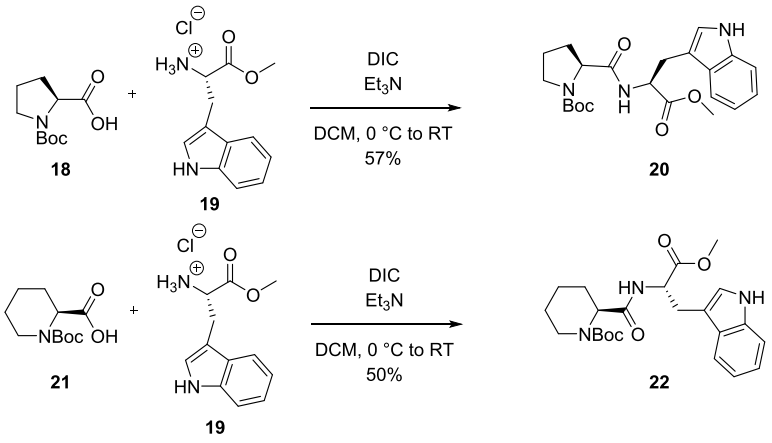
While performing the TBS-protection of compound **16c**, an unexpected side reaction was observed (Scheme 12). The presence of iodine during the protection procedure allowed the iodination of the aromatic ring. Since the hydroxyl moiety attached to the aromatic ring exhibits a positive mesomeric effect and therefore pushes electrons into the aromatic system, we could observe a significant amount of starting material being iodinated under described conditions. NMR indicated a distribution of compounds **16c*** and **17c** of almost 1:1. Purification attempts using flash chromatography turned out not to be successful, since R_f values of both compounds were almost the same. Since we could not perform flash chromatography as purification method of choice, we were forced to change our strategy. Therefore another reaction step was performed in which the mixture of compounds **16c*** and **17c** was treated with hydrogen, in the presence of triethylamine and palladium on charcoal in methanol, to furnish desired compound **17c** in acceptable 45% yield after an ultimate four-step sequence. Scheme 12 outlines our different strategy, compared to previous synthetic approaches.



Scheme 12: Unexpected side reaction and new synthetic approach to avoid undesired sideproduct.

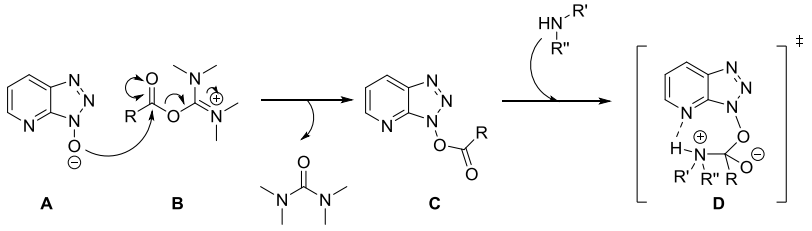
4.5 Elongation of the *C*-Terminus

With all the pseudodipeptide fragments in hand, the core structures were now ready for elongation at the *C*-terminus with the corresponding dipeptides. These dipeptides had first to be synthesized. For that purpose literature known procedures[60] were used, utilizing triethylamine as base and *N*,*N'*-diisopropylcarbodiimide as coupling agent (Scheme 13). Dipeptide fragments Boc-Pro-Trp-OMe and Boc-Pip-Trp-OMe were obtained in 57% and 50% yield respectively.



Scheme 13: Amino acid coupling in order to obtain desired dipeptides

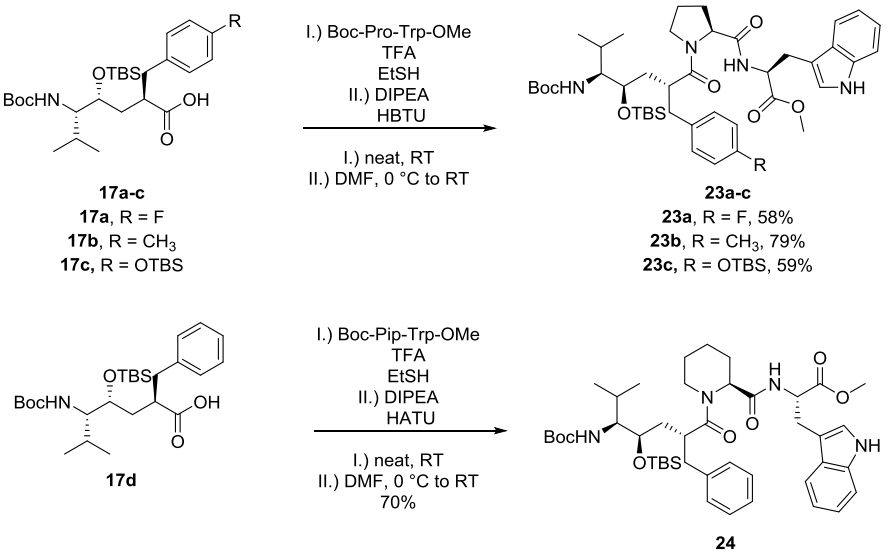
The elongation of the *C*-terminus was carried out under standard coupling conditions. The same strategy as described in previous work was applied. First Boc-deprotection of the dipeptide fragment was achieved by using TFA. Subsequent coupling was performed in the presence of DiPEA and HBTU in DMF, in case of the synthesis of compounds **23a-c**. First attempts to obtain compound **24**, with Boc-Pip-Trp-OMe as dipeptide fragment, failed. The usage of HBTU as coupling agent turned out not to be successful. According to literature HBTU exhibits the less activating property compared to HATU.[71] Increased activity of coupling additive HATU can possibly arise due to hydrogen bonding of the nitrogen of the pyridine moiety, stabilizing the seven-membered cyclic transition state (Scheme 14).[71]



Scheme 14: Reaction scheme of the activation of amino acids with HATU.

Therefore, the switch to HATU as activating agent was obvious. The need of HATU, being the more powerful coupling additive, instead of HBTU could also be explained, that the nitrogen of the pipecolic acid residue is not as accessible for activation as the nitrogen of the proline amino acid residue. Binding angles of the nitrogen in the Pip-residue are larger than in the Pro-residue. Hence, it

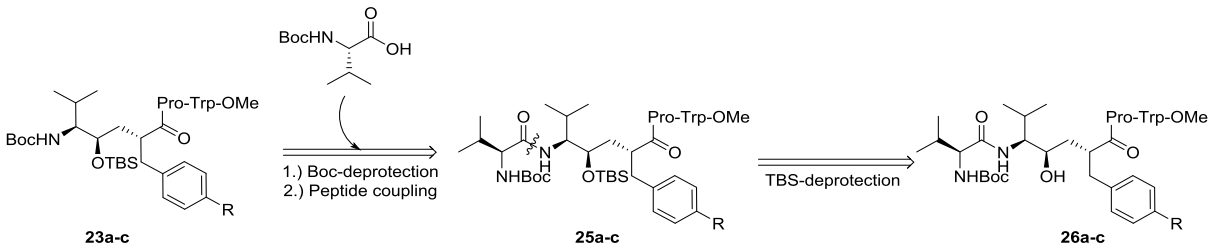
is much more sterically hindered since its part of six-membered ring compared to the five-membered ring of the proline amino acid residue. Furthermore, nucleophilicty of the pyrrolidine moiety is considered to be much higher than of the piperidine moiety.[72] Scheme 15 illustrates the synthetic approaches towards the different elongated pseudotetrapeptides.



Scheme 15: Synthetic route towards differently substituted pseudotetrapeptides.

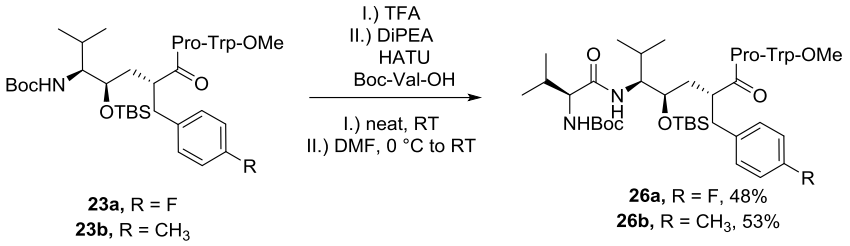
4.6 Elongation of the *N*-terminus and first attempts for TBS-deprotection

With the pseudotetrapeptides in hand, only one amino acid residue was left to be attached to get to the final structure of the newly designed inhibitors. For that purpose the idea was, to first couple the last amino acid residue and subsequently deprotect the hydroxy moiety of the molecule in a two-step sequence. The general synthetic approach is displayed in Scheme 16.



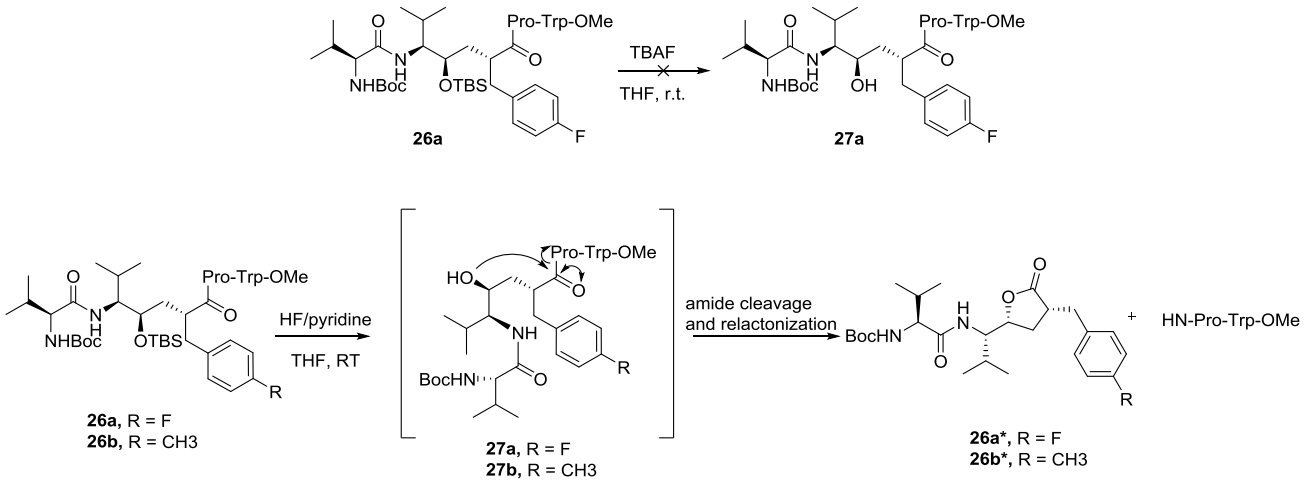
Scheme 16: Proposed reaction cascade for the synthesis of the desired pseudopentapeptides.

The coupling of the Val residue was achieved using the same conditions which were already established in the previous step - starting with the Boc-deprotection of the tetrapeptide using TFA, followed by coupling of the Val residue under standard conditions with HATU as coupling additive and DiPEA as base in DMF. Scheme 17 shows how TBS-protected pseudopentapetides **26a** and **26b** were obtained.



Scheme 17: First peptide couplings with Boc-Val-OH.

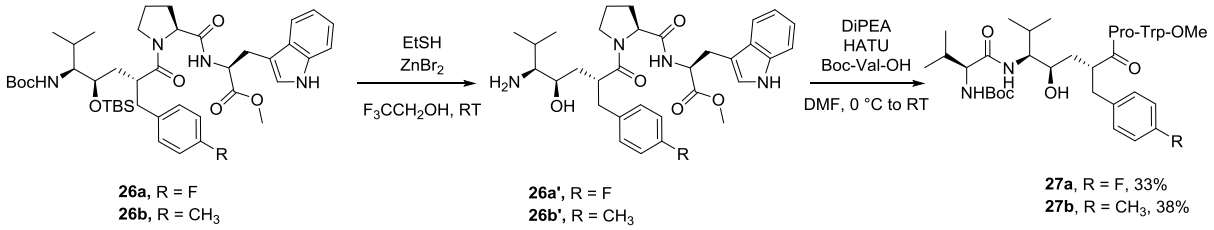
Even though only moderate yields with 48% (**26a**) and 53% (**26b**) respectively could be obtained, there was enough material to move on and pursue the TBS-deprotection of both compounds. The most common approach to easily cleave off the TBS-moiety is with tetrabutylammoniumfluoride in THF. Due to the strong affinity of the fluorine to silicone, reactions usually proceed very quick and efficient. Consequently, it was logical that the first approach was to deprotect the hydroxyl moiety under standard deprotection conditions.[73] Unfortunately, performing the reaction with compound **26a** did not lead to any observable conversion to the desired compound. Even prolonged reaction times up to 72 h and an excess of TBAF of 10 eq. did not promote reaction progress. Therefore, the strategy was changed to a different deprotection agent. Following the procedure of *Dias et al.*[74] with HF/pyridine in THF, complete consumption of the starting material could be observed after 20 h and after extractive work up and flash chromatography, two compounds could be isolated. Unfortunately, none of them turned out to be the desired compound, but only the decomposition fragments of the starting material. According to the mechanism shown in Scheme 18, amide cleavage and relactonization occurred to a full extent after successful cleavage of the TBS-moiety. It appears that under these conditions the reformation of the five-membered lactone ring is thermodynamically and kinetically favored compared to the original structure of the pseudopentapeptide. Since all of the material was used in the reaction, we had to start all over and come up with a new strategy in order to assemble the pseudopentapeptide.



Scheme 18: Attempts for TBS-deprotection of the hydroxyl moiety.

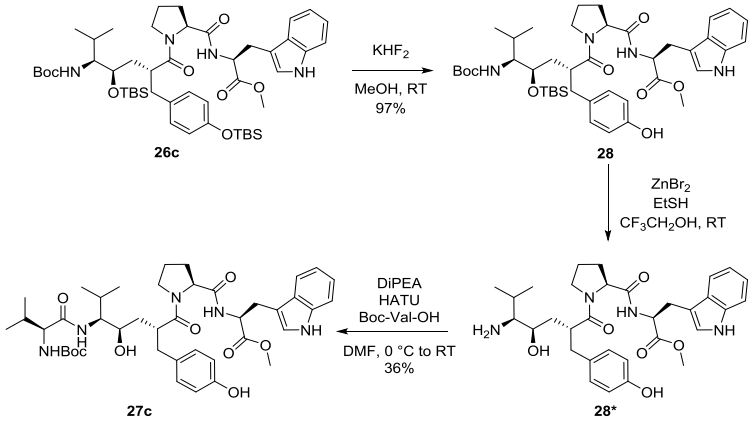
4.7 Different approach for elongation and coupling: tandem deprotection and subsequent coupling

The synthetic approach to first attach the L-valine amino acid residue and subsequently deprotect the hydroxyl functionality turned out to be not successful. A new strategy was pursued, based on previous work[60] and other examples.[75] The simultaneous deprotection of both the hydroxy and the amino moiety was successfully achieved by the use of zinc dibromide, ethanethiol in trifluoroethanol. After the tandem deprotection step no purification was performed, instead the intermediate was directly allowed to react with Boc-Val-OH, in the presence of HATU and DiPEA to furnish the desired pseudopentapeptides (Scheme 19).



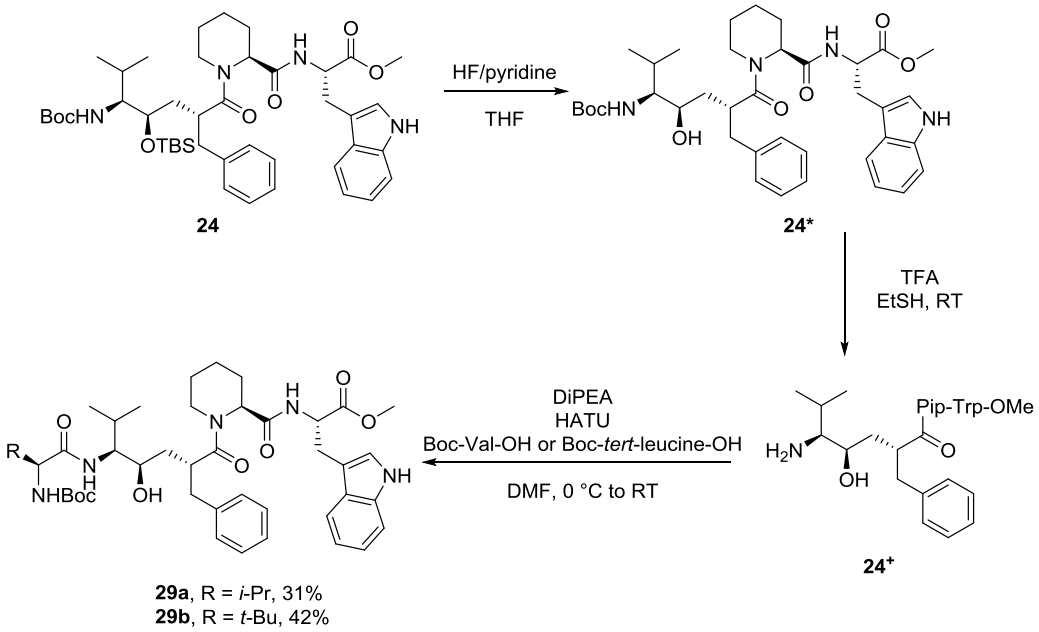
Scheme 19: Reaction scheme for the successful synthesis of two pseudopentapetides.

Before the Val residue could be attached to the tetrapeptide **26c**, the aryl silyl ether had first to be cleaved off. For that reason a synthetic route for the selective cleavage of the TBS-protected aryl alcohol was pursued. Among classic deprotection additives like TBAF, we also tried a DBU-mediated cleavage of the TBS-moiety,[76] which was not successful. Eventually, selective and very efficient deprotection of the aryl alcohol could be achieved following a procedure of *Lakshman et al.*[77] The selective deprotection of compound **26c** utilizing KHF_2 in methanol, led to pure compound **28** in excellent yield. The already established treatment with zinc dibromide and ethanethiol in trifluoroethanol led to the successful tandem deprotection of compound **28**. Subsequent coupling with Boc-Val-OH provided desired compound **27c** in 36% overall yield. Although the overall yield could have been better, enough material was obtained for the final deprotection step.



Scheme 20: Established reaction cascade for the synthesis of compound 27c.

Since this established procedure provided the desired compounds **27a-c** for the final deprotection step, it was logical to provide the same conditions for deprotection and coupling for our pseudopeptides with the pipecolic acid residue (**24**). Interestingly, applying the same conditions for deprotection and coupling turned out not to be successful. During tandem deprotection, decomposition of the starting material occurred once again. Amide cleavage and relactonization only led to the isolation of the lactone and the dipeptide fragment H-Pip-Trp-OMe. In the need of a new deprotection strategy, we tried once again different additives for selective deprotection. Eventually, we again attempted deprotection conditions using HF/pyridine in THF.[74] Even though not being successful for the silyl ether cleavage of compounds **26a** and **26b**, selective and quick deprotection of the hydroxyl moiety of compound **24** could be observed after 1 h, according to NMR. Nitrogen deprotection was achieved by using TFA in ethanethiol and subsequent coupling with either Boc-Val-OH or Boc-*tert*-leucine-OH provided compounds **29a** and **29b** in 31% and 42% overall yield respectively (Scheme 21).

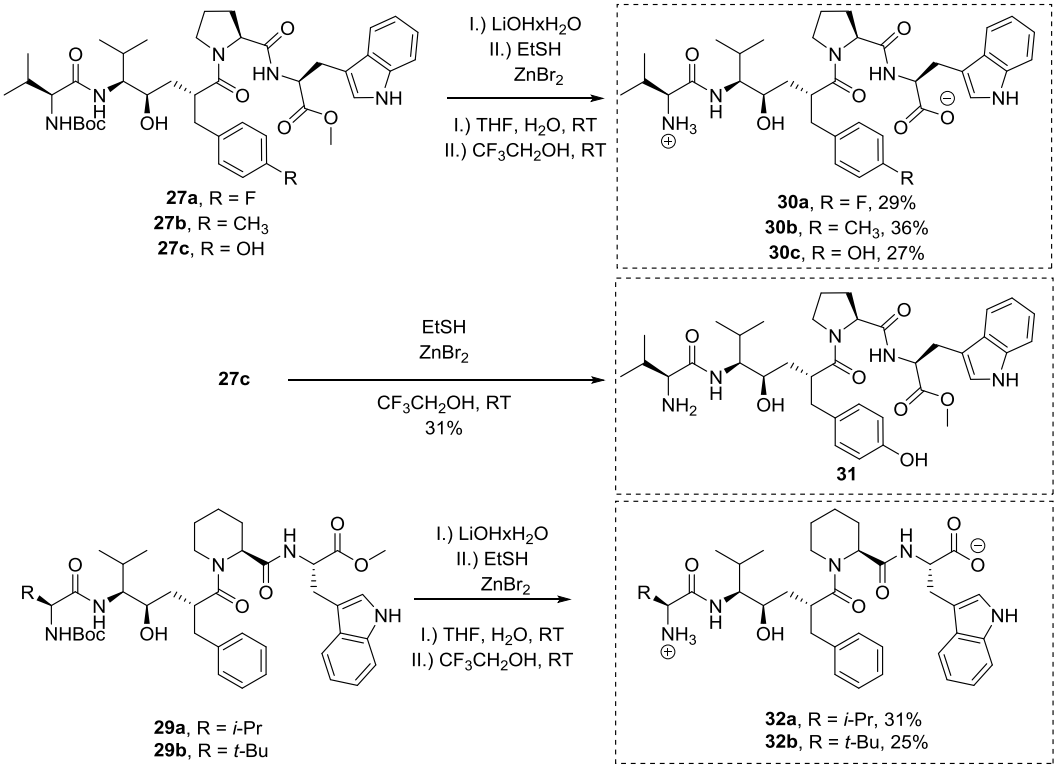


Scheme 21: Different approach in deprotection and subsequent coupling.

4.8 Final deprotection

With all the fully elongated pseudopeptides in hand, only the final deprotection steps had to be carried out. For the saponification of the methyl ester, $LiOHxH_2O$ in THF/H_2O was used. After the successful conversion into the corresponding acid, the only protecting group which had to be cleaved off was the Boc-moiety. In order to deprotect the amino functionality, we pursued two different synthetic strategies. On the one hand deprotection was carried out with TFA in ethanethiol and the other hand, zinc dibromide and ethanethiol in trifluoroethanol was used (Scheme 22). In order to achieve excellent purity of the molecules, the final purification involved preparative HPLC as method of choice.

Additionally to the already desired compounds, another compound (**31**) was synthesized. As displayed in Scheme 22, only the nitrogen was deprotected under already established conditions. This was performed in order to investigate, whether the still attached methyl group on the *C*-terminus exhibits any effect towards inhibition of DPP III.



Scheme 22: Successful deprotections for all desired compounds.

After two steps and purification via preparative HPLC, only moderated yields between 25-36% were obtained. However, enough material of all compounds could be provided to perform the biological assays. Worth mentioning is the fact that yields are dramatically low after only two steps, due to the reason that amide cleavage and relactonization occurred to a concerning extent. For all compounds, decomposition rates were drastically high. According to HPLC measurements, decomposition rates for some compounds (e.g. **27a**) were determined with values up to 60% with reaction times of the deprotection steps within 5 min and 40 h. Nevertheless, all desired compounds could finally be synthesized and tested towards inhibition of DPP III in fluorescence-based inhibition assays.

4.9 Biological assays

All of the six synthesized inhibitors were tested in fluorescence-based inhibition assays to determine their biological activity. The experiments for the inhibition of DPP III have been performed by Dr. Shalinee Jha at the Institute of Biochemistry, Graz University of Technology.

4.9.1 Fluorescence-based inhibition assay

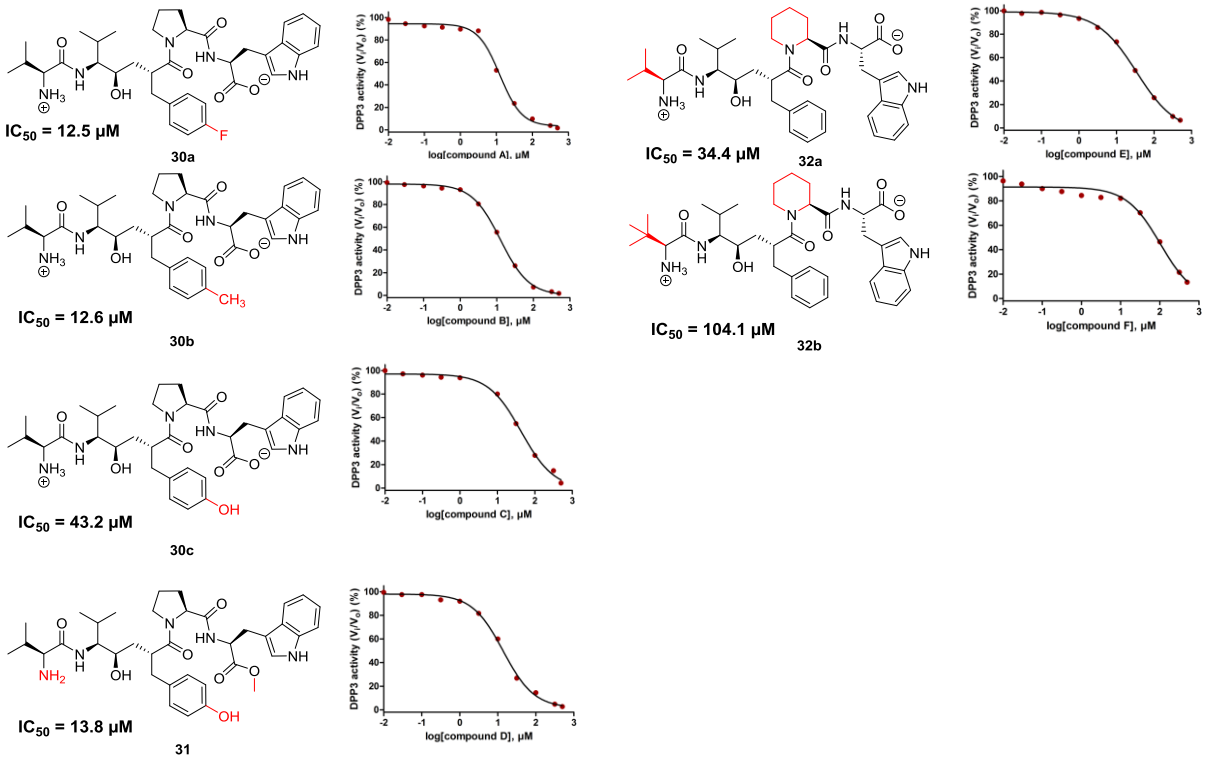
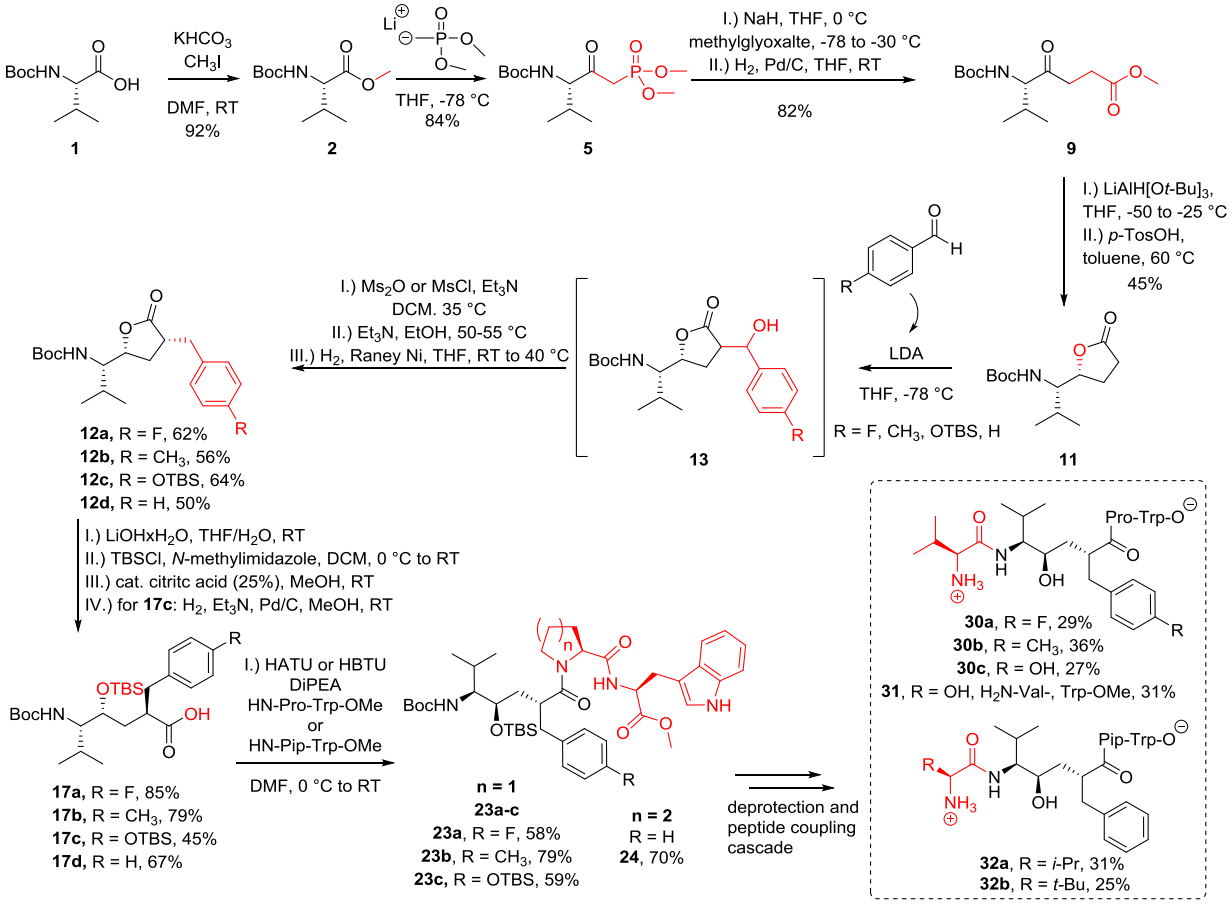


Figure 14: Results of the fluorescence-based inhibition assays for all six compounds.

All results of the fluorescence-based inhibition assays are depicted in Figure 14. As already expected compounds **30a** (12.5 µM) and **30b** (12.6 µM) did not exhibit any major deviations in IC_{50} values compared to lead structure ***HER*** (8.8 µM). Unfortunately, the results of compound **30c** with the attached hydroxyl moiety did not provide a significant boost in activity. On the contrary, activity dropped dramatically with an IC_{50} value of only 43.2 µM. Nevertheless, results of the inhibition assay of compound **31**, which is similar to **30c** with the difference that the *C*-terminus still remained methyl-protected and only *N*-terminus was unprotected, turned out to be very surprising. It appears that the methyl group does not have a crucial influence upon substrate binding. An IC_{50} value of 13.8 µM gave rise to question, whether it is even necessary, to fully deprotect future potential inhibitors. This would also provide a simplification in synthesis and more importantly purification, by avoiding a significant amount of decomposition. Unfortunately, exchanging the Pro amino acid residue to the pipecolic acid residue, while simultaneously varying the Val residue, did not improve activity towards inhibition. With IC_{50} values of 34.4 µM (**32a**) and 104.1 µM (**32b**), these results are too far away from the desired nM range.

5 Summary and outlook

In the course of this work, six different inhibitors for DPP III were synthesized and tested in biological assays towards inhibition of this peptide cutting enzyme. Based on the lead structure of ***HER*** (*R*)-hydroxyethylene, developed by J. Ivković during his PhD thesis,[60] several modifications were installed within this molecule with the ultimate goal to further increase activity of these inhibitors. Following the synthetic route of *Haug et al.*[63] and previous work on this topic,[60–62] allowed the introduction of new amino acid residues or other functional substituents. Scheme 23 outlines the synthetic approach of how the inhibitors were obtained.



Scheme 23: Overall reaction cascade for the synthesis of new potential inhibitors for DPP III.

The synthesis started with a simple methylation of Boc-Val-OH, yielding *O*-protected compound **2**. This was further converted to ketophosphonate **5** by the use of lithiated dimethyl methylphosphonate. Performing a *Horner-Wadsworth-Emmons reaction* and subsequent reduction gave access to keto ester **9**. After stereoselective reduction and lactonization, compound **11** could be used for the introduction of various new substituents. A four-step reaction cascade allowed the synthesis of alkylated lactones **12a-d**. After hydrolytic lactone opening and subsequent alcohol protection, compounds **17a-d** were ready to be elongated at the *C*-terminus with different dipeptide fragments. With pseudotetrapeptides **23a-c**

and **24** in hand, the last peptide coupling and a series of deprotection steps could be performed. After these deprotection steps all of the desired compounds were obtained in moderate yields between 25-36%.

All of the synthesized inhibitors were tested towards inhibition of DPP III in a fluorescence-based inhibition assay. The results are summarized in Table 1.

Table 1: Results of the biological fluorescence-based inhibition assays of all newly synthesized compounds.

Entry	Inhibitor	IC_{50} [µM]
	–Pro-Trp-O^- residue	
1	R = F (**30a**)	12.5
2	R = CH_3 (**30b**)	12.6
3	R = OH (**30c**)	43.2
	H_2N-Val-,-Pro-Trp-OMe residue	
4	R = OH (**31**)	13.8
	–Pip-Trp-O^- residue	
5	R = *i*-Pr (**32a**)	34.4
6	R = *t*-Bu (**32b**)	104.1

Compounds **30a** and **30b** with the already established amino acid sequence –Pro-Trp-O^-, but different aromatic residues, exhibit comparable IC_{50} values to the one of ***HER*** (8.5 µM). Interestingly, IC_{50} value of compound **30c** with the replaced Tyr residue instead of the original Phe residue, is far away from the expected boost in activity, since it is almost identical to lead compound tynorphin.[12] However, the still methyl protected compound **31**, exhibits a way better activity with an IC_{50} value of 13.8 µM. Changes of the –Pro-Trp-O^- to the –Pip-Trp-O^- residue did not give a significant boost in activity.

For the future it would be interesting, if still full deprotection of new potential inhibitors is as crucial as it was assumed so far. The results for activity of methyl protected compound **31** revealed that the ionic species might not be necessarily responsible for competitive inhibition. It would be a desirable goal to synthesize more compounds with different aromatic substituents and not fully deprotect the potential inhibitor. This would not only simplify the synthetic approach to at least a small extent, but could also provide a simplification in purification and of course give a significant increase in yields.

6 Experimental section

6.1 General aspects

All reactions with moisture or air sensitive compounds were carried out under inert atmosphere. Previous evacuation (oil pump; vacuum 10^{-2} to 10^{-3} mbar), heating and either nitrogen or argon purging was applied for the needed glassware, in order to exclude any moisture or oxygen from the reaction vessel. For all reactions a Teflon® coated magnetic stirring bar was used for stirring. All chemicals and reagents were purchased from the companies ABCR, Acros Organics, Alfa Aesar, Fisher Scientific, Fluka, Merck, Roth, Sigma Aldrich or VWR and were used without further purification, unless otherwise stated.

Catalysts for hydrogenations were carefully removed under argon atmosphere by filtration through a Schlenk-frit containing a plug of Celite®. The plug was first washed with the solvent of the reaction and then with H_2O. The remaining slurry was stored under water in a container dedicated for catalyst waste and disposed as hazardous waste.

6.1.1 Solvents and reagents

Acetone: Acetone used for non-inert conditions was purchased from Brenntag and distilled using a rotary evaporator.

Cyclohexane: Cyclohexane used for non-inert conditions was purchased from Fisher Scientific as analytical grade (99.99 %) and was used as obtained.

Ethyl acetate: Ethyl acetate used for non-inert conditions was purchased from Fisher Scientific as analytical grade (99.99 %) and was used as obtained.

Dichloromethane: Dichloromethane was treated with phosphorus pentoxide, distilled, then heated under reflux for two days over calcium hydride and then distilled under argon atmosphere into a dry brown 1 L Schlenk bottle with activated 4 Å molecular sieves. Dichloromethane used for non-inert conditions was purchased from Fisher Scientific as analytical grade (99.99 %) and was used as obtained.

Diethyl ether: Et_2O was distilled using a rotary evaporator and stored in a brown bottle over KOH.

Ethanol: EtOH was purchased from Merck (99 %, containing 1 % methylethyl ketone) and heated under reflux together with sodium and diethyl phthalate in an inert distillation apparatus under argon for 2 h. Subsequently, the dry ethanol was distilled and stored over activated 3Å molecular sieves in a brown 1 L Schlenk bottle under argon.

Methanol: Methanol used for non-inert conditions was purchased from Fisher Scientific as analytical grade (99.99 %) and was used as obtained.

***N,N*-Dimethylformamide**: DMF was purchased in extra dry quality from Alfa Aesar and transferred into a brown 1 L Schlenk bottle with activated 3Å molecular sieves and stored under argon.

Tetrahydrofuran: Anhydrous tetrahydrofuran was produced by heating it over Na under reflux for 48 h under argon atmosphere until benzophenone indicated its dryness by turning into deep blue color. It was distilled into an amber 1000 mL Schlenk bottle and stored over 4 Å MS and under argon atmosphere.

Toluene: Toluene used for non-inert conditions was purchased from Fisher Scientific as analytical grade (99.99 %) and was used as obtained.

Water: The water which was used in organic reactions and during workup procedures was deionized by an ion exchanger prior to use.

Molecular sieves: 3Å and 4Å molecular sieves (Sigma Aldrich, beads 8–12 mesh) were activated by heating them in a round bottom flask with a heating mantle under high vacuum for 8 h. Activated molecular sieves were stored at RT under Ar atmosphere.

6.2 Analytical methods and instruments

6.2.1 Thin layer chromatography (TLC)

For analytical thin layer chromatography, plates purchased from the company Merck (TLC- silica gel 60 F254, 20 x 20 cm) were used. R_f-values and used eluents are given in the corresponding experimental procedures.

UV-lamp: (λ = 254 nm, λ = 366 nm).

$KMnO_4$: 3.0 g $KMnO_4$ and 20.0 g K_2CO_3 dissolved in 300 mL 5 % aqueous NaOH solution.

CAM: 2.0 g Cer(IV)-sulfate, 50.0 g ammonium molybdate and 50 mL conc. H_2SO_4 were dissolved in 400 mL deionized water.

6.2.2 Flash column chromatography

Preparative column chromatography was performed using silica gel 60 from Acros Organics (particle size 35-70 μm). Further details such as amount of silica gel and the composition of the eluent are indicated in the corresponding experimental procedures.

6.2.3 Nuclear magnetic resonance (NMR)

All described and listed NMR spectra were acquired using a Bruker AVANCE III with auto sampler: 300.36 MHz-^{1}H NMR, 75.53 MHz-^{13}C-NMR or a or a Varian Inova NB high resolution spectrometer at 499.88 MHz (1H) and 125.70 MHz (13C).

Peak multiplicities are abbreviated as follows: s (singlet), d (duplet), dd (doublet of doublet), t (triplet), q (quadruplet), p (pentet), m (mulitplet). The deuterated solvent, the chemical shifts δ in ppm (parts per million), the coupling constants *J* in Hertz (Hz) and the integral and assignment of the respective signals are given. Whenever it was necessary, additional 1D and 2D techniques (APT, COSY, HSQC, NOESY, ^{19}F, ^{31}P) were recorded to determine the correct structure.

6.2.4 Gas chromatography with mass-selective detection

For gas chromatography a Hewlett Packard GC-System „HP 6890Series“ with a polar HP-5MS capillary column (length: 30 m, inner diameter: 0.25 mm, film thickness: 0.25 µm) was used. Helium 5.0 was used as carrier gas. As source for ionization an EI-ionization source with a potential of E = 70 eV was used. Ions were detected with the mass selective detector 5972 MSD. In this work two different methods were used:

MK_Standard: 50 °C, 1 min, ramp 40 °C min^{-1}, linear to 300 °C, 300 °C 1 min

MT_50_XS: 50 °C, 1 min, ramp 10 °C min^{-1} to 150 °C, 150 °C 2 min, ramp 40 °C min^{-1} to 300 °C

6.2.5 High-performance liquid chromatography

6.2.5.1 Analytical HPLC-MS

Analytical high-performance liquid chromatography was performed on an “Agilent Technologies 1200 Series” HPLC system with 1260 HiP Degasser G4225A, binary pump SL G1312, autosampler HiP-ALS SL G1367C, thermostated column compartment TCC SL G1316B, multiple wavelength detector G1365C MWD SL with deuterium lamp (λ = 190–400 nm) and subsequent connected mass detector (Agilent Technologies 6120 Quadrupole LC/MS) with an electrospray ionization (ESI) source. The components were separated on a RP Agilent Poroshell 120 SB-C18 column (3.0 × 100 mm, 2.7 µm) with a Merck LiChroCART® 4-4 pre-column. Signals were detected at 210 nm or 280 nm. As mobile phase acetonitrile (VWR HiPerSolv, HPLC-MS grade) and H_2O (deionized and purified by Barnstead™ Nanopure™ water purification system)) with 0.01 % trifluoroacetic acid were used. Following methods were used:

FAST_POROSHELL120_001HCOOH_8MINGRADIENT.M: 40 °C, flow rate 0.7 mL/min; 0.0–2.0 min MeCN/H_2O = 10:90 (v/v), 2.0–10.0 min linear increase to MeCN/H_2O = 95:5 (v/v), 10.0–16.0 min hold MeCN/H_2O = 95:5 (v/v).

Integrated peak areas were used for the calculation of conversion. Since no internal or external standard was used, these values are not exact.

6.2.5.2 Preparative HPLC

Isolation of polar compounds were performed on a "Thermo Scientific Dionex UltiMate 3000" system with UltiMate 3000 pump, UltiMate 3000 autosampler, UltiMate 3000 column compartment, UltiMate 3000 diode array detector (deuterium lamp, λ = 190–380 nm) and a UltiMate 3000 automatic fraction collector. The components were separated on a RP Macherey-Nagel 125/21 Nucleodur® 100-5 C18ec column (21 × 125 mm, 5.0 µm). Signals were detected at 210 nm and 280 nm. As mobile phase acetonitrile (VWR HiPerSolv, HPLC grade) and water (deionized and purified by Barnstead™ Nanopure™ water purification system) with 0.01 % formic acid were used. The following method was used:

CLF_NucleodurC18_pc001_HCOOH_5to95: 26 °C, flow rate 15 mL/min; 0.0–5.0 min MeCN/H_2O = 5:95 (v/v), 5.0–30.0 min linear increase to MeCN/H_2O = 95:5 (v/v), 30.0–35.0 min hold MeCN/H_2O = 95:5 (v/v).

6.2.6 High resolution mass spectroscopy

The high-resolution mass spectra were recorded using MALDI-TOF on a Waters Micromass® MALDI micro MX™ spectrometer. α-Cyano-4-hydroxycinnamic acid or dithranol (1,8-dihydroxy-9,10-dihydroanthracen-9-one) provided the matrix and PEG (polyethyleneglycol) served as an internal standard. Values are stated as m/z.

6.2.7 Melting point detection

Melting points are uncorrected and were determined using a "Mel-Temp®" apparatus with integrated microscopical support by the company Electrothermal. The temperature was measured using a mercury thermometer.

6.2.8 Optical rotation

A Perkin Elmer Polarimeter 341 was used for the determination of the specific rotation at λ = 589 nm (sodium D-line). Measurements were recorded at 23, 24 or 25 °C and repeated three times.

6.2.9 Fluorescence-based inhibition assay

The enzyme activity of hDPP III was determined by following the release of 2-naphthylamine in a plate reader (excitation at 332 and emission at 420 nm) at 37 °C in a mixture containing 25 µL of 200 µM Arg-Arg-2-naphthylamide as substrate in 50 mM Tris-HCl buffer, pH 8.0 and 0.05-0.1 µM of enzyme in a total reaction mixture of 235 µL (White, Tissue Culture treated Krystal 2000 96-well plate from Porvair sciences, Norfolk, UK). The activity assay was performed by continuous measurement of fluorescence of 2-naphthylamide for 30 min (Molecular Devices, Sunnyvale CA, USA). For the inhibition assay, the inhibitors were added to the mixture without the substrate and incubated for 10 min at RT. The reaction was started by the addition of the substrate. The concentration of an inhibitor that gave 50% inhibition (IC_{50}) was determined through a series of assays with a fixed substrate concentration but with various inhibitor concentrations.

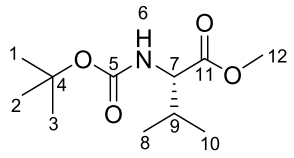
The activity (in %) in the presence of increasing concentrations of the inhibitor was calculated using the equation:

$$\% \text{ activity} = 100 \text{ X } (\Delta\text{fluorescence}/ \Delta\text{fluorescence of control})$$

The activity (in %) against concentration of inhibitor (log scale for inhibitor concentration [x-axis] and linear scale for percent activity [y-axis]) was plotted. The activity in % vs. log of concentration was fitted to a sigmoidal dose-response curve using the four parameter logistic equation entitled “log (inhibitor) vs. response -- variable slope” in GraphPad Prism (San Diego, CA, USA).

6.3 Experimental procedures

6.3.1 Methyl *(tert*-butoxycarbonyl)-L-valinate (1)



1

A 500 mL three-necked round bottom flask, equipped with gas valve, dropping funnel and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with 23.00 g (105.9 mmol, 1 eq.) Boc-Val-L-OH and 150 mL DMF followed by the addition of 21.20 g (211.7 mmol, 2 eq.) $KHCO_3$. The reaction mixture was stirred for 15 min at RT. 10.54 mL (169.4 mmol, 1.6 eq.) methyl iodide were added dropwise via a dropping funnel within 15 min. After addition was complete the yellowish reaction mixture was stirred for 3 h at RT. Afterwards, 400 mL H_2O were added and the product was extracted with a mixture of cyclohexane/EtOAc (1:1 (v/v), 3x100 mL). The combined organic layers were washed with H_2O (2x100 mL), 5% Na_2SO_3 (2x100 mL), brine (100 mL) and dried over Na_2SO_4. The solvents were removed under reduced pressure and the residue dried *in vacuo* to give the desired product as a colorless oil.

$C_{11}H_{21}NO_4$ [231.29 g/mol].

Yield: 22.5 g (97.2 mmol, 92%), colorless oil.

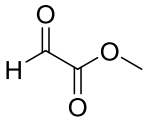
R_f = 0.85 (cyclohexane/EtOAc = 1:4 (v/v); staining: $KMnO_4$).

$[\alpha]_D^{24} =$ +6.8 (c = 1.08, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.01 (d, ^3J = 7.3 Hz, 1H, *H*-6), 4.21 (dd, ^3J = 8.6, 4.5 Hz, 1H, *H*-7), 3.73 (s, 3H, *H*-12), 2.22-1.99 (m, 1H, *H*-9), 1.44 (s, 9H, *H*-1, 2, 3), 0.95 (d, ^3J = 6.9 Hz, 3H, *H*-8), 0.92 (d, ^3J = 6.9 Hz, 3H, *H*-10).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.0 (*C*-11), 155.7 (*C*-5), 79.8 (*C*-4), 58.6 (*C*-7), 52.1 (*C*-12), 31.4 (*C*-9), 28.4 (*C*-3, 2, 1), 19.0 (*C*-8), 17.7 (*C*-10).

6.3.2 Methyl glyoxylate (2)

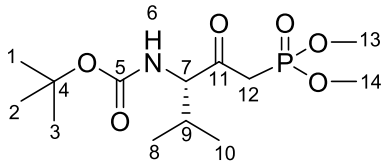


2

A 500 mL two-necked round bottom flask, equipped with gas valve and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with 11.72 g (65.8 mmol, 1 eq.) dimethyl tartrate and 130 mL Et_2O. 15.0 g (65.8 mmol, 1 eq.) H_5IO_6 were added portionwise within 30 min. After the addition was finished, the white suspension was stirred for additional 45 min. The suspension was filtered through a glass frit and the residue was washed with Et_2O (3x50 mL). The filtrate was dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure (T < 35 °C) and dried *in vacuo* for 30 min to furnish a pale orange oil. The crude product was stored in the freezer under Ar and used in the next step without further purification.

$C_3H_4O_3$ [88.06 g/mol].

6.3.3 *tert*-Butyl *(S)*-(1-(dimethoxyphosphoryl)-4-methyl-2-oxopentan-3-yl)carbamate (3)



3

A 1000 mL three-necked round bottom flask, equipped with gas valve, dropping funnel and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with 22.0 mL (206.0 mmol, 3.3 eq.) dimethyl methyl phosphonate and 250 mL abs. THF. The reaction mixture was cooled to -78 °C with a dry ice/acetone cooling bath and 80 mL (200.0 mmol, 3 eq.) of a 2.5 M *n*-butyllithium solution in hexane were added dropwise within a period of 30 min. The yellowish reaction mixture was stirred for additional 25 min at that temperature. A solution of 14.01 g (61.0 mmol, 1 eq.) **1** in 120 mL abs. THF was added dropwise via a dropping funnel within 30 min and the yellow reaction mixture was stirred at -78 °C. After 2.5 h TLC indicated full conversion, 200 mL satd. NH_4Cl were added and the reaction mixture was allowed to warm to RT. Additional 100 mL H_2O were added and the mixture was transferred into a separation funnel. The phases were separated and the aqueous layer was extracted with EtOAc (2x200 mL). The combined organic layers were washed with H_2O (2x250 mL), dried over Na_2SO_4 and the solvents removed under reduced pressure. The residue was dried *in vacuo* to give the desired product as yellowish oil.

$C_{13}H_{26}NO_6P$ [323.33 g/mol].

Yield: 17.0 g (53.0 mmol, 84%), yellowish oil.

R_f = 0.36 (cyclohexane/EtOAc = 4:1 (v/v); staining: $KMnO_4$).

