# Design, Synthesis and Biological Evaluation of Conformationally Constrained Helicokinin I Analogues


DISSERTATION

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## Abstract

Insect neuropeptides have been known as important components in regulating many physiological and behavioural processes in insect life such as growth, molting, metamorphosis, homeostasis, osmoregulation, water balance, reproduction and diuresis. Comprising of a short sequence of 6-15 amino acids, insect kinins and their receptors promise excellent targets for a novel generation of highly selective and environmental friendly insecticides.

Belonging to the myokinin family, helicokinin I displays a high biological activity against *Heliothis virescens* in a functional bioassay. Moreover, helicokinin I inhibits weight gain and increases mortality after injection into larvae of *Heliothis virescens*, a serious agricultural pest. Determining the preferred conformation of the receptor bound peptide is a crucial step for the design of selective ligands in developing novel insecticidal agents. It is therefore more and more important to develop suitable strategies that aim at mimicking the structure and activity of biologically interesting peptides in order to design new effective insecticides.

The first part of this thesis is dedicated to the design and synthesis of β-turn helicokinin I peptidomimetics. Herein, the synthesis and incorporation of 4-*trans*-aminopyroglutamates, as type VI β-turn inducers, into the native helicokinin I peptide backbone are presented. In addition, two convenient strategies for stabilizing the conformational preferences of helicokinin I analogues will be described.

In the second part the important role of the Trp side chain on the secondary structure of helicokinin I will be investigated. This includes the preparation of conformationally constrained building blocks derived from the Trp moiety using Pictet-Spengler reaction or xanthate radical transfer reagents.

The third part of this thesis deals with a cycloscan that utilizes the available hydroxy functional groups on Ser and Tyr residues. Formation of macrocyclic peptides using either a ring-closing metathesis reaction or Mitsunobu etherification is described.

The last section focuses on further investigations of the structure-activity relationships. The use of conformationally constrained structures, such as β-proline amino acid and spirolactam, for replacing selectively helicokinin I residues is investigated.

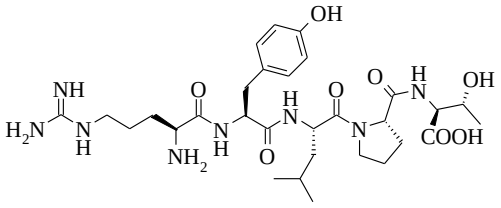
# I. Introduction

## 1.1 Insect neuropeptides

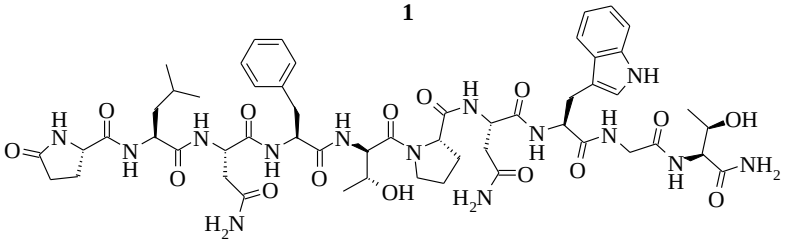
Insect neuropeptides are cellular components that are released from specialized neuroglandular cells and transported through the haemolymph to the target organs. Consisting of 5 to about 80 amino acids linked by amide bonds, insect neuropeptides create functionally and structurally diverse signaling substances in insects.

Early 1922, Stefan Kopec proposed that processes of moulting and metamorphosis in insects are controlled by a hormone in the brain,[1] as suggested by the concept of neurosecretion that was first described by Berta Scharrer and Ernst Scharrer, in 1937.[2] Some years later, in 1945, they defined the term of neurosecretory organs and gland-like nerve cell in both vertebrates and invertebrates, and described the similarities between the corpora cardiac-corpora allata system in insects.[3] However, until the middle of the 1970s, there have been only two insect neuropeptides isolated and identified. The first pentapeptide proctolin (**1**, Figure 1) was isolated, from an extraction of adult American cockroaches *Periplaneta Americana in 1975*.[4] It showed potent myotropic activity in the cockroach hindgut. The other peptide, adipokinetic hormone (AKH) (**2**, Figure 1), produced by glandular cells of the corpus cardiacum, was reported by Stone in 1976.[5] In the next ten years, there were only a few insect neuropeptides isolated due to difficulties in isolating of those small amounts at that time. Nevertheless, in the late 1980s and early 1990s with the rapid improvement of separation and identification methods, such as matrix-assisted laser desorption/ ionization time of flight mass spectroscopy (MALDI-TOP-MS), refined high-performance liquid chromatography (HPLC), microsequencing techniques, fast atom bombardment mass spectroscopy (Fab-MS) and genetic engineering technologies, the number of isolated and sequenced insect neuropeptides increased dramatically. Most of which were isolated from cockroaches (*Leucophaea maderae*, *Periplaneta Americana*, *Diploptera punctata*), locusts (*Losuta migratoria*) and moths (*Manduca sexta*, *Bombyx mori* and various Heliothinae species). In particular a significant achievement in insect neuropeptide receptor research on *Drosophila* genome, in the year 2000, opened a great opportunity for studying insect neuropeptides and their

receptors.[6] Recently, 44 genes of the G-protein coupled receptors (GPSRs) encoded by the *Drosophila* genome have been reported.[7]



**1**



**2**

**Figure 1:** Structure of proctolin **(1)** and adipokinetic hormone (AKH) **(2)**

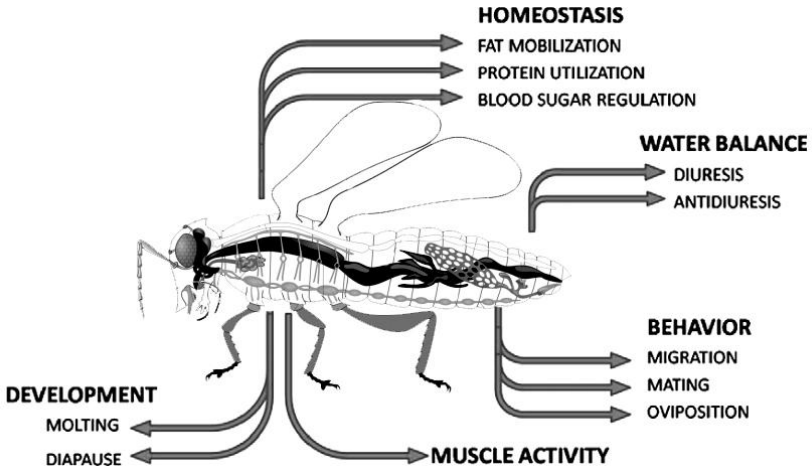



**Figure 2:** Overview on functions of insect neuropeptides[8]

Neuropeptides and peptide hormones are involved in many physiological processes and regulate every aspect of insect life. Based on their functional roles, neuropeptides can be grouped into four major categories (Figure 2).[8,9]

- Growth and development
- Behaviour and reproduction

- Metabolism and homeostasis
- Muscle activity

## 1.2 Neuropeptides as potential ligands for pest control

As the largest group of all animal species, insects have extremely important roles in the ecological system. Besides the multifaceted beneficial aspects, for instance the pollination of flowering plants or killing the pests, a large number of insect species cause severe damage to crop and transfer serious diseases to humans and animals, such as malaria and sleeping sickness. Due to their great impact on humans and agriculture, 50 insect genome projects have been initiated and half of which have been completed.[10,11]

Insecticides play a pivotal role in achieving high-yield crop production. Traditionally, insecticidal agents are neurotoxic compounds that contaminate the environment with toxic residues, endanger humans and disrupt the ecological balance. In addition, uncontrolled application of chemical insecticides has led to resistance in insect species.

Apart from conventional insecticides, several other methods are used in controlling insect pests, such as natural product extracts from plants, natural enemies, microbial insecticides and recombinant baculovirus strains.[12,13] However, all these applications have particular restrictions that prevent the replacement for neurotoxic organic insecticides. Thus, the requirements for developing ecofriendly, effective and non-toxic insecticides that could eventually replace the conventional insect control agents are essential.

Insect neuropeptides, as presented in Figure 2, regulate the most critical functions in insect such as embryonic and postembryonic development, osmoregulation, muscle activity, migration and oviposition. Understanding the mode of action of the key functional neuropeptides may provide the basic physiological mechanism of the general interest from which the use of superagonist or antagonist may intervene in the normal growth, development and behavior of insect life. Thus, insect neuropeptides and their receptors promise a prime target in the development of novel insectidal agents that present higher levels of selectivity and environmental compatibility, which are absent from neurotoxic insecticides. Despite their potential, insect neuropeptides have been not utilized as agricultural insecticides so far because of some major drawbacks:

- Due to their high molecular weight and hydrophobic nature, neuropeptides are poorly absorbed across membrane barriers of insects, especially across the cuticle.
- Neuropeptides are environmental fragile.
- Neuropeptides are only partially soluble in both organic solvents and water.
- Neuropeptides are highly susceptible to proteolytic degradation by enzymatic hydrolysis in the insect gut and hemolymph, and have a poor bioavailability.

To overcome these inherent limitations in the physiological properties of peptides, the development of peptidomimetics represents an effective strategy to improve their biological effects.

## 1.3 Neuropeptide receptors

### 1.3.1 Insect G-protein coupled receptors

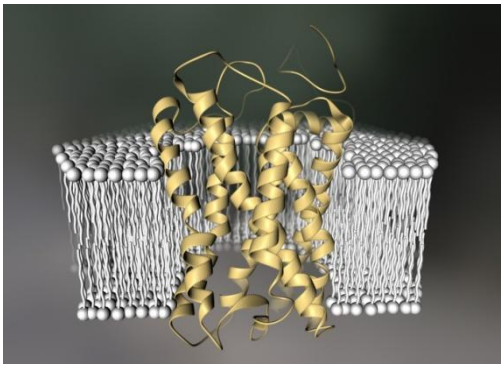


**Figure 3:** Structure of a membrane[a]

G-protein coupled receptors (GPCRs) constitute the largest family of integral membrane proteins (Figure 3) and are responsible for transmission of extracellular signals to intracellular responses. More than 5,000 different GPCRs have been identified, and many of them have been implicated as major therapeutic targets for the treatment of human diseases.[14] It is estimated that more than 50% of current therapeutic agents on the market are targeted to these receptors.[15]

GPCRs are composed of seven hydrophobic transmembrane-spanning (7TM) α-helices, from 20 to 25 amino acid residues, with an extracellular *N*-terminus, an intracellular *C*-terminus and three

[a] http://www.scientificimages.co.uk/Proteins.htm

interhelical loops on each side of the membrane (Figure 4).[16,17] Insect G-protein coupled receptors are classified into four families: family A, the largest group including rhodopsin-like receptors; family B, including secretin-like and adhesion receptors; family C, including metabotropic glutamate receptor; and atypical GPCRs such as Frizzled/Smoothened family.

Binding of ligands, such as hormones and neurotransmitters, to GPCRs from the extracellular side of the membrane may induce various conformational changes that propagate to the cytoplasmic surface, leading to an activation of G proteins and a consequent change in the level of intracellular messengers such as cAMP, $Ca^{2+}$ or signaling lipids.[18,19] Consequently, controlling these activation processes and conformational changes could be a useful approach for the development of potential drugs with fewer side effects and more favorable pharmacological properties.

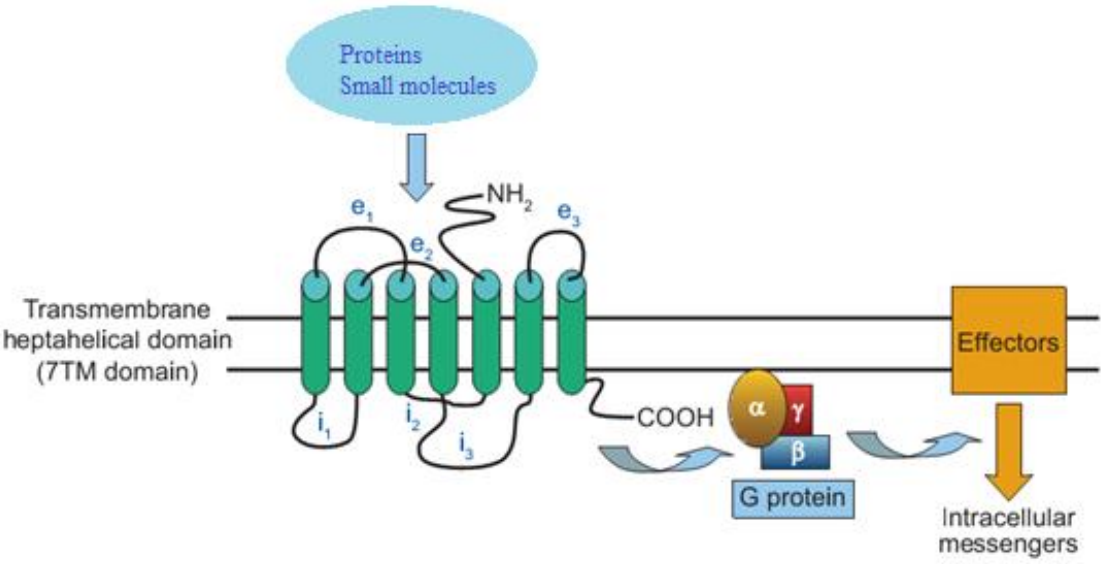



**Figure 4:** Representative structure of genetic GPCRs[17]

From the drug discovery and rational design point of view, GPCRs are important for drug design, particularly 3D-structural information of the GPCRs is considered as a key tool for structure-based drug designs. Although the advances in technologies for structure-based design have recently improved, the approaches to GPCR targets have remained challenging due to difficulties in purifying and crystallizing of membrane proteins.[20,21] To date, only a few 3D-structures characterized by high-resolution X-ray crystallography, namely bovine and squid

rhodopsin,[20,22] $\beta_1$- and $\beta_2$-adrenergic receptor,[23-25] and adenosine $A_{2A}$ receptor,[26] are available. In particular, the first NMR-based structural determination of a detergent-solubilized GPCR was published in 2010.[27]

### 1.3.2 Biologically active conformation

Research for the biologically active neuropeptide conformation is principally based on the receptor-bound state. Unfortunately, this work is extremely difficult to accomplish because insect neuropeptides bind to GPCRs. Much attention to identify receptor-bound conformations of GPCR ligands in aqueous solution by NMR spectroscopy has been focused so far, such as pituitary adenylate cyclase-activating peptide (PACAP),[28] neurotensin,[29] and the backbone of bradykinin.[30] For all other neuropeptides, however, only indirect information on the receptor bound conformations is currently available. The most popular strategy is based on conformationally restricted neuropeptide analogues as for instance cyclic peptides that can easily be analyzed by conventional NMR spectroscopy.[31,32]

Another approach, commonly employed to neuropeptides, is based on the determination of conformations in the membrane-bound state. According to the membrane-compartment theory, the target cell surface influences the receptor selection of regulatory peptides by increasing peptide concentration in the vicinity of the receptor and by inducing preferred conformations and orientations.[33-37] According to the model of ligand binding to membrane-embedded receptors (Figure 5),[38] the accumulation of ligands on the surface membrane is first directed by electrostatic interactions that regulate both negatively charged phospholipids in the membrane components and cationic ligand residues. In the next step, the ligand is reoriented in such a way that the hydrophobic side chains penetrate into the membrane. Subsequently, the ligand diffuses laterally along the membrane until a particular state from which the peptide is recognized by the receptor. The initial conformation of the peptide in this transition would be close to the membrane-bound state, but may undergo further conformational changes following an "induced-fit" mechanism. Another outcome of surface accumulation is the decrease in the dimensionality of the "receptor search" by the ligand from three-dimensional to two-dimensional diffusion, which may be beneficial to low surface concentrations of the receptors.

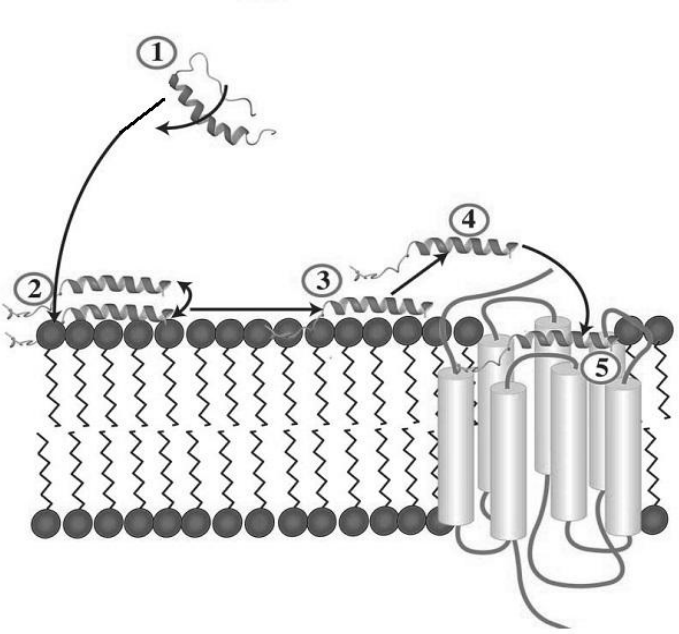



**Figure 5:** Schematic presentation of receptor-binding mechanism:[38] 1. Unligated peptide in bulk solution, 2. Movement to the membrane and reorientation, 3. Lateral diffusion, 4. Initial recognition by the receptor, 5. Receptor bound state.

During the past two decades, several membrane-bound conformations of signal peptides, such as neuropeptide Y,[38] cholecystokinin-8,[39] bradykinin,[40] the endomorphins,[41] enkephalins,[42] myosupressin, tachykinin and helicokinins[43] have been elucidated by NMR techniques using dodecylphosphocholine (DPC) or sodium dodecyl-sulfate (SDS) micelles as the membrane-mimicking environments.[44,45]

### 1.3.3 Insect kinin receptors as promising target sites for the development of pesticides

G-protein coupled receptors (GPCRs) are responsible for a large number of physiological processes, such as sensory transduction, mediation of hormonal activity, and cell-to-cell communication. Thus, modern drug developments are targeted on GPCRs discovery. Upon determining the preferred conformation of receptor-bound peptides, the design of selective peptidomimetic ligands could be accomplished by far more efficiently. However, because of the lack of three-dimensional structures, structure-based drug designs are difficult. Instead, receptor-binding characteristics that are deduced from structural analysis of ligands and their analogues

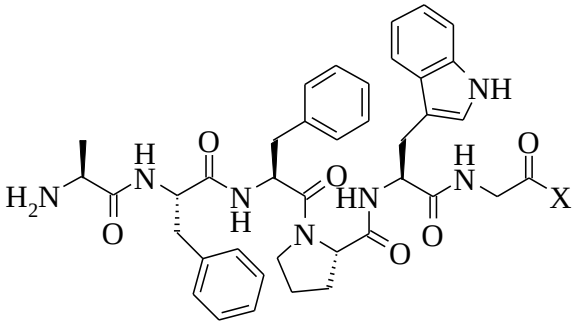
could provide a rational basis for probing receptor structures and designing conformationally restricted agonist or antagonists. Consequently, characterization of these designed analogues could offer further information on structural requirements for receptor binding and signal transduction.

**Table 1:** Selected myokinins from various insect species[46]

| Insect species | | Name | Sequence |
|---|---|---|---|
| Cricket | *Acheta domesticus* | Achetakinin I | Ser-Gly-Ala-Asp-**Phe**-Tyr-Pro-**Trp**-**Gly**-$NH_2$ |
| | | Achetakinin II | Ala-Tyr-**Phe**-Ser-Pro-T**rp**-**Gly**-$NH_2$ |
| | | Achetakinin III | Ala-Leu-Pro-**Phe**-Ser-Ser-**Trp**-**Gly**-$NH_2$ |
| Mosquito | *Aedes aegypti* | Aedeskinin I | Asn-Ser-Lys-Tyr-Val-Ser-Lys-Gln-Lys-**Phe**-Tyr-Ser-**Trp**-**Gly**-$NH_2$ |
| | | Aedeskinin II | Arg-Pro-**Phe**-His-Ala-**Trp**-**Gly**-$NH_2$ |
| | | Aedeskinin III | Arg-Arg-Pro-Arg-Val-**Phe**-Tyr-Pro-**Trp**-**Gly**-$NH_2$ |
| Mosquito | *Culex salinarius* | Culekinin I | Arg-Pro-**Phe**-His-Ser-**Trp**-**Gly**-$NH_2$ |
| | | Culekinin II | Arg-Arg-Ala-Arg-Val-**Phe**-Tyr-Pro-**Trp**-**Gly**-$NH_2$ |
| | | Culekinin III | Trp-Lys-Tyr-Val-Ser-Lys-Gln-**Phe**-Phe-Ser-**Trp**-**Gly**-$NH_2$ |
| Moth | *Heliothis zea* | Helicokinin I | Tyr-**Phe**-Ser-Pro-**Trp**-**Gly**-$NH_2$ |
| | | Helicokinin II | Val-Arg-**Phe**-Ser-Pro-**Trp**-**Gly**-$NH_2$ |
| | | Helicokinin III | Lys-Val-Lys-**Phe**-Ser-Ala-**Trp**-**Gly**-$NH_2$ |
| Cockroach | *Leucophaea maderae* | Leucokinin I | Asp-Pro-Ala-**Phe**-Asn-Ser-**Trp**-**Gly**-$NH_2$ |
| | | Leucokinin II | Asp-Pro-Gly-**Phe**-Ser-Ser-**Trp**-**Gly**-$NH_2$ |
| | | Leucokinin III | Asp-Gln-Gly-**Phe**-Asn-Ser-**Trp**-**Gly**-$NH_2$ |
| Locust | *Locusta migratoria* | Locustakinin | Ala-**Phe**-Ser-Ser-**Trp**-**Gly**-$NH_2$ |

Insect kinins share a highly conserved *C*-terminal pentapeptide sequence $Phe^1$-$Xaa^2$-$Xbb^3$-$Trp^4$-$Gly^5$-$NH_2$, where $Xaa^2$ is Tyr, His, Ser or Asn, and $Xbb^3$ is Ala, Ser, Pro (Table 1). The first member of insect kinin neuropeptides, Leukopyrokinin, was isolated from the cockroach *Leucophaea maderae* in 1986.[47] Since then, over 30 peptides have been isolated and identified from various species of Dictyoptera, Lepidoptera and Orthopera.[48] The other diuretic peptide family in insects are the corticotrophin-releasing factor (CRF)-related peptides, consisting of 30-37 amino acids in length, which were isolated from *Tenebrio molitor*, *Manduca sexta*, *Acheta domesticus*, *Locusta migratoria*. The role of the CRF-related peptides acts as diuretic hormones that stimulate Malpighian tubule fluid secretion through a cyclic-AMP as secondary messengers.

**Figure 6:** Myokinin mimetics with various substitutions at the *C*-terminus[49]

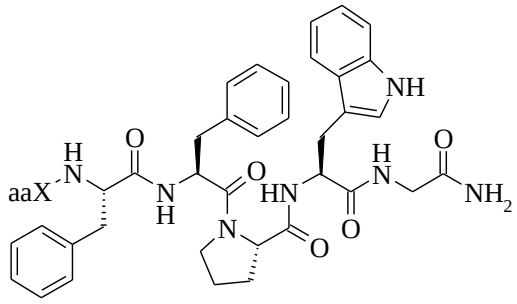


**3**

**Table 2:** Diuretic activity of derivatives **3**

| **3** | **X** | $EC_{50}$ (nM) |
|---|---|---|
| **a** | $NH_2$ | 0.004 |
| **b** | OH | 1000 |
| **c** | OMe | 20 |
| **d** | SMe | 5 |
| **e** | OEt | 100 |
| **f** | $OCH_2Ph$ | 50 |
| **g** | NHMe | 28 |
| **h** | $NMe_2$ | Inactive |

**Figure 7:** Myokinin mimetics with various substitutions at the *N*-terminus[50]



**4**

**Table 3:** *N*-Terminal variations of peptide **4**

| Xaa | $EC_{50}$ (nM) |
|---|---|
| H | 0.1 |
| Trp | 0.042 |
| Phe | 0.029 |
| Arg | 0.020 |
| Ala | 0.005 |
| Lys | 0.005 |
| Aib | 0.005 |
| Asp | 0.002 |
| Leu | 0.002 |
| Asn | 0.001 |
| Val | 0.001 |

Initial structure-activity relationship studies on the insect kinins revealed that the intact *C*-terminal pentapeptide sequences are required to elicit fully myotropic and diuretic activity, which therefore represents the active core sequence. Within the active domain, a series of alanine-substituted analogues proved that Trp and Phe are the most significant residues for full biological activity. Functional variations at the *C*-terminus of AFFPWG-X (**3**, Figure 6) in *Acheta domesticus*, for instance, demonstrated the importance of the *C*-terminal amidation (Table 2).[49] The diuretic and myotropic activity is completely lost when the *C*-terminal amide of insect kinins is replaced with a negatively charged acid moiety, whereas the replacements by ester groups (**3c** and **3e**) or thioester (**3d**) reduced remarkably the diuretic activity. When modified at the *N*-terminus, hexapeptide analogues aaX-FFPWG-$NH_2$ (**4**, Table 3) displayed high stimulation of fluid secretion in the isolated cricket Malpighian tubules Ramsay assay. In

particular when Xaa corresponded to Asp, Leu, Asn and Val, analogues **4** demonstrated the most superagonistic activity in comparison with the native achetakinin.[50]

Recent experiments demonstrated that several members of the insect kinins are easily hydrolyzed and therefore deactivated by both angiotensin-converting enzyme (ACE), which only cleaves at the primary hydrolysis site, and neprilysin (NEP) that cleaves insect kinins at both the primary and secondary hydrolysis sites.[51] The most sensitive sites for hydrolysis are the amide bonds between Trp and Xbb (mostly Ser and Pro). To overcome this restriction, the Xbb residues of the *C*-terminal active core sequence (Phe$^1$-Xaa$^2$-Xbb$^3$-Trp$^4$-Gly$^5$-$NH_2$) are replaced by non-proteinogenic amino acids. For instance, three kinin peptides, Phe-Phe-**Aib**-Trp-Gly-$NH_2$ (**5**), pGlu-Lys-Phe-Phe-**Aib**-Trp-Gly-$NH_2$ (**6**) and pGlu-Lys(pGlu)-Phe-Phe-**Aib**-Trp-Gly-$NH_2$ (**7**), containing sterically hindered aminoisobutyric acid (Aib), demonstrated potent stimulation of fluid secretion in isolated Malpighian tubules of the cricket *Acheta domesticus* with $EC_{50}$ values of 5.6 pM, 2.8 pM and 8.3 pM, respectively.[52] Notably, all analogues demonstrate similar potencies at a level two- to eight-fold more active than achetakinin IV (Asn-Phe-Lys-Phe-Asn-Pro-Trp-Gly-$NH_2$), one of the most potent insect kinins found in the cricket. The high activities could be explained by the turn-stabilizing effect of the sterically hindered Aib moiety that accelerates efficient receptor binding.

**Table 4:** Cricket Malpighian tubule secretion activity of $\beta$-amino acid containing insect kinin analogues and achetakinins[53]

| Peptide analogues | $EC_{50}$ (pM) |
|---|---|
| Natural achetakinins | 20-325 |
| Ac-Arg-Phe-Phe-[$\beta^3$Pro]-Trp-Gly-$NH_2$ **(8a)** | 30 |
| Ac-Arg-[$\beta^3$Phe]-Phe-Pro-Trp-Gly-$NH_2$ **(8b)** | 270 |
| Ac-Arg-Phe-Phe-Pro-[$\beta^3$Trp]-Gly-$NH_2$ **(8c)** | 20000 |
| Ac-Arg-[$\beta^2$homoPhe]-Phe-Pro-Trp-Gly-$NH_2$ **(8d)** | 22500 |
| Ac-Arg-Phe-Phe-Pro-[$\beta^2$Trp]-Gly-$NH_2$ **(8e)** | 40000 |
| Ac-Arg-[$\beta^3$Phe]-Phe-[$\beta^3$Pro]-Trp-Gly-$NH_2$ **(8f)** | 170000 |
| Ac-Arg-[$\beta^3$Phe]-Phe-Phe-[$\beta^3$Pro]-Trp-Gly-$NH_2$ **(8g)** | 100 |
| Ac-Arg-Phe-[$\beta^3$Phe]-[$\beta^3$Pro]-Trp-Gly-$NH_2$ **(8h)** | 1000 |

Another approach enhancing both resistance to peptidase hydrolysis and biological activity utilizes $\beta$-amino acids. Replacement of the critical residues Phe, Trp and Pro with their $\beta^3$- or $\beta^2$-amino acid counterparts was investigated. As shown in Table 4, the single-replacement analogue Ac-Arg-Phe-Phe-[$\beta^3$Pro]-Trp-Gly-$NH_2$ (**8a**) with an $EC_{50}$ value of 30 pM showed the most potent diuretic activity on isolated Malpighian tubules of the cricket *Acheta domesticus.* In addition, the peptide **8a** also enhanced resistance to

the endopeptidases ACE and neprilysin (NEP), which deactivate the natural insect kinins. On the other hand, the double-replacement analogue Ac-Arg-[$\beta^3$Phe]-Phe-[$\beta^3$Pro]-Trp-Gly-$NH_2$ (**8f**) proved to be about 5700 times less potent than (**8a**), with an $EC_{50}$ of 170,000 pM.[53] Notably, all analogues are also blocked at the *N*-terminus with an Ac group that contributes to an additional stabilization against peptidases and aminopeptidases.[54]

A more detailed knowledge of the receptor-bound conformation or a preferred conformation in solution is a prerequisite for the rational design of peptidomimetic analogues. Based on molecular dynamics simulations of leucokinin II (Asp-Pro-Gly-Phe-Ser-Ser-Trp-Gly-$NH_2$, **9**), a β-turn preference for the active core over residues Phe-Ser-Ser-Trp was established. The preferred β-turn conformations have also been proposed for the other insect kinins, such as leucokinins (Phe-Xaa-Ser-Trp-Gly-$NH_2$) and achetakinins (Phe-Xaa-Pro-Trp-Gly-$NH_2$), where Xaa is His, Tyr, Ser or Asn. The conformational preference of the insect kinins (type I, type II or type VI) depends largely on the amino acid sequence and the solvent. However, in all cases molecular dynamics simulations indicate that the essential side chains, $Phe^1$ and $Trp^4$, are always oriented on the same side of the mainchain backbone and interact with the receptor, whereas the variable residue in the position 2 (Tyr, His or Ser) points way from the Phe and Trp.[55]

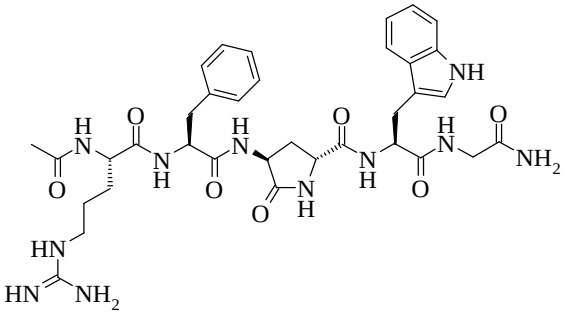
Owing to decreased conformational flexibility, formation of active cyclic analogues is thought an excellent strategy for defining the receptor-bound conformation. Based on aqueous NMR spectroscopy and molecular dynamics (MD) calculations to a head-to-tail cyclic peptide, Nachman and co-workers concluded that the cyclic achetakinin analogue cyclo($Ala^1$-$Phe^2$-$Phe^3$-$Pro^4$-$Trp^5$-$Gly^6$) (**10**) adopts two individual turn conformations within the *C*-terminal active pentapeptide core region.[55] The more rigid of the two conformations characterizes a *cis*-Xaa-Pro amide bond in the third position of a type VI β-turn over residues 1-4 ($Phe^2$-$Phe^3$-$Pro^4$-$Trp^5$). The other less rigid conformation has a type I β-turn over residues 2-5 ($Phe^3$-$Pro^4$-$Trp^5$-$Gly^6$), with a *trans*-Xaa-Pro amide bond. Furthermore, the aqueous NMR studies proved that the *cis*-Xaa-Pro amide bond predominates over 60%, while the other is about 40%. This agrees well with studies on $Pro^3$-containing linear peptides in which the critical aromatic residues, Phe and Trp, promote preferentially the formation of type VI β-turn.[55-57]

**Table 5:** Diuretic activity of insect kinin mimetics in the cricket *Acheta domesticus* and tick receptor[58]

| Peptide analogues | Tick receptor (BmLK3 cell line) | Cricket diuretic assay |
|---|---|---|
| | $EC_{50}$ (μM) | $EC_{50}$ ($10^{-8}$M) |
| Ac-Arg-Phe-(**2*S*,4*S***)-**APy**-Trp-Gly-$NH_2$ (**11a**) | 11.1 | 14 |
| Ac-Arg-Phe-(**2*R*,4*R***)-**APy**-Trp-Gly-$NH_2$ (**11b**) | 1.6 | 7 |
| Ac-Arg-Phe-(**2*R*,4*S***)-**APy**-Trp-Gly-$NH_2$ (**11c**) | N.D. | 0.7 |
| Ac-Arg-Phe-(**2*S*,4*R***)-**APy**-Trp-Gly-$NH_2$ (**11d**) | N.D. | 12 |
| Phe-Phe-ψ[**$CN_4$**]-**Ala**-Trp-Gly-$NH_2$ (L,L) (**11e**) | N.D. | 34 |
| Phe-**D-Phe**-ψ[**$CN_4$**]-**D-Ala**-Trp-Gly-$NH_2$ (D,D) (**11f**) | N.D. | 58 |
| Phe-**Phe**-ψ[**$CN_4$**]-**D-Ala**-Trp-Gly-$NH_2$ (L,D) (**11g**) | N.D. | 43 |
| Phe-**D-Phe**-ψ[**$CN_4$**]-**Ala**-Trp-Gly-$NH_2$ (D,L) (**11h**) | - | 22 |
| N.D.: An $EC_{50}$ could not be determined | | |

In order to obtain more clear evidence that the predominant conformation *cis*-Xaa-Pro type VI β-turn is indispensable to receptor interaction, insect kinin analogues containing either tetrazoles (ψ[$CN_4$]) or 4-aminopyroglutamate (APy) were synthesized and evaluated (Table 5). NMR and computer modeling studies indicated that tetrazole- and APy-containing analogues induce a type VI turn over the residues 1–4 in aqueous solution.[59,60] Like the head-to-tail cyclic achetakinin analogue **10**, molecular dynamics computation of tetrazole-containing peptides demonstrated that the most critical Phe and Trp side chains form an aromatic surface that is thought to be a key element to facilitate the receptor binding of insect kinins (Figure 9). Further experimental evidence supporting the significance of stereochemical requirements for the kinin receptor binding was obtained from the tick *Boophilus microplus* (Table 5). Three analogues Ac-Arg-Phe-**APy(2*S*,4*S***)-Trp-Gly-

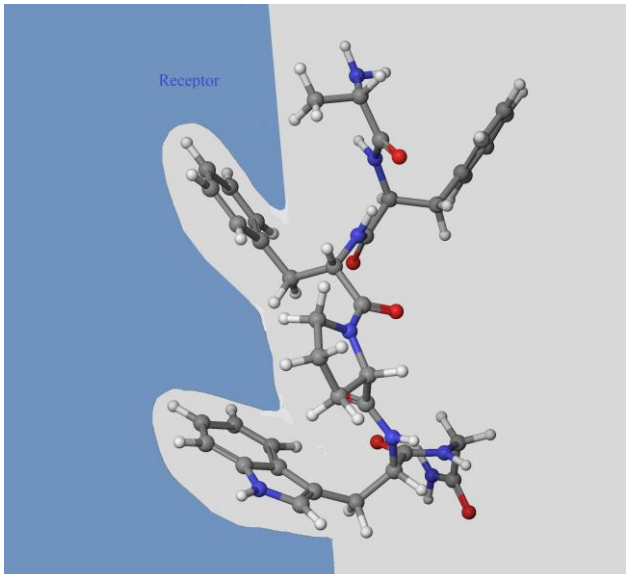
**Figure 8:** Insect kinin analogue **11** with a mimetic *cis*-amide bond



**11c**

$NH_2$ (**11a**), Ac-Arg-Phe-**APy**(**2*R*,4*R***)-Trp-Gly-$NH_2$ (**11b**) and Ac-Arg-Phe-**APy(2*R*,4*S***)-Trp-Gly-$NH_2$ (**11c**) demonstrated intrinsic activity, whereas the fourth peptide Ac-Arg-Phe-**APy**(**2*S*,4*R***)-Trp-Gly-$NH_2$ (**11d**) was found to be inactive. The two analogues, Phe-Phe-ψ[**$CN_4$**]-**Ala**-Trp-Gly-$NH_2$ (L,L) (**11e**) and Phe-**D-Phe**-ψ[**$CN_4$**]-**Ala**-Trp-Gly-$NH_2$ (D,L) (**11h**), showed agonist activity with maximal response of 32% and 47% compared to FFSWGa, respectively. Neither of these analogues were active enough to allow the calculation of an $EC_{50}$ value. An *in vitro* cricket diuretic assay showed that the most active kinin analogues are Phe-D-Phe-ψ[**$CN_4$**]-Ala-Trp-Gly-$NH_2$ (D,L) (**11h**) and Ac-Arg-Phe-**APy**(**2*R*,4*S***)-Trp-Gly-$NH_2$ (**11c**) with $EC_{50}$ values of 22 and 7 nM, respectively. Clearly, the tetrazole-containing analogues showed significantly lower diuretic activities than the APy mimetics. Thus, the use of tetrazole scaffold as *cis*-amide bond template for researching the receptor bound conformation is limited.

**Figure 9:** A receptor interaction model of an insect kinin *C*-terminal pentapeptide core region

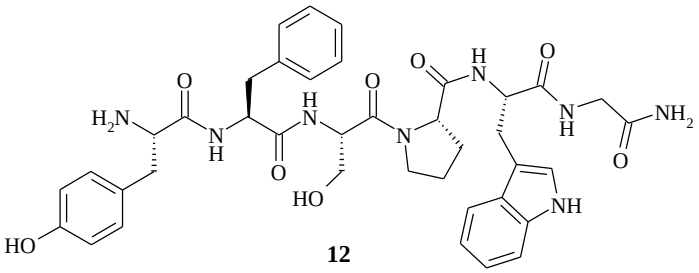



It must be emphasized that the most available biological data have been obtained so far from organ-assay (the isolated Malpighian tubule and cockroach hindgut) or receptor assays on different insect species. Therefore, the results have not provided clear interpretation whether a insect kinin or its derivative targets specifically only one receptor or a family of closely related receptors. Thus, it is essential to establish a suitable biological system for testing potential neuropeptide on only single receptor target of one insect species.

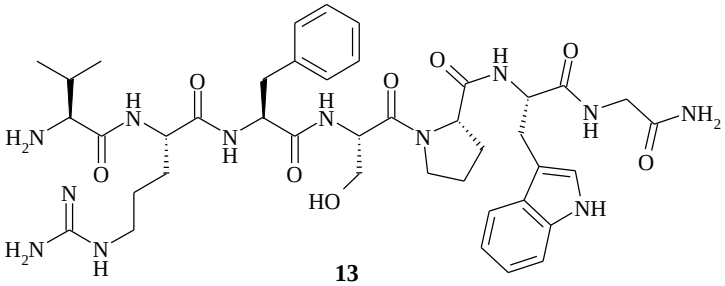
## 1.4 Helicokinin

### 1.4.1 Historical perspective

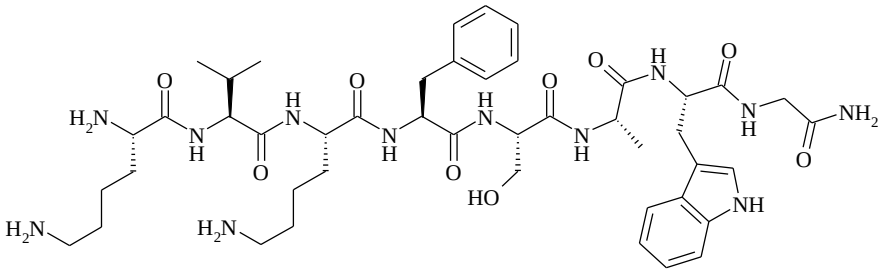
Helicokinins belong to the insect kinins which are characterized by a common *C*-terminal pentapeptide sequence Phe[1]-Xaa[2]-Xbb[3]-Trp[4]-Gly[5]-$NH_2$, where Xaa[2] is Ser, and Xbb[3] is Ala, Pro (Table 1). Helicokinins (Hez-KI, -KII, -KIII, Figure 10) were first isolated and sequenced by Blackburn, in 1995, from the insect *Helicoverpa* (*Heliothis) zea.*[61]



**12**



**13**



**14**

**Figure 10:** Various structures of helicokinin I **(12): Tyr-Phe-Ser-Pro-Trp-Gly-$NH_2$,** helicokinin II **(13): Val-Arg-Phe-Ser-Pro-Trp-Gly-$NH_2$** and helicokinin III **(14): Lys-Val-Lys-Phe-Ser-Ala-Trp-Gly-$NH_2$**

*In vitro* assays of helicokinins (I-III) on the isolated whole-gut of fifth instar larvae from *Spodoptera frugiperda* showed that all three peptides stimulate fluid secretion in a concentration-dependent manner with the $EC_{50}$ values of 1.0 nM, 0.2 nM and 0.6 nM, respectively.[62] When tested on the Malpighian tubules isolated from *Manduca sexta*, Hez-KII was found more effective than either Hez-KI or Hez-KIII at inducing fluid secretion with an $EC_{50}$ value of 0.6

pM.[61] These results suggest that Hez-KII is the most potent myotropic activity in both *M. sexta* and *S. frugiperda*.

**Figure 11:** Crop pest *Heliothis virescens* [b]

To investigate the effects of helicokinins on water balance in *Heliothis virescen*s (Figure 11), a modified Ramsay assay was developed by Seinsche.[63] This *in vitro* assay is based on the assessment of increased droplet secretion from single Malpighian tubules of the larvae. The Ramsay assay showed that all three helicokinins induced significant myotropic effects in a dose-dependent manner over a range from $10^{-12}$ to $10^{-7}$, with $EC_{50}$ values of $2.9x10^{-11}$ M (Hez-KI, **12**), $2.0x10^{-11}$ (Hez-KII, **13**) and $3.6x10^{-10}$ M (Hez-KIII, **14**).[63] At a concentration of $10^{-9}$ M these peptides increased fluid secretion by 410% (Hez-KI), 354% (Hez-KII) and 207% (Hez-KIII). The highest rates of the fluid secretion were observed at concentration of $10^{-8}$M with the increases of 6.1 times for Hez-KI, 5.3 times for Hez-KII and 4.2 times for Hez-KIII. In good agreement with the Ramsay assay, the receptor assay proved that Hez-KI is the most active kinin, followed by slightly less active Hez-KII and Hez-KIII, respectively.[64] These findings are in contrast to the results gained on *M. sexta* and *S. frugiperda*, where Hez-KII is the highest potency in stimulating fluid secretion in isolated Malpighian tubules. In the receptor assay, the helicokinin receptor (HKR) of *Heliothis virescens* cloned from a Malpighian tubule cDNA library is functionally expressed in a CHO line. The activation of the HKR is analysed in living

[b] http://mothphotographersgroup.msstate.edu/species.php?hodges=11071

cells by measuring the induced calcium ion flux in the cytosol after activation of the second messenger cascade via G-proteins. *In vivo* microinjections of helicokinins into developing larval *Heliothis viresens* caused weight reduction and increased mortality within 6 days. Moreover, the combinations of helicokinins with ACE inhibitors (captopril, enalapril or lisinopril), which suppress the proteolytic degradation of insect kinins, showed that helicokinin I has the highest potency in *in vivo* activity.[63] Due to its short sequence and the highest diuretic activity in *Heliothis virescens*, helicokinin I is the most promising lead structure for the development of metabolically stable peptidomimetics.

### 1.4.2 Structure-activity relationships

Among the helicokinins (I-III), Hez-KI displays the highest intrinsic activity compared to the others. Structure-activity relationships of Hez-KI were studied in both the Ramsay assay and the receptor assay.[64] The truncation of the single amino acid tyrosine from the *N*-terminus of Hez-KI (Phe-Ser-Pro-Trp-Gly-$NH_2$) only resulted in a slight decrease in fluid secretion in the larval Malpighian tubules (factor 1.4). This is good agreement with the previous studies from other insect species.[55] However, the receptor activity of the pentapeptide (Phe-Ser-Pro-Trp-Gly-$NH_2$) is reduced significantly by a factor of 120. Deletion of any other amino acid in the helicokinin I sequence causes a complete loss of receptor activity.[64]

Alanine-scans of helicokinin I demonstrate the significance of the amino acid side chains. Replacement of glycine or phenylalanine with alanine resulted in inactive peptides. When tryptophan was replaced by alanine, the biological activity decreased significantly with an $EC_{50}$ value 25 fold higher than that of the parent helicokinin I.[64] Substitution of tyrosine, serine and proline with alanine, respectively, did not show significant differences in $EC_{50}$ values, suggesting that residues Ser and Pro in the active core pentapeptide are not of great importance for binding to the receptor and therefore both residues probably function as a spacer to keep a correct distance between the crucial residues within *C*-terminus (Tyr-Phe and Trp-Gly-$NH_2$).

In order to evaluate the significance of the stereochemistry of the Hez-KI residues, a D-amino acid scan was performed. Except Tyr that could be substituted by its D-isomer, substitutions of the Phe and Ser residues with their D-enantiomers did not show any significant diuretic

activities, while a D-Trp replacement reduced receptor activity by a factor of 160. N-methylation of amide bond proved the importance of the *C*-terminal amide as hydrogen bond donor. Mono-N-methylation of the *C*-terminus reduced the receptor activity by a factor around 300, while bis-N,N-methylation led to a complete loss of biological activity. The structure-activity relationships of Hez-KI are summarized in Figure 12.[64,65]

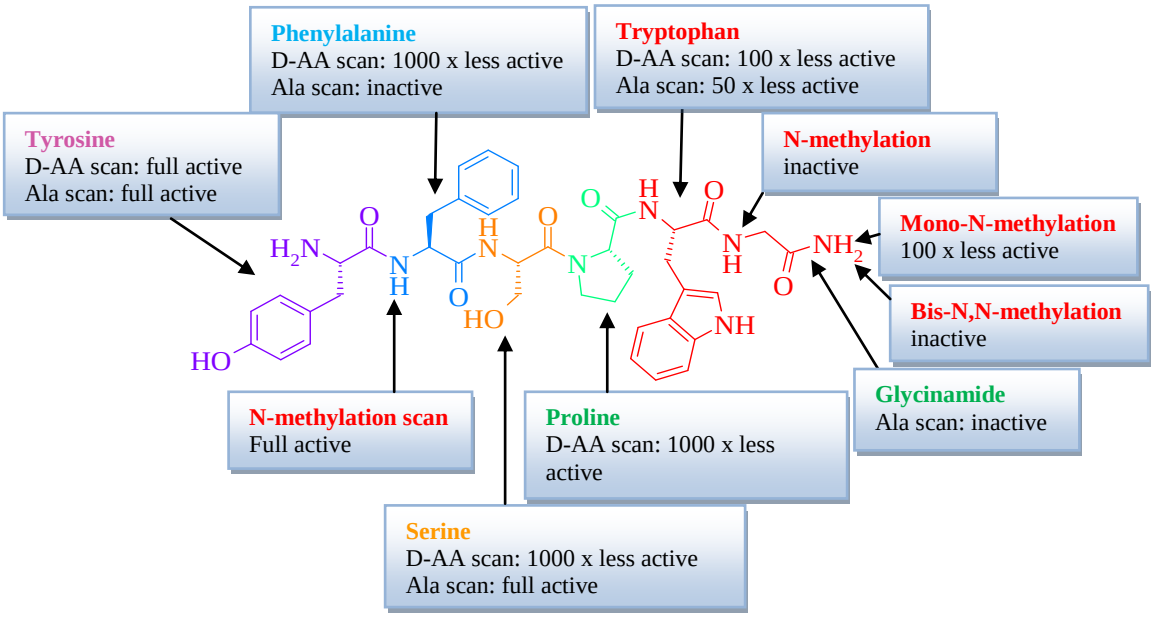
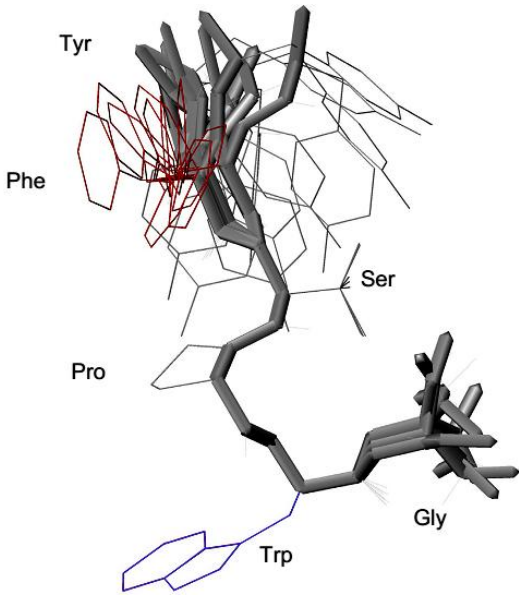



**Figure 12:** Overview of structure-activity relationships of helicokinin I [65]

### 1.4.3 Conformational analysis

Previous studies confirmed that the presence of the *C*-terminal pentapeptide in the insect kinins is crucial for full biological activity. Detailed NMR spectroscopic studies in both dodecylphosphocholine (DPC) and sodium dodecyl sulfate (SDS) micelles demonstrated that hexapeptide helicokinin I (Tyr$^1$-Phe$^2$-Ser$^3$-Pro$^4$-Trp$^5$-Gly$^6$NH$_2$, **12**) forms a *C*-terminal type-I β-turn, which comprises the amino acid residues Ser$^3$ (i), Pro$^4$ (i+1),Trp$^5$

**Figure 13:** Preferred conformation of helicokinin I in the presence of DPC micelles[43]



(i+2) and $Gly^6$ (i+3) with a *trans* Ser-Pro amide bond. Whereas the *N*-terminus seems highly unstructured and the flexible side chain Tyr points away from Phe and Trp residues (Figure 13).[43] As expected, in aqueous solution helicokinin I does not adopt any preferred conformation, proving that the β-turn is induced by the interaction of the peptide with the membrane. In addition, a spin-labeling experiment was observed that helicokinin I is anchored to the membrane by $Phe^2$ and $Trp^5$ residues, which are therefore postulated to be absolutely significant for receptor binding of helicokinin I.

## 1.5 Aims of the thesis

Preceding investigations of helicokinin I suggest that the type-I β-turn could be regarded as a prime target for peptidomimetic approaches. With the aim of getting a better insight into the conformational prerequisites for receptor binding of helicokinin I, the main objectives of this dissertation are:

- Synthesis of β-turn peptidomimetics and analysis of their preferred conformations.
- Macrocylic helicokinin I peptidomimetics in order to evaluate the effect of global conformational constraints on receptor activation.
- Design and synthesis of conformationally constrained building blocks derived from tryptophan moiety in order to investigate the significance of the Trp side chain to molecular recognition.
- Additional structure-activity relationships of helicokinin I
- Validating biological activity of all helicokinin I analogues synthesized in a functional *Heliothis virescens* receptor assay.

## II. Results and discussion

### 2.1 β-Turn peptidomimetics of helicokinin I

One of the most challenging aspects of modern drug development as well as neuropeptide chemistry is the design and synthesis of highly potent, selective and metabolically stable peptidomimetics. An efficient approach for digging such biologically active compounds is the use of molecular scaffolds (Figure 14) that induce conformational restraints and stabilize the secondary structure of peptides, which is a critical element for the effectiveness of protein-receptor interactions. [66-68]

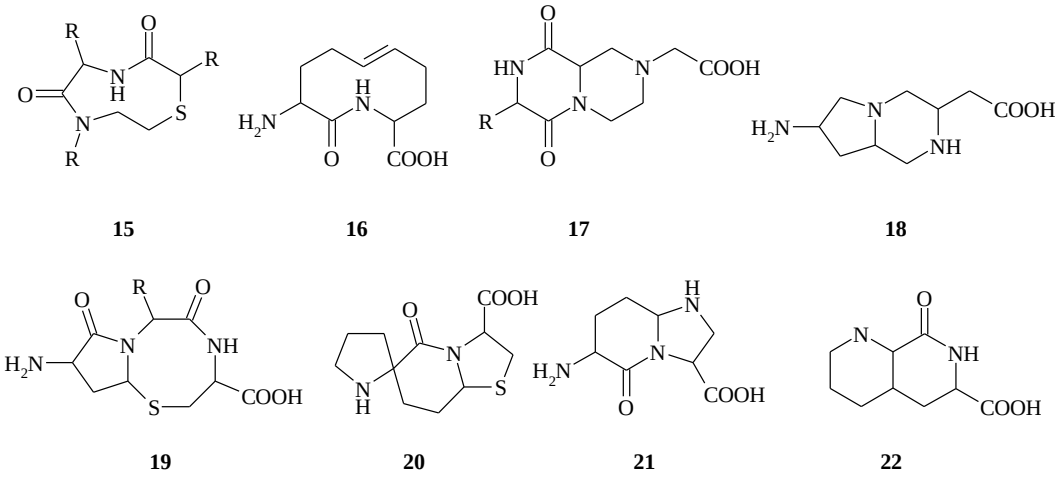



**Figure 14:** Mimetic β-turn scaffolds

Among the most important secondary motifs, the β-turns play a particularly important role in molecular recognition, biological activity and folding of peptides.[69] With the aim of determining potent and selective therapeutic agents, a great deal of efforts have focused on the design and synthesis of small constrained turn mimetics for a better understanding of the molecular mechanisms responsible for peptide-protein or peptide-receptor interactions. For this reason, the peptidomimetic approaches based on the β-turn structure are very fashionable and have attracted a large interest in the field of medicinal chemistry. Following this trend, the first primary goal of this dissertation deals with the construction of β-turn helicokinin I mimetics. Based on previous SAR studies, the chemical modification will mainly focus on the active core of the *C*-terminal type I β-turn of helicokinin I in such a way that:

- The *C*-terminal glycine amide will spatially orient in the same direction as the parent β-turn.
- The two important aromatic residues Trp and Phe are kept intact.
- The less important spacer residues either Ser or Pro are substituted by other amino acids.

### 2.1.1 4-Aminopyroglutamate containing helicokinin I analogues

It is well-known that the unique structural features of proline-containing peptides, possessing a *cis*/*trans*-Xaa-Pro amide bond (Figure 15), play a key role in the folding of peptides that influence their biological activity and in many cases only one of the two configurational isomers displays biological activity.[70-73] Particularly, the *cis*-geometry of the proline amide bond induces the type VI β-turn where the proline residue is located at the position (i+2) of the peptide bond commonly. Recently, pseudo-prolines (APy or ψ[**CN$_4$**]) fixed as a *cis*-amide bond were introduced to insect kinins which then adopt a type VI β-turn conformation.[59,60]

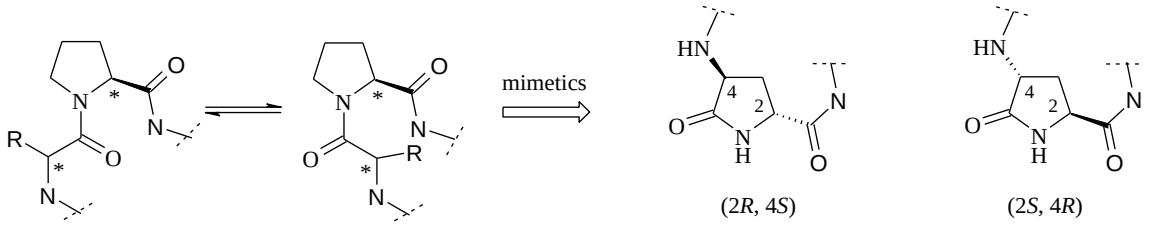



**Figure 15:** *trans*- and *cis*-Xaa-Proline peptide conformers and *trans*-4-aminopyroglutamic acids (APy)

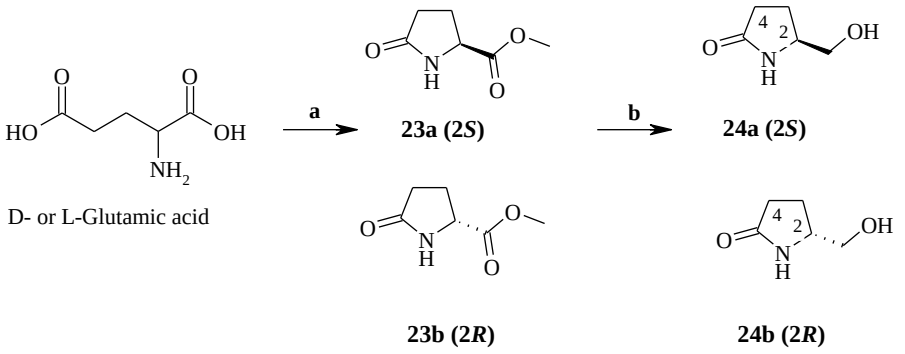
Inspired by the high activities of APy-containing achetakinin analogues reported by Nachman (Table 5), a modified efficient methodology to prepare 4-amino-pyroglutamates, which are then incorporated into helicokinin I, is presented.

#### 2.1.1.1 Synthesis of 4-azido-pyroglutamic acids

Based on Nachman's studies,[58] two *trans*-isomers of 4-azido-pyroglutamates **31a** (2*R*, 4*S*) and **31b** (2*S*, 4*R*) were prepared in enantiomerically pure form. Though several methods have been reported on the synthesis of substituted pyroglutamates,[74-78] most of them are inefficient synthetic routes to obtain an enantiopure product.

Our synthesis is based on the pyroglutaminol intermediates **24a** and **24b** (Scheme 1), which were easily obtained from D- and L-glutamic acids. Esterifications were carried out in methanol and $SOCl_2$,[79] followed by refluxing in toluene in the presence of triethylamine (TEA) to provide the methyl pyroglutamate esters **23a** and **23b**. In order to prevent racemization in the later synthetic steps, the methyl esters were converted into the corresponding alcohols to reduce the acidity of Hα. When **23a** and **23b** were treated with an excess of $NaBH_4$ in *i*PrOH for 20 h, the desired products **24a** and **24b** were obtained in 84% yield.[80]

**Scheme 1: Preparation of pyroglutaminols**

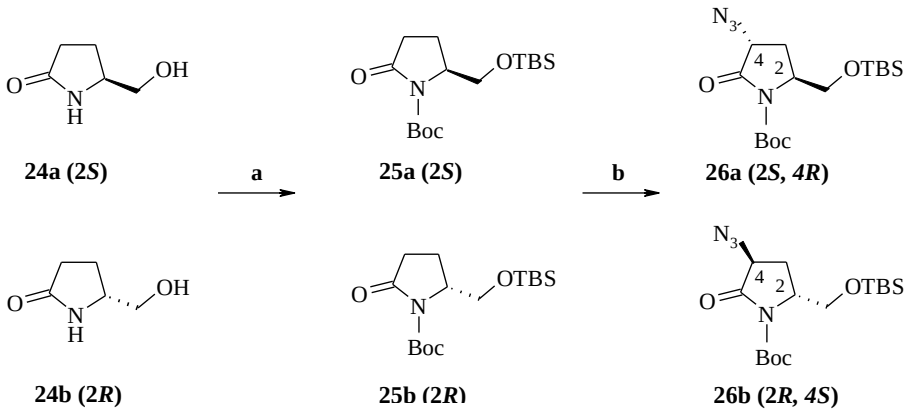



Reagents and conditions: a) MeOH, $SOCl_2$, rt, 18 h, toluene, TEA, reflux, 3 h, 73-97%; b) $NaBH_4$, *i*PrOH, rt, 20 h, 84%.