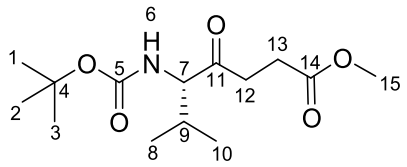
$[\alpha]_D^{24}$ = +20.0 (c = 1.09, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.31 (d, ^3J = 8.8 Hz, 1H, *H*-6), 4.31 (dd, ^3J = 8.8, 4.0 Hz, *H*-7), 3.83-3.73 (m, 6H, *H*-13, 14), 3.37-3.00 (m, 2H, *H*-12), 2.36-2.19 (m, 1H, *H*-9), 1.43 (s, 9H, *H*-1, 2, 3), 0.99 (d, ^3J = 6.8 Hz, 3H, *H*-8), 0.80 (d, ^3J = 6.8 Hz, 3H, *H*-10).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 201.4 (*C*-11), 156.0 (*C*-5), 80.0 (*C*-4), 65.2 (*C*-7), 53.3 (d, *J* = 2.5 Hz, *C*-13), 53.2 (d, *J* = 2.5 Hz, *C*-14), 39.8 (d, *J* = 130.8 Hz, *C*-12), 29.3 (*C*-7), 28.4 (*C*-3, 2, 1), 20.0 (*C*-8), 16.8 (*C*-10).

^{31}P NMR (202 MHz, $CDCl_3$): δ = 22.2 (s).

6.3.4 Methyl (*S*)-5-((*tert*-butoxycarbonyl)amino)-6-methyl-4-oxoheptanoate (4)



4

Horner-Wadsworth-Emmons reaction: A 1000 mL three-necked round bottom flask, equipped with gas valve, dropping funnel and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with 14.7 g (45.4 mmol, 1 eq.) compound **3** and 450 mL abs THF. After cooling to 0 °C in an ice bath, 3.63 g (90.8 mmol, 2 eq.) NaH (60% dispersion in mineral oil) were added portionwise within 30 min and the reaction was stirred for additional 30 min at 0 °C. Subsequently, the reaction mixture was cooled to -78 °C in a dry ice/acetone cooling bath. A second two-necked round bottom flask, equipped with gas valve and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with 10.0 g (113.5 mmol, 2.5 eq.) aldehyde **2** and 100 mL abs. THF. The solution of the aldehyde was also cooled to -78 °C (dry ice/acetone) and transferred to the deprotonated ketophosphonate via a cannula. The reaction mixture was stirred at -78 °C for 30 min. The flask was warmed to -30 °C (cooled in an ice/$CaCl_2$ slurry and liquid N_2) and stirred for additional 2 h. After TLC indicated full conversion, a solution of 7 mL glacial acetic acid in 12 mL THF was added dropwise via a dropping funnel within a period of 1 h. Subsequently, the reaction mixture was allowed to warm up to RT and concentrated under reduced pressure to a small volume. The residue was partitioned between 300 mL EtOAc and 150 mL H_2O. The phases were separated and the aqueous layer extracted with EtOAc (2x150 mL). The combined organic layers were washed with satd.

$NaHCO_3$ (150 mL), brine (150 mL), dried over Na_2SO_4 and the solvent was removed under reduced pressure to furnish a yellow oil.

Hydrogenation: In a 500 mL two-necked round bottom flask the oily residue was dissolved in 250 mL THF and 963 mg palladium on charcoal (5 % palladium; 1 mol-%) were added. The flask was evacuated and purged with H_2 (balloon) three times. The reaction mixture was stirred under H_2 balloon atmosphere at RT until GC-MS indicated full conversion. Subsequently, the catalyst was removed under an N_2 atmosphere by filtration through a Schlenk-frit containing a plug of Celite® and the filtrate was concentrated. The residue was purified via flash chromatography (450 g SiO_2, cyclohexane/EtOAc = 3:1 (v/v)) to give the desired product as a colorless oil.

$C_{14}H_{25}NO_5$ [287.36 g/mol].

Yield: 10.7 g (0.037 mol, 82%), colorless oil.

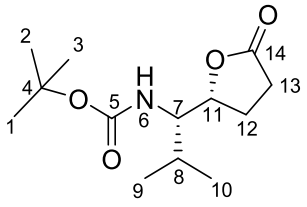
R_f = 0.38 (cyclohexane/EtOAc = 3:1 (v/v); staining: $KMnO_4$).

$[\alpha]_D^{24}$ = +33.6 (c = 0.89, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.09 (d, ^3J = 7.7 Hz, 1H, *H*-6), 4.26 (dd, ^3J = 8.0, 4.3 Hz, 1H, *H*-7), 3.65 (s, 3H, *H*-15), 2.98-2.48 (m, 4H, *H*-13, 12), 2.28-2.11 (m, 1H, *H*-4), 1.41 (s, 9H, *H*-3, 2, 1), 0.99 (d, ^3J = 6.8 Hz, 3H, *H*-8), 0.79 (d, ^3J = 6.8 Hz, 3H, *H*-10).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 208.0 (*C*-11), 173.1 (*C*-14), 156.1 (*C*-5), 79.8 (*C*-4), 64.1 (*C*-7), 51.9 (*C*-15), 35.5 (*C*-12), 30.3 (*C*-9), 28.4 (*C*-3, 2, 1), 27.6 (*C*-13), 19.9 (*C*-8), 16.8 (*C*-10).

6.3.5 *tert*-Butyl ((*S*)-2-methyl-1-((*R*)-5-oxotetrahydrofuran-2-yl)propyl)carbamate (5)



5

Stereoselective Reduction: A 1000 mL three-necked round bottom flask, equipped with gas valve and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with 10.0 g (34.8 mmol, 1 eq.) **4** and 325 mL abs. THF. The solution was cooled to -50 °C using a cooling bath with acetone and a cryostat in order to maintain a constant temperature. After 30 min 26.5 g (104.4 mmol, 3 eq.) lithium tri-*tert*-butoxyaluminum hydride were added in one portion and the reaction mixture was stirred vigorously at -50 °C for additional 60 min. Subsequently, the yellowish reaction mixture was allowed to warm to -40 °C. Reaction progress was monitored frequently via GC-MS measurements.

The temperature was then gradually set to -25 °C within 48 h. After that period of time, the reaction mixture was quenched by the addition of 300 mL citric acid solution (25% in H_2O). The reaction mixture was allowed to warm up to RT and EtOAc (200 mL) was added. Subsequently, the mixture was transferred into a separation funnel and the phases were separated. The aqueous layer was extracted with EtOAc (2x200 mL). The combined organic layers were washed with H_2O (2x150 mL), satd. $NaHCO_3$ (150 mL), brine (150 mL), dried over Na_2SO_4 and the solvents were removed under reduced pressure to furnish the reduced intermediate as a reddish oil.

Lactonization: In a 250 mL round bottom flask, equipped with magnetic stirring bar, the crude product of the previous step was dissolved in 150 mL toluene and 66 mg (0.35 mmol, 0.1 eq.) *p*-toluenesulfonic acid monohydrate were added to the reaction mixture. The reaction mixture was heated to 60 °C and stirred vigorously for 20 h. Subsequently, the reaction mixture was concentrated to a small volume. The crude oily residue was recrystallized from boiling *n*-pentane to furnish 4.00 g (15.5 mmol) of the desired diastereomer **5** as a white powder.

$C_{13}H_{23}NO_4$ [257.33 g/mol].

Yield: 4.00 g (15.5 mmol, 45%, 2 steps from **4**), colorless solid.

R_f = 0.22 (cyclohexane/EtOAc = 2:1 (v/v); staining: $KMnO_4$).

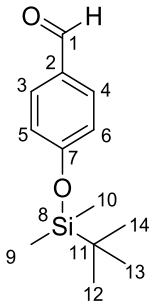
$[\alpha]_D^{24}$ = -16.1 (c = 0.87, $CHCl_3$).

m.p. = 104-106 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.48-4.22 (m, 2H, *H*-11, 6), 3.71-3.48 (m, 1H, *H*-7), 2.64-2.41 (m, 2H, *H*-13), 2.32-2.20 (m, 1H, *H*-8), 2.18-2.03 (m, 2H, *H*-12), 1.42 (s, 9H, *H*-3, 2, 1), 0.94 (d, ^3J = 6.8 Hz, 3H, *H*-9), 0.87 (d, ^3J = 6.8 Hz, 3H, *H*-10).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.0 (*C*-14), 156.3 (*C*-5), 79.9 (*C*-4), 57.9 (*C*-7), 28.4 (*C*-3, 2, 1), 28.2 (*C*-13, 8), 25.2 (*C*-12), 19.9 (*C*-9), 15.8 (*C*-10).

6.3.6 4-((*tert*-Butyldimethylsilyl)oxy)benzaldehyde (6)



6

A 250 mL three-necked round bottom flask, equipped with gas valve and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with 3.00 g (24.0 mmol, 1 eq.) 4-hydroxybenzaldehyde, 5.01 g (73.0 mmol, 3 eq.) imidazole and 50 mL abs. dichloromethane. The solution was cooled to 0 °C in an ice bath. 4.44 g (29.0 mmol, 1.2 eq.) TBSCl were added portionwise within a period of 20 min. The reaction mixture was stirred for additional 30 min at 0 °C and then at RT overnight. The yellow turbid reaction mixture was quenched by pouring the content in 100 mL of ice cold H_2O. The mixture was stirred for 15 min and subsequently transferred into a separation funnel. The phases were separated and the aqueous layer was extracted with dichloromethane (2x50 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified via flash chromatography (450 g SiO_2, cyclohexane/EtOAc 5:1 to 3:1 (v/v)) to give the desired product as a yellow oil.

$C_{13}H_{20}O_2Si$ [236.39 g/mol].

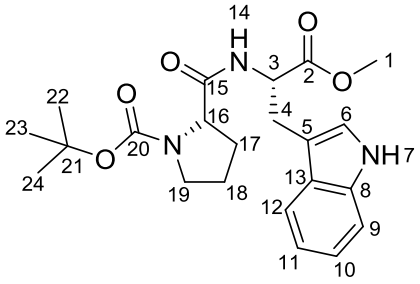
Yield: 3.88 g (16.4 mmol, 67%), yellow oil.

R_f = 0.70 (cyclohexane/EtOAc = 5:1 (v/v); staining: $KMnO_4$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.88 (s, 1H, *H*-1), 7.79 (d, ^3J = 8.6 Hz, 2H, *H*-4, 3), 6.94 (d, ^3J = 8.6 Hz, 2H, *H*-6, 5), 0.99 (s, 9H, *H*-14, 13, 12), 0.25 (s, 6H, *H*-10, 9).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 191.0 (*C*-1), 161.6 (*C*-7), 132.0 (*C*-4, 3), 130.5 (*C*-2), 120.6 (*C*-6, 5), 25.7 (*C*-14, 13, 12), 18.4 (*C*-11), -4.2 (*C*-10, 9).

6.3.7 *tert*-Butyl (*S*)-2-(((*S*)-3-(1*H*-indol-3-yl)-1-methoxy-1-oxopropan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (7)



7

A 250 mL three-necked round bottom flask, equipped with gas valve, dropping funnel and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with 4.23 g (19.6 mmol, 1 eq.) Boc-Pro-OH and 35 mL abs. dichloromethane. The reaction mixture was cooled to 0 °C in an ice bath and 3.65 mL (23.6 mmol, 1.2 eq.) *N,N'*-diisopropylcarbodiimide were added in one portion. Subsequently, 5.00 g (19.6 mmol, 1 eq.) H-Trp-OMe, dissolved in 5.44 mL (39.3 mmol, 2 eq.) triethylamine and 30 mL abs. dichloromethane, were added dropwise via a dropping funnel within 10 min. The reaction mixture stirred for additional 3 h at 0 °C. The white suspension was allowed to warm up to RT and stirred overnight at this temperature. After 20 h the reaction mixture was concentrated to a small volume and the residue was dissolved in 350 mL EtOAc. The organic layer was washed with 1 M HCl (200 mL), 0.1 M NaOH (200 mL), brine (200 mL), dried over Na_2SO_4 and concentrated. The residue was purified via flash chromatography (550 g SiO_2, cyclohexane/EtOAc = 1:1 (v/v)).

$C_{22}H_{29}N_3O_5$ [415.49 g/mol].

Yield: 4.65 g (11.2 mmol, 57%), colorless solid.

R_f = 0.23 (cyclohexane/EtOAc = 1:1 (v/v); staining: $KMnO_4$).

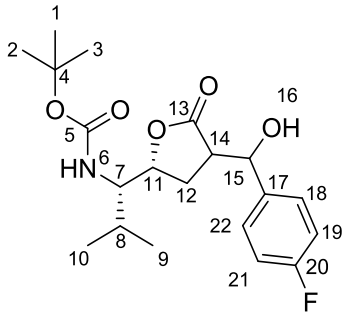
$[\alpha]_D^{24}$ = -31.0 (c = 1.03, $CHCl_3$).

m.p. = 58-60 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.18 (br s, 1H, *H*-7), 7.53 (d, ^3J = 7.8 Hz, 1H, *H*-12), 7.34 (d, ^3J = 7.8 Hz, 1H, *H*-9), 7.22-6.95 (m, 3H, *H*-11, 10, 6), 6.53 (br s, 1H, *H*-14), 4.89 (br s, 1H, *H*-3), 4.33-4.10 (m, 1H, *H*-16), 3.68 (s, 3H, *H*-1), 3.43-3.05 (m, 4H, *H*-19, 4), 2.31-1.62 (m, 4H, *H*-18, 17), 1.37 (s, 9H, *H*-24, 23, 22).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.2 (*C*-15, 2), 154.7 (*C*-20), 136.3 (*C*-8), 127.7 (*C*-13), 123.0 (*C*-6), 122.3 (*C*-10), 119.7 (*C*-11), 118.5 (*C*-12), 111.4 (*C*-9), 110.1 (*C*-5), 81.1-80.3 (m, *C*-21), 61.3-60.0 (m, *C*-16), 53.5-52.5 (m, *C*-3), 52.4 (*C*-1), 47.0 (*C*-19), 30.6 (*C*-17), 28.3 (*C*-24, 23, 22), 27.9 (*C*-4), 23.6-23.4 (m, *C*-18).

6.3.8 *tert*-Butyl ((1*S*)-1-((2*R*)-4-((4-fluorophenyl)(hydroxy)methyl)-5-oxotetrahydrofuran-2-yl)-2-methylpropyl)carbamate (8)



8

A 100 mL three-necked round bottom flask, equipped with gas valve and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with 700 mg (2.72 mmol, 1 eq.) lactone **5** and 15 mL abs. THF. The reaction mixture was cooled to -78 °C in a dry ice/acetone cooling bath followed by the dropwise addition of 4.08 mL (8.16 mmol, 3 eq.) LDA solution (2.0 M in THF). The deep orange reaction mixture was stirred at -78 °C for additional 60 min. Subsequently, 437 µL (4.08 mmol, 1.5 eq.) 4-fluorobenzaldehyde were added dropwise within 5 min. The reaction mixture turned yellow after a few min and was stirred for additional 50 min at -78 °C until TLC indicated full conversion. The reaction was quenched by the addition of 5 mL satd. NH_4Cl. The reaction mixture was allowed to warm to RT followed by the addition of 40 mL H_2O and 50 mL EtOAc. The phases were separated and the aqueous layer extracted with EtOAc (2x30 mL). The combined organic layers were washed with 1 M HCl (30 mL), brine (30 mL), dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified via flash chromatography (300 g SiO_2, cyclohexane/EtOAc 7:1 to 1:1 (v/v)) to furnish two diastereomers, which were separated and characterized separately.

$C_{20}H_{28}FNO_5$ [381.44 g/mol].

Yield: unidentified diastereomer A: 225 mg, unidentified diastereomer B: 672 mg, total: 897 mg (2.35 mmol, 86%), colorless solid.

R_f = 0.44, 0.38 (cyclohexane/EtOAc = 1:1 (v/v); staining: $KMnO_4$).

m.p. = 74-76 °C.

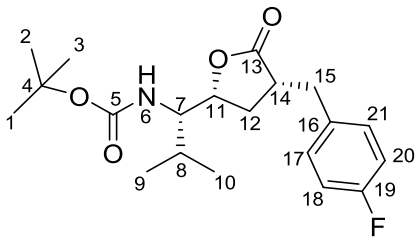
^{1}H NMR (300 MHz, $CDCl_3$, unidentified diastereomer A): δ = 7.24 (d, ^3J = 7.6 Hz, 2H, *H*-22, 21), 7.16 (d, ^3J = 7.6 Hz, 2H, *H*-19, 18), 5.38- 5.31 (m, 1H, *H*-15), 4.46-4.19 (m, 2H, *H*-11, 6), 3.68-3.26 (m, 1H, *H*-7), 3.14-2.96 (m, 1H, *H*-14), 2.12-1.81 (m, 3H, *H*-12, 8), 1.47-1.33 (m, 9H, *H*-3, 2, 1), 0.88 (dd, ^3J = 19.3, 7.0 Hz, 6H, *H*-10, 9).

^{1}H NMR (300 MHz, $CDCl_3$, unidentified diastereomer B): δ = 7.25 (d, ^{3}J = 7.5 Hz, 2H, *H*-22, 21), 7.15 (d, ^{3}J = 7.6 Hz, 2H, *H*-19, 18), 4.78 (d, ^{3}J = 8.3 Hz, 1H, *H*-15), 4.29 (d, ^{3}J = 10.0 Hz, 1H, *H*-11), 4.22-4.09 (m, 1H, *H*-6), 3.63-3.37 (m, 1H, *H*-7), 3.25-3.04 (m, 1H, *H*-14), 2.08-1.84 (m, 3H, *H*-12, 8), 1.43-1.33 (m, 9H, *H*-3, 2, 1), 0.95-0.78 (m, 6H, *H*-10, 9).

^{19}F NMR (470 MHz, $CDCl_3$): δ = -113.8 (s).

^{19}F NMR (470 MHz, $CDCl_3$): δ = -116.7 (s).

6.3.9 *tert*-Butyl ((*S*)-1-((2*R*,4*R*)-4-(4-fluorobenzyl)-5-oxotetrahydrofuran-2-yl)-2-methylpropyl)carbamate (9)



9

Mesylation: A 100 mL round bottom flask, equipped with a Schlenk adapter and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with 778 mg (2.04 mmol, 1 eq.) compound **8**, 20 mL abs. dichloromethane and 1.41 mL (10.2 mmol, 5 eq.) triethylamine. The reaction mixture was cooled to 0 °C in an ice bath followed by the dropwise addition of 474 µL (6.12 mmol, 3 eq.) methanesulfonyl chloride. The reaction mixture was stirred at 0 °C for additional 2 h and at RT overnight. Since the conversion was not complete, additional 848 µL (6.12 mmol, 3 eq.) triethylamine and 315 µL (4.08 mmol, 2 eq.) methanesulfonyl chloride were added and the reaction mixture heated to 35 °C and stirred overnight. After 22 h TLC indicated full conversion. The reaction was quenched by the addition of 50 mL H_2O. The reaction mixture was transferred into a separation funnel and 20 mL dichloromethane were added. The phases were separated and the aqueous layer was extracted with dichloromethane (3x20 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4 and the solvent was removed under reduced pressure to furnish a deep red solid residue which was used in the next step without further purification.

Elimination: A 100 mL round bottom flask, equipped with a Schlenk adapter and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with the crude residue of the previous step and 25 mL abs. ethanol were added, followed by the addition of 565 µL (4.08 mmol, 2 eq.) triethylamine. The reaction mixture was heated to 40 °C and stirred at this temperature for 22 h. Since no full conversion could be observed the temperature was raised to 45 °C and stirred for additional 20 h. After this period the reaction mixture was allowed to come to RT and concentrated to a small

volume. The residue was purified via flash chromatography (300 g SiO_2, cyclohexane/EtOAc 6:1 to 3:1 (v/v)) to give the corresponding olefin as a colorless solid.

Hydrogenation: In a two-necked round bottom flask, equipped with gas valve and magnetic stirring bar, the olefin was dissolved in 14 mL THF and 47 mg (0.82 mmol, 0.4 eq) Raney®-Nickel (slurry in H_2O) were added. Nitrogen atmosphere was exchanged by hydrogen (3×evacuation/purging) and the reaction mixture was stirred at RT for 2 d. After NMR indicated full conversion, the catalyst was removed under an argon atmosphere by filtration through a Schlenk-frit containing a plug of Celite® and the filter cake was washed with EtOAc (3×20 mL). The filtrate was concentrated and dried *in vacuo* to furnish the pure diastereomer as a colorless solid.

$C_{20}H_{28}FNO_4$ [365.44 g/mol].

Yield: 465 mg (1.27 mmol, 62%, 3 steps from **8**), colorless solid.

R_f (olefin) = 0.28 (cyclohexane/EtOAc = 3:1 (v/v); staining: $KMnO_4$, stains with $KMnO_4$ without heating).

$[\alpha]_D^{24}$ = -49.6 (c = 0.62, $CHCl_3$).

m.p. = 65-67 °C.

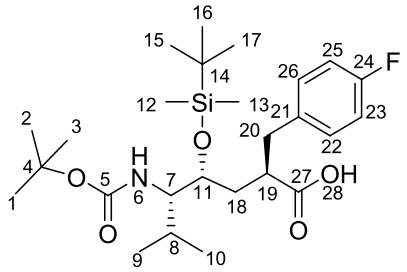
^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.22-7.11 (m, 2H, H-20, 18), 7.05-6.92 (m, 2H, H-21, 17), 4.39-4.11 (m, 2H, H-11, 6), 3.76-3.54 (m, 1H, H-7), 3.26 (d, ^3J = 12.9 Hz, 1H, H-15), 2.95-2.62 (m, 2H, H-15, 14), 2.33-2.17 (m, 1H, H-12), 2.16-2.01 (m, 1H, H-12), 1.86 (dd, ^3J = 16.2, 12.9 Hz, 1H, H-8), 1.43 (s, 9H, H-3, 2, 1), 0.95 (d, ^3J = 6.5 Hz, 3H, H-9), 0.86 (d, ^3J = 6.5 Hz, 3H, H-10).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.6 (*C*-13), 160.3 (*C*-19), 156.1 (*C*-5), 134.4 (*C*-16), 130.5 (d, $J_{C\text{-}F}$ = 7.9 Hz, *C*-21, 17), 115.6 (d, $J_{C\text{-}F}$ = 20.9 Hz, *C*-20, 18), 80.0 (*C*-11), 78.2 (*C*-4), 58.4 (*C*-7), 42.6 (*C*-14), 35.6 (*C*-15), 32.0 (*C*-12), 28.5-28.2 (m, *C*-8, 3, 2, 1), 19.9 (*C*-9), 15.9 (*C*-10).

^{19}F NMR (470 MHz, $CDCl_3$): δ = -116.4 (s).

HRMS (MALDI-TOF): *m/z* calcd. for $C_{20}H_{28}FNO_4Na$ $[M+Na]^+$: 388.1900; found: 388.1882.

6.3.10 (2*R*,4*R*,5*S*)-5-((*tert*-Butoxycarbonyl)amino)-4-((*tert*-butyldimethylsilyl)oxy)-2-(4-fluorobenzyl)-6-methylheptanoic acid (10)



10

Lactone opening: A 100 mL round bottom flask with magnetic stirring bar was charged with 465 mg (1.27 mmol, 1 eq.) alkylated lactone **9** and 4.5 mL THF. A solution of 214 mg (5.09 mmol, 4 eq.) $LiOHxH_2O$ in 6.7 mL H_2O was added and the turbid reaction mixture was stirred vigorously at RT. After 160 min TLC indicated full conversion and 12 mL Et_2O were added. The reaction mixture was cooled to 0 °C with an ice bath, followed by the careful addition of citric acid solution (25% in H_2O) until a pH of 4 was adjusted. Subsequently, the reaction mixture was transferred into a separation funnel and the phases were separated. The aqueous layer was extracted with Et_2O (3x20 mL). The combined organic layers were washed with brine (30 mL), dried over Na_2SO_4 and the solvent was evaporated under reduced pressure (T < 30 °C). The residue was dried *in vacuo* to furnish the intermediate product as a colorless solid. The crude product was used in the next step without further purification.

Silylation: A 100 mL three-necked round bottom flask, equipped with gas valve and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with the crude product of the previous step and 5 mL abs. dichloromethane were added. After the addition of 609 µL (7.63 mmol, 6 eq.) *N*-methylimidazole the reaction mixture was cooled to 0 °C in an ice bath. After 10 min 1.93 g (7.63 mmol, 6 eq.) iodine were added and the deep red reaction mixture stirred for additional 15 min. 767 mg (5.09 mmol, 4 eq.) TBSCl were added portionwise within a period of 5 min. After the addition was complete the reaction mixture was stirred for additional 3 h at 0 °C and at RT overnight. Afterwards, 20 mL Et_2O were added and the mixture was transferred into a separation funnel. 30 mL 1 M $Na_2S_2O_3$-solution were added and the organic layer was washed until total decoloration occurred. The phases were separated and the aqueous layer was extracted with Et_2O (2x30 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4 and the solvents removed under reduced pressure. The yellow oily residue was used in the next step without further purification.

Ester hydrolysis: In a 50 mL round bottom flask, equipped with magnetic stirring bar the residue of the previous step was dissolved in 3.5 mL MeOH and 100 µL (0.13 mmol, 0.1 eq.) citric acid (25% in H_2O) were added. The yellow reaction mixture was stirred at RT overnight. After 23 h the reaction

mixture was concentrated and purified via flash chromatography (375 g SiO_2, cyclohexane/EtOAc/AcOH 4:1:0.01 to 2:1:0.05 (v/v/v)) to give the protected acid **10** as a viscous oil.

$C_{26}H_{44}FNO_5Si$ [497.72 g/mol].

Yield: 538 mg (1.08 mmol, 85%, 3 steps from **9**), pale orange oil.

R_f = 0.37 (cyclohexane/EtOAc/AcOH = 2:1:0.05 (v/v/v); staining: $KMnO_4$, stains white with $KMnO_4$).

$[\alpha]_D^{24}$ = -8.3 (c = 0.51, $CHCl_3$).

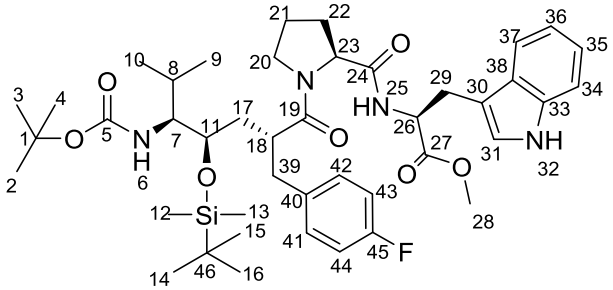
^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.20-7.08 (m, 2H, *H*-25, 23), 7.02-6.89 (m, 2H, *H*-26, 22), 4.60 (d, ^3J = 9.8 Hz, 1H, *H*-6), 3.86-3.64 (m, 1H, *H*-11), 3.52-3.25 (m, 1H, *H*-7), 3.04-2.86 (m, 1H, *H*-20), 2.84-2.62 (m, 2H, *H*-20, 19), 1.96-1.76 (m, 1H, *H*-18), 1.73-1.50 (m, 2H, *H*-18, 8), 1.41 (s, 9H, *H*-3, 2, 1), 0.89-0.74 (m, 15H, *H*-17, 16, 15, 10, 9), 0.10-0.02 (m, 6H, *H*-13, 12).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 179.9 (*C*-27),160.2 (*C*-24), 156.2 (*C*-5), 134.5 (*C*-21), 130.6 (*C*-22), 130.5 (*C*-26), 115.6 (*C*-23), 115.3 (*C*-25), 79.5 (*C*-4), 71.9 (*C*-11), 57.9 (*C*-7), 43.7 (*C*-19), 37.8 (*C*-20), 35.7 (*C*-18), 28.6 (*C*-3, 2, 1), 28.0 (*C*-8), 25.8 (*C*-14), 26.0 (*C*-17, 16, 15), 20.9 (*C*-9), 18.1 (*C*-10),-4.3 (*C*-13), -4,.6 (*C*-12).

^{19}F NMR (470 MHz, $CDCl_3$): δ = -116.7 (s).

HRMS (MALDI-TOF): *m/z* calcd. for $C_{26}H_{44}FNO_5SiNa$ $[M+Na]^+$: 520.2870; found: 520.2848.

6.3.11 Methyl ((2*R*,4*R*,5*S*)-5-((*tert*-butoxycarbonyl)amino)-4-((*tert*-butyldimethylsilyl)oxy)-2-(4-fluorobenzyl)-6-methylheptanoyl)-L-prolyl-L-tryptophanate (11)



11

Boc-Deprotection of Boc-Pro-Trp-OMe: In a 50 mL round bottom flask, equipped with a magnetic stirring bar, 516 mg (1.24 mmol, 1.2 eq.) peptide **7** were dissolved in 766 µL (10.4 mmol, 10 eq.) ethanethiol. Subsequently, 2.39 mL (31.0 mmol, 30 eq.) trifluoroacetic acid were added and the yellowish reaction mixture was stirred vigorously at RT. After 1 h the volatiles were removed under reduced pressure with a cooling trap. The residue was dissolved in 15 mL EtOAc, 25% aqueous ammonia (10 mL) were added and the reaction mixture was stirred for 5 min. The phases were

separated and the aqueous layer was extracted with EtOAc (2x10 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was dried *in vacuo* to furnish the deprotected intermediate as a colorless solid.

Coupling: A 50 mL round bottom flask, equipped with a Schlenk adapter and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with 515 mg (1.03 mmol, 1 eq.) compound **10** which was then dissolved in 5 mL abs. DMF, followed by the addition of 180 µL (1.03 mmol, 1 eq.) Hünig`s base. The flask was cooled to 0 °C in an ice bath and 471 mg (1.24 mmol, 1.2 eq.) HBTU were added to the reaction mixture. The previously deprotected peptide **7** was dissolved in 2.5 mL abs. DMF and 360 µL (2.06 mmol, 2 eq.) Hünig`s base were added. After 5 min, the solution of the deprotected intermediate was added to the solution of acid **10** with a syringe. After the addition was complete, the ice bath was removed and the yellow reaction mixture was stirred at RT for additional 2 h. Subsequently, the reaction mixture was quenched by the addition of brine (10 mL). Additionally, 20 mL of EtOAc were added and the mixture was stirred vigorously for 10 min. Afterwards, the mixture was transferred into a separation funnel and the phases were separated. The aqueous layer was extracted with EtOAc (2x20 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4 and the solvents were removed under reduced pressure. The residue was purified via flash chromatography (375 g SiO_2, dichloromethane/MeOH 100:1 to 30:1 (v/v)) to furnish a pale orange oily residue. The residue was dissolved in 50 mL EtOAc and the organic layer washed with H_2O (5x50 mL) in order to remove remaining DMF and tetramethylurea. The organic layer was dried over Na_2SO_4, the solvent removed under reduced pressure and the residue dried *in vacuo* to yield the desired product as a colorless solid.

$C_{43}H_{63}FN_4O_7Si$ [795.08 g/mol].

Yield: 471 mg (0.59 mmol, 58%, 2 steps from **10**), colorless solid.

R_f = 0.64 (dichloromethane/MeOH = 30:1 (v/v); staining: $KMnO_4$).

$[\alpha]_D^{24}$ = -0.6 (c = 0.99, $CHCl_3$).

m.p. = 79-81 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.14 (br s, 1H, *H*-32), 7.55 (d, ^3J = 7.0 Hz, 1H, *H*-37), 7.32-6.76 (m, 9H, *H*-44, 43, 42, 41, 36, 35, 34, 31, 25), 4.84-4.71 (m, 1H, *H*-26), 4.64-4.41 (m, 2H, *H*-23, 6), 3.71-3.60 (m, 4H, *H*-28, 11), 3.54-3.43 (m, 1H, *H*-20), 3.40-3.20 (m, 3H, *H*-29, 7), 2.99-2.88 (m, 1H, *H*-20), 2.73-2.42 (m, 3H, *H*-39, 18), 2.27-2.17 (m, 1H, *H*-22), 1.92-1.51 (m, 6H, *H*-22, 21, 17, 8), 1.40 (s, 9H, *H*-4, 3, 2), 0.91-0.78 (m, 15H, *H*-16, 15, 14, 10, 9), 0.09 (d, ^3J = 9.5 Hz, 6H, *H*-13, 12).

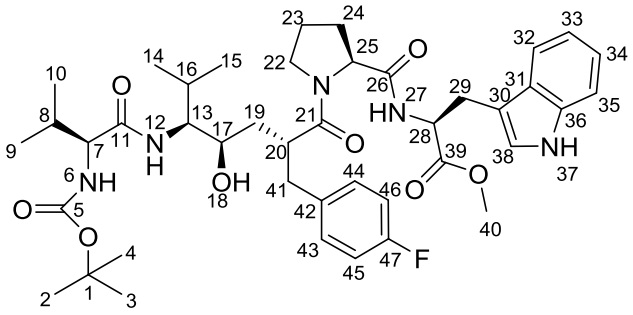
^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.4 (*C*-19), 172.5 (*C*-27), 171.2 (*C*-24), 163.3 (*C*-45), 156.0 (*C*-5), 136.2 (*C*-40), 134.9 (*C*-33), 130.5 (*C*-42), 130.4 (*C*-41), 127.7 (*C*-38), 123.5 (*C*-31), 122.2 (*C*-35), 119.7 (*C*-36), 118.7 (*C*-37), 115.4 (*C*-44), 115.1 (*C*-43), 111.3 *C*-34), 110.2 (*C*-30), 79.0 (*C*-1), 72.2

(*C*-11), 60.2 (*C*-23), 57.7 (*C*-7), 53.5 (*C*-26), 52.4 (*C*-28), 47.3 (*C*-20), 42.0 (*C*-18), 38.1 (*C*-39), 35.6 (*C*-17), 28.6 (*C*-8), 28.5 (*C*-4, 3, 2), 27.8 (*C*-29), 27.6 (*C*-22), 26.0 (*C*-16, 15, 14), 24.9 (*C*-21), 21.4 (*C*-10), 18.4 (*C*-9), 18.0 (*C*-46), -4.1 (*C*-12), (*C*-13).

^{19}F NMR (470 MHz, $CDCl_3$): δ = -116.5 (s).

HRMS (MALDI-TOF): *m/z* calcd. for $C_{43}H_{63}FN_4O_7SiNa$ $[M+Na]^+$: 817.4348; found: 817.4398.

6.3.12 Methyl ((2*R*,4*R*,5*S*)-5-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methylbutanamido)-2-(4-fluorobenzyl)-4-hydroxy-6-methylheptanoyl)-L-prolyl-L-tryptophanate (12)



12

Tandem deprotection of Boc- and TBS-moiety: In a 50 mL round bottom flask, equipped with magnetic stirring bar, 225 mg (0.283 mmol, 1 eq.) **11** were dissolved in 3 mL 2,2,2-trifluoroethanol. Afterwards, 83 µL (1.13 mmol, 4 eq.) ethanethiol were added, followed by the addition of 509 mg (2.26 mmol, 8 eq.) zinc dibromide. The reaction mixture was stirred vigorously at RT and after 15 min the formation of a white precipitate could be observed. The turbid reaction mixture was stirred for additional 90 min, followed by the addition of 25% aqueous ammonia (6 mL) and EtOAc (10 mL). The reaction mixture was stirred for 10 min and subsequently transferred into a separation funnel and the phases were separated. The aqueous layer was extracted with EtOAc (2x10 mL). The combined organic layers were dried over Na_2SO_4 and the solvents were removed under reduced pressure. The residue was dried *in vacuo* for 30 min to furnish the deprotected intermediate as a colorless solid.

Coupling: A 50 mL round bottom flask, equipped with magnetic stirring bar and Schlenk adapter, was dried (evacuated, heated, N_2-purged) and charged with 74 mg (0.340 mmol, 1.2 eq.) Boc-L-Val-OH and 0.8 mL abs. DMF, followed by the addition of 49 µL (0.283 mmol, 1 eq.) Hünig's base. The flask was cooled to 0 °C with an ice bath. Subsequently, 129 mg (0.340 mmol, 1.2 eq.) HATU were added to the reaction mixture and stirred for additional 6 min. In a second 50 mL round bottom flask, equipped with magnetic stirring bar and Schlenk adapter, the previously deprotected peptide was dissolved in 1.6 mL abs. DMF and 99 µL (0.566 mmol, 2 eq.) Hünig's base were added. Afterwards, the solution of the deprotected peptide was added to the solution of the activated acid using a syringe. After the addition was complete, the ice bath was removed and the deep yellow reaction mixture was

stirred for additional 90 min at RT. The reaction was quenched by the addition of 6 mL brine, followed by the addition of EtOAc (10 mL). The reaction mixture was stirred for 10 min and subsequently transferred into a separation funnel. The phases were separated and the aqueous layer was extracted with EtOAc (2x15 mL). The combined organic extracts were dried over Na_2SO_4 and the solvents removed concentrated under reduced pressure. The residue was purified via flash chromatography (375 g SiO_2, dichloromethane/MeOH = 20:1 (v/v)) to furnish a pale yellow oily residue. The residue was dissolved in 50 mL EtOAc and the organic layer washed with H_2O (3x50 mL) The organic layer was dried over Na_2SO_4, the solvent removed under reduced pressure and the residue dried *in vacuo* to yield the desired product **12** as a colorless solid.

$C_{42}H_{58}FN_5O_8$ [779.95 g/mol].

Yield: 107 mg (0.137 mmol, 48%, 2 steps from **11**), colorless solid.

R_f = 0.21 (dichloromethane/MeOH = 20:1 (v/v); staining: CAM).

$[\alpha]_D^{24}$ = -23.8 (c = 0.86, $CHCl_3$).

m.p. = 88-90 °C.

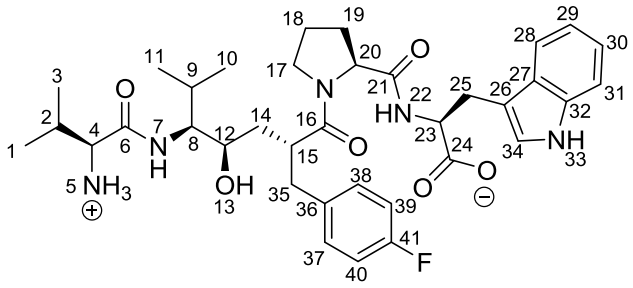
^{1}H NMR (300 MHz, $CDCl_3$, mixture of 2 rotamers in 5:1 ratio): δ = 8.96 (bs, 1H, *H*-37), 7.38 (d, ^3J = 7.6 Hz, 1H, *H*-32), 7.22-6.84 (m, 9H, *H*-46, 45, 44, 43, 38, 35, 34, 33, 27), 6.06 (d, ^3J = 9.8 Hz, 1H, *H*-12), 4.97 (d, ^3J = 8.6 Hz, 1H, *H*-6), 4-59-4.55 (m, 1H, *H*-28), 3.80-3.71 (m, 1H, *H*-7), 3.70-3.44 (m, 5H, *H*-40, 17, 13), 3.33-3.05 (m, 3H, *H*-29, 25), 2.99-2.82 (m, 1H, *H*-22), 2.76-2.60 (m, 1H, *H*-22), 2.57-2.40 (m, 2H, *H*-41), 2.39-2.25 (m, 1H, *H*-20), 2.16-1.54 (m, 5H, *H*-24, 19, 16, 8), 1.38-1.26 (m, 9H, *H*-4, 3, 2), 1.17-1.11 (m, 1H, *H*-22), 0.99-0.61 (m, 14H, *H*-24, 23, 15, 14, 10, 9).

^{13}C NMR (75 MHz, $CDCl_3$, mixture of 2 rotamers in 5:1 ratio): δ = 175.2 (*C*-21), 172.8 (*C*-39), 172.7 (*C*-26), 172.2 (*C*-11), 163.4 (*C*-47), 156.2 (*C*-5), 136.3 (*C*-42), 134.5 (*C*-36), 130.7 (*C*-44), 130.6 (*C*-43), 127.6 (*C*-31), 123.4 (*C*-28), 122.4 (*C*-34), 119.9 (*C*-33), 118.0 (*C*-32), 115.5 (*C*-46), 115.2 (*C*-45), 111.8 (*C*-35), 109.8 (*C*-30), 80.4 (*C*-1), 71.1 (*C*-17), 60.8 (*C*-7), 60.1 (*C*-25), 59.3 (*C*-13), 53.7 (*C*-28), 52.6 (*C*-40), 46.2 (*C*-22), 43.9 (*C*-20), 38.8 (*C*-41), 37.0 (*C*-19), 31.3 (*C*-24), 29.8 (*C*-8), 28.5 (*C*-4, 3, 2), 28.0 (*C*-16), 26.2 (*C*-29), 21.8 (*C*-23), 20.6 (*C*-14), 19.9 (*C*-10), 18.2 (*C*-9), 16.7 (*C*-15).

^{19}F NMR (470 MHz, $CDCl_3$): δ = -116.1 (s).

HRMS (MALDI-TOF): *m/z* calcd. for $C_{42}H_{58}FN_5O_8Na$ $[M+Na]^+$: 802.4167; found: 802.3501.

6.3.13 ((2*R*,4*R*,5*S*)-5-((*S*)-2-Ammonio-3-methylbutanamido)-2-(4-fluorobenzyl)-4-hydroxy-6-methylheptanoyl)-L-prolyl-L-tryptophanate (13)



13

Saponification: In a 25 mL round bottom flask, equipped with magnetic stirring bar, 84 mg (0.108 mmol, 1 eq.) of compound **12** were dissolved in 1 mL THF. Afterwards, a solution of 36 mg (0.862 mmol, 8 eq.) $LiOHxH_2O$ in 3 mL H_2O was added and the reaction mixture was stirred at RT. After 120 min TLC indicated full conversion. Then, 1 M HCl was added until a pH of ~3 was achieved. Subsequently, 10 mL of EtOAc were added and the mixture transferred into a separation funnel. The phases were separated and the aqueous layer was extracted with EtOAc (2x5 mL). The combined organic extracts were dried over Na_2SO_4 and the solvents removed under reduced pressure. The residue was dried *in vacuo* for 30 min to yield the saponified intermediate as a colorless solid.

Boc-Deprotection: In a 25 mL round bottom flask, equipped with magnetic stirring bar, the crude intermediate was dissolved in 1.5 mL 2,2,2-trifluoroethanol, followed by the addition of 32 µL (0.431 mmol, 4 eq.) ethanethiol and 194 mg (0.862 mmol, 8 eq.) zinc dibromide. The white turbid reaction mixture was stirred vigorously at RT for 40 h. Afterwards, the reaction mixture was concentrated and the residue dissolved in mixture of H_2O (500 µL), acetonitrile (490 µL) and DMSO (10 µL) and purified by preparative HPLC (*CLF_NucleodurC18_pc001_HCOOH_5to95*) to furnish compound **13** as colorless solid.

$C_{36}H_{48}FN_5O_6$ [665.81 g/mol].

Yield: 21.0 mg (0.032 mmol, 29%), colorless solid.

$[\alpha]_D^{24} =$ -19.0 (c = 0.53, MeOH).

m.p. = 168-170 °C.

^{1}H NMR (500 MHz, MeOD, complex mixture of 2 rotamers in 4:1 ratio): δ = 8.36 (br s, 1H, *H*-33), 7.59 (d, ^3J = 8.4 Hz, 1H, *H*-28), 7.33 (d, ^3J = 8.4 Hz, 1H, *H*-31), 7.13-6.91 (m, 7H, *H*-40, 39, 38, 37, 34, 30, 29), 4.66-4.58 (m, 1H, *H*-23), 3.83-3.64 (m, 3H, *H*-12, 8, 4), 3.52-3.35 (m, 2H, *H*-25, 20), 3.21-3.05 (m, 2H, *H*-25, 17), 2.98-2.90 (m, 1H, *H*-17), 2.74-2.53 (m, 3H, *H*-35, 15), 2.29-2.07 (m, 2H,

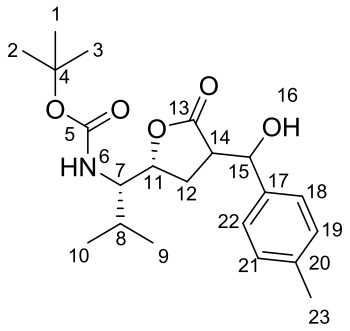
H-9, 2), 1.95-1.86 (m, 1H, *H*-14), 1.66-1.53 (m, 2H, *H*-19, 14), 1.18-0.77 (m, 15H, *H*-19, 18, 11, 10, 3, 1).

^{13}C NMR (125 MHz, MeOD, complex mixture of 2 rotamers in 4:1 ratio): δ = 177.5 (*C*-24, 16), 173.4 (*C*-21), 170.2 (*C*-6), 164.0 (*C*-41), 138.0 (*C*-6), 136.2 (*C*-36), 131.8 (*C*-38), 131.7 (*C*-37), 129.0 (*C*-27), 124.3 (*C*-34), 122.4 (*C*-30), 119.9 (*C*-29), 119.4 (*C*-28), 116.3 (*C*-40), 116.1 (*C*-39), 112.3 (*C*-31), 112.0 (*C*-26), 71.1 (*C*-12), 62.1 (*C*-20), 61.1 (*C*-8), 60.1 (*C*-4), 56.3 (*C*-23), 47.5 (*C*-17), 45.4 (*C*-15), 39.7 (*C*-35), 37.6 (*C*-14), 32.1 (*C*-2), 31.6 (*C*-19), 29.4 (*C*-9), 28.3 (*C*-25), 22.5 (*C*-18), 21.0 (*C*-11), 19.6 (*C*-3), 18.1 (*C*-10), 17.9 (*C*-1).

^{19}F NMR (470 MHz, $CDCl_3$): δ = -118.6 (s), -119.0 (s).

HRMS (MALDI-TOF): *m/z* calcd. for $C_{36}H_{48}FN_5O_6Na$ $[M+Na]^+$: 688.3486; found: 688.4046.

6.3.14 *tert*-Butyl ((1*S*)-1-((2*R*)-4-(hydroxy(p-tolyl)methyl)-5-oxotetrahydrofuran-2-yl)-2-methylpropyl)carbamate (14)



14

A 100 mL three-necked round bottom flask, equipped with gas valve and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with 500 mg (1.95 mmol, 1 eq.) lactone **5** and 20 mL abs. THF. The reaction mixture was cooled to -78 °C in a dry ice/acetone cooling bath followed by the dropwise addition of 2.90 mL (5.83 mmol, 3 eq.) LDA solution (2.0 M in THF). The deep orange reaction mixture was stirred at -78 °C for additional 45 min. Subsequently, 344 µL (2.93 mmol, 1.5 eq.) *p*-tolualdehyde were added dropwise within 5 min. The reaction mixture turned yellow after a few min and was stirred for additional 45 min at -78 °C until TLC indicated full conversion. The reaction was quenched by the addition of 10 mL satd. NH_4Cl and was allowed to warm up to RT followed by the addition of 30 mL H_2O and 50 mL EtOAc. The phases were separated and the aqueous layer extracted with EtOAc (2x30 mL). The combined organic layers were washed with 1 M HCl (50 mL), brine (50 mL), dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified via flash chromatography (270 g SiO_2, cyclohexane/EtOAc 7:1 to 2:1 (v/v)) to furnish two diastereomers, which were separated and characterized separately.

$C_{21}H_{31}NO_5$ [377.48 g/mol].

Yield: unidentified diastereomer A: 159 mg, unidentified diastereomer B: 310 mg, total: 469 mg (1.24 mmol, 63%), colorless solid.

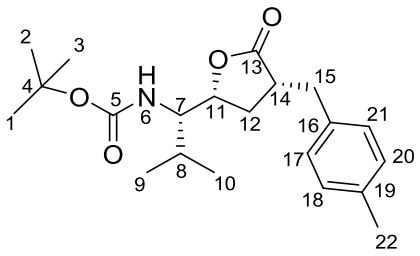
R_f = 0.29, 0.21 (cyclohexane/EtOAc = 3:1 (v/v); staining: $KMnO_4$).

m.p. = 92-94 °C.

^{1}H NMR (300 MHz, $CDCl_3$, unidentified diastereomer A): δ = 7.24 (d, ^3J = 7.6 Hz, 2H, *H*-22, 21), 7.16 (d, ^3J = 7.6 Hz, 2H, *H*-19, 18), 5.38- 5.31 (m, 1H, *H*-15), 4.46-4.19 (m, 2H, *H*-11, 6), 3.68-3.26 (m, 1H, *H*-7), 3.14-2.96 (m, 1H, *H*-14), 2.33 (s, 3H, *H*-23), 2.12-1.81 (m, 3H, *H*-12, 8), 1.47-1.33 (m, 9H, *H*-3, 2, 1), 0.88 (dd, ^3J = 19.3, 7.0 Hz, 6H, *H*-10, 9).

^{1}H NMR (300 MHz, $CDCl_3$, unidentified diastereomer B): δ = 7.25 (d, ^3J = 7.5 Hz, 2H, *H*-22, 21), 7.15 (d, ^3J = 7.6 Hz, 2H, *H*-19, 18), 4.78 (d, ^3J = 8.3 Hz, 1H, *H*-15), 4.29 (d, ^3J = 10.0 Hz, 1H, *H*-11), 4.22-4.09 (m, 1H, *H*-6), 3.63-3.37 (m, 1H, *H*-7), 3.25-3.04 (m, 1H, *H*-14), 2.32 (s, 3H, *H*-23), 2.08-1.84 (m, 3H, *H*-12, 8), 1.43-1.33 (m, 9H, *H*-3, 2, 1), 0.95-0.78 (m, 6H, *H*-10, 9).

6.3.15 *tert*-Butyl ((*S*)-2-methyl-1-((2*R*,4*R*)-4-(4-methylbenzyl)-5-oxotetrahydrofuran-2-yl)propyl)carbamate (15)



15

Mesylation: A 100 mL round bottom flask, equipped with a Schlenk adapter and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with 439 mg (1.16 mmol, 1 eq.) **14**, 12 mL abs. dichloromethane and 806 µL (5.81 mmol, 5 eq.) triethylamine. Subsequently, 607 mg (3.48 mmol, 3 eq.) methanesulfonic anhydride were added portionwise within 3 min and the reaction mixture heated to 35 °C and stirred overnight. After 23 h the reaction was quenched by the addition of 50 mL H_2O. The mixture was transferred into a separation funnel and additional 30 mL dichloromethane were added. The phases were separated and the aqueous layer was extracted with dichloromethane (3x40 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4 and the solvent was removed under reduced pressure to furnish a deep red solid residue which was used in the next step without further purification.

Elimination: A 50 mL round bottom flask, equipped with a Schlenk adapter and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with the crude residue of the previous step and 8 mL abs. ethanol were added, followed by the addition of 323 µL (2.33 mmol, 2 eq.) triethylamine.

The reaction mixture was heated to 50 °C and stirred at this temperature for 18 h. After this period the reaction mixture was allowed to warm up to RT and concentrated to a small volume. The residue was purified via flash chromatography (225 g SiO_2, cyclohexane/EtOAc 5:1 to 3:1 (v/v)) to furnish the corresponding olefin as a colorless solid

Hydrogenation: In a two-necked round bottom flask, equipped with gas valve and magnetic stirring bar, the olefin was dissolved in 10 mL THF and 27 mg (0.46 mmol, 0.4 eq) Raney®-Nickel (slurry in H_2O) were added. Nitrogen atmosphere was exchanged by hydrogen (3×evacuation/purging) and the reaction mixture was stirred under a H_2 balloon atmosphere at 40 °C for 24 h. After NMR indicated full conversion, the catalyst was removed under an argon atmosphere by filtration through a Schlenk-frit containing a plug of Celite® and the filter cake was washed with EtOAc (3×20 mL). The filtrate was concentrated and dried *in vacuo* to furnish the pure diastereomer as a colorless solid.

$C_{21}H_{31}NO_4$ [361.48 g/mol].

Yield: 236 mg (0.65 mmol, 56%, 3 steps from **14**), colorless solid.

R_f (olefin) = 0.42 (cyclohexane/EtOAc = 3:1 (v/v); staining: $KMnO_4$).

$[\alpha]_D^{24} =$ -45.8 (c = 2.03, $CHCl_3$).

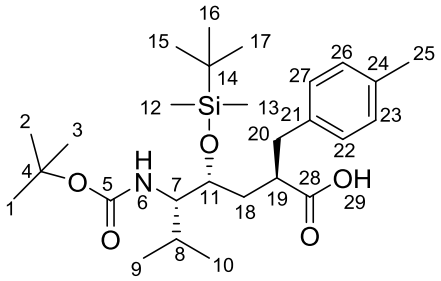
m.p. = 76-79 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.09 (s, 4H, *H*-21, 20, 18, 17), 4.33 (d, ^3J = 8.9 Hz, 1H, *H*-6), 4.23-4.10 (m, 1H, *H*-11), 3.75-3.49 (m, 1H, *H*-7), 3.26 (d, ^3J = 12.2 Hz, 1H, *H*-15), 2.89-2.74 (m, 1H, *H*-14), 2.73-2.57 (m, 1H, *H*-15), 2.36-2.19 (m, 4H, *H*-22, 12), 2.13-1.99 (m, 1H, *H*-12), 1.96-1.76 (m, 1H, *H*-8), 1.42 (s, 9H, *H*-3, 2, 1), 0.93 (d, ^3J = 6.5 Hz, 3H, *H*-9), 0.85 (d, ^3J = 6.5 Hz, 3H, *H*-10).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.8 (*C*-13), 156.6 (*C*-5), 136.3 (*C*-16), 135.7 (*C*-19), 129.4 (*C*-21, 17), 128.4 (*C*-20, 18), 79.9 (*C*-11), 78.2 (*C*-4), 58.3 (*C*-7), 42.7 (*C*-14), 36.0 (*C*-15), 32.2 (*C*-12), 28.6-28.2 (m, *C*-8, 3, 2, 1), 21.1 (*C*-22), 19.9 (*C*-9), 16.0 (*C*-10).

HRMS (MALDI-TOF): *m/z* calcd. for $C_{21}H_{31}NO_4Na$ $[M+Na]^+$: 384.2151; found: 384.2204.

6.3.16 (2*R*,4*R*,5*S*)-5-((*tert*-Butoxycarbonyl)amino)-4-((tert-butyldimethylsilyl)oxy)-6-methyl-2-(4-methylbenzyl)heptanoic acid (16)



16

Lactone opening: A 50 mL round bottom flask with magnetic stirring bar was charged with 221 mg (0.61 mmol, 1 eq.) alkylated lactone **15** and 2 mL THF. A solution of 103 mg (2.44 mmol, 4 eq.) $LiOHxH_2O$ in 3 mL H_2O was added and the turbid reaction mixture was stirred vigorously at RT. After 75 min TLC indicated full conversion and 6 mL Et_2O were added. The reaction mixture was cooled to 0 °C in an ice bath, followed by the careful addition of citric acid solution (25% in H_2O) until a pH of 4 was adjusted. Subsequently, the reaction mixture was transferred into a separation funnel and the phases were separated. The aqueous layer was extracted with Et_2O (2x10 mL). The combined organic layers were washed with H_2O (10 mL), brine (10 mL), dried over Na_2SO_4 and the solvent was evaporated under reduced pressure (T < 30 °C). The residue was dried *in vacuo* for 30 min to furnish the intermediate product as a colorless solid. The crude product was used in the next step without further purification.

Silylation: A 100 mL three-necked round bottom flask, equipped with gas valve and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with the crude product of the previous step and 3.5 mL abs. dichloromethane were added. After the addition of 293 µL (3.67 mmol, 6 eq.) *N*-methylimidazole the reaction mixture was cooled to 0 °C in an ice bath. After 10 min, 931 mg (3.67 mmol, 6 eq.) iodine were added and the deep red reaction mixture was stirred for additional 5 min. 277 mg (1.83 mmol, 3 eq.) TBSCl were added in one portion to the reaction mixture. After the addition, the ice bath was removed and the deep reaction solution stirred at RT overnight. Afterwards, 15 mL Et_2O were added and the mixture was transferred into a separation funnel. 15 mL 1 M $Na_2S_2O_3$ were added and the organic layer was washed until total decoloration occurred. The phases were separated and the aqueous layer was extracted with Et_2O (2x10 mL). The combined organic layers were washed with citric acid solution [(25% in H_2O), 10 mL], brine (10 mL), dried over Na_2SO_4 and the solvents removed under reduced pressure. The yellow oily residue was used in the next step without further purification.

Ester hydrolysis: In a 25 mL round bottom flask, equipped with magnetic stirring bar the residue of the previous step was dissolved in 2.5 mL MeOH and 80 µL (0.06 mmol, 0.1 eq.) citric acid (25% in

H_2O) were added. The yellow reaction mixture was stirred at RT overnight. After 23 h the reaction mixture was concentrated and purified via flash chromatography (120 g SiO_2, cyclohexane/EtOAc 6:1 to 2:1 (v/v)) to give the protected acid **16** as a colorless oil.

$C_{27}H_{47}NO_5Si$ [493.76 g/mol].

Yield: 238 mg (0.48 mmol, 79%, 3 steps from **15**), colorless oil.

R_f = 0.43 (cyclohexane/EtOAc = 2:1 (v/v); staining: $KMnO_4$, stains white with $KMnO_4$).

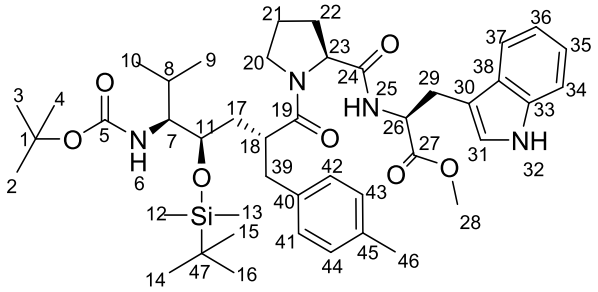
$[\alpha]_D^{24}$ = -7.0 (c = 0.35, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.07 (s, 4H, *H*-27, 26, 23, 22), 4.61 (d, ^3J = 9.9 Hz, 1H, *H*-6), 3.82-3.59 (m, 1H, *H*-11), 3.52-3.27 (m, 1H, *H*-7), 3.00-2.85 (m, 1H, *H*-20), 2.81-2.58 (m, 2H, *H*-20, 19), 2.30 (s, 3H, *H*-25), 1.96-1.77 (m, 1H, *H*-18), 1.73-1.53 (m, 2H, *H*-18, 8), 1.42 (s, 9H, *H*-3, 2, 1), 0.88-0.74 (m, 15H, *H*-17, 16, 15, 10, 9), 0.09-0.04 (m, 6H, *H*-13, 12).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 179.4 (*C*-28),155.2 (*C*-5), 135.2 (*C*-21), 128.3 (*C*-27, 22), 128.0 (*C*-26, 23), 127.9 (*C*-24), 78.3 (*C*-4), 70.8 (*C*-11), 57.2 (*C*-7), 42.8 (*C*-19), 37.2 (*C*-20), 34.4 (*C*-18), 27.6 (*C*-3, 2, 1), 27.4 (*C*-8), 25.0 (*C*-17, 16, 15), 20.2 (*C*-9), 20.0 (*C*-25), 18.1 (*C*-10),-4.3 (*C*-13), -4.6 (*C*-12).

HRMS could not be determined.

6.3.17 Methyl ((2*R*,4*R*,5*S*)-5-((*tert*-butoxycarbonyl)amino)-4-((*tert*-butyldimethylsilyl)oxy)-6-methyl-2-(4-methylbenzyl)heptanoyl)-L-prolyl-L-tryptophanate (17)



17

Boc-Deprotection of Boc-Trp-OMe: In a 50 mL round bottom flask, equipped with magnetic stirring bar, 183 mg (0.44 mmol, 1.2 eq.) peptide **7** were dissolved in 272 µL (4.4 mmol, 10 eq.) ethanethiol. Subsequently, 850 µL (mmol, 30 eq.) trifluoroacetic acid were added and the yellowish reaction mixture was stirred vigorously at RT. After 1 h the volatiles were removed under reduced pressure with a cooling trap. The residue was dissolved in 15 mL EtOAc and 25% aqueous ammonia (10 mL) were added and the reaction mixture was stirred for 5 min. The phases were separated and the aqueous layer was extracted with EtOAc (2x10 mL). The combined organic layers were dried over Na_2SO_4 and

the solvent was removed under reduced pressure. The residue was dried *in vacuo* to furnish the deprotected intermediate as a colorless solid.

Coupling: A 50 mL round bottom flask, equipped with Schlenk adapter and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with 181 mg (0.36 mmol, 1 eq.) **16** which was dissolved in 0.75 mL abs. DMF, followed by the addition of 64 µL (0.36 mmol, 1 eq.) Hünig`s base. The flask was cooled to 0 °C in an ice bath and 168 mg (0.44 mmol, 1.2 eq.) HBTU were added to the reaction mixture. The previously deprotected peptide **7** was dissolved in 1.5 abs. DMF and 128 µL (0.74 mmol, 2 eq.) Hünig`s base were added. After 5 min, the solution of the deprotected intermediate was added to the solution of acid **16** with a syringe. After the addition was complete, the ice bath was removed and the yellow reaction mixture was stirred at RT for additional 2 h. Subsequently, the reaction mixture was quenched by the addition of brine (5 mL). Additionally, 10 mL of EtOAc were added and the mixture was stirred vigorously for 10 min. Afterwards, the mixture was transferred into a separation funnel and the phases were separated. The aqueous layer was extracted with EtOAc (2x20 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4 and the solvents were removed under reduced pressure. The residue was purified via flash chromatography (150 g SiO_2, dichloromethane/MeOH 80:1 to 30:1 (v/v)) to furnish a pale orange oily residue. The residue was dissolved in 50 mL EtOAc and the organic layer washed with H_2O (5x50 mL) in order to remove remaining DMF and tetramethylurea. The organic layer was dried over Na_2SO_4, the solvent removed under reduced pressure and the residue dried *in vacuo* to yield the desired product as a colorless solid.

$C_{44}H_{66}N_4O_7Si$ [791.12 g/mol].

Yield: 105 mg (0.132 mmol, 36%, 2 steps from **16**), colorless solid.

R_f = 0.53 (dichloromethane/MeOH = 30:1 (v/v); staining: $KMnO_4$).

$[\alpha]_D^{24}$ = -12.9 (c = 0.47, $CHCl_3$).

m.p. = 77-79 °C.

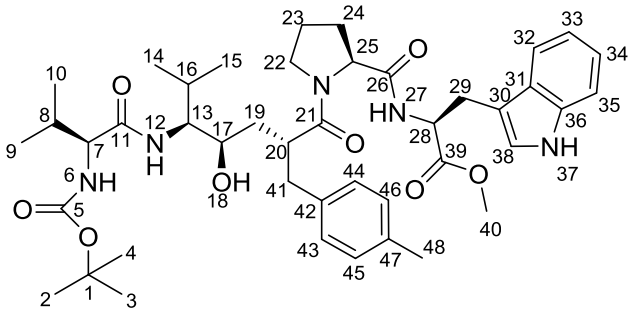
^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.11 (br s, 1H, *H*-32), 7.52 (d, ^3J = 7.5 Hz, 1H, *H*-37), 7.41-7.30 (m, 1H, *H*-25), 7.24-6.84 (m, 8H, *H*-44, 43, 42, 41, 36, 35, 34, 31), 4.76 (dd, ^3J = 8.9, 6.1 Hz, 1H, *H*-26), 4.63-4.46 (m, 2H, *H*-23, 6), 3.71-3.59 (m, 4H, *H*-28, 11), 3.56-3.46 (m, 1H, *H*-20), 3.40-3.16 (m, 3H, *H*-29, 7), 3.02-2.90 (m, 1H, *H*-20), 2.77-2.45 (m, 3H, *H*-39, 18), 2.34-2.19 (m, 4H, *H*-46, 22), 1.94-1.50 (m, 6H, *H*-22, 21, 17, 8), 1.41 (s, 9H, *H*-3, 2, 1), 0.91-0.78 (m, 24H, *H*-51, 49, 48, 16, 15, 14, 10, 9), 0.16-0.02 (m, 12H, *H*-47, 46, 13, 12).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 175.1 (*C*-19), 172.4 (*C*-27), 171.2 (*C*-24), 156.0 (*C*-5), 136.2 (*C*-33), 136.0 (*C*-45, 40), 129.2 (*C*-44, 43), 128.9 (*C*-42, 41), 127.7 (*C*-38), 123.4 (*C*-31), 122.1 (*C*-35), 119.6 (*C*-36), 118.7 (*C*-37), 111.3 (*C*-34), 110.3 (*C*-31), 79.0 (*C*-1), 72.1 (*C*-11), 60.1 (*C*-23), 57.8 (*C*-7),

53.5 (*C*-26), 52.4 (*C*-28), 47.3 (*C*-20), 41.9 (*C*-18), 38.6 (*C*-39), 35.5 (*C*-17), 28.6 (*C*-4, 3, 2), 27.9 (*C*-8), 27.5 (*C*-29), 27.4 (*C*-22), 26.0 (*C*-16, 15, 14), 24.8 (*C*-21), 21.4 (*C*-10), 21.1 (*C*-46), 18.4 (*C*-9), 18.0 (*C*-47), -4.1 (*C*-12), -4.7 (*C*-13).

HRMS (MALDI-TOF): *m/z* calcd. for $C_{44}H_{66}N_4O_7SiNa$ $[M+Na]^+$: 813.4598; found: 813.4868.

6.3.18 Methyl ((2*R*,4*R*,5*S*)-5-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methylbutanamido)-4-hydroxy-6-methyl-2-(4-methylbenzyl)heptanoyl)-L-prolyl-L-tryptophanate (18)



18

Tandem deprotection of Boc- and TBS-moeity: In a 50 mL round bottom flask, equipped with magnetic stirring bar, 141 mg (0.178 mmol, 1 eq.) **17** were dissolved in 2 mL 2,2,2-trifluoroethanol. Afterwards, 52 µL (0.713 mmol, 4 eq.) ethanethiol were added, followed by the addition of 321 mg (1.43 mmol, 8 eq.) zinc dibromide. The reaction mixture was stirred vigorously at RT and after 45 min the formation of a white precipitate could be observed. The turbid reaction mixture was stirred for additional 2 h, followed by the addition of 25% aqueous ammonia (5 mL) and EtOAc (15 mL). The reaction mixture was stirred for 15 min and subsequently transferred into a separation funnel and the phases were separated. The aqueous layer was extracted with EtOAc (2x10 mL). The combined organic layers were dried over Na_2SO_4 and the solvents were removed under reduced pressure. The residue was dried *in vacuo* for 30 min to furnish the deprotected intermediate as a colorless solid.

Coupling: A 50 mL round bottom flask, equipped with magnetic stirring bar and Schlenk adapter, was dried (evacuated, heated, N_2-purged) and charged with 47 mg (0.214 mmol, 1.2 eq.) Boc-L-Val-OH and 0.4 mL abs. DMF, followed by the addition of 31 µL (0.178 mmol, 1 eq.) Hünig's base. The flask was cooled to 0 °C in an ice bath. Subsequently, 81 mg (0.214 mmol, 1.2 eq.) HATU were added to the reaction mixture and stirred for additional 5 min. In a second 50 mL round bottom flask, equipped with magnetic stirring bar and Schlenk adapter, the previously deprotected peptide was dissolved in 1 mL abs. DMF and 62 µL (0.356 mmol, 2 eq.) Hünig's base were added. Afterwards, the solution of the deprotected peptide was added to the solution of the activated acid using a syringe. After the addition was complete, the ice bath was removed and the deep yellow reaction mixture was stirred for additional 3 h at RT. The reaction was quenched by the addition of 5 mL brine, followed by the addition of EtOAc (15 mL). The reaction mixture was stirred for 15 min and subsequently transferred

into a separation funnel. The phases were separated and the aqueous layer was extracted with EtOAc (2x15 mL). The combined organic extracts were dried over Na_2SO_4 and the solvents removed concentrated under reduced pressure. The residue was purified via flash chromatography (300 g SiO_2, dichloromethane/MeOH = 20:1 (v/v)) to furnish an oily residue. The residue was dissolved in 50 mL EtOAc and the organic layer washed with H_2O (4x40 mL) in order to remove remaining DMF. The organic layer was dried over Na_2SO_4, the solvent removed under reduced pressure and the residue dried *in vacuo* to yield the desired product **18** as a colorless solid.

$C_{43}H_{61}N_5O_8$ [775.99 g/mol].

Yield: 52 mg (0.067 mmol, 38%, 2 steps from **17**), colorless solid.

R_f = 0.21 (dichloromethane/MeOH = 20:1 (v/v); staining: CAM).

$[\alpha]_D^{24}$ = -26.4 (c = 0.89, $CHCl_3$).

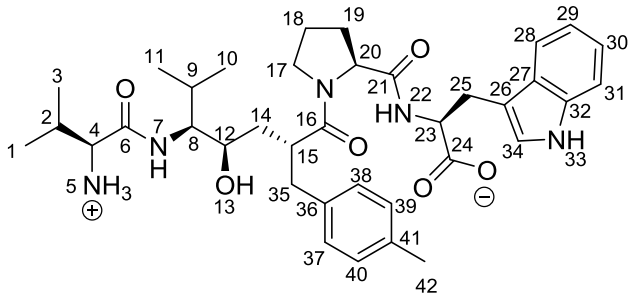
m.p. = 92-94 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.03 (bs, 1H, *H*-37), 7.51 (d, ^3J = 7.5 Hz, 1H, *H*-32), 7.36 (d, ^3J = 7.6 Hz, 1H, *H*-27), 7.22-6.84 (m, 8H, *H*-46, 45, 44, 43, 38, 35, 34, 33), 6.08 (d, ^3J = 9.9 Hz, 1H, *H*-12), 5.08 (d, ^3J = 8.7 Hz, 1H, *H*-6), 4-78-4.60 (m, 1H, *H*-28), 3.93-3.81 (m, 1H, *H*-7), 3.80-3.61 (m, 5H, *H*-40, 17, 13), 3.40-3.19 (m, 3H, *H*-29, 25), 3.12-2.99 (m, 1H, *H*-22), 2.93-2.78 (m, 1H, *H*-22), 2.69-2.52 (m, 2H, *H*-41), 2.51-2.37 (m, 1H, *H*-20), 2.33-1.64 (m, 8H, *H*-48, 24, 19, 16, 8), 1.49-1.42 (m, 9H, *H*-4, 3, 2), 1.37-1.32 (m, 1H, *H*-22), 1.08-0.83 (m, 14H, *H*-24, 23, 15, 14, 10, 9).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 175.6 (*C*-21), 172.8 (*C*-39), 172.7 (*C*-26), 172.4 (*C*-11), 156.2 (*C*-5), 136.3 (*C*-47, 42), 135.6 (*C*-36), 129.2 (*C*-46, 45), 129.0 (*C*-44, 43), 127.6 (*C*-31), 123.4 (*C*-38), 122.3 (*C*-34), 119.9 (*C*-33), 118.1 (*C*-32), 111.7 (*C*-35), 110.0 (*C*-30), 80.3 (*C*-1), 71.1 (*C*-17), 60.8 (*C*-7), 60.1 (*C*-25), 59.2 (*C*-13), 53.7 (*C*-28), 52.6 (*C*-40), 46.2 (*C*-22), 44.1 (*C*-20), 39.5 (*C*-41), 37.1 (*C*-19), 31.0 (*C*-24), 29.8 (*C*-8), 28.5 (*C*-4, 3, 2), 28.0 (*C*-16), 26.2 (*C*-29), 21.8 (*C*-23), 21.1 (*C*-48), 20.6 (*C*-14), 19.9 (*C*-10), 18.3 (*C*-9), 16.7 (*C*-15).

HRMS (MALDI-TOF): *m/z* calcd. for $C_{43}H_{61}N_5O_8Na$ $[M+Na]^+$: 798.4418; found: 798.5275.

6.3.19 ((2*R*,4*R*,5*S*)-5-((*S*)-2-Ammonio-3-methylbutanamido)-4-hydroxy-6-methyl-2-(4-methylbenzyl)heptanoyl)-L-prolyl-L-tryptophanate (19)



19

Saponification: In a 25 mL round bottom flask, equipped with magnetic stirring bar, 45 mg (0.058 mmol, 1 eq.) **18** were dissolved in 0.5 mL THF. Afterwards, a solution of 20 mg (0.464 mmol, 8 eq.) LiOHxH_2O in 1.5 mL H_2O was added and the reaction mixture was stirred at RT. After 90 min TLC indicated full conversion, followed by the addition of 1 M HCl until a pH of ~4 was achieved. Subsequently, 10 mL EtOAc were added and the mixture transferred into a separation funnel. The phases were separated and the aqueous layer was extracted with EtOAc (2x5 mL). The combined organic extracts were dried over Na_2SO_4 and the solvents removed under reduced pressure. The residue was dried *in vacuo* for 30 min to yield the saponified intermediate as a colorless solid.

Boc-Deprotection: In a 25 mL round bottom flask, equipped with magnetic stirring bar, the crude intermediate was dissolved in 1.5 mL 2,2,2-trifluoroethanol, followed by the addition of 17 µL (0.232 mmol, 4 eq.) ethanethiol and 105 mg (0.464 mmol, 8 eq.) zinc dibromide. The reaction mixture turned pink immediately and was stirred vigorously at RT for 2 h. Afterwards, the reaction mixture was concentrated and the residue dissolved in a mixture of H_2O (600 µL), acetonitrile (390 µL) and DMSO (10 µL) and purified by preparative HPLC (*CLF_NucleodurC18_pc001_HCOOH_5to95*) to furnish compound **19** as a colorless solid.

$C_{37}H_{51}N_5O_6$ [661.84 g/mol].

Yield: 13.8 mg (0.021 mmol, 36%, 2 steps from **18**), colorless solid.

$[\alpha]_D^{24}$ = -17.6 (c = 0.94, MeOH).

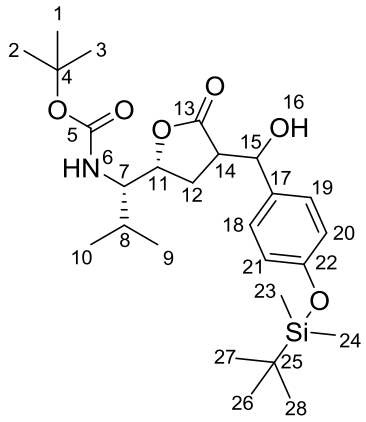
m.p. = 160-162 °C.

^{1}H NMR (500 MHz, MeOD): δ = 8.35 (br s, 1H, *H*-33), 7.57 (d, ^3J = 7.9 Hz, 1H, *H*-28), 7.32 (d, ^3J = 8.0 Hz, 1H, *H*-31), 7.11-7.00 (m, 5H, *H*-38, 37, 34, 30, 29), 6.95 (d, ^3J = 7.8 Hz, 2H, *H*-40, 39), 4.63-4.57 (m, 1H, *H*-23), 3.80-3.68 (m, 3H, *H*-12, 8, 4), 3.51-3.37 (m, 2H, *H*-25, 20), 3.23-3.03 (m, 2H, *H*-25, 17), 2.95-2.87 (m, 1H, *H*-17), 2.72-2.58 (m, 3H, *H*-35, 15), 2.30-2.21 (m, 5H, *H*-42, 9, 2), 2.00-1.94 (m, 1H, *H*-14), 1.63-1.52 (m, 2H, *H*-19, 14), 1.16-0.67 (m, 15H, *H*-19, 18, 11, 10, 3, 1).

^{13}C NMR (125 MHz, MeOD): δ = 177.8 (*C*-24, 16), 173.5 (*C*-21), 170.2 (*C*-6), 138.0 (*C*-6), 137.4 (*C*-36), 137.0 (*C*-41), 130.2 (*C*-38, 37), 130.0 (*C*-40, 39), 129.0 (*C*-27), 124.3 (*C*-34), 122.4 (*C*-30), 119.9 (*C*-29), 119.4 (*C*-28), 112.2 (*C*-31), 111.9 (*C*-26), 71.2 (*C*-12), 62.1 (*C*-20), 61.2 (*C*-8), 60.2 (*C*-4), 56.2 (*C*-23), 47.4 (*C*-17), 45.6 (*C*-15), 40.4 (*C*-35), 37.8 (*C*-14), 32.0 (*C*-2), 31.6 (*C*-19), 29.3 (*C*-9), 28.2 (*C*-25), 22.0 (*C*-18), 21.1 (*C*-11), 21.0 (*C*-42), 19.6 (*C*-3), 18.0 (*C*-10), 17.4 (*C*-1).

HRMS (MALDI-TOF): *m/z* calcd. for $C_{37}H_{51}N_5O_6Na$ $[M+Na]^+$: 684.3737; found: 684.3685.

6.3.20 *tert*-Butyl ((1*S*)-1-((2*R*)-4-((4-((*tert*-butyldimethylsilyl)oxy)phenyl)(hydroxy)methyl)-5-oxotetrahydrofuran-2-yl)-2-methylpropyl)carbamate (20)



20

A 100 mL three-necked round bottom flask, equipped with gas valve and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with 800 mg (3.11 mmol, 1 eq.) lactone **5** and 20 mL abs. THF. The reaction mixture was cooled to -78 °C in a dry ice/acetone cooling bath followed by the dropwise addition of 4.66 mL (9.33 mmol, 3 eq.) LDA solution (2.0 M in THF). The orange reaction mixture was stirred at -78 °C for additional 50 min. Subsequently, 1.14 mL (4.66 mmol, 1.5 eq.) aldehyde **6** were added in one portion. The reaction mixture turned yellow after a few min and was stirred for additional 45 min at -78 °C until TLC indicated full conversion. The reaction was quenched by the addition of 15 mL satd. NH_4Cl. The reaction mixture was allowed to come to RT, followed by the addition of 50 mL H_2O and 50 mL EtOAc. The phases were separated and the aqueous layer was extracted with EtOAc (2x50 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified via flash chromatography (375 g SiO_2, cyclohexane/EtOAc 7:1 to 2:1 (v/v)) to furnish two diastereomers, which were separated and characterized separately.

$C_{26}H_{43}NO_6Si$ [493.72 g/mol]

Yield: unidentified diastereomer A: 364 mg, unidentified diastereomer B: 647 mg, total: 1.01 g (2.05 mmol, 66%), colorless solid.

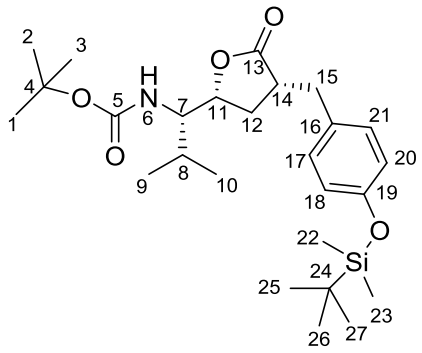
R_f = 0.21, 0.18 (cyclohexane/EtOAc = 3:1 (v/v); staining: $KMnO_4$).

m.p. = 96-98 °C.

^{1}H NMR (300 MHz, $CDCl_3$, unidentified diastereomer A): δ = 7.21 (d, ^3J = 8.3 Hz, 2H, *H*-21, 20), 6.81 (d, ^3J = 8.3 Hz, 2H, *H*-18, 19), 5.37-5.22 (m, 1H, *H*-15), 4.60-4.16 (m, 2H, *H*-11, 6), 3.84-3.32 (m, 1H, *H*-7), 3.12-2.74 (m, 1H, *H*-14), 2.47-2.28 (m, 1H, *H*-12), 2.16-1.77 (m, 2H, *H*-12, 8), 1.51-1.23 (m, 9H, *H*-3, 2, 1), 1.00-0.80 (m, 15H, *H*-28, 27, 26, 10, 9), 0.18 (s, 6H, *H*-24, 23).

^{1}H NMR (300 MHz, $CDCl_3$, unidentified diastereomer B): δ =7.23 (d, ^3J = 8.3 Hz, 2H, *H*-21, 20), 6.80 (d, ^3J = 8.3 Hz, 2H, *H*-18, 19), 4.81-4.64 (m, 1H, *H*-15), 4.40-4.05 (m, 2H, *H*-11, 6), 3.71-3.44 (m, 1H, *H*-7), 3.19-2.80 (m, 1H, *H*-14), 2.16-1.71 (m, 3H, *H*-12, 8), 1.47-1.30 (m, 9H, *H*-3, 2, 1), 1.0-0.78 (m, 15H, *H*-28, 27, 26, 10, 9), 0.17 (s, 6H, *H*-24, 23).

6.3.21 *tert*-Butyl ((*S*)-1-((2*R*, 4*R*)-4-(4-((*tert*-butyldimethylsilyl)oxy)benzyl)-5-oxotetrahydrofuran-2-yl)-2-methylpropyl)carbamate (21)



21

Mesylation: A 50 mL round bottom flask, equipped with a Schlenk adapter and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with 333 mg (0.67 mmol, 1 eq.) compound **20**, 10 mL abs. dichloromethane and 468 µL (3.37 mmol, 5 eq.) triethylamine. Subsequently, 353 mg (2.02 mmol, 3 eq.) methanesulfonic anhydride were added in one portion and the reaction mixture heated to 35 °C and stirred overnight. After 23 h the reaction mixture was allowed to warm to RT and was quenched by the addition of 20 mL H_2O. The reaction mixture was transferred into a separation funnel and additional 20 mL dichloromethane were added. The phases were separated and the aqueous layer was extracted with dichloromethane (2x20 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4 and the solvent was removed under reduced pressure to furnish a deep red solid residue, which was used in the next step without further purification.

Elimination: A 50 mL round bottom flask, equipped with a Schlenk adapter and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with the crude residue of the previous step and 12 mL abs. ethanol were added, followed by the addition of 187 µL (1.35 mmol, 2 eq.) triethylamine. The reaction mixture was heated to 55 °C and stirred at this temperature for 16 h. After this period the reaction mixture was allowed to warm to RT and concentrated to a small volume. The residue was

partioned between EtOAc (20 mL) and H_2O (20 mL). The aqueous layer was extracted with EtOAc (2x20 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4 and the solvent was removed under reduced pressure to furnish the intermediate olefin as a yellow oil.

Hydrogenation: In a two-necked round bottom flask, equipped with gas valve and magnetic stirring bar, the crude olefin was dissolved in 8 mL THF and 15 mg (0.24 mmol, 0.4 eq) Raney®-Nickel (slurry in H_2O) were added. Nitrogen atmosphere was exchanged by hydrogen (3×evacuation/purging) and the reaction mixture was stirred under H_2 balloon atmosphere at 40 °C for 48 h. After NMR indicated full conversion, the catalyst was removed under an argon atmosphere by filtration through a Schlenk-frit containing a plug of Celite® and the filter cake was washed with EtOAc (3×20 mL). The filtrate was concentrated and the residue was purified via flash chromatography (225 g SiO_2, cyclohexane/EtOAc 7:1 to 3:1) to furnish the pure diastereomer **21** as a colorless solid.

$C_{26}H_{43}NO_5Si$ [477.72 g/mol]

Yield: 205 mg (0.43 mmol, 64%, 3 steps from **20**), colorless solid.

R_f = 0.61 (cyclohexane/EtOAc = 2:1(v/v); staining: $KMnO_4$).

$[\alpha]_D^{24}$ = -40.7 (c = 0.73, $CHCl_3$).

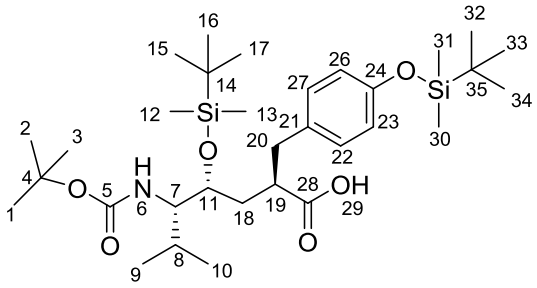
m.p. = 80-82 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.09 (s, 4H, *H*-21, 20, 18, 17), 4.33 (d, ^3J = 8.9 Hz, 1H, *H*-6), 4.23-4.10 (m, 1H, *H*-11), 3.75-3.49 (m, 1H, *H*-7), 3.26 (d, ^3J = 12.2 Hz, 1H, *H*-15), 2.89-2.74 (m, 1H, *H*-14), 2.73-2.57 (m, 1H, *H*-15), 2.36-2.19 (m, 4H, *H*-22, 12), 2.13-1.99 (m, 1H, *H*-12), 1.96-1.76 (m, 1H, *H*-8), 1.42 (s, 9H, *H*-3, 2, 1), 0.93 (d, ^3J = 6.5 Hz, 3H, *H*-9), 0.85 (d, ^3J = 6.5 Hz, 3H, *H*-10).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.9 (*C*-13), 156.1 (*C*-5), 154.4 (*C*-16), 130.2 (*C*-19), 129.9 (*C*-21, 17), 120.3 (*C*-20, 18), 79.9 (*C*-11), 78.2 (*C*-4), 58.3 (*C*-7), 42.9 (*C*-14), 35.7 (*C*-15), 32.2 (*C*-12), 29.8 (*C*-8), 28.4 (*C*-3, 2, 1), 25.8 (*C*-27, 26, 25), 20.0 (*C*-9), 18.3 (*C*-24), 16.0 (*C*-10), -4.3 (*C*-23, 22).

HRMS (MALDI-TOF): *m/z* calcd. for $C_{26}H_{43}NO_5SiNa$ $[M+Na]^+$: 500.2808; found: 500.2888.

6.3.22 (2*R*,4*R*,5*S*)-5-((*tert*-Butoxycarbonyl)amino)-4-((*tert*-butyldimethylsilyl)oxy)-2-(4-((*tert*-butyldimethylsilyl)oxy)benzyl)-6-methylheptanoic acid (22)



22

Lactone opening: A 100 mL round bottom flask with magnetic stirring bar was charged with 1.04 g (2.18 mmol, 1 eq.) alkylated lactone **21** and 11 mL THF. A solution of 366 mg (8.71 mmol, 4 eq.) $LiOHxH_2O$ in 7 mL H_2O was added and the turbid reaction mixture was stirred vigorously at RT. After 165 min TLC indicated full conversion and 22 mL Et_2O were added. The reaction mixture was cooled to 0 °C in an ice bath, followed by the careful addition of citric acid (25% in H_2O) until a pH of 4 was adjusted. Subsequently, the reaction mixture was transferred into a separation funnel and the phases were separated. The aqueous layer was extracted with Et_2O (2x20 mL). The combined organic layers were washed with brine (40 mL), dried over Na_2SO_4 and the solvent was evaporated under reduced pressure (T < 30 °C). The residue was dried *in vacuo* for 30 min to furnish the intermediate product as a colorless solid. The crude product was used in the next step without further purification.

Silylation: A 100 mL three-necked round bottom flask, equipped with gas valve and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with the crude product of the previous step and 9.0 mL abs. dichloromethane were added. After the addition of 1.04 mL (13.06 mmol, 6 eq.) *N*-methylimidazole the reaction mixture was cooled to 0 °C in an ice bath. After 10 min 3.31 g (13.06 mmol, 6 eq.) iodine were added and the deep red reaction mixture stirred for additional 10 min. 1.31 g (8.71 mmol, 4 eq.) TBSCl were added portionwise within a period of 5 min. After the addition was complete, the ice bath was removed and the deep reaction solution stirred at RT overnight. Afterwards, 30 mL Et_2O were added and the mixture was transferred into a separation funnel. 50 mL 1 M $Na_2S_2O_3$ were added and the organic layer was washed until total decoloration occurred. The phases were separated and the aqueous layer was extracted with Et_2O (2x50 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4 and the solvents removed under reduced pressure. The yellow oily residue was used in the next step without further purification.

Ester hydrolysis: In a 50 mL round bottom flask, equipped with a magnetic stirring bar the residue of the previous step was dissolved in 6.5 mL MeOH and 165 µL (0.13 mmol, 0.1 eq.) citric acid solution (25% in H_2O) were added. The yellow reaction mixture was stirred at RT overnight. After 23 h the reaction mixture was concentrated and used in the next step without further purification.

Hydrogenation: In a three-necked round bottom flask, equipped with magnetic stirring bar, the crude product was dissolved in 21 mL MeOH and 755 µL (5.45 mmol, 2.5 eq) triethylamine were added. Under nitrogen stream, 48 mg palladium on charcoal (5 % palladium; 1 mol-%) were added to the reaction mixture. Nitrogen atmosphere was exchanged by hydrogen (3×evacuation/purging) and the reaction mixture was stirred under H_2 balloon atmosphere at RT for 4 days. After NMR indicated full conversion and the absence of any iodinated byproduct, the catalyst was removed under an argon atmosphere by filtration through a Schlenk-frit containing a plug of Celite® and the filter cake was washed with MeOH (3×30 mL). The filtrate was concentrated and the residue was purified via flash chromatography (450 g SiO_2, cyclohexane/EtOAc/AcOH 6:1:0.01 to 3:1:0.01 (v/v/v)) to furnish compound **21** as a colorless oil.

$C_{32}H_{59}NO_6Si_2$ [610.00 g/mol].

Yield: 592 mg (0.97 mmol, 45%, 4 steps from **21**), colorless oil.

R_f = 0.30 (cyclohexane/EtOAc/AcOH = 3:1:0.01 (v/v); staining: $KMnO_4$, stains white with $KMnO_4$).

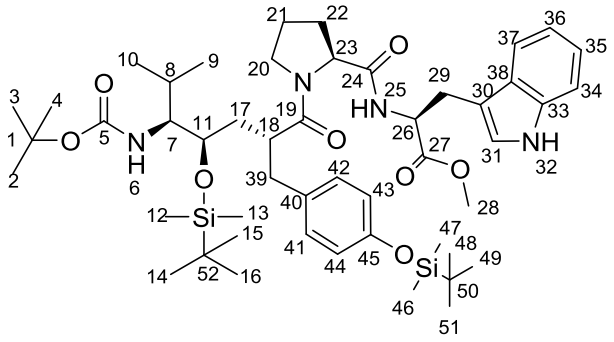
$[\alpha]_D^{24}$ = -8.3 (c = 0.62, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.03 (d, ^3J = 8.2 Hz, 2H, *H*-27, 22), 6.75 (d, ^3J = 8.2 Hz, 2H, *H*-26, 23), 4.60 (d, ^3J = 9.8 Hz, 1H, *H*-6), 3.85-3.67 (m, 1H, *H*-11), 3.49-3.26 (m, 1H, *H*-7), 3.01-2.86 (m, 1H, *H*-20), 2.76-2.55 (m, 2H, *H*-20, 19), 1.98-1.73 (m, 1H, *H*-18), 1.72-1.50 (m, 2H, *H*-18, 8), 1.42 (s, 9H, *H*-3, 2, 1), 0.98-0.72 (m, 24 H, *H*-35, 33, 32, 17, 16, 15, 10, 9), 0.18-0.05 (m, 12H, *H*-31, 30, 13, 12).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 178.9 (*C*-27), 156.2 (*C*-24), 154.3 (*C*-5), 131.5 (*C*-21), 130.1 (*C*-27, 22), 120.2 (*C*-26, 23), 79.4 (*C*-4), 72.1 (*C*-11), 57.9 (*C*-7), 43.8 (*C*-19), 38.0 (*C*-20), 35.2 (*C*-18), 28.6 (*C*-3, 2, 1), 28.1 (*C*-8), 26.0 (*C*-35, 14), 25.9 (*C*-17, 16, 15), 25.8 (*C*-34, 33, 32), 21.0 (*C*-9), 18.1 (*C*-10),-3.4 (*C*-31, 13), -4.3 (*C*-30, 12).

HRMS (MALDI-TOF): *m/z* calcd. for $C_{32}H_{59}NO_6Si_2Na$ $[M+Na]^+$: 632.3779; found: 632.3652.

6.3.23 Methyl ((2*R*,4*R*,5*S*)-5-((*tert*-butoxycarbonyl)amino)-4-((*tert*-butyldimethylsilyl)oxy)-2-(4-((*tert*-butyldimethylsilyl)oxy)benzyl)-6-methylheptanoyl)-L-prolyl-L-tryptophanate (23)



23

Boc-Deprotection of Boc-Pro-Trp-OMe: In a 50 mL round bottom flask, equipped with magnetic stirring bar, 459 mg (1.10 mmol, 1.2 eq.) peptide **7** were dissolved in 674 µL (9.21 mmol, 10 eq.) ethanethiol. Subsequently, 2.13 mL (27.6 mmol, 30 eq.) trifluoroacetic acid were added and the yellowish reaction mixture was stirred vigorously at RT. After 1 h the volatiles were removed under reduced pressure with a cooling trap. The residue was dissolved in 15 mL EtOAc and 25% aqueous ammonia (10 mL) were added and the reaction mixture was stirred for 10 min. The phases were separated and the aqueous layer was extracted with EtOAc (2x15 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was dried *in vacuo* to furnish the deprotected intermediate as a colorless solid.

Coupling: A 50 mL round bottom flask, equipped with a Schlenk adapter and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with 562 mg (0.92 mmol, 1 eq.) **22** and dissolved in 5 mL abs. DMF, followed by the addition of 161 µL (0.92 mmol, 1 eq.) Hünig`s base. The flask was cooled to 0 °C in an ice bath and 419 mg (1.10 mmol, 1.2 eq.) HBTU were added to the reaction mixture. The previously deprotected peptide **7** was dissolved in 2.5 abs. DMF and 322 µL (1.84 mmol, 2 eq.) Hünig`s base were added. After 7 min, the solution of the deprotected intermediate was added to the solution of acid **22** with a syringe. After the addition was complete, the ice bath was removed and the yellow reaction mixture was stirred at RT for additional 1 h. After that period another 161 µL (0.92 mmol, 1 eq.) Hünig`s base were added to the reaction mixture and stirred for additional 120 min. Subsequently, the reaction mixture was quenched by the addition of brine (15 mL). Additionally, 20 mL EtOAc were added and the mixture was stirred vigorously for 20 min. Afterwards, the mixture was transferred into a separation funnel and the phases were separated. The aqueous layer was extracted with EtOAc (2x30 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4 and the solvents were removed under reduced pressure. The residue was purified via flash chromatography (375 g SiO_2, dichloromethane/MeOH 100:1 to 30:1 (v/v)) to furnish a pale orange oily residue. The residue was dissolved in 50 mL EtOAc and the organic layer

washed with H_2O (5x50 mL) in order to remove remaining DMF and tetramethylurea. The organic layer was dried over Na_2SO_4, the solvent removed under reduced pressure and the residue dried *in vacuo* to yield the desired peptide **23** as a colorless solid.

$C_{49}H_{78}N_4O_8Si_2$ [907.35 g/mol].

Yield: 590 mg (0.65 mmol, 59%, 2 steps from **22**), colorless solid.

R_f = 0.28 (dichloromethane/MeOH = 30:1 (v/v); staining: $KMnO_4$).

$[\alpha]_D^{24}$ = -0.7 (c = 1.45, $CHCl_3$).

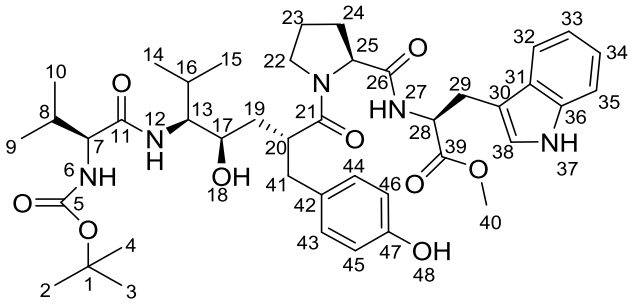
m.p. = 78-80 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.08 (br s, 1H, *H*-32), 7.56 (d, ^3J = 6.5 Hz, 1H, *H*-37), 7.37 (d, ^3J = 5.3 Hz, 1H, *H*-25), 7.25-7.00 (m, 4H, *H*-36, 35, 34, 31), 6.83 (d, ^3J = 8.1 Hz, 2H, *H*-42, 41), 6.66 (d, ^3J = 8.1 Hz, 2H, *H*-44, 43), 4.84-4.72 (m, 1H, *H*-26), 4.63-4.42 (m, 2H, *H*-23, 6), 3.71-3.63 (m, 4H, *H*-28, 11), 3.54-3.43 (m, 1H, *H*-20), 3.37-3.20 (m, 3H, *H*-29, 7), 2.99-2.87 (m, 1H, *H*-20), 2.71-2.35 (m, 3H, *H*-39, 18), 2.30-2.17 (m, 1H, *H*-22), 1.93-1.54 (m, 6H, *H*-22, 21, 17, 8), 1.40 (s, 9H, *H*-3, 2, 1), 0.99-0.79 (m, 24H, *H*-51, 49, 48, 16, 15, 14, 10, 9), 0.18-0.08 (m, 12H, *H*-47, 46, 13, 12).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 175.1 (*C*-19), 172.5 (*C*-27), 171.2 (*C*-24), 156.0 (*C*-5), 154.3 (*C*-45), 136.2 (*C*-33), 132.0 (*C*-40), 130.0 (*C*-42, 41), 127.8 (*C*-38), 123.5 (*C*-31), 122.2 (*C*-35), 120.2 (*C*-44, 43), 119.6 (*C*-36), 118.7 (*C*-37), 111.3 (*C*-34), 110.3 (*C*-31), 80.0 (*C*-1), 72.4 (*C*-11), 60.1 (*C*-23), 57.6 (*C*-7), 53.5 (*C*-26), 52.4 (*C*-28), 47.3 (*C*-20), 42.2 (*C*-18), 38.4 (*C*-39), 35.4 (*C*-17), 28.5 (*C*-4, 3, 2), 27.7 (*C*-8), 27.6 (*C*-29), 27.4 (*C*-22), 26.0 (*C*-16, 15, 14), 24.9 (*C*-21), 21.5 (*C*-10), 18.4 (*C*-9), 18.3 (*C*-50), 18.1 (*C*-52), -3.4 (*C*-47), -4.1 (*C*-46), -4.3 (*C*-13), -4.8 (*C*-12).

HRMS (MALDI-TOF): *m/z* calcd. for $C_{49}H_{78}N_4O_8Si_2Na$ $[M+Na]^+$: 929.5256; found: 929.3843.

6.3.24 Methyl ((2*R*,4*R*,5*S*)-5-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methylbutanamido)-4-hydroxy-2-(4-hydroxybenzyl)-6-methylheptanoyl)-L-prolyl-L-tryptophanate (24)



24

Aryl-TBS-Deprotection: In a 25 mL polypropylene vial, equipped with magnetic stirring bar, 178 mg (0.20 mmol, 1 eq.) **23** were dissolved in 2 mL MeOH. Afterwards, 61 mg (0.78 mmol, 4 eq.) potassium hydrogen difluoride were added in one portion. The vial was sealed and the reaction mixture was stirred at RT overnight. After 18 h the reaction mixture was concentrated and the residue was purified via flash chromatography (120 g SiO_2, dichloromethane/MeOH = 20:1 (v/v)) to furnish the deprotected aryl alcohol as a colorless solid (147 mg, 0.19 mmol). The product was used in the next step without further treatment.

Tandem deprotection of Boc- and TBS-moeity: In a 50 mL round bottom flask, equipped with a magnetic stirring bar, 147 mg (0.19 mmol 1 eq.) of the previously deprotected intermediate were dissolved in 2 mL 2,2,2-trifluoroethanol. Subsequently, 55 µL (0.75 mmol, 4 eq.) ethanethiol and 334 mg (1.48 mmol, 8 eq.) zinc dibromide were added in one portion and the mixture was stirred vigorously at RT. After 30 min the formation of a brownish precipitate could be observed. The reaction mixture was stirred for additional 3 h. Afterwards, the reaction mixture was quenched by the addition of 10 mL 25% aqueous ammonia, followed by the addition of EtOAc (20 mL). The mixture was stirred for 15 min and then transferred into a separation funnel. The phases were separated and the aqueous layer was extracted with EtOAc (2x10 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure and the residue dried *in vacuo* for 30 min to furnish the fully deprotcted intermediate as a colorless solid.