The introduction of an azide functional group on C-4 of the pyroglutaminols was carried out following two synthetic strategies. In the first route (Scheme 2), pyroglutaminols **24a** and **24b** were fully protected by silylation with (*tert*-butyl)dimethylsilyl chloride (TBSCl), followed by reaction with di(*tert*-butyl)dicarbonate ($Boc_2O$) in TEA/DMAP,[81] to obtain products **25a** and **25b**.

Next, a diastereoselective azidation of the protected lactams was performed according to the Evans methodology.[82,83] The resulting enolates, prepared by addition of 1.5 equivalents of potassium hexamethyldisilazide (KHDMS) to the lactams **25a** and **25b** in THF at -78 $^oC$ for 50 min, were treated with trisyl azide (2,4,6-triisopropylbenzenesulfonyl azide) at 0 $^oC$ for 3 min and quenched with AcOH. The azidation reactions were highly stereoselective, providing only single diastereomer **26a** and **26b** (Scheme 2). However, after chromatographic purifications, **26a** and **26b** were only obtained in 12% and 13 %, respectively.

**Scheme 2: Azidation of pyroglutaminol derivatives**




Reagents and conditions: a) (i) TBSCl, imidazole, DMAP, THF, 24 h, rt, 87-88%; (ii) $Boc_2O$, DMAP, TEA, 24 h, rt, 91%; b) KHMDS, trisyl azide, AcOH, 12-13%.

The low conversions may probably be caused by an extensive decomposition of the triazene intermediates to diazo compounds. The use of KOAc which enables to effectively prevent the decomposition of the triazene intermediate along with acetic acid afforded no improvement in the yield of the azidation reaction. The stereochemistry of the azidated products (**26a** and **26b**) was determined by NMR experiments. The NOESY data illustrated in Figure 16 confirm the required *trans*-products.

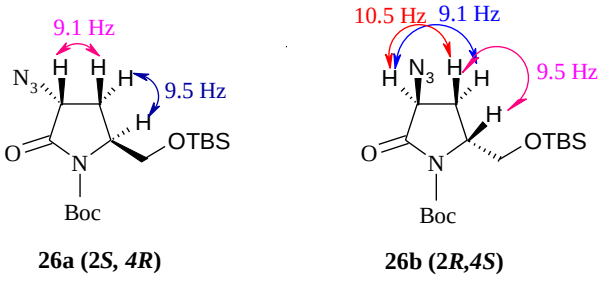
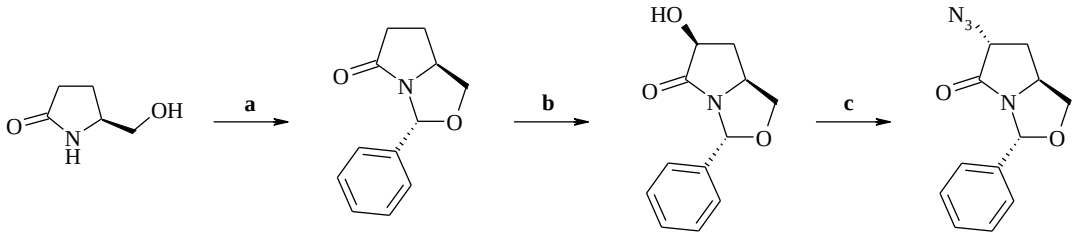



**Figure 16:** Observed NOESY correlations

The low yield of azido products from the first strategy made it impossible to continue with further chemical transformations. Therefore, an alternative path to obtain the target compounds was studied. This strategy involves a four-step protocol, including the selective protection of the pyroglutaminols, diastereoselective hydroxylation on the C-4 of the lactam ring, mesylations and

azide displacement. Practically, treatment of lithium enolates, derived from lactams **25a** and **25b** as starting materials for hydroxylation, with the Davis reagent provides exclusively *trans*-4-hydroxyl-pyroglutaminol derivatives.[84-86] Nonetheless, the required stereochemistry at the position C-4 is opposite to the *trans*-4-hydroxyl-pyroglutaminol. Thus, this synthetic approach is unsuitable for the preparation of *trans*-4-azidopyroglutamic acids.

**Scheme 3: A stereoselective hydroxylation reaction of bislactam 27a**




**24a (2*S*)** **27a** **28a** **29a**

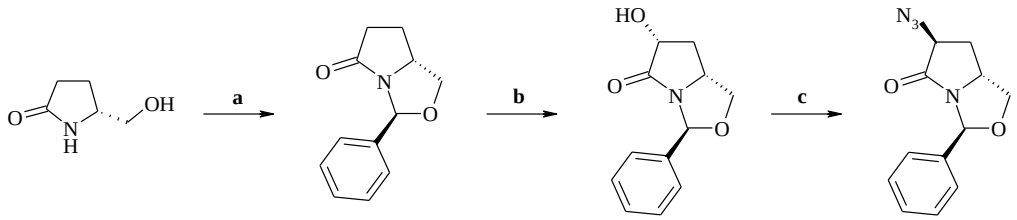
Reagents and conditions: a) Benzaldehyde, PTSA, toluene, reflux, 4 h, 72%; b) LDA, -78 $^{o}$C, 50 min, MoOPD, -40 - -35 $^{o}$C, 3 h, 72%; c) (i) MsCl, TEA, rt, 18 h, 88%; (ii) $NaN_3$, DMSO, 60 $^{o}$C, 1 h, 84%.

It was reported that bicyclic lactams derived from pyroglutamic acid can be used as a chiral synthons for a highly stereoselective hydroxylation reaction.[86] In addition, the selection of a suitable protecting group plays a crucial role for *endo*- or *exo*-attack because it serves as a stereocontrolling element in enolate addition reactions.[87] Following this approach, a highly diastereoseletive hydroxylation was achieved by sequential reactions (Scheme 3 and 4). Treatment of pyroglutaminols with benzaldehyde in refluxing toluene in the presence of p-toluenesulfonic acid (PTSA) as catalyst gave the corresponding hemiaminal ethers **27a** and **27b** as single diastereoisomers in 72% and 63% yields, after chromatographic purification.[88] Next, the lactam enolate anions were readily generated by treatment of the bicyclic lactams **27a** and **27b** with LDA (1.3 equiv) and subsequently oxidized with the molybdenum complex MoOPD ($MoO_5$.Py.DMPU), which facilitates an *endo*-selective attack, at -35 to -40 $^{o}$C for 3 h providing 72-73% yield of **28a** and **28b** in diastereomerically pure form (Scheme 3 and 4).[84,86]

The corresponding mesylate intermediates were prepared by treatment of alcohols **28a** and **28b** with MsCl in TEA,[89] which were subsequently reacted with a large excess of $NaN_3$ in DMSO at

65 $^{o}$C for 1 hour affording the desired azido products **29a** and **29b** with an inverted configuration at C-4.[90]

**Scheme 4: A stereoselective hydroxylation reaction of bislactam 27b**

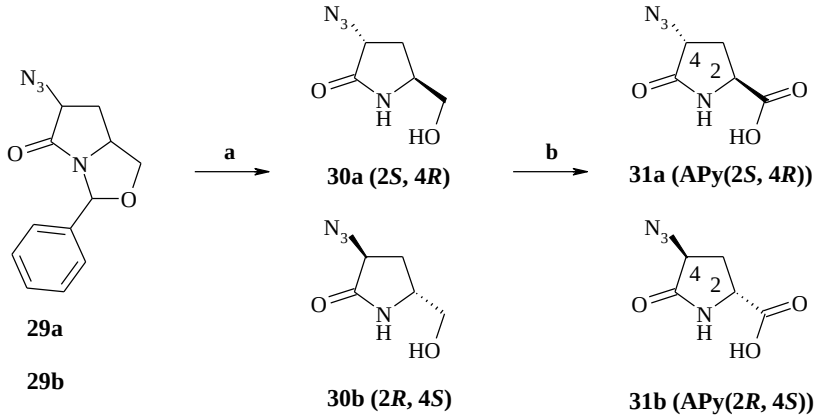



**24b (2*R*)** **27b** **28b** **29b**

Reagents and conditions: a) Benzaldehyde, PTSA, toluene, reflux, 4 h, 63%; b) LDA, -78 $^{o}$C, 50 min, MoOPD, -40 - -35 $^{o}$C, 3 h, 73%; c) (i) MsCl, TEA, rt, 18 h, 83%; (ii) $NaN_3$, DMSO, 60 $^{o}$C, 1 h, 82%.

Ring opening of the hemiaminal ethers **29a** and **29b** was carried out under acidic conditions of TFA in THF/$H_2O$ for 18 h leading to the 4-azido-pyroglutaminols **30a** (APy(2*S*, 4*R*)) and **30b** (APy(2*R*, 4*S*)) in 89% and 90% yields, respectively.[91] An efficient oxidation of the pyroglutaminols using ruthenium-mediated $RuCl_3$-$NaIO_4$ in $CCl_4$/ACN/$H_2O$ (2/2/3) yielded the corresponding *trans*-4-azidopyroglutamic acids **31a** and **31b** in excellent yields (88-92%) (Scheme 5).[92,93]

**Scheme 5: Preparation of 4-azido-pyroglutamic acids**




**29a**

**29b**

**30a (2*S*, 4*R*)**

**30b (2*R*, 4*S*)**

**31a (APy(2*S*, 4*R*))**

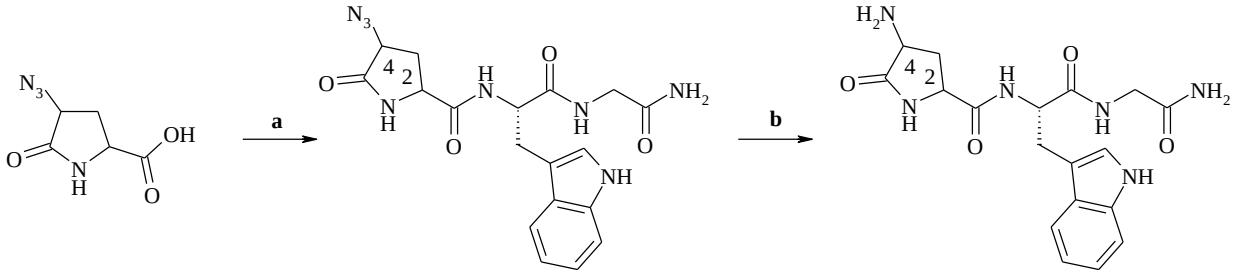
**31b (APy(2*R*, 4*S*))**

Reagents and conditions: a) TFA, THF/$H_2O$, rt, 18 h, 89-90%; b) $RuCl_3.xH_2O$ (2 mol%), $NaIO_4$, $CCl_4$/ACN/$H_2O$ (2/2/3), rt, 16 h, 88-92%.

### 2.1.1.2 Incorporation of 4-aminopyroglutamates into helicokinin I

With the *trans*-4-azidopyroglutamic acids in hand, two key tripeptides **33a** and **33b** were successfully synthesized. In the course of the reactions, the azido functional group of the acids was remained intact and only reduced prior to peptide-chain elongation to avoid unnecessary additional protecting group manipulations. Incorporation of the 4-azidopyroglutamic acids into dipeptide H-Trp-Gly-$NH_2$ using O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexa-fluorophosphate (HATU) as coupling reagent resulted in the key building blocks **32a** and **32b**, in moderate yields of 60% and 65%, respectively. Next, the azido tripeptides **32a** and **32b** were subjected to catalytic hydrogenation (10% Pd/C) in MeOH at room temperature for 16 h providing the free amines **33a** and **33b** in nearly quantitative yields (95-96%) (Scheme 6).

**Scheme 6: Incorporation of 4-azido-pyroglutamic acids into dipeptide Trp-Gly-$NH_2$**




**31a (APy(2*S*, 4*R*)**
**31b (APy(2*R*, 4*S*)**

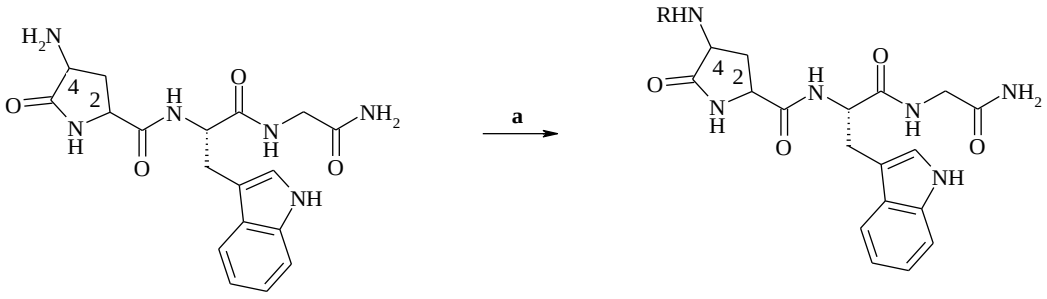
**32a (APy(2*S*, 4*R*))**
**32b (APy(2*R*, 4*S*))**

**33a (APy(2*S*, 4*R*))**
**33b (APy(2*R*, 4*S*))**

Reagents and conditions: a) H-Trp-Gly-$NH_2$, HATU, DIPEA, DMF, rt, 20 h, 60-65%; b) Pd/C, $H_2$, MeOH, rt, 16 h, 95-96%.

In order to evaluate the effect of *cis*-Pro amide bond on biological activity of helicokinin I, we used the APy residues as substituents for either a single Pro residue or the dipeptide Ser-Pro. Coupling of either dipeptide Boc-Tyr(*t*Bu)-Phe-OH or tripeptide Boc-Tyr(*t*Bu)-Phe-Ser(*t*Bu)-OH to tripeptides **33a** and **33b** using HATU as coupling reagent produced the corresponding penta- and hexapeptide helicokinin I analogues (**34a**, **34b**, **35a** and **35b**) in moderate yields (range 66-68%). The resulting peptides were subsequently treated with TFA/TIPS/$H_2O$ at 0 $^oC$ for 4 h yielding the desired helicokinin I analogues in high purity (95-98%) (Scheme 7).

**Scheme 7: Peptide-chain elongation**




**33a (APy(2*S*, 4*R*))**

**33b (APy(2*R*, 4*S*))**

**34a (APy(2*R*, 4*S*)): R = Tyr-Phe- CO-**

**34b (APy(2*S*, 4*R*)): R = Tyr-Phe-CO-**

**35a (APy(2*R*, 4*S*)): R = Tyr-Phe-Ser-CO-**

**35b (APy(2*S*, 4*R*)): R = Tyr-Phe-Ser-CO-**

Reagents and conditions: a) (i) Boc-Tyr(*t*Bu)-Phe-OH or Boc-Tyr(*t*Bu)-Phe-Ser(*t*Bu)-OH, HATU, DIPEA, DMF, rt, 22 h, 66-68%; (ii) TFA/TIPS/$H_2O$, 0 °C, 4 h, 85-92%.

### 2.1.2 Oxopiperazine-based helicokinin I mimic

As a part of our ongoing project for exploring the preferred conformation of helicokinin I analogues, a conformationally constrained six-membered ring scaffold is established by using an ethylene bridge to link two adjacent $N_{i+1}$ and $N_{i+2}$ atoms (Figure 17). In this regard, the Pro residue is replaced by a L-valine amino acid and the (*S*)-3-isopropylpiperazin-2-one ring is formed by ethylations of amide bonds of Val and Trp. Due to its higher flexibility in respect to Pro residue, the piperazine scaffold is thought to be an interesting template in structure activity research. The isopropyl side-chain is intended to mimic part of the lipophilic pyrrolidine ring of proline.[94]

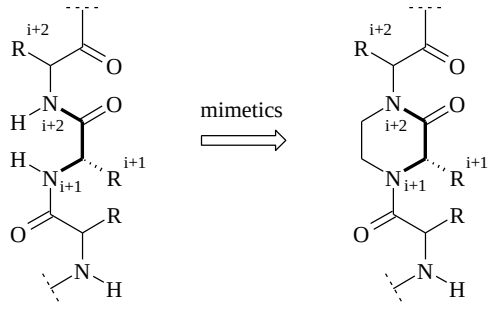
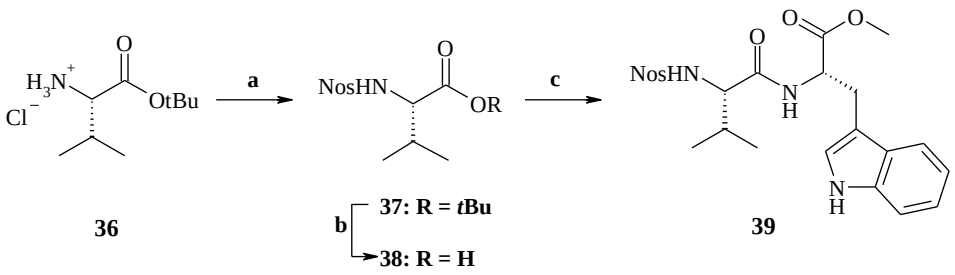



**Figure 17:** Protein β-turn (left) and β-turn mimetic (right)

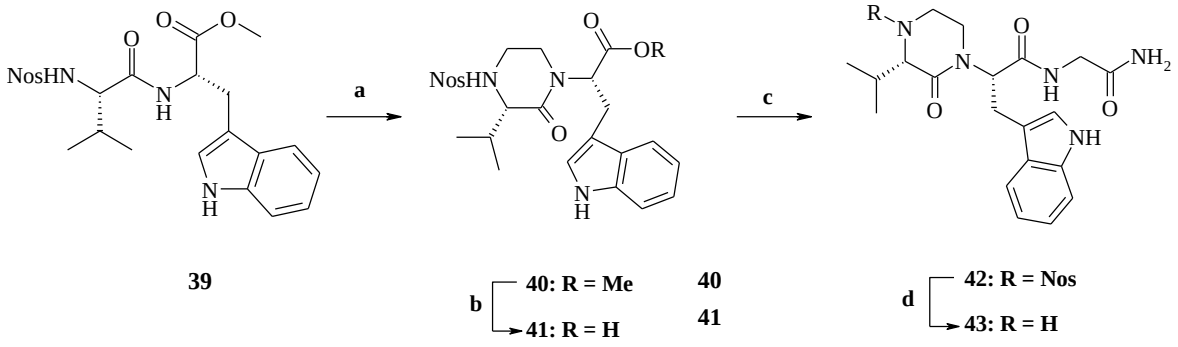
The (*S*)-3-isopropylpiperazin-2-one scaffold was prepared from L-valine-*tert*-butyl ester hydrochloride **36** as starting material. In the synthesis, 2-nitrophenyl sulfonylchloride (NosCl) was chosen as an orthogonal protecting group. Upon treatment of amino ester **36** with NosCl/TEA,[95] the corresponding sulfonamide **37** was obtained almost in quantitative yield (98%). Acidic hydrolysis of ester **37** with TFA gave the corresponding carboxylic acid **38** in 96% yield. Coupling of the acid with L-tryptophan methyl ester using a classical TBTU-mediated amidation resulted in the key sulfonamide dipeptide **39** in 92% yield.

**Scheme 8: Preparation of dipeptide 39**




Reagents and conditions: a) (i) 2-nitrophenylsulfonyl chloride, TEA, DCM, rt, 20 h, 98%; (ii) TFA:DCM (1:1), 0 ºC, 4 h, 96%; b) L-tryptophan methyl ester, TBTU, DIPEA, DCM, rt, 24 h, 92%.

**Scheme 9: Preparation of 2-oxo-piperazine**

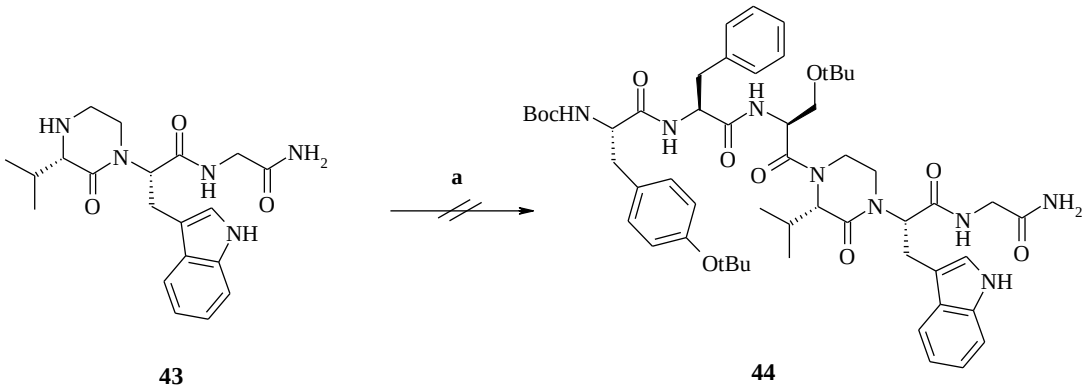



Reagents and conditions: a) 1,2-dibromoethane, $K_2CO_3$, DMF, 60 ºC, 48 h, 73%; b) 4% LiOH, THF, rt, 3 h, 100%; c) glycinamide hydrochloride, TBTU, DMF, rt, 24 h, 72%; d) PhSH, $K_2CO_3$, rt, 4 h, 96%.

The key cyclization reaction was successfully accomplished by treatment of the sulfonamide dipeptide **39** with a large excess of 1,2-dibromoethane and $K_2CO_3$ in dimethylformamide at 60 °C for 48 h.[96,97] The desired 2-oxo-piperazine analogue **40** was achieved in 73% yield

(Scheme 9). Hydrolysis of the methyl ester with 4% LiOH aqueous solution, followed by coupling with glycinamide hydrochloride, furnished the tripeptide **42** in 72% yield.[98] A successive treatment of the sulfonamide **42** with thiophenol and $K_2CO_3$ afforded the corresponding free secondary amine **43** in high yield (96%).[99]

**Scheme 10: Peptide chain extension of 43**



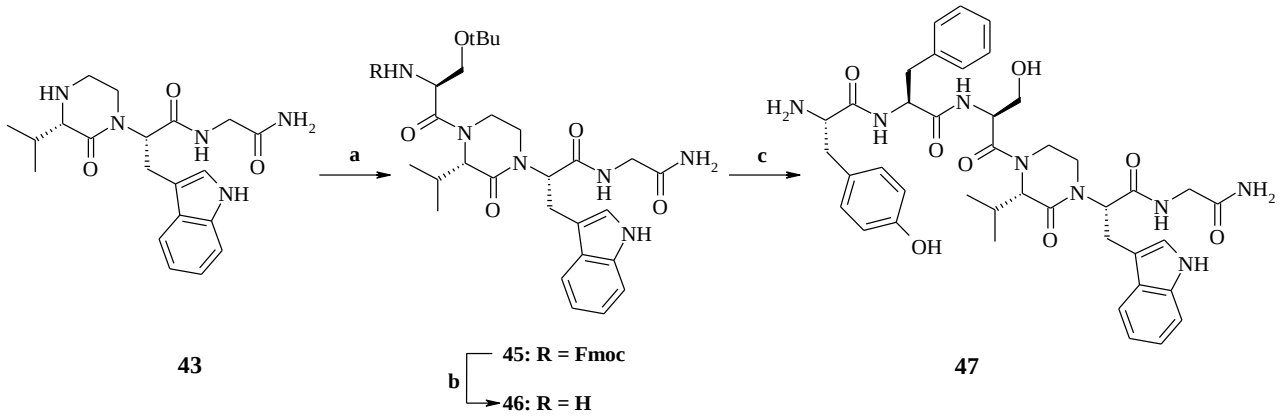

**43** **44**

Reagents and conditions: a) Boc-Tyr(*t*Bu)-Phe-Ser(*t*Bu)-OH, HATU, DIPEA, DMF.

With the key building block **43** in hand, our attention was focused on the convergent synthesis of the desired hexapeptide **44** (Scheme 10). Attempted reaction of tripeptide Boc-Tyr(*t*Bu)-Phe-Ser(*t*Bu)-OH with the secondary amine to produce hexapeptide **44** was unsuccessful, even at elevated temperature (90 $^{o}$C for 18 h) or with highly active coupling reagents such as O-(benzotriazol-1-yl)-*N*,*N*,*N*,*N*-tetramethyluronium tetrafluoroborate (TBTU), HATU and bromotri-(pyrrolidino)-phosphonium hexafluorophosphate (PyBrop). This is possibly caused by a steric hindrance of the bulky isopropyl substituent adjacent to the secondary amine group along with a steric side chain around the activated carboxylate.

After the failure of the first synthetic route, a stepwise synthesis was carried out (Scheme 11). Coupling of Fmoc-Ser(*t*Bu)-OH with the secondary amine **43** using TBTU and DIPEA in DMF produced the *N*-Fmoc protected tetrapeptide **45** in excellent yield (95%), which upon mild basic *N*-Fmoc deprotection with piperidine afforded the amine **46** (98%). Incorporation of dipeptide Boc-Tyr(*t*Bu)-Phe-OH into the amine **46** followed by removal of protecting groups afforded the 2-oxopiperazine-containing helicokinin I analogue **47** in 78% overall yield.

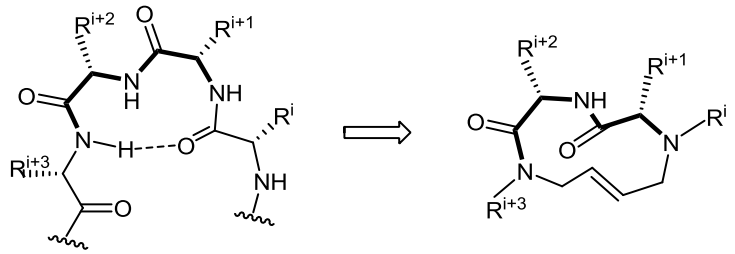
**Scheme 11: Synthesis of peptide 47**




Reagents and conditions: a) Fmoc-Ser(*t*Bu)-OH, TBTU, DIPEA, DMF, 95%; b) piperidine/DCM, 98%; c) (i) Boc-Tyr(*t*Bu)-Phe-OH, TBTU, DMF, DIPEA, 87%; (ii) TFA/TIPS/$H_2O$ (95/2.5/2.5), 0 ºC, 3 h, 90%.

### 2.1.3 Synthesis of macrocycle-bridged helicokinin I analogues

In our ongoing research directed toward controlling the folding of helicokinin I, two medium 11-membered ring containing helicokinin I analogues are prepared. Compared to the parent peptide, the novel helicokinin I analogues are formed by replacement of Ala for Pro and constrained by tetra-carbon spacers (Figure 18).[100]

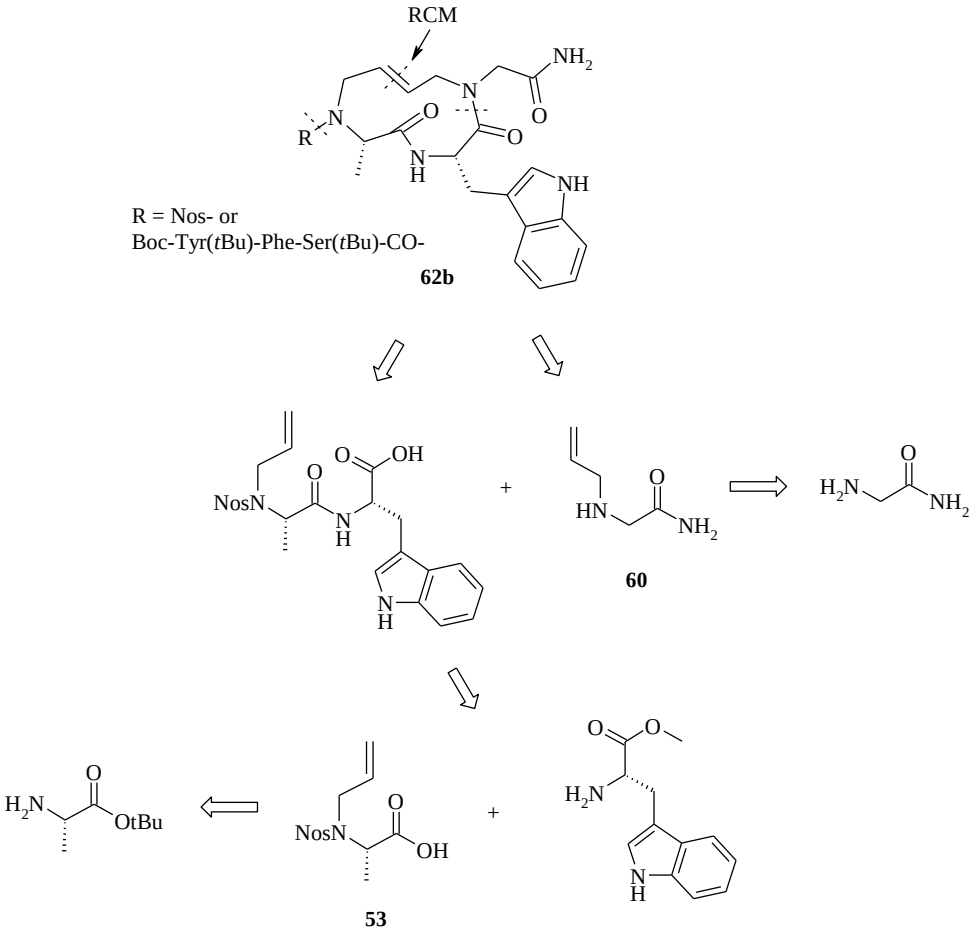


β-turn ‘‘amide-to-amide’’β-turn mimetics

**Figure 18:** β-Turn protein and β-turn mimetic

Retrosynthetically, the macrocyclic peptide **62b** is formed by utilizing a ring-closing metathesis (RCM) reaction from its open chain diene precursor. The diene is prepared from the coupling of

*N*-allyl-Ala-Trp and *N*-allyl-Gly-$NH_2$, which are readily synthesized by *N*-allylation of the commercially available corresponding valine ester and glycine amide (Scheme 12).

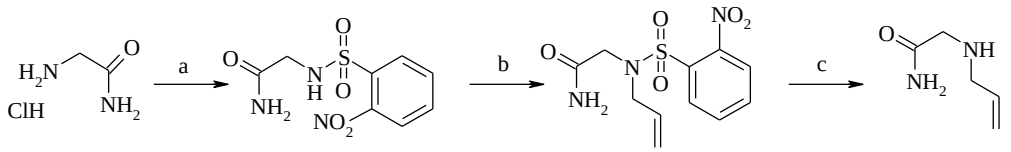



**Scheme 12:** Retrosynthesis of macrocyclic peptide

The most efficient method for the site-selective *N*-alkylation of peptides was developed by Fukuyama.[101] This involves a three-step procedure. The amine group is first activated by reaction with an *ortho*- or *para*-nitrobenzenesulfonyl group, followed by alkylation of alkyl halides on the activated nitrogen and a final deprotection of the Nosyl group. Using this standard protocol, the first fragment *N*-allyl-glycinamide **50** was obtained in good yield (77%) over a three-step sequence (Scheme 13). Glycinamide hydrochloride was first reacted with *o*-nitrobenzenesulfonyl chloride (NosCl) in the presence of TEA in DCM/DMF to give the sulfonamide **48**. Due to poor solubility of product **48**, it was recrystallized from hot solvent mixture of ethanol and water to obtain a pure solid in 74% yield. Subsequent allylation of the

sulfonamide **48** in the presence of $Cs_2CO_3$ furnished product **49**,[102] which upon treatment with thiophenol and $K_2CO_3$ in acetonitrile yielded the desired *N*-allyl glycine amide **50**.

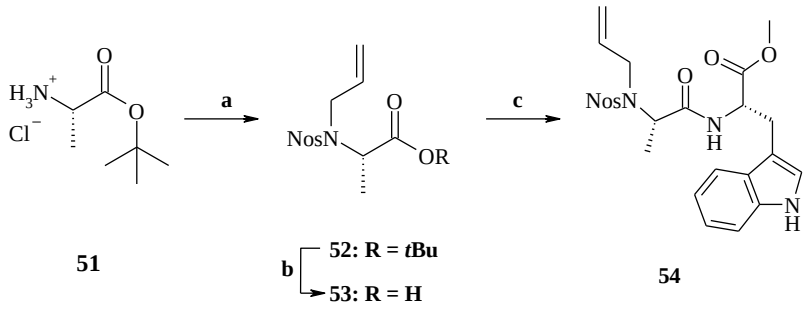
**Scheme 13: Synthesis of *N*-allyl-glycinamide**




Reagents and conditions: a) *o*-nitrobenzenesulfonyl chloride, DCM/DMF, TEA, 0 °C to rt, 24 h, 74%; b) allyl bromide, $Cs_2CO_3$, DMF, rt, 20 h, 98%; c) thiophenol, $K_2CO_3$, ACN, 20 h, 100%.

The other *N*-allylated building block was prepared similarly. Commercially available L-alanine *tert*-butyl ester hydrochloride **51** was first converted into the corresponding sulfonamide using NosCl/TEA in DCM, which was then reacted with allyl bromide and caesium carbonate in DMF to provide the *N*-allylated alanine ester **52** in excellent yield (98%). Acidic hydrolysis of the ester, followed by reaction with tryptophan methyl ester hydrochloride using PyBop-mediated coupling in DIPEA, gave the dipeptide **54** (82%) (Scheme 14).

**Scheme 14: Preparation of *N*-allylated sulfonamide 54**




Reagents and conditions: a) (i) *o*-nitrobenzenesulfonyl chloride, DCM, TEA, 20 h, rt, 98%; (ii) allylbromide, $Cs_2CO_3$, DMF, rt, 30 min, 100%; b) TFA, DCM, 4 h, rt, 100%; c) tryptophanmethylester hydrochloride, PyBop, DIPEA, ACN, 20 h, 82%.

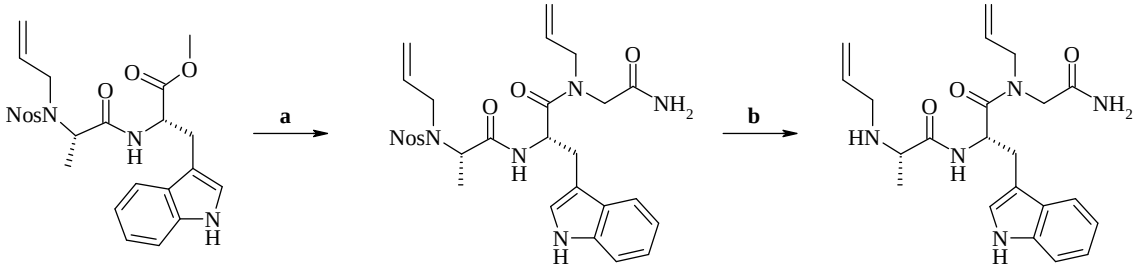
Saponification of the methyl ester with 4% LiOH in THF afforded the corresponding acid, which then reacted with the secondary amine **50** to provide the key product **55** in 60% yield (2 steps). However, the coupling reaction was problematic. The use of TBTU as the first coupling reagent

led to the racemization of the acid. To solve this problem, several coupling reagents were examined, including HATU, 2-bromo-1-ethyl pyridinium tetrafluoroborate (BEP), benzotriazol-1-yloxytri(pyrrolidino)-phosphonium hexafluorophosphate (PyBOP) and bis(2-oxo-3-oxazolidinyl) phosphinic chloride (BOP-Cl) (Table 6). Accordingly, the desired product **55** was obtained in 75% yield, without epimerization, when BOP-Cl was employed in acetonitrile for 16 h at room temperature (Scheme 15).

**Table 6:** Optimal reaction conditions of the diene **55**

| Coupling reagent | Solvent | Conditions | Racemization ratio (Based on HPLC-MS) |
|---|---|---|---|
| TBTU/DIPEA | ACN | 20 h, rt | 60:40 |
| HATU/DIPEA | DMF | 20 h, rt | 70:30 |
| BEP/DIPEA | DMF | 20 h, rt | 65:35 |
| BOP-Cl/DIPEA | ACN | 16 h, rt | 100:0 |
| PyBrOP/DIPEA | THF | 20 h, rt | 85:15 |

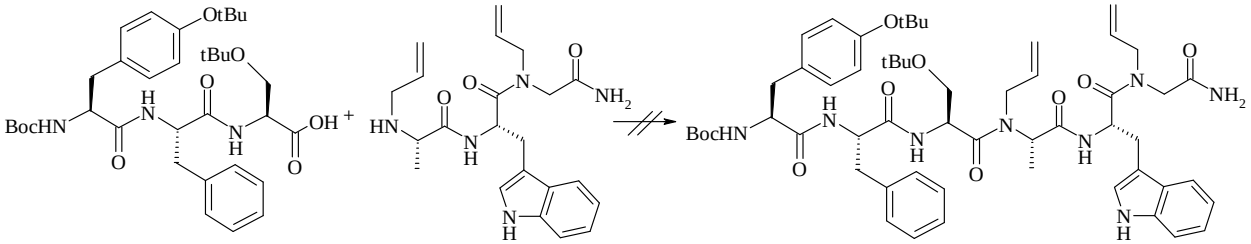
**Scheme 15: Synthesis of diene 55**




**54** **55** **56**

Reagents and conditions: a) (i) 4% LiOH, THF, rt, 4 h, 80%; (ii) *N*-allyl-glycinamide (**50**), BOP-Cl, DIPEA, ACN, -10 $^{o}$C to rt, 16 h, 75%; b) thiophenol, $K_2CO_3$, ACN, 20 h, 98%.

**Scheme 16: Convergent synthesis of hexapeptide 58**




**57** **56** **58**

Reagents and conditions: Shown in Table 7

Having the bis-*N*,*N*-allylated tripeptide **55** in hand, two synthetic strategies were used to obtain the final macrocyclic hexapeptide **66**. The first synthetic route involved in *N*-Nos removal, elongation of the *N*-terminal tripeptide and macrocyclization reaction. For this reason, the *N*-Nos protected tripeptide **55** was first treated with thiophenol and $K_2CO_3$ to yield the secondary amine **56** (98%) (Scheme 15).

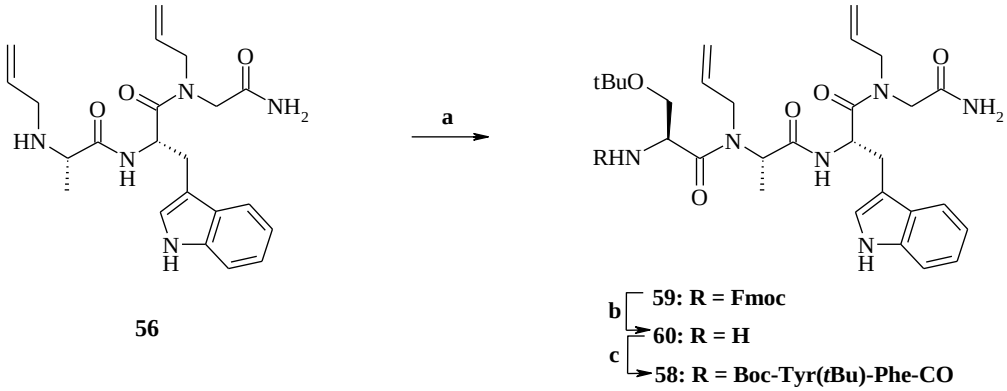
Attachment of tripeptide Boc-Tyr(*t*Bu)-Phe-Ser(*t*Bu)-OH to the secondary amine **56** was initially examined to get the hexapeptide **58** (Scheme 16). A number of standard peptide coupling reagents that are efficiently useful for coupling of *N*-alkylated amines were employed, such as bromotripyrrolidinophosphonium hexafluorophosphate (PyBrop), 2-bromo-3-ethyl-4-methylthiazolium tetrafluoroborate (BEMT), HATU, 2-bromo-1-ethylpyridinium tetrafluoroborate (BEP), bis(trichloromethyl)-carbonate (BTC), BMIT, and 4-(4,6-bis[2,2,2-trifluoroethoxy]-1,3,5-triazin-2-yl)-4-methylmorpholinium tetrafluoroborate (DFET)[103] (Table 7). However, the desired product **58** was not formed, even at elevated temperature and for prolonged reaction times. Probably, the sterically hindered side chains around the activated carboxylic acid intermediate prevent the coupling at the selective site.

**Table 7**: Optimization of the convergent synthesis of **58**

| Coupling reagent and base | Solvent | Conditions | Results (based on HPLC/MS analysis) |
|---|---|---|---|
| HATU/ DIPEA | ACN | 20 h, rt | No reaction (NR) |
| HATU/HOAt/NMM | NMP | 10 h, rt, then 50 $^{o}$C, 10 h | NR |
| HATU/HOAt/DIPEA | DMF or DCM/DMF | 10 h, rt, 50 $^{o}$C, 8 h, 65 $^{o}$C 6h | NR |
| BEP/HOAt/DIPEA | DMF or DCM/DMF | 48 h, rt, 50 $^{o}$C, 8 h, 65 $^{o}$C 8h | NR |
| PyBrOP/DIPEA | THF | 48 h, rt, then 50 $^{o}$C 8h | NR |
| BTC/ 2,4,6-colidine or 2,6-lutidine | THF | 24 h, rt, 50 $^{o}$C, 18 h | NR |
| BEMT/ DIPEA | DCM, | -10 $^{o}$C to rt, 36 h, then 40 $^{o}$C 24 h | NR |
| BMIT/ DIPEA | DCM | 24 h, rt, 45 $^{o}$C, 24 h | NR |
| DFET/NMM/$BF_4$ | THF | 48 h, rt, then 45 $^{o}$C, 6 h | NR |

Due to the unsuccessful convergent synthesis, the hexapeptide **58** was prepared stepwise. Incorporation of Fmoc-Ser(*t*Bu)-OH into tripeptide **56** produced the tetrapeptide **59** in a high yield (98%). Again, the use of HATU as a coupling reagent in acetonitrile at room temperature led to racemized products (in a ratio of 60:30). In order to determine the optimal reaction conditions, several highly active coupling reagents were tried in different solvents (Table 8). The results proved that PyBrop prevented efficiently the epimerization. Only 4% of racemized product was formed (based on HPLC-MC analysis). Upon exposure of the Fmoc-protected peptide **59** to DBU in DCM for 30 min followed by coupling with dipeptide Boc-Tyr(*t*Bu)-Phe-OH, the ring-closing metathesis precursor **58** was obtained in 52% overall yield (Scheme 17).

**Scheme 17: Elongation of peptide chain**



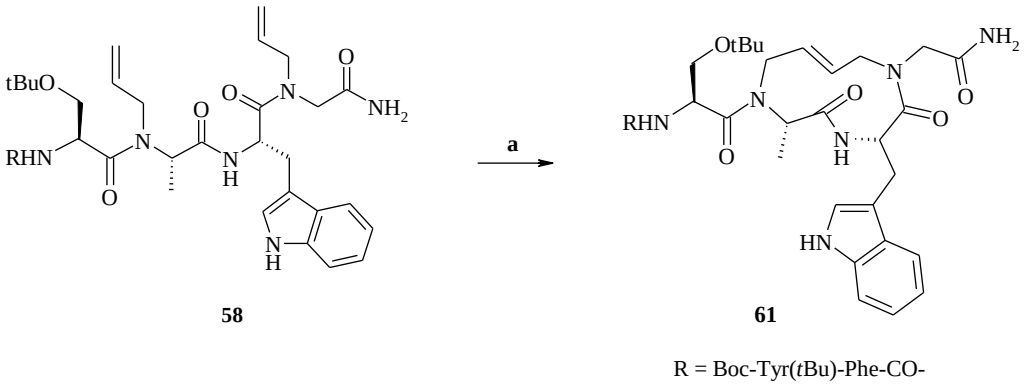

Reagents and conditions: a) Fmoc-Ser(*t*Bu)-OH, PyBrop, DIPEA, THF, rt, 20 h, 98%; b) DBU, DCM, 30 min, 82%; c) Boc-Tyr(*t*Bu)-Phe-OH, HATU, DIPEA, DMF, rt, 18 h, 63%.

**Table 8**: Optimizing the coupling reaction of **59**

| Coupling reagent | Solvent | Conditions | Racemization ratio (Based on HPLC-MS) |
|---|---|---|---|
| HATU/DIPEA | ACN | 20 h, rt | 70:30 |
| HATU/DIPEA | ACN/DMF (1/4) | 20 h, rt | 70:30 |
| HATU/DIPEA | DMF | 20 h, rt | 85:15 |
| BOPCl/DIPEA | DMF | 60 h, rt | No reaction |
| PyBrOP/DIPEA | THF | 20 h, rt | 96:4 |

The key step of the synthetic route was ring-closing metathesis reaction in order to form the 11-membered macrocycle **61** (Scheme 18). In the first attempt the macrocyclization was carried out with a Grubbs 1st generation catalyst (10 mol%) in refluxing $CH_2Cl_2$ for 72 h providing none of the desired product **61**. However, when the highly active Zhan's catalyst (10 mol%) was employed in the same manner, the intramolecular cyclization proceeded slowly.[104-106] The expected macrocycle **61** was obtained in 19% yield, after 96 h refluxing. The low conversion was reasoned due to the bulky substituents around the diene termini that prevent them from coming to close proximity. Thus, this synthetic strategy was disadvantageous for preparation of the targeted macrocyclic peptide.

**Scheme 18: Ring-closing metathesis of diene 58**



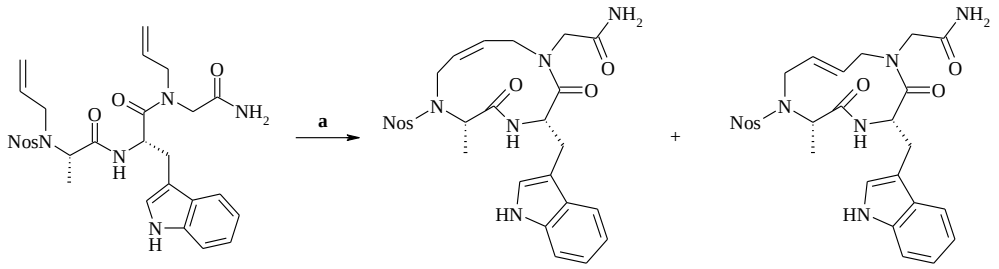

**58** **61**

R = Boc-Tyr(*t*Bu)-Phe-CO-

Reagents and conditions: a) Zhan catalyst (10 mol%), DCM, reflux, 94 h, 19%.

In the other approach, the olefin ring-closing metathesis reaction was performed with the diene **55** (Scheme 19). Upon treatment of **55** with the Grubbs 1st generation catalyst (10 mol% loading) in refluxing $CH_2Cl_2$ for 60 h, the desired macrocycle **62** was only obtained in 10% yield. On the other hand, ring-closing metathesis of diene **55** with highly diluted solution of 1 mM using the 2nd Hoveyda-Grubbs catalyst (10 mol% loading) in refluxing DCM for 56 h afforded two separable products **62a** and **62b** in 9% and 43% yields, respectively, after flash column chromatography. The *E*-geometry of the major product **62b** was established by the high coupling constant of the two adjacent alkene protons ($J_{Ha\text{-}Hb}$ = 15.5 Hz) based on COSY- and $^1$H-NMR decoupling experiments (Figure 19).

**Scheme 19: Ring-closing metathesis of diallylamine 55**




**55** **62a** **62b**

Reagents and conditions: a) Hoveyda-Grubbs 2nd catalyst (10 mol %), DCM, reflux, 56 h, 43% (**62b**), 9% (**62a**).

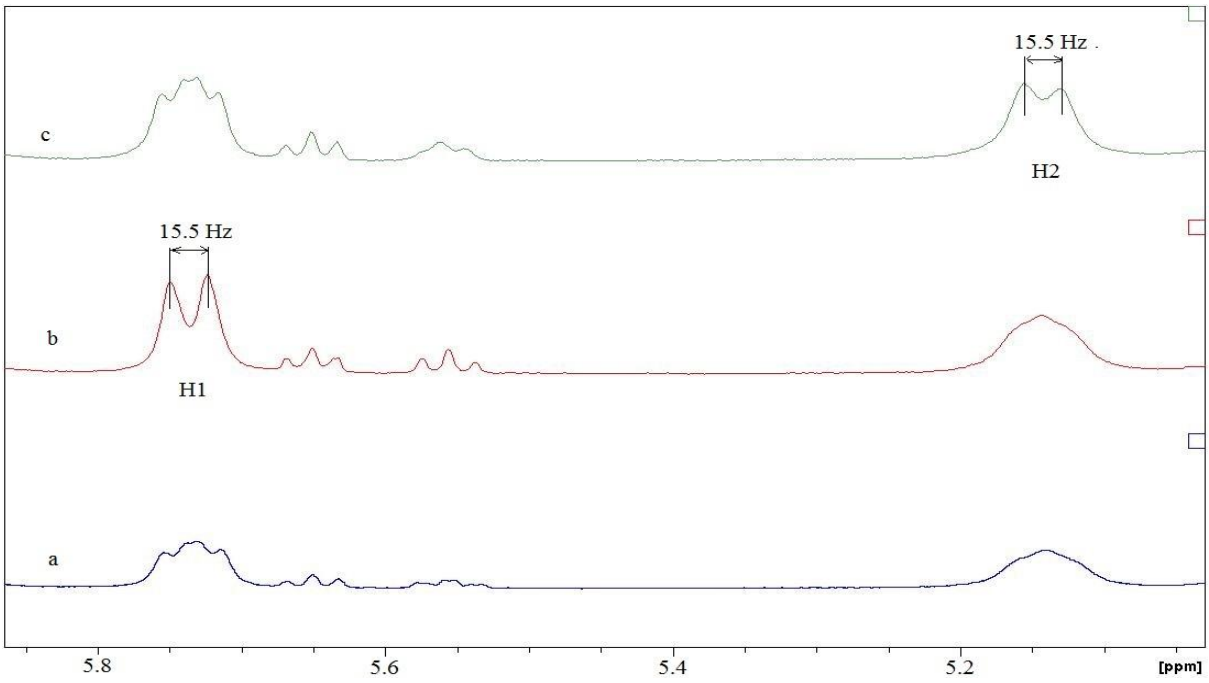
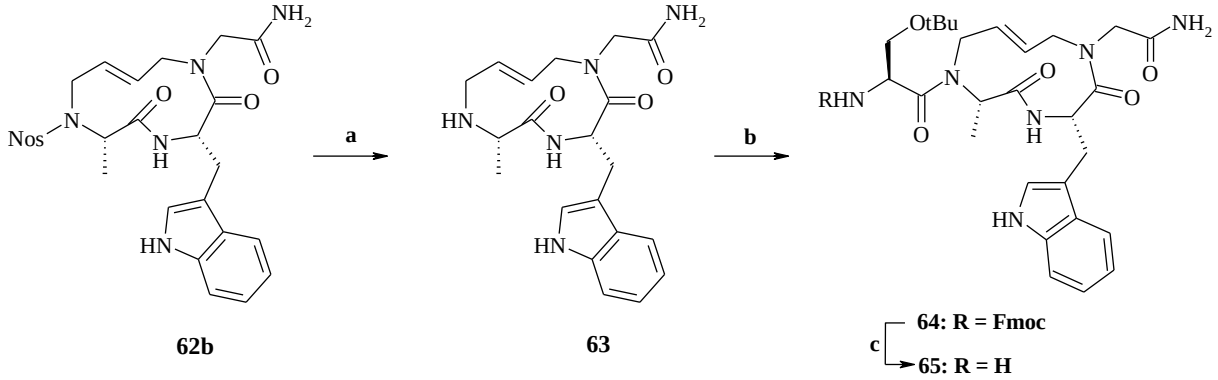



**Figure 19**: a) $^{1}$H-NMR expanded alkene area of alkene **62b**; b) Selective decoupling of proton at 3.91 ppm; c) Selective decoupling of proton at 3.58 ppm.

The *N*-Nosyl deprotection of compound **62b** was first tested with thiophenol and $K_2CO_3$ at room temperature for 18 h. However, only 10% conversion of the secondary amine **63** was found. Alternatively, when macrocycle **62b** was treated with mercaptoethanol in the presence of DBU (1,8-diaza-bicyclo[5.4.0]undec-7-ene) as catalyst at room temperature for 1 h, the desired amine **63** was obtained in excellent yield (95%). Acylation of Fmoc-Ser(*t*Bu)-OH to the secondary

amine using HATU as the coupling reagent and subsequent *N*-Fmoc deprotection with piperidine in DCM provided the cyclic peptide **65**, in 67% overall yield (Scheme 20).

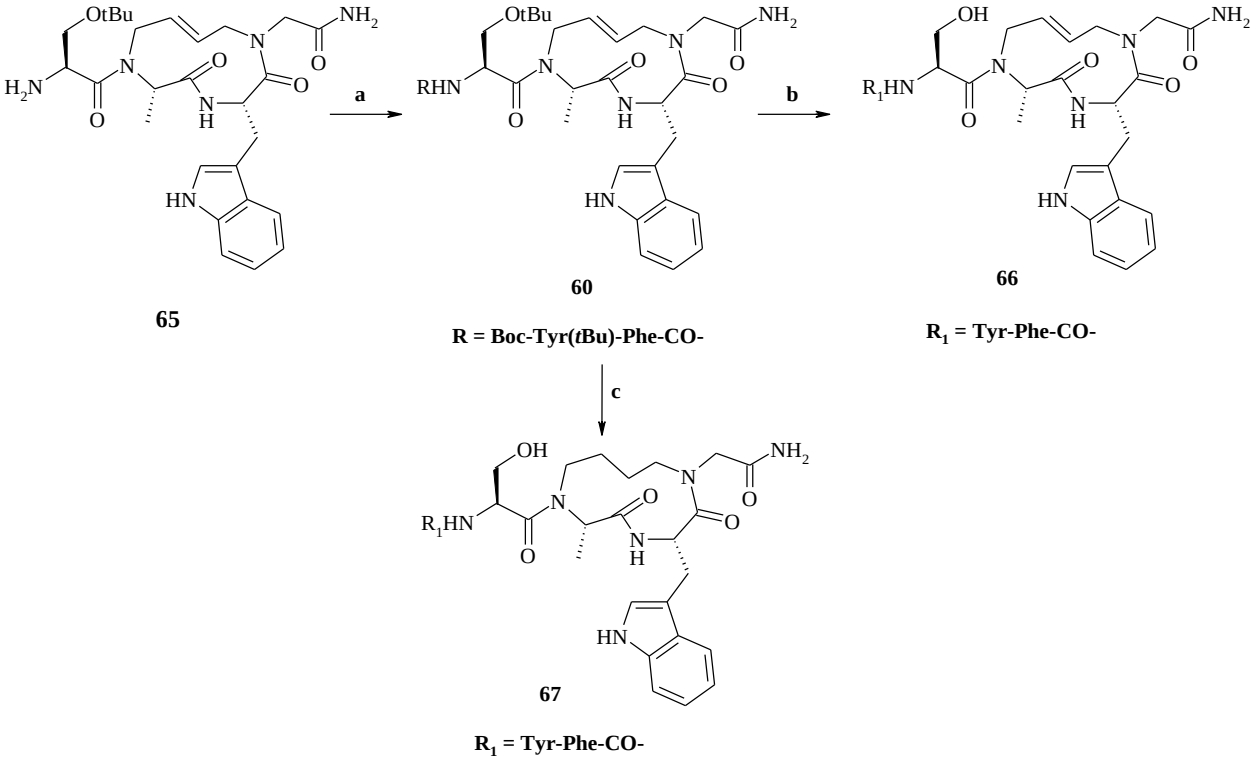
**Scheme 20: Extension of the *N*-terminal peptide chain of macrocyclic peptide 62b**




**62b** **63**

Reagents and conditions: a) $HSCH_2CH_2OH$, DBU, DMF, rt, 1 h, 95%; b) Fmoc-Ser(*t*Bu)-OH, HATU, DIPEA, DMF, rt, 67%; c) Piperidine, DCM, 4 h, rt, 99%.

**Scheme 21: Synthesis of peptides 66 and 67**




**65**

**60**

**R = Boc-Tyr(*t*Bu)-Phe-CO-**

**66**

**$R_1$ = Tyr-Phe-CO-**

**67**

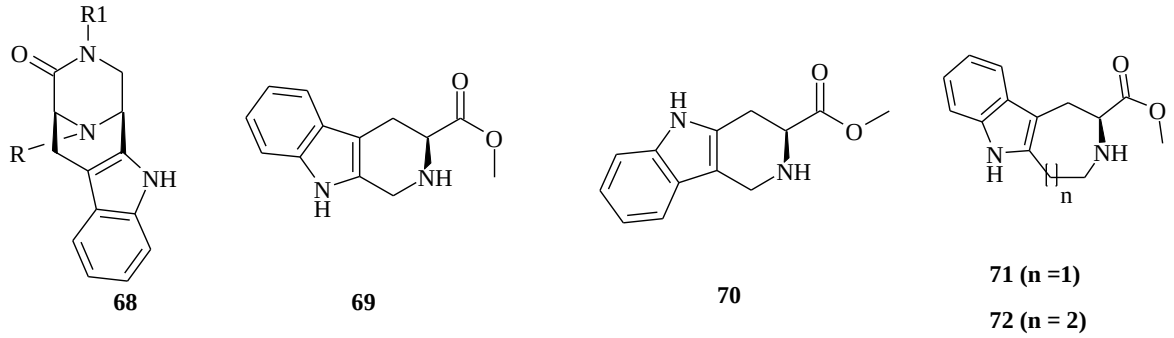
**$R_1$ = Tyr-Phe-CO-**

Reagents and conditions: a) Boc-Tyr (*t*Bu)-Phe-OH, HATU, DIPEA, DMF, 24 h, 76%; b) TFA/TIPS/$H_2O$, 0 $^oC$, 4 h, 95%; c) (i) Pd/C, $H_2$, MeOH/ EtOAc, rt, 24 h, 97%; (ii) TFA/TIPS/$H_2O$, 0 $^oC$, 4 h, 91%.

The key intermediate **60** was prepared in 76% yield from the coupling of dipeptide Boc-Tyr(*t*Bu)-Phe-OH to amine **65**, which was then converted into the desired product **66** by acidic deprotection. On the other hand, the saturated peptide **67** was readily obtained in 88% yield by a sequential reaction of hydrogenation and successive acidic deprotection (Scheme 21).

## 2.2 Tryptophan-derived helicokinin I peptidomimetics

Reduction of the conformational flexibility of the peptide backbone can be achieved not only by forming macrocyclic peptides, but also by constraining amino acid residues. Due to the restriction of the ϕ and ψ torsion angles within the amino acid residues, their incorporation into the peptide backbones will rigidify the backbone conformations and therefore influence on the orientation of the *N*- and *C*-terminus.