Coupling: A 50 mL round bottom flask, equipped with a Schlenk adapter and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with 48 mg (0.22 mmol, 1.2 eq.) Boc-Val-OH and dissolved in 0.45 mL abs. DMF, followed by the addition of 33 µL (0.19 mmol, 1 eq.) Hünig`s base. The flask was cooled to 0 °C in an ice bath and 84 mg (0.22 mmol, 1.2 eq.) HATU were added to the reaction mixture. The previously fully deprotected peptide was dissolved in 1 mL abs. DMF and 66 µL (0.38 mmol, 2 eq.) Hünig`s base were added. After 5 min, the solution of the deprotected intermediate was added to the solution of the activated Boc-L-Val-OH with a syringe. After the

addition was complete, the ice bath was removed and the yellow reaction mixture was stirred at RT overnight. Subsequently, the reaction mixture was quenched by the addition of brine (5 mL). Additionally, 15 mL of EtOAc were added and the mixture was stirred vigorously for 10 min. Afterwards, the mixture was transferred into a separation funnel and the phases were separated. The aqueous layer was extracted with EtOAc (2x10 mL). The combined organic layers were dried over Na_2SO_4 and the solvents removed under reduced pressure. The residue was purified via flash chromatography (375 g SiO_2, dichloromethane/MeOH = 20:1 (v/v)) to furnish a yellowish oily residue. The residue was dissolved in 50 mL EtOAc and the organic layer washed with H_2O (3x50 mL) in order to remove remaining DMF. The organic layer was dried over Na_2SO_4, the solvent removed under reduced pressure and the residue dried *in vacuo* to yield the desired peptide **24** as a colorless solid.

$C_{42}H_{59}N_5O_9$ [777.96 g/mol]

Yield: 55 mg (0.071 mmol, 36%, 3 steps from **23**), colorless solid.

R_f = 0.15 (dichloromethane/MeOH = 20:1 (v/v); staining: CAM).

$[\alpha]_D^{24}$ = -25.6 (c = 1.91, $CHCl_3$).

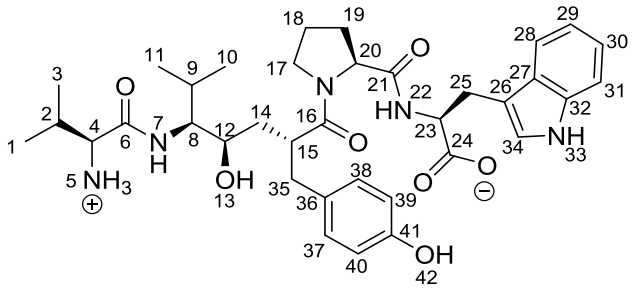
m.p. = 91-93 °C.

^{1}H NMR (300 MHz, $CDCl_3$, complex mixture of 2 rotamers in 3:1 ratio): δ = 9.07 (bs, 1H, *H*-37), 7.51 (d, ^3J = 7.5 Hz, 1H, *H*-32), 7.36-6.81 (m, 7H, *H*-44, 43, 38, 35, 34, 33, 27), 6.67 (d, ^3J = 8.1 Hz, 2H, *H*-46, 45), 6.15 (d, ^3J = 9.2 Hz, 1H, *H*-12), 5.14-5.03 (m, 1H, *H*-6), 4-72-4.61 (m, 1H, *H*-28), 3.88-3.81 (m, 1H, *H*-7), 3.80-3.59 (m, 5H, *H*-40, 17, 13), 3.50-3.17 (m, 3H, *H*-29, 25), 3.12-3.03 (m, 1H, *H*-22), 2.89-2.74 (m, 1H, *H*-22), 2.63-2.35 (m, 3H, *H*-47, 20), 2.24-1.55 (m, 5H, *H*-24, 19, 16, 8), 1.47-1.39 (m, 9H, *H*-4, 3, 2), 1.37-1.32 (m, 1H, *H*-22), 1.05-0.83 (m, 14H, *H*-24, 23, 15, 14, 10, 9).

^{13}C NMR (75 MHz, $CDCl_3$, complex mixture of 2 rotamers in 3:1 ratio): δ = 176.0 (*C*-21), 173.0 (*C*-39), 172.8 (*C*-26, 11), 156.2 (*C*-5), 155.5 (*C*-47), 136.4 (*C*-36), 130.2 (*C*-44, 43), 130.0 (*C*-42), 127.4 (*C*-31), 123.4 (*C*-38), 122.3 (*C*-34), 119.9 (*C*-33), 118.1 (*C*-32), 115.6 (*C*-46, 45), 111.8 (*C*-35), 109.9 (*C*-30), 80.4 (*C*-1), 71.2 (*C*-17), 61.2 (*C*-7), 60.8 (*C*-25), 59.4 (*C*-13), 53.7 (*C*-28), 52.7 (*C*-40), 46.3 (*C*-22), 44.4 (*C*-20), 39.3 (*C*-41), 36.9 (*C*-19), 31.2 (*C*-24), 29.8 (*C*-8), 28.5 (*C*-4, 3, 2), 28.1 (*C*-16), 26.2 (*C*-29), 21.7 (*C*-23), 20.6 (*C*-14), 19.9 (*C*-10), 18.3 (*C*-9), 16.7 (*C*-15).

HRMS (MALDI-TOF): *m/z* calcd. for $C_{42}H_{59}N_5O_9Na$ $[M+Na]^+$: 800.4210; found: 800.4656.

6.3.25 ((2*R*,4*R*,5*S*)-5-((*S*)-2-Ammonio-3-methylbutanamido)-4-hydroxy-2-(4-hydroxybenzyl)-6-methylheptanoyl)-L-prolyl-L-tryptophanate (25)



25

Saponification: In a 25 mL round bottom flask, equipped with magnetic stirring bar, 37.8 mg (0.049 mmol, 1 eq.) **24** were dissolved in 0.4 mL THF. Afterwards, a solution of 16 mg (0.388 mmol, 8 eq.) LiOHxH_2O in 1.2 mL H_2O was added and the reaction mixture was stirred at RT. After 45 min TLC indicated full conversion, Et_2O (2 mL) was added, followed by the careful addition of glacial acetic acid until a pH of ~4 was achieved. The mixture was transferred into a separation funnel. The phases were separated and the aqueous layer was extracted with Et_2O (2x5 mL). The combined organic extracts were dried over Na_2SO_4 and the solvents removed under reduced pressure. The residue was dried *in vacuo* for 45 min to yield the saponified intermediate as a colorless solid.

Boc-Deprotection: In a 25 mL round bottom flask, equipped with a magnetic stirring bar, the crude intermediate was dissolved in 1 mL 2,2,2-trifluoroethanol, followed by the addition of 14 µL (0.194 mmol, 4 eq.) ethanethiol and 88 mg (0.388 mmol, 8 eq.) zinc dibromide. The reaction mixture turned pink immediately and was stirred vigorously at RT overnight. Afterwards, the reaction mixture was concentrated and the residue dissolved in a mixture of H_2O (700 µL), acetonitrile (290 µL) and DMSO (10 µL) and purified via preparative HPLC (*CLF_NucleodurC18_pc001_HCOOH_5to95*) to furnish compound **25** as a colorless solid.

$C_{36}H_{49}N_5O_7$ [663.82 g/mol].

Yield: 8.6 mg (0.013 mmol, 27%, 2 steps from **24**), colorless solid.

$[\alpha]_D^{24}$ = -18.5 (c = 0.59 , MeOH).

m.p. = 172-174 °C.

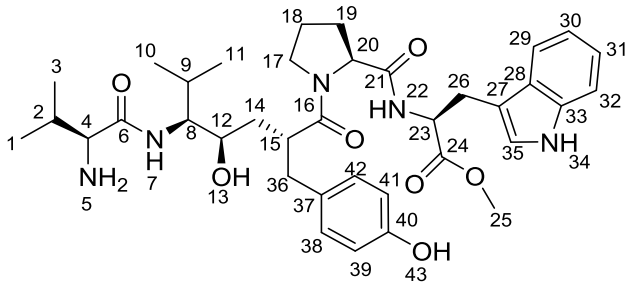
^{1}H NMR (300 MHz, MeOD, complex mixture of 2 rotamers in 4:1 ratio): δ = 8.41 (br s, 1H, *H*-33), 7.58 (d, ^3J = 7.6 Hz, 1H, *H*-28), 7.31 (d, ^3J = 7.9 Hz, 1H, *H*-31), 7.10-6.99 (m, 3H, *H*-34, 30, 29), 6.89 (d, ^3J = 8.3 Hz, 2H, *H*-38, 37), 6.64 (d, ^3J = 8.3 Hz, 2H, *H*-40, 39), 4.63-4.54 (m, 1H, *H*-23), 3.81-3.63 (m, 3H, *H*-12, 8, 4), 3.54 (d, ^3J = 7.2 Hz, 1H, *H*-20), 3.46-3.36 (m, 1H, *H*-25), 3.23-3.04 (m, 2H, *H*-

25, 17), 2.96-2.88 (m, 1H, *H*-17), 2.73-2.49 (m, 3H, *H*-35, 15), 2.31-2.09 (m, 2H, *H*-9, 2), 1.99-1.91 (m, 1H, *H*-14), 1.66-1.51 (m, 2H, *H*-19, 14), 1.19-0.60 (m, 15H, *H*-19, 18, 11, 10, 3, 1).

^{13}C NMR (75 MHz, MeOD): δ = 178.1 (*C*-24, 16), 173.4 (*C*-21), 170.2 (*C*-6), 157.3 (*C*-36), 138.0 (*C*-32), 131.0 (*C*-38, 37), 130.8 (*C*-41), 129.0 (*C*-27), 124.3 (*C*-34), 122.4 (*C*-30), 119.8 (*C*-29), 119.4 (*C*-28), 116.3 (*C*-40, 39), 112.2 (*C*-31), 112.0 (*C*-26), 71.2 (*C*-12), 62.2 (*C*-20), 61.3 (*C*-8), 60.2 (*C*-4), 56.5 (*C*-23), 47.4 (*C*-17), 45.7 (*C*-15), 39.9 (*C*-35), 37.6 (*C*-14), 32.0 (*C*-2), 31.6 (*C*-19), 29.3 (*C*-9), 28.3 (*C*-25), 22.0 (*C*-18), 21.0 (*C*-11), 19.6 (*C*-3), 18.0 (*C*-10), 17.4 (*C*-1).

HRMS (MALDI-TOF): *m/z* calcd. for $C_{36}H_{49}N_5O_7Na$ $[M+Na]^+$: 686.3530; found: 686.3739.

6.3.26 Methyl ((2*R*,4*R*,5*S*)-5-((*S*)-2-amino-3-methylbutanamido)-4-hydroxy-2-(4-hydroxybenzyl)-6-methylheptanoyl)-L-prolyl-L-tryptophanate (26)



26

In a 5 mL glass vial, equipped with magnetic stirring bar, 15 mg (0.019 mmol, 1 eq.) **24** were dissolved in 2,2,2-trifluoroethanol, followed by the addition of 5.6 µL (0.154 mmol, 4 eq.) ethanethiol. Afterwards, 35 mg (0.154 mmol, 8 eq.) zinc dibromide were added in one portion and the turbid reaction mixture was stirred vigorously at RT for 24 h. The reaction mixture was concentrated and the residue dissolved in H_2O (300 µL) and acetonitrile (200 µL). The crude product was purified via preparative HPLC (*CLF_NucleodurC18_pc001_HCOOH_5to95*) to yield compound **26** as a colorless solid.

$C_{37}H_{51}N_5O_7$ [677.84 g/mol].

Yield: 4.5 mg (0.006 mmol, 31%), colorless solid.

$[\alpha]_D^{24}$ = -17.8 (c = 0.06 , MeOH).

m.p. = 148-150 °C.

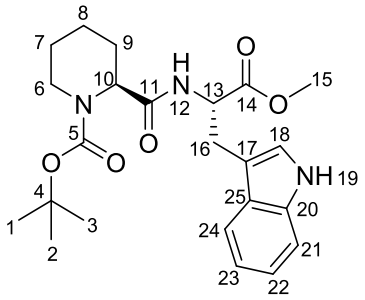
^{1}H NMR (500 MHz, MeOD, complex mixture of 2 rotamers in 2:1 ratio): δ = 8.50 (br s, 1H, *H*-34), 7.52 (d, ^3J = 7.8 Hz, 1H, *H*-29), 7.33 (d, ^3J = 8.0 Hz, 1H, *H*-32), 7.10-6.99 (m, 3H, *H*-35, 31, 30), 6.93 (d, ^3J = 8.4 Hz, 2H, *H*-42, 38), 6.66 (d, ^3J = 8.4 Hz, 2H, *H*-41, 39), 4.70-4.63 (m, 1H, *H*-23), 3.87-3.55 (m, 7H, *H*-25, 20, 12, 8 ,4), 3.40-3.33 (m, 1H, *H*-26), 3.20-3.15 (m, 1H, *H*-26), 3.03-2.92 (m, 2H, *H*-

17), 2.64-2.48 (m, 3H, *H*-36, 15), 2.27-2.10 (m, 2H, *H*-9, 2), 1.96-1.90 (m, 1H, *H*-14), 1.63-1.55 (m, 2H, *H*-19, 14), 1.34-0.88 (m, 15H, *H*-19, 18, 11, 10, 3, 1).

^{13}C NMR (125 MHz, MeOD): δ = 177.8 (*C*-16), 174.5 (*C*-24), 173.9 (*C*-21), 170.2 (*C*-6), 157.4 (*C*-37), 138.2 (*C*-33), 131.2 (*C*-40), 131.0 (*C*-42, 38), 128.4 (*C*-28), 124.6 (*C*-35), 122.6 (*C*-31), 120.1 (*C*-30), 119.1 (*C*-29), 116.4 (*C*-41, 39), 112.5 (*C*-32), 112.4 (*C*-27), 71.6 (*C*-12), 61.8 (*C*-20), 61.36 (*C*-8), 60.0 (*C*-4), 55.0 (*C*-23), 52.9 (*C*-25), 47.5 (*C*-17), 45.9 (*C*-15), 40.2 (*C*-36), 38.2 (*C*-14), 32.2 (*C*-2), 32.0 (*C*-19), 29.9 (*C*-9), 29.3 (*C*-26), 22.6 (*C*-11), 22.1 (*C*-18), 19.9 (*C*-3), 17.3 (*C*-10), 17.1 (*C*-1).

HRMS (MALDI-TOF): *m/z* calcd. for $C_{37}H_{51}N_5O_7Na$ $[M+Na]^+$: 700.3486; found: 700.3273.

6.3.27 *tert*-Butyl (*S*)-2-(((*S*)-3-(1*H*-indol-3-yl)-1-methoxy-1-oxopropan-2-yl)carbamoyl)piperidine-1-carboxylate (27)



27

A 250 mL three-necked round bottom flask, equipped with gas valve, dropping funnel and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with 5.00 g (22.0 mmol, 1 eq.) Boc-Pip-OH and 40 mL abs. dichloromethane. The reaction mixture was cooled to 0 °C in an ice bath and 4.05 mL (26.0 mmol, 1.2 eq.) *N,N'*-diisopropylcarbodiimide were added in one portion. Subsequently, 5.55 g (22.0 mmol, 1 eq.) H-Trp-OMe, dissolved in 6.05 mL (44.0 mmol, 2 eq.) triethylamine and 50 mL abs. dichloromethane, were added dropwise via a dropping funnel within 5 min. The reaction mixture was stirred for additional 15 min at 0 °C. The yellowish suspension was allowed to warm up to RT and stirred overnight at this temperature. After 22 h the reaction mixture was concentrated to a small volume and the residue was dissolved in 400 mL EtOAc. The organic layer was washed with 1 M HCl (200 mL), 0.1 M NaOH (200 mL), brine (200 mL), dried over Na_2SO_4 and concentrated. The residue was purified via flash chromatography (600 g SiO_2, cyclohexane/EtOAc = 2:1 (v/v)).

$C_{23}H_{31}N_3O_5$ [429.25 g/mol].

Yield: 4.71 g (11.0 mmol, 50%), colorless solid.

R_f = 0.28 (cyclohexane/EtOAc = 2:1(v/v); staining: CAM).

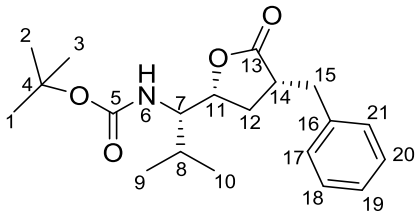
$[\alpha]_D^{24}$ = -29.5 (c = 1.20, $CHCl_3$).

m.p. = 66-68 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.24 (br s, 1H, *H*-19), 7.53 (d, ^{3}J = 7.6 Hz, 1H, *H*-24), 7.33 (d, ^{3}J = 8.0 Hz, 1H, *H*-21), 7.23-7.06 (m, 2H, *H*-23, 22), 7.04-6.92 (m, 1H, *H*-18), 6.63-6.37 (m, 1H, *H*-12), 5.00-4.57 (m, 2H, *H*-13, 10), 4.00-3.51 (m, 4H, *H*-15, 6), 3.40-3.21 (m, 2H, *H*-16), 2.60-2.10 (m, 2H, *H*-9, 6), 1.61-1.24 (m, 14H, *H*-9, 8, 7, 3, 2, 1).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.4 (*C*-14), 171.0 (*C*-11), 154.8 (*C*-5), 136.3 (*C*-20), 127.5 (*C*-25), 122.9 (*C*-18), 122.4 (*C*-22), 119.8 (*C*-23), 118.7 (*C*-24), 111.3 (*C*-21), 110.0 (*C*-17), 80.7 (*C*-4), 55.5 (*C*-10), 53.0 (*C*-13), 52.5 (*C*-15), 41.0 (*C*-6), 28.3 (*C*-3, 2, 1), 27.9 (*C*-16), 25.6 (*C*-7), 24.9 (*C*-9), 20.5 (*C*-8).

6.3.28 *tert-Butyl* ((*S*)-1-((2*R*, 4*R*)-4-benzyl-5-oxotetrahydrofuran-2-yl)-2-methylpropyl)carbamate (28)



28

Aldol Condensation: A 250 mL three-necked round bottom flask, equipped with gas valve and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with 2.00 g (7.77 mmol, 1 eq.) lactone **5** and 70 mL abs. THF. The reaction mixture was cooled to -78 °C in a dry ice/acetone cooling bath followed by the dropwise addition of 11.7 mL (23.3 mmol, 3 eq.) LDA solution (2.0 M in THF). The orange reaction mixture was stirred at -78 °C for additional 1 h. Subsequently, 1.18 mL (11.7 mmol, 1.5 eq.) benzaldehyde were added slowly within 3 min. The reaction mixture turned yellow after a few minutes and was stirred for additional 60 min at -78 °C until TLC indicated full conversion. The reaction was quenched by the addition of 30 mL satd. NH_4Cl. The reaction mixture was allowed to warm up to RT, followed by the addition of 70 mL H_2O and 70 mL EtOAc. The phases were separated and the aqueous layer was extracted with EtOAc (2x50 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified via flash chromatography (450 g SiO_2, cyclohexane/EtOAc 2:1 (v/v)) furnishing two diastereomers in 2.35 g yield (6.30 mmol). The product was used in the next step without further treatment.

Mesylation: A 100 mL round bottom flask, equipped with a Schlenk adapter and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with the previously obtained product, 60 mL abs. dichloromethane and 4.37 mL (31.5 mmol, 5 eq.) triethylamine. The reaction mixture was cooled to 0 °C in an ice bath followed by the dropwise addition of 1.47 mL (18.9 mmol, 3 eq.) methanesulfonyl chloride. The reaction mixture was stirred at 0 °C for additional 1 h and at 35 °C

overnight. Since the conversion was not complete, additional 1.75 mL (12.6 mmol, 2 eq.) triethylamine and 975 µL (12.6 mmol, 2 eq.) methanesulfonyl chloride were added. After 22 h TLC indicated full conversion. The reaction was quenched by the addition of 100 mL H_2O. The reaction mixture was transferred into a separation funnel and additional 50 mL dichloromethane were added. The phases were separated and the aqueous layer was extracted with dichloromethane (3x50 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4 and the solvent was removed under reduced pressure to furnish a brownish solid residue, which was used in the next step without further purification.

Elimination: A 100 mL round bottom flask, equipped with a Schlenk adapter and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with the crude residue of the previous step and 70 mL abs. ethanol were added, followed by the addition of 1.75 mL (6.12 mmol, 2 eq.) triethylamine. The reaction mixture was heated to 50 °C and stirred at this temperature. After 24 h additional 1.75 mL (12.6 mmol, 2 eq.) triethylamine were added. After additional 22 h the reaction mixture was cooled to RT, concentrated and the residue purified via flash chromatography (600 g SiO_2, cyclohexane/EtOAc 4:1 to 3:1 (v/v)) to yield the corresponding olefin as a colorless solid.

Hydrogenation: In a 100 mL three-necked round bottom flask, equipped with gas valve and magnetic stirring bar, the olefin was dissolved in 37 mL THF and 96 mg (1.54 mmol, 0.4 eq) Raney®-Nickel (slurry in H_2O) were added. Nitrogen atmosphere was exchanged by hydrogen (3×evacuation/purging) and the reaction mixture was stirred at RT for 72 h. After NMR indicated full conversion, the catalyst was removed under an argon atmosphere by filtration through a Schlenk-frit containing a plug of Celite® and the filter cake was washed with THF (3×50 mL). The filtrate was concentrated to furnish the pure diastereomer **28** as a colorless solid.

$C_{20}H_{29}NO_4$ [347.46 g/mol].

Yield: 1.36 g (3.91 mmol, 50%, 4 steps from **5**), colorless solid.

R_f (alcohols) = 0.55, 0.40 (cyclohexane/EtOAc = 1:1(v/v); staining: $KMnO_4$).

R_f (oelfin) = 0.38 (cyclohexane/EtOAc = 3:1(v/v); staining: $KMnO_4$).

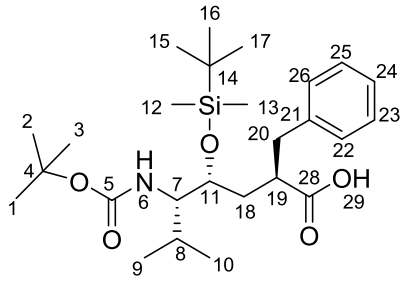
$[\alpha]_D^{24}$ = -62.9 (c = 1.10 , $CHCl_3$).

m.p. = 60-62 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.36-7.10 (m, 5H, *H*-21, 20, 19, 18, 17), 4.40-4.14 (m, 2H, *H*-11, 6), 3.77-3.45 (m, 1H, *H*-7), 3.31 (dd, ^3J = 14.0, 2.7 Hz, 1H, *H*-15), 2.96-2.57 (m, 2H, *H*-15, 14), 2.33-2.17 (m, 1H, *H*-12), 2.12-2.02 (m, 1H, *H*-12), 1.93-1.76 (m, 1H, *H*-8), 1.43 (s, 9H, *H*-3, 2, 1), 0.94 (d, ^3J = 6.8 Hz, 3H, *H*-9), 0.86 (d, ^3J = 6.8 Hz, 3H, *H*-10).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.6 (*C*-13), 156.1 (*C*-5), 136.8 (*C*-16), 129.0 (*C*-21, 17), 128.8 (*C*-20, 18), 126.8 (*C*-19), 79.9 (*C*-11), 78.1 (*C*-4), 58.3 (*C*-7), 42.7 (*C*-14), 36.5 (*C*-15), 32.2 (*C*-12), 28.6-28.3 (m, *C*-8, 3, 2, 1), 19.9 (*C*-9), 16.0 (*C*-10).

6.3.29 (2*R*,4*R*,5*S*)-2-Benzyl-5-((*tert*-butoxycarbonyl)amino)-4-((*tert*-butyldimethylsilyl)oxy)-6-methylheptanoic acid (29)



29

Lactone opening: A 100 mL round bottom flask with magnetic stirring bar was charged with 1.28 g (3.68 mmol, 1 eq.) alkylated lactone **28** and 18 mL THF. A solution of 618 mg (14.7 mmol, 4 eq.) $LiOHxH_2O$ in 11 mL H_2O was added and the turbid reaction mixture was stirred vigorously at RT. After 145 min TLC indicated full conversion and 25 mL Et_2O were added. The reaction mixture was cooled to 0 °C in an ice bath, followed by the careful addition of citric acid (25% in H_2O) until a pH of 4 was adjusted. Subsequently, the reaction mixture was transferred into a separation funnel and the phases were separated. The aqueous layer was extracted with Et_2O (2x20 mL). The combined organic layers were washed with H_2O (50 mL), brine (50 mL), dried over Na_2SO_4 and the solvent was removed under reduced pressure (T < 30 °C). The residue was dried *in vacuo* to furnish the intermediate product as a colorless solid. The crude product was used in the next step without further purification.

Silylation: A 100 mL three-necked round bottom flask, equipped with gas valve and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with the crude product of the previous step and 24 mL abs. dichloromethane were added. After the addition of 1.76 mL (22.1 mmol, 6 eq.) *N*-methylimidazole the reaction mixture was cooled to 0 °C with an ice bath. After 10 min, 5.61 g (22.1 mmol, 6 eq.) iodine were added and the deep red reaction mixture was stirred for additional 10 min. 1.67 g (11.1 mmol, 3 eq.) TBSCl were added portionwise within 5 min to the reaction mixture. After the addition the ice bath was removed and the deep reaction solution stirred at RT overnight. Afterwards, 50 mL Et_2O were added and the mixture was transferred into a separation funnel. 60 mL 1 M $Na_2S_2O_3$ were added and the organic layer was washed until total decoloration occurred. The phases were separated and the aqueous layer was extracted with Et_2O (2x50 mL). The combined organic layers were washed with citric acid [(25% in H_2O), 50 mL], brine (50 mL), dried over Na_2SO_4 and the solvents removed under reduced pressure. The yellow oily residue was used in the next step without further purification.

Ester hydrolysis: In a 50 mL round bottom flask, equipped with magnetic stirring bar the residue of the previous step was dissolved in 11 mL MeOH and 283 µL (0.36 mmol, 0.1 eq.) citric acid solution (25% in H_2O) were added. The yellow reaction mixture was stirred at RT overnight. After 22 h the reaction mixture was concentrated and purified via flash chromatography (600 g SiO_2, cyclohexane/EtOAc/AcOH = 3:1:0.01(v/v/v)) to furnish the protected acid **29** as a colorless oil.

$C_{26}H_{45}NO_5Si$ [479.73 g/mol].

Yield: 1.18 g (2.77 mmol, 67%, 3 steps from **28**), colorless oil.

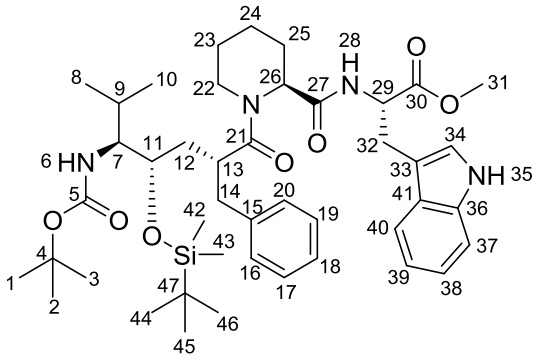
R_f = 0.46 (cyclohexane/EtOAc/AcOH = 3:1:0.01(v/v/v), staining: $KMnO_4$).

$[\alpha]_D^{24}$ = -14.3 (c = 0.93, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.33-7.10 (m, 5H, *H*-26, 25, 24, 23, 22), 4.60 (d, ^3J = 10.0 Hz, 1H, *H*-6), 3.85-3.58 (m, 1H, *H*-11), 3.52-3.21 (m, 1H, *H*-7), 3.07-2.89 (m, 1H, *H*-20), 2.83-2.60 (m, 2H, *H*-20, 19), 1.96-1.48 (m, 3H, *H*-18, 8), 1.42 (s, 9H, *H*-3, 2, 1), 0.95-0.75 (m, 15H, *H*-17, 16, 15, 10, 9), 0.11-0.03 (m, 6H, *H*-13, 12).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 178.4 (*C*-28), 156.3 (*C*-5), 138.8 (*C*-21), 129.1 (*C*-26, 22), 128.6 (*C*-25, 23), 126.7 (*C*-24), 79.4 (*C*-4), 72.1 (*C*-11), 58.1 (*C*-7), 43.5 (*C*-19), 38.6 (*C*-20), 35.3 (*C*-18), 28.6 (*C*-3, 2, 1), 28.4 (*C*-8), 26.0 (*C*-17, 16, 15), 20.8 (*C*-9), 18.1 (*C*-10),-3.4 (*C*-13), -4.6 (*C*-12).

6.3.30 Methyl ((*S*)-1-((2*R*,4*R*,5*S*)-2-benzyl-5-((*tert*-butoxycarbonyl)amino)-4-((*tert*-butyldimethylsilyl)oxy)-6-methylheptanoyl)piperidine-2-carbonyl)-L-tryptophanate (30)



30

Boc-Deprotection of Boc-Pip-Trp-OMe: In a 250 mL round bottom flask, equipped with magnetic stirring bar, 841 mg (1.96 mmol, 1.2 eq.) peptide **27** were dissolved in 1.20 mL (16.3 mmol, 10 eq.) ethanethiol. Subsequently, 3.77 mL (49.0 mmol, 30 eq.) trifluoroacetic acid were added and the yellowish reaction mixture was stirred vigorously at RT. After 30 min the volatiles were removed under reduced pressure with a cooling trap. The residue was dissolved in 50 mL EtOAc and 25% aqueous ammonia (70 mL) were added and the reaction mixture was stirred for 20 min. The phases were separated and the aqueous layer was extracted with EtOAc (2x50 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was dried *in vacuo* to furnish the deprotected intermediate as a colorless solid.

Coupling: A 50 mL round bottom flask, equipped with a Schlenk adapter and magnetic stirring bar, was dried (evacuated, heated, N_2-purged) and charged with 782 mg (1.63 mmol, 1 eq.) acid **29** and dissolved in 8 mL abs. DMF, followed by the addition of 568 µL (3.26 mmol, 2 eq.) Hünig`s base. The flask was cooled to 0 °C in an ice bath and 744 mg (1.96 mmol, 1.2 eq.) HATU were added to the reaction mixture. The previously deprotected peptide **27** was dissolved in 4 mL abs. DMF and 852 µL (4.90 mmol, 3 eq.) Hünig`s base were added. After 5 min, the solution of the deprotected intermediate was added to the solution of acid **29** with a syringe. After the addition was complete, the ice bath was removed and the yellow reaction mixture was stirred at RT overnight. After 18 h the reaction mixture was quenched by the addition of brine (25 mL). Additionally, 20 mL EtOAc were added and the mixture was stirred vigorously for 5 min. Afterwards, the mixture was transferred into a separation funnel and the phases were separated. The aqueous layer was extracted with EtOAc (2x30 mL). The combined organic layers were washed with brine (30 mL), dried over Na_2SO_4 and the solvents were removed under reduced pressure. The residue was purified via flash chromatography (400 g SiO_2, dichloromethane/MeOH = 30:1 (v/v)) to furnish an orange oily residue. The residue was dissolved in

50 mL EtOAc and the organic layer washed with H_2O (4x50 mL) in order to remove remaining DMF and tetramethylurea. The organic layer was dried over Na_2SO_4, the solvent removed under reduced pressure and the residue dried *in vacuo* to yield the desired peptide **30** as a colorless solid.

$C_{44}H_{66}N_4O_7Si$ [791.12 g/mol].

Yield: 920 mg (1.16 mmol, 70%, 2 steps from **29**), colorless solid.

R_f = 0.28 (dichloromethane/MeOH = 20:1(v/v); staining: CAM).

$[\alpha]_D^{24}$ = -30.9 (c = 0.91, $CHCl_3$).

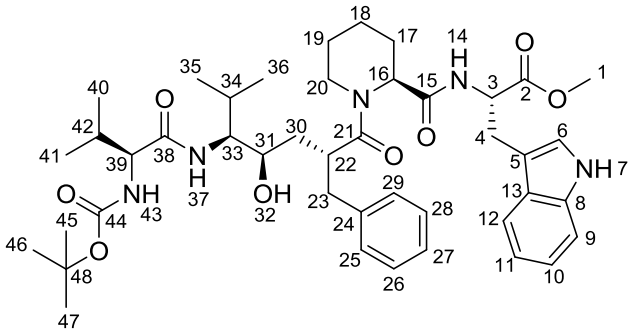
m.p. = 82-84 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.13 (br s, 1H, *H*-35), 7.51 (d, ^3J = 7.3 Hz, 1H, *H*-40), 7.33-6.84 (m, 9H, *H*-39, 38, 37, 34, 20, 19, 18, 17, 16), 6.20 (d, ^3J = 6.9 Hz, 1H, *H*-28), 5.25-5.10 (m, 1H, *H*-26), 4.82-4.65 (m, 1H, *H*-29), 4.54 (d, ^3J = 10.0 Hz, 1H, *H*-6), 3.80-3.57 (m, 4H, *H*-31, 11), 3.56-3.20 (m, 3H, *H*-32, 22, 7), 3.18-2.91 (m, 2H, *H*-32, 13), 2.87-2.54 (m, 2H, *H*-14), 2.44-2.25 (m, 1H, *H*-22), 2.21-1.98 (m, 1H, *H*-25), 1.93-1.26 (m, 17H, *H*-25, 24, 22, 12, 9, 3, 2, 1), 0.93-0.77 (m, 15H, *H*-46, 45, 44, 10, 8), 0.15-0.02 (m, 6H, *H*-43, 42).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 175.3 (*C*-21), 172.3 (*C*-30), 170.7 (*C*-27), 156.0 (*C*-5), 139.6 (*C*-15), 136.2 (*C*-36), 129.0 (*C*-20, 16), 128.7 (*C*-19, 17), 127.7 (*C*-41), 126.7 (*C*-18), 123.0 (*C*-34), 122.3 (*C*-38), 119.7 (*C*-39), 118.7 (*C*-40), 111.3 (*C*-37), 110.3 (*C*-33), 79.0 (*C*-4), 72.0 (*C*-11), 58.1 (*C*-7), 52.8 (*C*-29), 52.6 (*C*-26), 52.4 (*C*-31), 43.3 (*C*-22), 39.5 (*C*-13), 38.5 (*C*-14), 36.2 (*C*-12), 28.5 (*C*-3, 2, 1), 28.2 (*C*-9), 27.5 (*C*-32), 26.0 (*C*-46, 45, 44), 25.8 (*C*-25), 25.6 (*C*-23), 21.2 (*C*-8), 20.4 (*C*-24), 18.4 (*C*-10), 18.0 (*C*-47), -4.0 (*C*-43), -4.6 (*C*-42).

HRMS (MALDI-TOF): *m/z* calcd. for $C_{44}H_{66}N_4O_7SiNa$ $[M+Na]^+$: 813.4598; found: 813.5087.

6.3.31 Methyl ((*S*)-1-((2*R*,4*R*,5*S*)-2-benzyl-5-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methylbutanamido)-4-hydroxy-6-methylheptanoyl)piperidine-2-carbonyl)-L-tryptophanate (31)



31

*TBS-deprotection of **30**:* In a 50 mL polypropylene vial, equipped with a magnetic stirring bar, 400 mg (0.51 mmol, 1 eq.) **30** were dissolved in 5 mL THF. Afterwards, 1.30 mL (50.6 mmol, 100 eq.) HF-pyridine (~70% HF) were added in one portion. The reaction mixture turned yellow immediately and was stirred for 90 min, until TLC indicated full conversion. The reaction mixture was quenched by pouring it into 100 mL ice cold satd. $NaHCO_3$ solution. Subsequently, 50 mL EtOAc were added and the mixture transferred into a separation funnel. The phases were separated and the aqueous layer was extracted with EtOAc (2x50 mL). The combined organic extracts were washed with brine (100 mL), dried over Na_2SO_4 and the solvents removed under reduced pressure. The colorless residue was dried *in vacuo* for 15 min and used in the next step without further purification.

Boc-deprotection: In a 50 mL round bottom flask, equipped with magnetic stirring bar, the previously TBS-deprotected peptide was dissolved in 373 µL (5.05 mmol, 10 eq.) ethanethiol. Subsequently, 1.17 mL (15.1 mmol, 30 eq.) trifluoroacetic acid were added and the yellowish reaction mixture was stirred vigorously for 3 min at RT. The volatiles were removed under reduced pressure with a cooling trap. The residue was dissolved in 50 mL EtOAc and 25% aqueous ammonia (50 mL) were added and stirred for 5 min. The phases were separated and the aqueous layer was extracted with EtOAc (2x30 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4 and the solvent was removed under reduced pressure.

Coupling: A 50 mL round bottom flask, equipped with a magnetic stirring bar and Schlenk adapter, was dried (evacuated, heated, N_2-purged) and charged with 132 mg (0.61 mmol, 1.2 eq.) Boc-L-Val-OH and 1.3 mL abs. DMF, followed by the addition of 176 µL (0.10 mmol, 2 eq.) Hünig's base. The flask was cooled to 0 °C in an ice bath. Subsequently, 231 mg (0.61 mmol, 1.2 eq.) HATU were added to the reaction mixture and stirred for additional 5 min. In a second 50 mL round bottom flask, equipped with a magnetic stirring bar and Schlenk adapter, the previously deprotected peptide was dissolved in 2.6 mL abs. DMF and 264 µL (1.52 mmol, 3 eq.) Hünig's base were added. Afterwards,

the solution of the deprotected peptide was added to the solution of the activated acid using a syringe. After the addition was complete, the ice bath was removed and the deep yellow reaction mixture was stirred at RT overnight. The reaction was quenched by the addition of 20 mL brine, followed by the addition of EtOAc (25 mL). The reaction mixture was stirred for 5 min and subsequently transferred into a separation funnel. The phases were separated and the aqueous layer was extracted with EtOAc (2x20 mL). The combined organic extracts were washed with brine (50 mL), dried over Na_2SO_4 and the solvents removed under reduced pressure. The residue was purified via flash chromatography (450 g SiO_2, dichloromethane/MeOH = 30:1 (v/v)) to furnish an oily residue. The residue was dissolved in 50 mL EtOAc and the organic layer washed with H_2O (4x40 mL) The organic layer was dried over Na_2SO_4, the solvent removed under reduced pressure and the residue dried *in vacuo* to yield the desired product **31** as a colorless solid.

$C_{43}H_{61}N_5O_8$ [775.99 g/mol].

Yield: 122 mg (0.16 mmol, 31%, 3 steps from **30**), colorless solid.

R_f = 0.15 (dichloromethane/MeOH = 20:1(v/v); staining: CAM).

$[\alpha]_D^{24}$ = -36.8 (c = 1.36, $CHCl_3$).

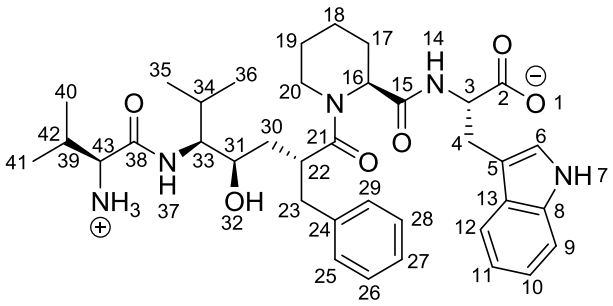
m.p. = 100-102 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.76 (br s, 1H, *H*-7), 7.60-7.42 (m, 2H, *H*-14, 12), 7.38-7.30 (m, 1H, *H*-9), 7.25-6.93 (m, 8H, *H*-29, 28, 27, 26, 25, 11, 10, 6), 6.08 (d, ^3J = 8.6 Hz, 1H, *H*-37), 5.16-4.99 (m, 1H, *H*-43), 4.85-4.71 (m, 1H, *H*-3), 4.25 (d, ^3J = 13.2 Hz, 1H, *H*-20), 4.09 (br s, 1H, *H*-16), 3.91-3.62 (m, 5H, *H*-39, 33, 1), 3.53-3.18 (m, 3H, *H*-31, 4), 2.84-2.57 (m, 3H, *H*-23, 22), 2.30-1.79 (m, 5H, *H*-42, 34, 30, 18, 17), 1.72-1.52 (m, 2H, *H*-30, 20), 1.47-1.40 (m, 9H, *H*-47, 46, 45), 1.29-0.78 (m, 15H, *H*-41, 40, 36, 35, 19, 18), -0.06-(-0.28) (m, 1H, *H*-17).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 175.3 (*C*-21), 173.2 (*C*-2), 172.8 (*C*-38), 170.6 (*C*-15), 156.2 (*C*-44), 138.8 (*C*-24), 136.4 (*C*-8), 129.4 (*C*-28, 26), 128.8 (*C*-29, 25), 127.4 (*C*-13), 126.8 (*C*-27), 123.4 (*C*-6), 122.2 (*C*-10), 119.7 (*C*-11), 118.6 (*C*-12), 111.5 (*C*-9), 110.5 (*C*-5), 80.2 (*C*-48), 72.1 (*C*-31), 61.1 (*C*-39), 59.3 (*C*-33), 57.3 (*C*-16), 53.4 (*C*-3), 52.5 (*C*-1), 41.3 (*C*-22), 40.7 (*C*-23), 39.6 (*C*-20), 37.6 (*C*-30), 29.9 (*C*-42), 28.5 (*C*-47, 46, 45), 28.0 (*C*-34), 26.6 (*C*-4), 25.0 (*C*-19), 24.5 (*C*-17), 20.6 (*C*-35), 20.4 (*C*-18), 19.9 (*C*-40), 18.2 (*C*-41), 17.1 (*C*-36).

HRMS (MALDI-TOF): *m/z* calcd. for $C_{43}H_{61}N_5O_8Na$ $[M+Na]^+$: 798.9629; found: 798.3790.

6.3.32 ((*S*)-1-((2*R*,4*R*,5*S*)-5-((*S*)-2-Ammonio-3-methylbutanamido)-2-benzyl-4-hydroxy-6-methylheptanoyl)piperidine-2-carbonyl)-L-tryptophanate (32)



32

Saponification: In a 50 mL round bottom flask, equipped with magnetic stirring bar, 94 mg (0.121 mmol, 1 eq.) **31** were dissolved in 1.2 mL THF. Afterwards, a solution of 41 mg (0.969 mmol, 8 eq.) LiOHxH_2O in 3.3 mL H_2O was added and the reaction mixture was stirred at RT. After 3 h TLC indicated full conversion. Then, 1 M HCl were carefully added, until a pH of ~4 was achieved. Additional 15 mL EtOAc were added and the mixture was transferred into a separation funnel. The phases were separated and the aqueous layer was extracted with EtOAc (3x10 mL). The combined organic extracts were dried over Na_2SO_4 and the solvents removed under reduced pressure. The residue was dried *in vacuo* for 2 h to yield the saponified intermediate as a colorless solid.

Boc-Deprotection: In a 10 mL round bottom flask, equipped with magnetic stirring bar, the crude intermediate was dissolved in 90 µL (1.21 mmol, 10 eq.) ethanethiol. Subsequently, 280 µL (3.63 mmol, 30 eq.) trifluoroacetic acid were added and the reaction mixture was stirred vigorously for 5 min. After this period the volatiles were removed with a preceding cooling trap and the residue was dried *in vacuo* for 1 h. The residue was dissolved in in mixture of acetonitrile (650 µL) and H_2O (350 µL) and purified via preparative HPLC (*CLF_NucleodurC18_pc001_HCOOH_5to95*) to yield compound **31** as a colorless solid.

$C_{37}H_{51}N_5O_6$ [661.84 g/mol].

Yield: 25.2 mg (0.038 mmol, 31%, 2 steps from **31**), colorless solid.

$[\alpha]_D^{24}$ = -41.2 (c = 0.84, MeOH).

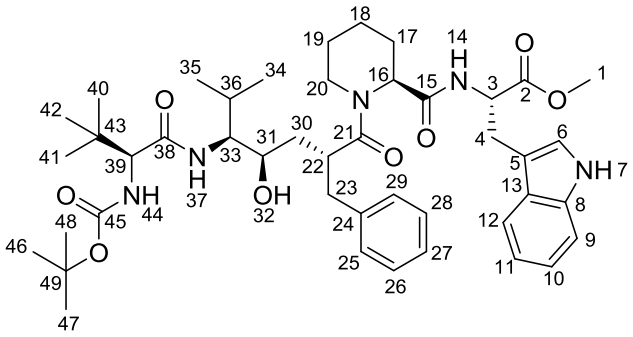
m.p. = 160-162 °C.

^{1}H NMR (300 MHz, MeOD): δ = 8.38 (br s, 1H, H-7), 7.57 (d, 1H, ^3J = 7.7 Hz, *H*-12), 7.33-6.93 (m, 9H, *H*-29, 28, 27, 26, 25, 11, 10, 9, 6), 4.80-4.66 (m, 1H, *H*-3), 4.37-4.25 (m, 1H, *H*-16), 4.03-3.88 (m, 1H, *H*-20), 3.87-3.65 (m, 3H, *H*-43, 33, 31), 3.50-3.06 (m, 3H, *H*-22, 4), 2.74-2.56 (m, 2H, *H*-23), 2.32-1.94 (m, 3H, *H*-42, 34, 30), 1.79-1.57 (m, 2H, *H*-30, 17), 1.48-0.57 (m, 17H, *H*-41, 40, 36, 35, 20, 19, 18), -0.06-(0.27) (m, 1H, *H*-17).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 178.1 (*C*-21), 177.5 (*C*-2), 171.2 (*C*-15), 170.4 (*C*-38), 140.6 (*C*-24), 138.0 (*C*-8), 130.4 (*C*-28, 26), 129.7 (*C*-29, 25), 128.9 (*C*-13), 127.6 (*C*-27), 124.4 (*C*-6), 122.3 (*C*-10), 119.7 (*C*-11), 119.6 (*C*-12), 112.1 (*C*-9), 112.0 (*C*-5), 71.7 (*C*-31), 61.4 (*C*-43), 60.1 (*C*-33), 58.6 (*C*-16), 56.0 (*C*-3), 42.4 (*C*-22), 40.9 (*C*-23), 40.5 (*C*-20), 38.0 (*C*-30), 31.6 (*C*-39), 29.4 (*C*-34), 28.6 (*C*-4), 26.4 (*C*-19), 25.4 (*C*-17), 21.3 (*C*-18), 20.9 (*C*-35), 19.5 (*C*-41), 18.4 (*C*-40), 17.3 (*C*-36).

HRMS (MALDI-TOF): *m/z* calcd. for $C_{37}H_{51}N_5O_6Na$ $[M+Na]^+$: 684.3737; found: 684.3716.

6.3.33 Methyl ((*S*)-1-((2*R*,4*R*,5*S*)-2-benzyl-5-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3,3-dimethylbutanamido)-4-hydroxy-6-methylheptanoyl)piperidine-2-carbonyl)-L-tryptophanate (33)



33

*TBS-deprotection of **30**:* In a 50 mL polypropylene vial, equipped with a magnetic stirring bar, 467 mg (0.59 mmol, 1 eq.) **30** were dissolved in 5 mL THF. Afterwards, 1.30 mL (59.0 mmol, 100 eq.) HF-pyridine (~70% HF) were added in one portion. The reaction mixture turned yellow immediately and was stirred for 100 min, until TLC indicated full conversion. The reaction mixture was quenched by pouring it into 100 mL of an ice cold satd. $NaHCO_3$ solution. Subsequently, 50 mL EtOAc were added and the mixture transferred into a separation funnel. The phases were separated and the aqueous layer was extracted with EtOAc (2x50 mL). The combined organic extracts were washed with brine (100 mL), dried over Na_2SO_4 and the solvents removed under reduced pressure. The colorless residue was dried *in vacuo* for 30 min and used in the next step without further purification.

Boc-deprotection: In a 50 mL round bottom flask, equipped with magnetic stirring bar, the previously TBS-deprotected peptide was dissolved in 440 µL (5.90 mmol, 10 eq.) ethanethiol. Subsequently, 1.37 mL (17.7 mmol, 30 eq.) trifluoroacetic acid were added and the yellowish reaction mixture was stirred vigorously for 2 min at RT. The volatiles were removed under reduced pressure with a cooling trap. The residue was dissolved in 50 mL EtOAc and 25% aqueous ammonia (50 mL) were added and the reaction mixture was stirred for 5 min. The phases were separated and the aqueous layer was extracted with EtOAc (2x50 mL). The combined organic layers were washed with brine (80 mL), dried over Na_2SO_4 and the solvent was removed under reduced pressure.

Coupling: A 50 mL round bottom flask, equipped with magnetic stirring bar and Schlenk adapter, was dried (evacuated, heated, N_2-purged) and charged with 164 mg (0.71 mmol, 1.2 eq.) Boc-L-*tert*-leucine and 1.4 mL abs. DMF, followed by the addition of 205 µL (1.18 mmol, 2 eq.) Hünig's base. The flask was cooled to 0 °C in an ice bath. Subsequently, 270 mg (0.71 mmol, 1.2 eq.) HATU were added to the reaction mixture and stirred for additional 5 min. In a second 50 mL round bottom flask, equipped with magnetic stirring bar and Schlenk adapter, the previously deprotected peptide was dissolved in 2.8 mL abs. DMF and 309 µL (1.77 mmol, 3 eq.) Hünig's base were added. Afterwards, the solution of the deprotected peptide was added to the solution of the activated acid using a syringe. After the addition was complete, the ice bath was removed and the yellow reaction mixture was stirred at RT overnight. The reaction was quenched by the addition of 20 mL brine, followed by the addition of EtOAc (25 mL). The reaction mixture was stirred for 5 min and subsequently transferred into a separation funnel. The phases were separated and the aqueous layer was extracted with EtOAc (2x30 mL). The combined organic extracts were washed with brine (50 mL), dried over Na_2SO_4 and the solvents removed under reduced pressure. The residue was purified via flash chromatography (500 g SiO_2, dichloromethane/MeOH = 30:1 (v/v)) to furnish an oily residue. The residue was dissolved in 50 mL EtOAc and the organic layer washed with H_2O (4x60 mL) The organic layer was dried over Na_2SO_4, the solvent removed under reduced pressure and the residue dried *in vacuo* to yield the desired product **33** as a colorless solid.

$C_{44}H_{63}N_5O_8$ [790.02 g/mol].

Yield: 195 mg (0.25 mmol, 42%, 3 steps from **30**), colorless solid.

R_f = 0.21 (dichloromethane/MeOH = 20:1(v/v); staining: CAM).

$[\alpha]_D^{24}$ = -39.3 (c = 0.99, $CHCl_3$).

m.p. = 106-108 °C.