**68** **69** **70** **71 (n =1)** **72 (n = 2)**

Diaza-heterocyclic derivative

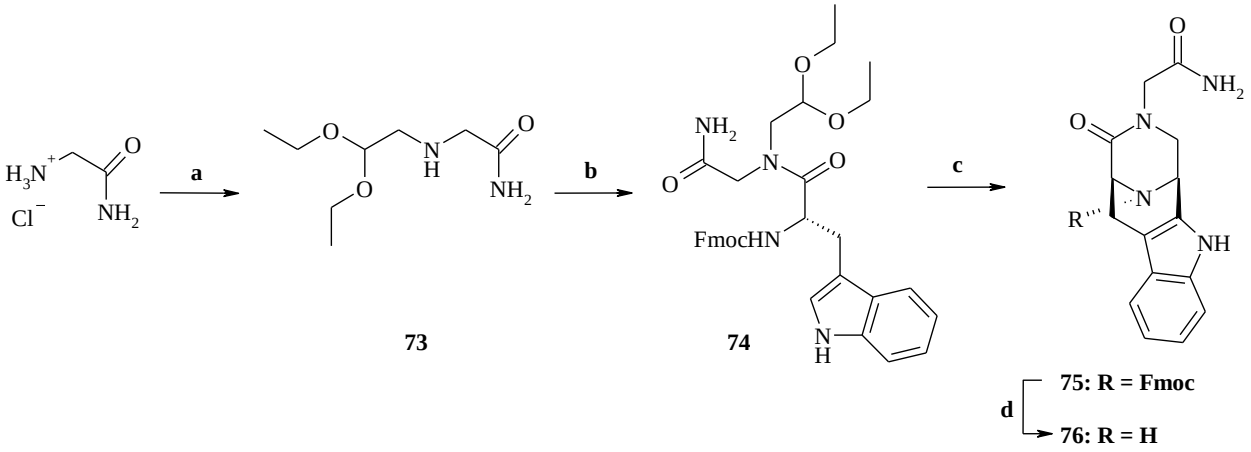
**Figure 20:** Conformationally constrained scaffolds derived from tryptophan and iso-tryptophan

It has been reported that the Trp residue is somewhat less important for triggering receptor activation,[64] and can be modified without complete loss of bioactivity. With the aim of exploring the significance of the Trp side chain for molecular recognition, several conformationally restricted structures derived from tryptophan and iso-tryptophan are aimed to synthesize (Figure 20). These building blocks are then incorporated into the helicokinin I sequence.

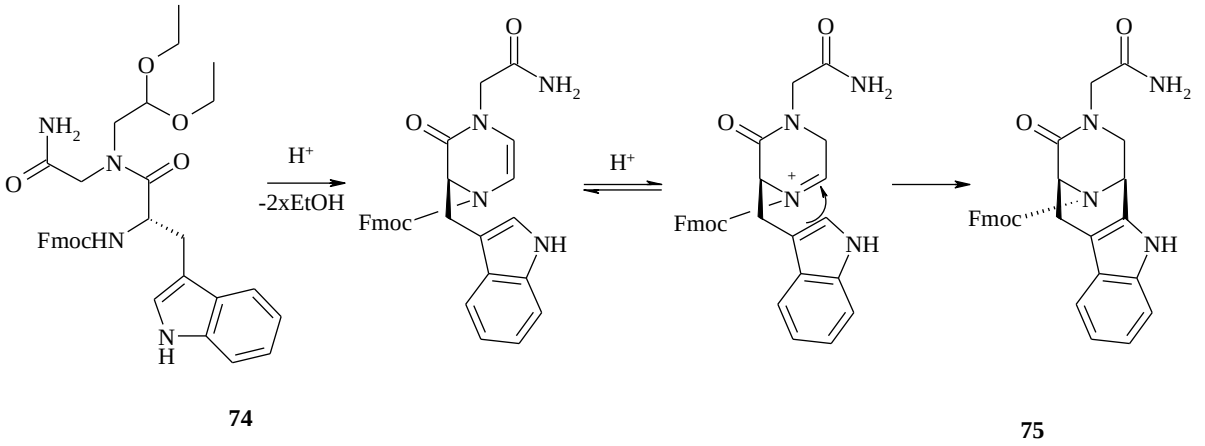
### 2.2.1 Helicokinin I analogue containing diaza-bridged heterocycle

The synthetic route used in the preparation of the key building block **76** is shown in Scheme 22. Treatment of glycinamide hydrochloride with 2-bromacetaldehyde diethylacetal and $NaHCO_3$ in refluxing acetonitrile for 48 h gave the product **73** in 37% yield. The low yield was due to the low solubility of the *N*-alkylated glycine amide which caused difficulty in working up and purifying by silica gel flash chromatography. Next, the dipeptide **74** was prepared in 98% yield by acylation of Fmoc-L-Trp-OH to the secondary amine **73** using TBTU as coupling reagent. Upon treatment of the acetal **74** with 1N HCl in acetonitrile at 0 ºC for 3 h, the tetraheterocycle **75** was obtained in 75% yield. The cyclization occurs in a sequential process (Scheme 23). The acetal deprotection under acidic conditions followed by stereospecific intramolecular cyclization *in situ* of the iminium intermediate afforded the tetracyclic product **75**. The secondary free amine **76** was obtained in 76% yield by treatment of the *N*-Fmoc protected product **75** with piperidine in DCM at room temperature for 14 h.

**Scheme 22: Preparation of piperazine-3-one building block**




Reagents and conditions: a) 2-Bromacetaldehyde diethylacetal, $NaHCO_3$, $CH_3CN$, reflux 48 h, 37%; b) Fmoc-L-Trp-OH, TBTU, DIPEA, DCM, rt, 24 h, 98%; c) 1N HCl, $CH_3CN$, 0 ºC, 3 h, 75%; d) piperidine, DCM, 14 h, 76%.

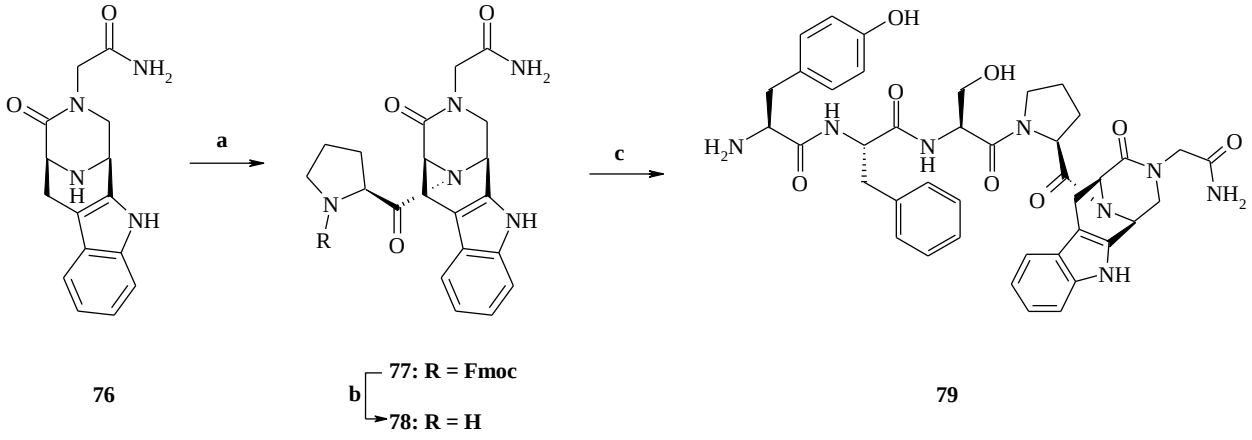


**74** **75**

**Scheme 23:** Mechanism of the intramolecular cyclization

The next steps were the elongation of the *N*-terminal tetrapeptide. Attachment of the Fmoc-Pro-OH to the amine **76** using HATU and DIPEA yielded the peptide **77** (89%), which upon *N*-Fmoc deprotection with piperidine in DCM afforded the tripeptide **78**. The corresponding hexapeptide was prepared in 67% yield by coupling of tripeptide Fmoc-Tyr(*t*Bu)-Phe-Ser(*t*Bu)-OH with the amine **78** using HATU. Finally, removal of *N*-Fmoc, Boc and *tert*-butyl protecting groups afforded the desired hexapeptide **79** in 99% yield (Scheme 24).

**Scheme 24: Synthesis of peptide 79**



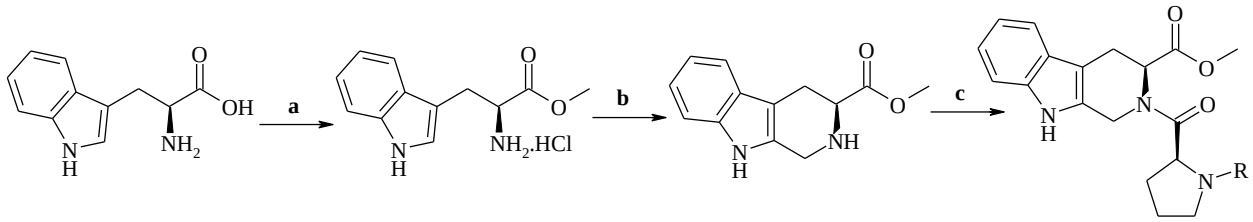
**76** **77: R = Fmoc** **78: R = H** **79**

Reagents and conditions: a) Fmoc-Pro-OH, HATU, DIPEA, DMF, 24 h, 89%; b) piperidine, DCM, rt, 5 h, 99%; c) (i) Fmoc-Tyr(*t*Bu)-Phe-Ser(*t*Bu)-OH, HATU, DIPEA, DMF, rt, 24 h, 67%; (ii) piperidine/ DCM, 75%; (iii) TFA/TIPS/$H_2O$, 0 °C, 3 h, 99%.

### 2.2.2 Helicokinin I analogue containing a tetrahydro-β-carboline moiety

Continuing to the derivatization of tryptophan residue, the tetrahydro-β-carboline **69** was similarly prepared according to Pictet–Spengler reaction. The acid-catalyzed intramolecular condensation between an iminium ion and an aromatic C-nucleophile of tryptophan ester was expected to give the desired product **69**. Surprisingly, upon treatment of L-tryptophan methyl ester with 36% formaldehyde in the presence of either 1N HCl or $CF_3COOH$ acid as catalyst, no expected product was formed.[107,108] Alternatively, exposure of 36% formaldehyde with L-tryptophan methyl ester hydrochloride,[109] which was readily prepared from esterification of tryptophan with methanol and thionyl chloride, afforded the key product *S*-methyl-1,2,3,4-tetrahydropyrido[3,4-b]indole-3-carboxylate **69** in 67% yield after 4 h at room temperature. Subsequent reaction of amine **69** with Fmoc-Pro-OH using TBTU as coupling reagent resulted in the dipeptide **82** (90%) (Scheme 25).

**Scheme 25: Synthesis of tetrahydro-β-carboline**

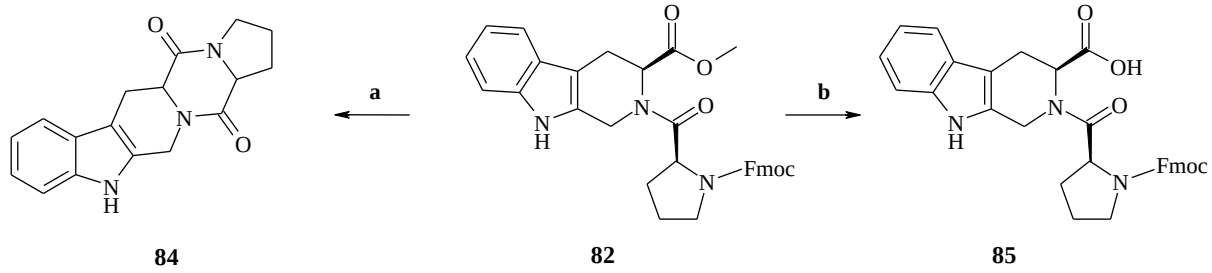



Reagents and conditions: a) $SOCl_2$, MeOH, rt, 20 h, 93%; b) 36% HCHO, MeOH, rt, 4 h, 67%; c) (i) **82**: Fmoc-L-Pro-OH, TBTU, DMF, DIPEA, 88%; (ii) **83**: Boc-L-Pro-OH, TBTU, DMF, DIPEA, 90%.

We next attempted to convert the methyl ester **82** into the corresponding acid **85** (Scheme 26). First treatment of ester **82** with 4% LiOH at 0 $^oC$ for 4 h followed by acidification with 1N HCl did not provide the desired product **85**, instead both methyl ester and Fmoc groups were simultaneously cleaved. Alternative conditions to hydrolysis were explored. Either lithium iodide (LiI) in refluxing ethyl acetate for 54 h or hydroxytrimethyltin ($Me_3SnOH$) in refluxing $C_2H_4Cl_2$ for 50 h was used for the reaction.[110-112] However, the conversion of the substrate was only 10% (based on HPLC/MS analysis). On the other hand, treatment of *N*-Fmoc protected product **82**

with piperidine in DCM to form the corresponding secondary amine was also unsuccessful. Under the mild basic conditions, an intramolecular cyclization product, diketopiperazine derivative **84**, was obtained in 97% yield.

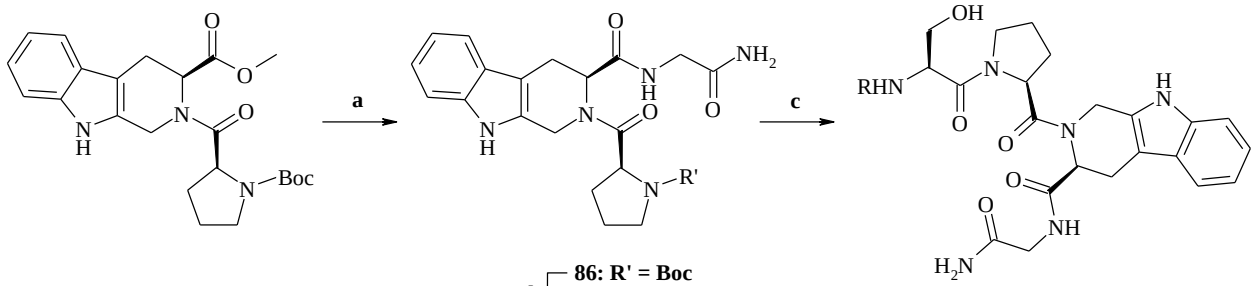
**Scheme 26: Attempted hydrolysis of the methyl ester 82**




**84** **82** **85**

Reagents and conditions: a) piperidine, DCM, rt, 4 h, 97%; b) 4% LiOH, THF, 0 ºC, 4 h.or LiI, AcOEt, reflux, 54 h; or $Sn(CH_3)_3OH$, $ClCH_2CH_2Cl$, 80 ºC, 50 h.

**Scheme 27: Synthesis of peptide 88**




**83** **86: R' = Boc** **87: R' = H** **88**

**R = Tyr-Phe-CO**

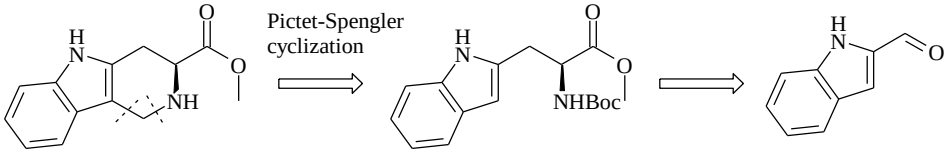
Reagents and conditions: a) (i) 4% LiOH, THF, rt, 4 h, 98%; (ii) glycinamide hydrochloride, TBTU, DMF, rt, 24 h, 90%; b) TFA:TIPS:$H_2O$ (95:2.5:2.5), 0 ºC, 3 h, 87%; c) (i) Boc-Tyr(*t*Bu)-Phe-Ser(*t*Bu)-OH, DMF, HATU, DIPEA, rt, 62%; (ii) TFA:TIPS:$H_2O$ (95:2.5:2.5), 0 ºC, 3 h, 97%.

Owing to the difficulty in removing selectively either methyl or Fmoc group, dipeptide **83** was prepared in 90% yield by coupling of amine **69** with Boc-Pro-OH (Scheme 25). Hydrolysis of the ester **83** with 4% LiOH afforded the corresponding carboxylic acid, which was then reacted with glycine amide hydrochloride using TBTU to give the tripeptide **86** in 88% overall yield. TFA-mediated deprotection and subsequent reaction with tripeptide Boc-Tyr(*t*Bu)-Phe-Ser(*t*Bu)-

OH using HATU as coupling reagent gave a full protected hexapeptide, which upon acidic deprotection afforded the desired hexapeptide **88** in 53% yield over three steps (Scheme 27).

### 2.2.3 Synthesis of iso-tryptophan

The next derivatization of tryptophan is the preparation of building block **70** (tetrahydro-5H-pyrido[4,3-b]indole analogue). Similarly, application of the Pictet Spengler cyclization reaction was intended to synthesize the targeted product through a condensation of formaldehyde with iso-tryptophan methyl ester **89** under acidic conditions (Scheme 28). Thus, our attention was first focused on preparation of iso-tryptophan methyl ester **89**.

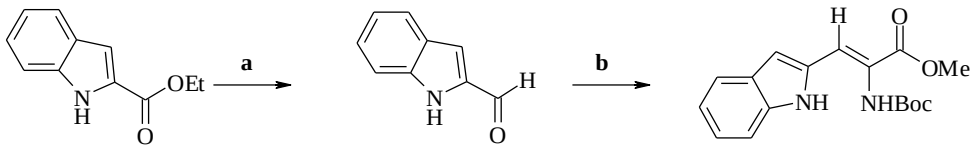



**70** Iso-tryptophan acid (**89**) **90**

**Scheme 28:** Retrosynthetic analysis of product **70**

Ethyl indole-2-carboxylate ester **91** served as starting material and was first converted into the corresponding primary alcohol by reduction with lithium aluminum hydride (LAH) in dry ether. Subsequently, the PCC-mediated oxidation of the primary alcohol furnished the 2-formylindole **90**.[113] Though a qualitative analysis (TLC monitoring) showed a complete consumption of the starting material, only 43% of the pure aldehyde **90** was obtained after extractive work-up and chromatographic purification. The main reason was the instability of the resulting aldehyde that was over-oxidized during workup process. In the next step, a Horner–Wadsworth–Emmons type olefination of the aldehyde **90** with Boc-α-phosphonoglycine trimethylester using 1,1,3,3-tetramethylguanidine as a base at room temperature for 3 days provided two separable products, the major *Z*-enamido ester **92** and its minor *E*-isomer in a ratio of 88/12, respectively. The *Z*-configuration of the major product **92** was assigned based on NOESY experiments by a medium-range interaction between the NH of the indole ring and the NH of enamide functional group (Figure 21).

**Scheme 29: Synthesis of isotryptophan**




**91** **90** **92**

Reagents and conditions: a) (i) LAH, ether, 1 h, rt, 86%; (ii) PCC, DCM, 0 $^{o}$C, 2 h, 43%; b) 1,1,3,3-tetramethyl-guanidine, Boc-α-phosphonoglycine trimethylester, THF, -78 $^{o}$C, 1 h, then rt, 3 days, 86%.

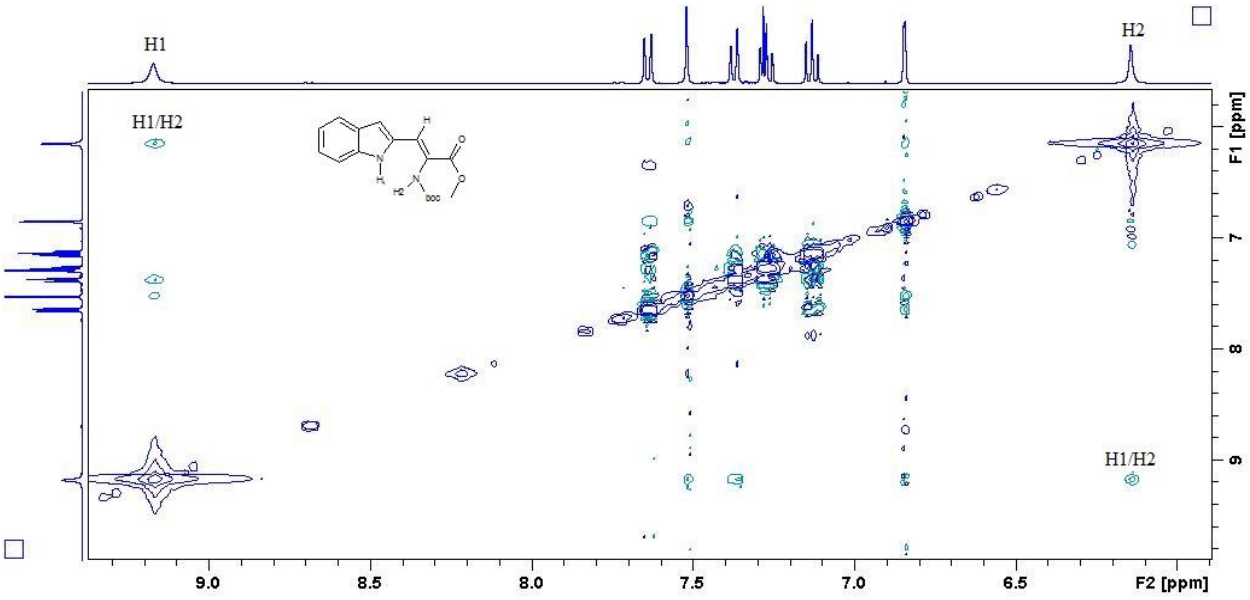
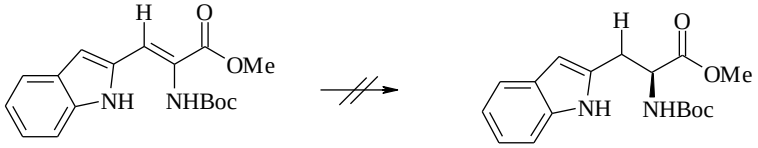



**Figure 21:** NOESY spectra of enamido ester **92**

**Scheme 30: Asymmetric hydrogenation**



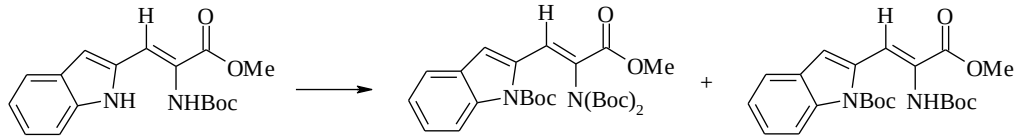

**92** **89**

Reagents and conditions: 1.2 mol% [Rh(COD)(S,S)-EtDuPhos]$^{+}$TfO$^{-}$, MeOH, $H_2$, rt, 3 days, or EtOAc, $H_2$, 5 atm, rt, 5 days.

The key step in the synthesis was the conversion of *Z*-enamido ester **92** into the desired (*S*)-iso-tryptophan methyl ester **89** by an asymmetric hydrogenation using Burk's catalyst

[Rh(COD)(*S*,*S*)-EtDuPhos]$^{+}$TfO$^{-}$ (1.2 mol% loading) in degassed MeOH or EtOAc. Unfortunately, no expected product was formed after 3-5 days at room temperature under hydrogen atmosphere (1-5 atm) (Scheme 30).

**Scheme 31: *N*-Boc protection reaction of 92**

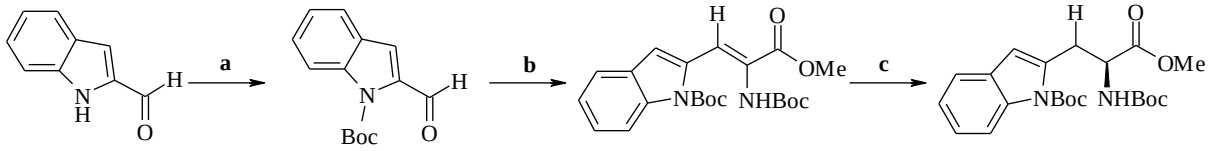



**92** **93** **94**

Reagents and conditions: $Boc_2O$, DMAP, ACN, rt, 2 h.

Owing to the failure of the hydrogenation of the alkene **92**, we decided to protect the NH of indole ring with Boc group prior to exposure to the hydrogenation conditions. A direct treatment of the *Z*-enamido ester **92** with $Boc_2O$ (1.0 equiv) in the presence of DMAP as a catalyst did not give the targeted product **94**, but undesired product **93** (Scheme 31).

**Scheme 32: Synthesis of iso-trytophan**




**90** **95** **94** **96**

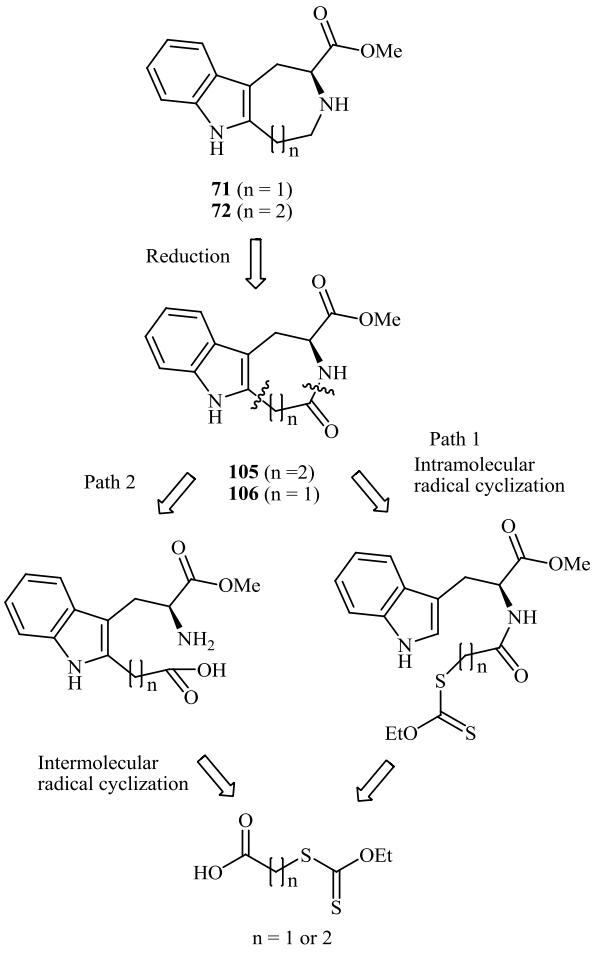
Reagents and conditions: a) $Boc_2O$, DMAP, ACN, rt, 99%; b) 1,1,3,3-tetramethylguanidine, Boc-α-phosphonoglycine trimethylester, THF, -78 $^{o}$C, 1 h, then rt, 3 days, 86%; c) 1.2 mol% [Rh(COD)(*S*,*S*)-EtDuPhos]$^{+}$TfO$^{-}$, MeOH, $H_2$, rt, 3 days, 5 atm (45% conversion, based on HPLC-MS).

On the other hand, treatment of 2-formylindole **90** with $Boc_2O$ and DMAP afforded product **95** in 99% yield. Under the same conditions as described above, the olefination reaction of aldehyde **95** with Boc-α-phosphonoglycine trimethylester and 1,1,3,3-tetramethylguanidine led to the *Z*-enamido ester **94** (86%) (Scheme 32). With the desired protected ester **94** in hand, we again explored the asymmetric hydrogenation using [Rh(COD)(*S*,*S*)-EtDuPhos]$^{+}$TfO$^{-}$ (1.2 mol%) as catalyst in degassed methanol. Interestingly, 45% conversion of the desired product **96** was found after 3 days at room temperature and 5 atm ($H_2$). Unfortunately, we were not able to obtain

the pure iso-tryptophan ester **96** due to the inability of separating it from the starting material by silica gel flash chromatography. The successful hydrogenation suggests that protection of the NH indole is necessary to facilitate the asymmetric hydrogenation. For further exploration of the hydrogenation, the *N*-Boc protected enamide should be exchanged for *N*-Cbz enamide to reduce its steric hindrance.

### 2.2.4 Synthesis of tryptophan-derived tricyclic analogues

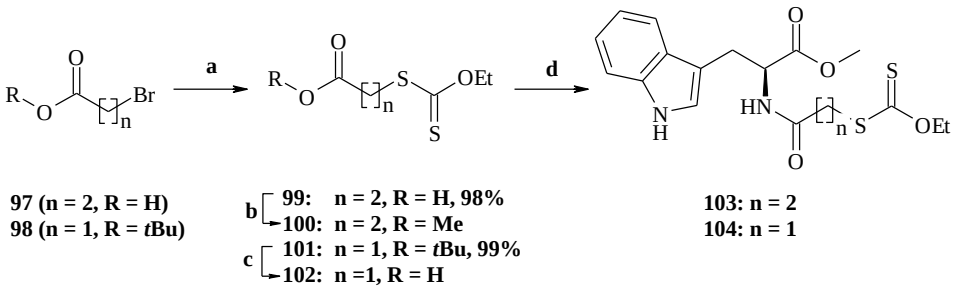
The next attention was focused on the preparation of two novel tryptophan-derived tricyclic analogues, azepinoindole (**71**) and azocinoindole (**72**). We intended two practical strategies to construct seven- and eight-membered rings. The carbon-carbon bond formation was accomplished by either intramolecular or intermolecular radical alkylation on C-2 of the indole ring utilizing radical reaction of xanthates (Scheme 33).




**Scheme 33: Retrosynthetic analysis of tricycles 106 and 105**

Xanthate radical transfers **99** and **101** were readily obtained in high yield by treatment of the commercially available potassium O-ethyl dithiocarbonate with the corresponding 3-bromopropanoic acid and *tert*-butyl-bromoacetate.[114] In the first synthetic route, intramolecular radical cyclization, the xanthate intermediates **103** and **104** were prepared in good yields (71-75%) by coupling of acids **99** and **102** with L-tryptophan methyl ester using TBTU and DIPEA in acetonitrile (Scheme 34).

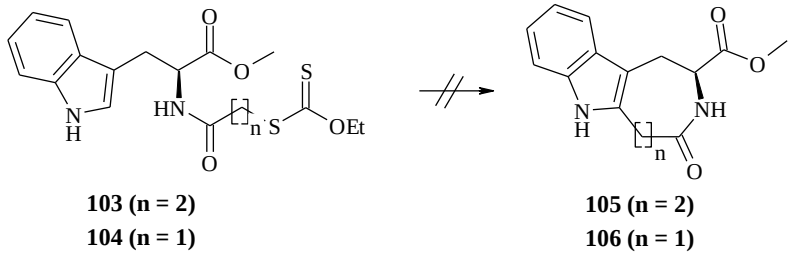
**Scheme 34: Synthesis of xanthates 103 and 104**




Reagents and conditions: a) Ethylxanthic acid potassium salt, ACN, rt, 18-20 h; b) THF, trimethylsilyldiazomethane, rt, 2 h, 97%; c) TFA, DCM, rt, 4 h, 98%; d) H-Trp-OMe, TBTU, DIPEA, ACN, rt, 20 h, 71-75%.

The key step in the synthesis was the xanthate-promoted intramolecular cyclization. Lactams **105** and **106** were initially attempted to be prepared by adding portionwise dilauroyl peroxide (DLP) to the boiling solution of the xanthate substrates (**103** and **104**) in degassed DCE for 4-6 h (Scheme 35).[115,116] However, the expected products **105** and **106** were not formed even after prolonged reaction time or excess addition of the radical initiator DLP.

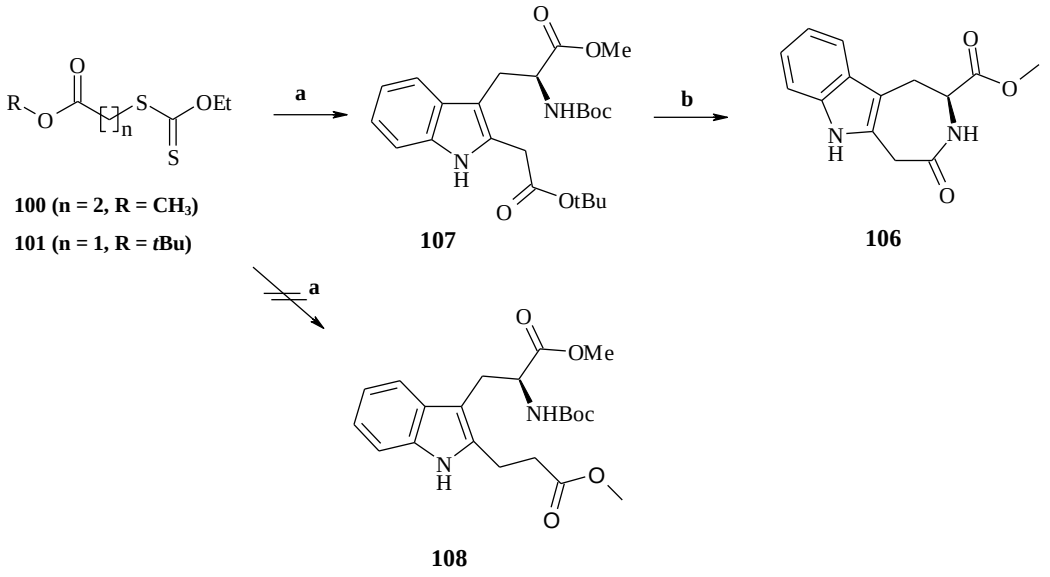
**Scheme 35: Attempted synthesis of 105 and 106 by an intramolecular radical cyclization**




Reagents and conditions: Lauroyl peroxide (1.3-1.8 equiv), 1,2-dichloroethane, reflux, 6-12 h.

As an alternative, the intermolecular radical alkylation of xanthate esters **101** and **100** on C-2 of tryptophan methyl ester, which was carried out under the same conditions as described above, provided the 2-alkylated indole **107** (n = 1) in 40% yield, while the other desired product **108** (n = 2) was not formed (Scheme 36). Despite a number of efforts, preparation of the expected tricycle **108** failed. A plausible explanation for the failure of the alkylation may be that stability of the radical intermediate stabilized by a conjugative resonance. For the xanthate **101**, the initial free radical formed is stabilized by a resonance of the adjacent carbonyl ester group and therefore reacts effectively with the tryptophan methyl ester, whereas the free radical derived from xanthate **100** is very unstable, so that the alkylation can not occur.

**Scheme 36: Intermolecular radical alkylation reaction**

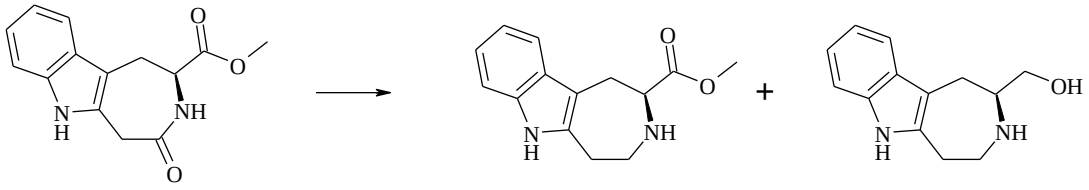



Reagents and conditions: a) Lauroyl peroxide (1.3-1.8 equiv.), 1,2-dichloroethane, reflux, 6 h; b) (i) TFA, DCM, rt, 3 h; (ii) HATU, DIPEA, DMF, rt, 18 h, 46% overall yield.

With the key product **107** in hand the attention was focused on the lactamization and chemoselective reduction to obtain the expected product **71**. Ester **107** was treated with TFA at room temperature for 3 h to remove the Boc/*t*Bu protecting groups and subjected to HATU as the coupling reagent in DIPEA and DMF to obtain the desired lactam **106** in 46% yield (Scheme 36). In the next step, we aimed to convert the lactam ester **106** into the ester **71** by a chemoselective reduction (Scheme 37). When lactam **106** was treated with $BH_3.Me_2S$ in anhydrous THF at room temperature for 6 h, a complete consumption of the starting material was observed (monitored by TLC).[117,118] However, the undesired compound **109** was obtained as a

major product along with 10% of the desired ester **71** (Scheme 37). Attempts to reduce chemoselectively the lactam ester **106** failed. Thus, we decided to convert product **106** into the amino alcohol **109** (86%) by treatment with a large excess of $BH_3.Me_2S$ in dry THF at room temperature for 6 h.

**Scheme 37: Chemoselective reduction of lactam ester 106**

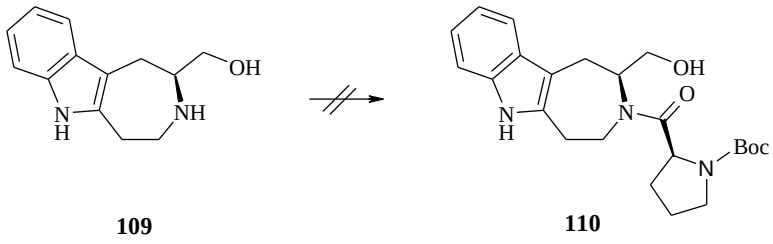


**106** **71** **109**

Reagents and conditions: a) $BH_3.Me_2S$/THF, 0 $^oC$ to rt, 6 h, **109** (86%).

Coupling of Boc-Pro-OH with amino acohol **109** was initially attempted to obtain the dipeptide **110** using HATU and DIPEA in DMF. However, none of the desired product was formed even with active coupling reagents BOP-Cl and PyBrop. From the failure of attempted couplings, it could be more advantageous if compound **109** is first converted into the corresponding *N*-Boc protected acid which is subsequently coupled with glycine amide and Boc-Pro-OH.

**Scheme 38: Attempted synthesis of peptide 110**



**109** **110**

Reagents and conditions: a) L-Boc-Pro-OH, HATU, DIPEA, DMF, rt, 12 h.

## 2.3 Stabilizing secondary structure by side-chain backbone macrocyclization

Constraint of highly flexible linear peptides by cyclization is one of the most widely used approaches to define the bioactive conformation of peptides. Reduction of the structural

flexibility can potentially enhance the selective molecular recognition and metabolic stability.[119,120] In addition, their rigid conformations provide a valuable tool in determining which conformational features are related to their biological activity.

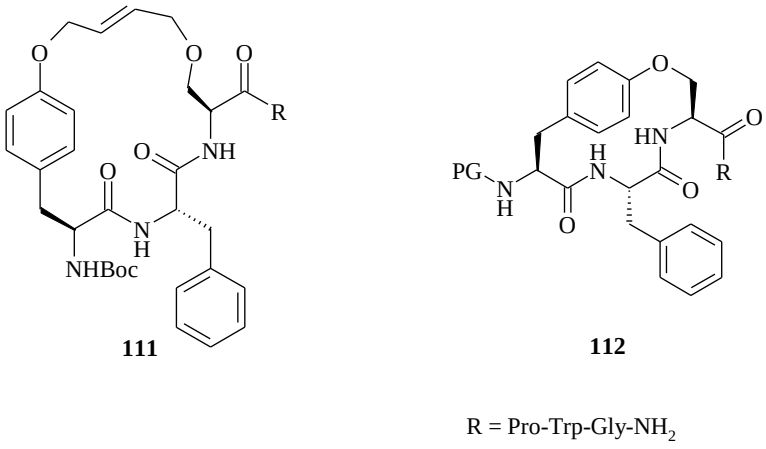
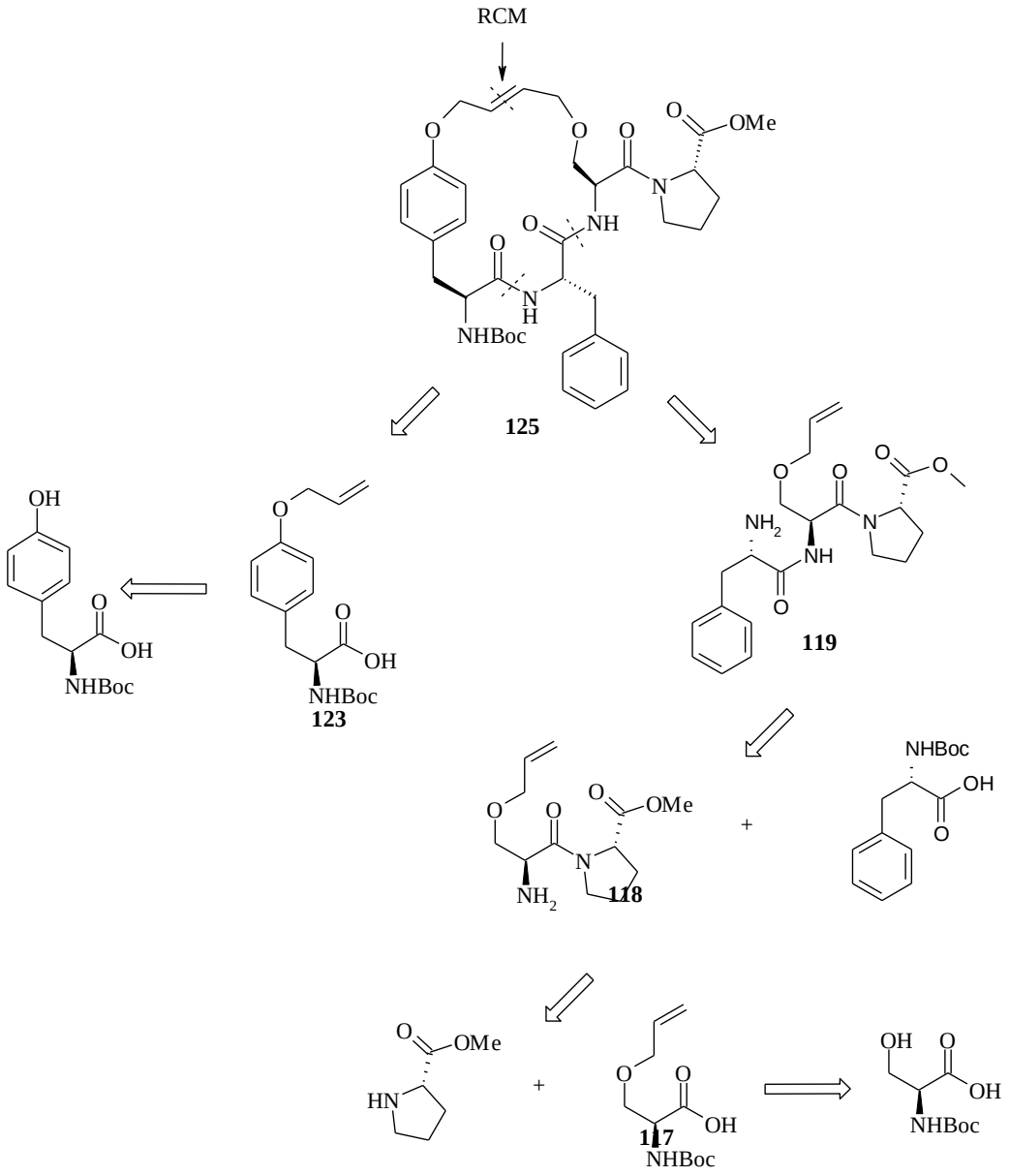



**Figure 22:** Selected macrocyclic helicokinin I analogues

It has been demonstrated that head to tail cyclizations of helicokinin I analogues provide inactive macrocyclic peptides. Moreover, methylations of the *C*-terminal amide almost reduce its receptor activation,[64] suggesting that any types of cyclization of *N*- or *C*-backbone to the *C*-terminal amide may provide weak or inactive peptides. One possibility of constraining the linear helicokinin I is based on the side chain backbone cyclization. In this study, two novel cyclic helicokinin I analogues are aimed to synthesize (Figure 22), in which all amino acids in the helicokinin sequence are intact and chemical modification is performed on the least important residues Ser and Tyr.

### 2.3.1 Macrocyclic analogues formed by a tetra-carbon bridge

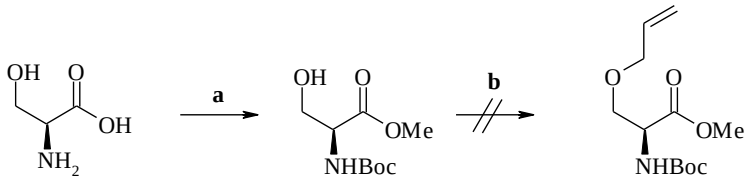
As depicted in Scheme 39, the macrocyclic peptide **125** is retrosynthetically prepared by a ring-closing metathesis reaction of the corresponding *O*-bisallyl precursor, which is derived from the incorporation of *O*-allyl-Tyr-OH into Phe-Ser(allyl)-Pro-OMe. These *O*-allylated building blocks are readily synthesized from commercially available amino acids Ser and Trp.




**Scheme 39:** Retrosynthetic analysis of macrocycle **125**

The starting point of the synthetic route was the selective *O*-allylation of serine moiety. The allylation was initially attempted by reaction of *N*-Boc protected serine methyl ester **114**, which was prepared by methyl esterification of serine amino acid and subsequent *N*-Boc protection, with allyl bromide and strong base NaH (1.1 equiv) in DMF (Scheme 40). However, no expected *O*-allylated product **115** was obtained.

**Scheme 40: Attempted allylation of *N*-Boc-protected serine methyl ester**

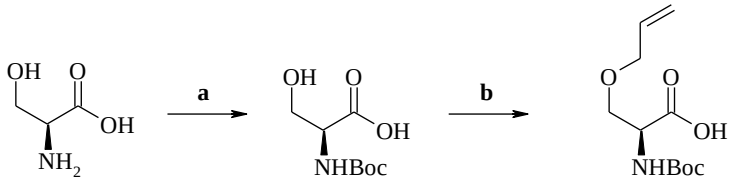



**113** **114** **115**

Reagents and conditions: a) (i) $SOCl_2$, MeOH; (ii) $Boc_2O$, $NaHCO_3$, THF; b) NaH, allyl bromide, DMF, 0 $^oC$ to rt.

Alternatively, when the *N*-Boc protected serine amino acid **116** was exposed with NaH (2 equiv) in DMF and followed by quenching with allyl bromide (1.1 equiv), the *O*-allylated serine acid **117** was obtained in 80% yield (Scheme 41). The unsuccessful allylation of methyl serine ester **114** is due to the easy deprotonation of the Hα in strong base NaH which causes a dehydration. In contrast, the resulting dianion derived from the treatment of *N*-Boc protected serine acid **116** with NaH prevents the C-allylation and dehydration, providing only the desired product **117**.

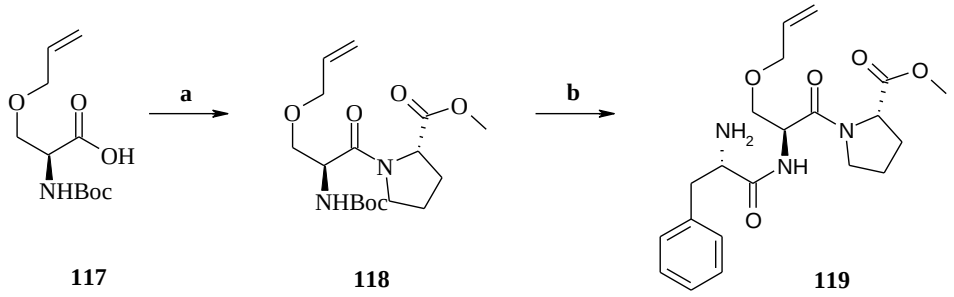
**Scheme 41: *O*-allylation of serine acid**




**113** **116** **117**

Reagents and conditions: a) $Boc_2O$, 1N NaOH, *t*BuOH, 54%; b) allyl bromide, NaH, DMF, 80%.

**Scheme 42: Preparation of tripeptide 119**



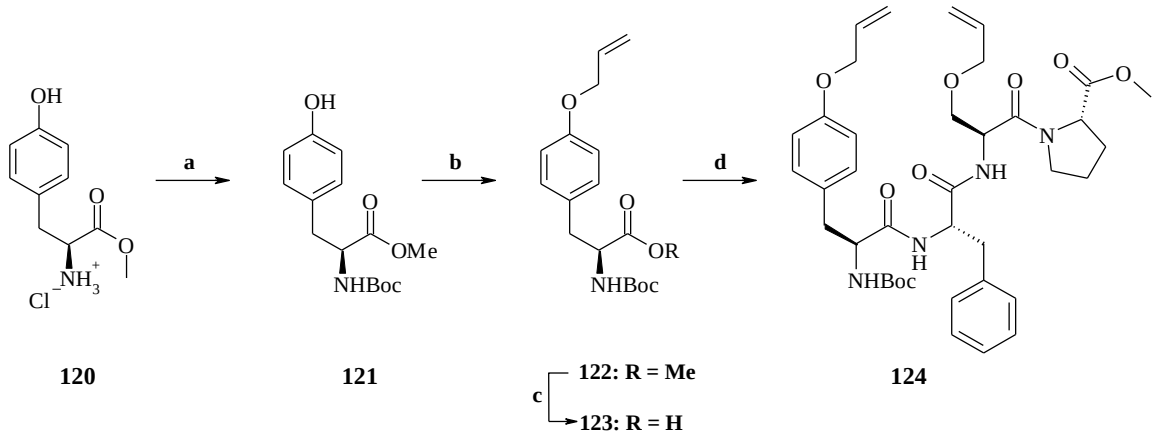

**117** **118** **119**

Reagents and conditions: a) Pro-OMe, TBTU, DIPEA, DCM, 86%; b) (i)TFA/TIPS/$H_2O$, 3 h, 88%; (ii) Boc-Phe-OH, TBTU, DIPEA, DCM, 91%; (iii) TFA/TIPS/$H_2O$, 3 h, 88%.

Dipeptide **118** was obtained in 86% yield from reaction of the *N*-Boc-*O*-allyl-serine acid **117** with L-proline methyl ester, which upon treatment with TFA afforded a primary amine intermediate. The key building block **119** was successfully prepared in 80% by coupling of the resulting amine with Boc-Phe-OH followed by *N*-Boc acidic deprotection (Scheme 42).

The synthesis of *O*-allyltyrosine **123** started from L-tyrosine methyl ester hydrochloride. The *N*-Boc protection of amine **120** and subsequent reaction with allyl bromide and potassium carbonate in DMF afforded the *O*-allylated tyrosine ester **122** in 95% yield. Hydrolysis of methyl ester with 1N NaOH in methanol provided the corresponding acid **123** in 98% yield, which was subsequently coupled with tripeptide **119** to furnish the key metathesis precursor **124** (94%) (Scheme 43).

**Scheme 43: Synthesis of diene 124**

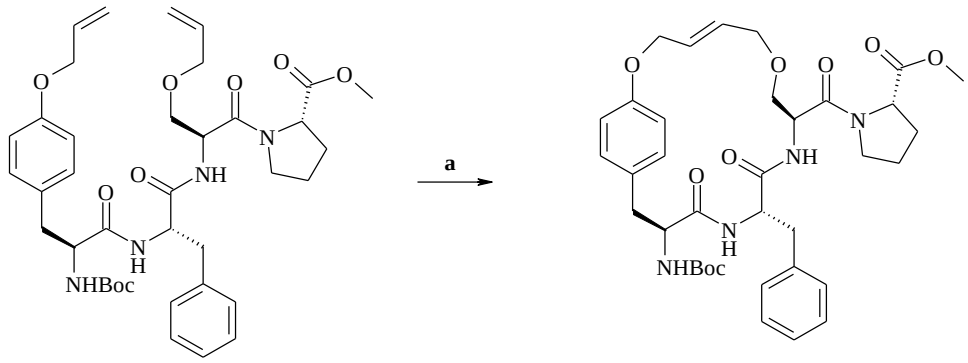



Reagents and conditions: a) $Boc_2O$, TEA, DCM, 97%; b) allyl bromide, $K_2CO_3$, DMF, 98%; c) 1N NaOH, MeOH, 18 h, rt, 98%; d) **119**, DIPEA, TBTU, DCM, 94%.

Having the required acyclic diene metathesis precursor **124**, the key ring-closing metathesis reaction was aimed to prepare macrocycle **125**. Exposure of the diene **124** (1 mM) to the Hoveyda-Grubbs 2$^{nd}$ catalyst (10 mol% loading) in refluxing $CH_2Cl_2$ for 50 hours afforded exclusively the corresponding macrocyclic peptide **125** in excellent yield (89%) (Scheme 44). The stereochemistry of the cyclized product was unable to elucidate at this stage due to the overlapping signals of the alkene protons. However, this work was easily done at the later step. Based on the NOESY and $^1$H-NMR decoupling experiments of the final product **128**, the *E*-geometry was clearly established by high coupling constant value ($J_{Ha\text{-}Hb}$ = 16.1 Hz) between

two adjacent alkene protons. Therefore, it could be concluded that the ring-closing metathesis reaction of diene **124** formed preferentially a *trans*-product (Figure 23).

**Scheme 44: Ring-closing metathesis of diene 124**




**124** **125**

Reagents and conditions: a) Hoveyda-Grubb catalyst (10 mol%), DCM, reflux, 50 h, 89%

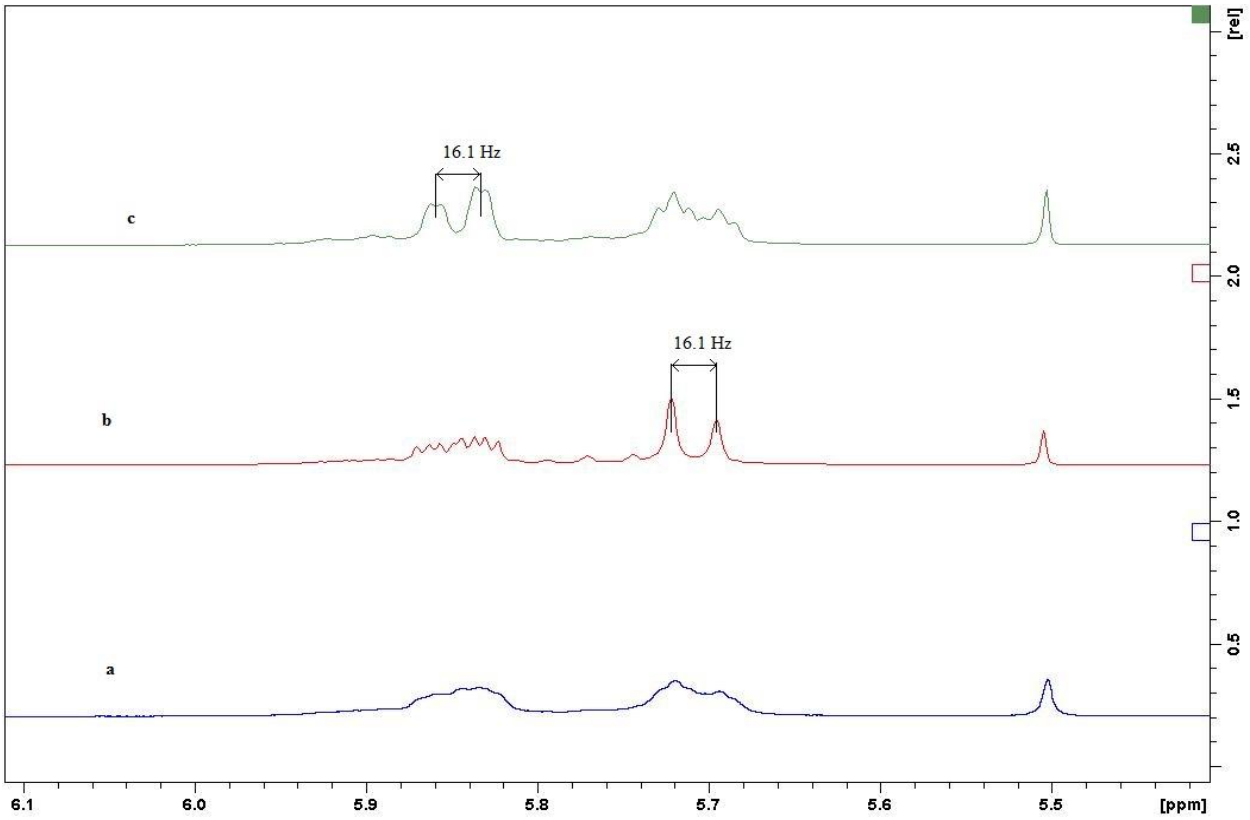
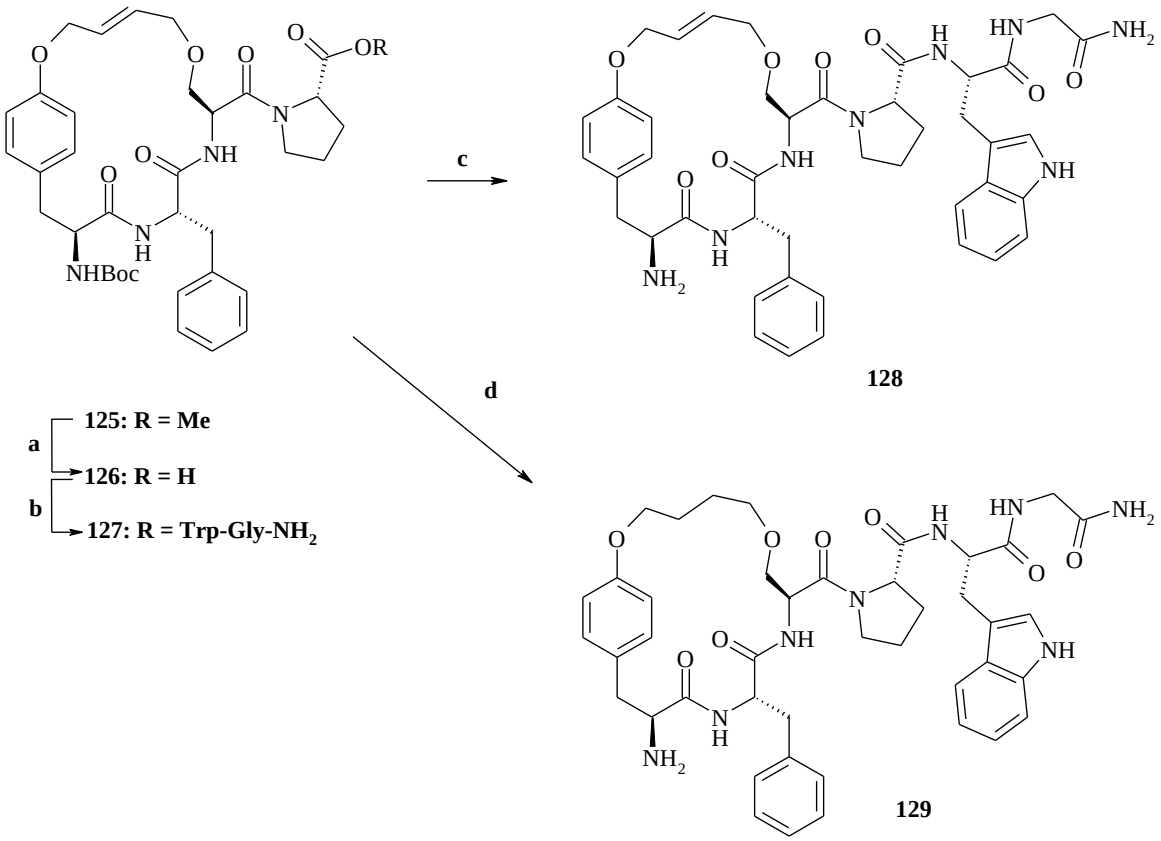



Figure 23:a) $^{1}$H-NMR expanded alkene area of macrocycle **128**; b) Selective decoupling of proton at 3.91 ppm; c) Selective decoupling of proton at 3.58 ppm.

After successfully achieving macrocycle **125**, our attention was focused on the extension of the *C*-terminal dipeptide Trp-Gly-$NH_2$. Upon treatment with 4% LiOH in THF/$H_2O$ (1/1), methyl ester **125** was converted into the corresponding carboxylic acid **126**, which was then reacted with dipeptide H-Trp-Gly-$NH_2$ using HATU-promoted coupling to provide the macrocyclic hexapeptide **127** in 98% yield (Scheme 45). With the aim of comparing the flexibility of the tetra-carbon bridge, two parallel synthetic pathways were carried out (Scheme 45). In one hand, the unsaturated cyclic peptide **127** was directly subjected to TFA/TIPS/$H_2O$ at 0 $^oC$ to yield the desired macrocyclic peptide **128** (94%). On the other hand, saturation of the double bond of the olefin **128** followed by acidic deprotection gave the desired saturated peptide **129** in 90% overall yield.

**Scheme 45: Extension of *C*-terminal dipeptide**

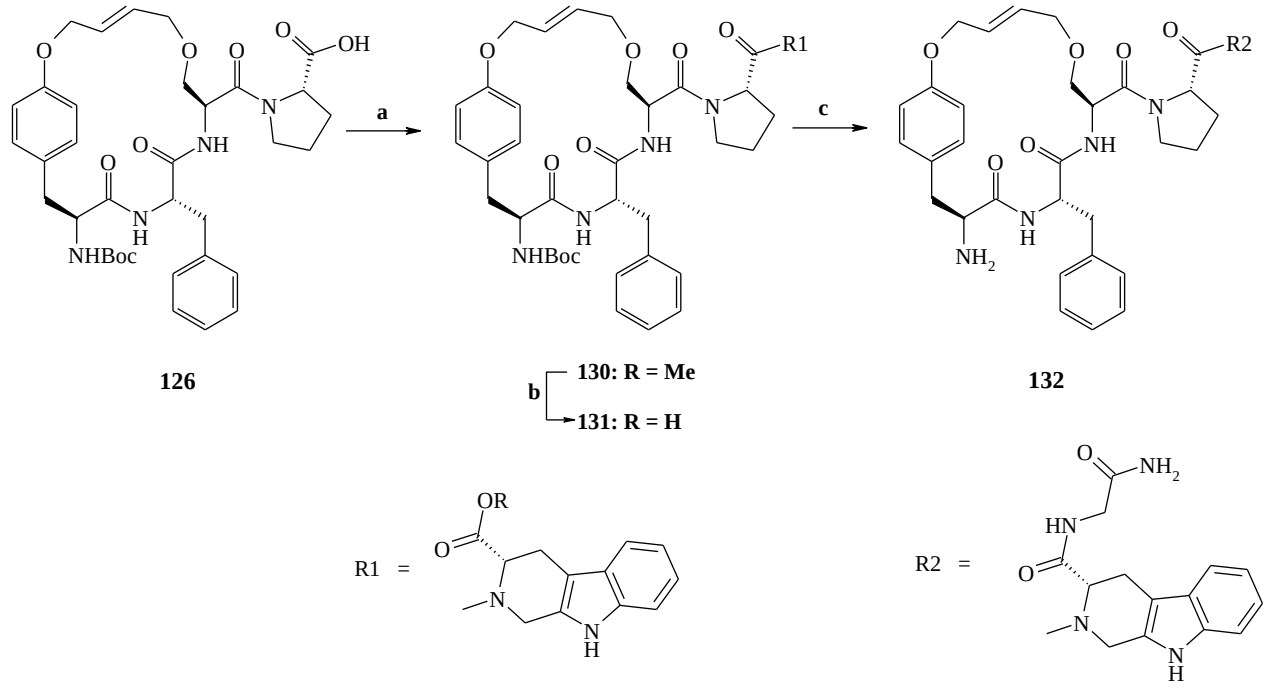



Reagents and conditions: a) LiOH, THF/$H_2O$, 18 h, 98%; b) H-Trp-Gly-$NH_2$, HATU, DIPEA, DMF, 98%; c) TFA/TIPS/$H_2O$, 0 $^oC$, 4 h, 90-94%; d) Pd/C, $H_2$, MeOH/ EtOAc (2/1), rt, 24 h, 98%.

### 2.3.2 Hybrid synthesis

Encouraged by high bioactivities of the β-carboline containing helicokinin I analogue **88** and the macrocyclic peptide **128** (Table 11), we decided to synthesize the analogue **132** that hybridizes the macrocyclic moiety in **128** with the conformationally constrained tetrahydro-β-carboline residue (Scheme 46).

**Scheme 46: Hybrid synthesis of 132**



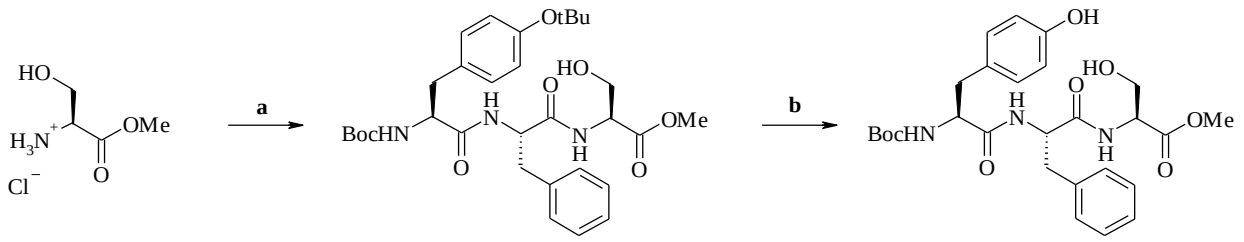

Reagents and conditions: a) tetrahydro-β-carboline (**69**), HATU, DIPEA, DMF, rt, 20 h, 78%; b) 4% LiOH, THF/$H_2O$ (2/1), 5 h, 91%; c) (i) H-Gly-$NH_2$.HCl, TBTU, DMF, DIPEA, rt, 20 h, 77%; (ii) TFA/TIPS/$H_2O$ (95:4:1), 0 $^oC$, 3 h, 86%.

First attachment of the acid **126** to the tetrahydro-β-carboline moiety **69** employing HATU as the coupling reagent afforded the pentapeptide **130** in good yield (78%). Next, saponification of the ester **130** with 4% LiOH followed by reaction with glycine amide hydrochloride yielded the corresponding hexapeptide. Final acidic deprotection furnished the desired peptide **132** in 86% yield.

### 2.3.3 Formation of macrocyclic ether

As continuation to our interest in the side chain backbone cyclization, a 14-membered ring ether was aimed to synthesize by a direct Tyr-Ser ether formation. The synthetic strategy was initially attempted by an intramolecular Mitsunobu cyclization reaction of tripeptide Boc-Tyr-Phe-Ser-OMe **135**, which was readily prepared in a three-step procedure. Coupling of serine methyl ester hydrochloride **133** with dipeptide Boc-Tyr(*t*Bu)-Phe-OH followed by TFA-mediated deprotection led to an amine intermediate. Upon treatment of the amine with $Boc_2O$, the cyclization precursor **135** was obtained in 92% yield (Scheme 47).

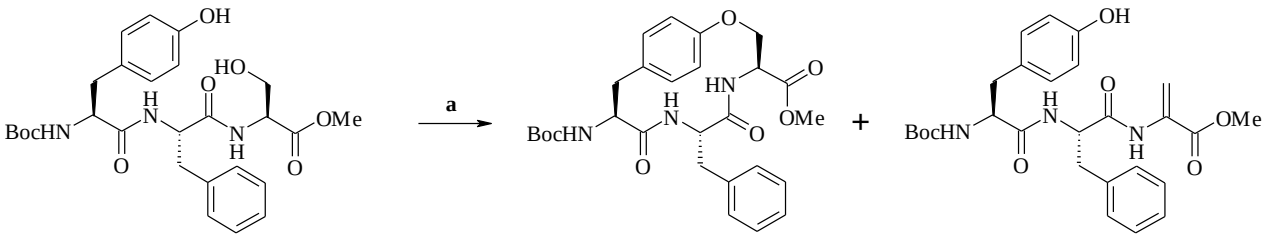
**Scheme 47: Preparation of Mitsunobu precursor 135**




**133** **134** **135**

Reagents and conditions: a) Boc-Tyr(*t*Bu)-Phe-OH, TBTU, DIPEA, DMF, rt, 89%; b) (i) TFA/TIPS/$H_2O$ (95:4:1); 0 $^oC$, 5 h, 96%; (ii) $Boc_2O$, $NaHCO_3$, THF/$H_2O$, rt, 18 h, 92%.

**Scheme 48: Attempted intramolecular Mitsunobu cyclization reaction**




**135** **136** **137**

Reagents and conditions: a) DIAD or DEAD, $PPh_3$, THF, 0 $^oC$, 3 h, **137** (85%).

The intramolecular etherification of the tripeptide **135** was carried out under Mitsunobu conditions using a mixture of triphenylphosphine and either diethyl dicarboxylate (DEAD) or diisopropyl azodicarboxylate (DIAD) in dry THF. Though the qualitative analysis (monitored by

TLC) indicated a complete consumption of the starting material, the cyclic ether **136** was not formed (Scheme 48). The $^{1}$H-NMR spectroscopy of the resulting product showed two proton signals at $\delta$ = 6.5 and 5.9 ppm, characterizing alkene protons. In addition, the $H_{\alpha}$ proton signal of Ser moiety was disappeared, suggesting that β-elimination occurred under Mitsunobu conditions (Figure 24).

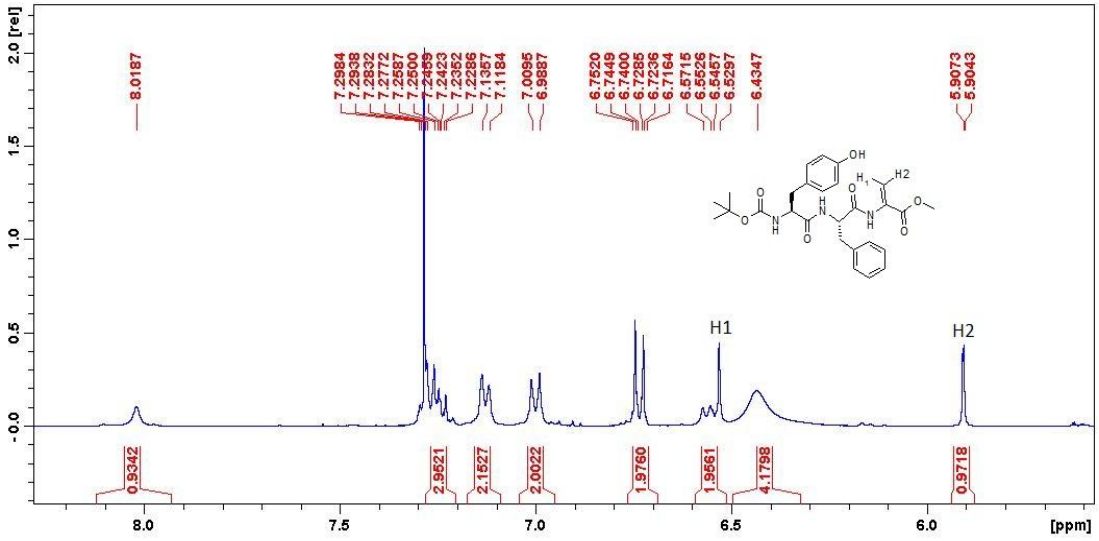
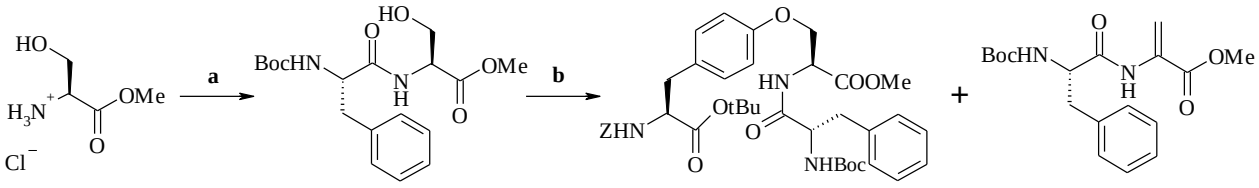



**Figure 24**: $^{1}$H-NMR of alkene **137**

Alternatively, the Z-Tyr-O*t*Bu and dipeptide Boc-Phe-Ser-OMe **138** was used to explore the intermolecular Mitsunobu etherification reaction in the same conditions as described above (Scheme 49). Instead of obtaining the desired ether **139**, again, only undesired β-elimination product **140** was formed in 49%. The byproduct was clearly confirmed by $^{1}$H-NMR spectroscopy (Figure 25).

**Scheme 49: Intermolecular Mitsunobu etherification reaction**




**133** **138** **139** **140**

Reagents and conditions: a) Boc-Phe-OH, TBTU, DIPEA, DMF, rt, 20 h, 89%; b) DIAD, toluene, rt, 48 h, **140** (49%).

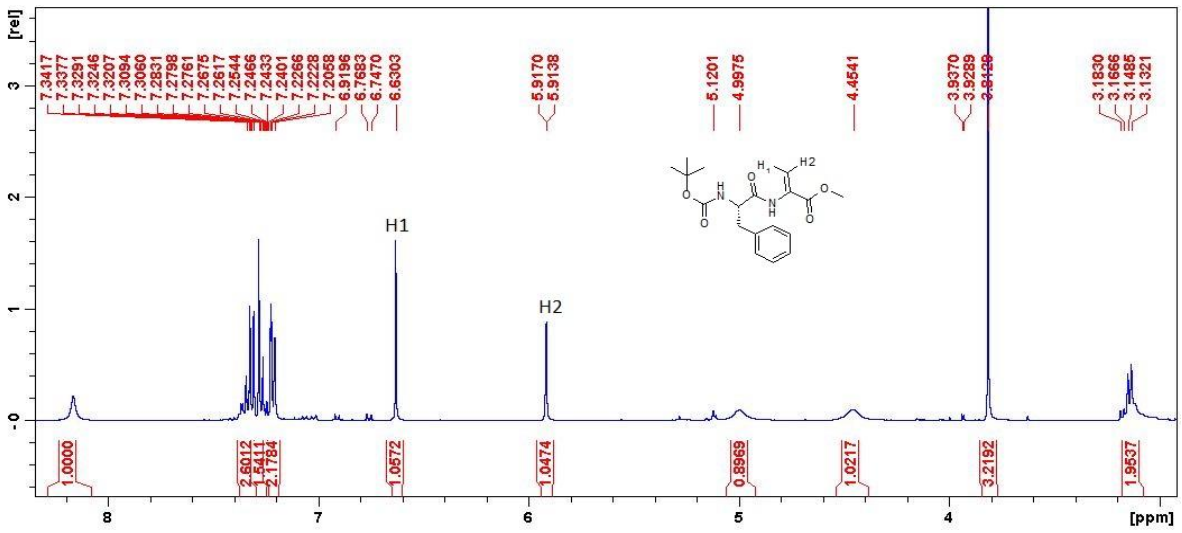
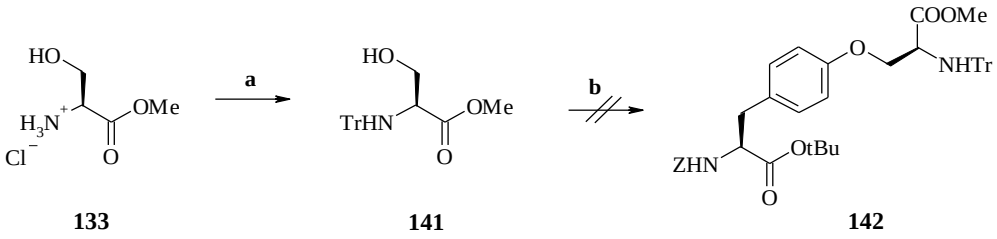



**Figure 25**: $^{1}$H-NMR of product **140**

It is evident that the use of *N*-carbamate protected serine ester analogues (**135** and **138**) to prepare Ser-Tyr ether under Mitsunobu conditions is impossible. The relative acidity of Hα of the serine moiety in ester analogues **135** and **138** along with a good leaving group of the alkoxy phosphonium formed during the Mitsunobu process facilitates the β-elimination.

**Scheme 50: Attempted etherification reaction**

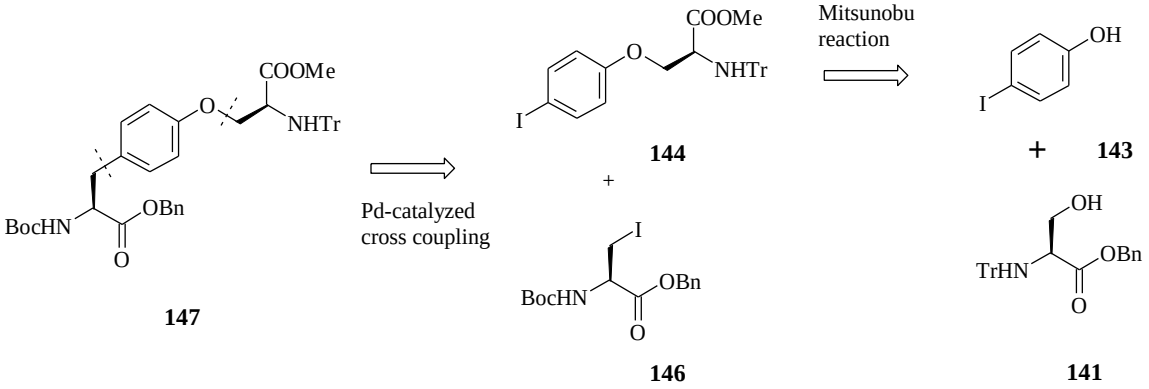



**133** **141** **142**

Reagents and conditions: a) Trityl chloride (TrCl), TEA, DCM, rt, 18 h, 69%; b) Z-Tyr-O*t*Bu, DIAD, toluene, 0 $^{o}$C to rt, 48 h.

In an effort to synthesize the Tyr-Ser ether under Mitsunobu conditions, serine methyl ester was alternatively protected with trityl group, a noncarbamate protecting group. The *N*-Tr serine methyl ester **141** was readily prepared in 69% yield by treatment of amine **133** with trityl chloride (TrCl) in the presence of TEA, which was then reacted with Z-Tyr-O*t*Bu under Mitsunobu conditions in either toluene or benzene. Again, no conversion of the expected ether **142** was found even for prolonged reaction times and heating up to 90 $^{o}$C for 48 h (Scheme 50). In this case, the β-elimination of serine methyl ester did not occur and the starting material was

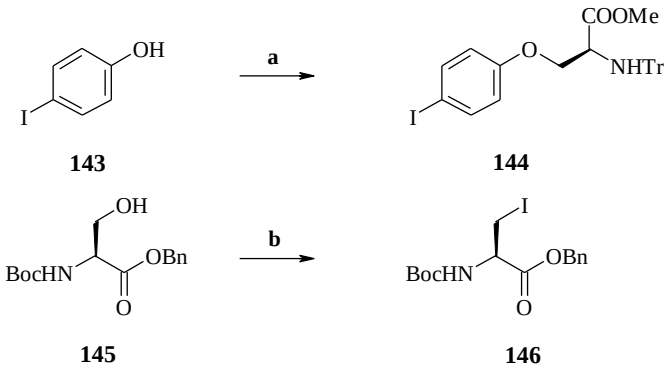
recovered. It was our opinion that the existence of the steric hindrance trityl group in Ser moiety makes it impossible to react with Z-Tyr-O*t*Bu. Stimulated by the reaction of hydroxyester **141** with 4-bromophenol under Mitsunobu conditions to give an aryl-Ser ether,[121] an alternative synthesis was pursued. This approach involves a carbon-carbon bond formation utilizing Pd-catalyzed cross-coupling reaction between iodo aryl compound and iodo-alanine ester (Scheme 51).




**Scheme 51:** Retrosynthesis of Tyr-Ser ether

The synthetic strategy for preparation of ether **147** was shown in Scheme 51. We used 4-iodophenol **143**, which can facilitate the later cross-coupling reaction, as starting material for the synthetic route. Upon treatment of the alcohol **141** with 4-iodophenol under Mitsunobu conditions using DEAD/$PPh_3$ in benzene at room temperature for 20 h, the desired ether **144** was successfully obtained in 83% yield, after extractive work-up and chromatographic purification (Scheme 52).

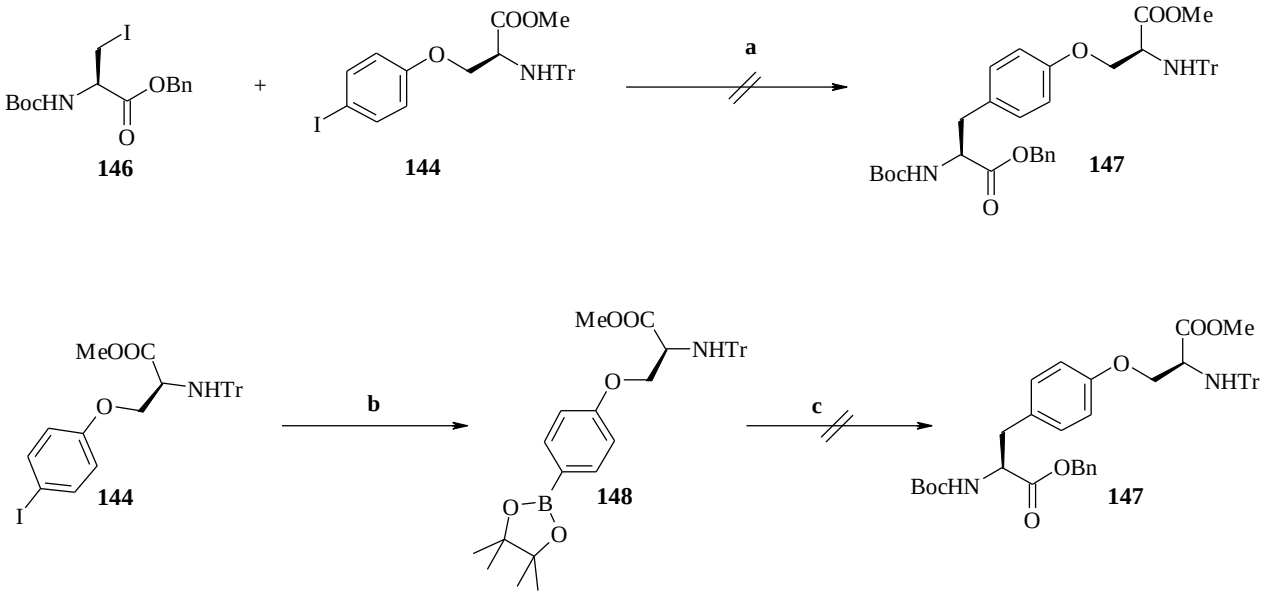
**Scheme 52: Preparation of the cross coupling precursors 144 and 146**




Reagents and conditions: a) Tr-*N*-Ser-OMe, DEAD, $PPh_3$, benzene, 20 h, rt, 83%; b) (i) MsCl, TEA, DCM, 0 $^{o}$C, 30 min, 98%; (ii) NaI, acetone, rt, 14 h, 89%.

The other cross-coupling precursor iodoalanine **146** was prepared in a two-step procedure.[122] The serine ester **145** was first converted into its mesylate in 98% yield by reaction with methanesulfonyl chloride under mild basic conditions TEA, which was then treated with NaI to afford the iodoalanine benzyl ester **146** (Scheme 52).

**Scheme 53: Attempted cross coupling reactions**



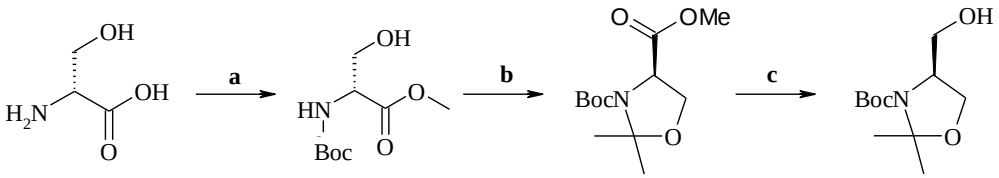

Reagents and conditions: a) $Pd_2(dba)_3$, P(o-Tol)$_3$, Zn, $I_2$, DMF, rt to 90 $^oC$, 48 h; b) $Pd(dppf)_2Cl_2$.DCM, AcOK, DMSO, 3 h, 80 $^oC$, 56%; c) $Pd(OAc)_2$, P(o-Tol)$_3$, *N*-Boc-iodoalanine benzyl ester, KF, DMF, 60 $^oC$, 18 h.

Coupling of iodoalanine **146** with iodoaryl ether **144** was initially attempted by using the Pd-catalyzed Negishi cross coupling reaction in dry DMF at high temperature (Scheme 53). In the course of the reaction, zinc powder was first activated by treatment with iodide in dry DMF under argon atmosphere for 15 min followed by addition of *N*-Boc protected iodoalanine **146** to generate *in situ* the organozinc intermediate. Next, **144** and the catalytic system of $Pd_2dba_3$ (2.5 mol%) and P(o-tol)$_3$ (10 mol%) were added.[123-125] Nevertheless, the Neghishi coupling reaction did not form the desired product **147**, even after heating up to 90 $^oC$ for 48 h. Another alternative route to the carbon-carbon bond formation was application of the Pd-catalyzed Suzuki-Miyaura cross coupling reaction (Scheme 53). Following this strategy, 4-iodo-arylether **144** was first

converted into the boronate **148** (56%) by reaction with pinacolborane in the catalysis of $Pd(dppf)_2Cl_2$.DCM complex and AcOK in DMSO at 80 $^oC$ for 3 h.[126] Coupling of the boronate **148** with the iodoalanine **146** was carried out in the presence of $Pd(OAc)_2$ (3 mol%) and $P(o\text{-}tol)_3$ (9 mol%) as catalysts.[127] Again, the expected product **147** was not formed.

Due to the unsuccessful approach of the carbon-carbon bond formation, our attention turned towards the Mitsunobu etherification reaction. As described above the β-elimination of the *N*-carbamate protected serine ester prevented the formation of Ser-Tyr ether under Mitsunobu conditions. In order to reduce the relative acidity of the Hα, serine ester was converted into its serinol derivatives. Our synthetic strategy used D-serine amino acid **149** as starting material for chemical transformation (Scheme 54). Esterification of acid **149** was carried out by treatment with $SOCl_2$ and MeOH followed by *N*-Boc protection to afford the protected serine ester **150** in 89% overall yield. The oxazolidine **151** was readily prepared in 98% upon reaction of the hydroxyester **150** with 2,2-dimethoxypropane (DMP) in the catalysis of $BF_3.Et_2O$. Reduction of ester **151** using lithium aluminum hydride (LAH) in dry THF gave the desired serinol acetonide **152** (99%).

**Scheme 54: Preparation of *N*-Boc-D-serinol acetonide**

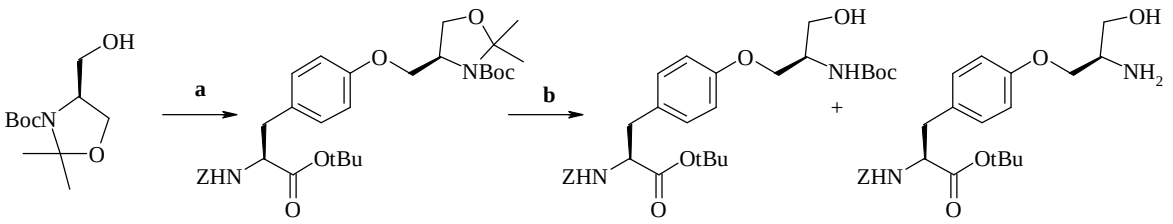



**149** **150** **151** **152**

Reagents and conditions: a) (i) MeOH, $SOCl_2$, rt, 18 h; (ii) $Boc_2O$, $NaHCO_3$, THF, rt, 12 h (98% two steps); b) 2,2-dimethoxypropane, $BF_3.Et_2O$, acetone, 98%; c) LAH, THF, rt, 20 min, 99%.

With the serinol analogue **152** in hand, the etherification reaction was initially explored. When the alcohol **152** was treated with Z-Tyr-O*t*Bu under Mitsunobu conditions in toluene at 90 °C for 48 h, the desired ether **153** was successfully obtained in 64% yield (Scheme 55). Removal of the isopropylidene group with either p-toluenesulfonic acid (PTSA, 5 mol%) in methanol or AcOH-$H_2O$ at room temperature afforded not only the expected product **154** but also the undesired peptide **155** in ratio of 55:45, respectively.

**Scheme 55: Etherification of serinol acetonide**

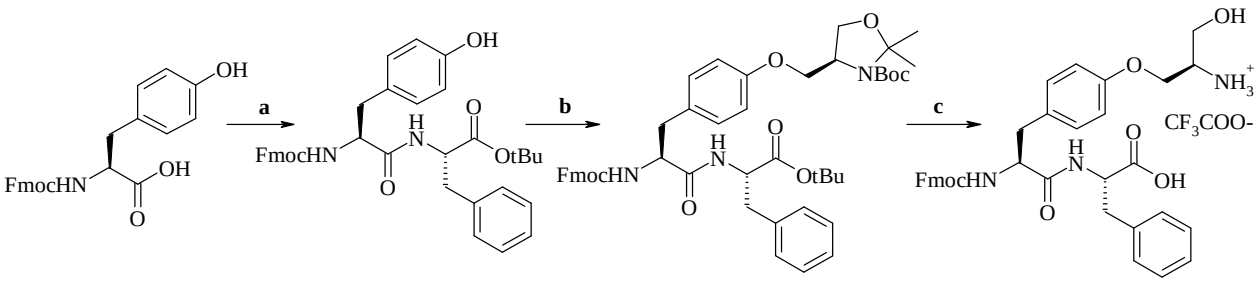



**152** **153** **154** **155**

Reagents and conditions: a) Z-Tyr-O*t*Bu, DEAD, $PPh_3$, toluene, 90 $^oC$, 48 h, 64%; b) PTSA (5 mol%), MeOH, rt, 5 h.

The unselective hydrolysis of the isopropylidene protecting group prompted us an alternative synthetic strategy, in which the serinol acetonide **152** was coupled with dipeptide Fmoc-Tyr-Phe-O*t*Bu **157** to produce the ether **158** (54%). Treatment of the acetonide ester **158** with TFA resulted the key desired product **159** in a quantitative yield (Scheme 56).

**Scheme 56: Alternative etherification of serinol**




**156** **157** **158** **159**

Reagents and conditions: a) HATU, PheO*t*Bu, DMF, rt, 86%; b) DEAD, $PPh_3$, toluene, 90 $^oC$, 48 h, 54%, b) TFA/TIPS/$H_2O$, 0 $^oC$, 3 h, 100%.

The key step of the synthetic route is the intramolecular cyclization to form the 14-membered macrocycle **160** (Scheme 57). The macrolactamization reaction was attempted under different conditions employing the highly active coupling reagents at ambient temperatures (Table 9). HPLC/MS analyses proved evidence that adducts with the reagents were formed, but neither intramolecular

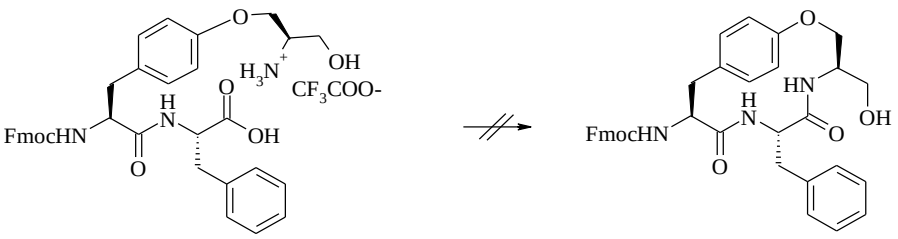
**Table 9:** Optimizing the macrolactamization

| Reagents and conditions | Temperature |
|---|---|
| HATU/DIPEA/ ACN | rt, 40 $^oC$,54 h |
| HATU/DIPEA/ DMF | rt; 40 $^oC$ or 80 $^oC$ |
| BOP-Cl/DIPEA/DMF | rt; 40 $^oC$ or80 $^oC$ |
| PyBop/DIPEA/THF | rt; 40 $^oC$ or 80 $^oC$ |
| $SOCl_2$/Pyridine/DCM | rt, 48 h |
| CMPI/DIPEA/DCM | rt, 48 h |

CMPI: 2-chloro-1-methylpyridinium iodide

cyclization nor dimerization was found. The unsuccessful lactamization through the sidechain residues Ser and Phe was reasoned due to the inability of the backbone to occupy a necessary conformation for amide bond formation.

**Scheme 57: Attempted lactamization of compound 159**




**159** **160**

Reagents and conditions: Presented in Table 8

## 2.4 Structure-activity relationships

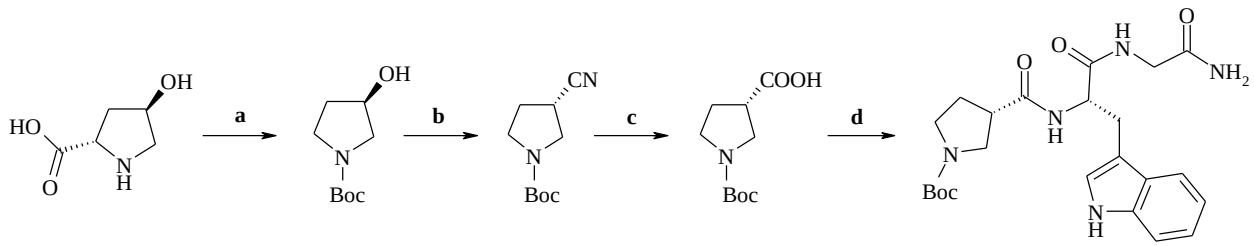
### 2.4.1 Substitution of proline by β-proline

Like proline residue, the β-proline has also been an interesting target in peptide synthesis due to its ability to rigidify the secondary structure.[128] In the course of our project directed towards the synthesis of modified helicokinin I, a β-proline amino acid is prepared and incorporated into helicokinin I as substituent for proline moiety. This study is aimed to evaluate how the backbone conformation of the modified helicokinin I analogue effects on the receptor activation.

For preparation of *N*-Boc-(*S*)-β-proline amino acid **164**, 4-(*R*)-hydroxy-(*S*)-proline (**161**) was chosen as starting material. The key intermediate **162** was prepared by a one-pot procedure.[129,130] Decarboxylation of 4-(*R*)-hydroxy-(*S*)-proline was accomplished in refluxing cyclohexanol with the catalysis of 2-cylohexanone for 6 h, providing an intermediate product 3-(*R*)-hydroxypyrrolidine. Treatment of the pyrrplidine analogue with $Boc_2O$ and $NaHCO_3$ in THF afforded the *N*-Boc-pyrrolidinol **162** in 69% yield (two steps) after silica gel flash chromatography (Scheme 59). In the next step, the hydroxyl group of **162** was mesylated and subsequently displaced with a nitrile group by reaction with NaCN in DMSO at 80 $^{o}$C for 21 h. The 3-cyano derivative **163** was obtained in 60% yield with a complete inversion of the configuration at C-3. The desired *N*-Boc-(*S*)-β-proline acid **164** was obtained in 78% yield by

refluxing the nitrile **163** in concentrated HCl for 5 h and subsequent reaction with $Boc_2O$ and $NaHCO_3$.

**Scheme 59: Synthesis of (3*S*)-carboxy pyrrolidine**

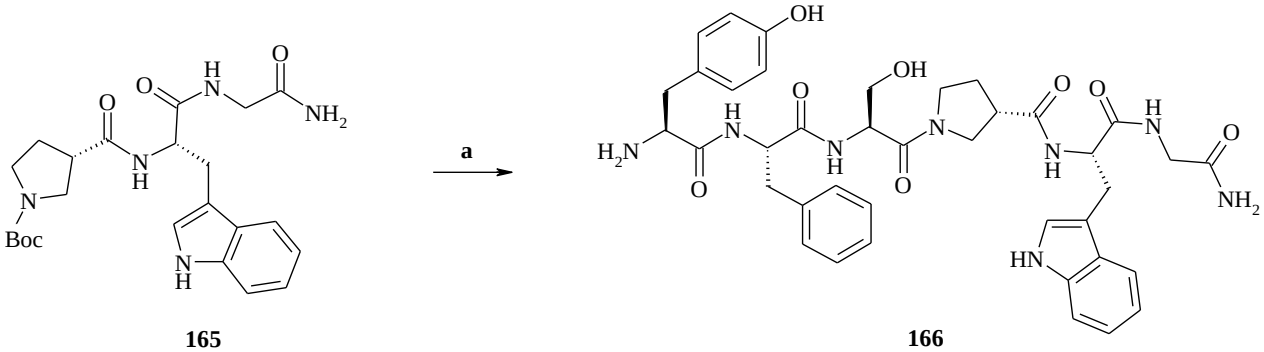



**4-(*R*)-hydroxy-(*S*)-proline** **161** **162** **163** **164** **165**

Reagents and conditions: a) (i) Cyclohexanol, 2-cyclohexanone, reflux, 6 h; (ii) $Boc_2O$, $NaHCO_3$, THF, rt, 18 h, 69% (2 steps); b) (i) MsCl, TEA, DCM, 3 h, 0 $^oC$, 97%; (ii) NaCN, DMSO, 80 $^oC$, 21 h, 60%; c) (i) con. HCl, reflux, 5 h; (ii) $Boc_2O$, $NaHCO_3$, THF, rt, 18 h, 78% (2 steps); d) TBTU, DIPEA, ACN, H-Trp-Gly-$NH_2$, rt, 18 h, 71%.

Tripeptide **165** was readily prepared in 71% yield by reaction of the *N*-Boc protected β-proline acid **164** with dipeptide H-Trp-Gly-$NH_2$ using HATU as coupling reagent and DIPEA in acetonitrile (Scheme 59). The *N*-Boc acidic deprotection of peptide **165** followed by reaction with tripeptide Boc-Tyr(*t*Bu)-Phe-Ser(*t*Bu)-OH resulted an intermediate hexapeptide, which upon treatment with TFA gave the final product **166** (86%) (Scheme 60).

**Scheme 60: Synthesis of hexapeptide 166**




**165** **166**

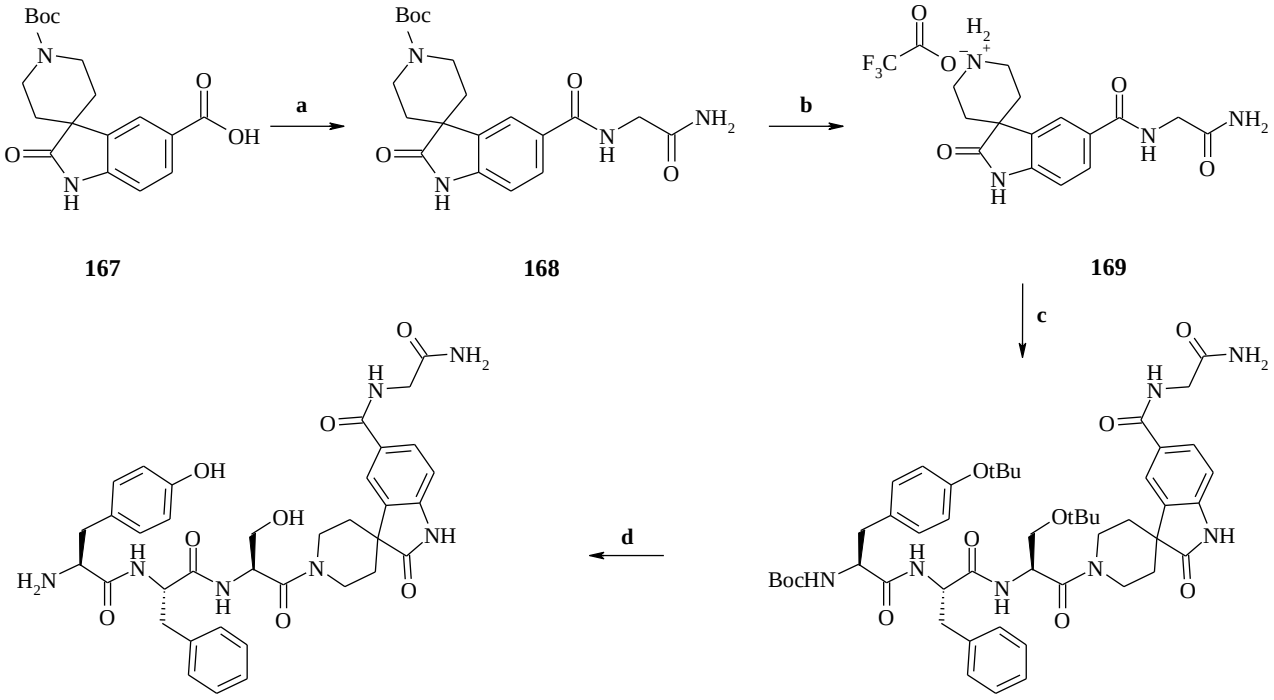
Reagents and conditions: a) (i) TFA/TIPS/$H_2O$ (95/2.5/2.5), 0 $^oC$, 4 h, 100%; (ii) Boc-Tyr(*t*Bu)-Phe-Ser(*t*Bu)-OH, HATU, DIPEA, DMF, 87%; (iii) TFA/TIPS/$H_2O$, (95/2.5/2.5), 0 $^oC$, 4 h, 86%.

### 2.4.2 Spirolactam-Scan

The use of constrained building blocks is one of the most popular approach for peptidomimetic design because they can enforce or stabilize a particular type of β-turn when inserted into a peptide backbone. In this context the conformationally constrained spirolactam **167** is used for directed screening of helicokinin I residues, except Gly and Ser, in order to investigate their defined secondary structures in receptor binding.

#### 2.4.2.1 Substitution of Pro-Trp dipeptide with spirolactam

**Scheme 61: Substitution of Pro-Trp with spirolactam**



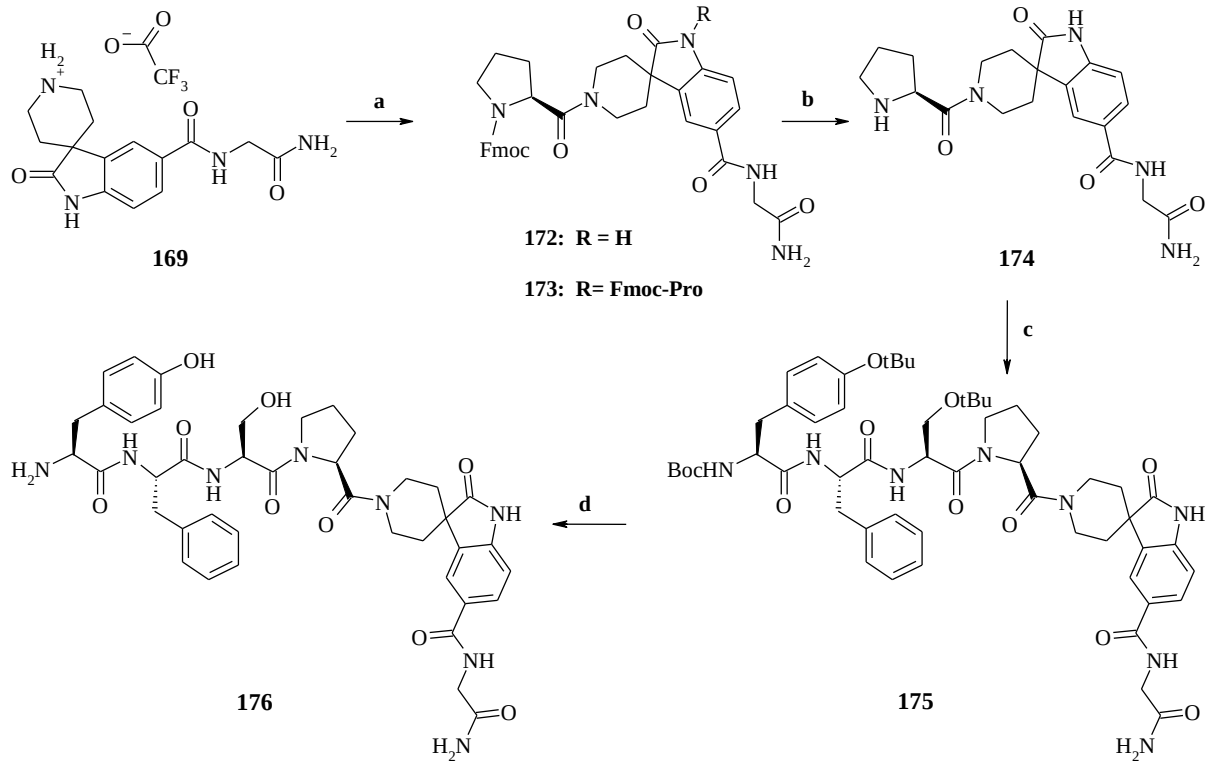

Reagents and conditions: a) H-Gly-$NH_2$.HCl, TBTU, DIPEA, DMF, rt, 18 h, 93%; b) TFA/TIPS/$H_2O$ (95:4:1); 0 $^oC$, 3 h, 98%; c) Boc-Tyr(*t*Bu)-Phe-Ser(*t*Bu)-OH, HATU, DIPEA, DMF, rt, 20 h, 69%; d) TFA/TIPS/$H_2O$ (95:4:1), 0 $^oC$, 5 h, 88%.

It can be envisioned that spirolatam **167** is somewhat similar to the dipeptide Pro-Trp sequence. Thus, our first synthesis used the constrained spirolactam **167** as substituent for the Pro-Trp

sequence in the active core domain of helicokinin I. Derived from the *N*-Boc-2-oxo-1,2-dihydrospiro[indole-3,4'-piperidine]-5-carboxylic acid, dipeptide **169** was readily obtained in 91% yield by TBTU-mediated amidation with a large excess of glycine amide hydrochloride, followed by TFA-mediated *N*-Boc deprotection. Attachment of tripeptide Boc-Tyr(*t*Bu)-Phe-Ser(*t*Bu)-OH to amine **169** using HATU/DIPEA furnished the pentapeptide **170** (69%), which was then subjected to TFA/TIPS/$H_2O$ (95/4/1) at 0 $^oC$ for 5 hours providing the final pentapeptide **171** in 88% yield, after chromatographic purification (Scheme 61).

### 2.4.2.2 Substitution of Trp with spirolactam

**Scheme 62: Substitution of Trp with spirolactam**



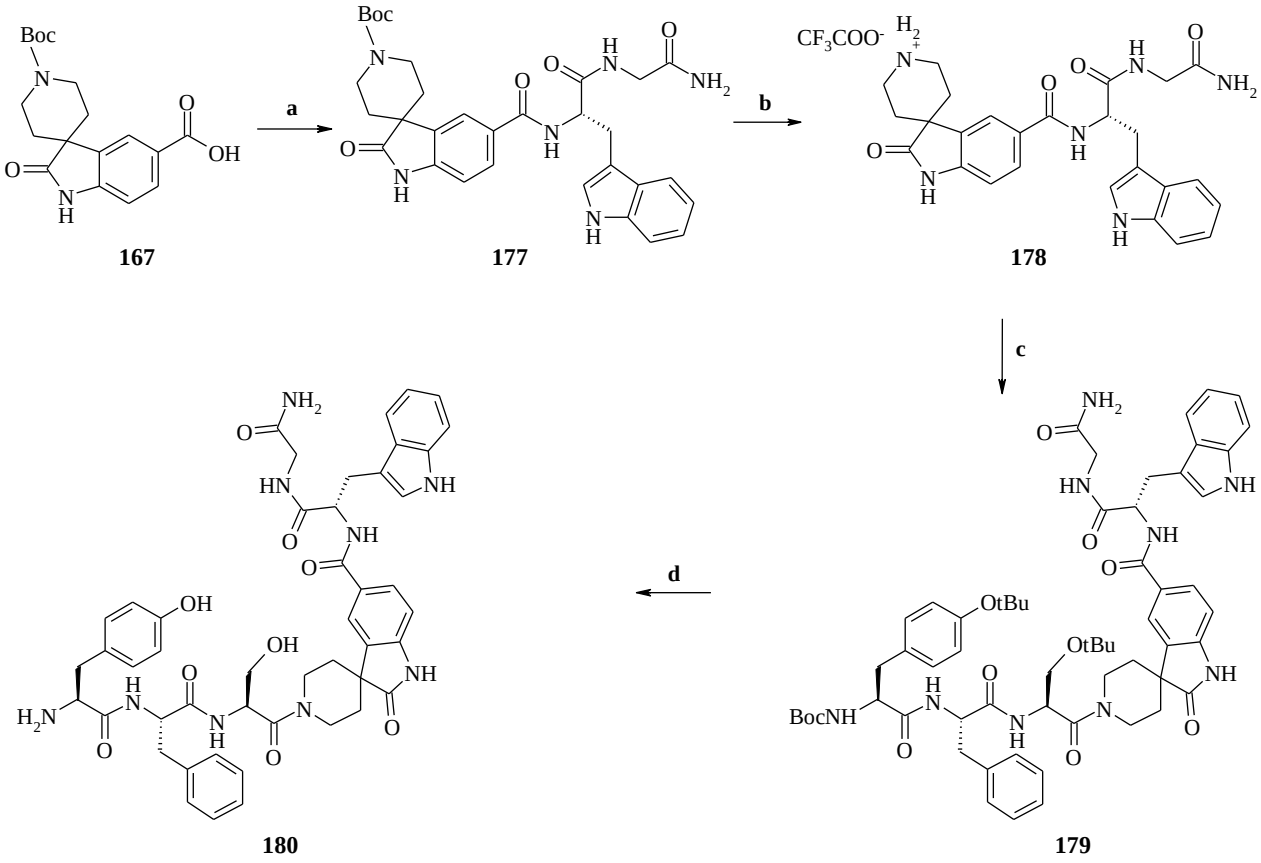

Reagents and conditions: a) Fmoc-L-Pro-OH, HATU, DIPEA, DMF, rt, 20 h, 86%; b) Piperidine, DCM, rt, 1 h, 97%; c) Boc-Tyr(*t*Bu)-Phe-Ser(*t*Bu)-OH, HATU, DIPEA, DMF, rt, 18 h, 57%; d) TFA/TIPS/$H_2O$ (95:4:1), 0 $^oC$, 5 h, 88%.

Tripeptide **172** was prepared from the coupling of amino acid Fmoc-Pro-OH with dipeptide **169**. In this coupling reaction, it was noted that the use of more than one molar equivalents of Fmoc-Pro-OH led to the decrease in the yield of the amidation. Only 42% yield of the desired peptide

**172** was obtained when using 1.3 molar equivalents of Fmoc-Pro-OH, whereas the yield increased up to 86% if 0.9 equiv was used. The reason was clear that the NH of the lactam ring also underwent the condensation reaction to form the undesired *N*,*N*-bisacylated product **173** (Scheme 62)**.** Upon treatment of peptide **172** with piperidine in DCM for 1 h and subsequent reaction with tripeptide Boc-Tyr(*t*Bu)-Phe-Ser(*t*Bu)-OH, the hexapeptide **175** was obtained in 55% yield. Finally, exposure of the full protected hexapeptide to TFA/TIPS/$H_2O$ (95/4/1) led to the desired peptide **176** (88%).

### 2.4.2.3 Substitution of Pro with spirolactam

**Scheme 63: Spirolactamscan at Pro position**



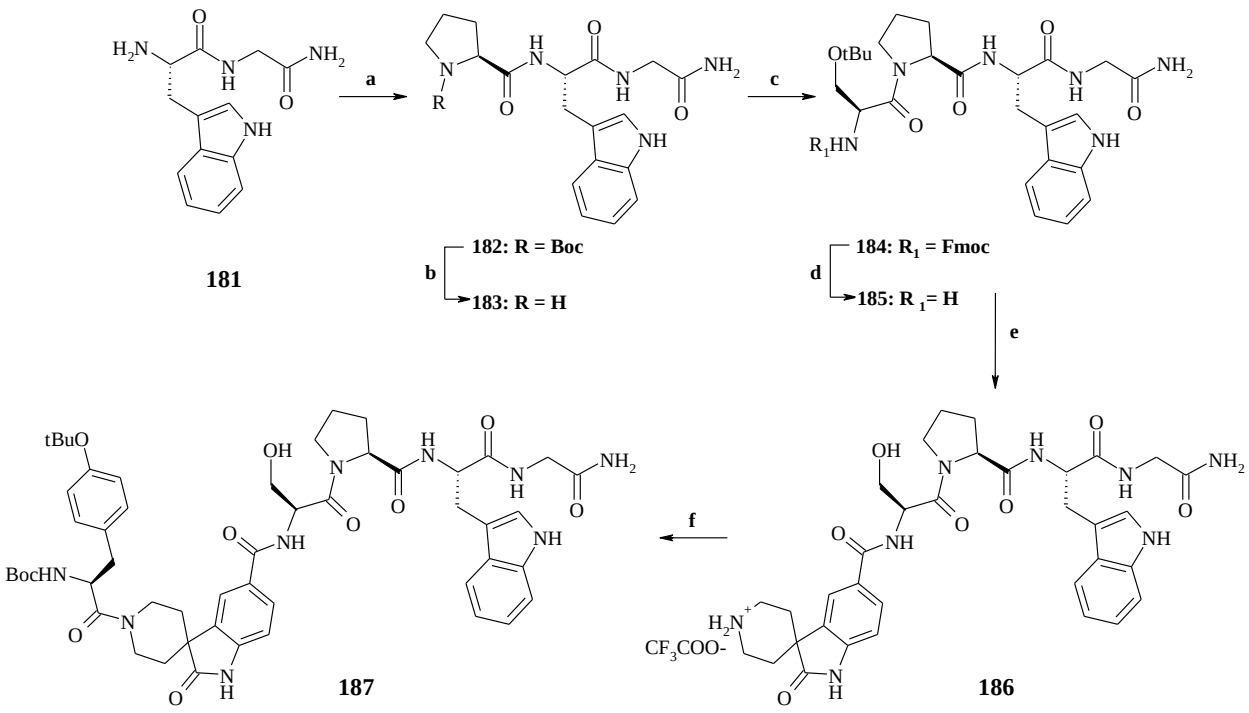

**167** **177** **178** **179** **180**

Reagents and conditions: a) H-Trp-Gly-$NH_2$, TBTU, DIPEA, DMF, rt, 20 h, 85%; b) TFA/TIPS/$H_2O$ (95:4:1), 0 $^oC$, 4 h, 95%; c) Boc-Tyr(*t*Bu)-Phe-Ser(*t*Bu)-OH, HATU, DIPEA, DMF, rt, 20 h, 65%; d) TFA/TIPS/$H_2O$ (95:4:1), 0 $^oC$, 5 h, 62%.

The desired peptide **180** was synthesized according to Scheme 63. The key building block **178** was prepared from the coupling of acid **167** with dipeptide H-Trp-Gly-$NH_2$ to generate the full protected tripeptide **177** (85%). The yield of the coupling reaction increased from 41% to 85% when 0.9 equiv of the acylating reagent was used instead of 1.2 equiv. Treatment of peptide **177** with TFA followed by reaction with tripeptide Boc-Tyr(*t*Bu)-Phe-Ser(*t*Bu)-OH using HATU-mediated coupling gave the hexapeptide **179**. Removal of protecting groups with TFA led to the desired product **180** in 62% yield, after chromatographic purification.

### 2.4.2.4 Substitution of Phe with spirolactam

**Scheme 64: Synthesis of peptide 187**

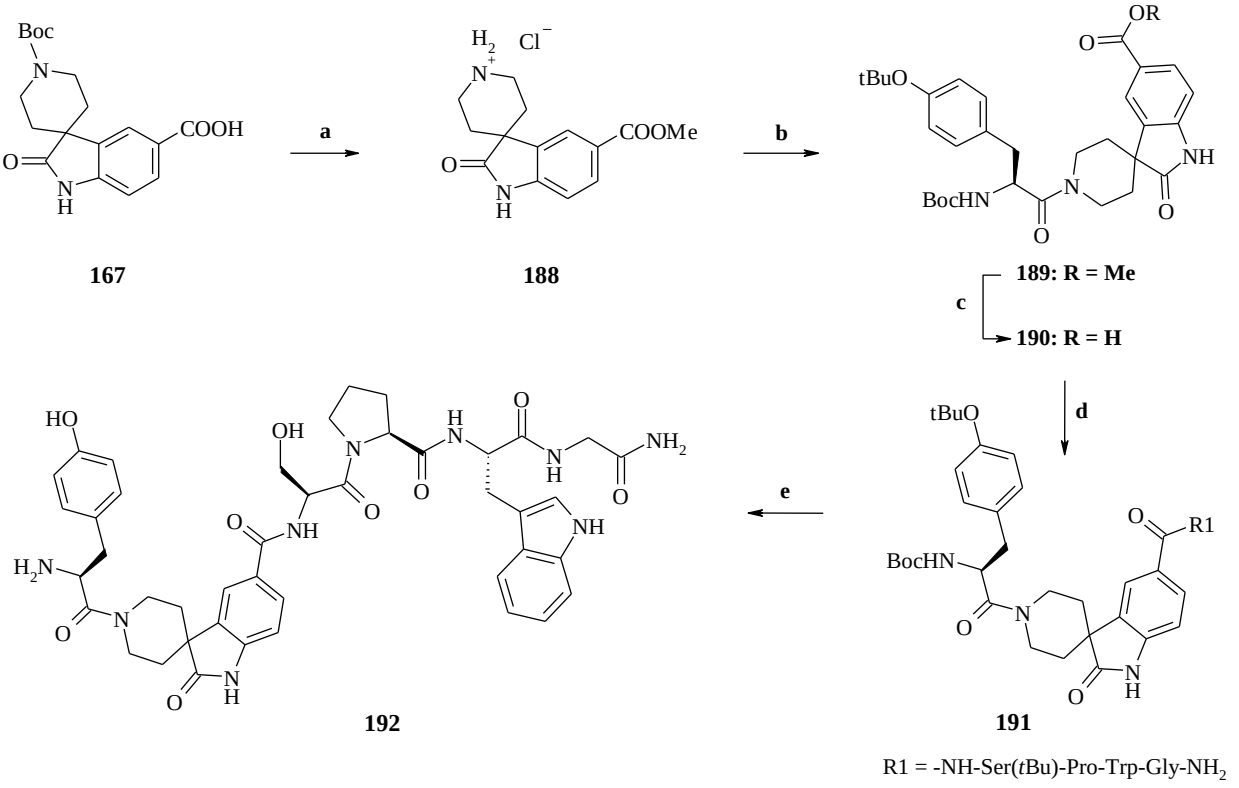



Reagents and conditions: a) Boc-Pro-OH, TBTU, DIPEA, DMF, rt, 20 h, 92%; b) TFA/TIPS/$H_2O$ (95:4:1), 0 $^oC$, 4 h, 96%; c) Fmoc-Ser(*t*Bu)-OH, HATU, DIPEA, DMF, rt, 20 h, 82%; d) Piperidine, DCM, rt, 20 min, 92 %; e) (i) HATU, DIPEA, DMF, rt, 20 h, 62%; (ii) TFA/TIPS/$H_2O$ (95:4:1), 0 $^oC$, 4 h, 86%; f) Boc-Tyr(*t*Bu)-OH, HATU, DIPEA, DMF, rt, 20 h, 10%.

The *N*-Boc protected tripeptide **182** derived from the acylation of Boc-Pro-OH with dipeptide H-Trp-Gly-$NH_2$ was treated with TFA to form the secondary amine **183** (96%). Attachment of

Fmoc-Ser(*t*Bu)-OH to the amine afforded the tetrapeptide **184** in 82% yield. Subsequent treatment of the *N*-Fmoc protected compound **184** with piperidine in DCM followed by reaction with acid **167** gave a full protected pentapeptide. Acidic deprotection of the protected peptide **167** afforded the secondary amine **186** in 86% yield. Notably, the *tert*-butyl protecting group on Ser residue was also removed under acidic conditions. This caused problem for the next coupling reaction. Introduction of Boc-Tyr(*t*Bu)-OH to peptide **186** using HATU as coupling reagent only provided the desired peptide **187** in 10% yield. For this reason, we decided to prepare the dipeptide **190** which was then incorporated into tetrapeptide **185** (Scheme 65).