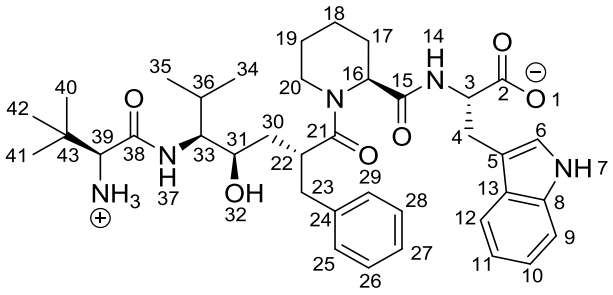
^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.73 (br s, 1H, *H*-7), 7.60-7.42 (m, 2H, *H*-14, 12), 7.38-7.30 (m, 1H, *H*-9), 7.25-6.93 (m, 8H, *H*-29, 28, 27, 26, 25, 11, 10, 6), 5.77 (d, ^3J = 9.1 Hz, 1H, *H*-37), 5.18 (d, ^3J = 9.4 Hz, 1H, *H*-44), 4.89-4.75 (m, 1H, *H*-3), 4.24 (d, ^3J = 12.9 Hz, 1H, *H*-20), 4.11 (br s, 1H, *H*-16), 3.88-3.61 (m, 5H, *H*-39, 33, 1), 3.54-3.16 (m, 3H, *H*-31, 4), 2.89-2.58 (m, 3H, *H*-23, 22), 2.27-1.78 (m, 4H, *H*-36, 30, 18, 17), 1.72-1.54 (m, 2H, *H*-30, 20), 1.50-1.35 (m, 9H, *H*-48, 46, 47), 1.33-0.77 (m, 18H, *H*-42, 41, 40, 35, 34, 19, 18), -0.05-(-0.22) (m, 1H, *H*-17).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 175.2 (*C*-21), 173.3 (*C*-2), 172.4 (*C*-38), 170.5 (*C*-15), 156.2 (*C*-45), 138.8 (*C*-24), 136.4 (*C*-8), 129.4 (*C*-28, 26), 128.8 (*C*-29, 25), 127.5 (1*C*-13), 126.8 (*C*-27), 123.2 (*C*-6), 122.3 (*C*-10), 119.8 (*C*-11), 118.6 (*C*-12), 111.5 (*C*-9), 110.4 (*C*-5), 80.2 (*C*-49), 72.0 (*C*-31), 63.0 (*C*-39), 59.4 (*C*-33), 57.3 (*C*-16), 53.2 (*C*-3), 52.5 (*C*-1), 41.3 (*C*-22), 40.5 (*C*-23), 39.6 (*C*-20), 37.4

(*C*-30), 33.9 (*C*-43), 28.5 (*C*-48, 47, 46), 28.2 (*C*-36), 26.8 (*C*-42, 41, 40), 26.7 (*C*-4), 25.0 (*C*-19), 24.5 (*C*-17), 20.7 (*C*-35), 20.4 (*C*-18), 17.1 (*C*-34).

HRMS (MALDI-TOF): *m/z* calcd. for $C_{44}H_{63}N_5O_8NaH$ $[M+Na]^+$: 812.4574; found: 813.3661.

6.3.34 ((*S*)-1-((2*R*,4*R*,5*S*)-5-((*S*)-2-Ammonio-3,3-dimethylbutanamido)-2-benzyl-4-hydroxy-6-methylheptanoyl)piperidine-2-carbonyl)-L-tryptophanate (34)



34

Saponification: In a 50 mL round bottom flask, equipped with a magnetic stirring bar, 146 mg (0.185 mmol, 1 eq.) **33** were dissolved in 1.8 mL THF. Afterwards, a solution of 62 mg (1.48 mmol, 8 eq.) $LiOHxH_2O$ in 5.4 mL H_2O was added and the reaction mixture was stirred at RT. After 3 h TLC indicated full conversion. Then, 1 M HCl were carefully added, until a pH of ~4 was achieved. Additional 15 mL EtOAc were added and the mixture was transferred into a separation funnel. The phases were separated and the aqueous layer was extracted with EtOAc (3x15 mL). The combined organic extracts were dried over Na_2SO_4 and the solvents removed under reduced pressure. The residue was dried *in vacuo* for 2 h to yield the saponified intermediate as a colorless solid.

Boc-Deprotection: In a 10 mL round bottom flask, equipped with a magnetic stirring bar, the crude intermediate was dissolved in 136 µL (1.85 mmol, 10 eq.) ethanethiol. Subsequently, 427 µL (5.55 mmol, 30 eq.) trifluoroacetic acid were added and the reaction mixture was stirred vigorously for 5 min. After this period the volatiles were removed with a preceding cooling trap and the residue was dried *in vacuo* for 1 h. The residue was dissolved in a mixture of acetonitrile (650 µL), H_2O (330 µL) and DMSO (20 µL) and purified via preparative HPLC (*CLF_NucleodurC18_pc001_HCOOH_5to95*) to yield compound **34** as a colorless solid.

$C_{38}H_{53}N_5O_6$ [675.87 g/mol].

Yield: 30.5 mg (0.045 mmol, 25%, 2 steps from **33**), colorless solid.

$[\alpha]_D^{24}$ = -32.3 (c = 1.02, MeOH).

m.p. = 164-166 °C.

^{1}H NMR (300 MHz, MeOD): δ = 8.40 (br s, 1H, H-7), 7.61-7.47 (m, 1H, *H*-12), 7.34-6.85 (m, 9H, *H*-29, 28, 27, 26, 25, 11, 10, 9, 6), 4.78-4.64 (m, 1H, *H*-3), 4.36-4.22 (m, 1H, *H*-16), 4.04-3.55 (m, 4H,

H-43, 33, 31, 20), 3.50-3.06 (m, 3H, *H*-22, 4), 2.80-2.66 (m, 2H, *H*-23), 2.10-1.93 (m, 2H, *H*-36, 30), 1.80-1.56 (m, 2H, *H*-30, 17), 1.47-0.51 (m, 20H, *H*-42, 41, 40, 34, 35, 20, 19, 18), -0.04-(0.26) (m, 1H, *H*-17).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 178.4 (*C*-21), 177.5 (*C*-2), 171.0 (*C*-15), 169.4 (*C*-38), 140.7 (*C*-24), 138.0 (*C*-8), 130.4 (*C*-28, 26), 129.6 (*C*-29, 25), 129.0 (*C*-13), 127.5 (*C*-27), 124.4 (*C*-6), 122.3 (*C*-10), 119.7 (*C*-12, 11), 112.1 (*C*-9), 112.0 (*C*-5), 71.4 (*C*-31), 63.0 (*C*-39), 61.8 (*C*-33), 58.6 (*C*-16), 56.2 (*C*-3), 42.4 (*C*-22), 40.5 (*C*-23, 20), 38.9 (*C*-30), 34.4 (*C*-43), 29.7 (*C*-36), 28.7 (*C*-4), 27.0 (*C*-42, 41, 40), 26.4 (*C*-19), 25.4 (*C*-17), 21.3 (*C*-18), 20.8 (*C*-35), 19.2 (*C*-34).

HRMS (MALDI-TOF): *m/z* calcd. for $C_{38}H_{53}N_5O_6Na$ $[M+Na]^+$: 698.8471; found: 698.3853.

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8 Abbreviations

$[\alpha]_D^{24}$	specific rotation
°C	degree celsius
abs.	absolute
Ac	acetyl
AcOH	acetic acid
ADAM	membrane-anchored disintegrin metalloproteinases
APP	amyloid precursor protein
APT	attached proton test
Ar	aryl or argon
ARE	antioxidant response element
Arg	arginine
Asp	aspartic acid
Boc	*tert*-butoxycarbonyl
bs	broad singlet
c	concentration
calcd.	calculated
CAM	cerium ammonium molybdate
$CDCl_3$	deuterated chloroform
$CHCl_3$	chloroform
cm	centimeter
COSY	correlation spectroscopy
CSF	cerebrospinal fluid
d	day(s) or doublet
DCI	3,4-dichloroisocoumarin
DCM	dichloromethane
dd	doublet of doublet
DFP	diisopropylfluorophosphate
DiPEA	*N*,*N*-diisopropylethylamine (Hünig's base)
DMF	*N*, *N*-dimethyl formamide

DMSO	dimethyl sulfoxide
DPP III	dipeptidyl-peptidase III
e.g.	*exempli gratia*
EDTA	ethylenediaminetetraacetic acid
EGF	epidermal growth factor
eq	equivalents
ER	endoplasmic reticulum
ESI	electron spray ionisation
et al.	*et alii*
Et_2O	diethyl ether
Et3N	trimethylamine
EtOAc	ethyl acetate
EtOH	ethanol
EtSH	ethanethiol
eV	electron volt
g	gram
GC	gas chromatography
GC-MS	gas chromatography with mass spectroscopy
Glu	glutamic acid
Gly	glycine
h	hour(s)
HATU	1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate
HBTU	*O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate
hDPP III	human dipeptidyl-peptidase III
HER	(*R*)-hydroxyethylene pseudopeptide
His	histidine
HIV	human immunodeficiency virus
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectroscopy
HSQC	heteronuclear single quantum coherence
HWE	Horner-Wadsworth-Emmons
IC_{50}	half maximal inhibitory concentration

i-Pr	*iso*-propyl
J	coupling constant
Leu	leucine
lit.	literature
Lys	lysine
m	meter(s) or multiplet
m.p.	melting point
m/z	mass/charge
MALDI	matrix-assisted laser desorption/ionization
Me	methyl
MeCN	acetonitrile
MeOD	deuterated methanol
MeOH	methanol
mg	milligram
MHz	megahertz
MMP	matrix metalloproteases
mol/L	mole(s) per liter
MS	mass spectroscopy
MWD	multiple wavelength detector
NAD(P)	nicotinamide adenine dinucleotide (phosphate)
n-BuLi	*n*-butyllithium
nm	nanometer
NMR	nuclear magnetic resonance
NOESY	nuclear Overhauser effect spectroscopy
pCMB	*p*-chloromercuribenzoic acid
pCMS	*p*-chloromercuriphenylsulfate
pH	negative logarithm of the hydronium ion concentration
Ph	phenyl
Phe	phenylalanine
pHMB	*p*-hydroxy-mercuribenzoate
Pip	pipecolic acid
PMSF	phenylmethanesulfonylfluoride
Pro	proline

p-TsOH	*para*-toluenesulfonic acid (4-methylbenzenesulfonic acid)
R_f	retention factor
ROS	reactive oxygen species
RP	reversed phase
RT	room temperature
s	singlet
satd.	saturated
SHE	(*S*)-hydroxyethylene pseudopeptide
SiO_2	silica gel
TBS	*tert*-Butyldimethylsilyl
TBSCl	*tert*-Butyldimethylsilylchloride
t-Bu	*tert*-Butyl
tert	tertiary
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TOF	time-of-flight (mass spectrometry)
TOP	thimet oligopeptidase
TPCK	tosyl-phenylalanyl chloromethyl ketone
TPP II	tripeptidylpeptidase II
Trp	tryptophan
Tyr	tyrosine
UV	Ultraviolet
v/v	volume/volume ratio
v/v/v	volume/volume/volume
Val	valine
w/w	weight/weight ratio
yDPP III	yeast dipeptidyl-peptidase III
βNA	2-naphthylamide
δ	delta (chemical shift in ppm)
λ	lambda (wavelength)

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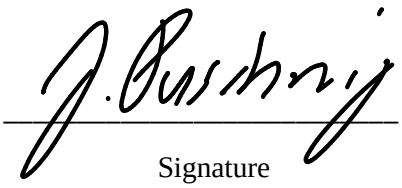
10 Affidavit

I declare that I have authored this thesis independently, that I have not used other than the declared sources/resources, and that I have explicitly indicated all material which has been quoted either literally or by content from the sources used. The text document uploaded to TUGRAZonline is identical to the present master‘s thesis.

08.05.2019

Date



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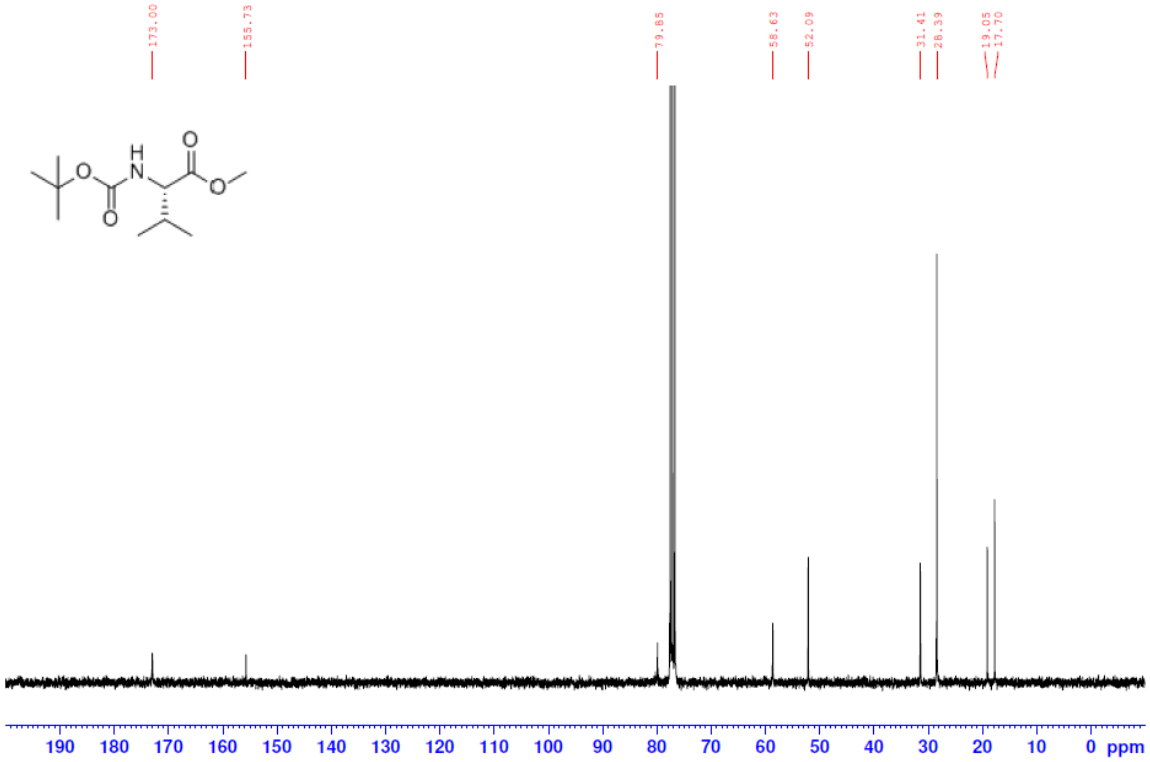
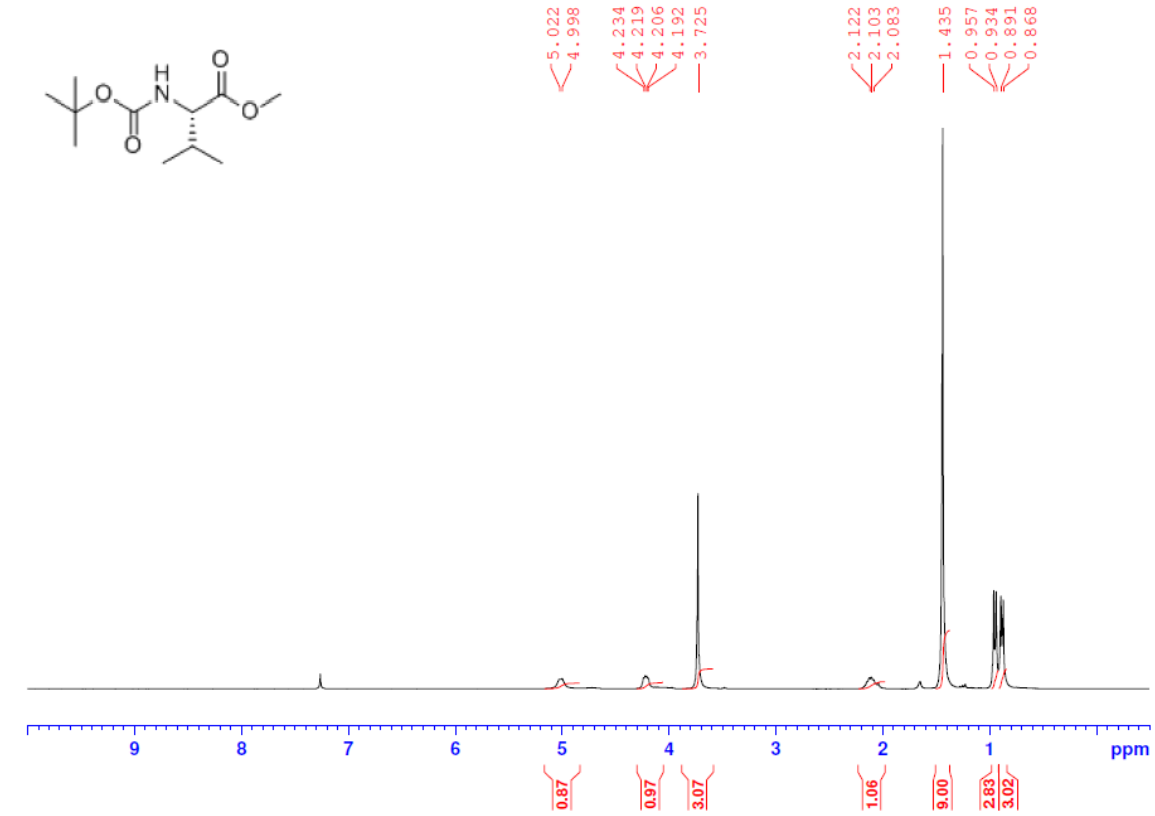
11 Appendix: NMR spectra

^{1}H and ^{13}C spectra of methyl *(tert*-butoxycarbonyl)-L-valinate (1)





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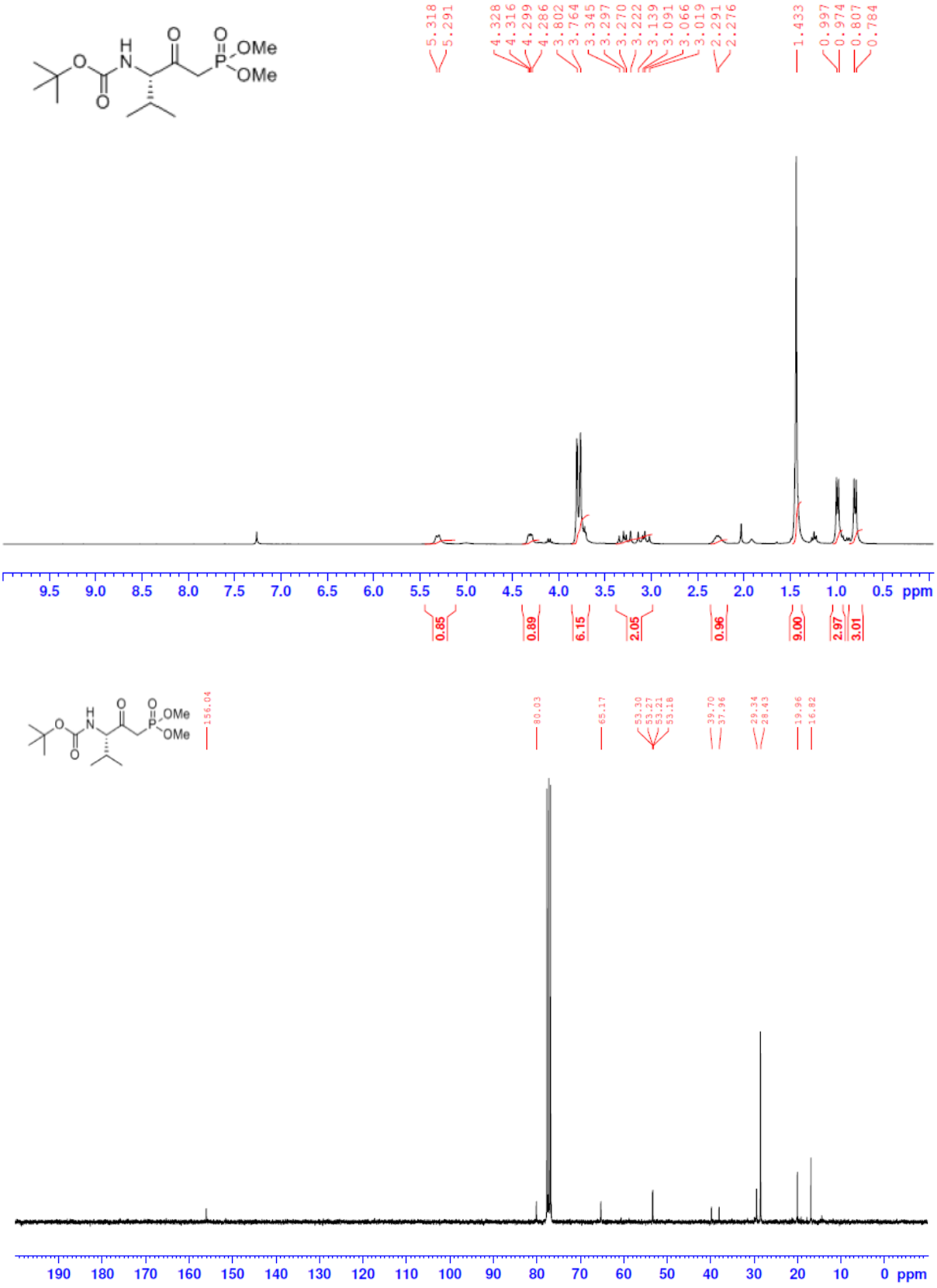

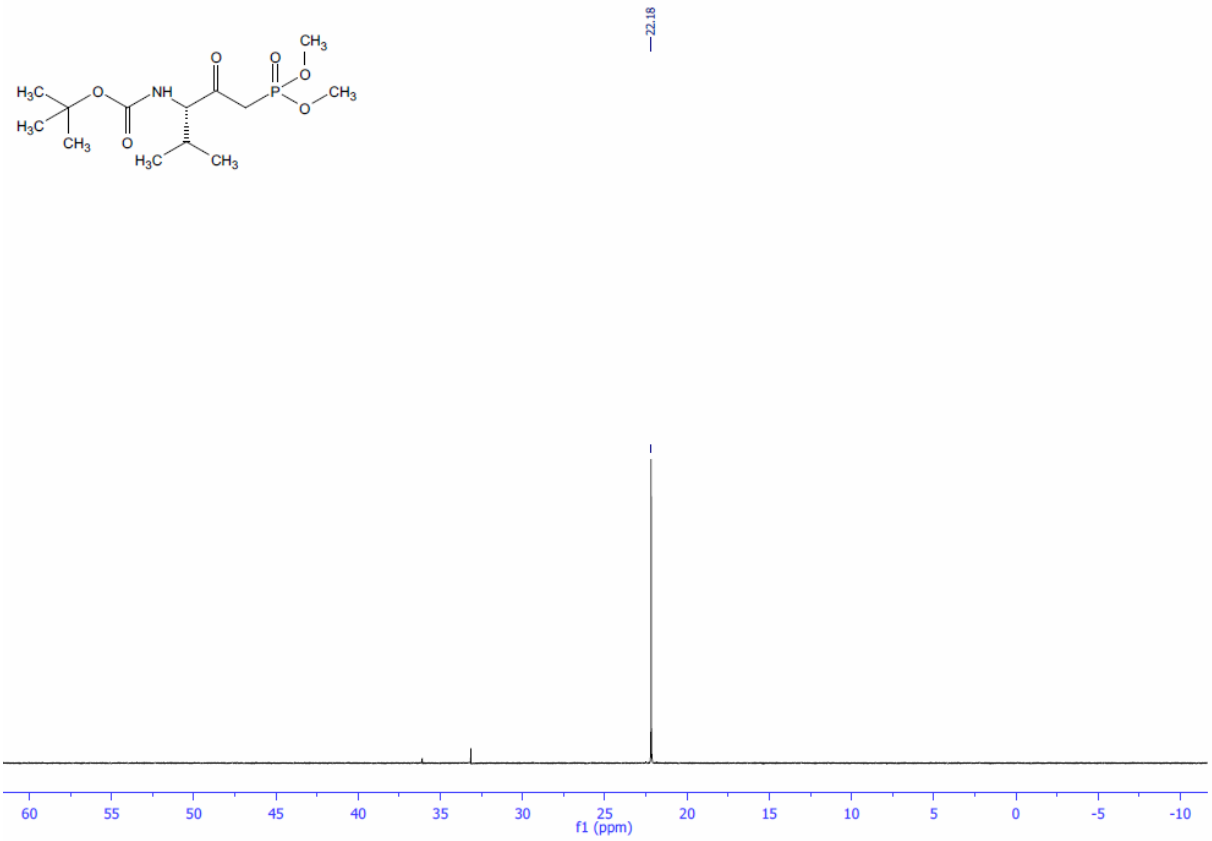
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55
50
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40
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30
25
20
15
10
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-5
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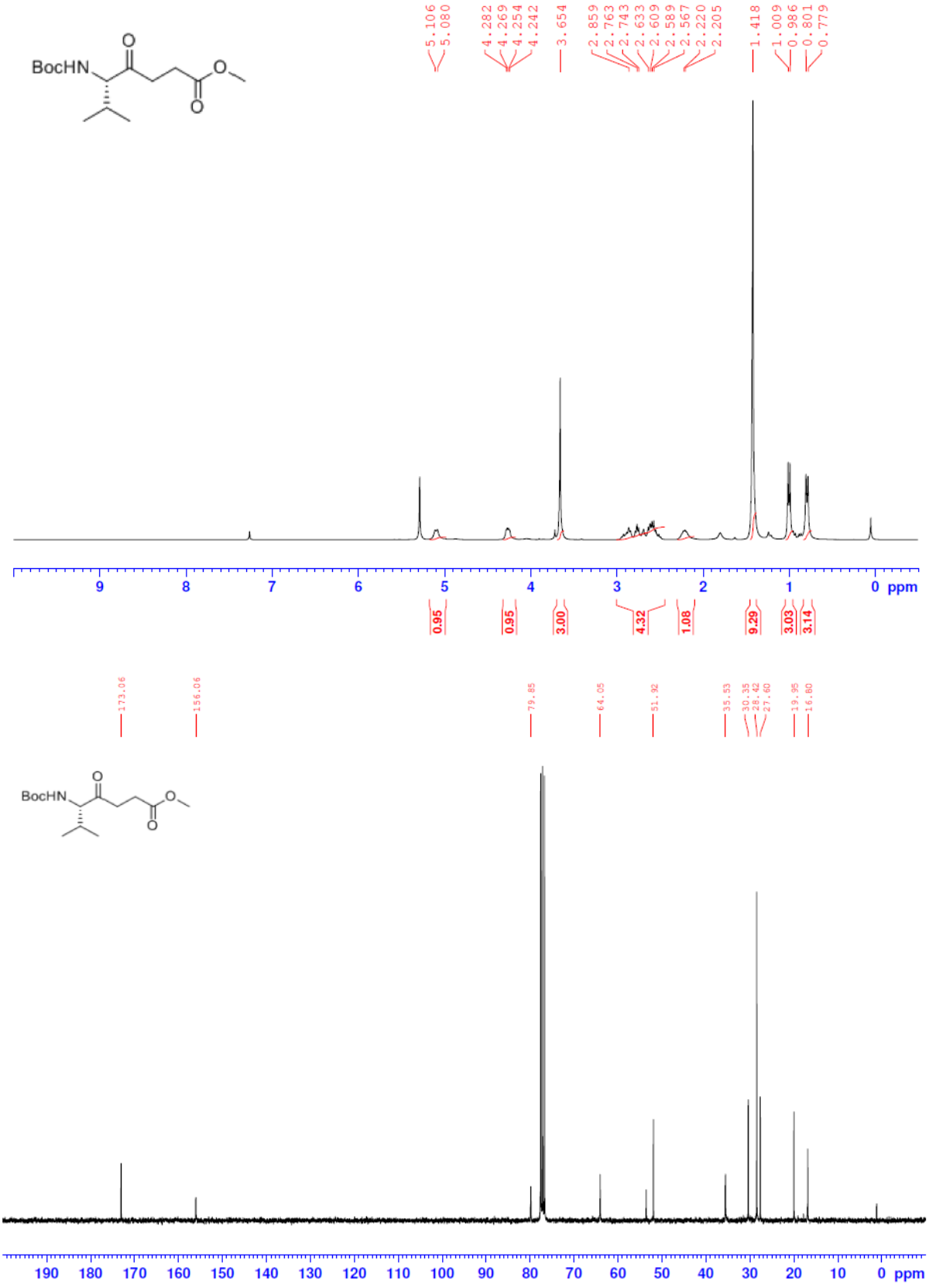
f1 (ppm)

^{1}H and ^{13}C spectra of methyl (*S*)-5-((*tert*-butoxycarbonyl)amino)-6-methyl-4-oxoheptanoate (4)



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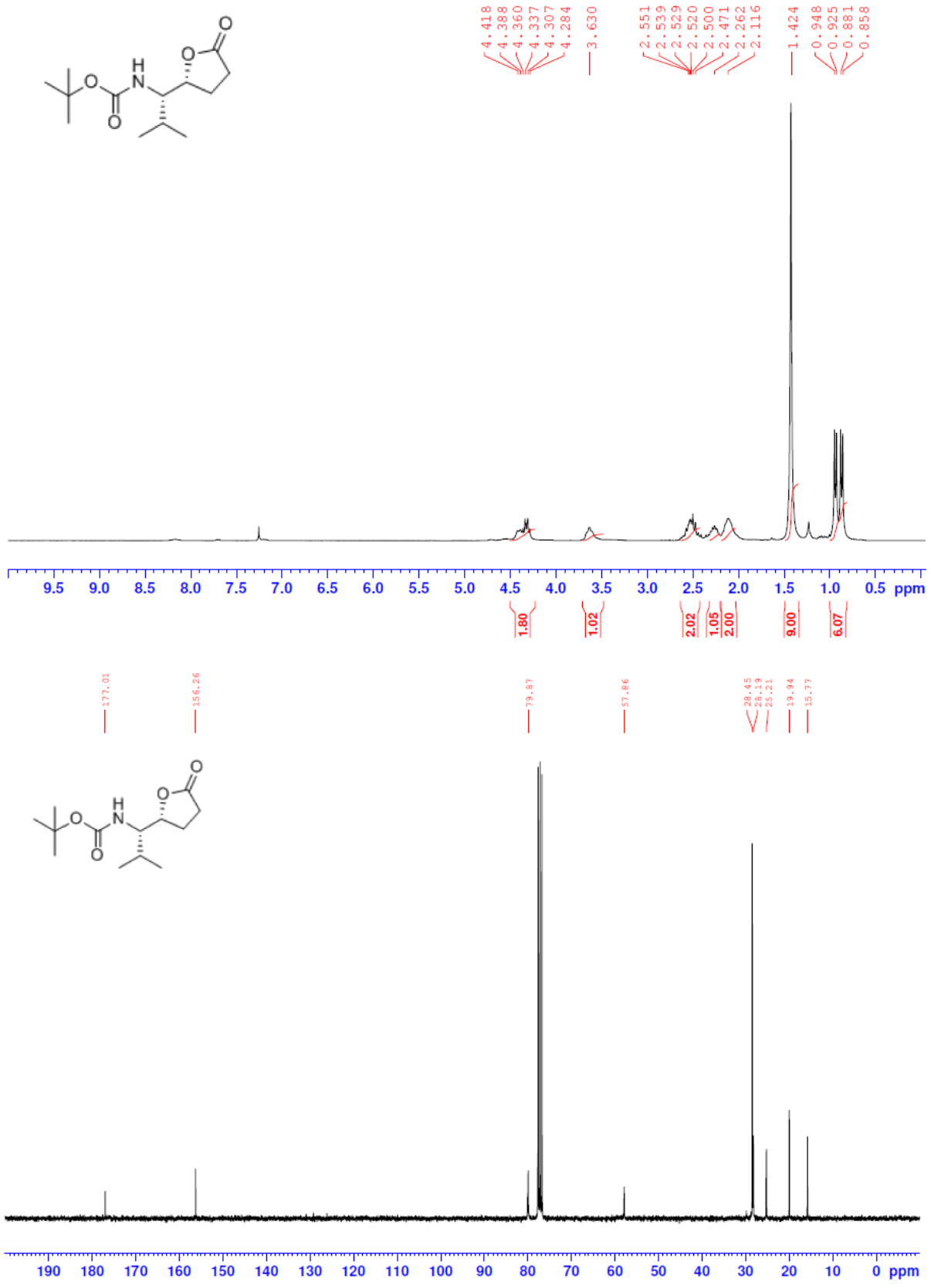

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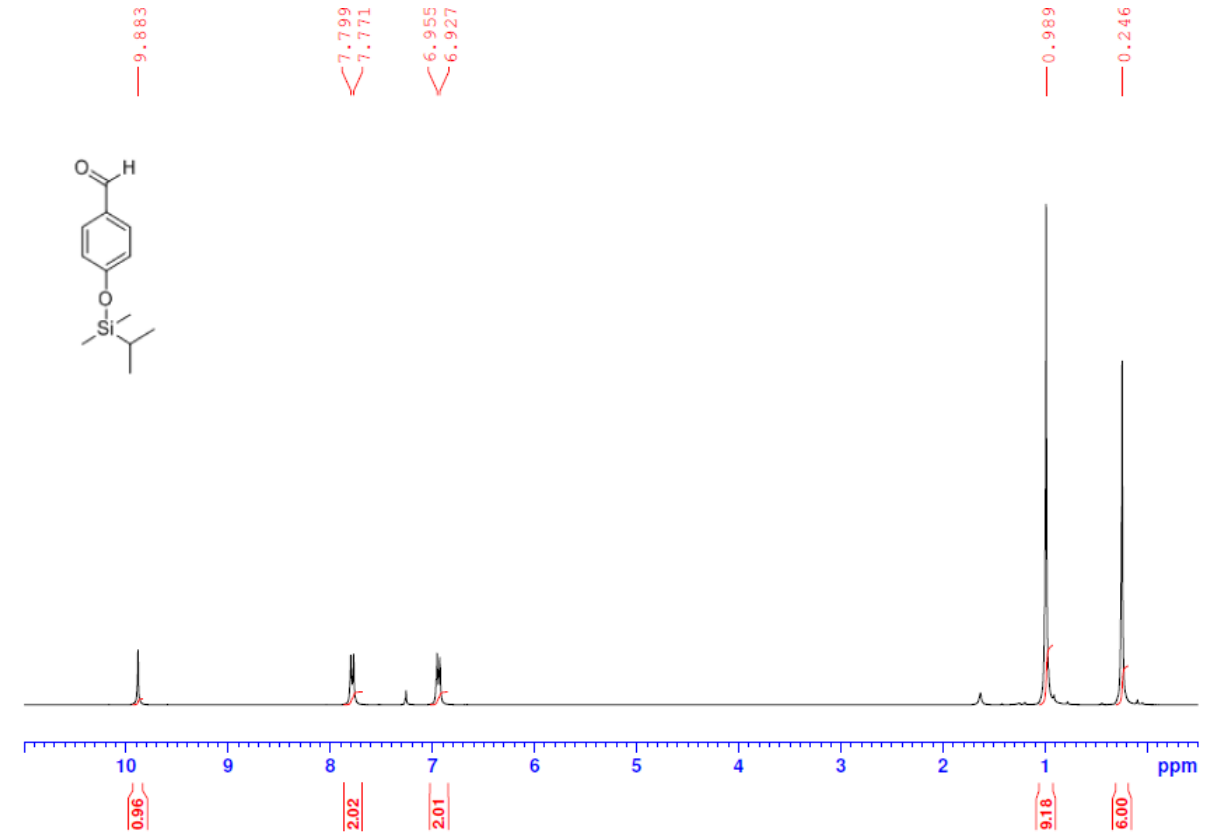
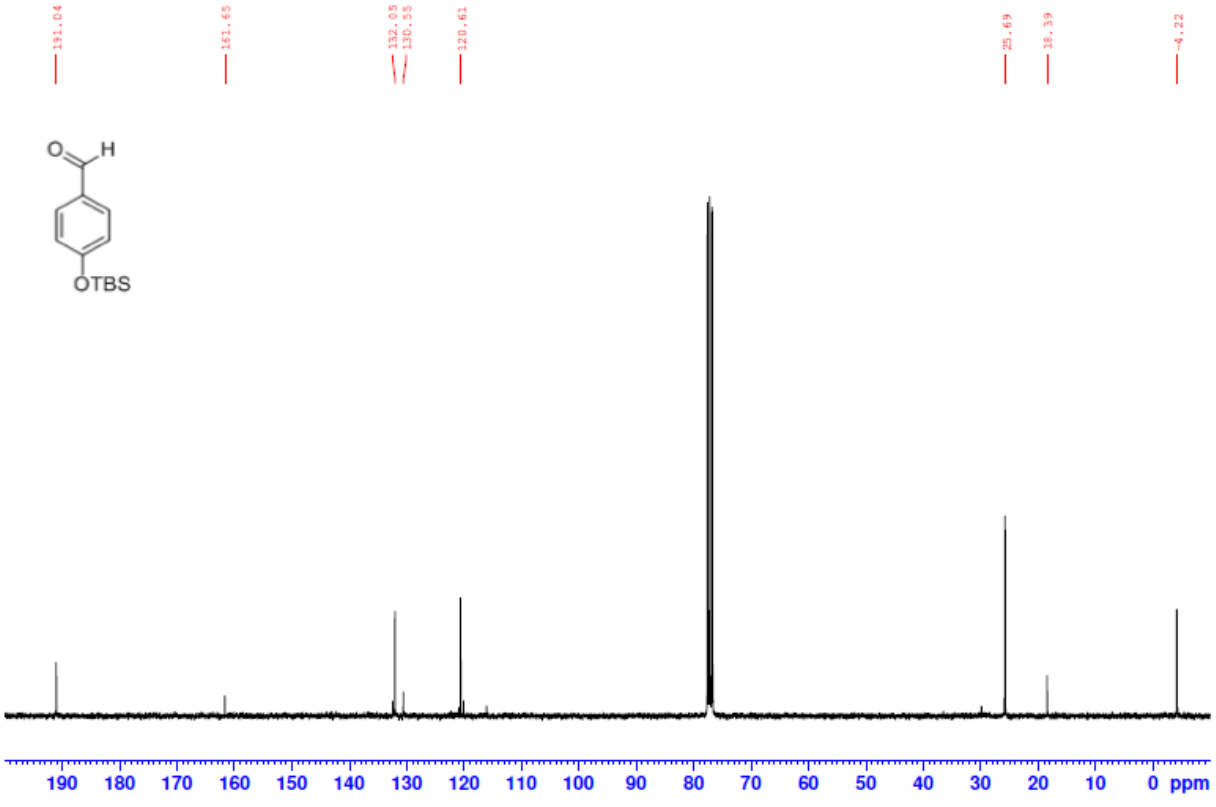
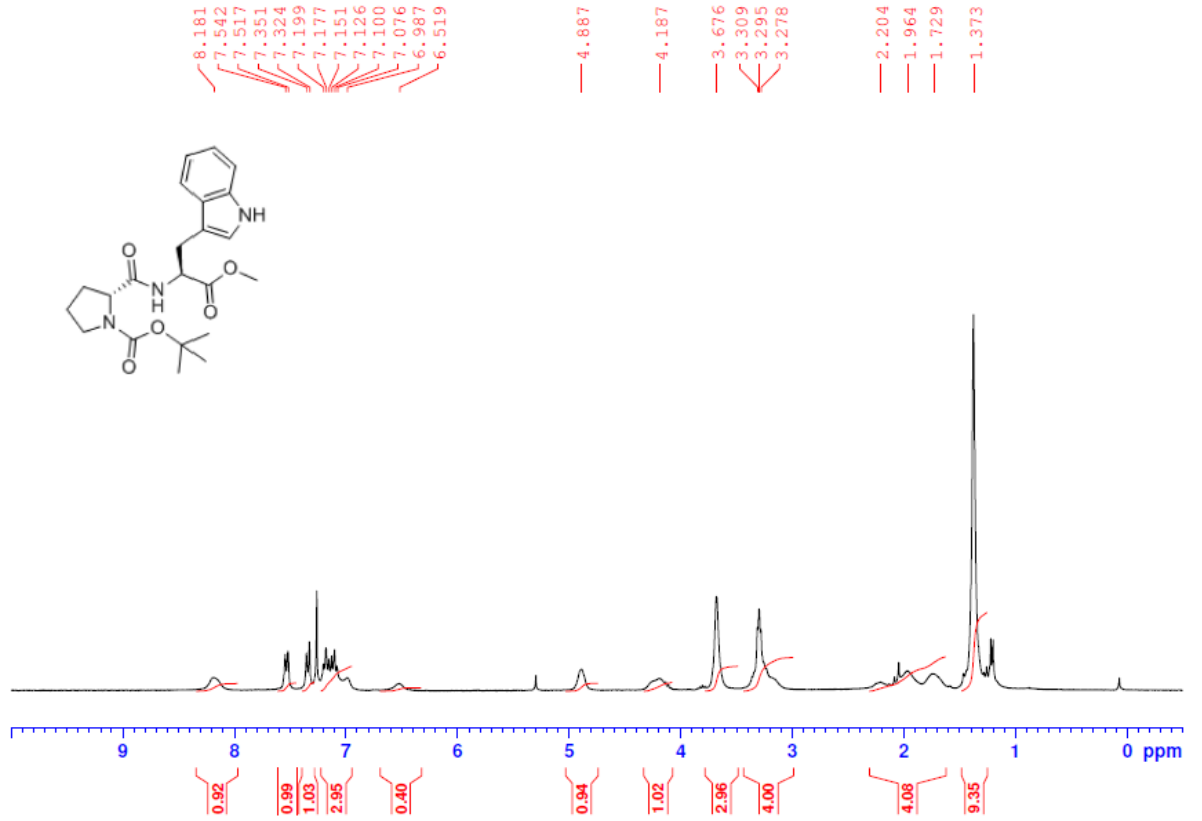
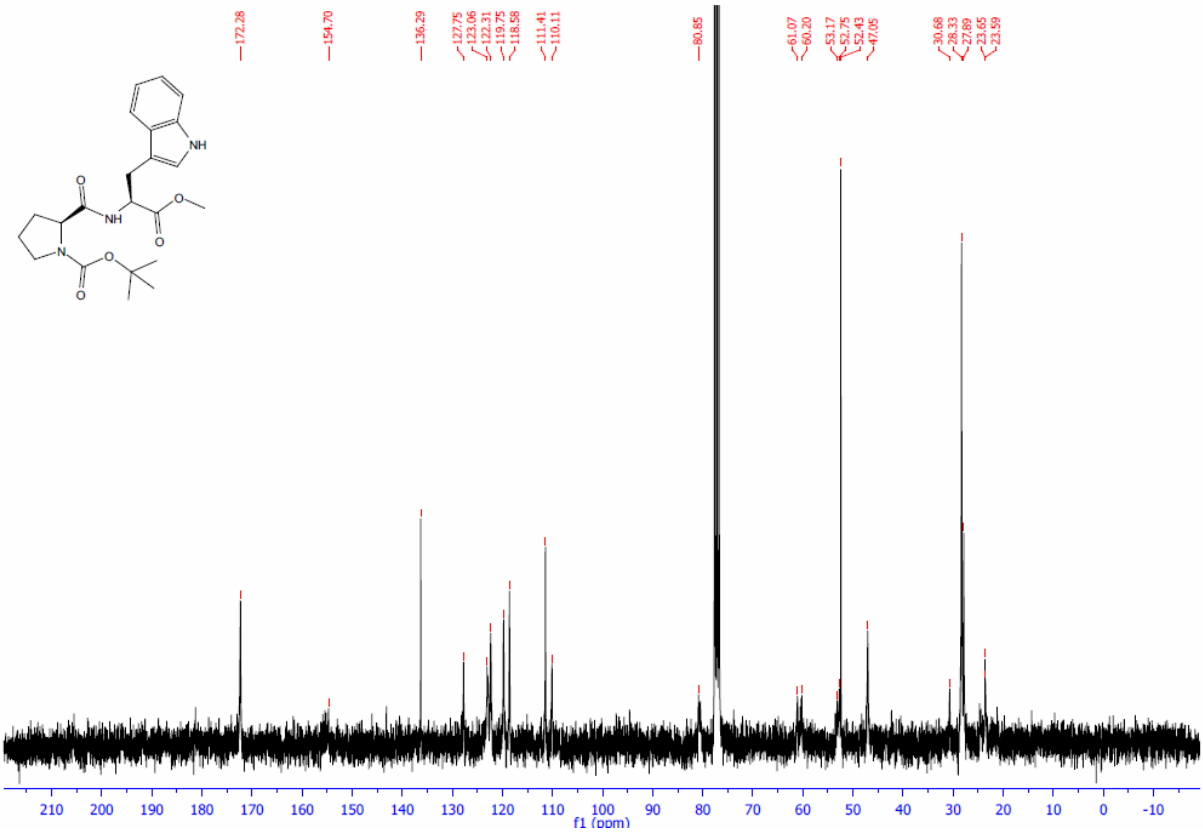


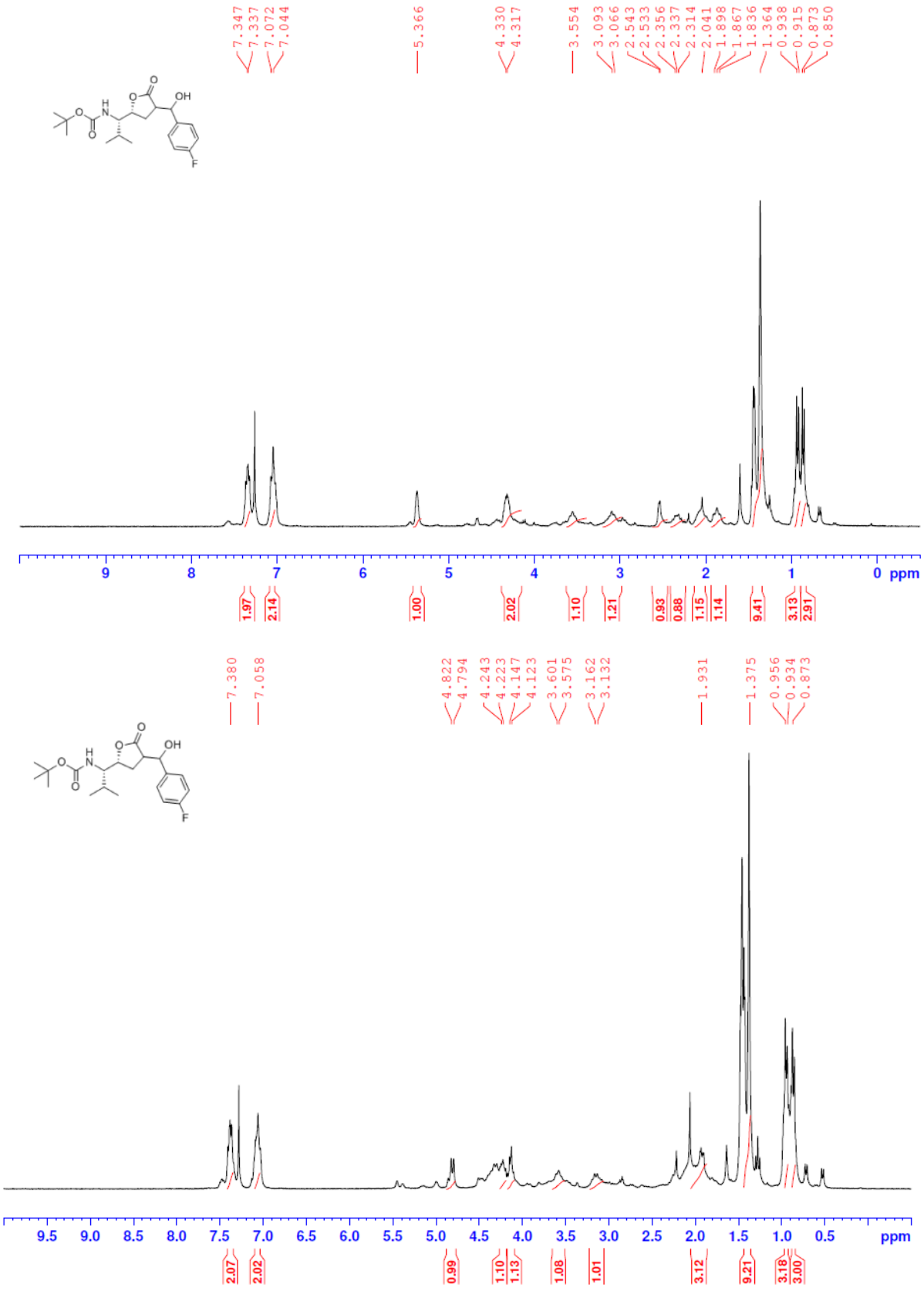


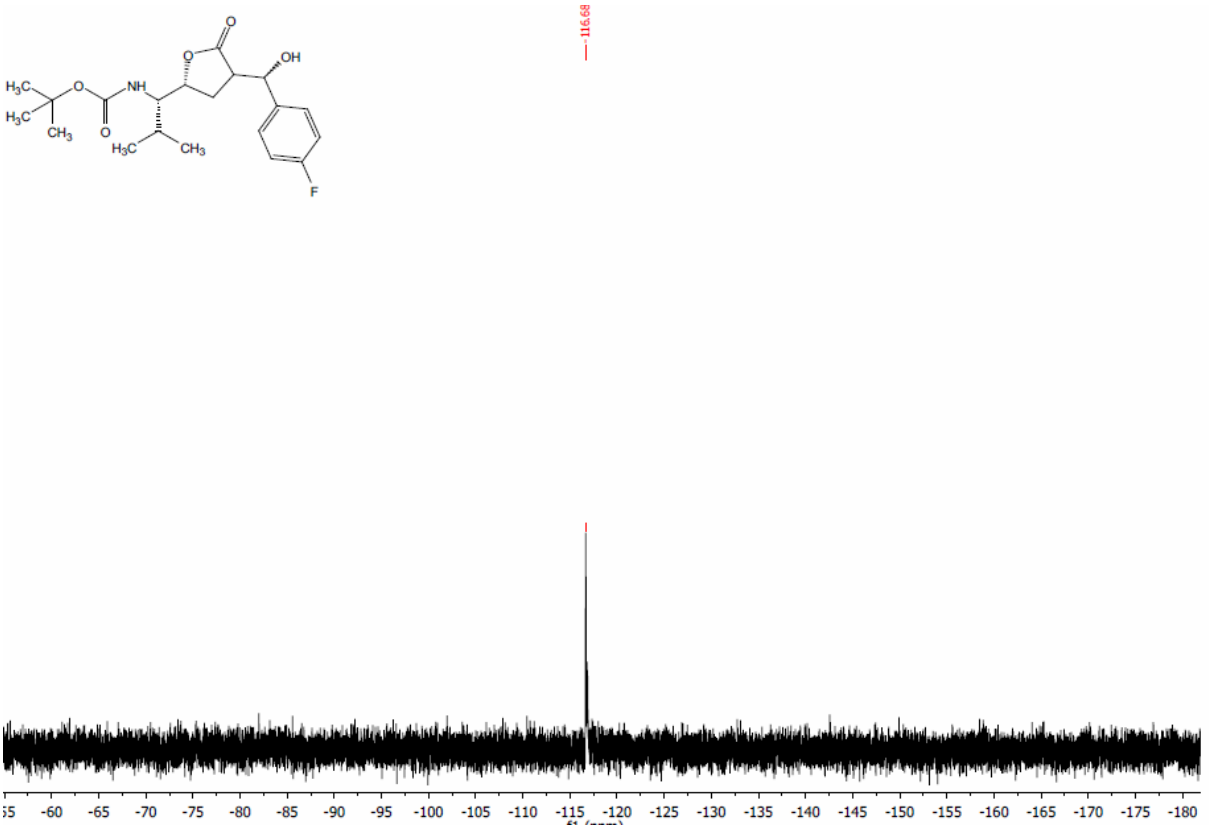
^{1}H and ^{13}C spectra of *tert*-butyl (*S*)-2-(((*S*)-3-(1H-indol-3-yl)-1-methoxy-1-oxopropan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (7)