**Scheme 65: Spiroscan at Phe position**




Reagents and conditions: a) MeOH, $SOCl_2$, 18 h, rt, 88%; b) Boc-Tyr(*t*Bu)-OH, HATU, DIPEA, DMF, 74%; c) 4% NaOH, MeOH, 46 h, 94%; d) **185**, TBTU, DIPEA, DMF, 52%; e) TFA/TIPS/$H_2O$ (95:4:1), 0 $^oC$, 5 h, 96%.

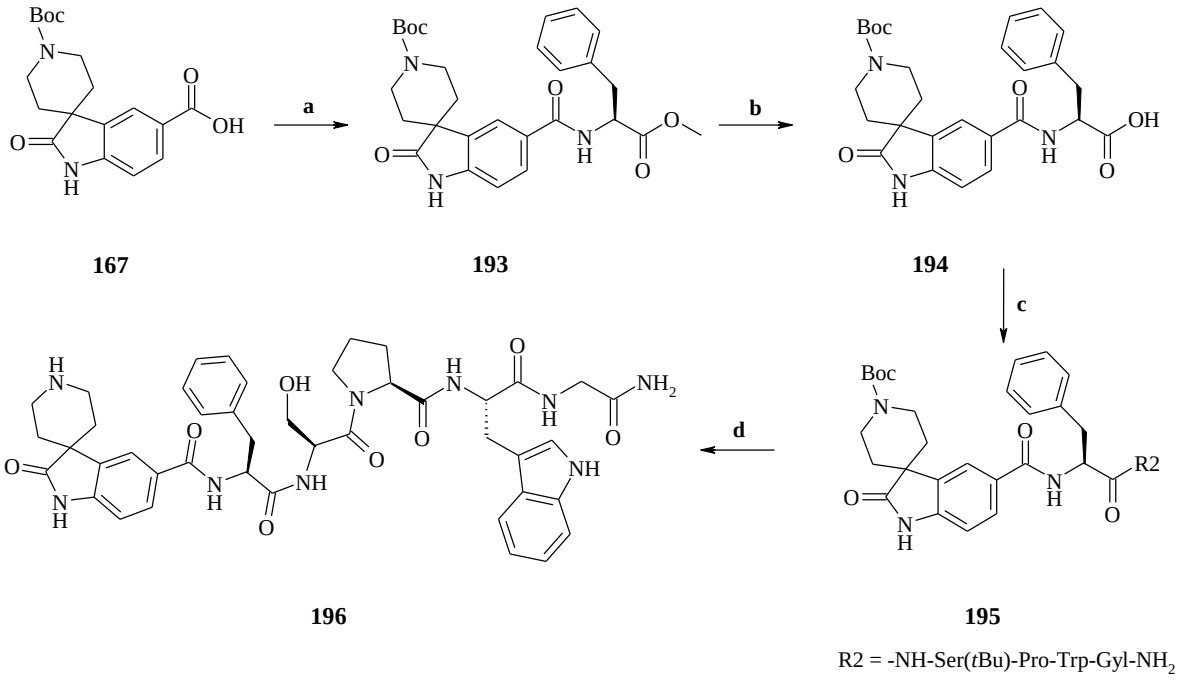
Esterification of the spirolactam acid **167** was carried out in MeOH and $SOCl_2$ furnishing the corresponding methyl ester **188** (88%). Under this conditions, the *N*-Boc protecting group was also removed to form a hydrochloride salt. The resulting methyl ester was then reacted with Boc-Tyr(*t*Bu)-OH using HATU/DIPEA to provide the dipeptide **189** (74%), which upon exposure

with 4% NaOH in MeOH for 46 hours at room temperature led to the corresponding acid **190** in 94% yield. Coupling of two peptides **185** and **190** resulted the desired product **191** in moderate yield (52%). Finally, TFA-mediated deprotection furnished the hexapeptide **192** in 96% yield, after silica gel flash chromatography (Scheme 65).

### 2.4.2.5 Substitution of Tyr with spirolactam

In the similar manner the replacement of Tyr with the spirolactam **167** was a convergent synthesis. The dipeptide ester **193** was prepared in 98% yield from acylation of the acid **167** to phenylalanine methyl ester. Hydrolysis of the ester with 4% NaOH in MeOH gave the corresponding acid **194** (Scheme 66). Attachment of the acid **194** to amine **185** provided the peptide **195** (62%), which after acidic deprotection furnished the desired hexapeptide **196** in 72% yield.

**Scheme 66: Spirolactamscan at Tyr position**



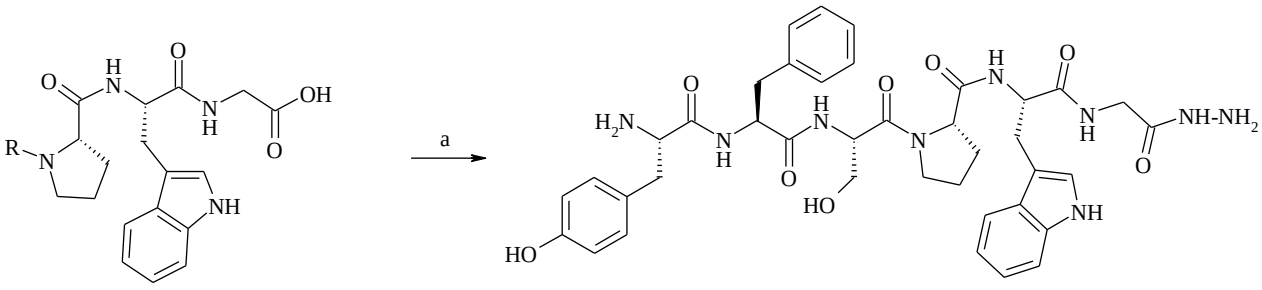

**167** **193** **194** **196** **195**

Reagents and conditions*:* a) Phe-OMe. HCl, TBTU, DIPEA, DMF, 20 h, rt, 98%; b) 4% NaOH, MeOH, 18 h, rt, 93%; c) TBTU, **185**, DIPEA, DMF, rt, 20 h, 62%; d) TFA/TIPS/$H_2O$ (95:4:1), 0 $^oC$, 5 h,72%.

### 2.4.3 *C*-Terminal modification

The importance of the amidated carbonyl group of helicokinin I for its receptor binding is studied by replacing the amide group with hydrazine to form a hydrazide functional group. Chemical transformation of **197** to **198** was involved in a two-step procedure. First, the carboxylic acid **197** was coupled with *tert*-butyl carbazate using TBTU/DIPEA to furnish the full protected hydrazide intermediate in 90% yield. Subsequently, removal of all protecting groups under acidic conditions afforded the hexapeptide **198** in excellent yield (95%).

**Scheme 67: *C*-terminal hydrazide modification**




**197**

R = Boc-Tyr(*t*Bu)-Phe-Ser(*t*Bu)-CO

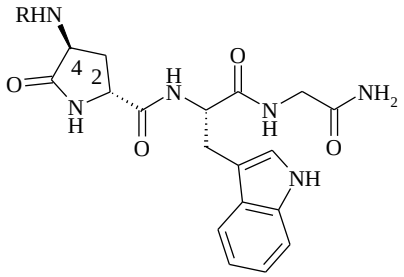
**198**

Reagents and conditions: a) (i) TBTU, DIPEA, $NH_2NHBoc$, DMF, 90%; (ii) TFA/TIPS/$H_2O$, 95%.

## 2.5 Conformational analysis

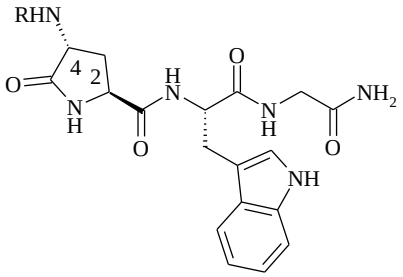
Structure determination of peptides is a decisive step to fully understand the mode of action in biological events. In order to gain a deeper insight into the conformational prerequisites for receptor binding of peptides, several techniques were developed, such as X-ray crystallography,[131] solution NMR,[132] solid-state NMR spectroscopy of oriented static samples of membrane protein in phospholipid bilayers, dodecylphosphocholine (DPC) and sodium dodecyl sulfate (SDS).[133]. Our preceding studies on determining structure of several insect kinins in the membrane-mimicking environment (DPC or SDS) were successfully achieved. As continuation of interest in interpreting relationships between the biological activity and

conformational preference, detailed NMR study of several helicokinin I analogues; namely **34a**, **34b**, **35a**, **35b**, **47**, **66**, **67** and **79** (Figure 26); were carried out using solution NMR in membrane-mimicking environment DPC. In addition, molecular dynamics simulations were also performed in explicit water, in the core membrane or in lipid bilayer in order to characterize these highly flexible peptides.



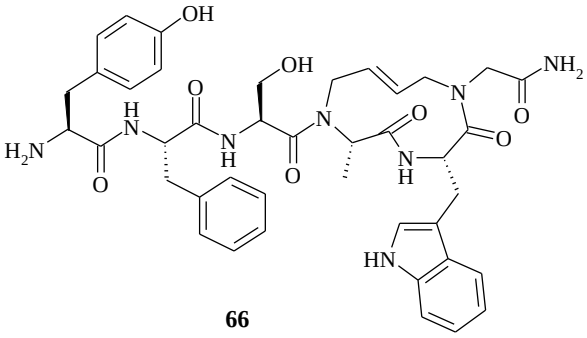
**34a (APy(2*R*, 4*S*)): R = Tyr-Phe- CO-**

**35a (APy(2*R*, 4*S*)): R = Tyr-Phe-Ser-CO-**

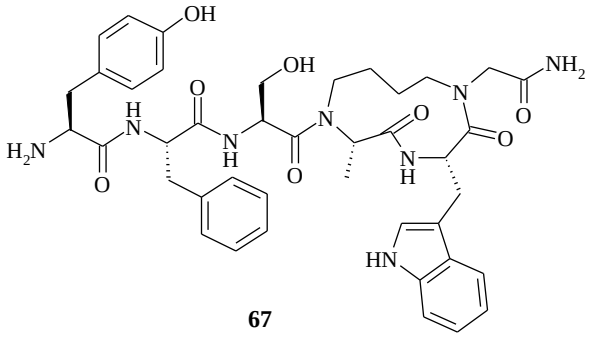


**34b (APy(2*S*, 4*R*)): R = Tyr-Phe-CO-**

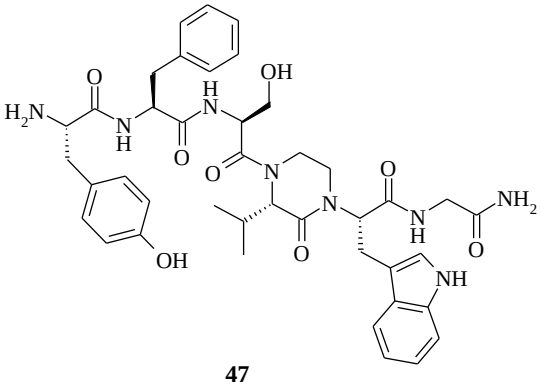
**35b (APy(2*S*, 4*R*)): R = Tyr-Phe-Ser-CO-**



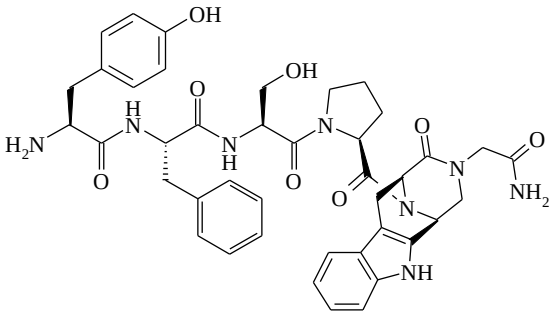
**66**



**67**



**47**



**79**

**Figure 26:** Selected helicokinin I analogues for conformational analysis

### 2.5.1 Conformational characterization of the APy-containing helicokinin analogues

The NOESY spectrum of the APy-containing pentapeptides **34a** and **34b** display a series of strong sequential NOEs. The analogue **34a** presents a high number of sequential NOEs between protons of APy and NH, α-, β- and δ-protons of Phe. Similarly, a significant number of NOEs between protons of APy moiety and the *δ*, NH protons of tryptophan as well as strong correlations between protons of Trp and Gly are found in the spectra of the peptide **34b**. Molecular dynamics simulations based on the NOE provided insights into the structure of the APy-containing helicokinin I analogues bound to DPC micelles. The best-fit superpositions of the lowest energy conformers that satisfy all of the distance and dihedral angle restraints are presented in Figure 27.

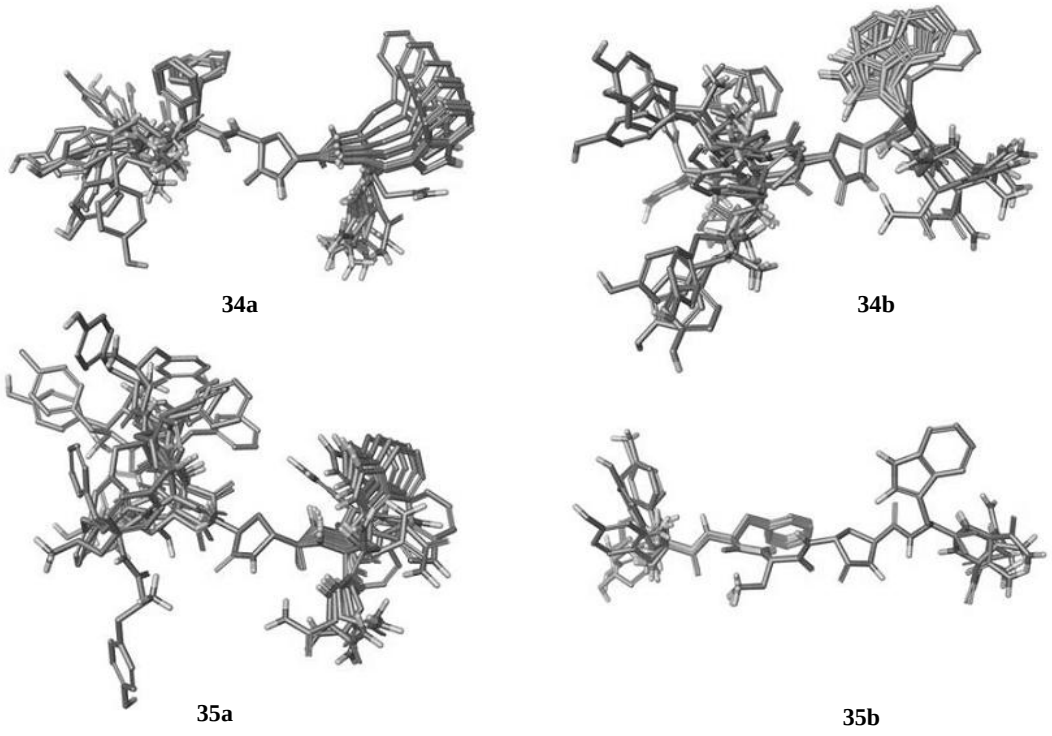


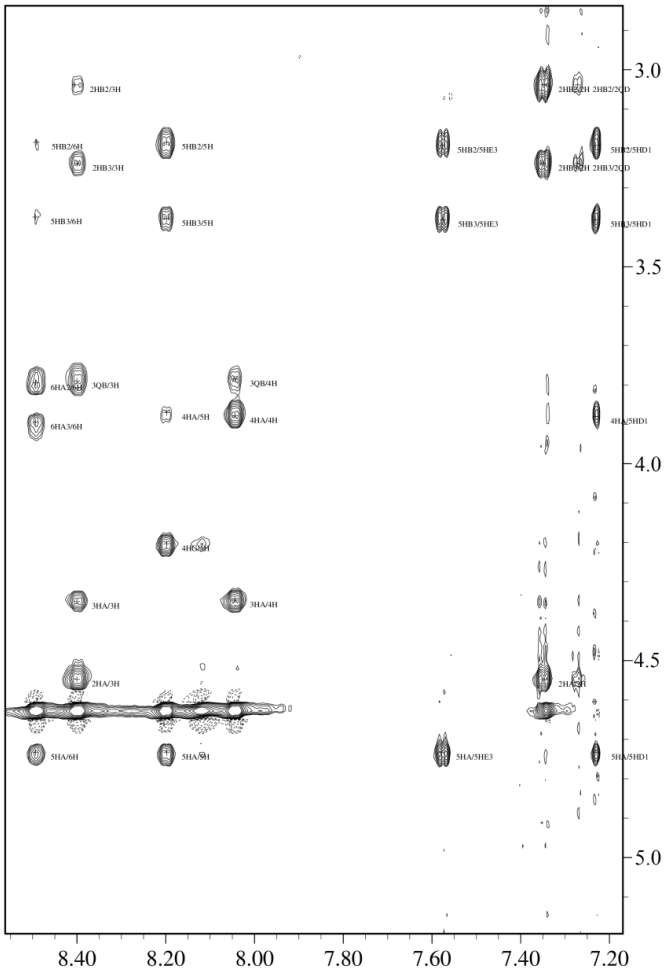

**Figure 27**: Superpositions of the lowest-energy conformers of APy containing helicokinin I analogues

Structure calculations revealed that both peptides **34a** and **34b** form linear and flexible peptide chains with little conformational preferences, in which the *N*-terminal tyrosine residue occupied two different conformational clusters. These are contributed to the 2,4-*trans*-arrangement of the carboxyl and amino functional groups in the APy residue, thus they are unable to induce the β-turn structures. Furthermore, the molecular dynamics calculations also confirmed the higher

average values of the distances between the α-protons of Phe and Trp with 9.2 Å and 8.0 Å for **34a** and **34b**, respectively. It could be concluded that a β-turn formation for both **34a** and **34b** is impossible.

In the NOESY spectrum of peptides **35a** and **35b**, only sequential NOEs were observed corresponding to a stretched conformation with an exception of NOEs between the δ and NH protons of Trp and the APy moiety in **35b** (Figure 28). The helicokinin analogue **35a** mainly displays medium- and long-range contacts between the β-protons of Ser and the NH of APy as well as between the NH of tryptophan and the protons of APy ring. Neither mimetic **35a** nor **35b** induces predominantly unique structure. However, both peptides clearly adopt preferentially extended conformations, which therefore confirm no turn-like structures to be formed.

**Figure 28**: An expansion of the NOESY-spectrum between sequential amide protons of peptide **35b**



### 2.5.2 Conformational characterization of the 2-oxo-piperazine-containing mimetic

For the helicokinin analogue **47**, a number of medium-range interactions between the β- and δ-protons of Phe residue and the isopropyl group on the piperazine moiety were observed. In addition, a significant number of NOEs between the side chain Trp and the isopropyl group are found. The molecular dynamics (MD) simulations in micelles confirm two distinct conformers for peptide **47** that induce a turn-like structure at the *C*-terminus and an unstructured *N*-terminus (Figure 29).

**Figure 29:** Low energy conformation of 2-oxo-piperazine containing helicokinin I analogue **47**

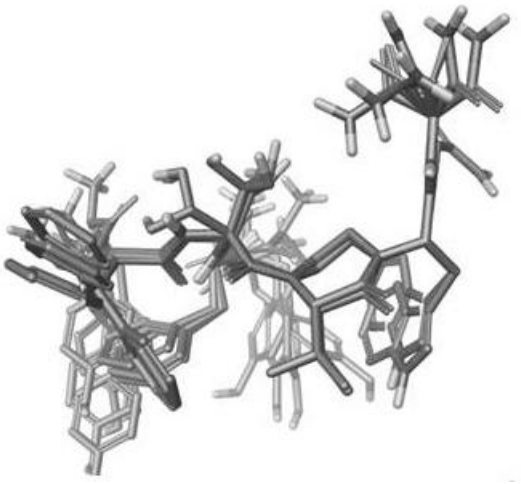


**Table 10**: Dihedral angles of the main conformer of helicokinin analogue **47**

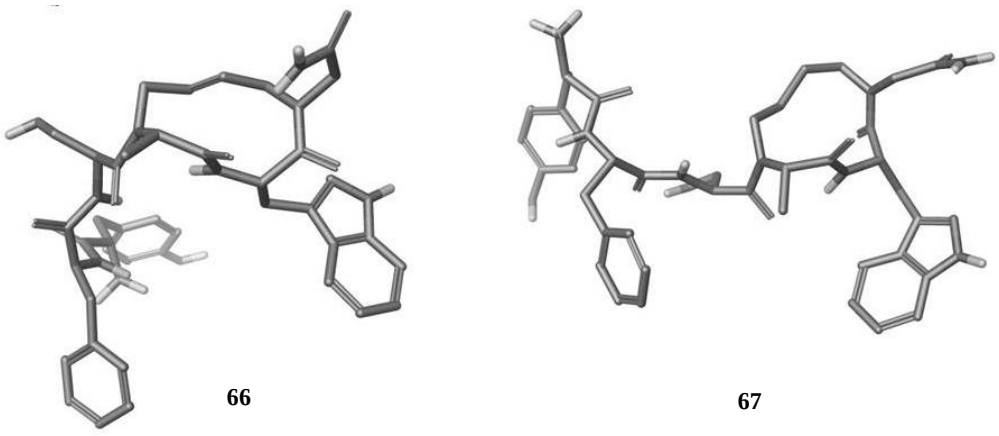
| | ϕ | ψ |
|---|---|---|
| **Tyr** | | 143.7 ±100.4 |
| **Phe** | -106.8 ± 108.0 | 149.8 ± 7.9 |
| **Ser** | -97.1 ±5.7 | 157.5 ± 1.4 |
| **Oxopiperazine** | -126.0 | -20.9 |
| **Trp** | -150.3, -136.9 | -87.5; 92.0 |
| **Gly** | -171.5 ± 35.9 | |

The two conformers are reasoned due to the tertiary amide bond between serine and 2-oxo-piperazine ring. The changes in dihedral angles within the critical turn region (Ser to $GlyNH_2$) resulted from the substitution of the 2-oxo-piperazine moiety with Pro. In parent helicokinin I the ϕ torsion angle of Pro is fixed at -75°, while it is -126° in the oxo-piperazine moiety of analogue **47**. Moreover, the ϕ and ψ torsion angles of Trp (Table 10) are also significantly different from native helicokinin I, which are fixed at -105.3$^{o}$ and -18.6$^{o}$, respectively.[43] These analyses drive a conclusion that the 2-oxo-piperazine containing helicokinin analogue is unsuitable either to the type I β-turn of helicokinin I or to any other standard β-turns.

### 2.5.3 Conformational analysis of the flexible macrocyclic analogues

The NOESY spectrum of macrocyclic peptides **66** and **67** display a reduced number of sequential NOEs arising from exchange-mediated line broadening regardless of temperature and field. These are due to the fast interchange between different conformers that lead to very low intensities of many signals in both TOCSY and NOESY spectra, especially of inherently weak amide signals. In spite of several efforts, we were unable to identify the defined structures of peptides **66** and **67**. Therefore, their three-dimensional structures (3D) were established by utilizing YASARA Structure molecular modeling package (version 13.5.10) with helicokinin I as reference. These calculations were performed by Prof. Dr. Guiscard Seebohm at the Department of Myocellular Electrophysiology, University of Münster. After parameterizing the new building blocks by means of semi-empirical quantum mechanics in AM1/NOVA and minimizing energy of the conformers in AMBER using the AMBER03 force field, the preferred conformations of peptides **66** and **67** were constructed (Figure 30). MD simulations were carried out following three different models: Type A) in water, Type B) into the core of the membrane, and Type C) into the region of the membrane head groups (phosphate region).

**Figure 30**: Low energy conformations of flexible macrocyclic scaffolds (**66** and **67**)



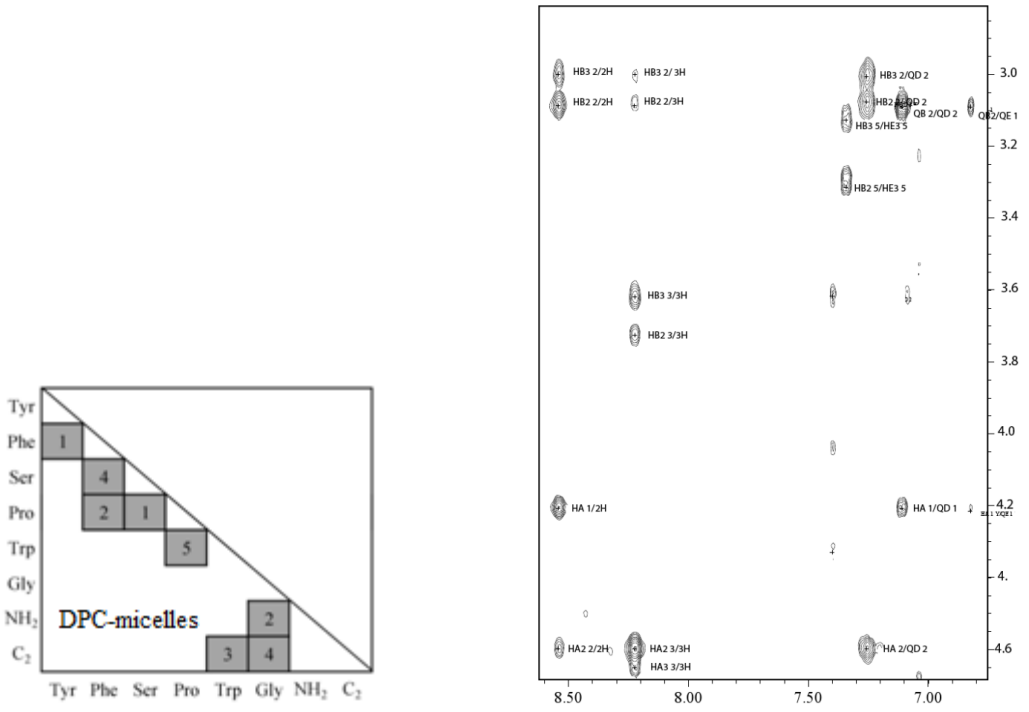
**66** **67**

Energy analyses suggest that simulations of helicokinin I, peptides **66** and **67** reach stable energies within 50 psec. All three peptides have similar energies in both membrane regions B and C, but they are significantly higher in aqueous environment confirming that three peptides tend to dissolve within different membrane regions rather than in water. When simulated the native helicokinin I was slowly expelled from the membrane core (within 0.5 ns) and remained stable among phosphates of the lipid head groups (5 ns simulations), whereas the peptidomimetics **66** and **67** stayed stable in the membrane core (within 1 ns). Rout-Mean-

Square-Deviation analyses (RMSD) suggest that helicokinin I residues fluctuate stronger in water (2.2 – 3.8Å) and slightly stronger among phosphates of the lipid head groups (0.8 – 2.2Å) than in the membrane core (0.7 – 1.4Å). In all cases, the helicokinin I *C*-terminal region (Ser-Pro-Trp-Gly$NH_2$) is more stable than the *N*-terminal residues Tyr and Phe as observed in the NMR studies. As expected, helicokinin I formed a type I β-turn over the residues Ser-Pro-Trp-Gly$NH_2$. This result is in good accordance with the previous study on helicokinin by NMR measurement in DPC micelles.[43] It was also found that the type I β-turn conformation was the most stable in the membrane core (RMSD in simulation type A: 2,79 ± 0,37 Å; type B: 1,24 ± 0,14 Å; type C: 0,87 ± 0,06 Å; errors as SEM). On the other hand, both the alkenyl- and alkyl-bridged macrocyclic peptides **66** and **67** stabilize the loop-structures at the *C*-terminal domain (Figure 30). However, due to the higher flexibility of the alkyl-spacer macrocycle **67** adopts a more stretched conformation in the membrane, while the more restricted analogue **66** induces a loop conformation that is very similar to helicokinin I conformation in the membrane mimicking environment.

### 2.5.4 Conformational analysis of the Trp-derived helicokinin I analogue

**Figure 31**: The number of significant inter-residual distance constraints of peptide **79** in DPC-micelles (left); An expansion of the NOESY-spectrum between sequential amide protons (right).



The NOESY spectroscopy of peptide **79** displays again a decreased number of sequential NOEs. Only a number of medium-range contacts between the β- and δ-protons of Phe residue and the NH amide of Ser were found corresponding to a disordered conformation (Figure 31). In this case, MD simulations were not carried out. Thus, its defined conformation was not established. However, due to the *cis*-amide bond between Pro and Trp residues, it is assumed that formation of any turn-like structures is impossible.

## 2.6 Biological data

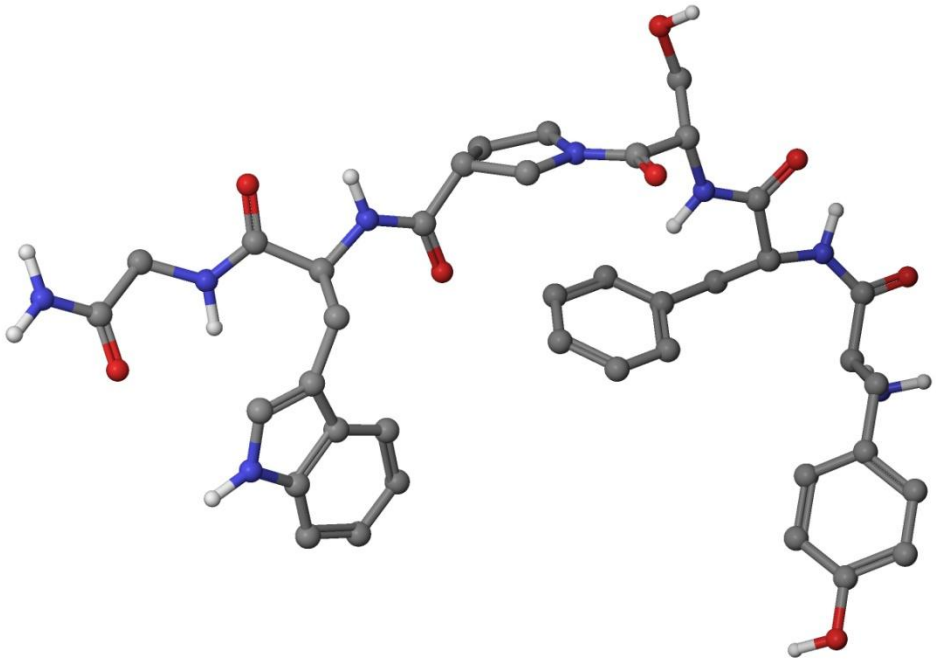
In order to evaluate structure-activity relationships of the helicokinin analogues for their receptor binding potency, all the peptidomimetics synthesized were tested on functional receptor assay of *Heliothis virescens* cloned from Mapighian tubules cDNA library. Within the scope of the biological validation, an inactive compound is defined as an $EC_{50}$ value higher than 100 µM corresponding to a complete loss of receptor-binding by a factor of more than 30.000 compared to the parent helicokinin I, which is chosen as reference (Table 11).

**Table 11: $EC_{50}$ values of helicokinin I analogues**

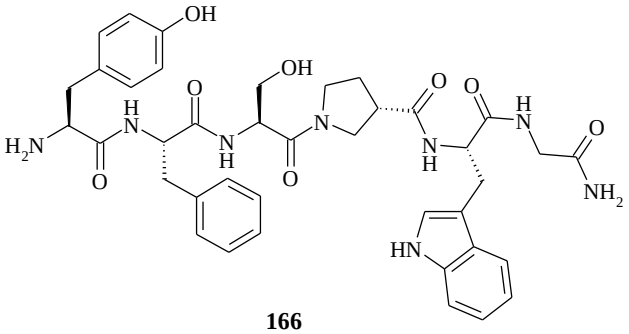
| Compd. | Receptor assay $EC_{50}$ [µM] |
|---|---|
| **YFSPWG-$NH_2$** (helicokinin I) | 0.003 |
| **34a** | >100 |
| **34b** | >100 |
| **35a** | >100 |
| **35b** | 25.12 |
| **47** | >100 |
| **66** | 1.99 |
| **67** | 12.5 |
| **79** | >100 |
| **88** | 1.0-1.58 |
| **128** | 0.0398 |
| **129** | 0.501 |
| **132** | 0.63-1.25 |
| **166** | >100 |
| **171** | >100 |
| **176** | 15.84 |
| **180** | 3.1 |
| **192** | 15.85 |
| **196** | 0.003 |
| **198** | 0.1-0.158 |

Encouraged by highly biological activities of 4-*trans*-aminopyroglutamate-achetakinin analogues reported by Nachman (Table 4), four diastereomeric helicokinin I analogues **34a**, **34b**, **35a** and **35b** were synthesized (Figure 26), Surprisingly, our results found that both helicokinin I peptidomimetics **34a** (APy(2*R*,4*S*)) and **34b** (APy(2*S*,4*R*)) were inactive, even up to an $EC_{50}$ value of 100 μM (Table 11). Most importantly, a prerequisite for any β-turn formations is the spatial orientation of the *N*- and *C*-terminal peptide chains in the same directions. However, this appears not true to the APy-containing helicokinin analogues **34a** and **34b**, where the *trans*-APy core moiety orients the attached peptide chains into opposite directions. Thus, it prevents formation of a *C*-terminal β-turn. The NMR spectroscopic studies evidently confirmed flexible and stretched conformations for both diastereoisomers **34a** and **34b**. The results of inactive pentapeptides arose again a question whether the stereochemistry or the length of peptide chains has much influence on receptor binding. It is known that the truncated helicokinin I analogues with any amino acids exhibited a complete loss of bioactivity, except for the *N*-terminal tyrosine. [64] In order to clarify these issues the hexapeptides **35a** and **35b** were prepared, which ensure sufficiently the length of peptide chains in comparison with the parent helicokinin I. However, only the peptide **35b** (2*S*,4*R*) exhibited weak activity ($EC_{50}$ = 25 μM), whereas its diastereomer **35a** was completely inactive (Table 11).

**Figure 32**: Preferred conformation of peptide **166**

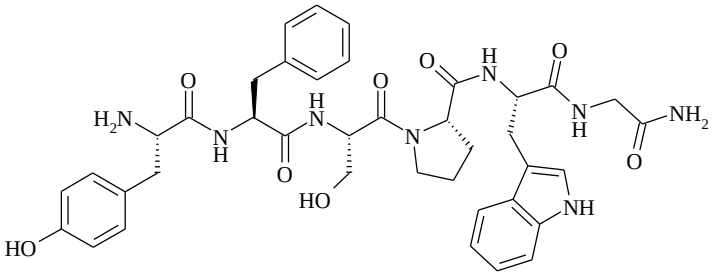


Incorporation of β-proline in the helicokinin I provided additional evidence of the 1,3-arrangements in the pseudo-proline moiety that plays important role for receptor binding. In this case analogue **166** (Figure 33) is considered as more opened conformation than helicokinin I because the β-Pro moiety arranges the attached peptide chains in positions 1 and 3. Not surprisingly, peptide **166** displays again a complete loss of biological activity (Table 11). Like the 1,3-connectivities in the *trans*-APy containing analogues, the β-Pro moiety probably orients the *N*- and *C*-terminus into inappropriate directions. Therefore, peptide **166** tends to adopt an extended conformation (Figure 32) rather than form a turn-like structure. It could be concluded that the main reason leading to the loss of biological activity is due to the inappropriate orientations of the *N*- and *C*-terminus and not simply insufficient length of the peptide chains.



**166**

**$EC_{50}$ >100 µM**



**Helicokinin I**

**$EC_{50}$ = 0.003 µM**

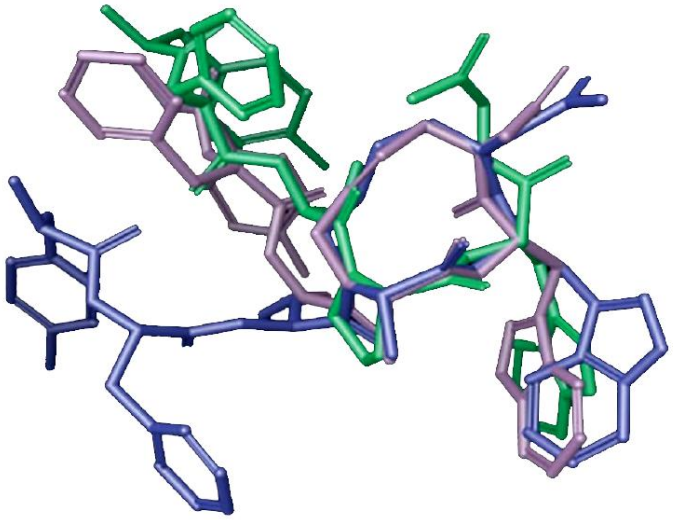
**Figure 33**: Chemical structure of helicokinin I and analogue **166**

Formed by an ethylene bridge between two adjacent amide bonds within the active core domain, the 2-oxo-piperazine moiety in the helicokinin analogues **47** serves as 1,4-connectivity motif that forces the attached *N*- and *C*-terminal peptide-chains in opposite directions. As expected, the helicokinin I analogue **47** was found inactive, too (Table 11). The NMR spectroscopic data

revealed that helicokinin I analogue **47** induces a bowl-like structure that is absent from a β-turn accompanied by complete loss of receptor binding. It is noteworthy that the spatial orientations of the *N*- and *C*-terminal peptide-chains actually play a crucial role in receptor binding. The 1,3- or 1,4-arrangements of the attached *C*- and *N*-terminal peptide chains almost result in a loss of receptor binding potency.

In an effort to bring the *N*- and *C*-terminus into close promixity, the macrocyclic helicokinin I analogues **66** and **67** were synthesized (Figure 26). In these regards, the amide bonds of Ala and Gly are fixed by tetra-carbon bridges and the critical Phe, Trp and Gly-$NH_2$ residues required for receptor binding are retained. The difference between peptides **66** and **67** (Figure 26) displays through the flexibility of the bridges. The more rigid *trans*-alkenyl bridge in analogue **66** induces a loop conformation that is very similar to the helicokinin I conformation in micelles, while the saturated analogue **67** presents a higher degree of conformational freedom (Figure 30). The more constrained macrocyclic helicokinin I analogue **66** retains considerable activity in the low micromolar range ($EC_{50}$ = 1.99 μM, Table 11) suggesting that it is possible to freeze the biologically active conformation needed for receptor binding in cyclic structure which can be analyzed conveniently with conventional NMR spectroscopic methods. Surprisingly, the more flexible macrocyclic helicokinin I analogue **67** demonstrates a six-fold reduced activity ($EC_{50}$ = 12.5 μM), suggesting a very specific receptor bound conformation tolerating only minor deviations in plasticity. Obviously, the *trans*-alkene bridge constrains the Trp residue in a direction that facilitates receptor binding and preserves the required flexibility of the critical Phe and Gly$NH_2$ residues, while the larger flexibility of the alkyl-spacer in cyclic peptide **67** induces a more disordered conformation. Consequently, in the lowest-energy conformation the *N*-terminal tripeptide-chain of macrocycle **67** adopts a considerably different position related to helicokinin I or macrocycle **66** (Figure 34). Differences can also be found within the macrocycles. For instance, the Trp carbonyl group in helicokinin I occupies an *exo*-position, whereas the corresponding carbonyl groups of Trp in peptides **66** and **67** are found in *endo*-positions. In addition, the double bond in the bridge of the macrocycle **66** adopts a position almost identical to the Ser carbonyl group in helicokinin I, suggesting that the double bond is able to mimic the carbonyl group and thus contributes to the remarkable activity of compound **66** in contrast to the saturated macrocycle **67**.

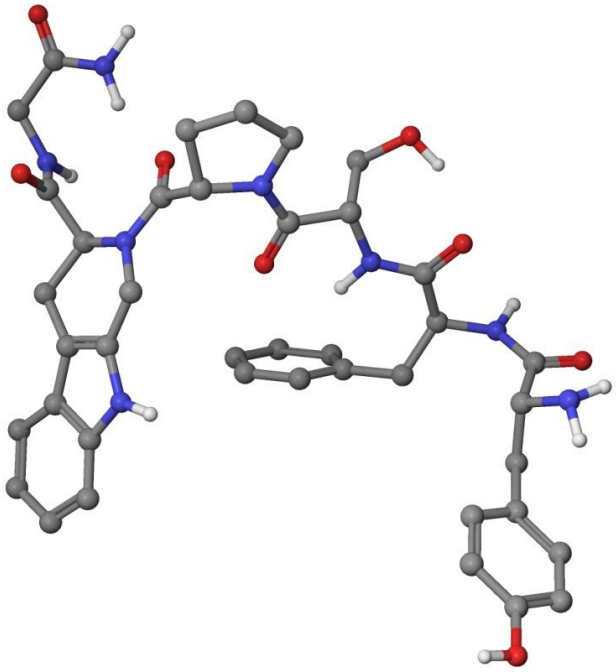
**Figure 34**: Superpositions of helicokinin I (green) with macrocycles **66** (purple) and **67** (blue)



In another aspect, the significance of the Trp side chain for receptor binding is clearly displayed by two helicokinin I peptidomimetics **79** and **88**. Incorporation of the tryptophan-constrained analogues, in which a methylene-bridge is placed between the C-2 of the indole ring and $N^{\alpha}$, into helicokinin I has great influence on their abilities to interact with the biological target. The resulting analogue **79** (Figure 26) was found inactivity (Table 11). The highly conformational constraint of the Trp moiety perturbs drastically the backbone conformation of the helicokinin I analogue and does not allow the peptide backbone and side chain Trp some degree of flexibility. Probably, the restriction of the motional freedom of indole ring disrupts largely its shape, making analogue **79** unable to fit into the binding pockets of receptor. In addition, NMR spectroscopic studies also confirm that the peptide **79** is unable to adopt any turn-like structures. In contrast, the tetrahydro-β-carboline containing helicokinin analogue **88** retains considerably biological activity in the low micromolar range ($EC_{50}$ = 1.0-1.5 μM). This suggests that the side chain modification is an interesting aspect in structure-activity relationship studies of helicokinin I. The difference between two peptidomimetics mainly involves in the limitation of ϕ and ψ torsion angles within the Trp moiety. It seems that the larger constraint of the ψ dihedral angle of the diaza-bridged heterocyclic system in analogue **79** orients the *N*- and *C*-terminal tripeptide in opposite directions, while the more flexible ψ torsion angle of the tetrahydro-β-carboline moiety in analogue **88** deviates slightly the *N*- and *C*-terminal orientation. In addition, the appropriate

orientations of the indole moiety and Phe residue in the same direction also facilitate the receptor binding potency of peptide **88** (Figure 35).

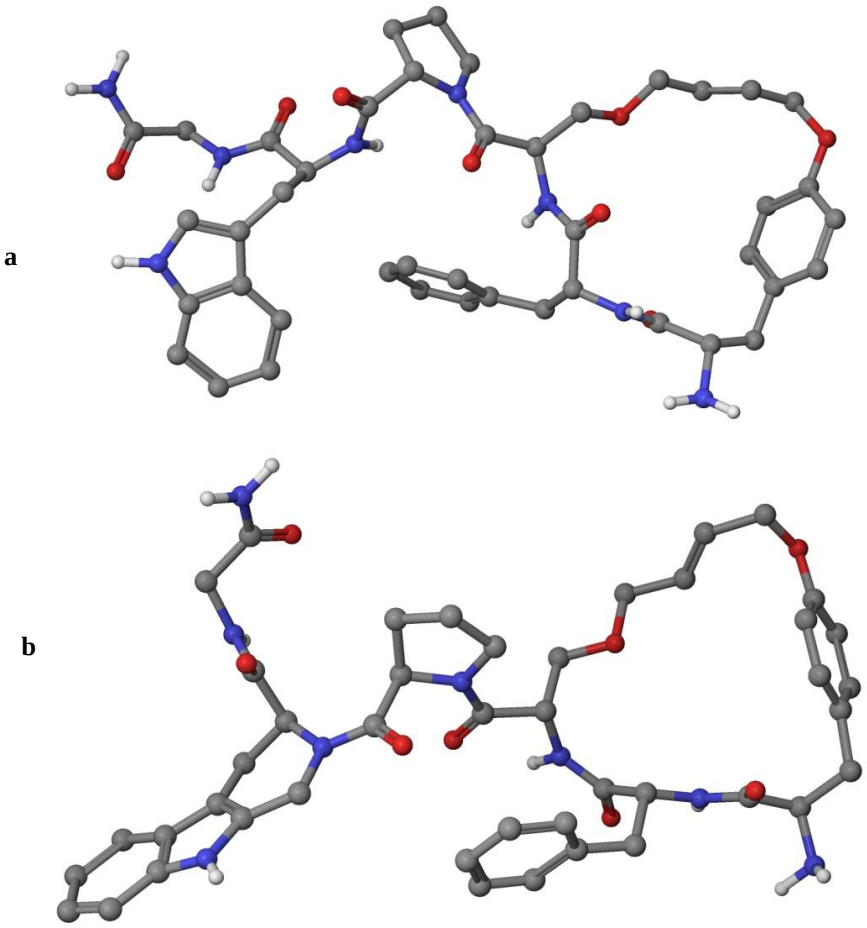
**Figure 35:** Preferred conformation of peptide **88**



Stimulated by considerable bioactivity of the macrocyclic peptide **66,** our attention focused on constructing macrocyclic helicokinin I analogues. For this purpose, the macrocycles **128**, **129** and **132** were successfully synthesized (Figure 37). Unlike the previous studies on head to tail backbone cyclization of the linear helicokinin I analogues, which provided all inactive peptides, the side chain to side chain cyclization analogues exhibit significantly biological activity. Remarkably, the flexible *trans*-alkene macrocycle **128** demonstrates greatly biological activity in nanomolar range ($EC_{50}$ = 39.8 nM). Formation of the 19-membered ring probably does not disturb the backbone conformation of helicokinin drastically. Probably, the constraint of the dihedral angles of Ser residue within the turn center causes slightly deviation of the *N*- and *C*-terminus in which the critical side chains Trp and Phe are still allowed to orient towards in the same side of the peptide backbone (Figure 36a). In addition, the maintenance of the correct distance between the *N*-terminal Tyr-Phe dipeptide and the *C*-terminal Trp-Gly$NH_2$ dipeptide through Ser-Pro spacer may play a significant element to retain its biological activity. Not

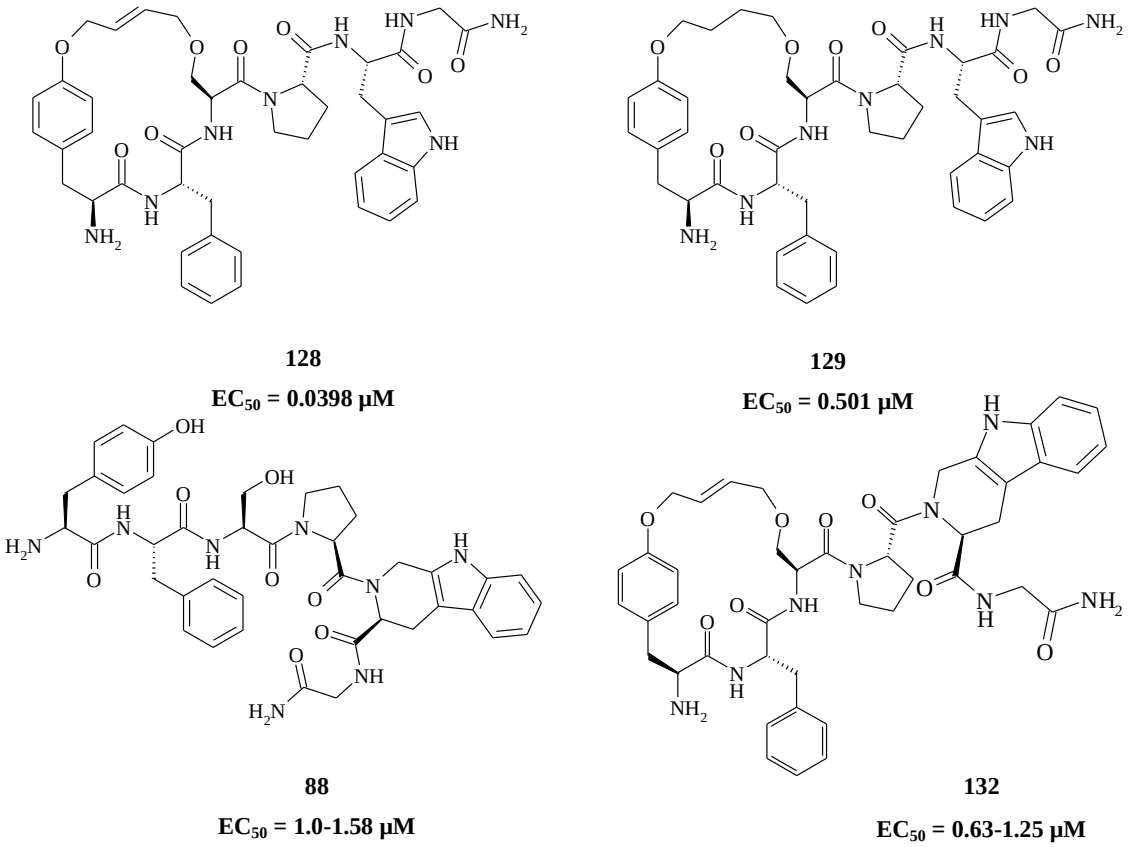
surprisingly, the alkyl-bridge containing analogue **129** displays a considerable reduction in the biological activity. The saturated bridge analogue **129** shows over 12-fold decreased bioactivity ($EC_{50}$ = 0.50 µM). These findings are consistent with the case of peptides **66** and **67**. Obviously, the more constrained-macrocyclic-bridged analogues (**66** and **128**) have significant roles in inducing the conformational preference of helicokinin I analogues that are capable of enhancing the potency of receptor binding.

**Figure 36:** Preferred conformation of peptide **128** (a) and peptide **132** (b)




Interestingly, the hybridized analogue **132** also retains a considerable bioactivity in very low micromolar range ($EC_{50}$ = 0.63-1.15 µM). Compared to the helicokinin I analogue **128**, the analogue **132** displays largely a disturbed conformation at the *C*-terminus (Figure 36b). The tertiary amide bond of Pro residue and the carboline moiety deviates the *C*-terminus in inappropriate direction. Perhaps, both side chains Phe and Trp tend to point towards in the same

direction of the peptide backbone along with constant distance of the *N*- and *C*-terminal dipeptide accelerating the receptor binding potency.



**128**

**$EC_{50}$ = 0.0398 µM**

**129**

**$EC_{50}$ = 0.501 µM**

**88**

**$EC_{50}$ = 1.0-1.58 µM**

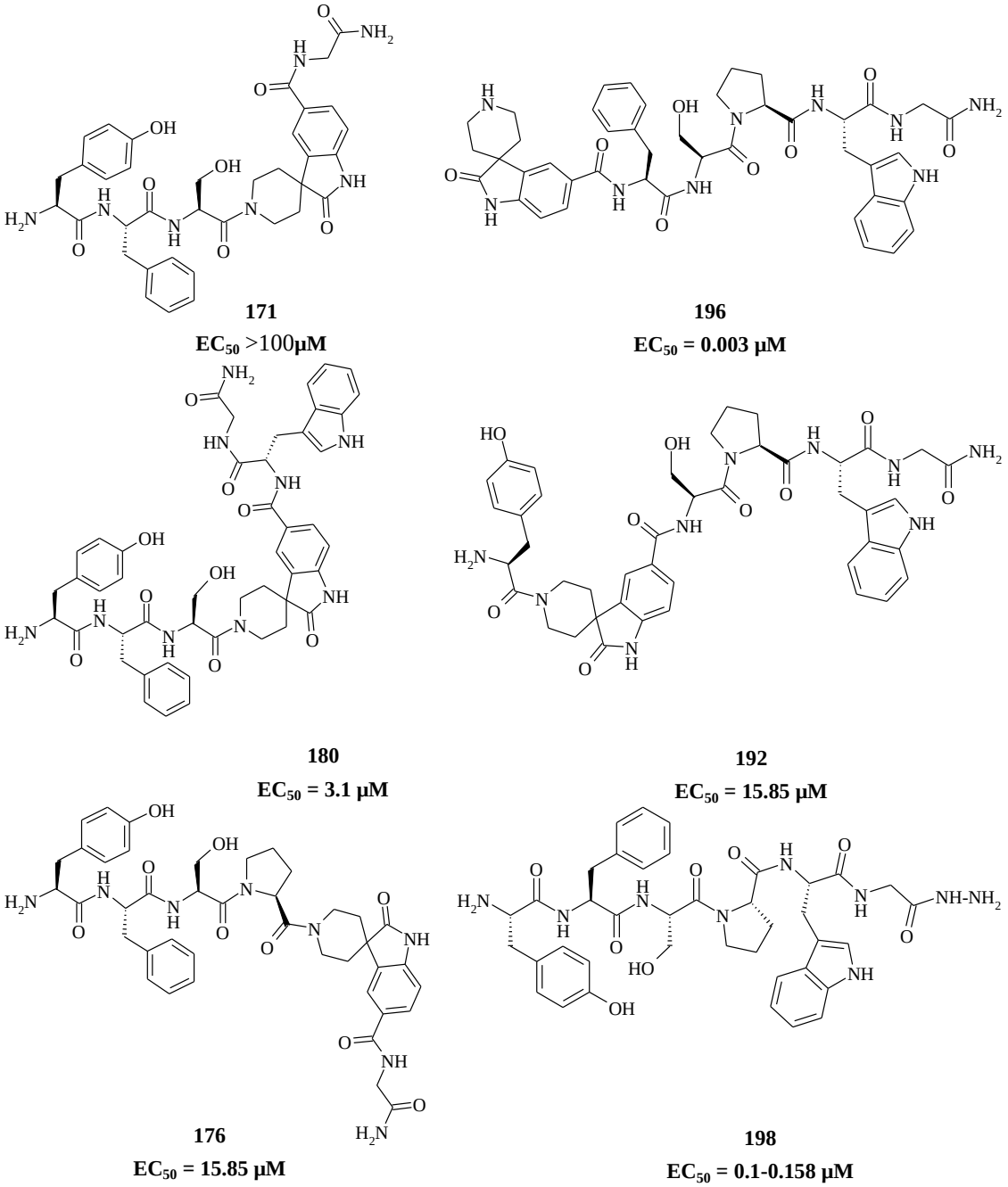
**132**

**$EC_{50}$ = 0.63-1.25 µM**

**Figure 37**: Chemical structure of helicokinin derivatives **88**, **128**, **129** and **132**

In order to obtain more detailed general picture on three-dimensional structure-activity relationships of helicokinin I, a highly constrained spirolactam **167** was used as β-turn motif for scanning the ability of receptor binding. Five helicokinin analogues (Figure 38) were synthesized and tested on receptor assay. The results of biological assay confirmed that the Tyr residue is uncritical for receptor activation (Figure 38, **192**). In addition, substitution of Pro-Trp dipeptide sequence with the constrained spirolactam (Figure 38, **171**) leads to a complete loss of biological activity. These findings are in good agreement with the previous studies on helicokinin I, in which the deletion of any amino acid in helicokinin I sequence to form pentapeptides provided inactive compounds, except Tyr.[64] Surprisingly, substitution of spirolactam for either Phe or Trp (Figure 38, **176**, **192**) exhibits the similar receptor binding potency in low micromolar ranges ($EC_{50}$ = 15.84 µM). These findings are contrast to the preceding study, suggesting that Phe

residue can be replaced somewhat by other amino acids without immediately complete loss of bioactivity. The spirolactamscan at position 4 reduced considerably receptor binding potency by a factor of 150. It demonstrated that the distance between the *N*-terminal dipeptide Tyr-Phe and the *C*-terminal Trp-Gly appears very important for remaining the biological activity of helicokinin I.



**171**
**$EC_{50}$** >100**µM**

**196**
**$EC_{50}$ = 0.003 µM**

**180**
**$EC_{50}$ = 3.1 µM**

**192**
**$EC_{50}$ = 15.85 µM**

**176**
**$EC_{50}$ = 15.85 µM**

**198**
**$EC_{50}$ = 0.1-0.158 µM**

**Figure 38**: Chemical structure of helicokinin I derivatives

Functional variation at the *C*-terminus again demonstrates the importance of the *C*-terminal amidation. In agreement with the published data, peptide **198** (Figure 38) shows a reduced bioactivity by a factor of 50-80, indicating that the amidated *C*-terminus is required for remaining its receptor binding.

## 2.7 Conclusion and outlook

We have presented several approaches to defining the secondary structure of helicokinin I required for receptor binding. The primary attention was focused on establishing β-turn peptidomimetics. A convenient synthetic approach for preparation of the pure diastereomeric 4-*trans*-aminopyroglutamates, as inducers of type VI β-turn, and their incorporation into helicokinin I have been described. In addition, two efficient strategies for stabilizing the secondary structure of helicokinin I analogues using hydrophobic linkers, ethylene or tetra-carbon bridges, have been successful. The detailed NMR spectroscopic studies and MD calculations of several helicokinin I peptidomimetics provided a deeper understanding of the conformational flexibility and orientation of the *N*- and *C*-terminal peptide chains required for receptor binding. All the 1,3- and 1,4-connectivites of the *N*- and *C*-terminal peptide chains (**34a**, **34b**, **35a**, **35b**, **47**, **166**) lead to the complete loss of biological activity because of their incapability of either adopting a β-turn structure at the *C*-terminus or inducing appropriate orientations. Notably, the macrocyclic helicokinin I analogue **66** with appropriate scaffold retains significant biological activity in low-micromolar receptor binding, indicating specific conformational freedom and spatial orientation of the *N*- and *C*-terminal peptides.

The global constraint on the secondary structure of helicokinin I by constructing macrocycles (**128**, **129** and **132**) provides the most interesting results in the biological activity. The chemical modification on the side chains Tyr and Ser, which are not involved directly in molecular recognition, shows highly potent agonist in receptor activation. Remarkably, the macrocyclic peptide **128** is the first modified helicokinin I analogue that exhibits in nanomolar range in the receptor binding. Thus, macrocycle **128** may provide excellent starting-points for probing conformational changes in helicokinin I analogues. Surprisingly, the saturated macrocyclic peptides **67** and **129** formed by hydrogenation of the alkenyl-bridged peptides **66** and **128**,

respectively, show considerably reduced receptor activation by a factor of 6-12 (Table 11). Probably, the higher flexibility of the alkyl spacers causes more stretched or disordered conformations in the membrane, while the more constrained *trans*-alkenyl linkers in analogues **66** and **128** may adopt preferentially loop conformations that are highly similar to helicokinin I in membrane-mimicking state.

On the other hand, the function of side chain Trp with respect to its role as anchoring tool on the membrane is also studied. Though several conformationally constrained structures derived from tryptophan residue were targeted to prepare (Figure 20), but only two analogues **79** and **88** were successfully synthesized. The restriction of side chain tryptophan affects obviously the global secondary structure of helicokinin I. The loss of the receptor binding of analogue **79** is contributed to the incapability of inducing any turn-like structures in which the *N*- and *C*-terminus are extended in different direction. In contrast, the analogue **88** retains considerable bioactivity. This suggests that the use of conformationally constrained side chains offers promising aspects for structure-activity relationship researches on helicokinin I.

Undoubtedly, the opportunity for obtaining well-defined conformations required for receptor binding is very narrow. Only slightly inappropriate orientations of the *N*- and *C*-terminus can lead to weak or inactive peptides. Expectedly, the novel helicokinin I analogues that show highly selective activities in the low micro- or nanomolar range may provide a useful approach for further exploitation of structure activity relationships and optimization of helicokinin I. The global or local constraints of conformation by forming macrocyclic peptidomimetics (**66**, **128** and **88**) are the most interesting aspects of the conformational research. In addition, the spirolactamscan revealed that both Phe and Trp residues can be somewhat replaced by their modified-analogues. Thereby, the use of conformationally constrained structures derived from Phe and Trp residues may present promising targets for evaluating the importance of Phe and Trp required for receptor binding.

# III Experimental

## 3.1 General chemistry

All chemical reagents were purchased from commercial suppliers and used without purification. Dichloromethane (DCM), acetonitrile (ACN), N,N-dimethylformamide (DMF) and N,N-diisopropylethylamine (DIPEA) were heated at reflux for 1 h over calcium hydride and distilled. Tetrahydrofuran (THF) was heated at reflux for several hours over $LiAlH_4$ and then distilled. The instrumentation was used as follows, $^{1}$H-NMR: Bruker Avance 400, Bruker Avance III 600. $^{13}$C-NMR: Bruker Avance 400, Bruker Avance III 600. Infrared (IR) spectra were obtained using a FTIR: Nicolet PROTÉGÉ 460 E.S.P; data are presented as the frequency of absorption ($cm^{-1}$). MS: Bruker microTOF, ESI-MS: Varian IT 500-MS Iontrap. LC: Preparative low-pressure chromatography (PLPC) was performed by using silica gel 60 μm (230–400 mesh, Macherey–Nagel) The abbreviations are used for the proton spectra multiplicities are: s, singlet; br, broad; bs, broad singlet; d, doublet; dd, double of doublet; dt, double of triplet; t, triplet; q, quartet; m, multiplet. Coupling constants (*J*) are reported in Hertz (Hz). TLC analyses were performed on silica gel 60 $F_{254}$ (Merck). Detection was conducted under UV light (254 nm) and staining with potassium permanganate. Flash column chromatography was performed on silica gel (0.063-0.200 mm).

## 3.2 Abbreviations

Boc, *tert*-butoxycarbonyl; Fmoc, fluorenylmethyloxycarbonyl; HATU, 2-(1H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium; PyBOP, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluoro-phosphate; HOAt, 1-Hydroxy-7-Azabenzotriazole; PyBOP, (Benzotriazol-1-yloxy)tripyrro-lidinophosphoniunhexafluorophosphate; PyBrOP, Brom-tris-pyrrolidinophosphoniumhexafluoro-phosphate; BOP-Cl, Bis(2-oxo-3-oxazolidinyl)phosphinic chloride; TBTU, *O*-(1H-benzotriazole-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium tetrafluoroborate; TFA, trifluoroacetic acid; TIPS, triisopropylsilane; DIPEA, *N*,*N*-Diisopropylethylamine; TEA, triethyl amine; DBU; 1,8-diazabicyclo[5.4.0]undec-7-en; DIPEA, *N*,*N*-Diisopropylethylamine; LDA, Lithium diisopropylamide; PTSA, *p*-toluenesulfonic acid; PMB, para methoxybenzyl; MoOPD, Oxodiperoxymolybdenum (pyridine)-1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone; DMF, *N*,*N*-dimethylformamide; ACN, acetonitrile; DCM, dichloromethane; THF, tetrahydrofuran; EtOAc, ethyl acetate; *t*Bu, *tert*-Butyl; TBDPS, *tert*-butylchlorodiphenylsilane; Cbz (Z),

benzyloxycarbonyl; DMSO, dimethylsulfoxide; TIPS, triisopropylsilan; BEP, 2-Brom-1-ethyl-pyridinium-tetrafluoroborate; Bn, Benzyl; TFE, 2,2,2-trifluorethanol; TFA, trifluoroacetic acid.

### 3.3 Receptor assay

The helicokinin receptor of *Heliothis virescens* was cloned from a Malpighian tubule cDNA library and showed about 50% homology (amino acids) to the leucokinin receptor from *Lymnea stagnalis*.[134,135] The helicokinin receptor (HKR) was functionally expressed in a Chinese hamster ovary (CHO) line. The activation of the HKR was analyzed in living cells by measuring the induced calcium ion flux in the cytosol after activation of the second messenger cascade via G-proteins. Calcium ion dyes were obtained from Molecular Devices and were used according to the supplier's protocol. Cells were plated in 384-well plates ($4x10^3$ cells per well) and incubated overnight ($37^oC$, 5% $CO_2$). Cells were removed from the incubator, allowed to reach room temperature over the course of 10 min, and then washed with 50 µl of HBSS. Subsequently, the medium was replaced with 50 µl of calcium ion dye solution in HBSS, and cells were loaded for 1 h in the dark. Then, the plates were read on a Flex station fluorescence plate reader (Molecular Devices). Excitation and emission wavelengths were set to 485 nm and 525 nm, respectively. Measurements were made every 2.7s intervals for 100s. Basal fluorescence was determined for 15–30 s, followed by addition of 25µl test compounds (to assess agonist activity). Helicokinins I or II were used as standards and were tested in four independent experiments for evaluating $EC_{50}$ values. Standard deviations were 2 nM for helicokinin I, 3 nM for helicokinin II and 3 nM for helicokinin III. $EC_{50}$ values were calculated after concentration-dependent induction of calcium flux analyzed in a Flexstation (Molecular Devices). Finally, $EC_{50}$-values were calculated as Fmax (maximal agonist signal) minus Fmin (baseline) according to Softmax-Pro 5.0 software (Molecular Devices). All tests compounds were measured in two independent experiments in ten dilution steps (in duplicates) from 0.5 nM to 10 µM.

### 3.4 NMR measurements and spectra evaluation

The NMR measurements were performed by using 2 mM solutions of the peptide in DPC or SDS micelles (300 mM), corresponding to a 5 mM concentration of micelles, and in MES buffer (20 mM, pH 5.5).[136] All NMR measurements were performed at these high DPC concentrations, well beyond the critical micelle concentration of 1.1 mM and at micelle/peptide ratios well above unity. This has been shown to significantly improve the quality of NMR spectra.[137] Structures were computed based on data derived from NOESY spectra obtained at100 ms (DPC) mixing times. The software XEASY was used for signal integration and spectra interpretation.[138] Resonances were assigned by using the sequential

resonance-assignment procedure developed by Wüthrich.[139] Structures based solely on NOE-derived upper distance in the program CYANA 2.1 and, because of the problematic nature of *J* couplings in nonrigid systems, restraints derived from scalar couplings were excluded. 100 structures were initially computed based on the NOE restrains by using the standard simulated-annealing protocol of CYANA with its simplified force field in the absence of explicit solvent.[140] The 20 lowest-energy conformers were selected to represent the NMR ensemble without further refinement. Structures were visualized in the program MOLMOL, which was also used to extract the values of dihedral angles.[141]

### 3.5 MD simulations

The models were placed into a simulation boxes and the solvent boxes were filled with water using the TIP3P water model, A). In case of the membrane simulations Yasaras run_membrane.mcr was used. In this approach a dense phosphatidylethanolamine (PEA) membrane is built, the peptide/peptidomimetic is placed into B) the membrane core or C) membrane lipid head region. Peptide/peptidomimetic insertion is done by placing a spacially "shrunken" place holder into the chosen position. Gradually increasing the size of the peptide/peptidomimetic to its normal size with intermediate energy minimizations guarantees dense packing of the model. Unrestrained high-resolution refinements and MD-simulation with explicit solvent molecules were run, using AMBER03 force field. The simulation parameters of the all-atoms-mobile MD simulations were as follows: force field AMBER03, time steps 1.2 fs, temperature was 298K, pressure at 1 bar, pH 7.0, Coulomb electrostatics at a cut-off 7.86, 0.9% NaCl, solvent density 0.997. The average structures, RMSDs, secondary structures and side-chain fluctuations of heavy atoms (RMSFs) were analysed using macros "md_analyze", "md_analyzers", "md_analyzemul" and "md_analyzesecstr" in Yasara Structure.

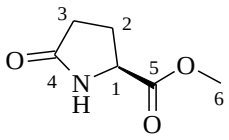
### 3.6 Synthesis of APy-containing helicokinin I analogues

**General procedure for synthesis of methyl 5-oxopyrrolidine-2-carboxylate**

A stirred suspension solution of glutamic acid (1 equiv) in MeOH (50 mL) was added dropwise $SOCl_2$ (6 equiv) at 0 $^{o}$C. After stirring at room temperature for 20 h, the reaction mixture was concentrated under reduced pressure to provide the methyl glutamate ester as a colorless oil. This raw material was then dissolved in toluene (150 mL) and refluxed for 2 h in the presence of TEA (1.0 equiv). The solvent was removed to yield a colorless oil product.

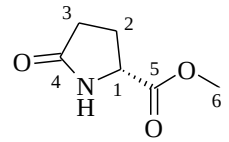
**(*S*)-Methyl-5-oxopyrrolidine-2-carboxylate (TVC-063, 23a)**

Starting material *S*-glutamic acid (2 g, 13.59 mmol) yielded **23a** (1.57 g, 80%).



**$^1$H-NMR** (600 MHz, $CDCl_3$): $\delta$ = 4.2 (m, 1H, 1-H), 3.7 (s, 3H, 6-H), 2.4 (m, 1H, 2-H), 2.2 (m, 1H, 2-H), 2.3 (m, 2H, 3-H). **$^{13}$C-NMR** (150 MHz, $CDCl_3$): $\delta$ = 178.1 (5-C), 172.4 (4-C), 55.3 (1-C), 52.5 (6-C), 29.2 (3-C), 24.7 (2-C). **IR** (neat) ν ($cm^{-1}$): 3244.3 (NH), 1669.9 (CO). **HPLC-ESI-MS**: m/z (%) = 144.0 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_6H_{10}NO_3$ $[M+H]^+$ 144.0655; found 144.0657. $R_f$ = 0.21 (EtOAc).

**(*R*)-Methyl-5-oxopyrrolidine-2-carboxylate (TVC-064, 23b)**



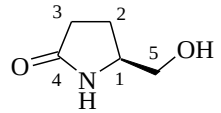
Starting material *R*-glutamic acid (10 g, 67.97 mmol) afforded **23b** (8.4 g, 100%).

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 4.2 (m, 1H, 1-H), 3.7 (s, 3H, 6-H), 2.4 (m, 1H, 2-H), 2.2 (m, 1H, 2-H), 2.3 (m, 2H, 3-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 177.9 (5-C), 172.4 (4-C), 55.2 (1-C), 52.5 (6-C), 29.3 (3-C), 24.8 (2-C). **IR** (neat) ν ($cm^{-1}$): 3242.3 (NH), 1667.3 (CO). **HPLC-ESI-MS**: m/z (%) = 144.0 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_6H_{10}NO_3$ $[M+H]^+$ 144.0655; found 144.0657. $R_f$ = 0.22 (EtOAc).

**General procedure for reduction of methyl-5-oxopyrrolidine-2-carboxylate**

A solution of methyl-5-oxopyrrolidine-2-carboxylate (1.0 equiv) in isopropanol was treated with $NaBH_4$ (4.0 equiv) for 20 h at room temperature. The reaction mixture was then acidified with 5% citric acid. The solvent was removed to dryness and the residue was dissolved in 60% EtOAc/MeOH. The precipitate was filtered off and the filtrate was evaporated to obtain a solid. The solid was redissolved in DCM and filtered again. Removal of the solvent afforded an oily product.

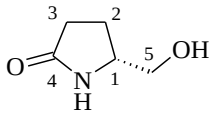
**(*S*)-5-(Hydroxymethyl)pyrrolidin-2-one (TVC-069, 24a)**



Starting material (*S*)-methyl-5-oxopyrrolidine-2-carboxylate **23a** (9.8 g, 68.46 mmol) in isopropanol (60 mL) gave **24a** (6.54 g, 83%).

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 3.7 (br, 1H, 1-H), 3.67 (m, 1H, 5-H), 3.5 (m, 1H, 5-H), 2.3 (m, 2H, 3-H), 2.12 (m, 1H, 2-H), 1.7 (m, 1H, 2-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 179.3 (4-C), 66.7 (5-C), 56.3 (1-C), 30.1 (3-C), 22.5 (2-C). **IR** (neat) ν ($cm^{-1}$): 3229.3 (NH), 1669.3 (CO). **HPLC-ESI-MS**: m/z (%) = 116.0 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_5H_{10}NO_2$ $[M+H]^+$ 116.0706; found 116.0706. $R_f$ = 0.18 (EtOAc/EtOH, 5/1).

**(*R*)-5-(Hydroxymethyl)pyrrolidin-2-one (TVC-067, 24b)**



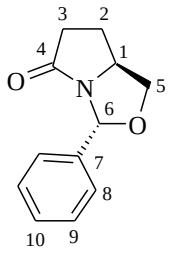
Starting material (*R*)-methyl-5-oxopyrrolidine-2-carboxylate **23b** (42 mg, 0.29 mmol) in isopropanol (8 mL) afforded product **24b** (28 mg, 83%).

**$^1$H-NMR** (600 MHz, $CDCl_3$): $\delta$ = 3.7 (bs, 1H, 1-H), 3.56 (dd, $J$ = 3.4 Hz, $J$ = 11.4 Hz, 1H, 5-H), 3.37 (dd, $J$ = 7.0 Hz, $J$ = 11.4 Hz, 1H, 5-H), 3.0 (br, 1H, NH), 2.3 (m, 2H, 3-H), 2.12 (m, 1H, 2-H), 1.7 (m,1H, 2-H). $^{13}$C-NMR (150 MHz, $CDCl_3$): $\delta$ = 179.3 (4-C), 65.4 (5-C), 56.2 (1-C), 30.0 (3-C), 22.4 (2-C). **IR** (neat) ν ($cm^{-1}$): 3369.3 (NH), 1675.3 (CO). **HPLC-ESI-MS**: m/z (%) = 116.0 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_5H_{10}NO_2$ $[M+H]^+$ 116.0706; found 116.0706. $R_f$ = 0.18 (EtOAc/EtOH, 5/1).

**General procedure for synthesis of 3-phenyl-hexahydropyrrolo[1,2-c]oxazol-5(3H)-one**

A mixture of compound **24a** or **24b** (1.0 equiv), benzaldehyde (1.3 equiv) and p-toluensulfonic acid monohydrate (0.05 equiv) in toluene (30 mL) was stirred at room temperature overnight and afterward refluxed under a Dean-Stark water separator for 6 h. The solvent was then removed under reduced pressure. The residue was purified by silica gel column chromatography using EtOAc/CyH (1/1) as eluent to afford desired product as colourless oil.

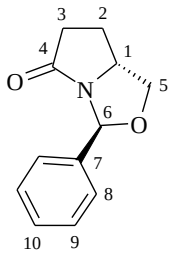
**(3*R*,7a*S*)-3-Phenyltetrahydropyrrolo[1,2-c]oxazol-5(3H)-one (TVC-086, 27a)**



Starting material **24a** (100 mg, 0.87 mmol) provided **27a** (136 mg, 77%). $[\alpha]_D^{20}$ = + 2.1 (c 1.158, MeOH).

**$^1$H-NMR** (600 MHz, $CDCl_3$): $\delta$ = 7.4 (d, $J$ = 7.2 Hz, 2H, 9-H), 7.33 (t, $J$ = 7.0 Hz, 2H, 8-H), 7.3 (m, 1H, 10-H), 6.3 (s, 1H, 6-H), 4.2 (t, $J$ = 8 Hz, 1H, 5-H), 4.1 (m, 1H, 1-H), 3.5 (t, $J$ = 8 Hz, 1H, 5-H), 2,8 (m, 1H, 3-H), 2,6 (m, 1H, 3-H), 2,3 (m, 1H, 2-H), 1.9 (m, 1H, 2-H). **$^{13}$C-NMR** (150 MHz, $CDCl_3$): $\delta$ = 178.0 (4-C), 138.7 (7-C), 128.5 (9-C), 128.4 (8-C), 125.9 (10-C), 87.1 (6-C), 71.6 (5-C), 58.7 (1-C), 33.4 (3-C), 23.0 (2-C). **IR** (neat) ν ($cm^{-1}$): 1662.9 (CO). **HPLC-ESI-MS**: m/z (%) = 204.1 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{12}H_{14}NO_2$ $[M+H]^+$ 204.1019; found 204.1018. $R_f$ = 0.35 (CyH/EtOAc, 1/1).

**(*3S,7aR*)-3-Phenyltetrahydropyrrolo[1,2-c]oxazol-5(3H)-one (TVC-098, 27b)**



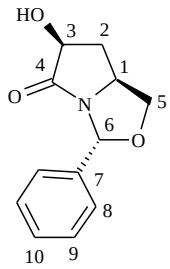
Starting material **24b** (780 mg, 6.77 mmol) afforded product **27b** (860 mg, 63%). $[\alpha]_D^{20}$ = -7.2 (c 0.575, MeOH).

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 7.4 (d, $J$ = 7.2 Hz, 2H, 9-H), 7.36-7.26 (m, 3H, 8-H, 10-H), 6.3 (s, 1H, 6-H), 4.2 (t, $J$ = 8 Hz, 1H, 4-H), 4.1 (m, 1H, 1-H), 3.5 (t, $J$ = 8 Hz, 1H, 5-H), 2,8 (m, 1H, 3-H), 2,6 (m, 1H, 3-H), 2,3 (m, 1H, 2-H), 1.9 (m, 1H, 2-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 178.0 (4-C), 138.8 (7-C), 128.4 (9-C), 128.4 (8-C), 125.9 (10-C), 87.1 (6-C), 71.6 (5-C), 58.7 (1-C), 33.3 (3-C), 23.1 (2-C). **IR** (neat) ν ($cm^{-1}$): 1673.9 (CO). **HPLC-ESI-MS**: m/z (%) = 204.1 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{12}H_{14}NO_2$ $[M+H]^+$ 204.1019; found 204.1017. $R_f$ = 0.4 (CyH/EtOAc, 1/1)

**General procedure for hydroxylation reaction**

Diisopropylamine (1.4 equiv) was added dropwise to a solution of n-BuLi (2.5 M in n-hexane, 1.4 equiv) in dry THF at 0 $^oC$. After stirring for 20 min, the resulting solution was cooled to -78 $^oC$ and a precooled solution of 3-phenyltetrahydropyrrolo[1,2-c]oxazol-5(3H)-one (1.0 equiv) in THF was added. The reaction mixture was stirred for 50 min and then pumped into a suspension of MoOPD (1.3 equiv) in THF. The reaction mixture was allowed to warm up to -40 - -35 $^oC$ and stirred for 3 h. The resulting solution was quenched with a saturated solution of $Na_2SO_3$, extracted with EtOAc, washed with 5% HCl and dried over $MgSO_4$. Removal of the solvent and column chromatography using EtOAc/CyH (1/1) as eluent solvent provided an oily product.

**(3*R*,6*S*,7a*S*)-6-Hydroxy-3-phenyltetrahydropyrrolo[1,2-c]oxazol-5(3H)-one (TVC-094, 28a)**

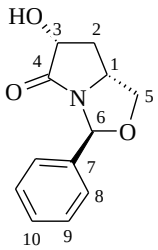


Starting material **27a** (450 mg, 2.21 mmol) afforded **28a** (350 mg, 72%).

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 7.45 (m, 2H, 8-H), 7.4-7.36 (m, 3H, 9-H, 10-H), 6.34 (s, 1H, 6-H), 5.6 (dd, $J$ = 8.5 Hz, $J$ = 10.5 Hz, 1H, 3-H), 4.4 (dd, $J$ = 7 Hz, $J$ = 10.5 Hz, 1H, 5-H), 3.6 (dd, $J$ = 1.5 Hz, $J$ = 8 Hz, 1H, 5-H), 4.05 (m, 1H, 1-H), 2,98 (m, 1H, 2-H), 2,14 (m, 1H, 2-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 169.8 (4-C), 137.3 (7-C), 129.1 (10-C), 128.6 (9-C), 125.9 (8-C), 87.2 (6-C), 78.7 (3-C), 72.4 (5-C), 54.1 (1-C), 33.3 (3-C). **IR** (neat) ν ($cm^{-1}$): 1683.3 (CO). **HPLC-ESI-MS**: m/z (%) = 220.1 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{12}H_{13}NO_3Na$ $[M+Na]^+$ 242.0786; found 242.0787. $R_f$ = 0.17 (CyH/EtOAc, 1/1).

**(3*S*,6*R*,7a*R*)-6-Hydroxy-3-phenyltetrahydropyrrolo[1,2-c]oxazol-5(3H)-one (TVC-099, 28b)**

Starting material **27b** (750 mg, 3.69 mmol) yielded **28b** (590 mg, 73%).



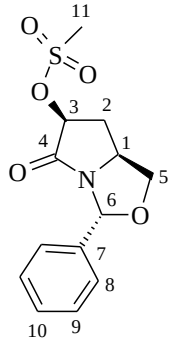
**$^{1}$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 7.46 (m, 2H, 8-H), 7.44-7.36 (m, 3H, 9-H, 10-H), 6.35 (s, 1H, 6-H), 4.76 (dd, *J* = 8.5 Hz, *J* = 10.5 Hz, 1H, 3-H), 4.3 (dd, *J* = 8 Hz, 1H, 5-H), 3.64 (t, *J* = 8 Hz, 1H, 5-H), 4.03 (dt, *J* = 1.5 Hz, *J* = 7.3 Hz, 1H, 1-H), 2,86 (m, 1H, 2-H), 1.87 (m, 1H, 2-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 176.5 (4-C), 137.8 (7-C), 128.8 (10-C), 128.5 (9-C), 126.0 (8-C), 86.8 (6-C), 72.5 (3-C), 72.4 (5-C), 54.5 (1-C), 33.3 (3-C). **IR** (neat) ν ($cm^{-1}$): 1683.3 (CO). **HPLC-ESI-MS**: m/z (%) = 220.1 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{12}H_{13}NO_3Na$ $[M+Na]^+$ 242.0788; found 242.0787. $R_f$ = 0.32 (EtOAc).

**General procedure for mesylation reaction**

Methanesulfonyl chloride (2.0 equiv) was added dropwise into a solution of 6-hydroxy-3-phenyltetrahydropyrrolo[1,2-c]oxazol-5(3H)-one (1.0 equiv) and TEA (5.0 equiv) in DCM at 0 °C. The reaction mixture was allowed to stir at room temperature for 20 h, then it was diluted with DCM, washed with $H_2O$, brine solution and dried over $MgSO_4$. The solvent was removed and the crude material was purified by silica gel column chromatography (CyH/EtOAc, 3/1) to yield the desired product.

**(3*R*,6*S*,7a*S*)-5-Oxo-3-phenylhexahydropyrrolo[1,2-c]oxazol-6-yl-methanesulfonate (TVC-096)**

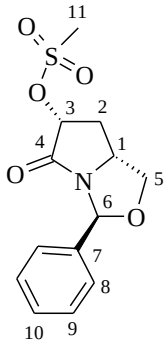
Starting material (3*R*,6*S*,7a*S*)-6-hydroxy-3-phenyltetrahydropyrrolo[1,2-c]oxazol-5(3H)-one **28a** (340 mg, 1.55 mmol) gave **TVC-096** (408 mg, 89%) as a colourless oily product.



**$^{1}$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 7.45 (m, 2H, 8-H), 7.40-7.38 (m, 3H, 9-H, 10-H), 6.35 (s, 1H, 6-H), 5.59 (dd, *J* = 8.5 Hz, *J* = 10.5 Hz, 1H, 3-H), 4.38 (dd, *J* = 8.5 Hz, 1H, 5-H), 3.67 (t, *J* = 8.2 Hz, 1H, 5-H), 4.06 (t, *J* = 7.3 Hz, 1H, 1-H), 3.32 (s, 3H, 11-H), 2,99 (m, 1H, 2-H), 2.15 (m, 1H, 2-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 169.8 (4-C), 137.3 (7-C), 129.1 (10-C), 128.6 (9-C), 126.0 (8-C), 87.2 (6-C), 78.6 (3-C), 72.4 (5-C), 54.1 (1-C), 39.9 (11-C), 33.5 (3-C). **IR** (neat) ν ($cm^{-1}$): 1689.9 (CO). **HPLC-ESI-MS**: m/z (%) = 298.1 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{13}H_{15}NO_5SNa$ $[M+Na]^+$ 320.0563; found 320.0563. $R_f$ = 0.38 (EtOAc).

**(3*S*,6*R*,7a*R*)-5-Oxo-3-phenylhexahydropyrrolo[1,2-c]oxazol-6-yl-methanesulfonate (TVC-101)**

Alcohol (3*S*,6*R*,7a*R*)-6-hydroxy-3-phenyltetrahydropyrrolo[1,2-c]oxazol-5(3H)-one **28b** (590 mg, 2.69 mmol) yielded product **TVC-101** (670 mg, 84%) as a colourless oil.

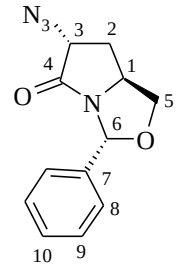


**$^{1}$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 7.45 (m, 2H, 8-H), 7.40-7.38 (m, 3H, 9-H, 10-H), 6.35 (s, 1H, 6-H), 5.59 (dd, *J* = 8.5 Hz, *J* = 10.5 Hz, 1H, 3-H), 4.39 (dd, *J* = 8.5 Hz, 1H, 5-H), 3.66 (t, *J* = 8.2 Hz, 1H, 5-H), 4.05 (t, *J* = 7.1 Hz, 1H, 1-H), 3.32 (s, 3H, 11-H), 2,99 (m, 1H, 2-H), 2.15 (m, 1H, 2-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 169.8 (4-C), 137.3 (7-C), 129.1 (10-C), 128.6 (9-C), 126.0 (8-C), 87.2 (6-C), 78.7 (3-C), 72.4 (5-C), 54.1 (1-C), 39.9 (11-C), 33.3 (3-C). IR (neat) ν ($cm^{-1}$): 1714.9 (CO). **HPLC-ESI-MS**: m/z (%) = 298.1 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{13}H_{15}NO_5SNa$ $[M+Na]^+$ 320.0563; found 320.0563. $R_f$ = 0.26 (CyH/EtOAc, 3/1).

**General procedure for azido formation**

A stirred solution of mesylate **TVC-096** or **TVC-101** (1.0 equiv) and $NaN_3$ (2.0 equiv) in DMSO was warmed to 60 $^{o}$C for 1 h. The reaction mixture was then quenched with EtOAc and the organic phase was washed with $H_2O$, brine solution and dried over $MgSO_4$. Flash chromatography (EtOAc/CyH, 4/1) afforded an oily product.

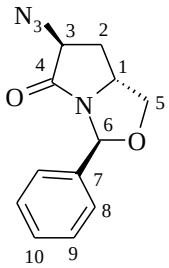
**(3*R*,6*R*,7a*S*)-6-Azido-3-phenyltetrahydropyrrolo[1,2-c]oxazol-5(3H)-one (TVC-104, 29a)**



Mesylate **TVC-096** (383 mg, 1.29 mmol) yielded **29a** (267 mg, 85%).

**$^{1}$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 7.47 (m, 2H, 8-H), 7.41-7.38 (m, 3H, 9-H, 10-H), 6.32 (s, 1H, 6-H), 4.33 (dd, *J* = 3.2 Hz, *J* = 8.3 Hz, 1H, 3-H), 4.28 (d, *J* = 8.0 Hz, 1H, 5-H), 3.5 (t, *J* = 8.0 Hz, 1H, 5-H), 4.23 (t, *J* = 7.0 Hz, 1H, 1-H), 2.3-2.2 (m, 2H, 2-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 172.9 (4-C), 137.7 (7-C), 128.9 (10-C), 128.6 (9-C), 125.9 (8-C), 87.1 (6-C), 71.4 (5-C), 63.3 (3-C), 57.1 (1-C), 30.3 (3-C). **IR** (neat) ν ($cm^{-1}$): 2103.3 ($N_3$), 1697.5 (CO). **HPLC-ESI-MS**: m/z (%) = 245.0 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{12}H_{12}N_4O_2Na$ $[M+Na]^+$ 245.1033; found 267.0855. $R_f$ = 0.25 (CyH/EtOAc, 4/1).

**(3*S*,6*S*,7a*R*)-6-Azido-3-phenyltetrahydropyrrolo[1,2-c]oxazol-5(3H)-one (TVC-102, 29b)**



Mesylate **TVC-101** (587 mg, 1.97 mmol) afforded **29b** (397 mg, 82%).

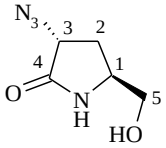
**$^{1}$H-NMR** (600 MHz, $CDCl_3$): $\delta$ = 7.47 (m, 2H, 8-H), 7.41-7.38 (m, 3H, 9-H, 10-H), 6.33 (s, 1H, 6-H), 4.33 (dd, *J* = 3.2 Hz, *J* = 8.3 Hz, 1H, 3-H), 4.3 (dd, *J* = 8.0 Hz, 1H, 5-H), 3.5 (t, *J* = 8.3 Hz, 1H, 5-H), 4.2 (t, *J* = 7.3 Hz, 1H, 1-H), 2.32-2.22 (m, 2H, 2-H). **$^{13}$C-NMR** (150 MHz, $CDCl_3$): $\delta$ = 172.9 (4-C), 137.7 (7-C), 128.8 (10-C), 128.5 (9-C),

125.9 (8-C), 87.1 (6-C), 71.4 (5-C), 63.3 (3-C), 57.1 (1-C), 30.5 (3-C). **IR** (neat) ν ($cm^{-1}$): 2105.3 ($N_3$), 1704.5 (CO). **HPLC-ESI-MS**: m/z (%) = 245.0 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{12}H_{13}N_4O_2$ $[M+H]^+$ 245.1033; found 245.1039. $R_f$ = 0.27 (CyH/EtOAc, 4/1).

**General procedure for ring-opening of oxazolidine**

A solution of 6-azido-3-phenyltetrahydropyrrolo[1,2-c]oxazol-5(3H)-one (1.0 equiv) in THF/$H_2O$ (1/1) was treated with TFA at room temperature for 18 h. The reaction mixture was concentrated under reduced pressure and subjected to silica gel column chromatography using EtOAc (with 1% TEA) as eluent to yield product as a yellow oil.

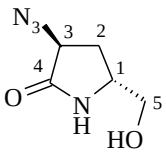
**(3*R*,5*S*)-3-Azido-5-(hydroxymethyl)pyrrolidin-2-one (TVC-110, 30a)**




Starting material **29a** (100 mg, 0.41 mmol) gave **30a** (54.2 mg, 85%).

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 4.3 (d, $J$ = 8.5 Hz, 1H, 3-H), 3.74 (dd, $J$ = 2.8 Hz, $J$ = 11.3 Hz, 1H, 5-H), 3.54 (dd, $J$ = 5.2 Hz, $J$ = 11.3 Hz, 1H, 5-H), 3.8 (m, 1H, 1-H), 2.3 (m, 1H, 2-H), 2.08 (m, 1H, 2-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 175.1 (4-C), 64.9 (5-C), 59.1 (3-C), 53.8 (1-C), 30.2 (2-C). **IR** (neat) ν ($cm^{-1}$): 2105.5 ($N_3$), 1688.5 (CO). **HPLC-ESI-MS**: m/z (%) = 179.0 (100) $[M+Na]^+$. **HR-ESI-MS** (m/z): calcd. for $C_5H_8N_4O_2Na$ $[M+Na]^+$ 179.0539; found 179.0538. $R_f$ = 0.16 (EtOAc, 1% TEA).

**(3*S*,5*R*)-3-Azido-5-(hydroxymethyl)pyrrolidin-2-one (TVC-103, 30b)**




Starting material **29b** (100 mg, 0.41 mmol) afforded **30b** (55.4 mg, 86%).

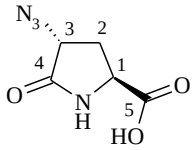
**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 4.3 (t, $J$ = 8.0 Hz, 1H, 3-H), 3.75 (dd, $J$ = 2.6 Hz, $J$ = 11 Hz, 1H, 5-H), 3.52 (dd, $J$ = 5.0 Hz, $J$ = 11 Hz, 1H, 5-H), 3.8 (m, 1H, 1-H), 2.3 (m, 1H, 2-H), 2.1 (m, 1H, 2-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 175.1 (4-C), 64.9 (5-C), 59.1 (3-C), 53.8 (1-C), 30.2 (2-C). **IR** (neat) ν ($cm^{-1}$): 2103.3 ($N_3$), 1683.5 (CO). **HPLC-ESI-MS**: m/z (%) = 179.0 (100) $[M+Na]^+$, 335.1 (15) $[2M+Na]^+$. $R_f$ = 0.17 (EtOAc, 1% TEA).

**General procedure for oxidation reaction**

To a solution of 3-azido-5-(hydroxymethyl)pyrrolidin-2-one (1.0 equiv) and $NaIO_4$ (3.2 equiv) in the mixture of solvent ACN/$CCl_4$/$H_2O$ (7 mL, 1/1/1.5), $RuCl_3.xH_2O$ (2 mol%). was added. The reaction was

stirred at room temperature for 14 h, then *i*PrOH was added. After stirring further 30 min, the reaction mixture was filtered through a Celite pad and the filtrate was concentrated under reduced pressure to dryness. Flash chromatography eluting DCM/MeOH (6/1, 1% AcOH) afforded a solid product.

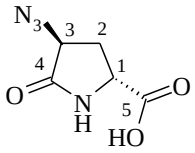
**(2*S*,4*R*)-4-azido-5-oxopyrrolidine-2-carboxylic acid (TVC-111, 31a)**



Alcohol **30a** (46.7 mg, 0.3 mmol) furnished acid **31a** (47 mg, 92%).

**$^1$H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 4.3 (t, $J$ = 8.2 Hz, 1H, 3-H), 4.09 (d, $J$ = 7.2 Hz, 1H, 1-H), 2.52 (m, 1H, 2-H), 2.2 (m, 1H, 2-H). $^{13}$C-NMR (100 MHz, $CD_3OD$): $\delta$ = 176.3 (4-C), 60.2 (3-C), 55.9 (1-C), 33.4 (2-C). **IR** (neat) ν ($cm^{-1}$): 2103.5 ($N_3$), 1687.5 (CO). **HPLC-ESI-MS**: m/z (%) = 193.0 (100) $[M+Na]^+$. **HR-ESI-MS** (m/z): calcd. for $C_5H_6N_4O_3Na$ $[M+Na]^+$ 193.0332; found 193.0332. $R_f$ = 0.23 (DCM/MeOH, 6/1, 0.5% AcOH).

**(2*R*,4*S*)-4-Azido-5-oxopyrrolidine-2-carboxylic acid (TVC-107, 31b)**



Alcohol **30b** (110.0 mg, 0.7 mmol) yielded acid **31b** (105 mg, 88%).

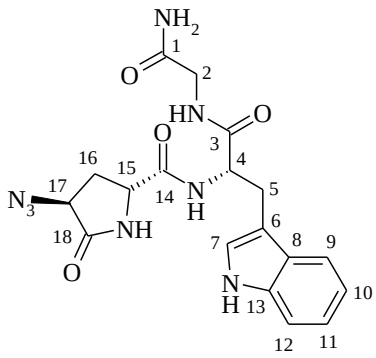
**$^1$H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 4.3 (t, $J$ = 8.2 Hz, 1H, 3-H), 4.14 (d, $J$ = 8.2 Hz, 1H, 1-H), 2.53 (m, 1H, 2-H), 2.2 (m, 1H, 2-H). **$^{13}$C-NMR** (100 MHz, $CD_3OD$): $\delta$ = 176.1 (4-C), 60.0 (3-C), 55.9 (1-C), 33.2 (2-C). **IR** (neat) ν ($cm^{-1}$): 2108.5 ($N_3$), 1695.5 (CO). **HPLC-ESI-MS**: m/z (%) = 193.0 (100) $[M+Na]^+$. **HR-ESI-MS** (m/z): calcd. for $C_5H_6N_4O_3Na$ $[M+Na]^+$ 193.0332; found 193.0332. $R_f$ = 0.42 (DCM/MeOH, 6/1, 1% AcOH).

**General procedure for synthesis of TVC-109 and TVC-112**

To a solution of acid **31a** or **31b** (1.0 equiv), dipeptide TFA*H-Trp-Gly-$NH_2$ (1.1 equiv) and HATU (1.1 equiv) in DMF, DIPEA (4.0 equiv) was added dropwise. The reaction mixture was stirred at room temperature for 18 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography silica gel, using DCM/MeOH (10/1) as eluent, to provide solid products.

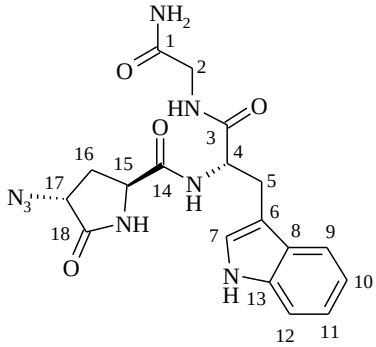
**(2*R*,4*S*)-N-((*S*)-1-((2-Amino-2-oxoethyl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)-4-azido-5-oxopyrrolidine-2-carboxamide (TVC-109, 32b)**

Starting material **31b** (46 mg, 0.27 mmol) afforded tripeptide **TVC-109** (67 mg, 60%).



**$^{1}$H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 7.6 (d, $J$ = 7.8 Hz, 1H, 9-H), 7.36(d, $J$ = 7.8 Hz, 1H, 12-H), 7.14 (s, 1H, 7-H), 7.12 (t, $J$ = 7.4 Hz, 1H, 11-H), 7.04 (t, $J$ = 7.4 Hz, 1H, 10-H), 4.7 (dd, $J$ = 6.5 Hz, $J$ = 8.8 Hz, 1H, 4-H), 4.1 (dd, $J$ = 3.0 Hz, $J$ = 8.4 Hz, 1H, 17-H), 4.0 (t, $J$ = 8.4 Hz, 1H, 15-H), 3.9 (d, $J$ = 17 Hz, 1H, 2-H), 3.7 (d, $J$ = 17 Hz, 1H, 2-H), 3.40 (m, 1H, 5-H), 3.2 (m, 1H, 5-H), 2.0-1.9 (m, 2H, 16-H). **$^{13}$C-NMR** (100 MHz, $CD_3OD$): $\delta$ = 176.8 (18-C), 174.4 (14-C),174.2 (3-C), 174.1 (1-C), 138.0 (13-C), 128.7 (8-C), 124.6 (7-C), 122.6 (11-C), 119.9 (10-C), 119.0 (9-C), 112.4 (12-C), 110.8 (6-C), 59.4 (15-C), 55.8 (4-C), 54.9 (17-C), 43.2 (2-C), 33.2 (16-C), 28.6 (5-C). **IR** (neat) ν ($cm^{-1}$): 2112.5 ($N_3$), 1653.5 (CO). **HPLC-ESI-MS**: m/z (%) = 413.2 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{18}H_{20}N_8O_4Na$ $[M+Na]^+$ 435.1500; found 435.1498. $R_f$ = 0.23 (DCM/MeOH, 6/1).

**(2*S*,4*R*)-N-((*S*)-1-((2-Amino-2-oxoethyl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)-4-azido-5-oxopyrrolidine-2-carboxamide (TVC-112, 32a)**



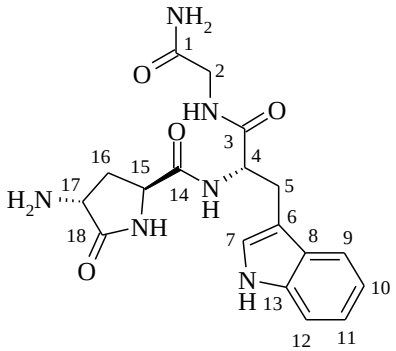
Starting material **31a** (37 mg, 0.21 mmol) yielded peptide **TVC-112** (58.7 mg, 66%).

**$^{1}$H-NMR** (600 MHz, $CD_3OD$): $\delta$ = 7.6 (d, $J$ = 7.8 Hz, 1H, 9-H), 7.36(d, $J$ = 7.8 Hz, 1H, 12-H), 7.14 (s, 1H, 7-H), 7.12 (t, $J$ = 7.4 Hz, 1H, 11-H), 7.04 (t, $J$ = 7.4 Hz, 1H, 10-H), 4.7 (dd, $J$ = 6.5 Hz, $J$ = 8.8 Hz, 1H, 4-H), 4.1 (dd, $J$ = 3.0 Hz, $J$ = 8.4 Hz, 1H, 17-H), 4.0 (t, $J$ = 8.4 Hz, 1H, 15-H), 3.9 (d, $J$ = 17 Hz, 1H, 2-H), 3.7 (d, $J$ = 17 Hz, 1H, 2-H), 3.40 (m, 1H, 5-H), 3.2 (m, 1H, 5-H), 2.0-1.9 (m, 2H, 16-H). **$^{13}$C-NMR** (150 MHz, $CD_3OD$): $\delta$ = 176.7 (18-C), 174.4 (14-C),174.3 (3-C), 174.2 (1-C), 138.0 (13-C), 128.7 (8-C), 124.7 (7-C), 122.6 (11-C), 119.9 (10-C), 119.3 (9-C), 112.5 (12-C), 110.7 (6-C), 59.1 (15-C), 56.2 (4-C), 55.0 (17-C), 43.2 (2-C), 33.2 (16-C), 28.3 (5-C). **IR** (neat) ν ($cm^{-1}$): 2112.5 ($N_3$), 1653.5 (CO). **HPLC-ESI-MS**: m/z (%) = 413.2 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{18}H_{20}N_8O_4Na$ $[M+Na]^+$ 435.1500; found 435.1498. $R_f$ = 0.20 (DCM/MeOH, 7/1).

**General procedure for hydrogenation**

A suspension of azide compound and Pd/C (10 wt%) in MeOH was stirred under hydrogen atmosphere for 16 h. The Pd/C catalyst was removed by filtration through a Celite pad. Removal of the solvent gave the desired product as a solid.

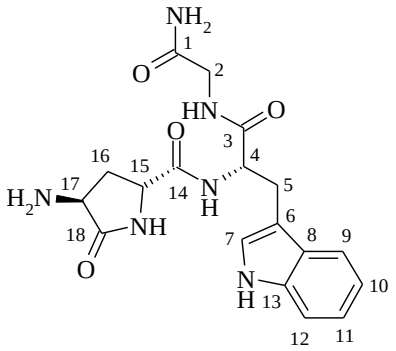
**(2*S*,4*R*)-4-Amino-N-((*S*)-1-((2-amino-2-oxoethyl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)-5-oxo-pyrrolidine-2-carboxamide (TVC-114, 33a)**



Azide **TVC-112** (60 mg, 0.15 mmol) provided amine **33a** (53.8 mg, 96%).

**$^1$H-NMR** (400 MHz, DMSO-$d_6$): $\delta$ = 10.8 (s, 1H, NH), 8.3 (bs. 2H, NH), 8.26 (s, 1H, NH), 7.6 (d, $J$ = 7.6 Hz, 1H, 9-H), 7.3 (d, $J$ = 7.6 Hz, 1H, 12-H), 7.1 (bs, 1H, 7-H), 7.0 (br, 1H, 11-H), 6.9 (t, $J$ = 7.4 Hz, 1H, 10-H), 4.5 (m, 1H, 4-H), 4.0 (d, $J$ = 8.5 Hz, 1H, 15-H), 3.7 (dd, $J$ = 2.0 Hz, $J$ = 17 Hz, 1H, 2-H), 3.6 (dd, $J$ = 2.0 Hz, $J$ = 17 Hz, 1H, 2-H), 3.5 (overlap, 1H, 17-H), 3.17 (m, 1H, 5-H),.3.0 (m, 1H, 5-H). 2.2-2.1 (m, 2H, 16-H). **$^{13}$C-NMR** (100 MHz, DMSO-$d_6$): $\delta$ = 174.3 (18-C), 171.9 (14-C),171.5 (3-C), 170.8 (1-C), 136.0 (13-C), 127.2 (8-C), 125.7 (7-C), 120.8 (11-C), 118.4 (10-C), 118.2 (9-C), 111.3 (12-C), 109.7 (6-C), 54.8 (4-C), 53.8 (15-C), 49.1 (17-C), 41.9 (2-C), 32.2 (16-C), 27.4 (5-C). **IR** (neat) ν ($cm^{-1}$):1673.8 (CO). **HPLC-ESI-MS**: m/z (%) = 387.1 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{18}H_{23}N_6O_4$ $[M+H]^+$ 387.1775; found 387.1774. $R_f$ = 0.11 (DCM/MeOH, 1/1).

**(2*R*,4*S*)-4-Amino-N-((*S*)-1-((2-amino-2-oxoethyl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)-5-oxo-pyrrolidine-2-carboxamide (TVC-115, 33b)**



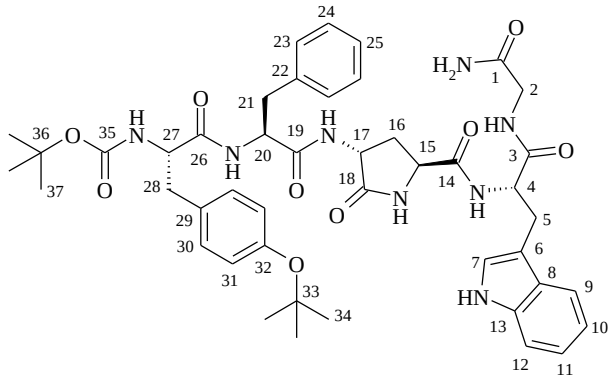
Azide **TVC-109** (90 mg, 0.22 mmol) yielded free amine **33b** (80.4 mg, 95%).

**$^1$H-NMR** (600 MHz, $CD_3OD$): $\delta$ = 7.6 (d, $J$ = 7.6 Hz, 1H, 9-H), 7.3 (d, $J$ = 7.6 Hz, 1H, 12-H), 7.1 (bs, 1H, 7-H), 7.0 (br, 1H, 11-H), 6.9 (t, $J$ = 7.4 Hz, 1H, 10-H), 4.5 (m, 1H, 4-H), 4.0 (d, $J$ = 8.5 Hz, 1H, 15-H), 3.7 (dd, $J$ = 2.0 Hz, $J$ = 17 Hz, 1H, 2-H), 3.6 (dd, $J$ = 2.0 Hz, $J$ = 17 Hz, 1H, 2-H), 3.5 (overlap, 1H, 17-H), 3.17 (m, 1H, 5-H),.3.0 (m, 1H, 5-H). 2.2-2.1 (m, 2H, 16-H). **$^{13}$C-NMR** (150 MHz, $CD_3OD$): $\delta$ = 179.3 (18-C), 174.8 (14-C),174.5 (3-C), 174.6 (1-C), 138.5 (13-C), 128.8 (8-C), 125.1 (7-C), 121.4 (11-C), 120.4 (10-C), 119.7 (9-C), 112.5 (12-C), 111.2 (6-C), 56.5 (4-C), 55.2 (15-C), 51.6 (17-C), 42.6 (2-C), 34.9 (16-C), 29.0 (5-C). **IR** (neat) ν ($cm^{-1}$):1674.4 (CO). **HPLC-ESI-MS**: m/z (%) = 387.1 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{18}H_{23}N_6O_4$ $[M+H]^+$ 387.1775; found 387.1774. $R_f$ = 0.09 (DCM/MeOH, 1/1).

**General procedure for preparation of penta- or hexapeptide**

To a solution of tripeptide H-APy-Trp-Gly-$NH_2$ (1.0 equiv) and dipeptide Boc-Tyr(*t*Bu)-Phe-OH (1.2 equiv) or tripeptide Boc-Tyr(*t*Bu)-Phe-Ser(*t*Bu)-OH (1.2 equiv) in DMF, HATU (1.3 equiv) and DIPEA (5 equiv) were added. The resulting solution was stirred for 20 h at room temperature and the solvent was removed to dryness. The residue was purified by silica gel column chromatography eluting DCM/MeOH (8/1).

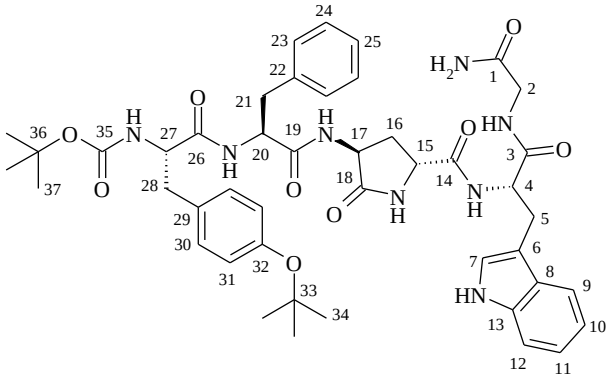
***tert*-Butyl-((*S*)-1-(((*S*)-1-(((3*R*,5*S*)-5-(((*S*)-1-((2-amino-2-oxoethyl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)carbamoyl)-2-oxopyrrolidin-3-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-(4-(*tert*-butoxy)phenyl)-1-oxopropan-2-yl)carbamate (TVC-132)**



Amine **33a** (40 mg, 103.5 µmol) yielded product **TVC-132** (58 mg, 66%) as a solid.

**$^1$H-NMR** (400 MHz, DMSO-$d_6$): $\delta$ = 7.6 (d, $J$ = 8.0 Hz, 1H, 9-H), 7.32 (d, $J$ = 8.0 Hz, 1H, 12-H), 7.25-7.24 (m, 4H, 25-H, 26-H), 7.18 (m, 1H, 25-H), 7.17 (s, 1H, 7-H), 7.06 (overlap, 3H, 11-H, 30-H), 6.98 (t, $J$ = 7.2 Hz, 1H, 10-H), 6.85 (d, $J$ = 8.0 Hz, 2H, 31-H), 4.57 (overlap, 1H, 20-H), 4.54 (t, $J$ = 7.0 Hz, 1H, 4-H), 4.30 (q, $J$ = 9.0 Hz, 1H, 17-H), 4.13 (m, 1H, 27-H), 4.02 (m, 1H, 15-H), 3.7 (dd, $J$ = 5.0 Hz, $J$ = 17.0 Hz, 1H, 2-H), 3.6 (dd, $J$ = 5.0 Hz, $J$ = 17.0 Hz, 1H, 2-H), 3.17-3.0 (m, 2H, 5-H), 2.94 (m, H, 21-H), 2.83 (overlap, 1H, 21-H), 2.82 (m, 1H, 28-H), 2.59 (t, $J$ = 10.0 Hz, 1H, 28-H), 2.11 (t, $J$ = 9.2 Hz, 1H, 16-H), 1.83 (t, $J$ = 9.3 Hz, 1H, 16-H), 1.28 (s, 9H, 34-H), 1.25 (s, 9H, 37-H). **$^{13}$C-NMR** (100 MHz, DMSO-$d_6$): $\delta$ = 176.5 (18-C), 172.0 (3-C), 171.5 (19-C), 171.4 (14-C), 171.2 (26-C), 170.8 (1-C), 155.0 (35-C), 153.3 (32-C), 139.4 (22-C), 136.0 (13-C), 130.2 (30-C), 129.8 (24-C), 128.4 (23-C), 126.8 (25-C), 127.2 (8-C), 123.8 (31-C), 124.2 (7-C), 121.4 (11-C), 118.8 (9-C), 118.2 (10-C), 111.7 (12-C), 109.7 (6-C), 78.0 (33-C), 77.5 (36-C), 56.2 (27-C), 53.9 (4-C), 52.7 (15-C), 48.6 (17-C), 42.4 (2-C), 37.4 (28-C), 33.0 (16-C), 29.0 (37-C), 28.4 (34-C), 27.8 (5-C). **IR** (KBr) ν ($cm^{-1}$): 1643.8 (CO). **HPLC-ESI-MS**: m/z (%) = 870.5 (100) $[M+NH_4]^+$, 853.4 (50) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{45}H_{56}N_8O_9Na$ $[M+Na]^+$ 875.4062; found 875.4065. $R_f$ = 0.23 (DCM/MeOH, 7/1).

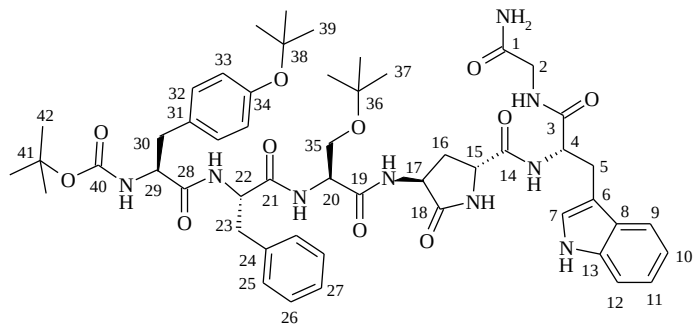
***tert*-Butyl-((*S*)-1-(((*S*)-1-(((3*S*,5*R*)-5-(((*S*)-1-((2-amino-2-oxoethyl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)carbamoyl)-2-oxopyrrolidin-3-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-(4-(*tert*-butoxy)phenyl)-1-oxopropan-2-yl)carbamate (TVC-131)**



Amine **33b** (50 mg, 129.4 µmol) afforded product **TVC-131** (75 mg, 68 %) as a solid.

**$^{1}$H-NMR** (600 MHz, $CD_3OD$): $\delta$ = 7.6 (d, $J$ = 8.0 Hz, 1H, 9-H), 7.35 (d, $J$ = 8.0 Hz, 1H, 12-H), 7.28-7.25 (m, 4H, 23-H, 24-H), 7.21 (m, 1H, 25-H), 7.16 (s, 1H, 7-H), 7.1 (overlap, 2H, 11-H, 30-H), 7.03 (t, $J$ = 7.2 Hz, 1H, 10-H), 6.89 (d, $J$ = 8.0 Hz, 2H, 31-H), 4.68 (t, $J$ = 7.0 Hz, 1H, 4-H), 4.36 (t, $J$ = 9.0 Hz, 1H, 17-H), 4.23 (dd, $J$ = 5.0 Hz, $J$ = 10.0 Hz, 1H, 27-H), 4.19 (dd, $J$ = 2.0 Hz, $J$ = 9.0 Hz, 1H, 15-H), 3.9 (d, $J$ = 17.0 Hz, 1H, 2-H), 3.68 (d, $J$ = 17.0 Hz, 1H, 2-H), 3.36 (m, 1H, 5-H), 3.17 (overlap, 2H, 5-H, 21-H), 2.98 (m, 1H, 21-H), 2.96 (m, 1H, 28-H), 2.67 (m, 1H, 21-H), 2.18 (m, 1H, 16-H), 2.08 (m, 1H, 16-H), 1.36 (s, 9H, 34-H), 1.31 (s, 9H, 37-H). **$^{13}$C-NMR** (150 MHz, $CD_3OD$): $\delta$ = 174.7 (3-C), 174.2 (14-C), 174.1 (26-C), 173.2 (1-C), 157.8 (35-C), 148.6 (32-C), 138.2 (22-C), 138.0 (13-C), 129.4 (30-C), 129.1 (24-C), 128.0 (23-C), 126.2 (25-C), 127.7 (8-C), 123.7 (31-C), 123.1 (7-C), 121.1 (11-C), 119.9 (10-C), 119.2 (9-C), 112.4 (12-C), 110.7 (6-C), 80.8 (33-C), 79.5 (36-C), 56.3 (27-C), 54.0 (4-C), 53.3 (15-C), 49.3 (17-C), 41.8 (2-C), 36.8 (28-C), 31.8 (16-C), 27.8 (34-C), 27.2 (37-C), 27.0 (5-C). **IR** (KBr) ν ($cm^{-1}$): 1639.8 (CO). **HPLC-ESI-MS**: m/z (%) = 870.5 (100) $[M+NH_4]^+$, 853.4 (50) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{45}H_{56}N_8O_9Na$ $[M+Na]^+$ 875.4062; found 875.4065. $R_f$ = 0.25 (DCM/MeOH, 7/1).

***tert*-Butyl-((*S*)-1-(((*S*)-1-(((*S*)-1-(((3*S*,5*R*)-5-(((*S*)-1-((2-amino-2-oxoethyl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)carbamoyl)-2-oxopyrrolidin-3-yl)amino)-3-(*tert*-butoxy)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-(4-(*tert*-butoxy)phenyl)-1-oxopropan-2-yl)carbamate (TVC-118)**

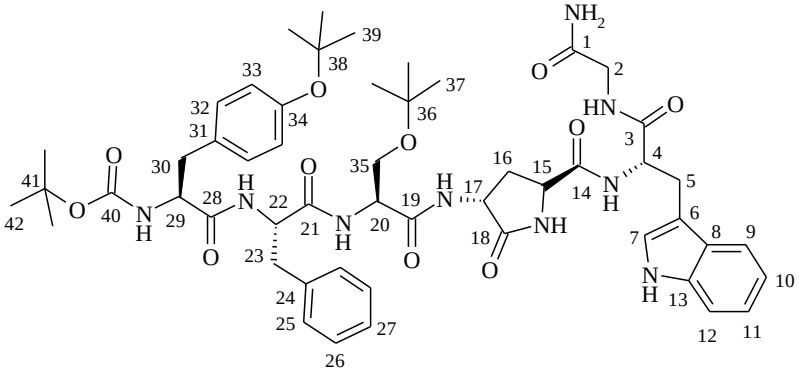


Starting material **33b** (46 mg, 119.0 µmol) provided the solid product **TVC-118** (79 mg, 66%).

**$^{1}$H-NMR** (600 MHz, DMSO-$d_6$): $\delta$ = 10.8 (s, 1H, NH), 8.3 (m, 2H, NH), 8.1 (bs, 2H, NH), 8.0 (d, $J$ = 8.0 Hz, 1H, NH), 7.9 (d, $J$ = 8.0 Hz, 1H, NH), 7.58 (d, $J$ = 8.0 Hz, 1H,

9-H), 7.3 (d, *J* = 8.0 Hz, 1H, 12-H), 7.25-7.23 (m, 5H, 25-H, 26-H, 27-H), 7.16 (overlap, 1H, 7-H), 7.05 (overlap, 2H, 32-H), 7.03 (overlap, 1H, 11-H), 6.96 (t, *J* = 7.0 Hz, 1H, 10-H), 6.84 (t, *J* = 8.0 Hz, 2H, 33-H), 4.65 (m, 1H, 22-H), 4.58 (q, *J* = 8.3 Hz, 1H, 4-H), 4.33 (q, *J* = 5.0 Hz, 1H, 20-H), 4.23 (q, *J* = 9.0 Hz, 1H, 17-H), 4.08 (overlap, 2H, 15-H, 29-H), 3.7(dd, *J* = 6.0 Hz, 17.0 Hz, 1H, 2-C), 3.6 (dd, *J* = 6.0 Hz, 17.0 Hz, 1H, 2-C), 3.46 (bs, 1H, 35-H), 3.17 (dd, *J* = 5.0 Hz, *J* = 14.0 Hz, 1H, 5-H), 3.06 (dd, *J* = 5.0 Hz, *J* = 14.0 Hz, 1H, 23-H), 2.99 (m, 1H, 5-H), 2.8 (m, 2H, 23-H, 30-H), 2.6 (t, *J* = 10.0 Hz, 1H, 30-H), 1.98 (m, 2H, 16-H), 1.28 (s, 9H, 37-H), 1.25 (s, 9H, 37-H), 1.12 (s, 9H, 42-H). **$^{13}$C-NMR** (150 MHz, DMSO-$d_6$): $\delta$ = 177.4 (18-C), 174.3 (3-C), 172. 7 (19-C), 171.3 (21-C), 170.2 (14-C), 169.5 (28-C), 154.9 (40-C), 153.3 (34-C), 137.5 (24-C), 136.0 (13-C), 130.1 (32-C), 129.9 (26-C), 128.6 (25-C), 127.3 (8-C), 126.6 (27-C), 126.1 (31-C), 124.1 (7-C), 123.4 (33-C), 121.3 (11-C), 118.9 (9-C), 118.7 (10-C), 111.8 (12-C), 109.7 (6-C), 77.9 (38-C), 77.5 (36-C), 72.8 (41-C), 62.5 (35-C), 56.3 (29-C), 54.0 (4-C), 53.8 (20-C), 52.8 (15-C), 49.2 (17-C), 42.5 (2-C), 38.1 (23-C), 37.4 (30-C), 32.8 (16-C), 28.4 (37-C), 28.9 (39-C), 27.8 (5-C). **IR** (neat) ν ($cm^{-1}$): 1668.4 (CO). **HPLC-ESI-MS**: m/z (%) = 996.5 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{52}H_{69}N_9O_{11}Na$ $[M+Na]^+$ 1018.5009; found 1018.4996. $R_f$ = 0.26 (DCM/MeOH, 15/1).