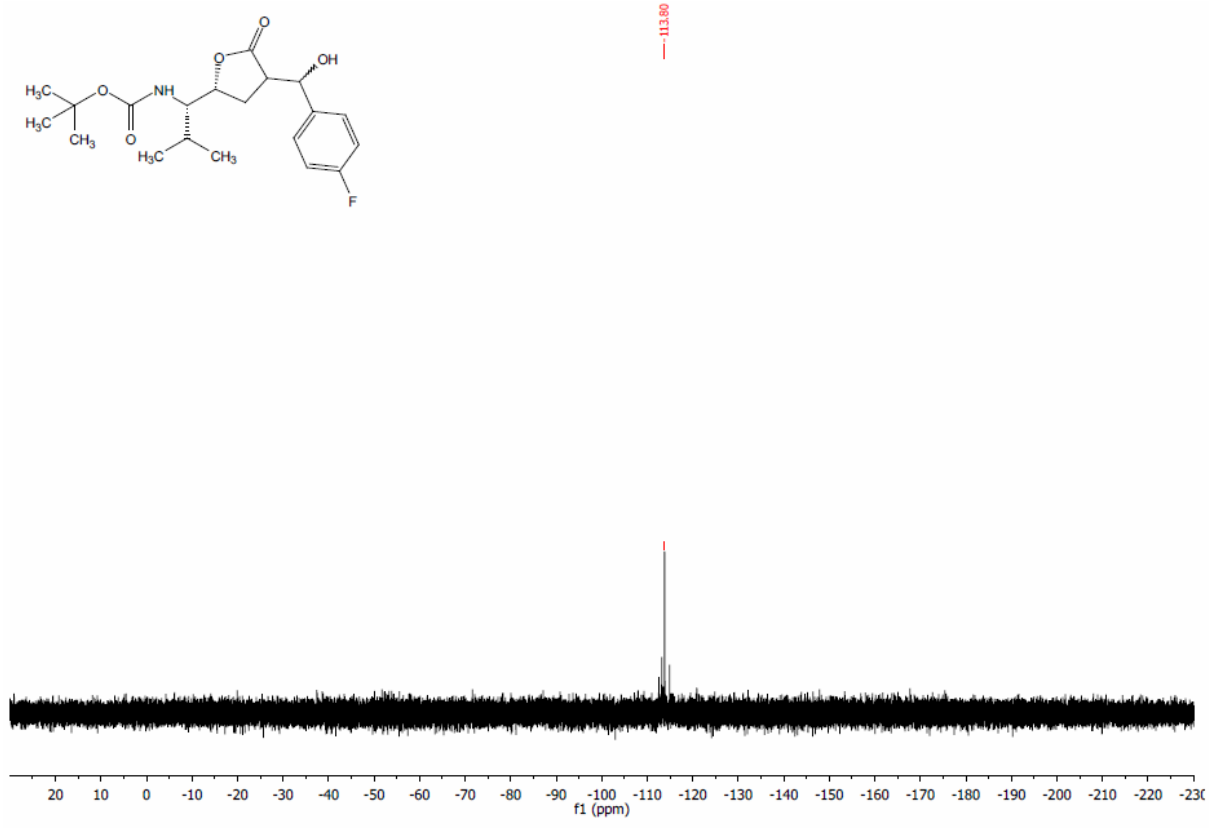




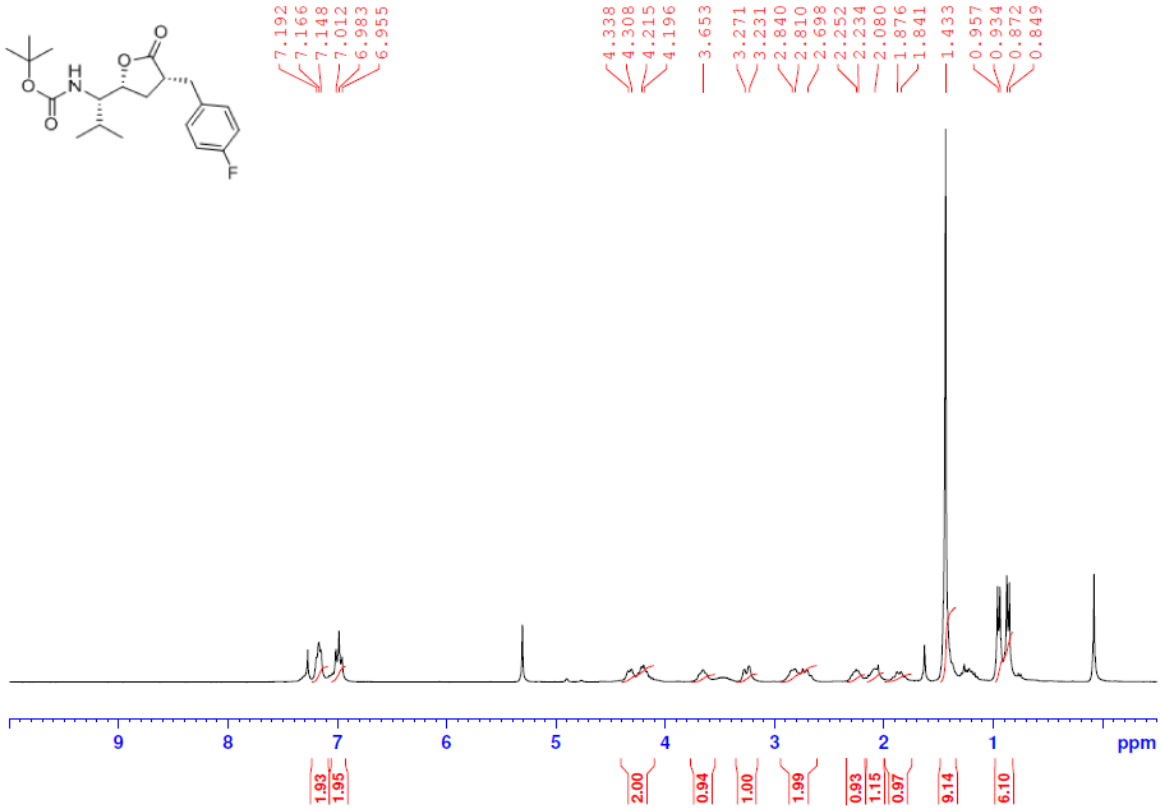
^{1}H and ^{19}F spectra of *tert*-Butyl ((1*S*)-1-((2*R*)-4-((4-fluorophenyl)(hydroxy)methyl)-5-oxotetrahydrofuran-2-yl)-2-methylpropyl)carbamate (8)







^{1}H, ^{13}C and ^{19}F spectra of *tert*-butyl ((*S*)-1-((2*R*, 4*R*)-4-(4-fluorobenzyl)-5-oxotetrahydrofuran-2-yl)-2-methylpropyl)carbamate (9)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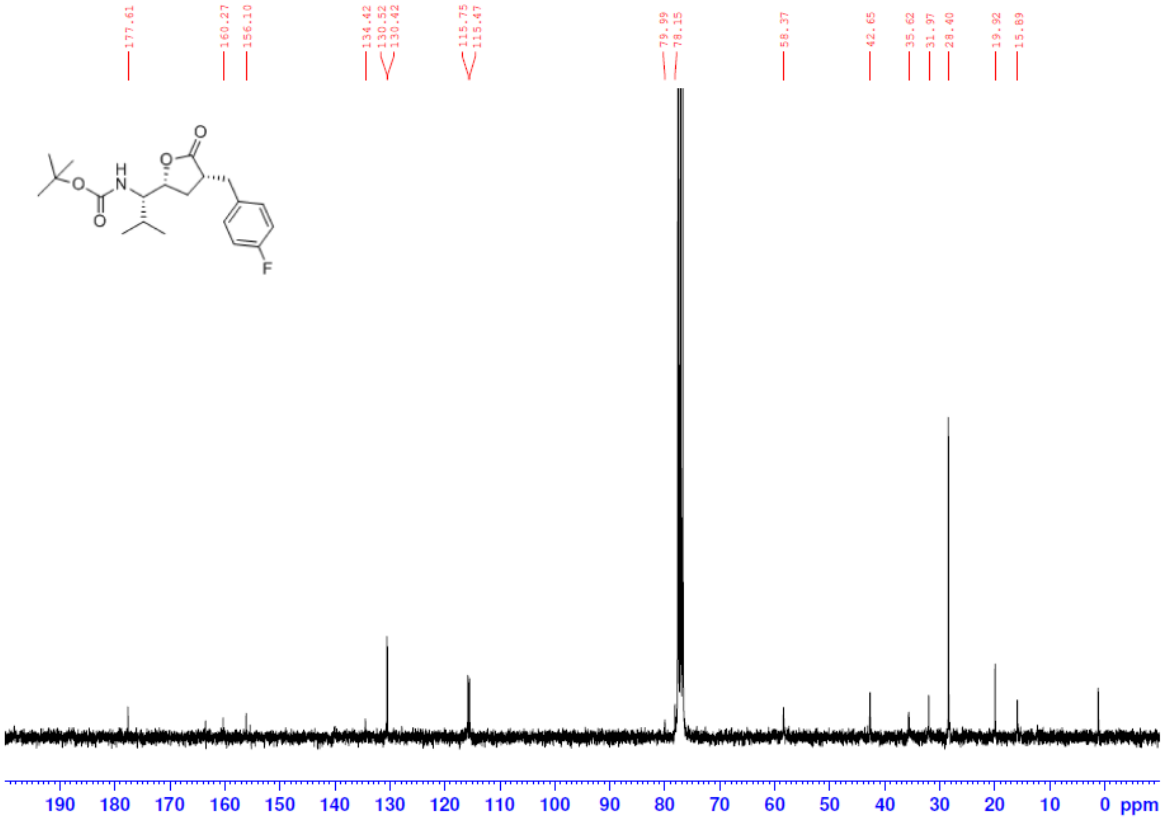
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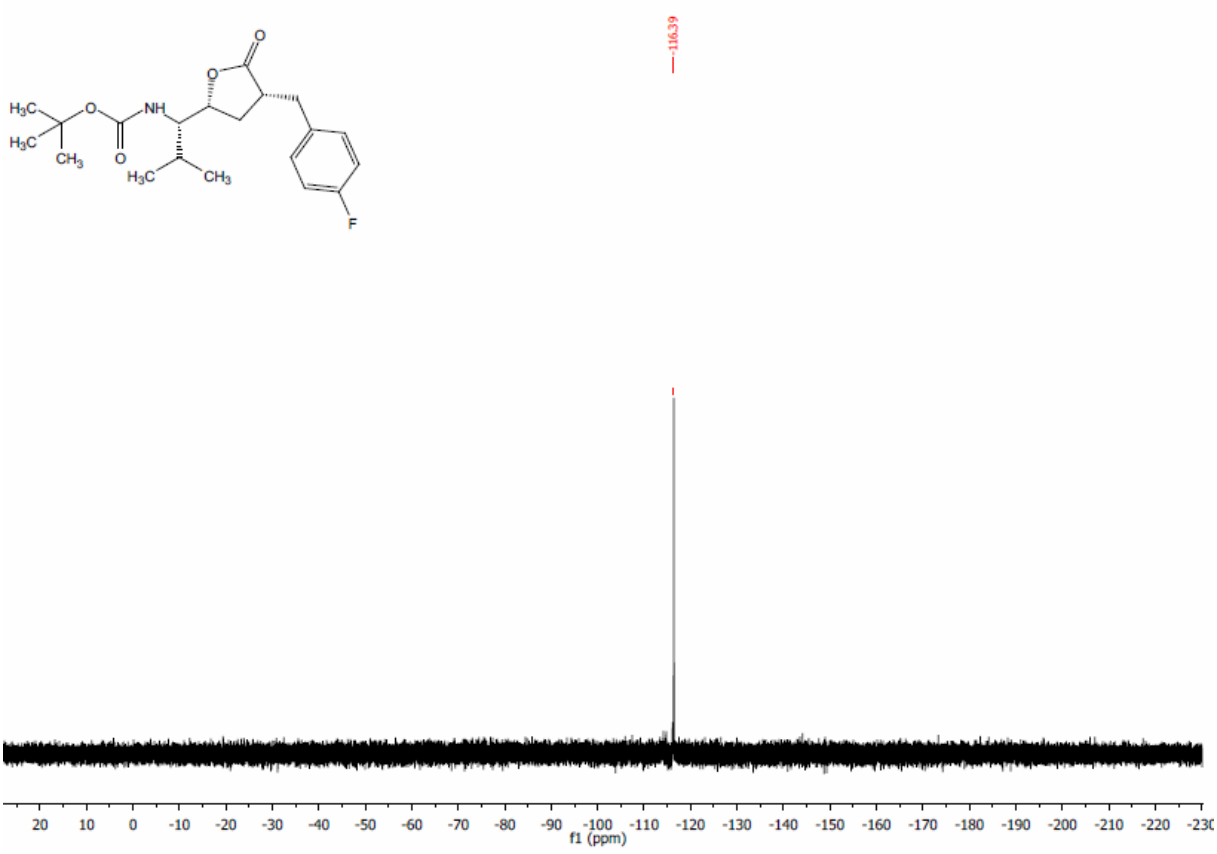
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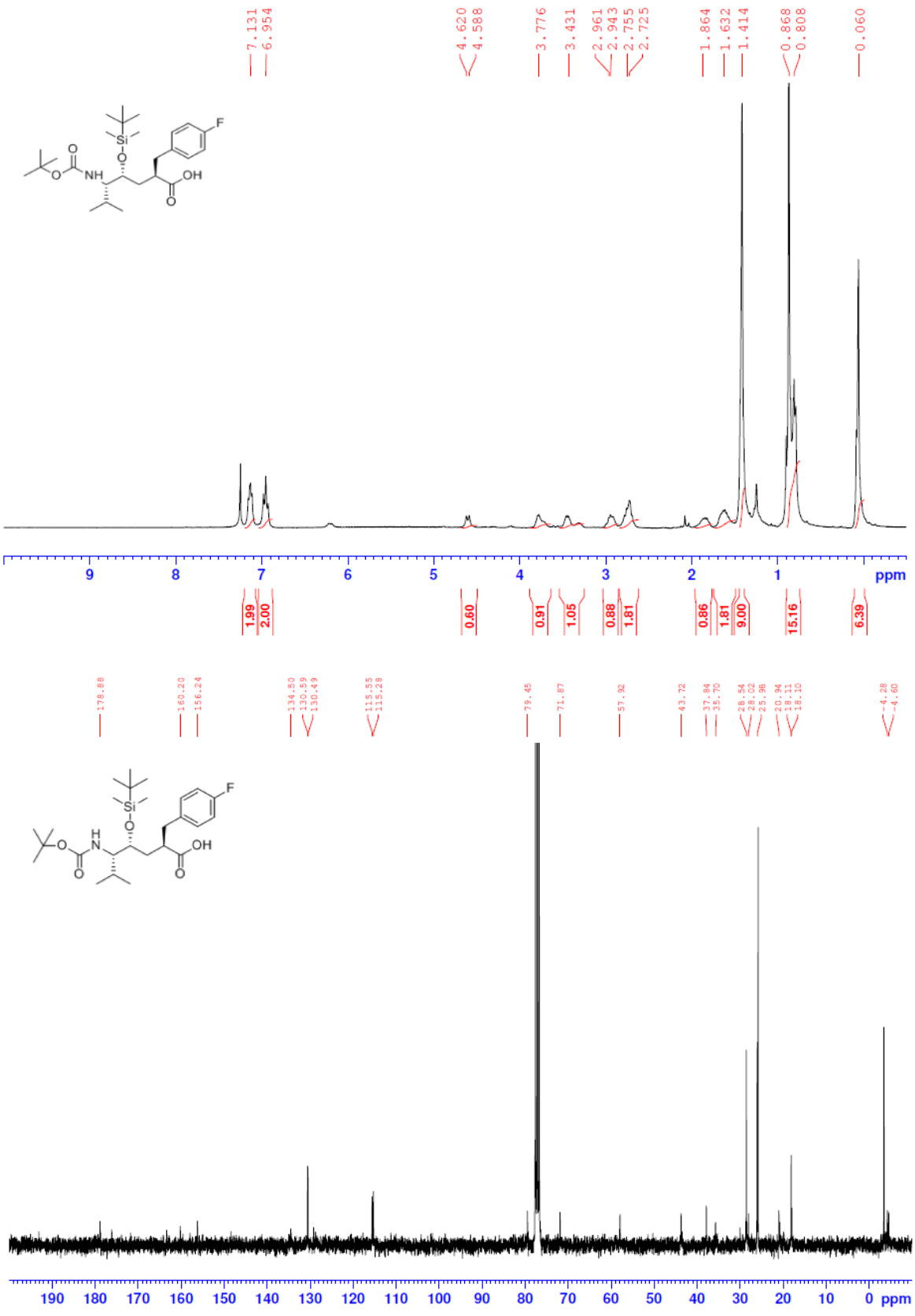
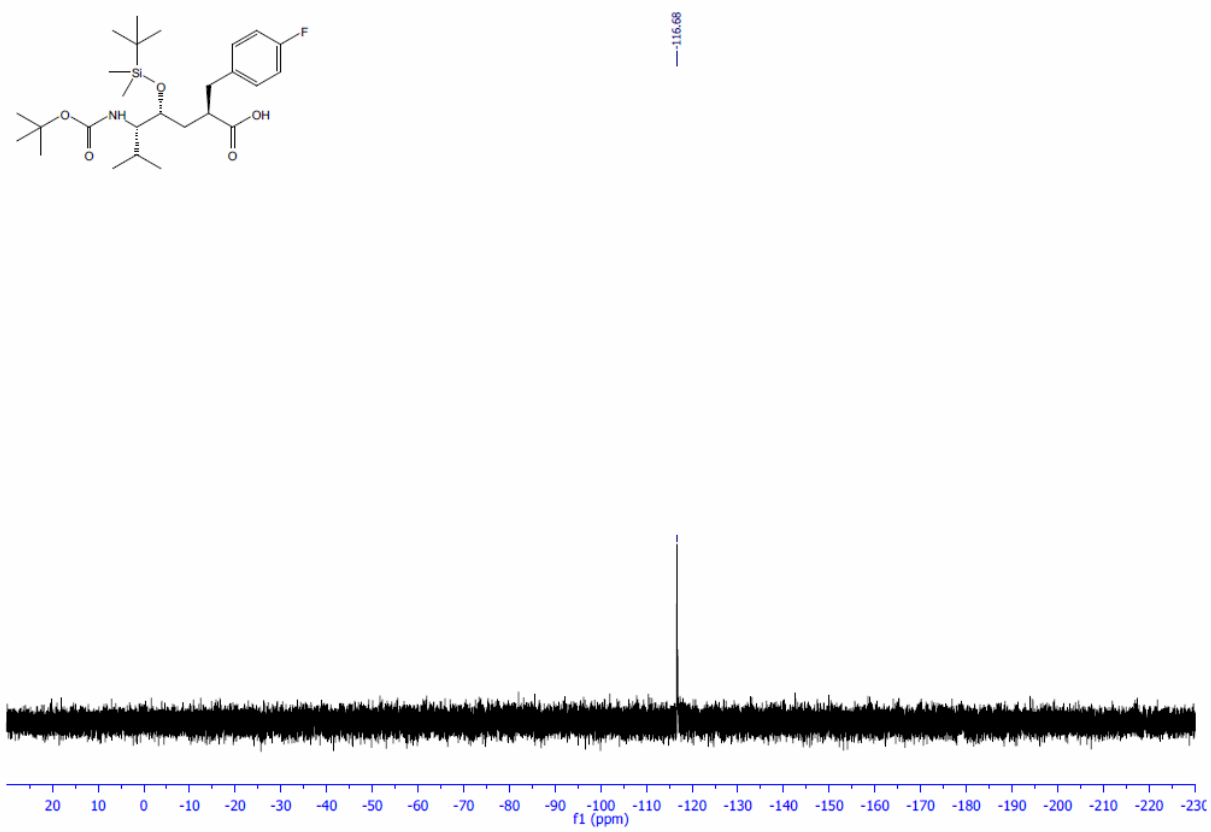
f1 (ppm)

^{1}H, ^{13}C and ^{19}F spectra of (2*R*,4*R*,5*S*)-5-((*tert*-Butoxycarbonyl)amino)-4-((*tert*-butyldimethylsilyl)oxy)-2-(4-fluorobenzyl)-6-methylheptanoic acid (10)

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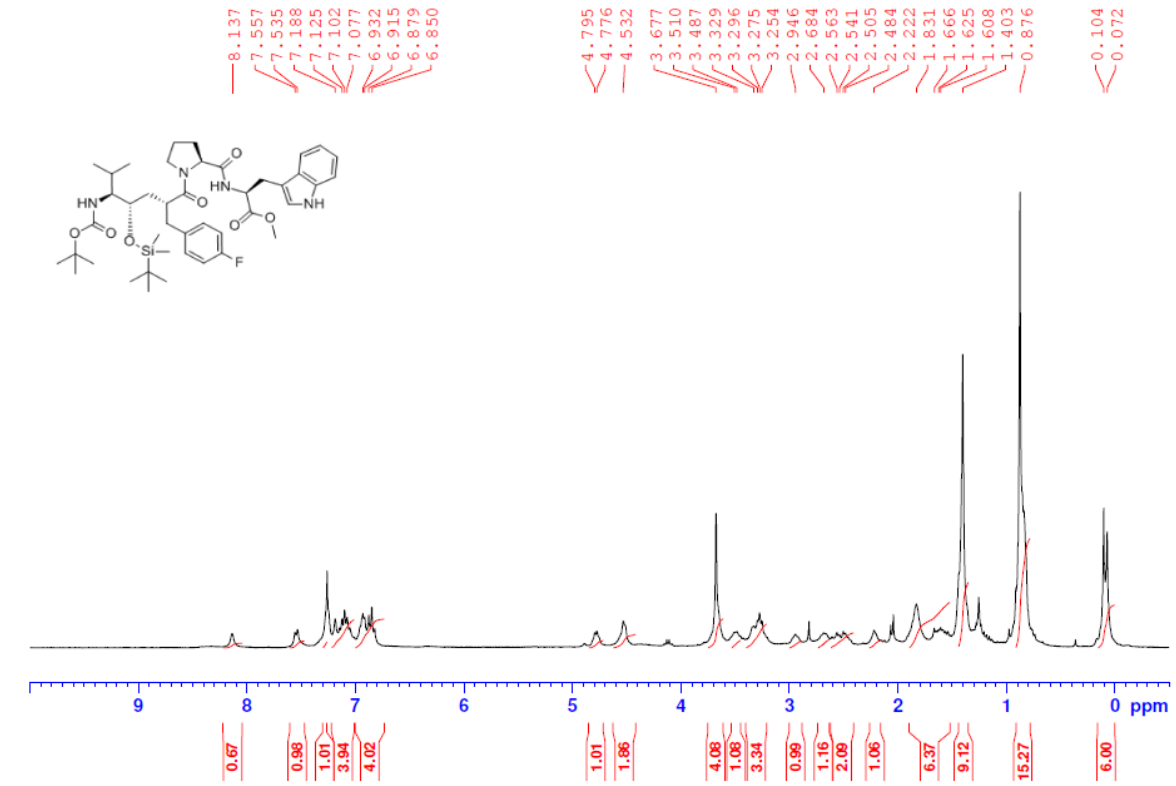
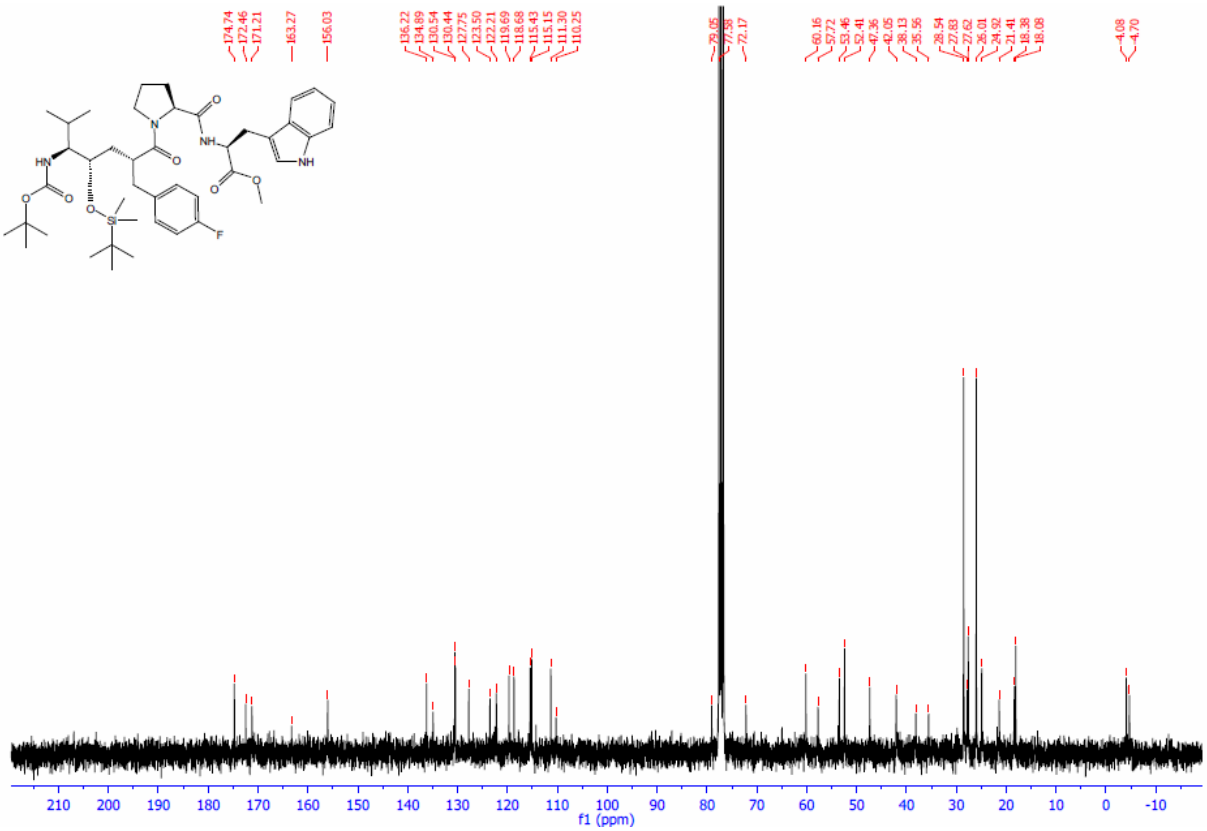
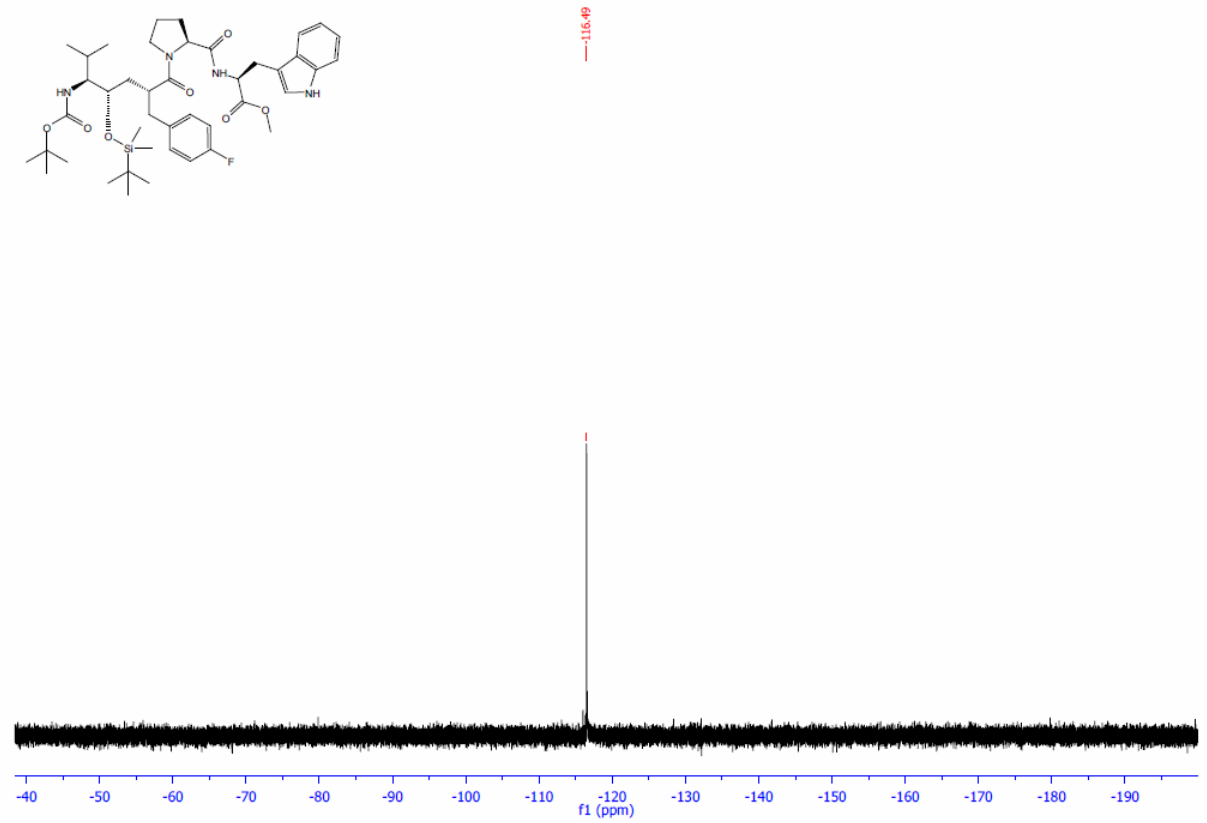
f1 (ppm)

^{1}H, ^{13}C and ^{19}F spectra of methyl ((2*R*, 4*R*, 5*S*)-5-((*tert*-butoxycarbonyl)amino)-4-((*tert*-butyldimethylsilyl)oxy)-2-(4-fluorobenzyl)-6-methylheptanoyl)-L-prolyl-L-tryptophanate (11)



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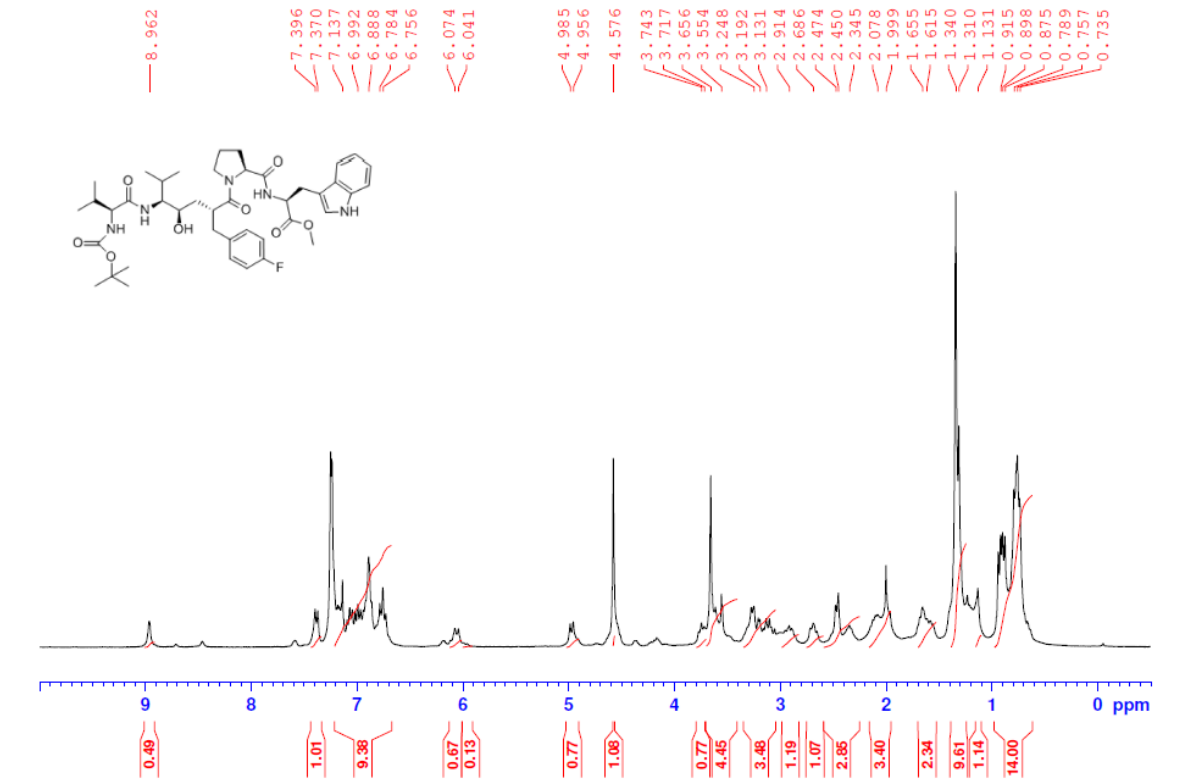
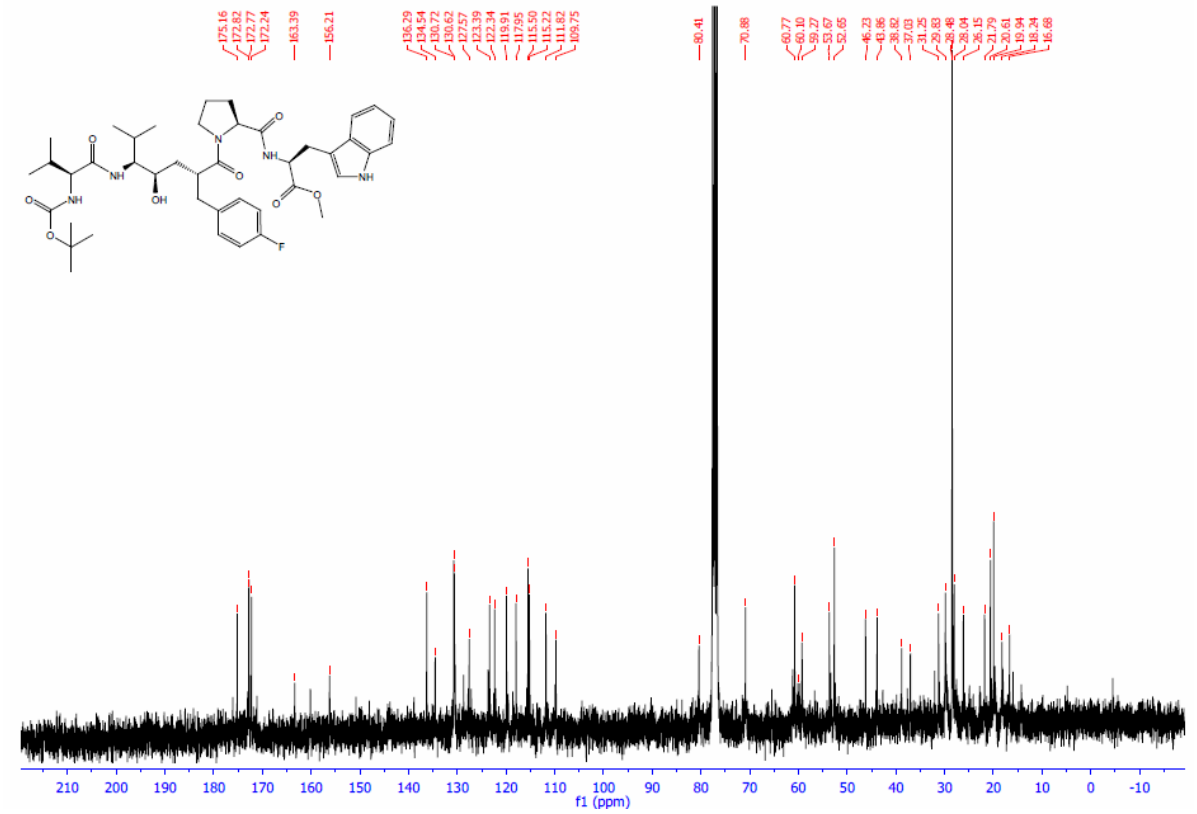
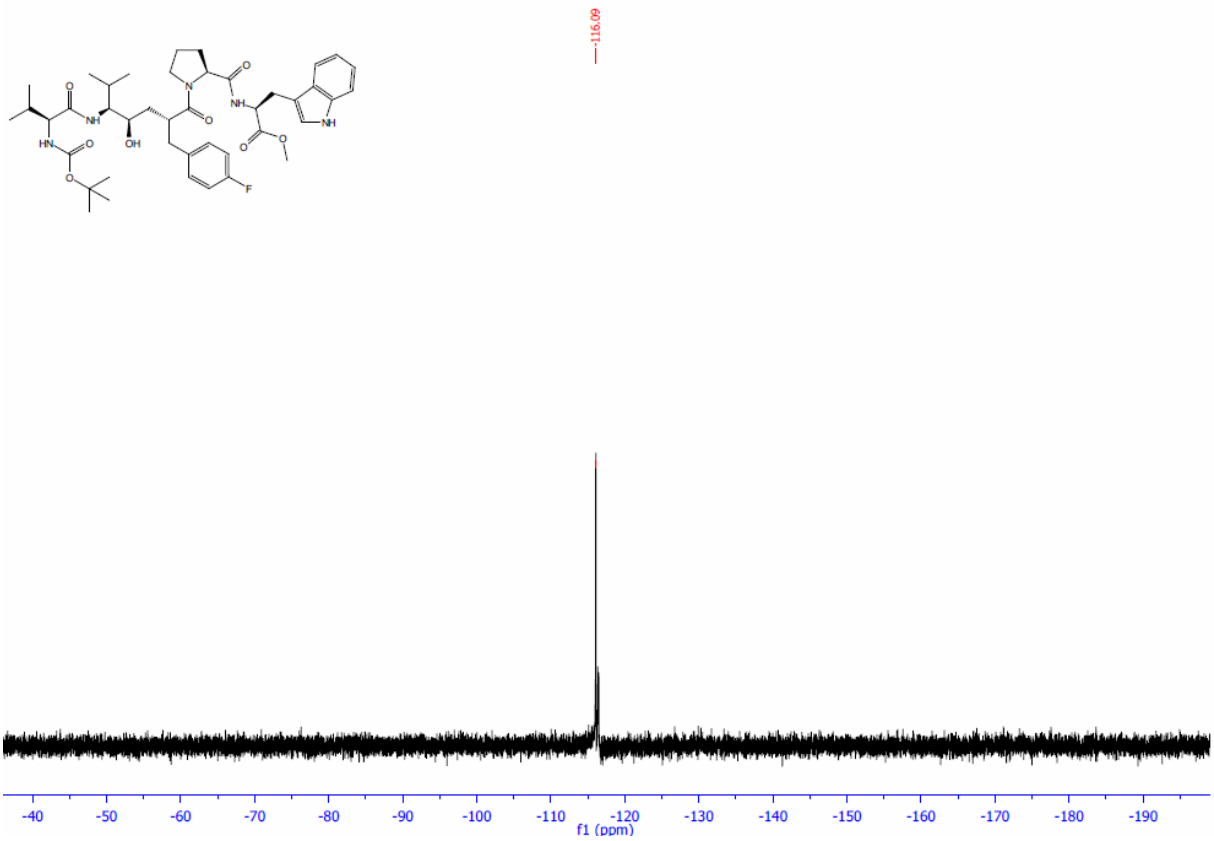
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
f1 (ppm)

^{1}H, ^{13}C and ^{19}F spectra of methyl ((2*R*,4*R*,5*S*)-5-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methylbutanamido)-2-(4-fluorobenzyl)-4-hydroxy-6-methylheptanoyl)-L-prolyl-L-tryptophanate (12)



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-100
-110
-120
-130
-140
-150
-160
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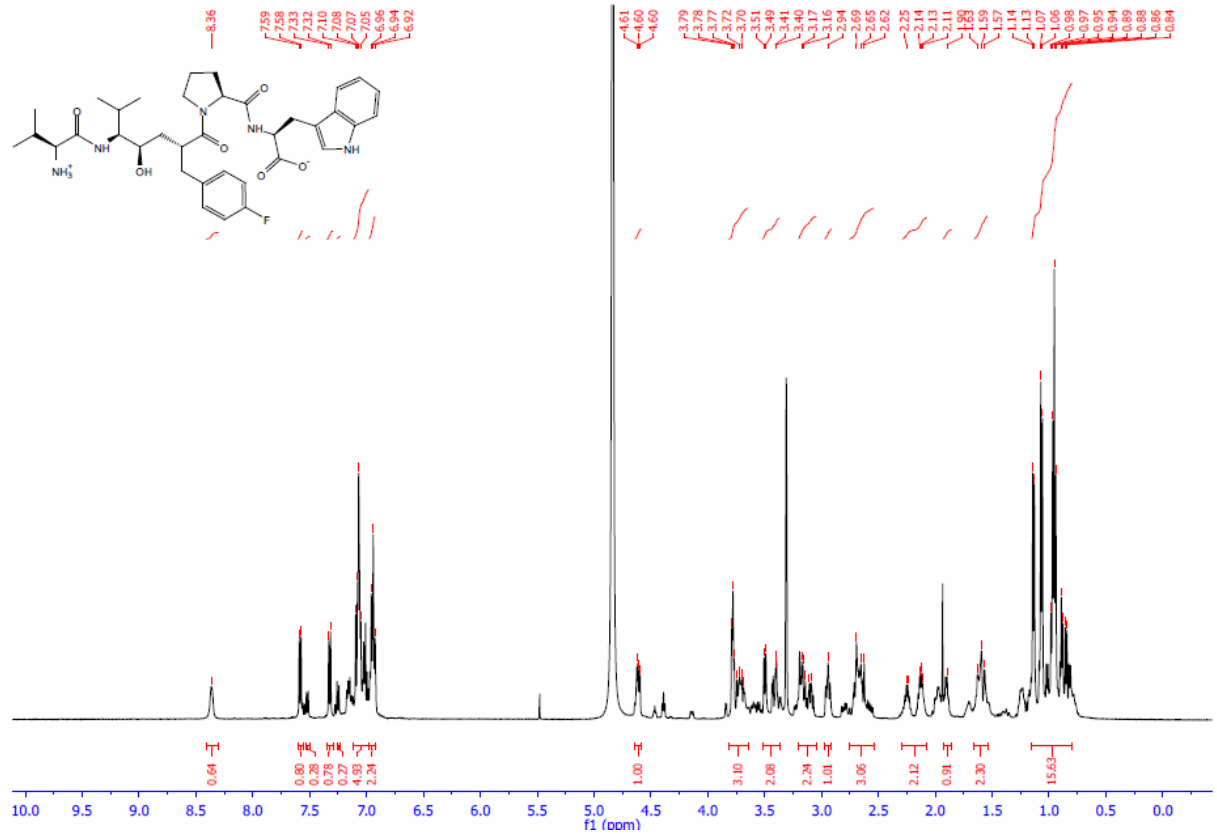
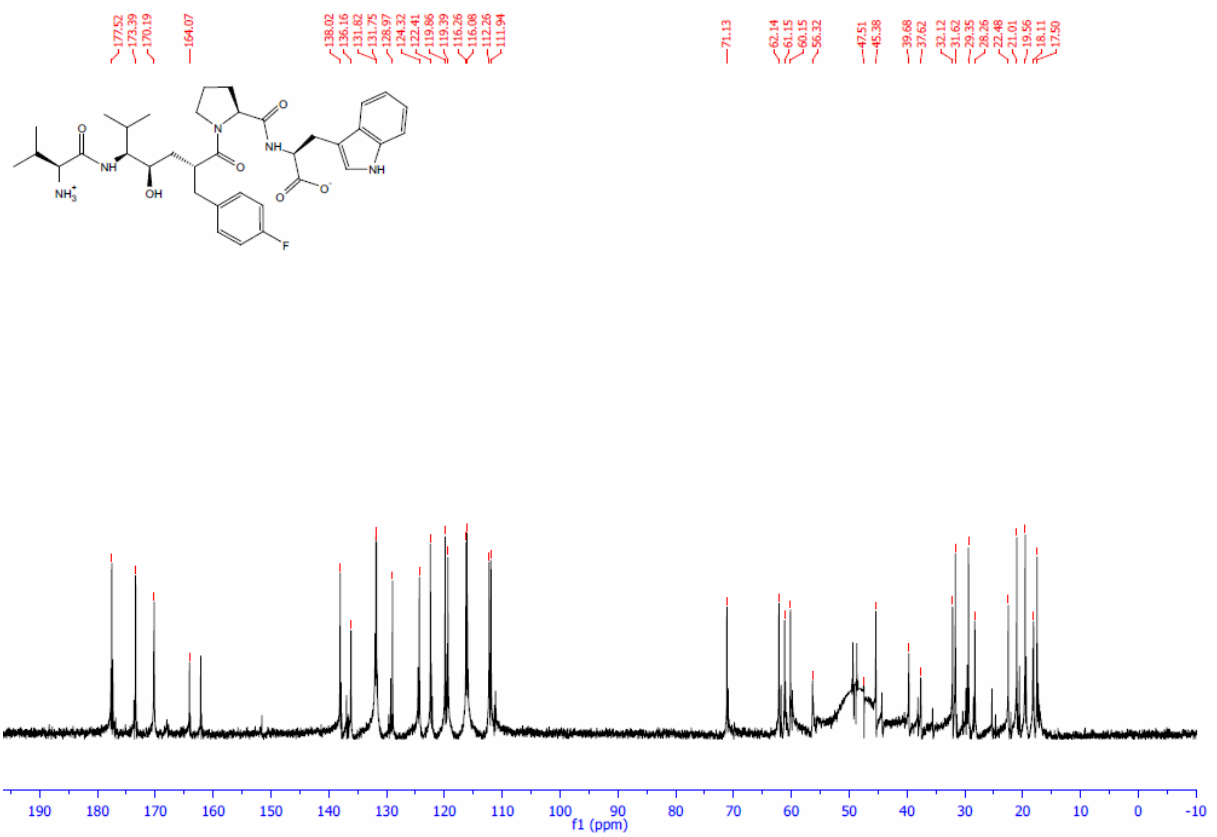
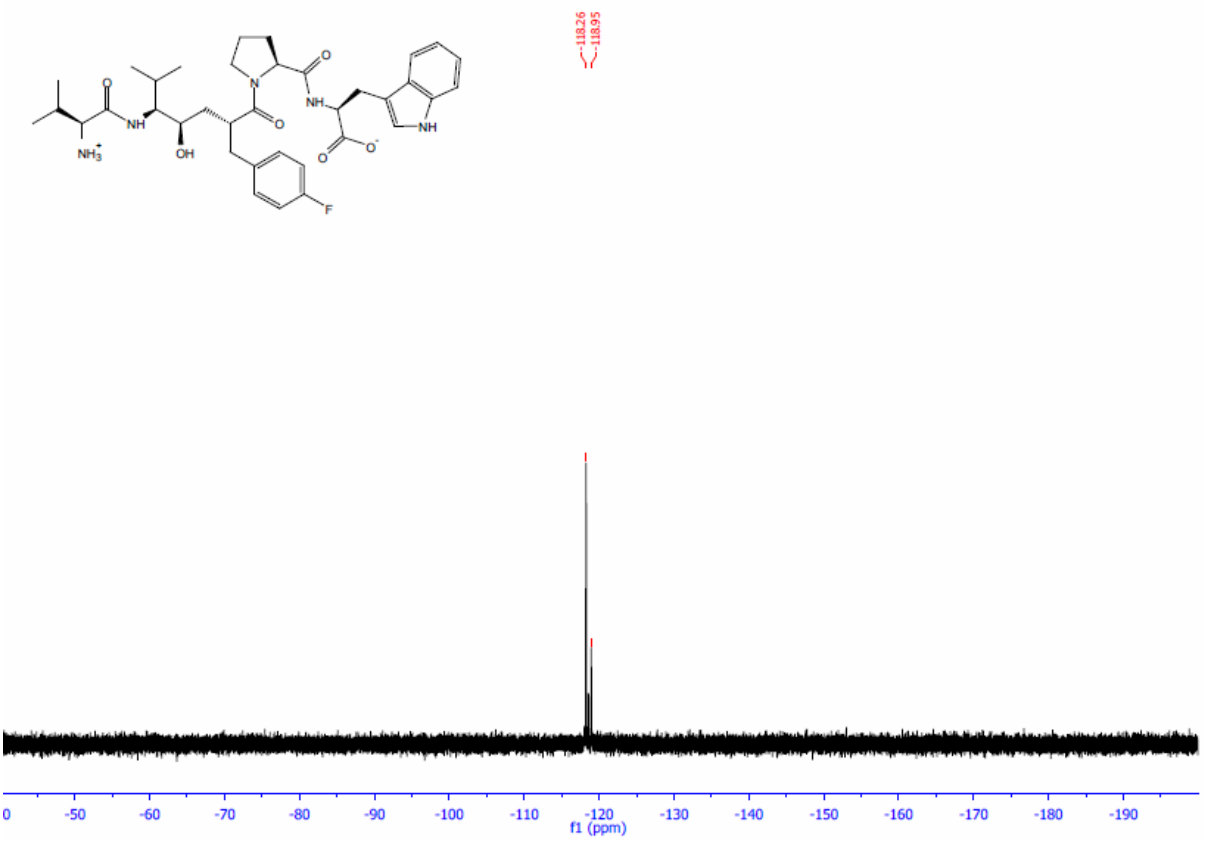
-180
-190
f1 (ppm)