***tert*-Butyl-((*S*)-1-(((*S*)-1-(((*S*)-1-(((3*R*,5*R*)-5-(((*S*)-1-((2-amino-2-oxoethyl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)carbamoyl)-2-oxopyrrolidin-3-yl)amino)-3-(*tert*-butoxy)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-(4-(*tert*-butoxy)phenyl)-1-oxopropan-2-yl)carbamate (TVC-116)**



Starting material **33a** (50 mg, 129.4 µmol) furnished the solid product **TVC-116** (127.3 mg, 98%).

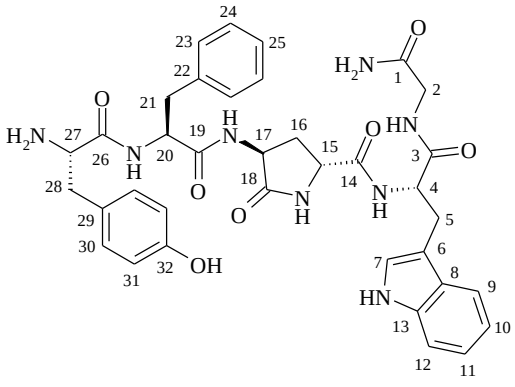
**$^1$H-NMR** (600 MHz, $CD_3OD$): $\delta$ = 7.6 (d, *J* = 7.6 Hz, 1H, 9-H), 7.3 (d, *J* = 7.6 Hz, 1H, 12-H), 7.3-7.2 (m, 5H, 25-H, 26-H, 27-H), 7.16 (s, 1H, 7-H), 7.12 (overlap, 1H, 32-H), 7.1 (overlap, 1H, 11-H), 7.05 (t, *J* = 7.6 Hz, 1H, 10-H), 6.9 (d, *J* = 7.7 Hz, 1H, 33-H), 4.67 (t, *J* = 7.5 Hz, 1H, 22-H), 4.64 (t, *J* = 7.0 Hz, 1H, 29-H), 4.44 (t, *J* = 5.0 Hz, 1H, 20-H), 4.4 (t, *J* = 9.0 Hz, 1H, 17-H), 4.29 (dd, *J* = 5 Hz, *J* = 10.0 Hz, 1H, 4-H), 4.19 (d, *J* = 7.5 Hz, 1H, 15-H), 3.9 (d, *J* = 17 Hz, 1H, 2-H), 3.66 (overlap, 1H, 35-H), 3.57 (t, *J* = 5.0 Hz, 1H, 35-H), 3.6 (d, *J* = 17 Hz, 1H, 2-H), 3.02 (overlap, 1H, 5-H),.2.71 (t, *J* = 11.0 Hz, 1H, 5-H). 2.3-2.2 (m, 2H, 16-H), 1.32 (18H, 39-H, 37-H), 1.28 (s, 9H, 42-H).

**$^{13}$C-NMR** (150 MHz, $CD_3OD$): $\delta$ = 177.4 (18-C), 174.3 (21-C), 174.3 (28-C),174.4 (19-C), 173 (3-C), 172.2 (14-C), 171.6 (1-C), 155.2 (40-C), 148.7 (34-C), 138.1 (13-C), 133.7 (24-C), 130.8 (26-C), 130.4 (31-C), 130.0 (33-C), 129.6 (25-C), 128.6 (27-C), 126.1 (8-C), 124.7 (7-C), 123.0 (32-C), 122.7 (11-C), 119.9 (10-C), 119.3 (9-C), 112.4 (12-C), 110.7 (6-C), 80.7 (38-C), 79.5 (36-C), 61.1 (35-C), 55.8 (4-C), 54.4 (29-C), 53.7 (20-C), 53.4 (15-C), 49.8 (17-C), 41.9 (2-C), 38.9 (23-C), 38.5 (30-C), 32.3 (16-C), 29.2 (37-C, 37-C), 28.7 (42-C), 27.8 (5-C). **IR** (neat) ν ($cm^{-1}$): 1670.3 (CO). **HPLC-ESI-MS**: m/z (%) = 996.5 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{52}H_{69}N_9O_{11}Na$ $[M+Na]^+$ 1018.5009; found 1018.4996. $R_f$ = 0.29 (DCM/MeOH, 10/1).

**General procedure for *N*-Boc and *O*-*t*Bu deprotection**

Peptides were treated with TFA/TIPS/$H_2O$ (2 mL, 95:4:1) at 0 °C for 4-5 h. The reaction mixtures were concentrated under reduced pressure, then purified by silica gel column chromatography using DCM/MeOH (gradient from 8/1 to 5/1, 0.1% TEA). The products were lyophilized in ACN/$H_2O$ to obtain white powders.

**(2*R*,4*S*)-N-((*S*)-1-((2-Amino-2-oxoethyl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)-4-((*S*)-2-((*S*)-2-amino-3-(4-hydroxyphenyl)propanamido)-3-phenylpropanamido)-5-oxo-pyrrolidine-2-carbox-amide (TVC-135, 34a)**

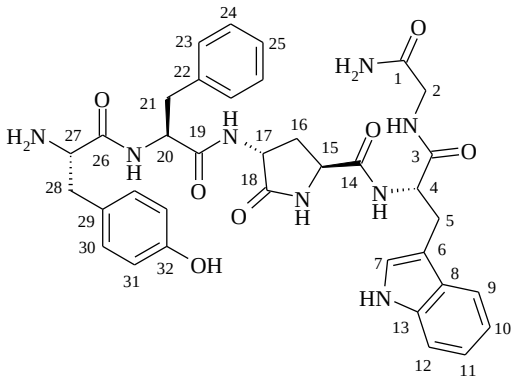


Pentapeptide **TVC-131** (16 mg, 19 µmol) yielded product **34a** (11.2 mg, 86%).

**$^1$H-NMR** (600 MHz, $CD_3OD$): $\delta$ = 7.6 (d, *J* = 7.7 Hz, 1H, 9-H), 7.36 (d, *J* = 8.2 Hz, 1H, 12-H), 7.29-7.28 (m, 4H, 23-H, 24-H), 7.22 (m, 1H, 25-H), 7.16 (s, 1H, 7-H), 7.1 (overlap, 2H, 11-H, 30-H), 7.04 (t, *J* = 7.7 Hz, 1H, 10-H), 6.78 (d, *J* = 9.0 Hz, 2H, 31-H), 4.68 (t, *J* = 7.0 Hz, 1H, 4-H), 4.65 (t, *J* = 6.0 Hz, 1H, 20-H), 4.37 (t, *J* = 9.0 Hz, 1H, 17-H), 4.02 (dd, *J* = 5.0 Hz, *J* = 8.0 Hz, 1H, 27-H), 4.19 (dd, *J* = 2.0 Hz, *J* = 9.0 Hz, 1H, 15-H), 3.9 (d, *J* = 17.0 Hz, 1H, 2-H), 3.66 (d, *J* = 17.0 Hz, 1H, 2-H), 3.34 (m, 1H, 5-H), 3.19 (overlap, 3H, 5-H, 21-H, 28-H), 2.98 (m, 1H, 21-H), 2.93 (m, 1H, 28-H), 2.20- 2.18 (m, 2H, 16-H). **$^{13}$C-NMR** (150 MHz, $CD_3OD$): $\delta$ = 177.5 (18-C), 174.6 (3-C), 174.4 (19-C), 174.2 (14-C), 172.9 (26-C), 169.6 (1-C), 158.3 (32-C), 138.1 (22-C), 138.0 (13-C), 130.3 (30-C), 128.8 (24-C), 128.0 (23-C), 126.4 (25-C), 128.7 (8-C), 123.0 (7-C), 121.1 (11-C), 118.4 (10-C), 117.4 (9-C), 112.4 (12-C), 115.4 (31-C), 110.7 (6-C), 54.8 (4-

C), 54.1 (27-C), 53.3 (15-C), 49.3 (17-C), 41.7 (2-C), 36.1 (28-C), 31.7 (16-C), 27.1 (5-C). **HPLC-ESI-MS**: m/z (%) = 697.3 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{36}H_{41}N_8O_7$ $[M+H]^+$ 697.3093; found 697.3094.

**(2*S*,4*R*)-N-((*S*)-1-((2-Amino-2-oxoethyl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)-4-((*S*)-2-((*S*)-2-amino-3-(4-hydroxyphenyl)propanamido)-3-phenylpropanamido)-5-oxopyrro-lidine-2-carbox-amide (TVC-134, 34b)**



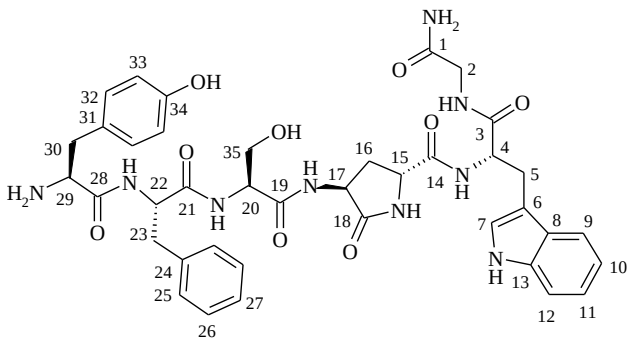
Peptide **TVC-132** (16.5 mg, 19.3 µmol) provided **34b** (12.4 mg, 92%).

**$^1$H-NMR** (600 MHz, $CD_3OD$): $\delta$ = 7.6 (d, *J* = 7.8 Hz, 1H, 9-H), 7.35 (d, *J* = 8.0 Hz, 1H, 12-H), 7.27-7.24 (m, 4H, 23-H, 24-H), 7.2 (m, 1H, 25-H), 7.15 (s, 1H, 7-H), 7.09 (overlap, 2H, 11-H, 30-H), 7.03 (t, *J* = 7.3 Hz, 1H, 10-H), 6.78 (d, *J* = 9.0 Hz, 2H, 31-H), 4.62 (t, *J* = 7.0 Hz, 1H, 4-H), 4.63 (t, *J* = 7.0 Hz, 1H, 20-H), 4.35 (t, *J* = 9.2 Hz, 1H, 17-H), 4.02 (d, *J* = 7.0 Hz, 1H, 27-H), 4.1 (d, *J* = 8.0 Hz, 1H, 15-H), 3.9 (d, *J* = 17.0 Hz, 1H, 2-H), 3.63 (d, *J* = 17.0 Hz, 1H, 2-H), 3.32 (m, 1H, 5-H), 3.18 (t, *J* = 8.0 Hz, 1H, 5-H), 3.13 (dd, *J* = 4 Hz, *J* = 13.4 Hz, 1H, 28-H), 3.07 (m, 1H, 21-H), 2.98 (t, *J* = 5.0 Hz, 1H, 21-H), 2.93 (dd, *J* = 4 Hz, *J* = 13.4 Hz, 1H, 28-H), 2.18 (t, *J* = 10.0 Hz, 1H, 16-H), 1.94 (m, 1H, 16-H). $^{13}$C-NMR (150 MHz, $CD_3OD$): $\delta$ = 177.4 (18-C), 174.7 (3-C), 174.5 (19-C), 174.2 (14-C), 172.6 (26-C), 169.4 (1-C), 158.2 (32-C), 138.0 (22-C), 137.9 (13-C), 130.2 (30-C), 129.0 (24-C), 128.1 (23-C), 126.4 (25-C), 127.7 (8-C), 123.2 (7-C), 121.1 (11-C), 118.4 (10-C), 117.9 (9-C), 112.4 (12-C), 116.9 (31-C), 110.6 (6-C), 54.8 (4-C), 54.3 (27-C), 53.3 (15-C), 48.9 (17-C), 41.8 (2-C), 36.2 (28-C), 32.1 (16-C), 27.0 (5-C). **HPLC-ESI-MS**: m/z (%) = 697.3 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{36}H_{41}N_8O_7$ $[M+H]^+$ 697.3093; found 697.3094.

**(2*R*,4*S*)-N-((*S*)-1-((2-Amino-2-oxoethyl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)-4-((*S*)-2-((*S*)-2-((*S*)-2-amino-3-(4-hydroxyphenyl)propanamido)-3-phenylpropanamido)-3-hydroxypropanamido)-5-oxopyrrolidine-2-carboxamide (TVC-119, 35a)**

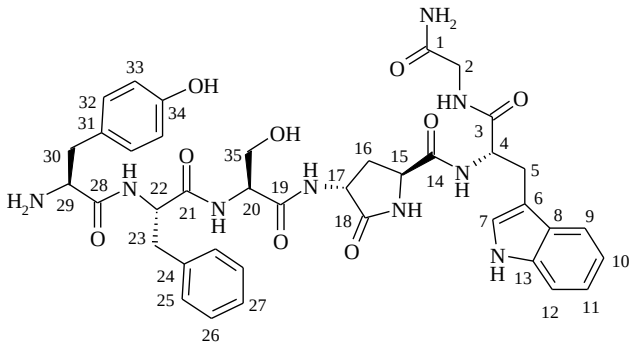
Peptide **TVC-118** (30 mg, 30 µmol) yielded product **35a** (19 mg, 81%).

**$^1$H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 7.6 (d, *J* = 8.1 Hz, 1H, 9-H), 7.35 (d, *J* = 7 Hz, 1H, 12-H), 7.28-7.25 (m, 4H, 25-H, 26-H), 7.2 (m, 1H, 27-H), 7.16 (s, 1H, 7-H), 7.1 (d, *J* = 8.0 Hz, 2H, 32-H), 7.1 (overlap, 1H, 11-H), 7.05 (t, *J* = 7.0 Hz, 1H, 10-H), 6.8 (d, *J* = 8.0 Hz, 2H, 33-H), 4.74 (dd, *J* = 6.0 Hz, *J* = 9.0 Hz,



1H, 22-H), 4.68 (t, *J* = 8.0 Hz, 1H, 4-H), 4.41 (overlap, 1H, 17-H, 20-H), 4.18 (d, *J* = 7.3 Hz, 1H, 15-H), 4.02 (dd, *J* = 5.0 Hz, *J* = 8.4 Hz, 29-H), 3.9 (d, *J* = 17.0 Hz, 1H, 2-H), 3.83-3.78 (m, 2H, 35-H), 3.68 (d, *J* = 17.0 Hz, 1H, 2-H), 3.35 (d, *J* = 6.0 Hz, 1H, 5-H), 3.18 (m, 1H, 5-H), 3.22 (overlap, 1H, 23-H), 3.0 (m, 1H, 23-H), 3.18 (overlap, 1H, 30-H), 2.92 (m, 1H, 30-H), 2.2-2.16 (m, 2H, 16-H). **$^{13}$C-NMR** (100 MHz, $CD_3OD$): $\delta$ = 177.8 (18-C), 174.5 (3-C), 174.1 (21-C), 172.9 (19-C), 172.1(14-C) , 172.2 (28-C), 169.9 (1-C), 158.3 (34-C), 138.2 (24-C), 138.0 (13-C), 131.6 (31-C), 130.3 (32-C), 128.9 (26-C), 128.3 (25-C), 127.9 (8-C), 126.5 (27-C), 123.2 (7-C), 121.3 (11-C), 118.5 (10-C), 118.0 (9-C), 115.6 (33-C), 112.0 (12-C), 110.7 (6-C), 61.7 (35-C), 55.3 (20-C), 55.0 (22-C), 54.7 (4-C), 54.3 (29-C), 53.4 (15-C), 49.5 (17-C), 41.8 (2-C), 37.2 (23-C), 36.2 (30-C), 32.0 (16-C), 27.2 (5-C). **HPLC-ESI-MS**: m/z (%) = 784.2 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{39}H_{46}N_9O_9$ $[M+H]^+$ 784.3413; found 784.3410.

**(2*S*,4*R*)-N-((*S*)-1-((2-Amino-2-oxoethyl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)-4-((*S*)-2-((*S*)-2-((*S*)-2-amino-3-(4-hydroxyphenyl)propanamido)-3-phenylpropanamido)-3-hydroxypropanamido)-5-oxopyrrolidine-2-carboxamide (TVC-117, 35b)**



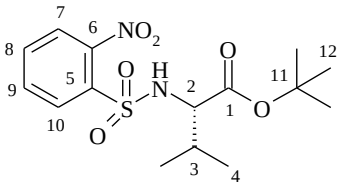
Peptide **TVC-116** (40 mg, 0.04 mmol) afforded **35b** (30 mg, 95%).

**$^1$H-NMR** (600 MHz, $CD_3OD$): $\delta$ = 7.61 (d, *J* = 8.1 Hz, 1H, 9-H), 7.36 (d, *J* = 7 Hz, 1H, 12-H), 7.3-7.25 (m, 4H, 25-H, 26-H), 7.23 (m, 1H, 27-H), 7.16 (s, 1H, 7-H), 7.12 (d, *J* = 8.0 Hz, 2H, 32-H), 7.11 (overlap, 1H, 11-H), 7.05 (t, *J* = 7.0 Hz, 1H, 10-H), 6.8 (d, *J* = 8.0 Hz, 2H, 33-H), 4.7 (dd, *J* = 6.0 Hz, *J* = 9.0 Hz, 1H, 22-H), 4.64 (t, *J* = 8.0 Hz, 1H, 4-H), 4.43 (t, *J* = 5.0 Hz, 1H, 20-H), 4.18 (dd, *J* = 1.2 Hz, *J* = 9.0 Hz, 1H, 15-H), 4.02 (dd, *J* = 5.0 Hz, *J* = 8.4 Hz, 29-H), 3.9 (d, *J* = 17.0 Hz, 1H, 2-H), 3.89 (dd, *J* = 5.0 Hz, *J* = 11.0 Hz, 1H, 35-H), 3.76 (overlap, 1H, 35-H), 3.6 (d, *J* = 17.0 Hz, 1H, 2-H), 3.34 (m, 1H, 5-H), 3.2 (m, 1H, 5-H), 3.22 (overlap, 1H, 23-H), 3.0 (m, 1H, 23-H), 3.19 (overlap, 1H, 30-H), 2.94 (m, 1H, 30-H), 2.32 (m, 1H, 16-H), 2.25 (m, 1H, 16-H). **$^{13}$C-NMR** (150 MHz, $CD_3OD$): $\delta$ = 177.7 (18-C), 174.7 (3-C), 174.4 (19-C), 174.3 (21-C), 173.0 (14-C) , 172.2 (28-C), 171.0 (1-C), 158.1 (34-C), 138.1 (13-C), 138.0 (24-C), 130.1 (32-C),

129.6 (31-C), 128.6 (26-C), 128.1 (25-C), 127.9 (8-C), 126.5 (27-C), 123.7 (7-C), 121.0 (11-C), 118.3 (10-C), 117.6 (9-C), 115.4 (33-C), 112.4 (12-C), 110.7 (6-C), 61.5 (35-C), 55.1 (22-C), 55.0 (20-C), 54.8 (4-C), 54.2 (29-C), 53.4 (15-C), 49.4 (17-C), 41.9 (2-C), 37.0 (23-C), 36.2 (30-C), 32.0 (16-C), 26.8 (5-C). **HPLC-ESI-MS**: m/z (%) = 784.2 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{39}H_{46}N_9O_9$ $[M+H]^+$ 784.3413; found 784.3410.

### 3.7 Synthesis of 2-oxo-piperazine containing helicokinin I analogue TVC-051 (47)

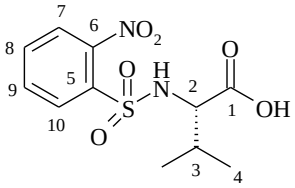
#### *tert*-Butyl-(2*S*)-3-methyl-2-(2-nitrobenzenesulfonamido)butanoate (TVC-002, 37)



To a stirred solution of L-valine-*tert*-butylester hydrochloride (2.20 g, 10.49 mmol) and TEA (2.9 ml, 20.98 mmol) in DCM (15 mL) at 0 °C, a solution of 2-nitrophenylsulfonyl chloride (2.79 g, 12.59 mmol) in DCM (20 mL) was added dropwise. After stirring at room temperature for 20 h, DCM (100 mL) was added. The organic phase was washed with $H_2O$ (2x10 mL), brine solution and dried over $Na_2SO_4$. Removal of the solvent and flash chromatography (eluent: CyH/EtOAc, 20/1) provided **37** (3.7 g, 98%) as a brown solid.

**$^1$H-NMR** (600 MHz, $CDCl_3$): $\delta$ = 8.07 (m, 1H, 7-H), 7.91 (m, 1H, 10-H), 7.70 (m, 2H, 8-H, 9-H), 6.05 (d, $J$ = 9.79 Hz, NH), 3.88 (dd, $J$ = 9.82 Hz, $J$ = 5.1 Hz, 1H, 2-H), 2.13 (m, 1H, 3-H), 1.4 (s, 9H, 12-H), 1.03 (d, $J$ = 6.82 Hz, 3H, 4-H), 0.94 (d, $J$ = 6.82 Hz, 3H, 4'-H). **$^{13}$C-NMR** (150 MHz, $CDCl_3$): $\delta$ = 169.7 (C-1), 147.8 (C-6), 134.4 (C-5), 133.4 (C-8), 132.8 (C-9), 130.5 (C-10), 124.5 (C-7), 82.4 (C-11), 62.7 (C-2), 31.6, 31.3 (C-3), 27.6 (C-12), 17.3 (C-4). **IR** (KBr) ν ($cm^{-1}$): 3340.5 (NH), 1712.4 (CO). **HPLC-ESI-MS**: m/z (%) = 257.0 (100), 376.1 (71) $[M+NH_4]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{15}H_{22}N_2O_6SNa$ $[M+Na]^+$ 381.1096; found 381.1091. $R_f$ = 0.4 (CyH/EtOAc, 15/1)

#### (2*S*)-3-Methyl-2-(2-nitrobenzenesulfonamido)butanoic acid (TVC-003, 38)

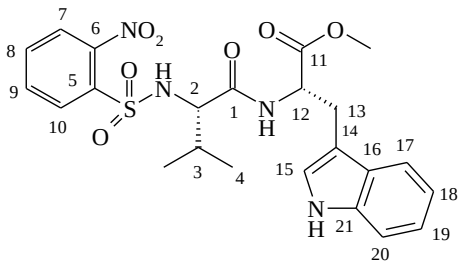


A solution of (*S*)-*tert*-butyl-3-methyl-2-(2-nitrophenylsulfonamido)butanoate **37** (100 mg, 0.28 mmol) in TFA/DCM (2 mL, 1/1) was stirred at 0 °C for 3 h. The solvent was evaporated under diminished pressure. The crude product was purified by silica gel column chromatography (EtOAc, 1% AcOH) to afford acid **38** (74 mg, 87%) as a yellow solid.

**$^1$H-NMR** (600 MHz, $CDCl_3$): $\delta$ = 8.10 (m, 1H, 7-H), 7.94 (m, 1H, 10-H), 7.75 (m, 2H, 8-H. 9-H), 6.04 (d, $J$ = 9.67 Hz, 1H, NH), 4.08 (dd, $J$ = 9.46 Hz, $J$ = 4.78 Hz, 1H, 2-H), 2.25 (m, 1H, 3-H), 1.06 (d, $J$ =

6.83 Hz, 3H, 4-H), 0.96 (d, *J* = 6.83 Hz, 3H, 4'-H). **$^{13}$C-NMR** (150 MHz, $CDCl_3$): $\delta$ = 175.6 (1-C), 147.6 (6-C), 134 (5-C), 133.8 (8-C), 132.9 (9-C), 130.3 (10-C), 125.5 (7-C), 61.7 (2-C), 31.3 (3-C), 18.9 (4-C), 17.2 (4'-C). **IR** (KBr) ν ($cm^{-1}$): 3350.2 (NH), 1713.9 (CO). **HPLC-ESI-MS**: m/z (%) = 320.1 (100) $[M+NH_4]^+$, 303.1 (24) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{11}H_{14}N_2O_6SNa$ $[M+Na]^+$ 325.0470, found 325.0465. $R_f$ = 0.21 (EtOAc/AcOH, 10/0.1).

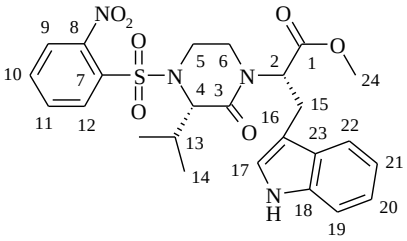
**Methyl-(2*S*)-3-(1H-indol-3-yl)-2-[(2*S*)-3-methyl-2-(2-nitrobenzenesulfonamido)butanamido] propanoate (TVC-005, 39)**



To a solution of (2*S*)-3-methyl-2-(2-nitrophenyl-sulfonamido)-butanoic acid **38** (70 mg, 0.23 mmol), L-trytophanmethyl ester (60.6 mg, 0.28 mmol) and TBTU (89.2 mg, 0.28 mmol) in DCM (5 mL), DIPEA (0.11 mL, 0.69 mmol) was added dropwise. After stirring at room temperature for 24 h, the solvent was removed under reduced pressure to dryness. The residue was dissolved in EtOAc (150 mL), washed with $H_2O$ (2x10 mL), brine solution and dried over $MgSO_4$. Evaporation of the solvent and flash chromatography (eluent: CyH/EtOAc, 5/2) afforded product **39** (106 mg, 91%) as a slightly yellow solid.

**$^1$H-NMR** (600 MHz, $CDCl_3$): $\delta$ = 8.16 (s, 1H, NH), 7.91 (dd, *J* = 7.89 Hz, *J* = 1.34 Hz, 1H, 7-H), 7.75 (dd, *J* = 8.0 Hz, *J* = 1.15 Hz, 1H, 10-H), 7.56 (dt, *J* = 7.7 Hz, *J* = 1.3 Hz, 1H, 9-H), 7.48 (d, *J* = 7.90 Hz, 1H, 17-H), 7.42 (dt, *J* = 7.8 Hz, *J* = 1.3 Hz, 1H, 8-H), 7.38 (d, *J* = 8.4 Hz, 1H, 20-H), 7.23 (dt, *J* = 7.2 Hz, *J* = 1.0 Hz, 1H, 4-H), 7.15 (dt, *J* = 7.9 Hz, *J* = 1.0 Hz, 1H, 18-H), 7.03 (d, *J* = 2.30 Hz, 1H, 15-H), 4.67 (m, 1H, 12-H), 3.77 (dd, *J* = 8.45 Hz, *J* = 5.17 Hz, 1H, 2-H), 3.65 (s, 3H, $OCH_3$), 3.15 (d, *J* = 5.80 Hz, 2H, 13-H), 2.10 (m, 1H, 3-H), 0.85 (d, *J* = 6.81 Hz, 3H, 4-H), 0.77 (d, *J* =6.81 Hz, 4'-H). **$^{13}$C-NMR** (150 MHz, $CDCl_3$): $\delta$ = 171.7 (1-C), 169.6 (11-C), 147 (6-C), 136.2 (21-C), 133.8 (5-C), 133.6 (9-C), 132.7 (8-C), 130.2 (10-C), 127.3 (16-C), 125.4 (7-C), 122.9 (15-C), 122.4 (4-C), 114.7 (18-C), 118.4 (17-C), 111.4 (20-C), 109.6 (14-C), 63.02 (2-C), 52.8 (-$OCH_3$), 52.4 (12-C), 31.25 (3-C), 27.6 (13-C), 24.0 (4-C), 17.1 (4'-C). **IR** (KBr) ν ($cm^{-1}$): 3358 (NH), 1735.6, 1669.9 (CO). **HPLC-ESI-MS**: m/z (%) = 503.2 (100) $[M+H]^+$, 525.2 $[M+Na]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{23}H_{26}N_4O_7SNa$ $[M+Na]^+$ 525.1414; found 525.1414. $R_f$ = 0.15 (CyH/EtOAc, 3/2).

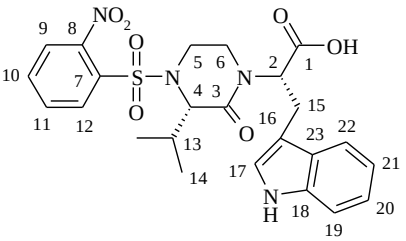
**Methyl-(2*S*)-3-(1H-indol-3-yl)-2-[(3*S*)-4-(2-nitrobenzenesulfonyl)-2-oxo-3-(propan-2-yl)piperazin-1-yl]propanoate (TVC-010, 40)**



A suspension of **39** (110 mg, 0.22 mmol), $K_2CO_3$ (136 mg, 0.98 mmol) and 1,2-dibromoethane (0.4 mL, 2.4 mmol) in DMF (5 mL) was stirred at 60 $^oC$ for 48 h. After that time EtOAc (50 mL) was added. The resulting solution was washed with $H_2O$, brine solution and dried over $Na_2SO_4$. The solvent was evaporated *in vacuo*. Flash chromatography (EtOAc/CyH, 3/2) provided a solid product **40** (83 mg, 74%).

**$^1$H-NMR** (600 MHz, $CDCl_3$): $\delta$ = 8.2 (s, 1H, NH), 7.96 (dd, *J* = 7.69 Hz, *J* = 1.55 Hz, 1H, 9-H), 7.68 (m, 1H, 12-H), 6.67 (m, 1H, 11-H), 7.61 (dd, *J* = 7.5 Hz, *J* = 1.7 Hz, 1H, 10-H), 7.52 (d, *J* = 7.9 Hz, 1H, 22-H), 7.33 (d, *J* = 8.2 Hz, 1H, 4-H), 7.17 (dt, *J* = 7.5 Hz, *J* = 1 Hz, 1H, 20-H), 7.09 (dt, *J* = 7.6 Hz, *J* = 0.8 Hz, 1H, 21-H), 7.02 (d, *J* = 2.2 Hz, 1H, 17-H), 5.18 (dd, *J* = 11 Hz, *J* = 5 Hz, 1H, 2-H), 4.08 (dd, *J* = 7.8 Hz, *J* = 1.2 Hz, 1H, 4-H), 3.77 (dt, *J* = 3.8 Hz, *J* = 1 Hz, 1H, 6-H), 3.64 (s, 3H, 24-H) 3.44 (dd, *J* = 4.9 Hz, *J* = 0.75 Hz, 1H, 15-H), 3.33 (m, 1H, 6-H), 3.20 (dd, *J* = 15.5 Hz, *J* = 11.2 Hz, 1H, 15-H), 3.14 (dd, *J* = 7 Hz, *J* = 4.7 Hz, 2H, 5-H), 1.83 (sept, *J* = 7 Hz, 1H, 13-H), 0.72 (dd, *J* = 6.8 Hz, 6H, 14-H). **$^{13}$C-NMR** (150 MHz, $CDCl_3$): $\delta$ = 170.5 (1-C), 167.1 (3-C), 148.1 (8-C), 136.2 (18-C), 133.8 (11-C), 132.9 (7-C), 131.9 (10-C), 130.8 (12-C), 126.9 (23-C), 124.2 (9-C), 122.8 (17-C), 122.2 (20-C), 14.6 (21-C), 118.1 (22-C), 111.4 (4-C), 110.5 (16-C), 64.5 (4-C), 57.5 (2-C), 52.1(24-C), 43.2 (5-C), 40.6 (6-C), 31.4 (13-C), 24.1 (15-C), 14.5 (14-C), 14.3 (14'-C). **IR** (KBr) ν ($cm^{-1}$): 3411.3 (NH), 1739.6, 1652.5 (CO). **HPLC-ESI-MS**: m/z (%) = 529.2 $[M+H]^+$, 546.2 $[M+NH_4]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{25}H_{28}N_4O_7SNa$ $[M+Na]^+$ 551.1576; found 551.1571. $R_f$ = 0.11 (EtOAc/CyH, 3/2).

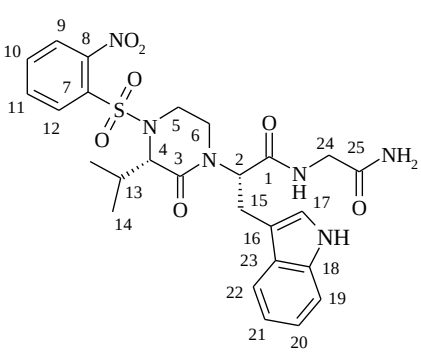
**(2*S*)-3-(1H-Indol-3-yl)-2-[(3*S*)-4-(2-nitrobenzenesulfonyl)-2-oxo-3-(propan-2-yl)piperazin-1-yl]-propanoic acid (TVC-018, 41)**



Methyl ester **40** (1.5 g, 2.84 mmol) in THF (30 mL) was treated with a solution of 4% LiOH (28.38 mmol). After stirring at room temperature for 3 h, the reaction mixture was acidified with 1N HCl to pH ≈ 4 and extracted with EtOAc (3x100 mL). The combined organic phases were washed with $H_2O$ (2x10 mL), brine solution and dried over $Na_2SO_4$. Removal of the solvent under reduced pressure gave pure solid product **41** (1.42 g, 97%).

**$^1$H-NMR** (600 MHz, DMSO-$d_6$): $\delta$ = 10.77 (s, 1H, NH), 7.96 (dd, *J* = 7.96 Hz, *J* = 1.12 Hz, 1H, 9-H), 7.91 (dd, *J* = 7.95 Hz, *J* = 1.16 Hz, 1H, 12-H), 7.86 (dt, *J* = 7.6 Hz, *J* = 1.23 Hz, 1H, 11H) ,7.77 (dt, *J* = 7.85 Hz, *J* = 1.3 Hz, 1H, 10-H), 7.48 (d, *J* = 7.9 Hz, 1H, 22-H), 7.3 (d, *J* = 8.1 Hz, 1H, 4-H), 7.07 (d, *J* = 2,1 Hz, 1H, 17-H), 7.04 (dt, *J* = 7.96 Hz, *J* = 0.87 Hz, 1H, 20-H), 6.95 (dt, *J* = 7.8 Hz, *J* = 0.74 Hz, 1H, 21-H), 5.20 (dd, *J* = 11.3 Hz, *J* = 4.7 Hz, 1H, 2-H), 3.84 (d, *J* = 7.65 Hz, 1H, 4-H), 3.68 (m, 1H, 6-H), 3.35 (m, 1H, 6-H), 3.34 (m, 1H, 5-H), 3.25 (dd, *J* = 15.3 Hz, *J* = 4.5 Hz, 1H, 15-H), 3.1 (m, 1H, 15-H), 3.08 (m, 1H, 5-H), 1.70 (sept, *J* = 7 Hz, 1H, 13-H), 0.6 (d, *J* = 6.8 Hz, 3H, 14-H), 0.51 (d, *J* = 6.7 Hz, 3H, 14'-H). **$^{13}$C-NMR** (150 MHz, DMSO-$d_6$): $\delta$ = 171.4 (1-C), 165.0 (2-C), 147.0 (8-C), 136.0 (18-C), 134.9 (11-C), 132.4 (10-C), 130.9 (7-C), 130 (12-C), 126.7 (23-C), 124.2 (9-C), 123.3 (17-C), 120.8 (20-C), 118.2 (21-C), 117.9 (22-C), 111.2 (4-C), 109.4 (16-C), 63.6 (4-C), 56.0 (2-C), 40.8 (5-C), 40.7 (6-C), 30.8 (13-C), 23.6 (15-C), 19.8 (14-C), 18.9 (14'-C). **IR** (KBr) ν ($cm^{-1}$): 3418.9 (NH), 1716.9, 1646.4 (CO). **HPLC-ESI-MS**: m/z (%) = 515.2 (100) $[M+H]^+$, 532.2 (10) $[M+NH_4]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{24}H_{26}N_4O_7SNa$ $[M+Na]^+$ 537.1412; found 537.1414. $R_f$= 0.22 (DCM/MeOH. 5/1).

**(2*S*)-N-(Carbamoylmethyl)-3-(1H-indol-3-yl)-2-[(3*S*)-4-(2-nitrobenzenesulfonyl)-2-oxo-3-(propan-2-yl)piperazin-1-yl]propanamide (TVC-026, 42)**

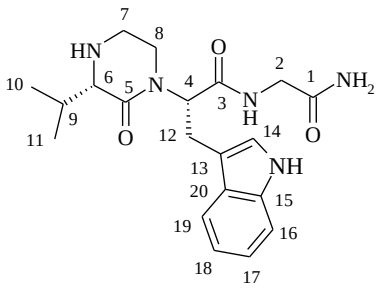



To a solution of (*S*)-3-(1H-indol-3-yl)-2-((*S*)-3-isopropyl-4-((2-nitrophenyl)sulfonyl)-2-oxopiperazin-1-yl)propanoic acid **41** (730 mg, 1.42 mmol), H-Gly-$NH_2$*HCl (235 mg, 2.13 mmol), TBTU (592 mg, 1.84 mmol) and HOBt*$H_2O$ (282 mg, 1.84 mmol) in DMF (10 mL), DIPEA (0.7 mL, 4.26 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 18 h, then diluted with EtOAc (250 mL). The organic layer was washed with $H_2O$ (3x15 mL), brine solution (2x15 mL) and dried over $MgSO_4$. Flash chromatography (EtOAc/EtOH, 10/1) yielded product **42** (580 mg, 72%) as a solid.

**$^1$H-NMR** (600 MHz, $CDCl_3$): $\delta$ = 8.25 (s, 1H, NH), 8.03 (d, *J* = 8.1 Hz, 1H, 9-H), 7.7 (m, 1H, 11-H), 7.67 (overlap, 1H, 17-H), 7.6 (m, 1H, 10-H), 7.52 (d, *J* = 7.7 Hz, 1H, 22-H), 7.33 (d, *J* = 8.1 Hz, 1H, 4-H), 7.18 (t, *J* = 7.1 Hz, 1H, 20-H), 7.10 (t, *J* = 7.1 Hz, 1H, 21-H), 7.01 (d, *J* = 2.2 Hz, 1H, 12-H), 5.33 (q, *J* = 7.3 Hz, *J* = 8.9 Hz, 1H, 2-H), 4.0 (dd, *J* = 1.1 Hz, *J* = 7.8 Hz, 1H, 4-H), 3.78-3.73 (m, 2H, 5-H), 3.82 (m, 1H, 6-H), 3.36 (m, 1H, 6-H), 3.32 (m, 2H, 15-H), 3.27 (m, 2H, 24-H), 1.97-1.92 (m, 1H, 13-H), 0.85 (d, *J* = 6.7 Hz, 3H, 14-H), 0.71 (d, *J* = 6.8 Hz, 3H, 14-H). **$^{13}$C-NMR** (150 MHz, $CDCl_3$): $\delta$ = 174.0 (25-C), 172.4 (1-C), 169.8 (3-C), 149.5 (8-C), 138.2 (7-C), 135.8 (12-C), 133.5 (11-C), 133.4 (18-C), 132.2 (9-C), 128.4 (23-C), 125.9 (10-C), 124.5 (17-C), 122.6 (20-C), 114.9 (21-C), 114.2 (22-C), 112.4 (4-C),

110.6 (16-C), 65.8 (4-C), 58.6 (2-C), 43.3 (5-C), 43.2 (24-C), 41.7 (6-C), 32.6 (13-C), 25.2 (15-C), 20.02 (14-C). **IR** (KBr) ν ($cm^{-1}$): 3392.0 (NH), 1733.5 (CO). **HPLC-ESI-MS**: m/z (%) = 571.2 (100) $[M+H]^+$, 588.2 (27) $[M+NH_4]^+$, 593.2 (28) $[M+Na]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{26}H_{30}N_6O_7SNa$ $[M+Na]^+$ 593.1794; found 593.1789. $R_f$ = 0.32 (DCM/MeOH, 20/1).

**(2*S*)-N-(Carbamoylmethyl)-3-(1H-indol-3-yl)-2-[(3*S*)-2-oxo-3-(propan-2-yl)piperazin-1-yl]propanamide (TVC-032, 43)**



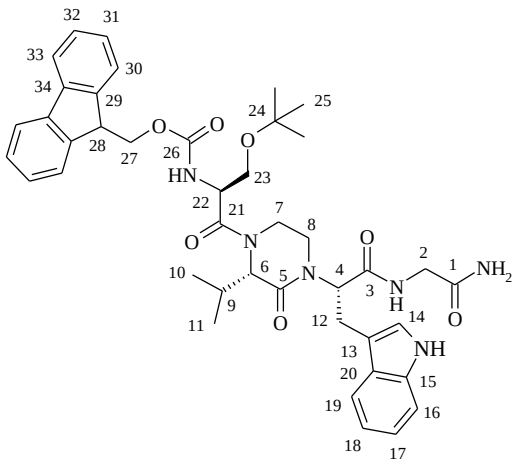

A suspension of compound **42** (20 mg, 0.04 mmol) and $K_2CO_3$ (14.5 mg, 0.11 mmol) in ACN (3 mL) was treated with thiophenol (8 mg, 0.07 mmol) for 4 h at room temperature. The reaction mixture was then filtered through a Celite pad and washed with EtOAc. The filtrate was concentrated and the residue was purified by silica gel column chromatography eluting DCM/MeOH (5/1) to afford desired product **43** (13 mg, 96%) as a solid.

**$^1$H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 7.52 (d, *J* = 7.8 Hz, 1H, 4-H), 7.32 (d, *J* = 8.0 Hz, 1H, 16-H), 7.11 (s, 1H, 14-H), 7.08 (dt, *J* = 1.1 Hz, *J* = 8.0 Hz, 1H, 18-H), 7.01 (dt, *J* = 1 Hz, *J* = 8 Hz, 1H, 17-H), 5.18 (dd, 5.9 Hz, 10 Hz, 1H, 4-H), 3.83 (q, *J* = 17 Hz, *J* = 4 Hz, 2H, 7-H), 3.41 (d, *J* = 4.3 Hz, 1H, 8-H), 3.14 (d, *J* = 10.8 Hz, 1H, 8-H), 3.38 (br, 2H, 12-H), 3.29 (overlap, 1H, 6-H), 2.94 (dd, *J* = 2.1 Hz, *J* = 13 Hz, 1H, 2-H), 2.65 (dd, *J* = 3.7 Hz, *J* = 11.4 Hz, 1H, 2-H), 2.23-2.27 (m, 1H, 9-H), 0.87 (d, *J* = 7.1 Hz, 3H, 11-H), 0.54 (d, *J* = 6.9 Hz, 3H, 10-H). **$^{13}$C-NMR** (100 MHz, $CD_3OD$): $\delta$ = 174.4 (1-C), 173.3 (3-C), 173.1 (5-C), 138.2 (15-C), 128.6 (20-C), 124.2 (14-C), 122.5 (17-C), 114.8 (19-C), 114.2 (18-C), 112.4 (16-C), 111.1 (13-C), 65.3 (6-C), 60.1 (4-C), 47.04 (8-C), 43.3 (7-C), 42.6 (2-C), 31.2 (9-C), 24.6 (12-C), 18.7 (10-C), 16.7 (11-C). **IR** (KBr) ν ($cm^{-1}$): 3405.7 (NH), 1668.2, 1623.5 (CO). **HPLC-ESI-MS**: m/z (%) = 386.2 (100) $[M+H]^+$, 408.2 (45) $[M+Na]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{20}H_{27}N_5O_3Na$ $[M+Na]^+$ 408.2012; found 408.2006. $R_f$ = 0.136 (DCM/MeOH, 6/1).

**9H-Fluoren-9-ylmethyl-N-[(2*S*)-3-(*tert*-butoxy)-1-[(2*S*)-4-[(1*S*)-1-[(carbamoylmethyl)-carbamoyl]-2-(1H-indol-3-yl)ethyl]-3-oxo-2-(propan-2-yl)piperazin-1-yl]-1-oxopropan-2-yl]carbamate (TVC-042, 45)**

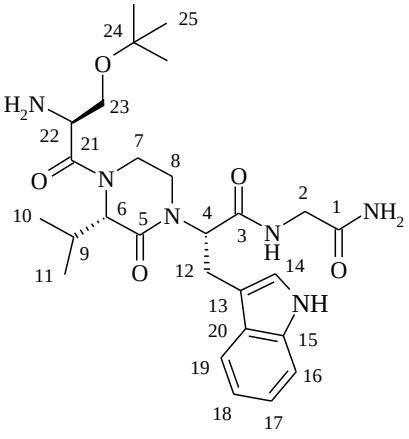
To a stirred solution of Fmoc-L-Ser(*t*Bu)-OH (180 mg, 0.47 mmol) and amine **43** (145 mg, 0.38 mmol) in DMF (12 mL), HATU (46 mg, 0.52 mmol) and DIPEA (0.23 mL, 1.4 mmol) were added. After stirring at room temperature overnight, EtOAc (300 mL) was added. The mixture was washed with $H_2O$ and brine

solution. The solvent was removed and the residue was purified by silica gel column chromatography (EtOAc/EtOH, 20/1) to obtain product **TVC-042** (335 mg, 95%) as a solid.



**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 8.67 (s, 1H, NH), 8.0 (s, 1H, NH), 7.74 (d, *J* = 7.32 Hz, 2H, 33-H), 7.55 (d, *J* = 7.3 Hz, 2H, 30-H), 7.55 (d, *J* = 7.3 Hz, 1H, 4-H), 7.38 (t, *J* = 7.32 Hz, 2H, 32-H), 7.3 (m, 1H, 16-H), 7.28 (overlap, 2H, 31-H), 7.1 (t, *J* = 7.6 Hz, 1H, 17-H), 7.09 (t, *J* = 7.5 Hz, 1H, 18-H), 7.0 (s, 1H, 14-H), 6.1 (s, 1H, NH), 5.9 (s, 1H, NH), 5.6 (d, *J* = 8.1 Hz, 1H, NH), 5.3 (m, 1H, 4-H), 4.78 (d, *J* = 6.9 Hz, 1H, 6-H), 4.71 (d, *J* = 5.4 Hz, 1H, 22-H), 4.35 (d, *J* = 4 Hz, 2H, 27-H), 4.2 (t, *J* = 6.8 Hz, 1H, 28-H), 3.96 (m, 1H, 8-H), 3.84 (m, 1H, 2-H), 3.73 (m, 1H, 2-H), 3.54 (m, 1H, 7-H), 3.41 (m, 1H, 7-H), 3.41 (overlap, 2H, 23-H), 3.38 (m, 1H, 8-H), 3.37 (m, 1H, 12-H), 3.22 (m, 1H, 12-H), 1.92 (br, 1H, 9-H), 1.1 (s, 9H, 25-H), 0.82 (d, *J* = 6.3 Hz, 3H, 11-H), 0.77 (d, *J* = 6.3 Hz, 3H, 10-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 171.5 (3-C), 171.1 (1-C), 170.6 (5-C), 170.6 (21-C), 169.0 (26-C), 155.0 (15-C),143.6 (20-C),141.3 (29-C), 136.2 (34-C), 127.7 (30-C), 127.0 (31-C), 125 (32-C), 122.9 (14-C), 122.6 (17-C), 14.9 (33-C), 14.7 (18-C), 110 (13-C), 74.0 (24-C), 67.1 (27-C), 63 (23-C), 60.7 (6-C), 57.4 (4-C), 51.1 (22-C), 47.1 (28-C), 43 (7-C), 42.7 (2-C), 41.6 (8-C), 32.2 (9-C), 27.3 (25-C), 24.1 (12-C), 14.6 (11-C, 10-C). **IR** (KBr) $\nu$ ($cm^{-1}$): 3428.0 (NH), 1656.9 (CO). **HPLC-ESI-MS**: m/z (%) = 751.4 (100) $[M+H]^+$, 769.4 (4) $[M+NH_4]^+$, 773.4 (11) $[M+Na]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{42}H_{50}N_6O_7Na$ $[M+Na]^+$ 773.3639; found 773.3633. $R_f$ = 0.22 (EtOAc/EtOH, 15/1).

**(2*S*)-2-[(3*S*)-4-[(2*S*)-2-Amino-3-(*tert*-butoxy)propanoyl]-2-oxo-3-(propan-2-yl)piperazin-1-yl]-N-(carbamoylmethyl)-3-(1H-indol-3-yl)propanamide (TVC-046, 46)**

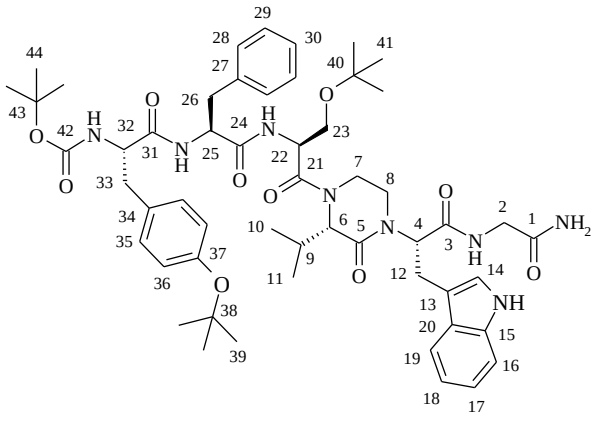


A solution of **TVC-042** (170 mg, 226.4 µmol) in DCM (10 mL) was treated with piperidine (335 µL, 340 µmol) for 5 h at room temperature. The reaction mixture was concentrated to dryness, then purified by silica gel column chromatography (EtOAc/EtOH, 5/1) to provide **TVC-046** (116 mg, 98%) as a solid.

**$^1$H-NMR** (600 MHz, $CD_3OD$): $\delta$ = 7.6 (d, *J* = 7.9 Hz, 1H, 4-H), 7.32 (d, *J* = 8.1 Hz, 1H, 16-H), 7.12 (s, 1H, 14-H), 7.08 (dt, *J* = 0.8 Hz, *J* = 7.8 Hz, 1H, 17-H), 7.0 (dt, *J* = 1.1 Hz, *J* = 7.1 Hz, 1H, 18-

H), 5.3 (t, *J* = 7.7 Hz, 1H, 4-H), 4.55 (d, *J* = 8.8 Hz, 1H, 6-H), 3.9 (m, 1H, 8-H), 3.84 (overlap, 1H, 22-H), 3.84 (overlap, 2H, 2-H), 3.51 (m, 1H, 8), 3.49 (br, 2H, 7-H), 3.37 (d, *J* = 8.4 Hz, 2H, 12-H), 3.32 (m, 2H, 23-H), 1.76 (m, 1H, 9-H), 1.12 (s, 9H, 25-H), 0.70 (d, *J* = 6.9 Hz, 3H, 11-H), 0.59 (d, *J* = 6.7 Hz, 3H, 10-H). **$^{13}$C-NMR** (150 MHz, $CD_3OD$): $\delta$ = 174.2 (3-C), 174.1 (1-C), 172.8 (5-C), 170.7 (21-C), 138.1 (15-C), 128.6 (20-C), 124.0 (14-C), 122.0 (17-C), 120.0 (18-C), 14.3 (4-C), 112.5 (16-C), 110.8 (13-C), 74.7 (24-C), 65.7 (23-C), 62.3 (6-C), 59.4 (4-C), 52.6 (22-C), 44.3 (7-C), 43.2 (2-C), 42.7 (8-C), 33.1 (9-C), 27.7 (25-C), 25.3 (12-C), 4.95 (11-C, 10-C). **IR** (KBr) ν ($cm^{-1}$): 3416.2 (NH), 1657.1, 1641.1 (CO). **HPLC-ESI-MS**: m/z (%) = 529.3 (100) $[M+H]^+$, 551.3 (16) [M+Na]. **HR-ESI-MS** (m/z): calcd. for $C_{27}H_{41}N_6O_5$ $[M+H]^+$ 529.3138; found 529.3133. $R_f$ = 0.18 (EtOAc/EtOH, 2/1).

**_tert_-Butyl-N-[(1*S*)-1-{[(1*S*)-1-{[(2*S*)-3-(_tert_-butoxy)-1-[(2*S*)-4-[(1S)-1-[(carbamoylmethyl)-carbamoyl]-2-(1H-indol-3-yl)ethyl]-3-oxo-2-(propan-2-yl)piperazin-1-yl]-1-oxopropan-2-yl]carbamoyl}-2-phenylethyl]carbamoyl}-2-[4-(_tert_-butoxy)phenyl]ethyl]carbamate (TVC-048, 44)**

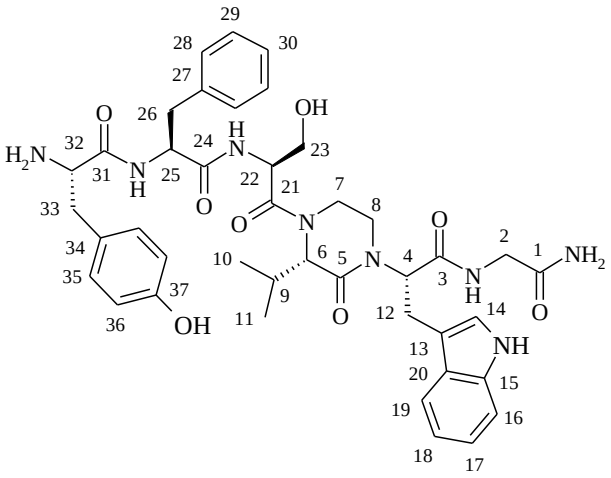


To a solution of amine **TVC-046** (70 mg, 132.4 µmol) and Boc-Tyr(*t*Bu)-Phe-OH (81 mg, 165.5 µmol) in DMF/ACN (8 mL, 6/4), HATU (68 mg, 179 µmol) and DIPEA (66 µl, 397 µmol) were added. After stirring at room temperature for 24 h, the solvent was removed under reduced pressure. Flash chromatography (EtOAc/EtOH, 20/1) afforded product **44** (115 mg, 87%) as a yellow solid.

**$^1$H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 7.97 (s, 1H, NH), 7.62 (d, *J* = 7.9 Hz, 1H, 419-H), 7.32 (d, *J* = 8.0, 1H, 16-H), 7.2 (m, 2H-35-H), 7.2 (m, 2H, 29-H), 7.1 (m, 2H, 28-H), 7.1 (s, 1H, 14-H), 7.08 (overlap, 1H, 18-H), 7.08 (m, 1H, 30-H) 7.03 (t, *J* = 7,1 Hz, 1H, 17-H), 6.9 (d, *J* = 8.3 Hz, 2H, 36-H), 5.36 (d, *J* = 8.25, 1H, 4-H), 4.76 (overlap, 1H, 22-H), 4.6 (t, *J* = 6.9 Hz, 1H, 25-H), 4.47 (d, *J* = 8.4 Hz, 1H, 6-H), 4.21 (d, *J* = 5 Hz, 1H, 33-H), 3.84-3.87 (m, 2H, 2-H), 3.82-3.86 (m, 1H, 7-H), 3.46-3.47 (m, 1H, 7-H), 3.48-3.52 (m, 2H, 8-H), 3.45-3.43 (m, 2H, 23-H), 3.36-3.38 (m, 2H, 12-H), 3.03 (m, 1H, 26-H), 2.85 (m, 1H, 26-H), 2.95 (m, 1H, 33-H), 2.66 (m, 1H, 33-H), 1.49-1.53 (m, 1H, 9-H, 39-H), 1.34 (s, 9H, 41-H), 1.30 (s, 9H, 44-H), 1.11 (s, 9H), 0.61 (d, *J* = 6.8 Hz, 3H, 10-H), 0.53 (d, *J* = 6.6 Hz, 3H, 11-H). **$^{13}$C-NMR** (100 MHz, $CD_3OD$): $\delta$ = 174.1 (24-C), 174.0 (31-C), 172.8 (3-C), 172.6 (1-C), 171.8 (21-C), 170.7 (5-C), 157.6 (42-C), 155.3 (37-C), 138.1 (15-C), 138 (27-C), 133.7 (34-C), 130.8 (28-C), 130.4 (29-C), 129.5 (35-C), 128.6 (20-C), 127.8 (30-C), 125.2 (6-C), 124.5 (14-C), 122.6 (18-C), 120 (17-C), 114.3 (19-C), 112.5

(16-C), 110.8 (13-C), 80.7 (38-C), 79.5 (40-C), 75.0 (43-C), 63.1 (23-C), 62.6 (6-C), 58.8 (4-C), 57.4 (32-C), 55.4 (25-C), 43.7 (7-C), 42.9 (8-C), 43.2 (2-C), 38.8 (26-C), 38.5 (33-C), 33.0 (9-C), 29.2 (44-C), 28.7 (39-C), 27.7 (41-C), 25.3 (12-C), 20.2 (1-C), 14.9 (11-C). **IR** (KBr) ν ($cm^{-1}$): 3834.2 (NH), 1655.2 (CO). **HPLC-ESI-MS**: m/z (%) = 995.6 (39.4) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{54}H_{75}N_8O_{10}$ $[M+H]^+$ 995.5606; found 995.5601. $R_f$ = 0.26 (EtOAc/EtOH, 15/1).

**(2*S*)-2-[(2*S*)-2-Amino-3-(4-hydroxyphenyl)propanamido]-N-[(2*S*)-1-[(2*S*)-4-[(1*S*)-1-[(carbamoyl-methyl)carbamoyl]-2-(1H-indol-3-yl)ethyl]-3-oxo-2-(propan-2-yl)piperazin-1-yl]-3-hydroxy-1-oxopropan-2-yl]-3-phenylpropanamide (TVC-051, 47)**

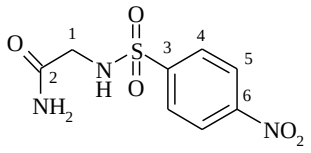


Hexapeptide **44** (50 mg, 50.2 µmol) was treated with TFA/TIPS/$H_2O$ (2 mL, 95/2.5/2.5) at 0 °C for 4 h. After removal of the solvent, the residue was dissolved in ACN/$H_2O$ (2 mL, 1/1) and lyophilized to provide product **47** (35.5 mg, 90%) as a white powder.