^{1}H, ^{13}C and ^{19}F spectra of ((2*R*,4*R*,5*S*)-5-((*S*)-2-ammonio-3-methylbutanamido)-2-(4-fluorobenzyl)-4-hydroxy-6-methylheptanoyl)-L-prolyl-L-tryptophanate (13)



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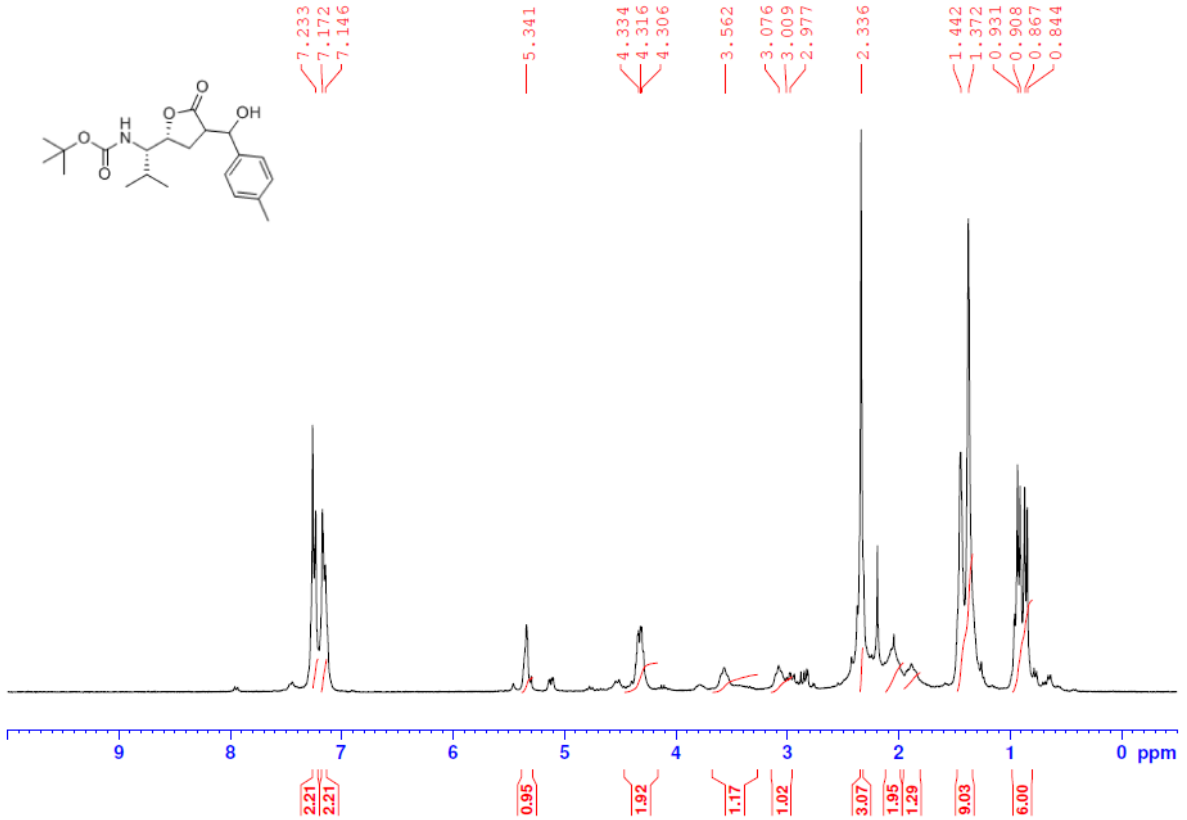
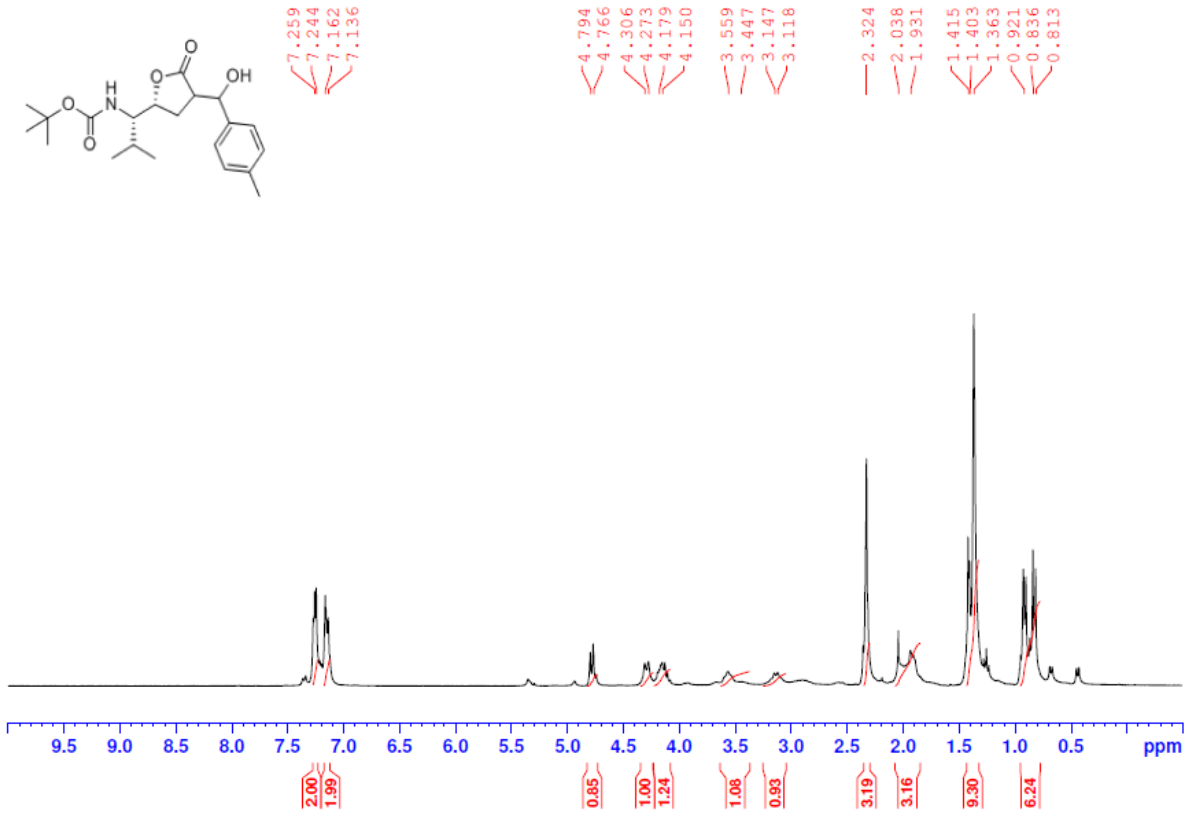
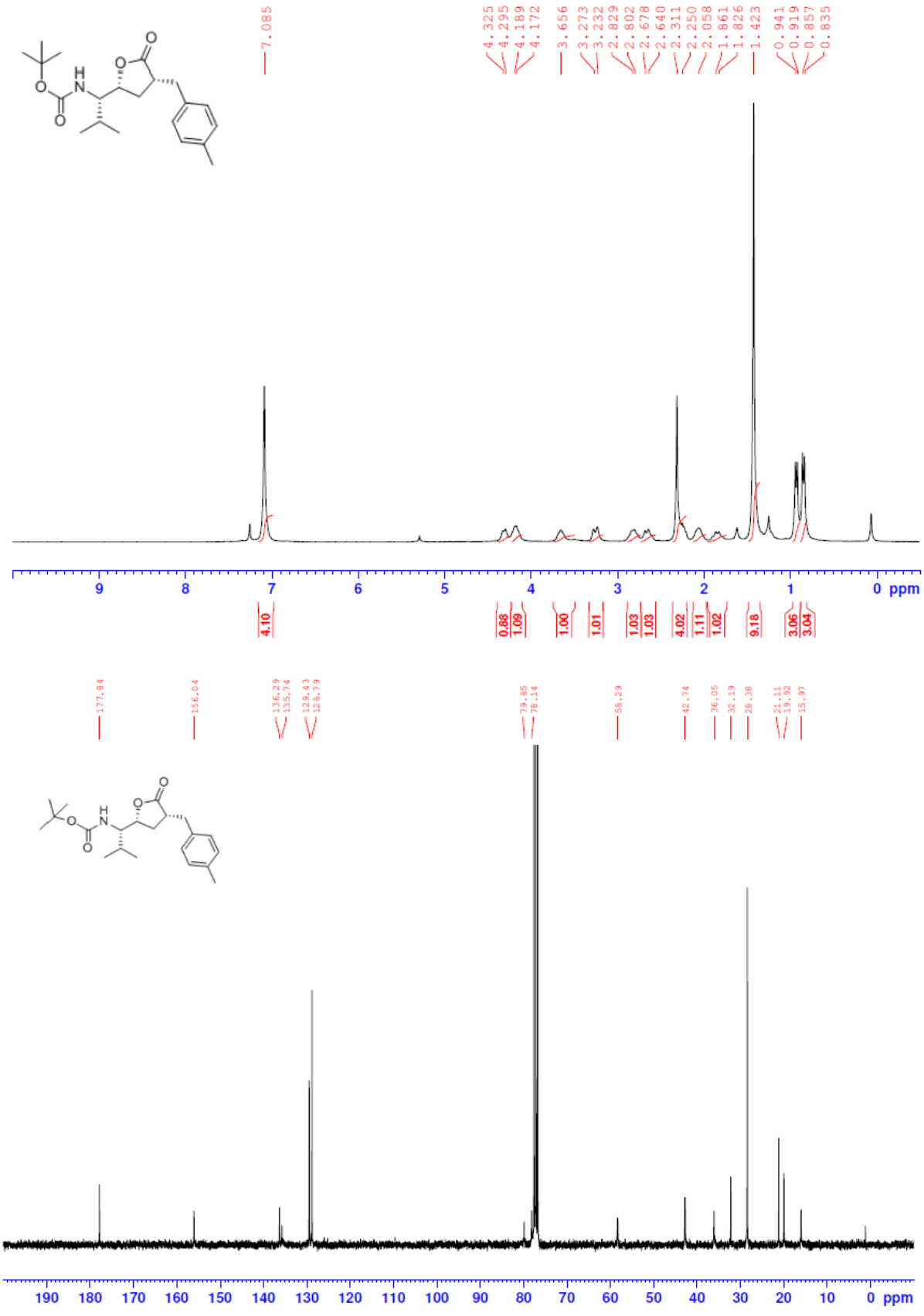
-118.95
0
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
f1 (ppm)

^{1}H and ^{13}C spectra of *tert*-butyl ((1*S*)-1-((2*R*)-4-(hydroxy(*p*-tolyl)methyl)-5-oxotetrahydrofuran-2-yl)-2-methylpropyl)carbamate (14)

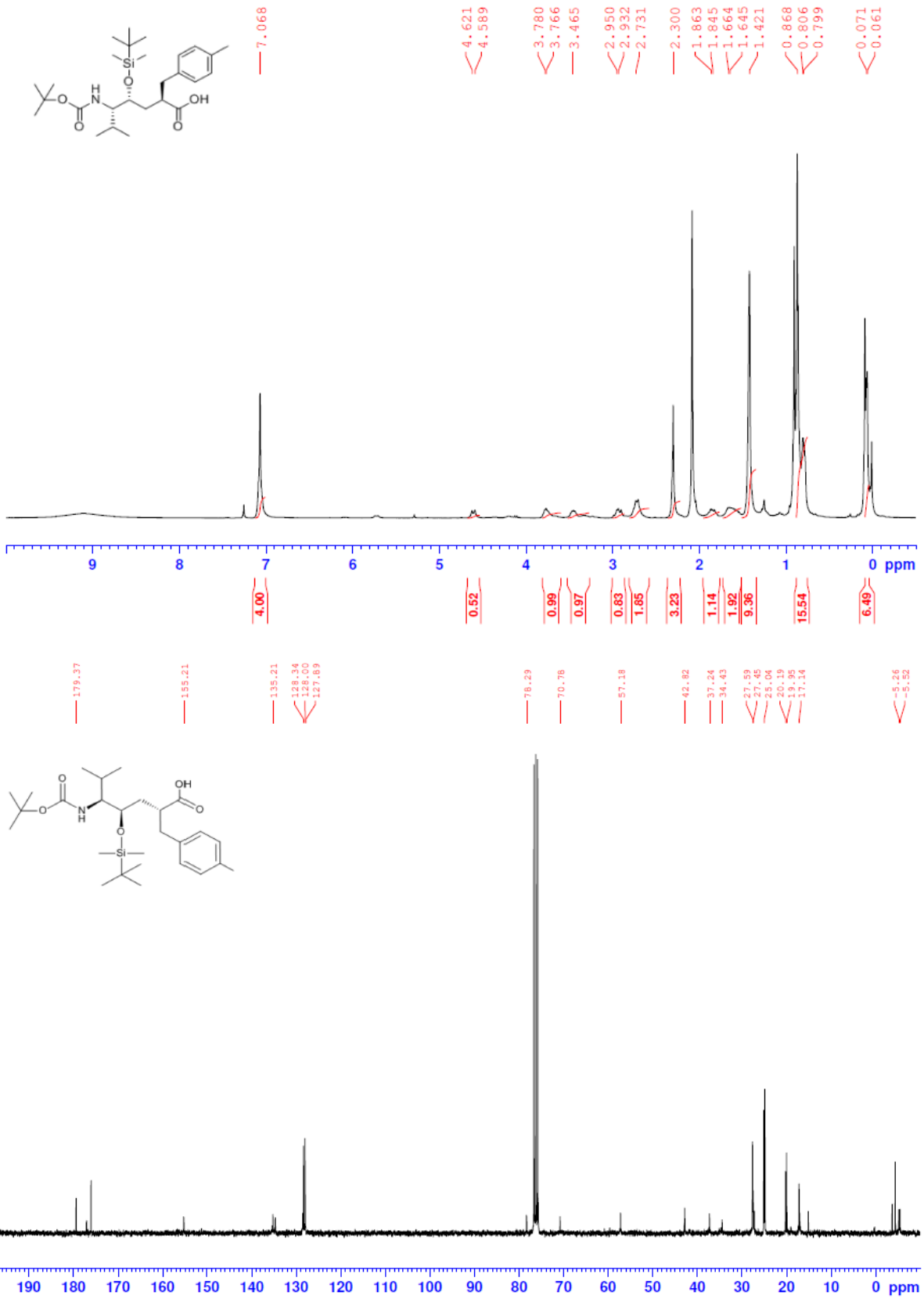




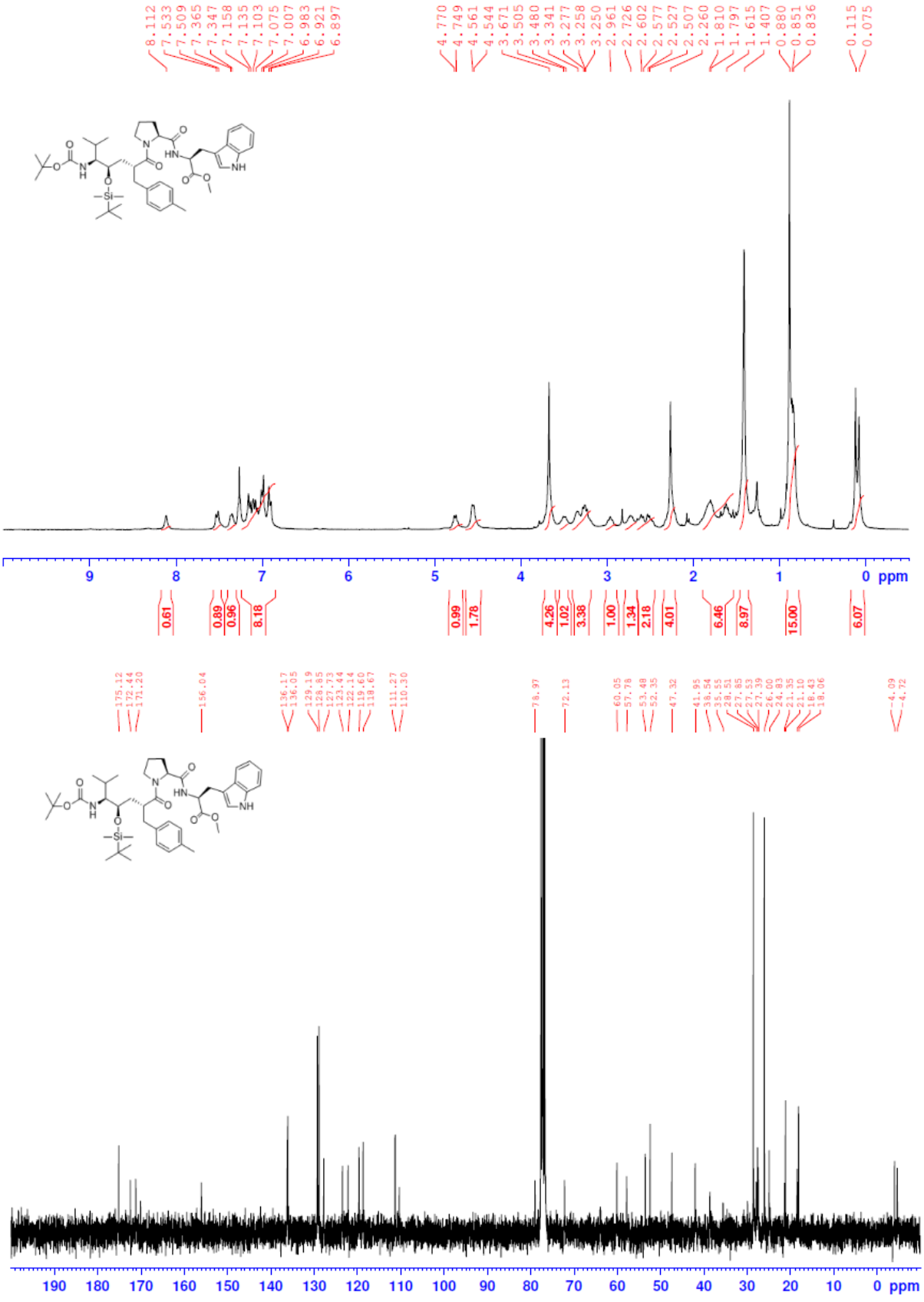
^{1}H and ^{13}C spectra of *tert*-butyl ((*S*)-2-methyl-1-((2*R*,4*R*)-4-(4-methylbenzyl)-5-oxotetrahydrofuran-2-yl)propyl)carbamate (15)



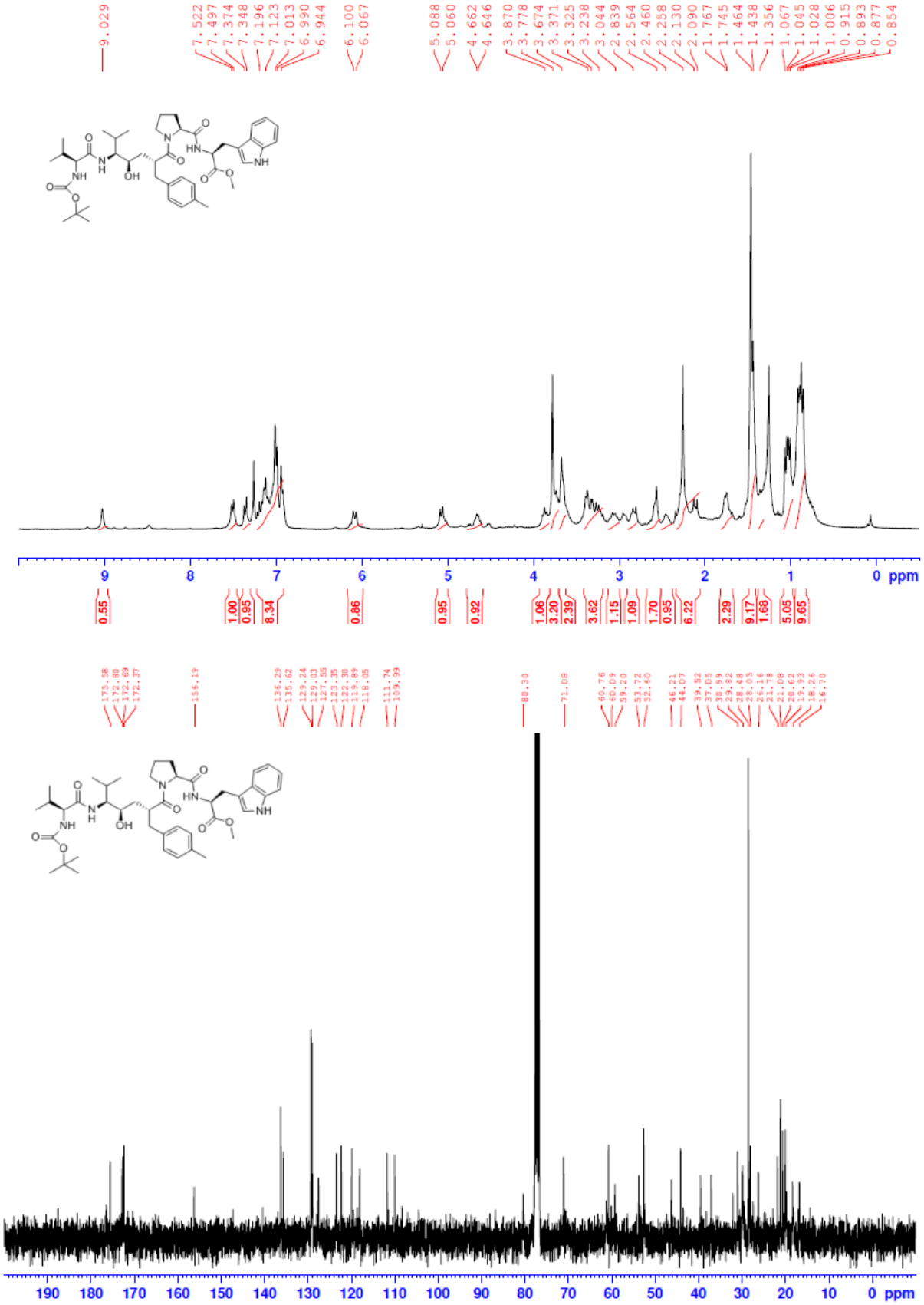
^{1}H and ^{13}C spectra of (2*R*,4*R*,5*S*)-5-((*tert*-butoxycarbonyl)amino)-4-((*tert*-butyldimethylsilyl)oxy)-6-methyl-2-(4-methylbenzyl)heptanoic acid (16)



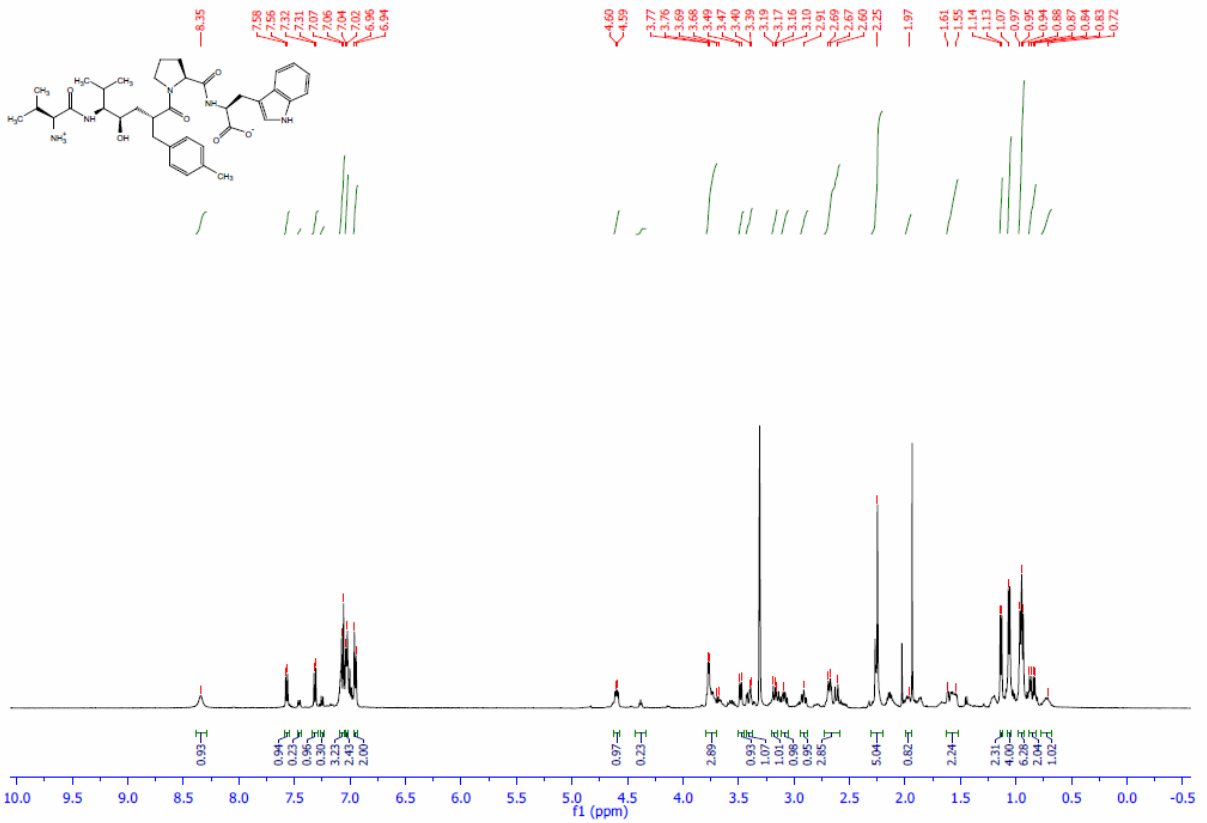
^{1}H and ^{13}C spectra of methyl ((2*R*,4*R*,5*S*)-5-((*tert*-butoxycarbonyl)amino)-4-((*tert*-butyldimethylsilyl)oxy)-6-methyl-2-(4-methylbenzyl)heptanoyl)-L-prolyl-L-tryptophanate (17)

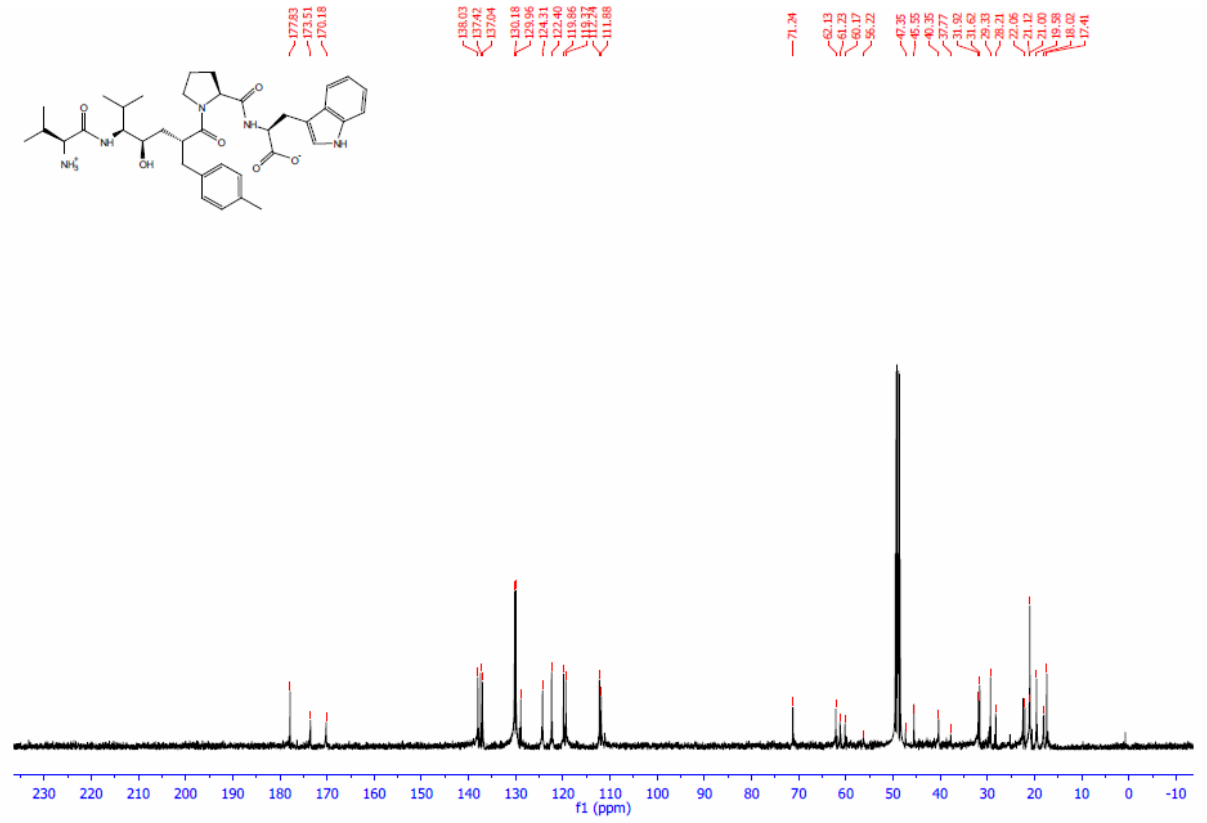


^{1}H and ^{13}C spectra of methyl ((2*R*,4*R*,5*S*)-5-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methylbutanamido)-4-hydroxy-6-methyl-2-(4-methylbenzyl)heptanoyl)-L-prolyl-L-tryptophanate (18)

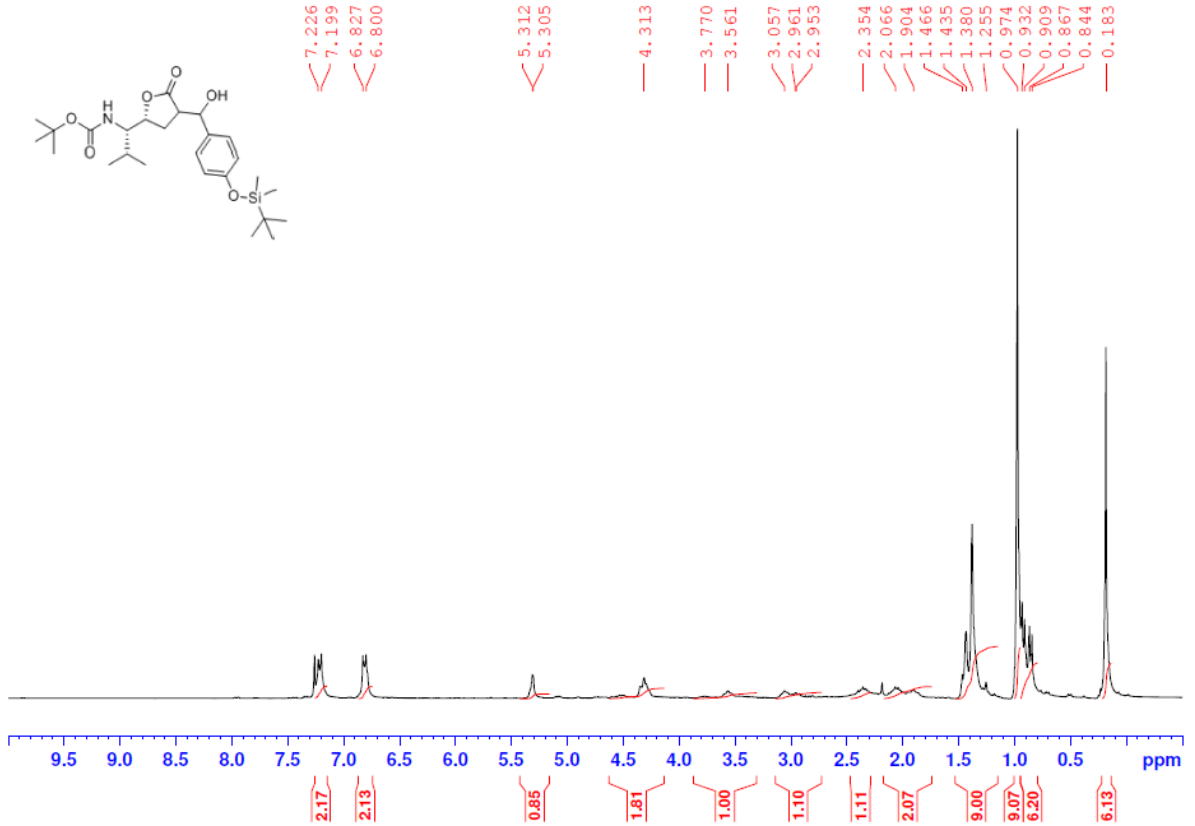


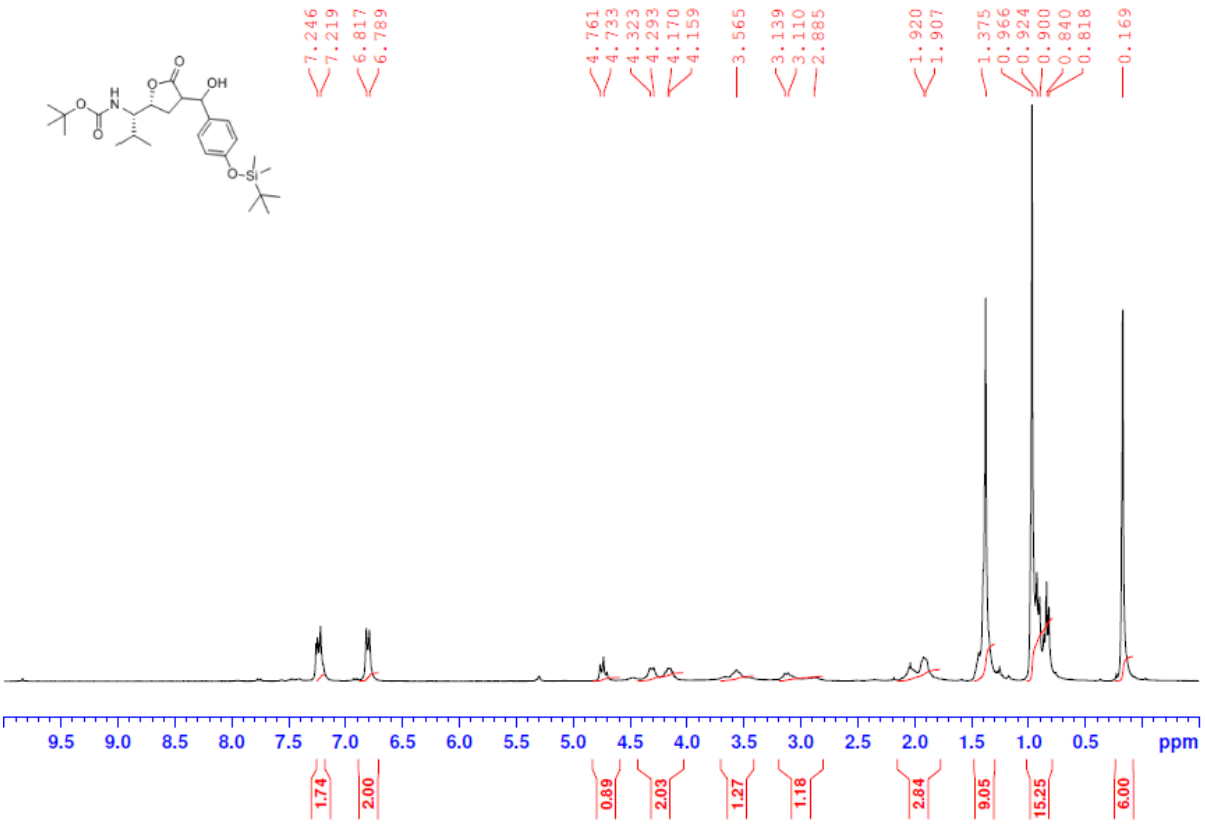
^{1}H and ^{13}C spectra of ((2*R*,4*R*,5*S*)-5-((*S*)-2-ammonio-3-methylbutanamido)-4-hydroxy-6-methyl-2-(4-methylbenzyl)heptanoyl)-L-prolyl-L-tryptophanate (19)



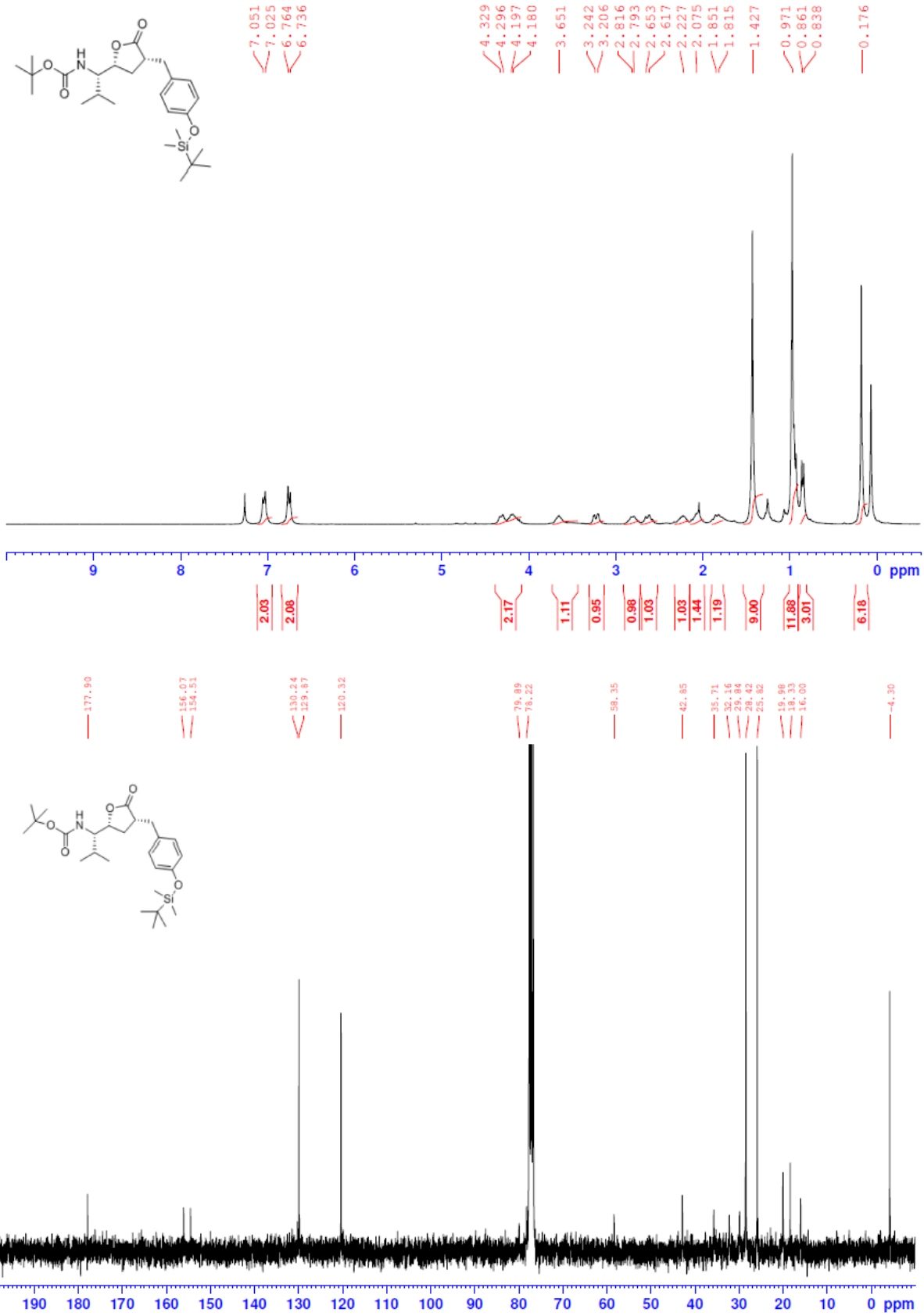


^{1}H and ^{13}C spectra of *tert*-butyl ((1*S*)-1-((2*R*)-4-((4-((*tert*-butyldimethylsilyl)oxy)phenyl)(hydroxy)methyl)-5-oxotetrahydrofuran-2-yl)-2-methylpropyl)carbamate (20)

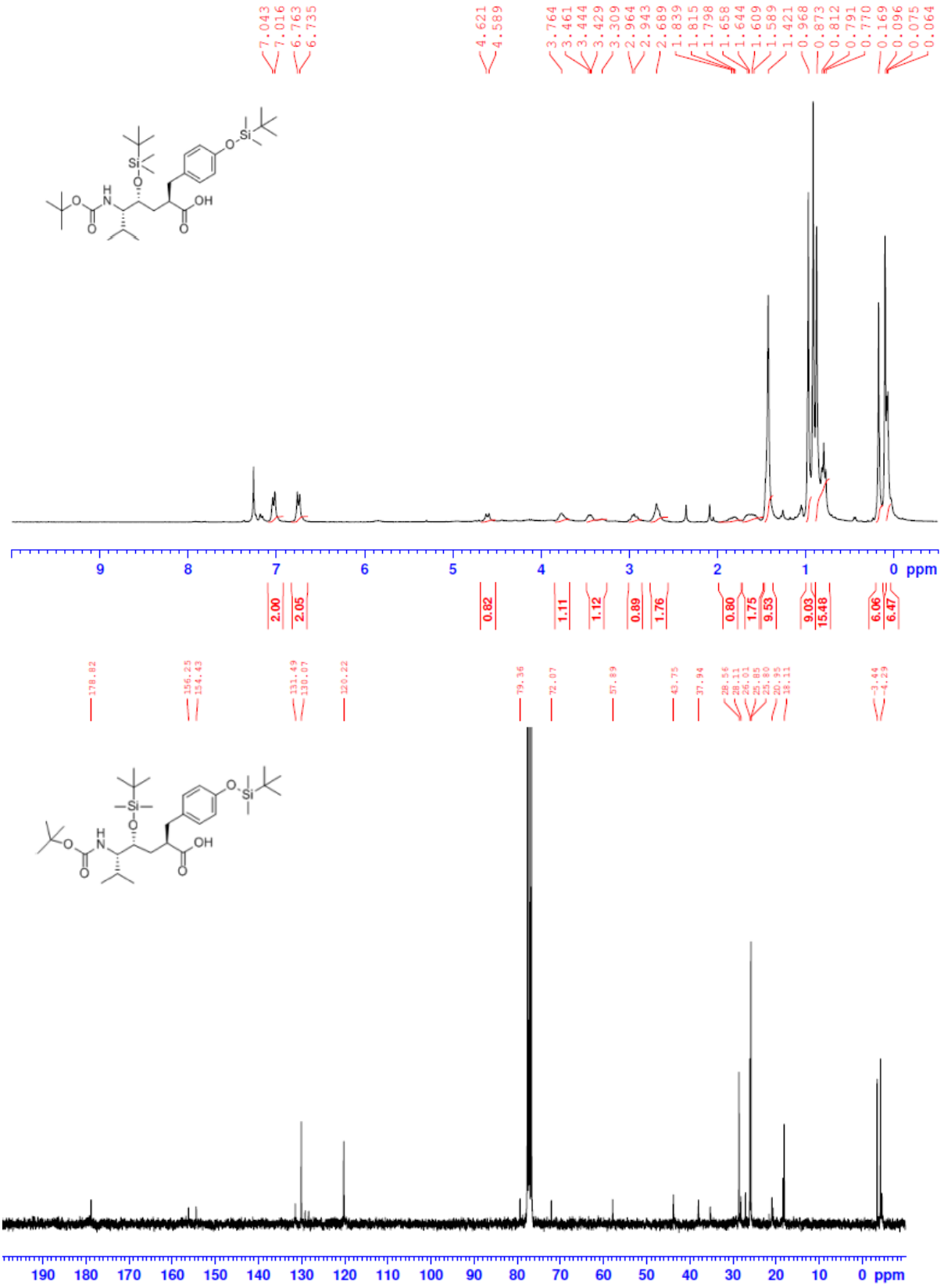




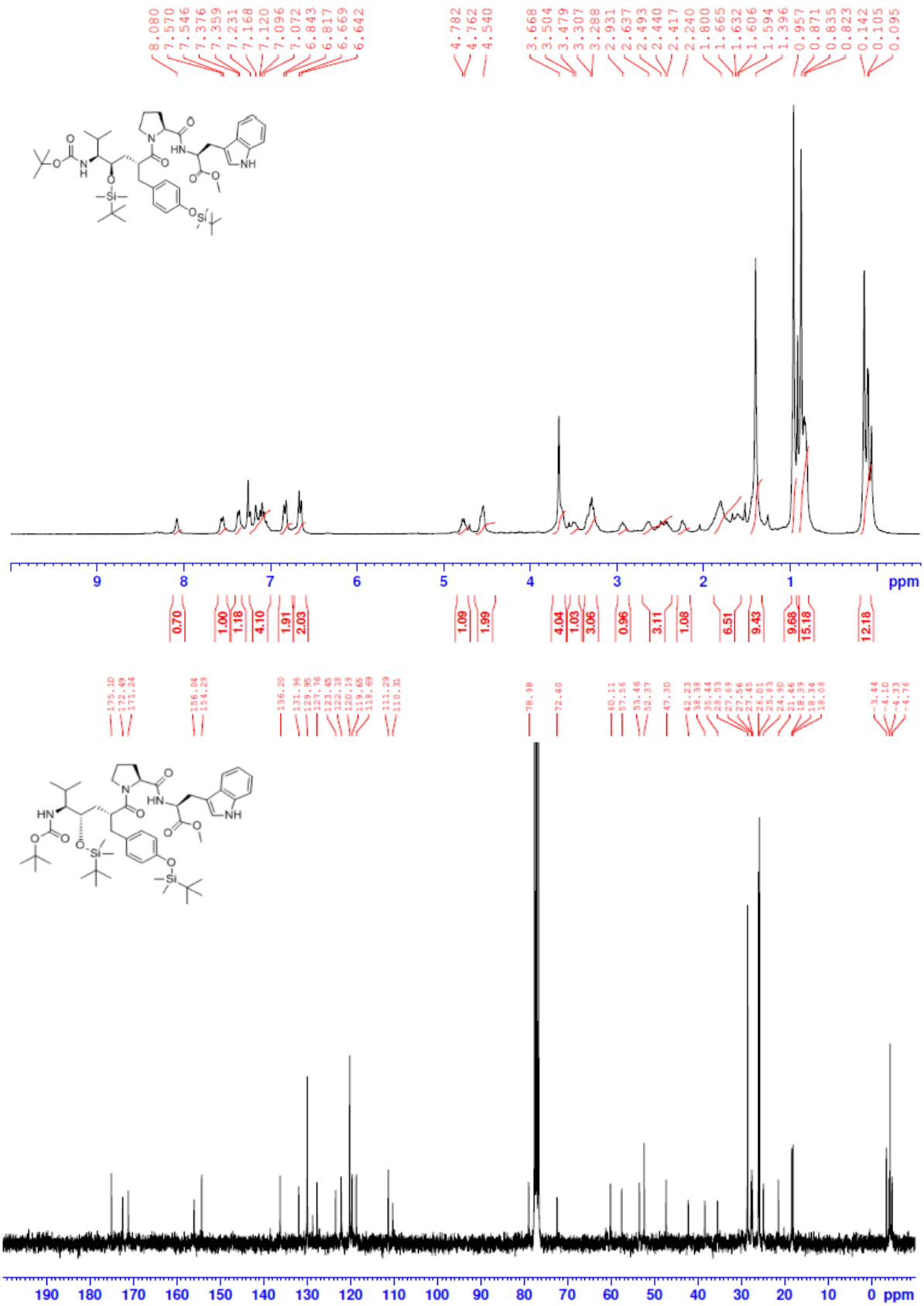
^{1}H and ^{13}C spectra of *tert*-butyl ((*S*)-1-((2*R*,4*R*)-4-(4-((*tert*-butyldimethylsilyl)oxy)benzyl)-5-oxotetrahydrofuran-2-yl)-2-methylpropyl)carbamate (21)



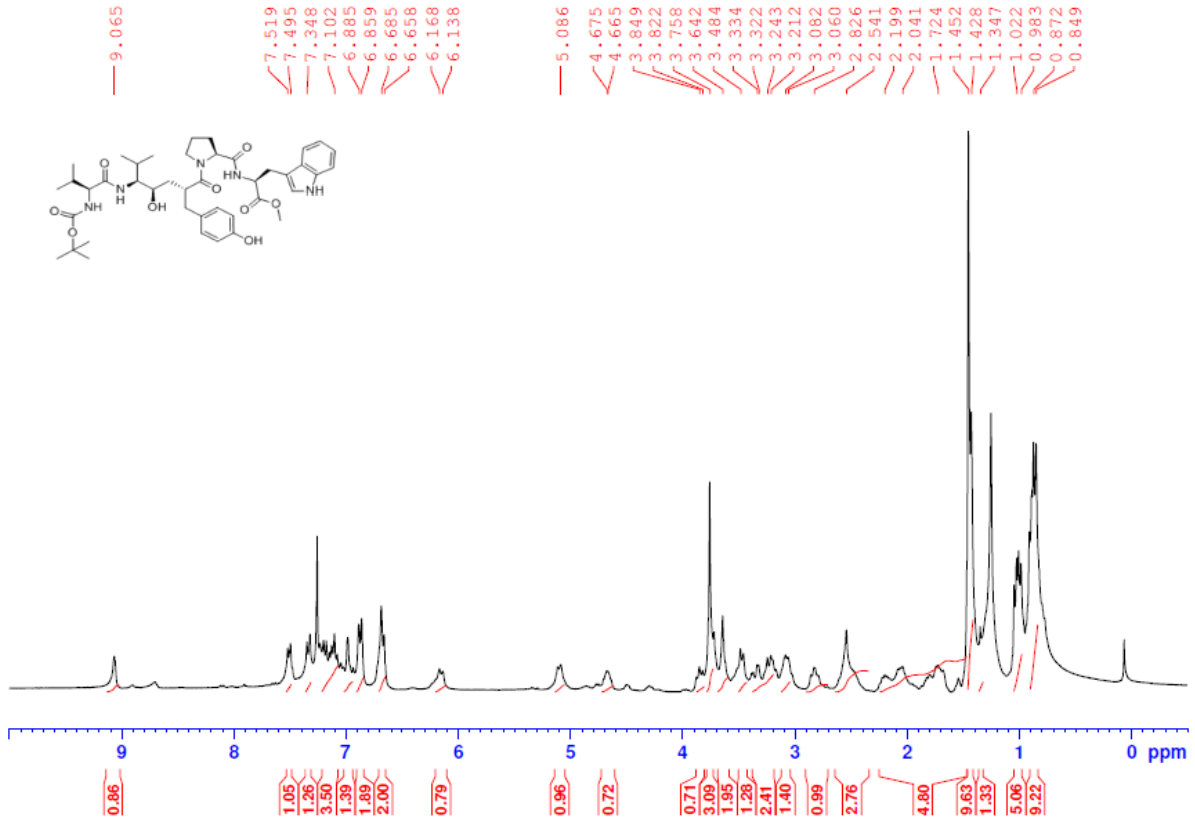
^{1}H and ^{13}C spectra of (2*R*,4*R*,5*S*)-5-((*tert*-butoxycarbonyl)amino)-4-((*tert*-butyldimethylsilyl)oxy)-2-(4-((*tert*-butyldimethylsilyl)oxy)benzyl)-6-methylheptanoic acid (22)

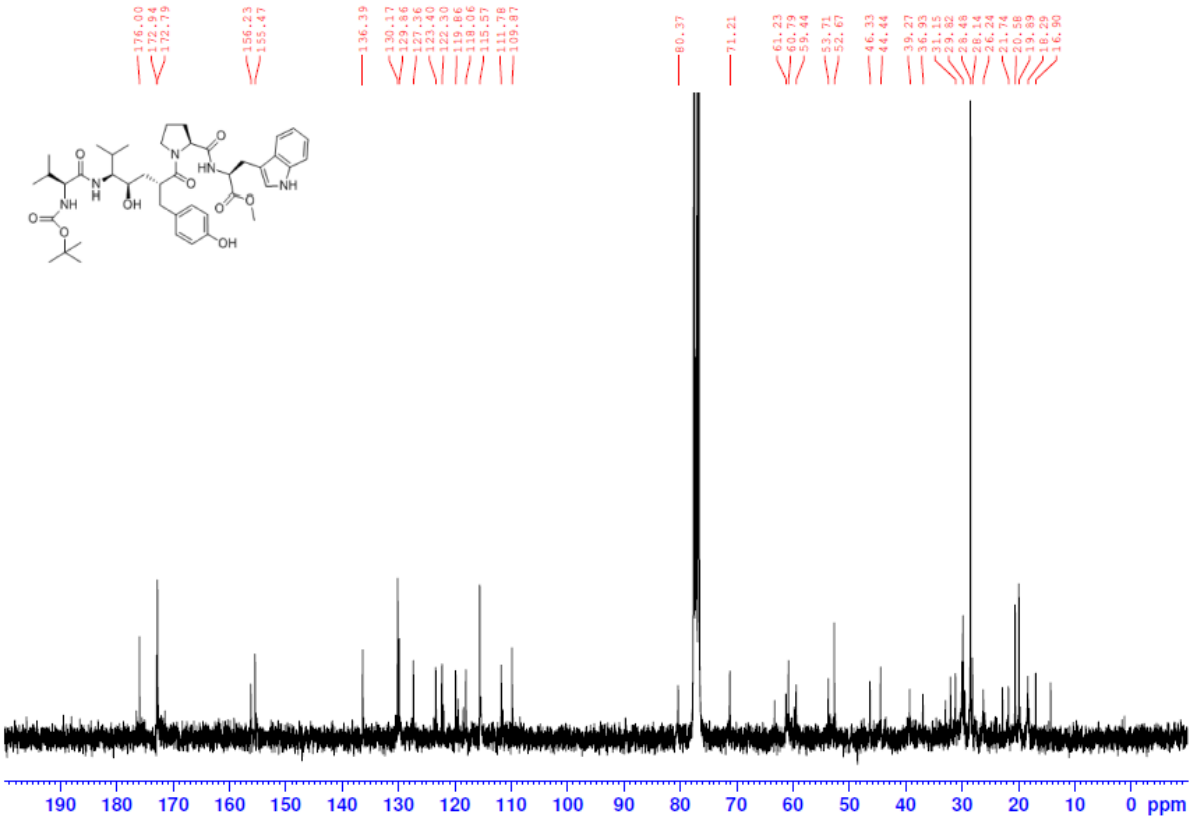


^{1}H and ^{13}C spectra of methyl ((2*R*,4*R*,5*S*)-5-((*tert*-butoxycarbonyl)amino)-4-((*tert*-butyldimethylsilyl)oxy)-2-(4-((*tert*-butyldimethylsilyl)oxy)benzyl)-6-methylheptanoyl)-L-prolyl-L-tryptophanate (23)

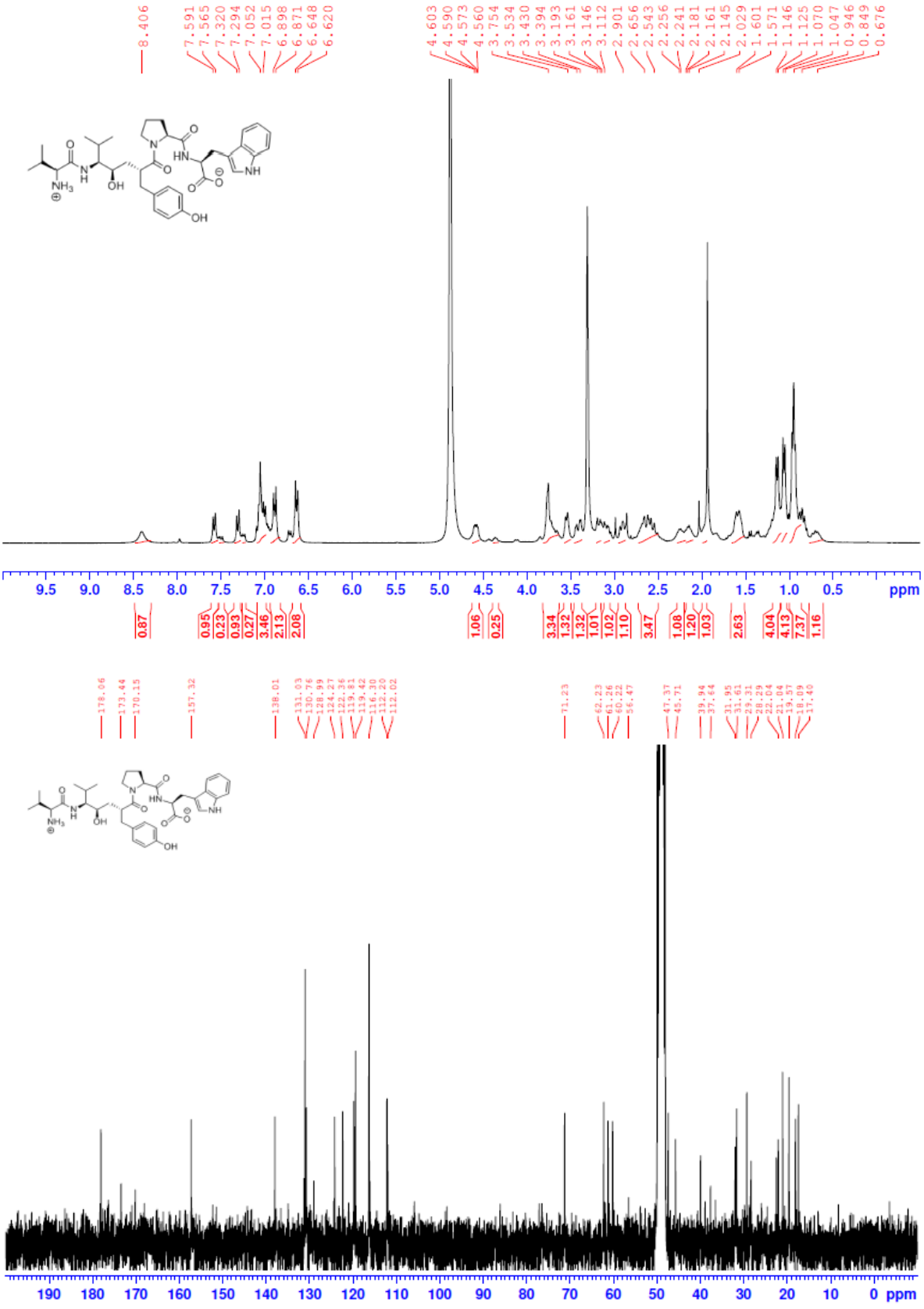


^{1}H and ^{13}C spectra of methyl ((2*R*,4*R*,5*S*)-5-((*S*)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-4-hydroxy-2-(4-hydroxybenzyl)-6-methylheptanoyl)-L-prolyl-L-tryptophanate (24)

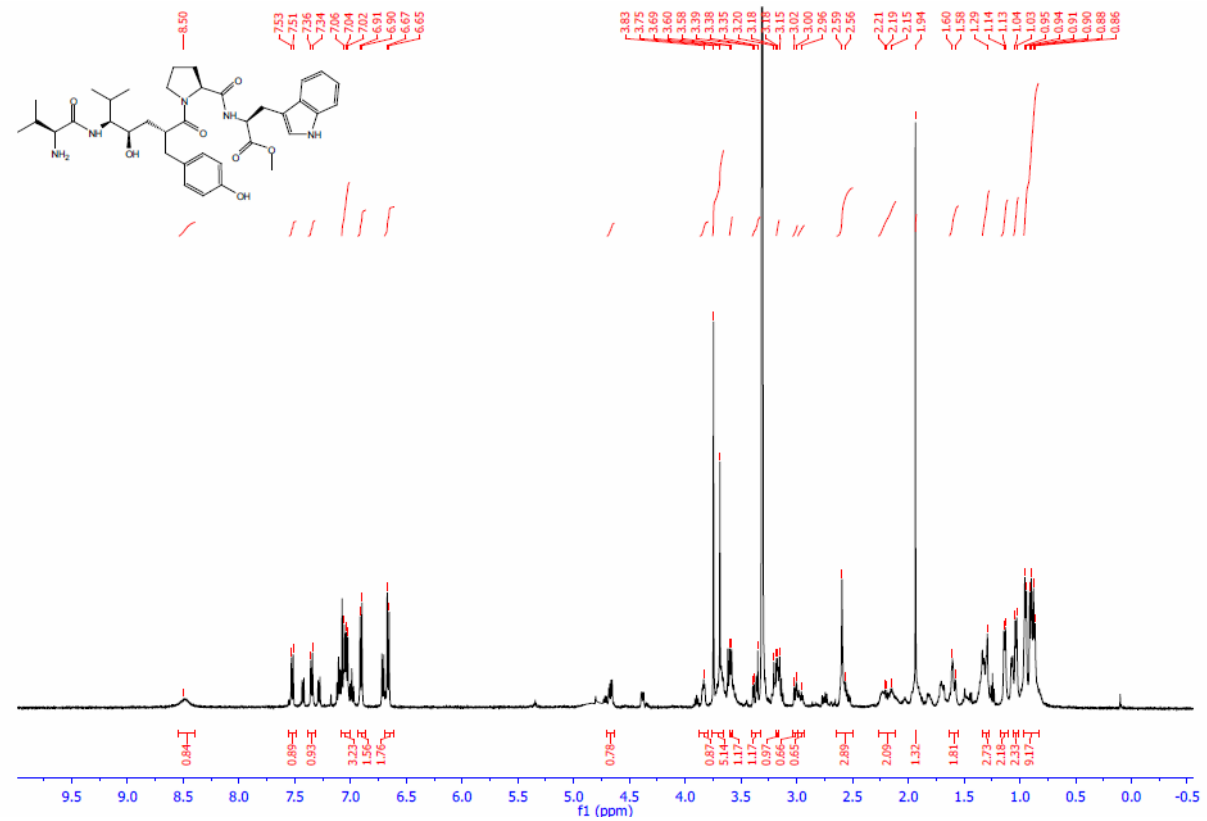


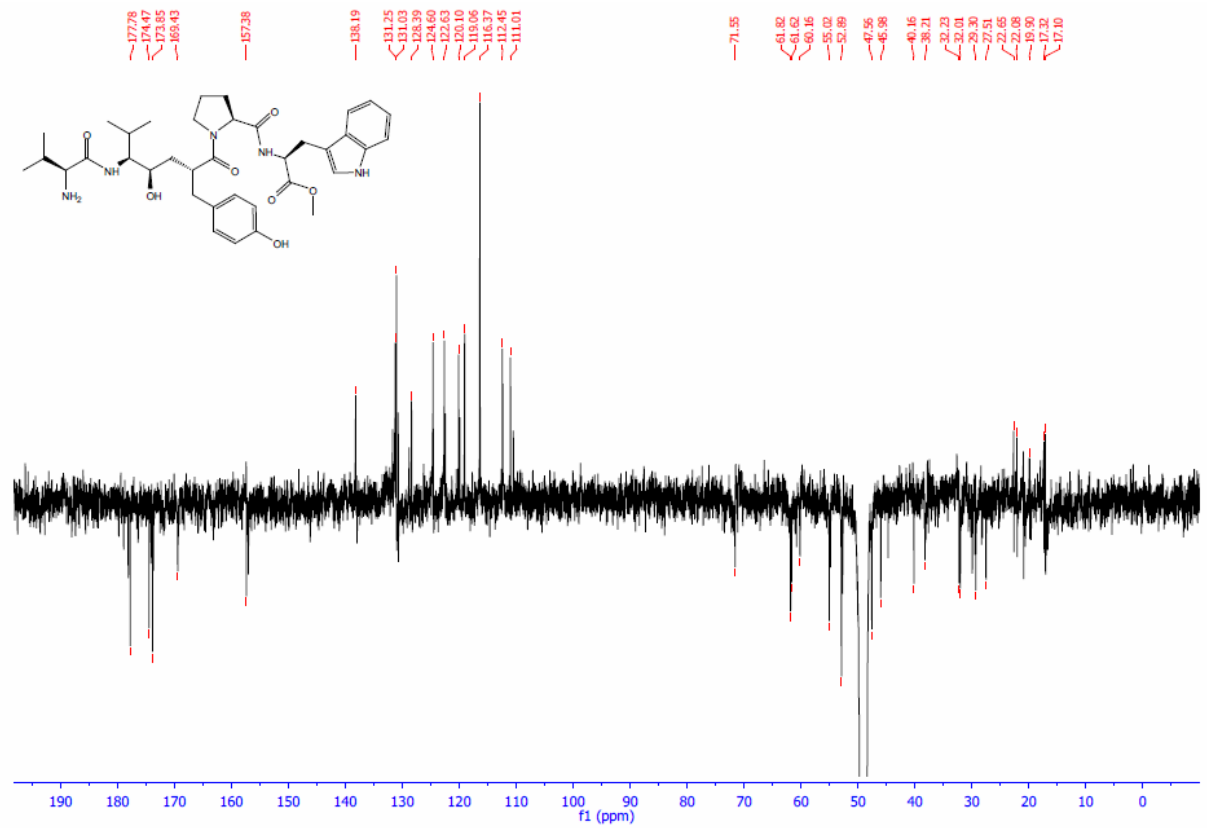


^{1}H and ^{13}C spectra of ((2*R*,4*R*,5*S*)-5-((*S*)-2-ammonio-3-methylbutanamido)-4-hydroxy-2-(4-hydroxybenzyl)-6-methylheptanoyl)-L-prolyl-L-tryptophanate (25)

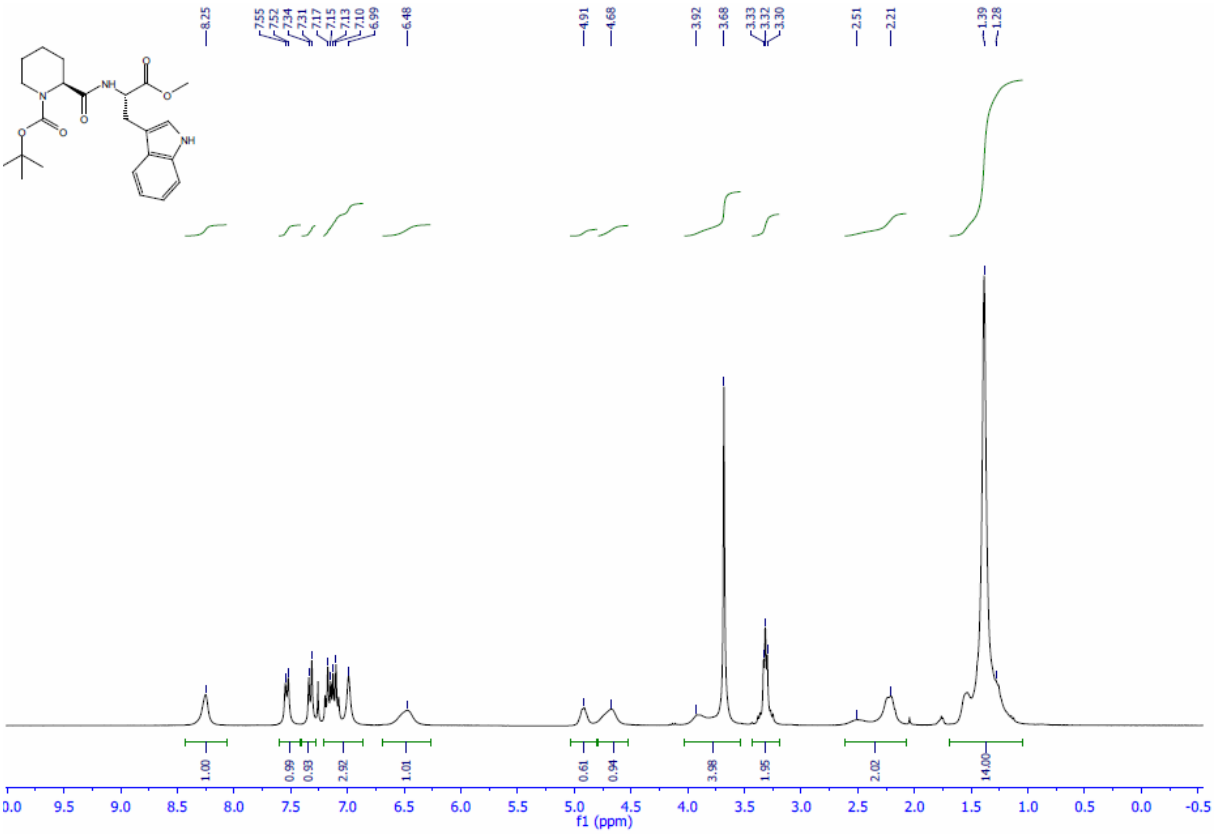


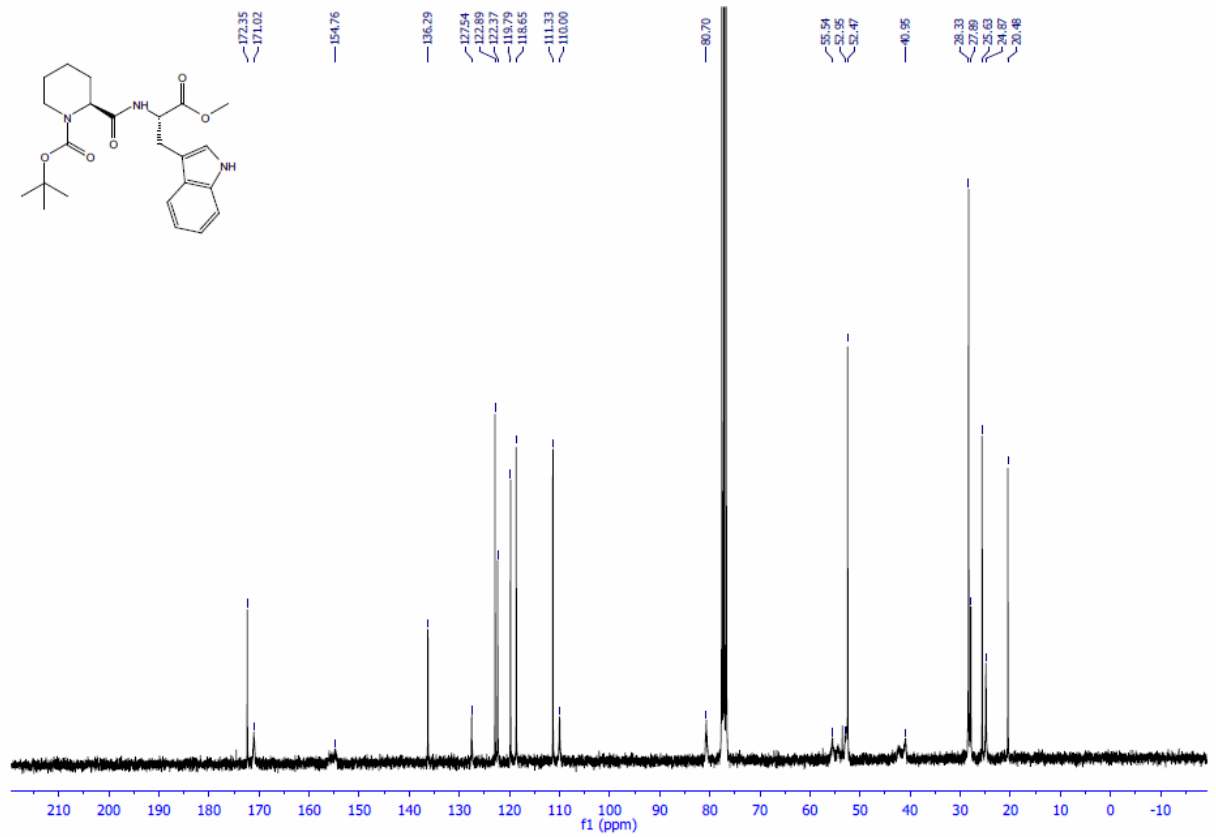
^{1}H and ^{13}C spectra of methyl ((2*R*,4*R*,5*S*)-5-((*S*)-2-amino-3-methylbutanamido)-4-hydroxy-2-(4-hydroxybenzyl)-6-methylheptanoyl)-L-prolyl-L-tryptophanate (26)



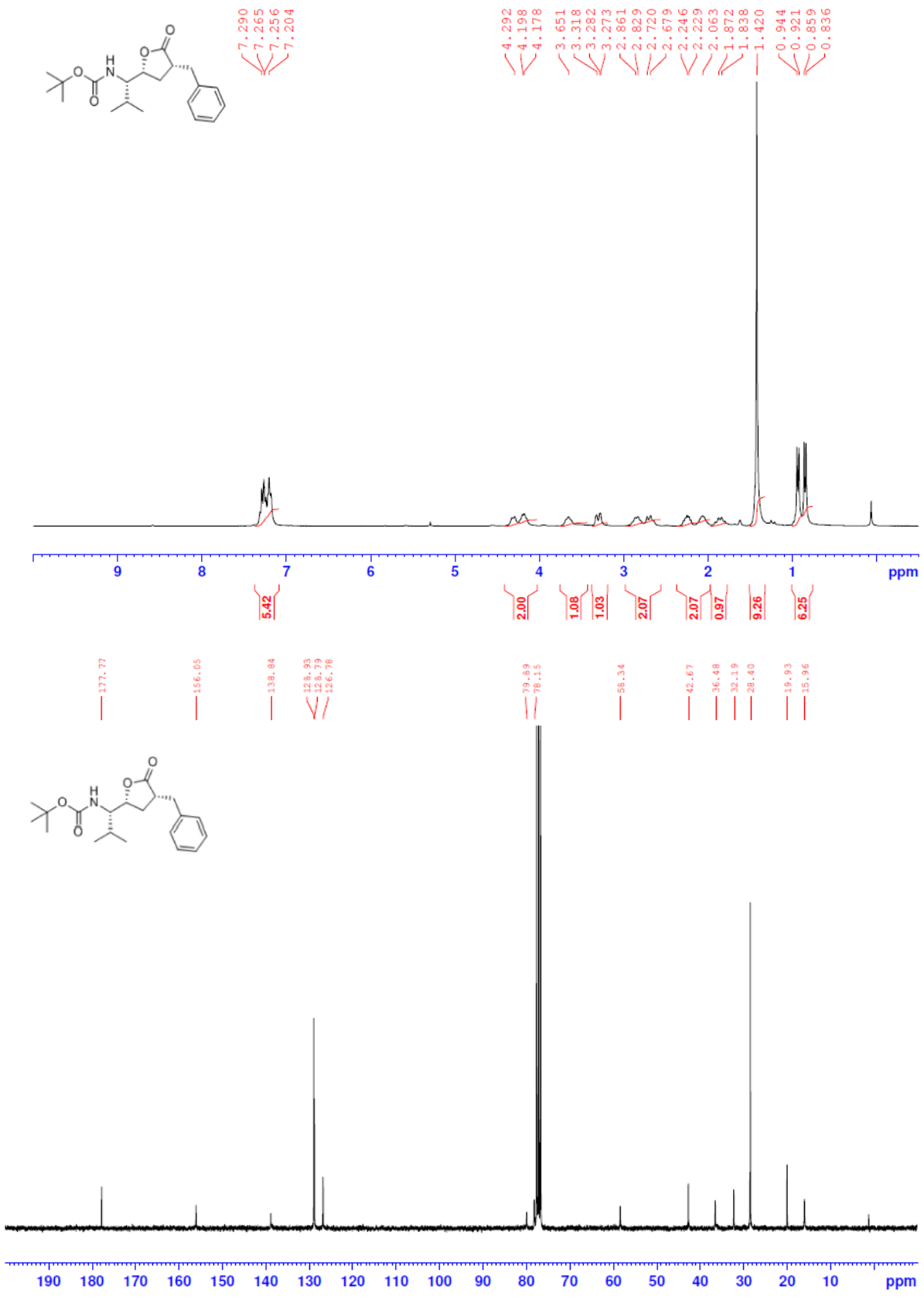


^{1}H and ^{13}C spectra of *tert*-butyl (*S*)-2-(((*S*)-3-(1*H*-indol-3-yl)-1-methoxy-1-oxopropan-2-yl)carbamoyl)piperidine-1-carboxylate (27)

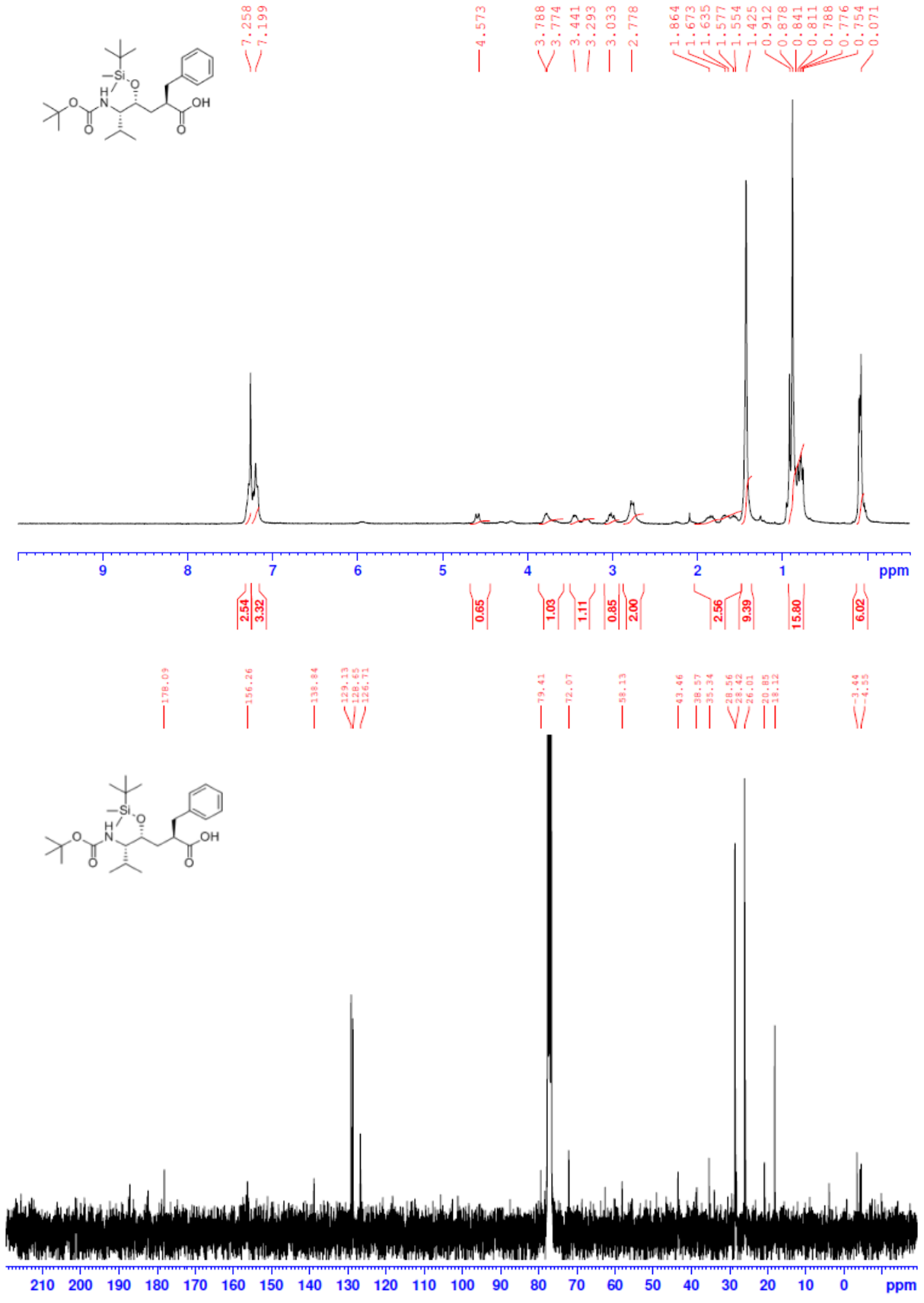




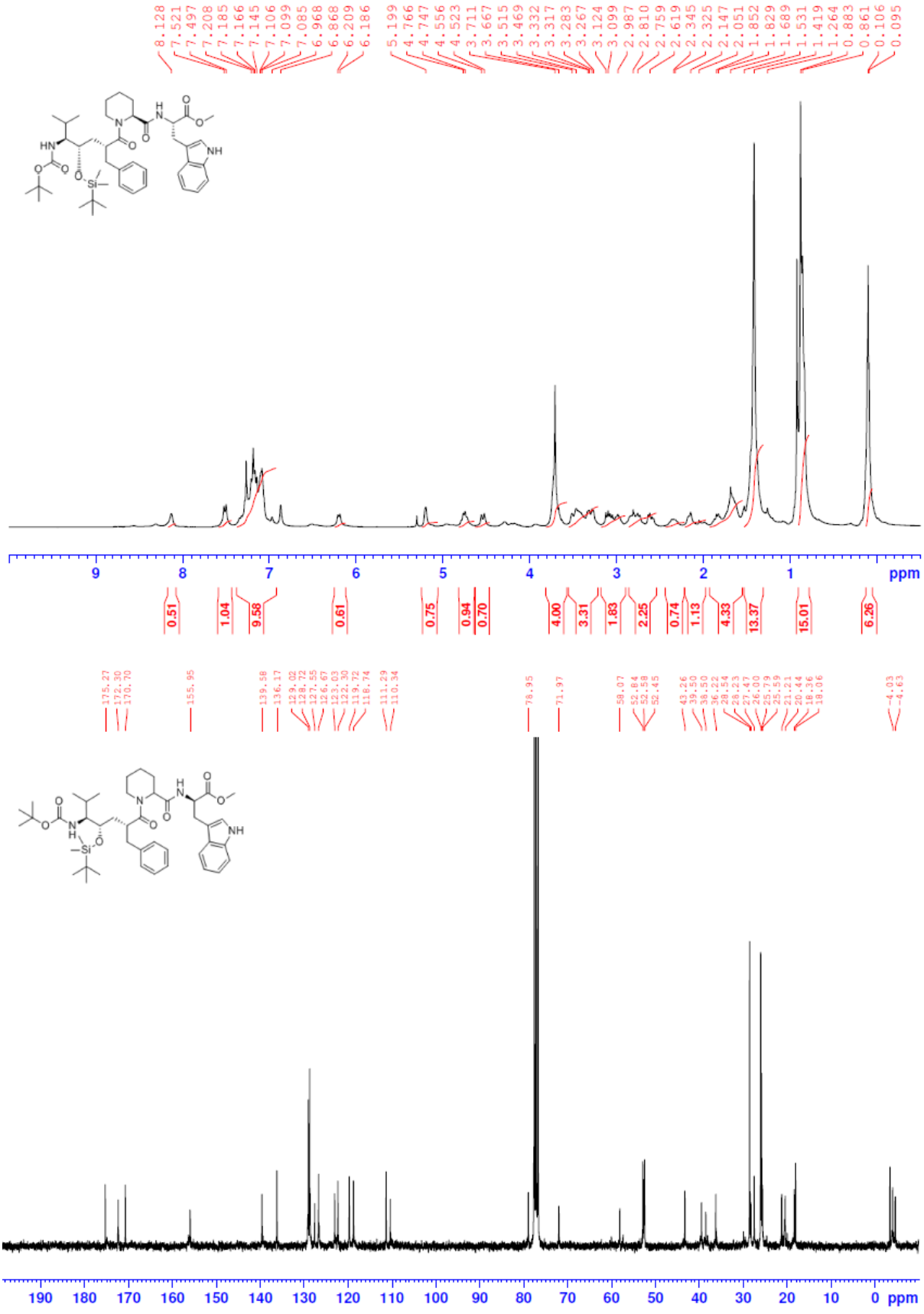
^{1}H and ^{13}C spectra of *tert*-butyl ((*S*)-1-((2*R*,4*R*)-4-benzyl-5-oxotetrahydrofuran-2-yl)-2-methylpropyl)carbamate (28)



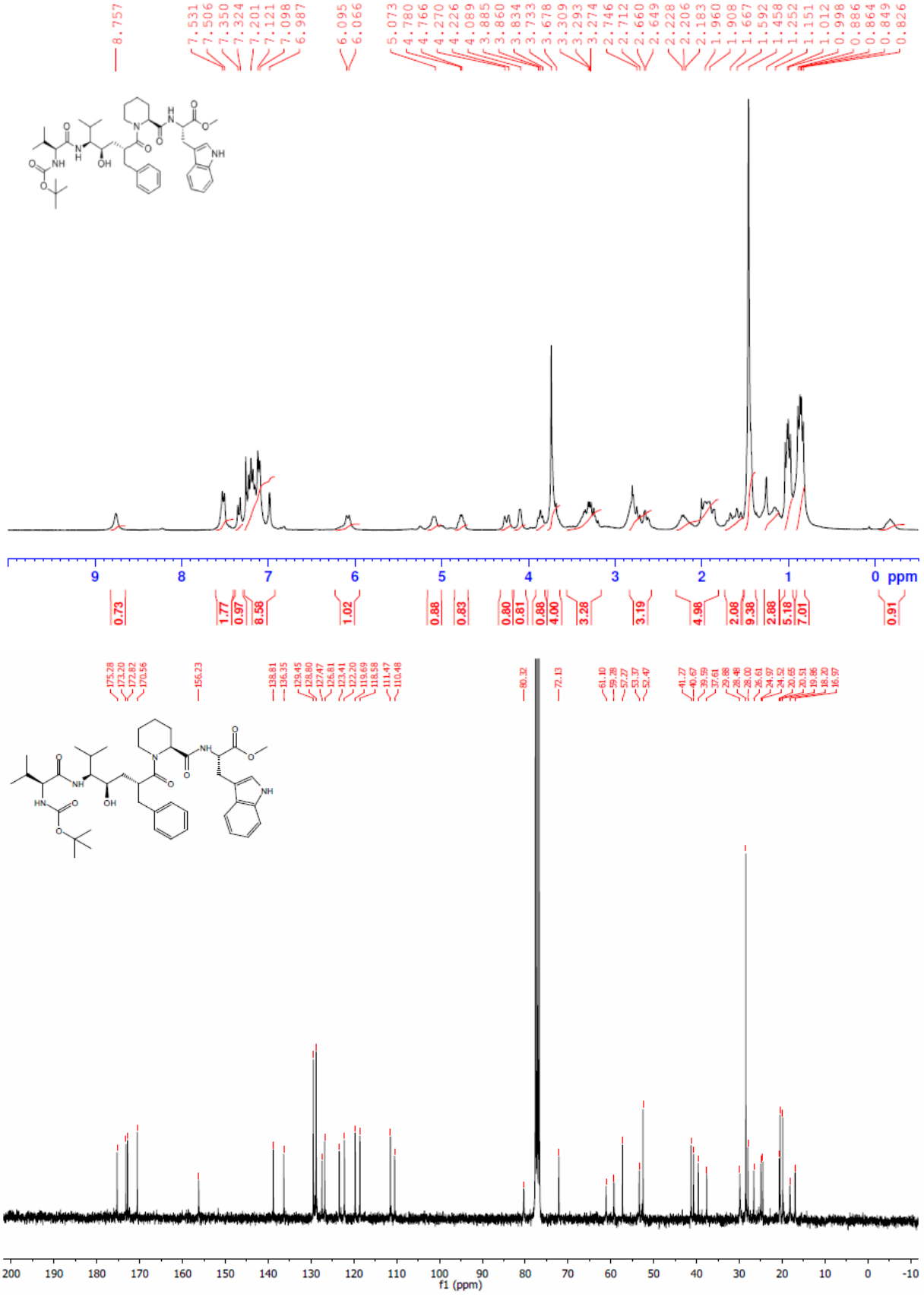
^{1}H and ^{13}C spectra of (2*R*,4*R*,5*S*)-2-benzyl-5-((*tert*-butoxycarbonyl)amino)-4-((*tert*-butyldimethylsilyl)oxy)-6-methylheptanoic acid (29)



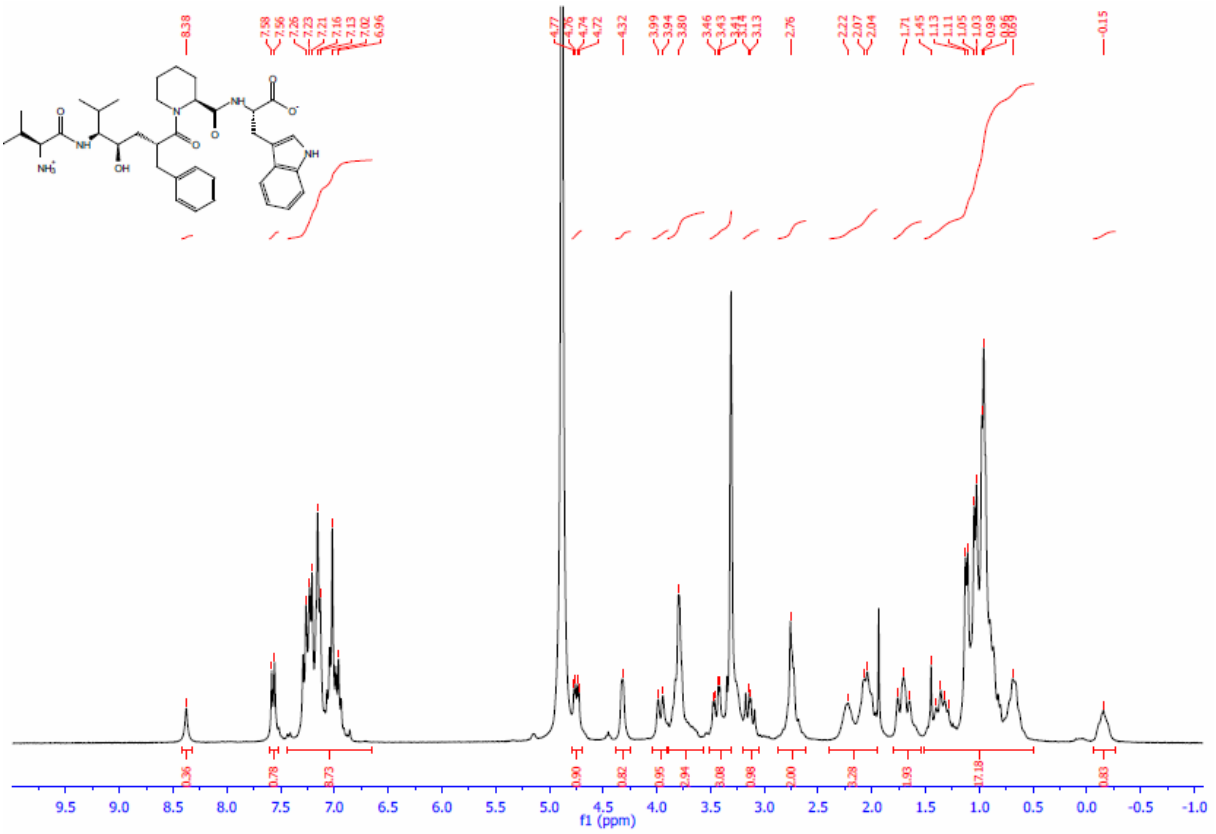
^{1}H and ^{13}C spectra of methyl ((*S*)-1-((2*R*,4*R*,5*S*)-2-benzyl-5-((*tert*-butoxycarbonyl)amino)-4-((*tert*-butyldimethylsilyl)oxy)-6-methylheptanoyl)piperidine-2-carbonyl)-L-tryptophanate (30)

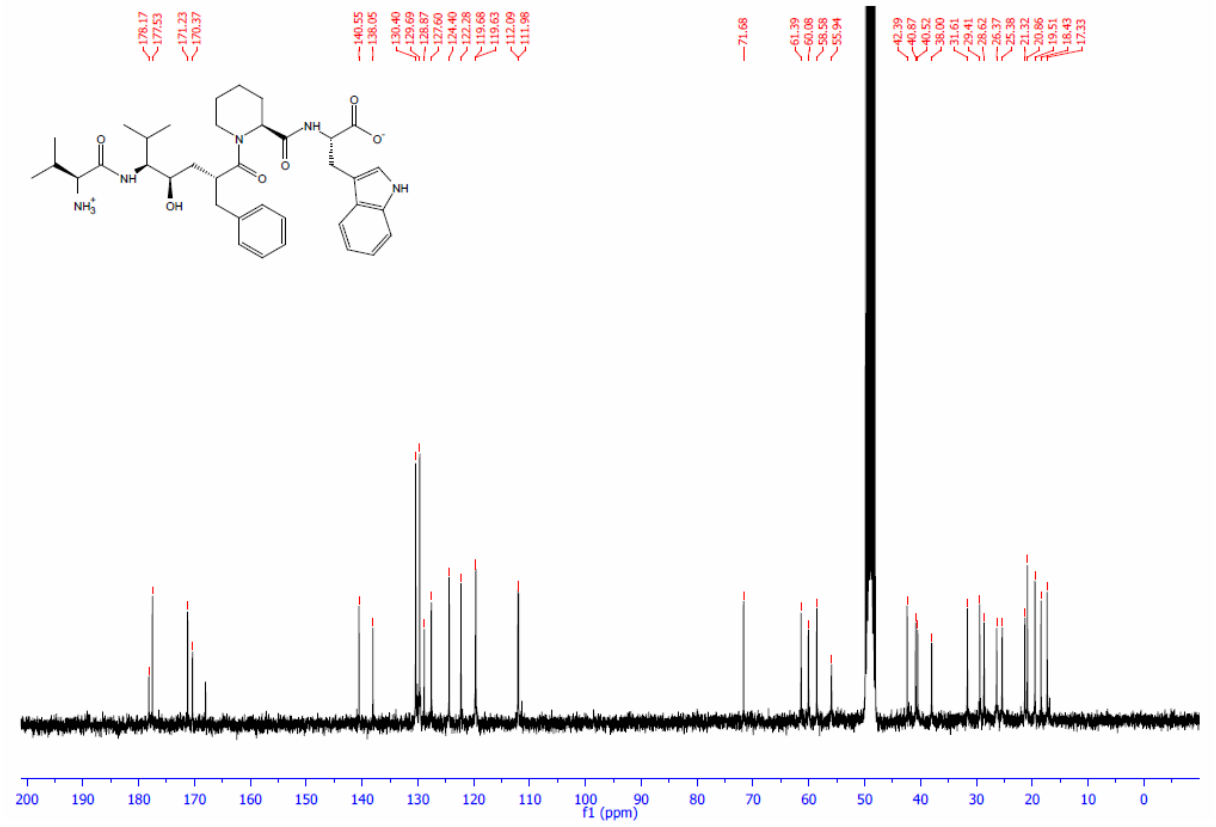


^{1}H and ^{13}C spectra of methyl ((*S*)-1-((2*R*,4*R*,5*S*)-2-benzyl-5-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methylbutanamido)-4-hydroxy-6-methylheptanoyl)piperidine-2-carbonyl)-L-tryptophanate (31)

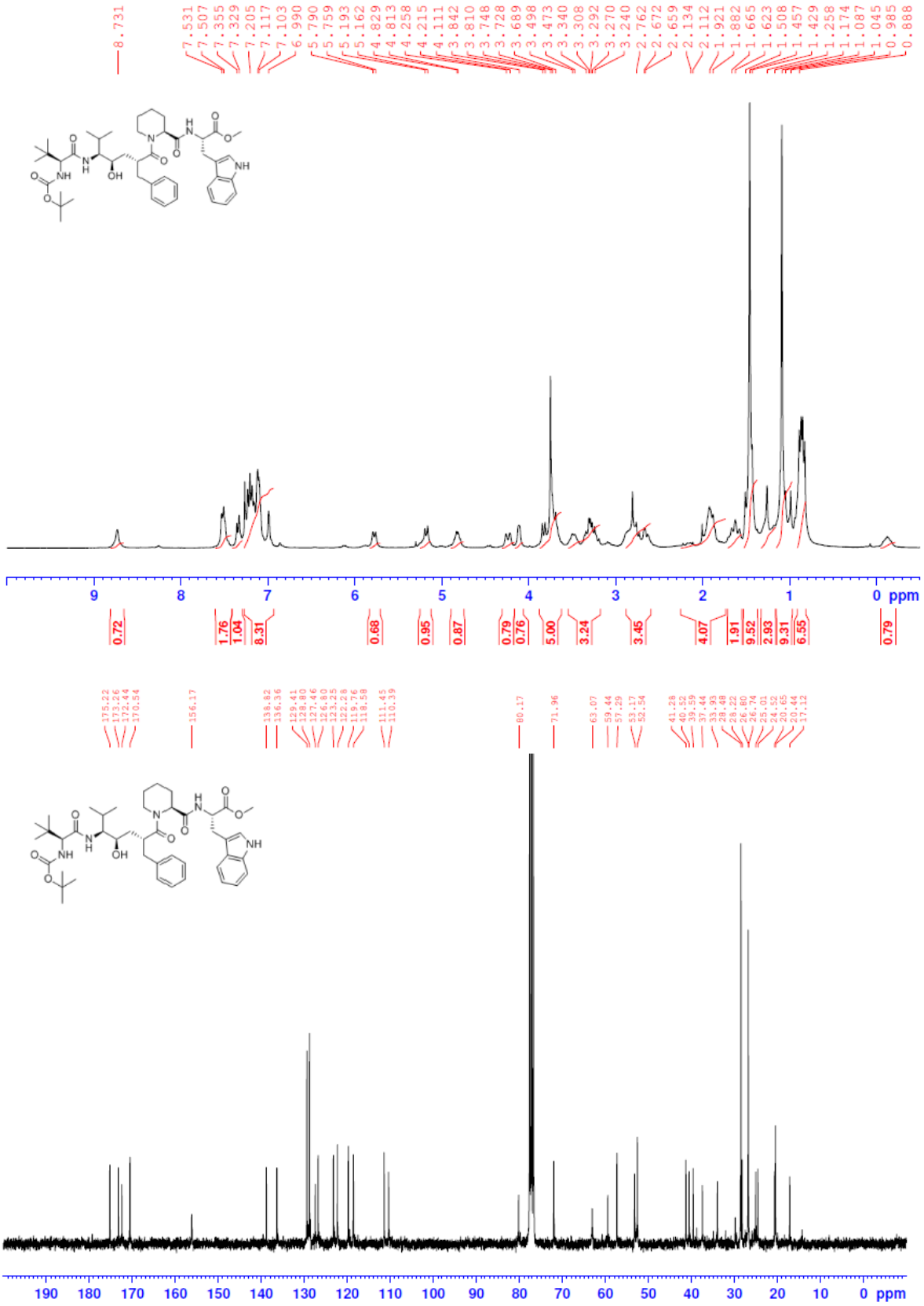


^{1}H and ^{13}C spectra of ((*S*)-1-((2*R*,4*R*,5*S*)-5-((*S*)-2-ammonio-3-methylbutanamido)-2-benzyl-4-hydroxy-6-methylheptanoyl)piperidine-2-carbonyl)-L-tryptophanate (32)





^{1}H and ^{13}C spectra of methyl ((*S*)-1-((2*R*,4*R*,5*S*)-2-benzyl-5-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3,3-dimethylbutanamido)-4-hydroxy-6-methylheptanoyl)piperidine-2-carbonyl)-L-tryptophanate (33)



^{1}H and ^{13}C spectra of ((*S*)-1-((2*R*,4*R*,5*S*)-5-((*S*)-2-ammonio-3,3-dimethylbutanamido)-2-benzyl-4-hydroxy-6-methylheptanoyl)piperidine-2-carbonyl)-L-tryptophanate (34)