**$^1$H-NMR** (400 MHz, DMSO-$d_6$): $\delta$ = 10.8 (d, $J$ = 2 Hz, 1H, NH), 9.2 (br, s, 1H, NH), 8.4 (d, $J$ = 8 Hz, 1H, NH), 8.2 (t, $J$ = 5.7 Hz, 1H, NH), 8.1 (br, s, 1H, NH), 7.27 (s, 1H, NH), 7.64 (d, $J$ = 7.8 Hz, 1H, 19-H), 7.31 (d, $J$ = 8.0 Hz, 16-H), 7.4-7.13 (m, 6H, 14-H, 28-H, 29-H, 30-H), 7.05 (t, $J$ = 8.0 Hz, 19-H), 6.9 (overlap, 1H, 18-H), 6.96 (d, $J$ = 8.5 Hz, 2H, 35-H), 6.66 (d, $J$ = 8.5 Hz, 2H, 36-H), 5.4 (dd, $J$ = 4.9 Hz, $J$ = 11 Hz, 1H, 4-H), 4.7 (q, $J$ = 6.7 Hz, 1H, 22-H), 4.6 (q, $J$ = 8 Hz, 1H, 25-H), 4.3 (d, $J$ = 8.5 Hz, 1H, 6-H), 3.8 (m, 1H, 7-H), 3.66 (d, $J$ = 5.8 Hz, 1H, 2-H), 3.58 (m, 1H, 23-H), 3.50 (m, 1H, 2-H), 3.5 (m, 1H, 8-H), 3.41 (m, 1H, 32-H), 3.4 (m, 1H, 7-H), 3.38 (m, 1H, 8-H), 3.3 (m, 1H, 12-H), 3.12 (m, 1H, 12-H), 2.92 (m, 1H, 26-H), 2.77 (m, 1H, 33-H), 2.73 (m, 1H, 26-H), 2.45 (m, 1H, 33-H), 1.44 (m, 1H, 9-H), 0.53 (d, $J$ = 7.0 Hz, 3H, 10-H), 0.40 (d, $J$ = 7.0 Hz, 3H, 11-H). **$^{13}$C-NMR** (100 MHz, DMSO-$d_6$): $\delta$ = 155.9 (37-C), 136.1 (15-C), 130.1 (34-C), 129.2 (28-C), 127.8 (29-C), 127.0 (35-C), 126.2 (30-C), 126.8 (20-C), 123.4 (14-C), 120.7 (17-C), 118.5 (19-C), 118.1 (18-C), 114.9 (36-C), 111.5 (16-C), 106.7 (13-C), 61.0 (23-C), 60.3 (6-C), 55.6 (4-C), 55.4 (32-C), 52.8 (25-C), 50.9 (22-C), 41.8 (2-C), 41.2 (7-C), 40.8 (8-C), 38.5 (33-C), 37.7 (26-C), 30.9 (9-C), 24.2 (12-C), 14.3 (10-C), 14.2 (11-C). **IR** (KBr) ν ($cm^{-1}$): 3423.41 (NH), 1651.7 (CO). **HPLC-ESI-MS**: m/z (%) = 783.4 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{41}H_{51}N_8O_8$ $[M+H]^+$ 783.3830; found 783.3824.

### 3.8 Synthesis of flexible cyclic helicokinin analogues TVC-113 (66) and TVC-158 (67)

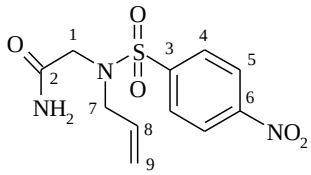
**2-(4-Nitrophenylsulfonamido)acetamide (TVC-052, 48)**



To a suspension of glycinamide hydrochloride (500 mg, 4.52 mmol) and TEA (1.57 mL, 11.3 mmol) in DCM (30 mL) at - 20 to -15 °C, a solution of 4-nitrobenzenesulfonyl chloride (982 mg, 4.43 mmol) in DCM/DMF (17 mL, 15/2) was added dropwise. The reaction mixture was stirred further for 1 h, and then the solid was collected and crystallized from EtOH/$H_2O$ to obtain product **48** (850 mg, 72%) as a white solid.

**$^1$H-NMR** (400 MHz, DMSO-$d_6$):$\delta$ = 8.39 (d, *J* = 8.97 Hz, 2H, 5-H), 8.27 (s, 1H, NH), 8.05 (d, *J* = 8.97 Hz, 2H, 4-H), 7.31 (s, 1H, NH), 7.06 (s, 1H, NH), 3.49 (s, 2H, 1-H). **$^{13}$C-NMR** (100 MHz, DMSO-$d_6$): $\delta$ = 169.1 (2-C), 149.4 (6-C), 146.1 (3-C), 128.1 (4-C), 124.3 (5-C), 44.7 (1-C). **IR** (KBr) ν ($cm^{-1}$): 3415.41 (NH), 1688.7 (CO). **HPLC-ESI-MS**: m/z (%) = 260.0 (8) $[M+H]^+$ 282.0 (12) $[M+Na]^+$. **HPLC-ESI-MS**: m/z (%) = 260.0 (100) $[M+H]^+$. $R_f$ = 0.23 (EtOAc).

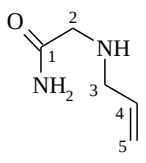
**2-(*N*-Allyl-4-nitrophenylsulfonamido)acetamide (TVC-055, 49)**



A suspension of 2-(4-nitrophenylsulfonamido)acetamide **48** (30 mg, 0.12 mmol) and $Cs_2CO_3$ (40 mg, 0.21 mmol) in DMF (4 mL) was stirred for 30 min at room temperature, followed by dropwise addition of allyl bromide (20 µL, 0.23 mmol). After stirring for 20 h, the mixture was quenched with AcOEt (100 mL), washed with $H_2O$, brine solution and dried over $MgSO_4$. Removal of the solvent gave product **49** (35.4 mg, 100%) as a white solid.

**$^1$H-NMR** (400 MHz, DMSO-$d_6$): $\delta$ = 8.39 (d, *J*= 8.9 Hz, 2H, 5-H), 8.1 (d, *J* = 8.9 Hz, 2H, 4-H), 7.4, 7.1 (s, 2H, $NH_2$), 5.7 (m, 1H, 8-H), 5.21-5.15 (m, 2H, 9-H), 3.9 (d, *J* = 6.4 Hz, 2H, 7-H), 3.81 (s, 2H, 1-H). **$^{13}$C-NMR** (100 MHz, DMSO-$d_6$):$\delta$ = 172.3 (2-C), 151.6 (6-C), 146.7 (3-C), 132.1 (8-C), 128.5 (4-C), 125.1 (5-C), 120.0 (9-C), 51.3 (7-C), 48.6 (2-C). **IR** (KBr) ν ($cm^{-1}$): 3204.8, 3434.8, 1675.5. **HPLC-ESI-MS**: m/z (%) = 300.1 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{11}H_{13}N_3O_5SNa$ $[M+Na]^+$ 322.0468; found 322.0465. $R_f$ = 0.28 (CyH/EtOAc, 3/2).

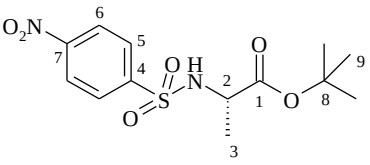
**2-(Allylamino)acetamide (TVC-060, 50)**



To a stirred suspension of 2-(N-allyl-4-nitrophenylsulfonamido)acetamide **49** (4.20 g, 14.03 mmol) and $K_2CO_3$ (5.8 g, 42.1 mmol) in ACN (120 mL), thiophenol (2.87 mL, 28.1 mmol) was added. After 20 h, the solvent was removed under reduced pressure. The residue was extracted with EtOAc by using a Soxhlet extractor to obtain a crude product which was purified by silica gel column chromatography using DCM/MeOH (6/1) providing **50** (1.62 g, 98%) as an oily product.

**$^1$H-NMR** (600 MHz, DMSO-$d_6$): $\delta$ = 7.26 (bs, 1H, NH), 7.02 (bs, 1H, NH), 5.8 (m, 1H, 4-H), 5.1 (dd, *J* = 1.8 Hz, *J* = 17.2 Hz, 1H, 5-H), 5.04 (dd, *J* = 1.8 Hz, *J* = 17.2 Hz, 1H, 5-H), 3.1 (d, *J* = 5.7 Hz, 2H, 3-H), 3.0 (s, 2H, 2-H). **$^{13}$C-NMR** (150 MHz, DMSO-$d_6$): $\delta$ = 173.0 (1-C), 136 (4-C), 115 (5-C), 51.2 (2-C), 50.8 (3-C). **IR** (neat) ν ($cm^{-1}$): 3304.9, 1653.5. **HPLC-ESI-MS**: m/z (%) = 115.09 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_5H_{10}N_2O$ $[M+H]^+$ 115.0870; found 115.0866. $R_f$ = 0.16 (EtOH/EtOAc, 1/1).

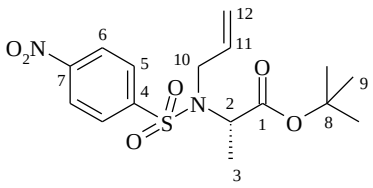
**(*S*)-*tert*-Butyl-2-(4-nitrophenylsulfonamido)propanoate (TVC-053)**



To a solution of H-Ala-O*t*Bu*HCl (5 g, 27.52 mmol) and 4-nitrobenzenesulfonyl chloride (6.7 g, 30.28 mmol) in DCM (100 mL) at 0 $^o$C, TEA (8.2 mL, 63.31 mmol) was added dropwise. The reaction mixture was allowed to warm up to room temperature and stirred for 20 h. After that time, the solution was diluted with DCM (300 mL), washed with $H_2O$ (2x25 mL), brine solution (30 mL) and dried over $MgSO_4$. Removal of the solvent and flash chromatography (CyH/EtOAc, 5/1) yielded desired product *(S)-tert*-butyl-2-(4-nitrophenylsulfonamido)propanoate **TVC-053** (8.74 g, 98%.) as a solid.

**$^1$H-NMR** (600 MHz, $CDCl_3$): $\delta$ = 8.33 (d, *J* = 8.9 Hz, 2H, 6-H), 8.04 (d, *J* = 8.9 Hz, 2H, 5-H), 5.46 (d, *J* = 8.5 Hz, 1H, NH), 3.93 (q, *J* = 7.1 Hz, 1H, 2-H), 1.38 (d, *J* = 7.1 Hz, 3H, 3-H), 1.3 (s, 9H, 9-H). **$^{13}$C-NMR** (150 MHz, $CDCl_3$): $\delta$ = 170.9 (1-C), 150.1 (7-C), 146.1 (4-C), 128.5 (5-C), 124.2 (6-C), 83.0 (8-C), 52.2 (2-C), 27.7 (9-C), 19.9 (3-C). **IR** (neat) ν ($cm^{-1}$): 3304.9, 1653.5. **HPLC-ESI-MS**: m/z (%) = 348 (80) $[M+NH_4]^+$, 683.2 (30) $[2M+Na]^+$. $R_f$ = 0.16 (CyH/EtOAc, 5/1).

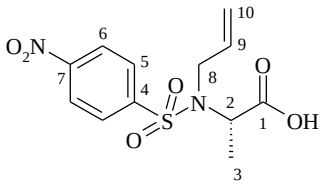
**(*S*)-*tert*-Butyl-2-(*N*-allyl-4-nitrophenylsulfonamido)propanoate (TVC-054, 52)**



To a stirred suspension of (*S*)-*tert*-butyl-2-(4-nitrophenylsulfonamido)-propanoate **TVC-053** (6.3 g, 19.07 mmol) and $Cs_2CO_3$ (6.6 g, 34.33 mmol) in DMF (100 mL) at 0 °C, a solution of allyl bromide (3.13 mL, 38.14 mmol) in DCM (20 mL) was added dropwise within 30 min. The reaction mixture was stirred for 20 h at room temperature and diluted with AcOEt (500 mL). The resulting mixture was washed with $H_2O$, brine solution and dried over $Na_2SO_4$. Removal of the solvent under diminished pressure afforded (*S*)-*tert*-butyl-2-(N-allyl-4-nitrophenylsulfonamido)propanoate **52** (7.19 g, 100%) as a yellow oil.

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 8.31 (d, *J* = 8.9 Hz, 2H, 6-H), 8.01 (d, *J* = 8.9 Hz, 2H, 5H), 5.86-5.79 (m, 1H, 11-H), 5.23 (dd, *J* = 1.3 Hz, *J* = 17.2 Hz, 1H, 12-H), 5.14 (dd, *J* = 1.2 Hz, *J* = 10.2 Hz, 1H, 12-H), 4.59 (q, *J* = 7.3 Hz, 1H, 2-H), 1.45 (d, *J* = 7.3 Hz, 3H, 3-H), 1.35 (s, 9H, 9-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 169.9 (1-C), 149.9 (7-C), 146.2 (4-C), 134.8 (11-C), 128.6 (5-C), 124.0 (6-C), 116.1 (12-C), 82.4 (8-C), 56.4 (2-C), 48.4 (10-C), 27.8 (9-C), 17.2 (3-C). **IR** (neat) ν ($cm^{-1}$): 2980.2, 1730.3 (CO). **HPLC-ESI-MS**: m/z (%) = 371.2 (50) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{16}H_{22}N_2O_6SNa$ $[M+Na]^+$ 393.1091; found 393.1073. $R_f$ = 0.28 ( CyH/EtOAc, 10/1).

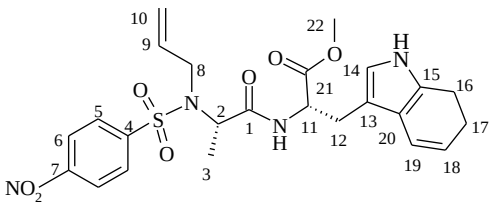
**(*S*)-2-(*N*-Allyl-4-nitrophenylsulfonamido)propanoic acid (TVC-056, 53)**



TFA (20 mL, 271 mmol) was added dropwise to a cooled solution of (S)-*tert*-butyl-2-(N-allyl-4-nitrophenyl-sulfonamido)propanoate **52** (6.7 g, 18.1 mmol) in DCM (40 mL) at 0 °C and continuously stirred for 4 h. Evaporation of the solvent provided pure acid **53** (5.6 g, 100%) as a brown oil.

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 8.33 (d, *J* = 8.93 Hz, 2H, 6-H), 8.02 (d, *J* = 8.90 Hz, 2H, 5-H), 5.84-5.74 (m, 1H, 9-H), 5.24 (dd, *J* = 1.2 Hz, *J* = 17.2 Hz, 1H, 10-H), 5.16 (dd, *J* = 1.2 Hz, *J* = 10.2 Hz, 1H, 10-H), 4.68 (q, *J* = 7.3 Hz, 1H, 2-H), 1.51 (d, *J* = 7.3 Hz, 3H, 3-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 175.3 (1-C), 150.0 (7-C), 145.8 (4-C), 133.9 (9-C), 128.6 (5-C), 124.1 (6-C), 118.8 (10-C), 55.5 (2-C), 48.7 (8-C), 16.6 (3-C). **IR** (KBr) ν ($cm^{-1}$): 3456.2, 3112.5, 1719.5 (CO). **HPLC-ESI-MS**: m/z (%) = 315 (70) $[M+H]^+$, 337.0 (100) $[M+Na]^+$. $R_f$ = 0.28 (CyH/EtOAc, 10/1).

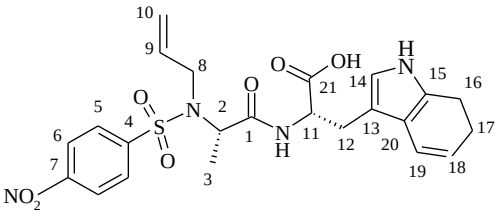
**Methyl-2-((*S*)-2-(*N*-allyl-4-nitrophenylsulfonamido)propanamido)-3-(6,7-dihydro-1H-indol-3-yl)-propanoate (TVC-057, 54)**



To a suspension of acid **53** (3 g, 9.54 mmol), L-Trp-OMe*HCl (2.92 g, 11.5 mmol) and PyBOP (5.96 g, 11.5 mmol) in ACN (40 mL), DIPEA (5.5 mL, 33.11 mmol) was added dropwise. After stirring at room temperature for 20 h, AcOEt (400 mL) was added. The resulting solution was washed with $H_2O$, brine solution and dried over $MgSO_4$. Flash chromatography (EtOAc/CyH, 1/3) gave **54** (4.1 g, 84%) as a yellow solid.

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 8.30 (s, 1H, NH), 8.24 (d, *J* = 8.9 Hz, 2H, 6-H), 7.87 (d, *J* = 8.9 Hz, 2H, 5-H), 7.56 (d, *J* = 7.8 Hz, 1H, 19-H), 7.37 (d, *J* = 8.0 Hz, 1H, 16-H), 7.16 (t, *J* = 7.1 Hz, 1H, 17-H), 7.13 (m, 2H, 14-H, 18-H), 6.66 (d, *J* = 7.0 Hz, 1H, NH), 5.24 (m, 1H, 9-H), 5.0 (dd, *J* = 1.5 Hz, *J* = 17.0 Hz, 1H, 10-H), 4.81 (dd, *J* = 1.0 Hz, *J* = 10.0 Hz, 1H, 10-H), 4.72 (q, *J* = 7.3 Hz, 1H, 2-H), 4.43 (q, *J* = 7.2 Hz, 1H, 11-H), 3.73 (s, 3H, 22-H), 3.59 (dd, *J* = 5.7 Hz, *J* = 16 Hz, 2H, 8-H), 3.37 (dd, *J* = 5.0 Hz, *J* = 14.8 Hz, 2H, 12-H), 1.2 (d, *J* = 7.2 Hz, 3H, 3-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 172.5 (21-C), 170.1 (1-C), 150.5 (7-C), 146.0 (4-C), 136.8 (15-C), 133.5 (9-C), 128.7 (5-C), 127.6 (20-C), 124.8 (6-C), 123.8 (14-C), 122.9 (17-C), 120.3 (18-C), 119.5 (10-C), 118.9 (19-C), 111.8 (16-C), 110.1 (13-C), 56.1 (11-C), 53.2 (2-C), 52.9 (22-C), 48.0 (8-C), 27.8 (12-C), 14.7 (3-C). **IR** (KBr) ν ($cm^{-1}$): 3414.41 (NH), 1720.6, 1683.7 (CO). **HPLC-ESI-MS**: m/z (%) = 515.2 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{24}H_{26}N_4O_7SNa$ $[M+Na]^+$ 537.1413; found 537.1414. $R_f$ = 0.24 (CyH/EtOAc, 3/2).

**2-((*S*)-2-(*N*-Allyl-4-nitrophenylsulfonamido)propanamido)-3-(6,7-dihydro-1H-indol-3-yl)propanoic acid (TVC-059)**

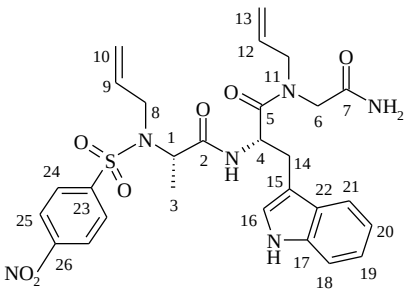


Methyl ester **54** (2.5 g, 4.86 mmol) in THF (40 mL) was treated with a solution of 4% LiOH (35 mL, 1.63 g, 38.9 mmol). After stirring for 4 h, the reaction mixture was acidified with 1N HCl to pH ≈ 4 and extracted with EtOAc (2x200 mL). The combined organic phases were washed with $H_2O$ (2x20 mL), brine (20 mL) and dried over $MgSO_4$. Removal of the solvent under reduced pressure afforded acid **TVC-059** (1.93 g, 79%) as a yellow solid.

**$^1$H-NMR** (600 MHz, $CDCl_3$): $\delta$ = 8.05 (d, *J* = 8.8 Hz, 2H, 6-H), 7.72 (d, *J* = 8.8 Hz, 2H, 5-H), 7.53 (d, *J* = 7.9 Hz, 1H, 19-H), 7.29 (d, *J* = 9.2 Hz, 1H, 16-H), 7.10 (t, *J* = 7.9 Hz, 1H, 17-H), 7.07 (s, 1H, 14-H), 7.04 (t, *J* = 7.8 Hz, 1H, 18-H), 5.21 (m, 1H, 9-H), 4.90 (d, *J* = 17.0 Hz, 1H, 10-H), 4.73 (d, *J* = 10.0 Hz, 1H, 10-H), 4.55 (m, 1H, 11-H), 4.40 (q, *J* = 7.2 Hz, 1H, 2-H), 3.50 (d, *J* = 6 Hz, 1H, 8-H), 3.45 (dd, *J* = 6.8 Hz, 1H, 8-H), 3.35 (dd, *J* = 5 Hz, 1H, 12-H), 3.12 (dd, *J* = 8.0 Hz, 1H, 12-H), 1.08 (d, *J* = 7.2 Hz,

3H, 3-H). **$^{13}$C-NMR** (150 MHz, $CDCl_3$): $\delta$ = 173.4 (21-C), 170 (1-C), 149.9 (7-C), 145.1 (4-C), 136.2 (15-C), 133.4 (9-C), 128.2 (6-C), 127.1 (20-C), 124.1 (5-C), 123.4 (14-C), 121.9 (17-C), 119.2 (18-C), 118.5 (19-C), 118.2 (10-C), 111.3 (16-C), 109.2 (13-C), 55.5 (2-C), 52.8 (11-C), 47.5 (8-C), 27.0 (12-C), 14.6 (3-C). **IR** (KBr) ν ($cm^{-1}$): 3420.8 (NH), 1722.6, 1670.7 (CO). **HPLC-ESI-MS**: m/z (%) = 501.1 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{24}H_{26}N_4O_7SNa$ $[M+Na]^+$ 523.1258; found 523.1254. $R_f$ = 0.44 (EtOAc, 0.1% AcOH).

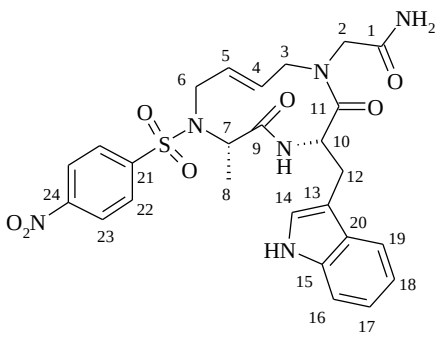
**(*S*)-*N*-Allyl-2-((*S*)-2-(*N*-allyl-4-nitrophenylsulfonamido)propanamido)-N-(2-amino-2-oxoethyl)-3-(1H-indol-3-yl)propanamide (TVC-065, 55)**



A solution of acid **TVC-059** (228 mg, 2.0 mmol), 2-(allylamino)-acetamide **50** (1.0 g, 2.0 mmol) and BOP-Cl (570 mg, 2.24 mmol) in ACN (40 mL) at -10 $^oC$ was added dropwise DIPEA (0.73 mL, 4.4 mmol). The reaction mixture was allowed to stir at room temperature for 16 h and diluted with EtOAc (300 mL). The resulting solution was washed with $H_2O$ (2x30 mL), brine solution and dried over $MgSO_4$. Removal of the solvent and flash chromatography using EtOAc as eluent provided **55** (897 mg, 75%) as a yellow solid.

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 8.23 (d, *J* = 8.9 Hz, 2H, 25-H), 7.85 (d, *J* = 8.9 Hz, 2H, 24-H), 7.5 (d, *J* = 7.8 Hz, 1H, 21-H), 7.4 (d, *J* = 8 Hz, 1H, 18-H), 7.25 (overlap, 1H, 16-H), 7.2 (m, 1H, 19-H), 7.1 (t, *J* = 7 Hz, 1H, 20-H), 5.7 (m, 1H, 12-H), 5.25 (d, *J* = 10 Hz, 1H, 13-H), 5.18 (d, *J* = 17 Hz, 1H, 13-H), 5.08 (m, 1H, 9-H), 4.9 (d, *J* = 16 Hz, 1H, 10-H), 4.7 (d, *J* = 10 Hz, 1H, 10-H), 4.77 (overlap, 1H, 4-H), 4.4 (overlap, 1H, 1-H), 4.32 (d, *J* = 16 Hz, 1H, 6-H), 3.7 (d, *J* = 16 Hz, 1H, 6-H), 4.2 (m, 1H, 11-H), 3.8 (m, 1H, 11-H), 3.6 (dd, *J* = 7.4 Hz, *J* = 15 Hz, 1H, 8-H), 3.46 (dd, *J* = 7.4 Hz, *J* = 15 Hz, 1H, 8-H), 3.2 (dq, *J* = 6 Hz, *J* = 14 Hz, 2H, 14-H), 1.02 (d, *J* = 7.2 Hz, 3H, 3-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 172.8 (2-C), 171.2 (5-C), 170.4 (7-C), 150.1 (26-C), 145.3 (23-C), 136.5 (17-C), 133.0 (9-C), 131.9 (12-C), 128.3 (24-C),126.9 (22-C), 124.4 (25-C), 124.0 (16-C), 122.6 (19-C), 119.9 (20-C), 118.9 (10-C), 118.2 (13-C), 118.0 (21-C), 111.7 (18-C), 109.3 (15-C), 55.1 (1-C), 51.5 (11-C), 50.0 (6-C), 49.7 (4-C), 47.0 (8-C), 28.1 (14-C), 14.2 (3-C). **IR** (KBr) ν ($cm^{-1}$): 3411.8 (NH), 1686.2, 1647.4 (CO). **HPLC-ESI-MS**: m/z (%) = 597.0 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{28}H_{32}N_6O_7SNa$ $[M+Na]^+$ 619.1945; found 619.1945. $R_f$ = 0.43 (EtOAc).

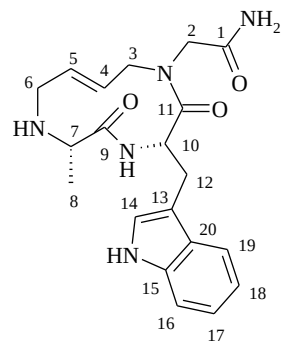
**2-((3*S*,6*S*,*E*)-3-((1H-Indol-3-yl)methyl)-6-methyl-7-(4-nitrophenylsulfonyl)-2,5-dioxo-1,4,7-triaza-cycloundec-9-en-1-yl)acetamide (TVC-126, 62b)**



To a solution of compound **55** (220 mg, 368 µmol) in degassed (40 min with argon) DCM (240 mL), Hoveyda-Grubbs catalyst 2nd generation (1,3-bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene) dichloro(*o*-isoprop-oxyphenylmethylene)-ruthenium **(23** mg, 38.6 µmol) was added. The reaction mixture was refluxed for 66 h, then concentrated and subjected to silica gel column chromatography (DCM/MeOH, 30/1). The desired product 2-((*3S,6S,E*)-3-((1H-indol-3-yl)methyl)-6-methyl-7-(4-nitrophenylsulfonyl)-2,5-dioxo-1,4,7-triazacyclo-undec-9-en-1-yl)acetamide **TVC-126** (90 mg, 43%) was obtained as a yellow solid.

**$^1$H-NMR** (600 MHz, DMSO-$d_6$): $\delta$ = 10.7 (s, 1H, NH), ), 8.4 (d, *J* = 8.7 Hz, 2H, 23-H), 8.2 (d, *J* = 8.7 Hz, 2H, 22-H), 7.85 (bs, 1H, NH), 7.6 (bs, 1H, 19-H), 7.3 (overlap, 1H, 16-H), 7.06 (t, *J* = 7.5 Hz, 1H, 17-H), 7.02 (s, 1H, 14-H), 6.97 (t, *J* = 7.5 Hz, 1H, 18-H), 5.75 (bs, 1H, 5-H), 5.15 (bs, 1H, 4-H), 4.73 (m, 1H, 10-H), 4.58 (d, *J* = 7.0 Hz, 1H, 7-H), 4.16 (d, *J* = 14.0 Hz, 1H, 3-H), 3.92 (br, 2H, 6-H), 3.9 (m, 2-H), 3.67 (d, *J* = 16 Hz, 1H, 2-H), 3.58 (t, *J* = 12 Hz, 1H, 3-H), 3.24 (m, 1H, 12-H), 2.8 (d, *J* = 11.0 Hz, 1H, 12-H), 1.51 (s, 3H, 8-H). **$^{13}$C-NMR** (150 MHz, DMSO-$d_6$): $\delta$ = 170.7 (9-C), 170.4 (11-C), 169.9 (1-C), 149.6 (24-C), 146.3 (21-C), 133.1 (5-C), 129.2 (22-C), 124.7 (23-C), 127.5 (20-C), 124.4 (4-C), 123.8 (14-C), 121.1 (17-C), 118.9 (19-C), 118.5 (18-C), 111.5 (16-C), 110.6 (13-C), 54.8 (7-C), 51.9 (6-C), 51.4 (2-C), 50.9 (10-C), 46.1 (3-C), 27.6 (12-C), 13.8 (8-C). **IR** (KBr) ν ($cm^{-1}$): 2978.8 (NH), 1656.2 (CO). **HPLC-ESI-MS**: m/z (%) = 569.2 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{26}H_{28}N_6O_7SNa$ $[M+Na]^+$ 591.1634; found 591.1632. $R_f$ = 0.17 (DCM/MeOH, 20/1).

**2-((3*S*,6*S*,*E*)-3-((1H-Indol-3-yl)methyl)-6-methyl-2,5-dioxo-1,4,7-triazacycloundec-9-en-1-yl)-acetamide (TVC-133, 63)**

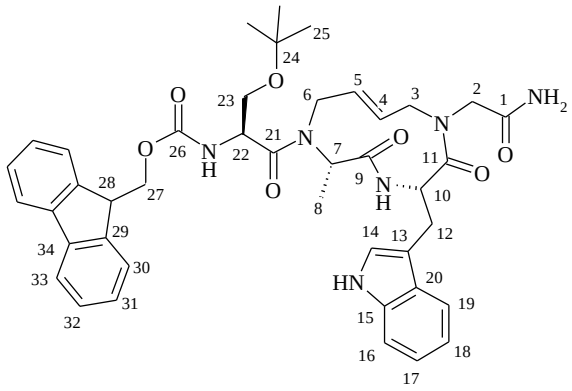


A solution of **TVC-126** (64 mg, 112.5 µmol) and DBU (46 µml, 340 mmol) in DMF (5 mL) was treated with 2-mercaptoethanol (98 µmL, 120 µmol) at room temperature for 1 h. The solvent was then removed and the crude material was purified by silica gel column chromatography (EtOAc/EtOH, 1/1) to provide product **63** (41.1 mg, 95%) as a solid.

**$^1$H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 7.55 (d, *J* = 8.1 Hz, 1H, 19-H), 7.34 (d, *J* = 8.15 Hz, 1H, 16-H), 7.09 (t, *J* = 7.0 Hz, 1H, 17-H), 7.01 (t, *J* = 7.0 Hz, 1H, 18-H), 5.62 (dd, *J* = 3.0 Hz, *J* = 15.7 Hz, 1H, 5-H), 5.39 (t, *J* = 13.0 Hz, 1H, 4-H), 4.29 (dd, *J* = 4.0 Hz, *J* = 11.0 Hz, 1H, 10-H), 4.12 (m, 1H, 6-H), 3.8 (d, *J* = 14.0 Hz, 1H, 6-H), 3.53 (m, 1H, 3-H), 3.46 (m, 1H, 12-H), 3.29 (dd, *J* = 4.0 Hz, *J* = 15.6 Hz, 1H, 12-H), 3.07 (t, *J* = 11.3 Hz, 1H, 3-H), 2.84 (m, 1H, 7-H),

1.08 (d, *J* = 7.5 Hz, 3H, 8-H). **$^{13}$C-NMR** (100 MHz, $CD_3OD$): $\delta$ = 174.1 (9-C), 173.6 (11-C), 164.9 (1-C), 138.1 (15-C), 131.1 (5-C), 129.5 (20-C), 124.7 (4-C), 123.0 (14-C), 122.7 (17-C), 119.7 (19-C), 119.5 (18-C), 112.7 (16-C), 112.2 (13-C), 55.5 (7-C), 54.4 (10-C), 50.9 (2-C), 49.9 (3-C), 49.2 (6-C), 25.1 (12-C), 17.6 (8-C). **IR** (KBr) ν ($cm^{-1}$): 3420.7 (NH), 1652.2 (CO). **HPLC-ESI-MS**: m/z (%) = 569.2 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{20}H_{25}N_5O_3Na$ $[M+Na]^+$ 406.1851; found 406.1850. $R_f$ = 0.13 (EtOH/EtOAc, 1/1).

**(9H-Fluoren-9-yl)methyl-(*S*)-1-((2*S*,5*S*,*E*)-5-((1H-indol-3-yl)methyl)-7-(2-amino-2-oxoethyl)-2-methyl-3,6-dioxo-1,4,7-triazacycloundec-9-en-1-yl)-3-*tert*-butoxy-1-oxopropan-2-yl-carbamate (TVC-141, 64)**

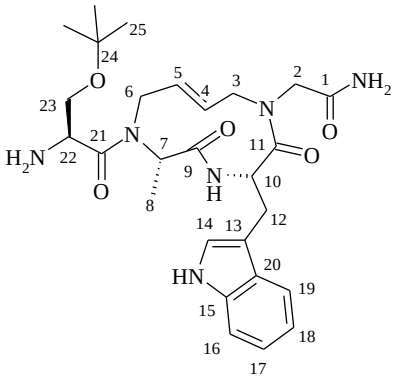


To a solution of 2-*((3S,6S,E)*-3-((1H-indol-3-yl)-methyl)-6-methyl-2,5-dioxo-1,4,7-triazacycloun-dec-9-en-1-yl)acetamide **63** (46 mg, 120 µmol) and Fmoc-L-Ser(*t*Bu)-OH (92 mg, 240 µmol) in DMF (4 mL), HATU (95.6 mg, 252 µmol) and DIPEA (80 µmL, 480 µmol) were added. The reaction mixture was stirred for 24 h at room temperature and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/EtOH, 25/1) to afford **64** (60 mg, 67%) as a solid.

**$^1$H-NMR** (600 MHz, $CD_3OD$): $\delta$ = 7.83 (m, 2H, 33-H), 7.7 (d, *J* = 7.1 Hz, 1H, 19-H), 7.65 (d, *J* = 8.6 Hz, 2H, 30-H), 7.28 (m, 1H, 16-H), 7.41 (m, 2H, 32-H), 7.23 (m, 2H, 31-H), 7.0 (m, 1H, 17-H), 6.98 (m, 1H, 18-H), 5.82 (bs, 1H, 5-H), 5.65 (bs, 1H, 4-H), 4.73 (br, 1H, 10-H), 4.72 (m, 1H, 7-H), 4.4 (bs, 3H, 22-H, 27-H), 4.2 (br, 1H, 28-H), 4.01 (m, 2H, 6-H), 3.96-3.7 (m, 2H, 2-H), 3.6-3.5 (m, 2H, 23-H), 3.33 (m, 2H, 3-H), 3.35-3.23 (m, 2H, 12-H), 3.29 (dd, *J* = 4.0 Hz, *J* = 15.6 Hz, 1H, 12-H), 3.07 (t, *J* = 11.3 Hz, 1H, 3-H), 1.45 (s, 3H, 8-H), 1.2 (s, 9H, 25-H). **$^{13}$C-NMR** (150 MHz, $CD_3OD$): $\delta$ = 174.4 (9-C), 174.0 (21-C), 173.4 (11-C), 157.9 (26-C), 145.3 (29-C), 142.6 (34-C), 137.5 (15-C), 131.1 (5-C), 128.3 (4-C), 127.6 (20-C), 127.4 (32-C), 126.6 (31-C), 125.0 (30-C), 124.8 (19-C), 123.0 (14-C), 120.7 (17-C), 119.3 (33-C), 118.2 (18-C), 110.9 (16-C), 110.2 (13-C), 74.8 (24-C), 66.6 (27-C), 62.2 (23-C), 54.0 (10-C), 53.8 (7-C), 52.8 (C-6), 52.6 (2-C), 47.2 (22-C), 46.9 (28-C), 42.4 (3-C), 26.8 (12-C), 26.2 (25-C), 13.3 (8-C). **IR** (KBr) ν ($cm^{-1}$): 3422.7 (NH), 1637.9 (CO). **HPLC-ESI-MS**: m/z (%) = 749.4 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{42}H_{48}N_6O_7Na$ $[M+Na]^+$ 771.3479; found 771.3477. $R_f$ = 0.39 (EtOAc/EtOH, 25/1).

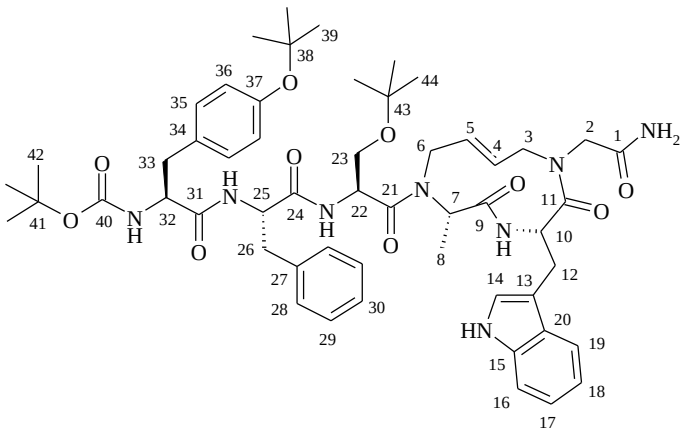
**2-((3*S*,6*S*,*E*)-3-((1H-Indol-3-yl)methyl)-7-((S)-2-amino-3-*tert*-butoxypropanoyl)-6-methyl-2,5-dioxo-1,4,7-triazacycloundec-9-en-1-yl)acetamide (TVC-144, 65)**

A solution of (9H-fluoren-9-yl)methyl-(*S*)-1-((*2S,5S,E*)-5-((1H-indol-3-yl)methyl)-7-(2-amino-2-oxoethyl)-2-methyl-3,6-dioxo-1,4,7-tri-azacyclo-undec-9-en-1-yl)-3-*tert*-butoxy-1-oxopropan-2-yl-carbamate **64** (50 mg, 66.8 µmol) in DCM (6.0 mL) was treated with piperidine (57 mg, 668 µmol) for 4 h at room temperature. Removal of the solvent and flash chromatography (EtOAc/EtOH, 1/1) yielded product **65** (35 mg, 99%) as a solid.



**$^{1}$H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 7.7 (d, $J$ = 8.1 Hz, 1H, 19-H), 7.32 (d, $J$ = 8.1 Hz, 1H, 16-H), 7.08 (m, 1H, 17-H), 7.07 (overlap, 1H, 14-H), 7.02 (t, $J$ = 7.1 Hz, 1H, 18-H), 5.85 (bs, 1H, 5-H), 5.33 (bs, 1H, 4-H), 4.61 (d, $J$ = 7.2 Hz, 1H, 7-H), 4.29 (br, 1H, 10-H), 4.01 (m, 1H, 6-H), 4.00 (br, 1H, 22-H), 3.92 (m, 1H, 2-H), 3.89 (m, 1H, 3-H), 3.78 (overlap, 2H, 6-H, 23-H), 3.63 (m, 1H, 3-H), 3.57 (m, 1H, 2-H), 3.53 (m, 1H, 23-H), 3.37 (m, 1H, 12-H), 3.06 (m, 1H, 12-H), 1.6 (s, 3H, 8-H), 1.2 (s, 9H, 25-H). **$^{13}$C-NMR** (100 MHz, $CD_3OD$): $\delta$ = 174.4 (9-C), 173.8 (11-C), 168.2 (21-C), 166.7 (1-C), 137.9 (15-C), 131.6 (5-C), 129.3 (4-C), 128.2 (20-C), 123.5 (14-C), 120.9 (17-C), 118.2 (19-C), 118.0 (18-C), 110.8 (16-C), 108.8 (13-C), 74.8 (24-C), 62.9 (23-C), 56.2 (22-C), 56.1 (10-C), 54.0 (7-C), 51.9 (C-6), 52.0 (2-C), 45.9 (3-C), 27.0 (12-C), 26.2 (25-C), 12.3 (8-C). **IR** (KBr) ν ($cm^{-1}$): 3446.7 (NH), 1646.9 (CO). **HPLC-ESI-MS**: m/z (%) = 527.2 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{27}H_{39}N_6O_5$ $[M+H]^+$ 527.2975; found 527.2976. $R_f$ = 0.36 (EtOAc/EtOH, 1/1).

***tert*-Butyl-(*S*)-1-((*S*)-1-((*S*)-1-((2*S*,5*S*,*E*)-5-((1H-indol-3-yl)methyl)-7-(2-amino-2-oxoethyl)-2-methyl-3,6-dioxo-1,4,7-triazacycloundec-9-en-1-yl)-3-*tert*-butoxy-1-oxopropan-2-ylamino)-1-oxo-3-phenyl-propan-2-ylamino)-3-(4-*tert*-butoxyphenyl)-1-oxopropan-2-ylcarbamate (TVC-146, 60)**

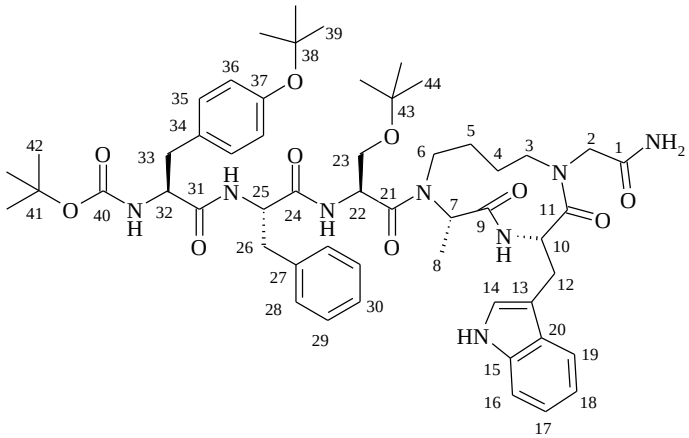


DIPEA (50 µl, 303 µmol) was added to a solution of **65** (32 mg, 60.8 µmol), Boc-Tyr(*t*Bu)-Phe-OH (35.3 mg, 73 µmol) and HATU (30 mg, 80 µmol) in DMF (2 mL). The reaction mixture was stirred for 20 h and followed by evaporation of the solvent. Flash chromatography (EtOAc/EtOH, 10/1)

gave pure product **60** (46 mg, 76%) as a solid.

**$^1$H-NMR** (600 MHz, DMSO-$d_6$): $\delta$ = 7.68 (m, 1H, 19-H), 7.3 (m, 1H, 16-H), 7.25-7.20 (m, 4H, 28-H, 29-H), 7.14 (t, *J* = 7.0 Hz, 1H, 30-H), 7.09 (overlap, 2H, 35-H), 7.03 (overlap, 1H, 17-H), 7.01 (s, 1H, 14-H), 6.96 (t, *J* = 7.5 Hz, 1H, 18-H), 6.83 (d, *J* =8.4 Hz, 2H, 36-H), 5.76 (br, 1H, 5-H), 5.2 (br, 1H, 4-H), 4.78 (br, 1H, 22-H), 4.7 (m, 1H, 25-H), 4.68 (bs, 1H, 7-H), 4.6 (m, 1H, 10-H), 4.1 (br, 1H, 32-H), 3.92 (m, 2H, 6-H), 3.9 (overlap, 1H, 2-H), 3.7 (overlap, 1H, 2-H), 3.58 (br, 1H, 23-H), 3.37 (overlap, 1H, 23-H), 3.27 (m, 2H, 3-H), 3.2 (overlap, 1H, 12-H), 2.8 (m, 1H, 12-H), 3.1 (m, 1H, 26-H), 2.98 (m, 1H, 26-H), 2.8 (m, 1H, 33-H), 2.6 (t, *J* = 11.3 Hz, 1H, 33-H), 1.45 (s, 3H, 8-H), 1.26 (s, 9H, 39-H), 1.15 (s, 9H, 42-H), 1.26 (s, 9H, 44-H). **$^{13}$C-NMR** (150 MHz, DMSO-$d_6$): $\delta$ = 174.3 (9-C), 173.7 (11-C), 173.2 (24-C), 172.9 (31-C), 154.2 (37-C), 138.4 (27-C), 136.6 (15-C), 131.8 (5-C), 130.4 (35-C), 129.8 (29-C), 128.3 (28-C), 126.5 (30-C), 126.0 (4-C), 123.6 (36-C), 123.4 (14-C), 121.2 (17-C), 118.7 (19-C), 118.5 (18-C), 111.5 (16-C), 77.5 (38-C), 72.8 (43-C), 72.2 (41-C), 62.8 (23-C), 56.2 (32-C), 53.8 (7-C), 53.6 (25-C), 53.3 (10-C), 52.2 (2-C), 49.9 (22-C), 46.6 (6-C), 43.1 (3-C), 38.0 (26-C), 37.3 (33-C), 28.5 (39-C), 28.3 (44-C), 27.7 (12-C), 27.2 (42-C), 13.8 (8-C). **IR** (KBr) ν ($cm^{-1)}$: 3419.3 (NH), 1653.9 (CO). **HPLC-ESI-MS**: m/z (%) = 1015.5 (100) $[M+Na]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{54}H_{72}N_8O_{10}Na$ $[M+Na]^+$ 1015.5264; found 1015.5264. $R_f$ = 0.17 ( DCM/ MeOH, 20/1).

***tert*-Butyl-(*S*)-1-((*S*)-1-((*S*)-1-((2*S*,5*S*)-5-((1H-indol-3-yl)methyl)-7-(2-amino-2-oxoethyl)-2-methyl-3,6-dioxo-1,4,7-triazacycloundecan-1-yl)-3-*tert*-butoxy-1-oxopropan-2-ylamino)-1-oxo-3-phenylpropan-2-ylamino)-3-(4-*tert*-butoxyphenyl)-1-oxopropan-2-ylcarbamate (TVC-155)**



A suspension of **60** (28 mg, 28.19 µmol) and Pd/C (20 wt%, 5.6 mg) in MeOH/EtOAc (6 mL, 2/1) was stirred at room temperature under a hydrogen atmosphere for 20 h. The Pd/C catalyst was removed by filtration over a Celite pad. Removal of the solvent yielded pure product **TVC-155** (18 mg, 97%) as a brown solid.

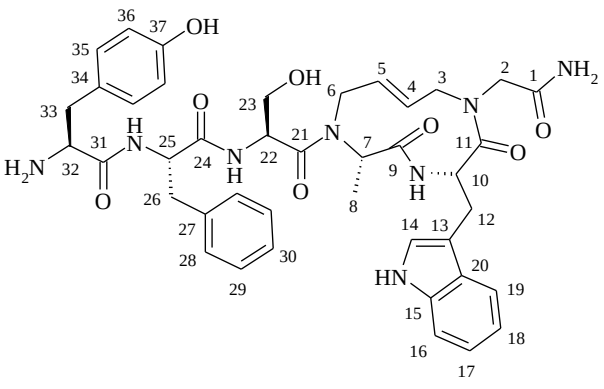
**$^1$H-NMR** (600 MHz, $CD_3OD$): $\delta$ = 7.62 (m, 1/3H, 19-H), 7.43 (d, *J* = 8.3 Hz, 2/3H, 19-H), 7.37 (d, *J* = 7.5 Hz, 1H, 16-H), 7.27-7.20 (m, 5H), 7.12 (overlap, 2H, 35-H), 7.1 (overlap, 1H, 17-H), 7.09 (s, 1H, 14-H), 7.03 (t, *J* = 7.5 Hz, 1H, 18-H), 6.91 (d, *J* =8.4 Hz, 2H, 36-H), 4.8 (m, 1H, 22-H), 4.59 (t, *J* = 7.3 Hz, 1H, 25-H), 4.55 (d, *J* = 7.0 Hz, 1H, 7-H), 4.36 (dd, *J* = 4.0 Hz, *J* = 10.3 Hz, 1H, 32-H), 4.22 (m, 1H, 10-

H), 4.06 (d, *J* = 16.2 Hz, 1H, 2-H), 3.86 (d, *J* = 16.2 Hz, 1H, 2-H), 3.62 (m, 1H, 23-H), 3.49 (overlap, 1H, 12-H), 3.48 (overlap, 1H, 23-H), 3.47 (t, *J* = 6.0 Hz, 2H, 3-H), 3.39 (m, 2H, 6-H), 3.18 (m, 1H, 12-H), 3.17 (m, 1H, 26-H), 3.07 (dd, *J* = 4.0 Hz, *J* = 14.3 Hz, 1H, 33-H), 2.84 (m, 1H, 26-H), 2.61 (t, *J* = 11.3 Hz, 1H, 33-H), 1.6 (m, 2H, 5-H), 1.55 (m, 2H, 4-H), 1.36 (s, 9H, 39-H), 1.15 (s, 9H, 42-H), 1.38 (overlap, 3H, 8-H), 1.3 (s, 9H, 44-H). **$^{13}$C-NMR** (150 MHz, $CD_3OD$): $\delta$ = 174.0 (9-C), 173.2 (11-C), 173.0 (24-C), 172.6 (31-C), 155.2 (37-C), 138.0 (27-C), 137.6 (15-C), 129.4 (35-C), 129.2 (29-C), 127.8 (28-C), 126.3 (30-C), 123.8 (36-C), 123.4 (14-C), 121.0 (17-C), 118.5 (18-C), 117.3 (19-C), 111.0 (16-C), 79.5 (43-C), 74.7 (41-C), 61.5 (23-C), 56.6 (32-C), 56.0 (10-C), 54.7 (7-C), 52.7 (25-C), 50.2 (22-C), 49.8 (2-C), 46.6 (6-C), 40.1 (3-C), 37.5 (26-C), 37.1 (33-C), 27.6 (44-C), 27.3 (39-C), 26.4 (5-C), 26.2 (42-C), 25.6 (12-C), 24.9 (4-C), 13.5 (8-C). **IR** (KBr) ν ($cm^{-1}$): 3420.3 (NH), 1654.9 (CO). **HPLC-ESI-MS**: m/z (%) = 996.6 (80) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{54}H_{74}N_8O_{10}Na$ $[M+Na]^+$ 1017.5420; found 1017.5440. $R_f$ = 0.22 (DCM/MeOH, 20/1).

**General procedure for *N*-Boc and *O*-*t*Bu deprotection**

Peptide **60** or **TVC-155** was treated with TFA/TIPS/$H_2O$ (2 mL, 95/3.75/1.25) at 0 $^oC$ for 4-5 h. After that time, the solution was evaporated and the residue was purified by silica gel column chromatography (DCM/MeOH, 4/1, 0.1% TEA). The pure products were dissolved in ACN/$H_2O$ (2 mL, 1/1) and lyophilized to provide white powders.

**(*S*)-N-((*S*)-1-((2*S*,5*S*,*E*)-5-((1H-Indol-3-yl)methyl)-7-(2-amino-2-oxoethyl)-2-methyl-3,6-dioxo-1,4,7-triazacycloundec-9-en-1-yl)-3-hydroxy-1-oxopropan-2-yl)-2-((S)-2-amino-3-(4-hydroxyphenyl) propanamido)-3-phenylpropanamide (TVC-113, 66)**

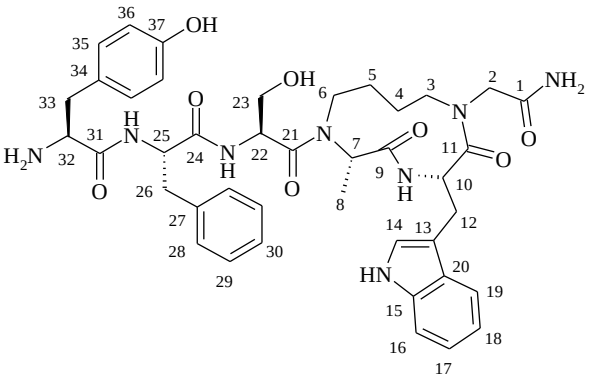


Starting material **60** (10 mg, 2.01 µmol) afforded product **66** (6.8 mg, 86%).

**$^1$H-NMR** (400 MHz, DMSO-$d_6$): $\delta$ = 7.6 (m, 1H, 19-H), 7.3 (m, 1H, 16-H), 7.3-7.24 (m, 4H), 7.18 (t, *J* = 7.0 Hz, 1H, 30-H), 7.03 (overlap, 2H, 35-H), 7.03 (overlap, 1H, 17-H), 7.01 (s, 1H, 14-H), 6.97 (t, *J* = 7.5 Hz, 1H, 18-H), 6.69 (d, *J* =8.4 Hz, 2H, 36-H), 5.78 (br, 1H, 5-H), 5.44 (br, 1H, 4-H), 4.88 (m, 1H, 25-H), 4.79 (br, 1H, 22-H), 4.69 (bs, 1H, 7-H), 4.67 (m, 1H, 10-H), 4.07 (overlap, 1H, 23-H), 4.05 (m, 2H, 6-H), 3.96 (m, 1H, 2-H), 3.94 (br, 1H, 32-H), 3.89 (br, 1H, 23-H), 3.88 (m. 1H, 2-H): 3.52

(overlap, 1H, 3-H), 3.41 (overlap, 1H, 3-H), 3.2 (overlap, 1H, 12-H), 3.16 (m, 1H, 12-H), 3.1 (m, 2H, 26-H, 33-H), 2.99 (m, 1H, 26-H), 2.9 (m, 1H, 33-H), 1.44 (s, 3H, 8-H). **$^{13}$C-NMR** (100 MHz, DMSO-$d_6$): $\delta$ = 174.1 (9-C), 173.8 (11-C), 173.6 (24-C), 173.2 (31-C), 155.2 (37-C), 134.4 (27-C), 136.2 (15-C), 131.9 (5-C), 131.8 (35-C), 129.8 (29-C), 129.4 (4-C), 128.5 (28-C), 126.8 (30-C), 123.9 (14-C), 121.2 (17-C), 118.9 (19-C), 118.6 (18-C), 115.8 (36-C), 111.7 (16-C), 64.1 (23-C), 57.2 (32-C), 54.7 (22-C), 54.3 (10-C), 54.1 (7-C), 51.4 (25-C), 52.0 (2-C), 51.7 (6-C), 47.8 (3-C), 37.3 (26-C), 36.3 (33-C), 27.7 (12-C), 15.3 (8-C). **HPLC-ESI-MS**: m/z (%) = 781.4 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{41}H_{49}N_8O_8$ $[M+H]^+$ 1017.5420; found 781.3626.

**(*S*)-N-((*S*)-1-((2*S*,5*S*)-5-((1H-Indol-3-yl)methyl)-7-(2-amino-2-oxoethyl)-2-methyl-3,6-dioxo-1,4,7-triazacycloundecan-1-yl)-3-hydroxy-1-oxopropan-2-yl)-2-((S)-2-amino-3-(4-hydroxy-phenyl)-propanamido)-3-phenylpropanamide (TVC-158, 67)**

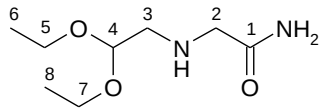


Starting material **TVC-155** (24 mg, 26.46 µmol) yielded product **67** (22 mg, 91%).

**$^1$H-NMR** (600 MHz, $CD_3OD$): $\delta$ = 7.61 (d, $J$ = 7.5 Hz, 1H, 19-H), 7.35 (d, $J$ = 7.5 Hz, 1H, 16-H), 7.28-7.19 (m, 5H), 7.19 (s, 1H, 14-H), 7.1 (overlap, 1H, 17-H), 7.08 (d, $J$ = 8.0 Hz, 2H, 35-H), 7.03 (t, $J$ = 7.5 Hz, 1H, 18-H), 6.78 (d, $J$ =8.4 Hz, 2H, 36-H), 4.81 (m, 1H, 22-H), 4.67 (t, $J$ = 7.3 Hz, 1H, 25-H), 4.63 (m, 1H, 7-H), 4.10 (m, 1H, 10-H), 4.01 (m, 1H, 32-H), 4.0 (m, 1H, 2-H), 3.85 (m, 1H, 2-H), 3.79 (m, 1H, 23-H), 3.65 (overlap, 1H, 23-H), 3.36 (overlap, 2H, 12-H), 3.47 (t, $J$ = 6.0 Hz, 2H, 3-H), 3.38 (m, 2H, 6-H), 3.15 (m, 1H, 26-H), 3.13 (m, 1H, 33-H), 2.98 (m, 1H, 26-H), 2.94 (m, 1H, 33-H), 1.59 (m, 2H, 5-H), 1.54 (m, 2H, 4-H), 1.36 (s, 3H, 7-H). **$^{13}$C-NMR** (150 MHz, $CD_3OD$): $\delta$ = 174.0 (9-C), 173.2 (11-C), 173.0 (24-C), 172.6 (31-C), 155.2 (37-C), 138.0 (27-C), 137.6 (15-C), 129.4 (35-C), 129.2 (29-C), 127.8 (28-C), 126.3 (30-C), 123.8 (36-C), 123.4 (14-C), 121.0 (17-C), 118.5 (18-C), 117.3 (19-C), 111.0 (16-C), 79.5 (43-C), 74.7 (41-C), 61.5 (23-C), 56.6 (32-C), 56.0 (10-C), 54.7 (7-C), 52.7 (25-C), 50.2 (22-C), 49.8 (2-C), 46.6 (6-C), 40.1 (3-C), 37.5 (26-C), 37.1 (33-C), 27.6 (44-C), 27.3 (39-C), 26.4 (5-C), 26.2 (42-C), 25.6 (12-C), 13.5 (8-C). **HPLC-ESI-MS**: m/z (%) = 783.4 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{41}H_{50}N_8O_8Na$ $[M+Na]^+$ 805.3644; found 805.3664.

### 3.9 Synthesis of hexapeptide TVC-044 (79)

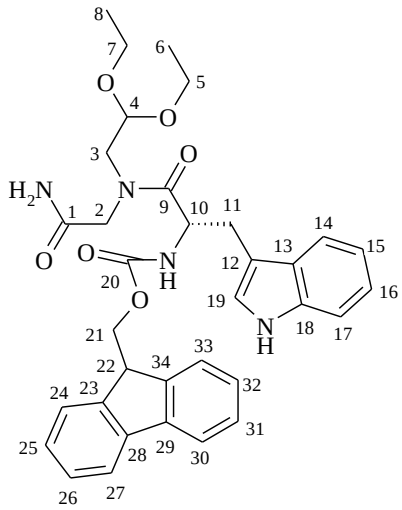
**2-[(2,2-Diethoxyethyl)amino]acetamide (TVC-001, 73)**



A suspension solution of glycinamide hydrochloride (2.5 g, 22.16 mmol) and $NaHCO_3$ (4.84g, 57.62 mmol) in ACN (120 mL) was added 2-bromacetaldehyde diethylacetal (3.33 mL, 22.16 mmol). After refluxing for 44 h, the hot reaction mixture was filtered through a Celite pad and washed with EtOAc. The filtrate was concentrated under reduced pressure. The residue was subjected to silica gel column chromatography using EtOAc/EtOH (4/1) to afford product **73** (1.56 g, 37%) as a brown oil.

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 7.18 (s, 1H, NH), 5.61 (s, 1H, NH), 4.53 (t, $J$ = 5.11 Hz, 1H, 4-H), 3.69 (quin, $J$ = 7.06 Hz, 2H, 5-H), 3.53 (quin, $J$ = 7.05 Hz, 7-H), 3.29 (s, 2H, 2-H), 2.75 (d, $J$ = 5.12 Hz, 3-H), 1.22 (t, $J$ = 7.05 Hz, 6H, 6,8-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 114.87 (1-C), 101 (4-C), 62.56 (5-C, 7 -C), 52.15 (2-C), 52.09 (3-C), 15.31 (6-C, 8-C). **IR** (KBr) ν ($cm^{-1}$): 3350.6, 1714.5, 1168.1. **HPLC-ESI-MS**: m/z (%) = 145 (100), 213 (20) $[M+Na]^+$. **HR-ESI-MS** (m/z): calcd. for $C_8H_{18}N_2O_3Na$ $[M+Na]^+$ 213.1215; found 213.1210. $R_f$ = 0.37 (EtOAc/EtOH, 4/1).

**9H-Fluoren-9-ylmethyl-N-[(1*S*)-1-[(carbamoylmethyl)(2,2-diethoxyethyl)carbamoyl]-2-(1H-indol-3-yl)ethyl]carbamate (TVC-004, 74)**

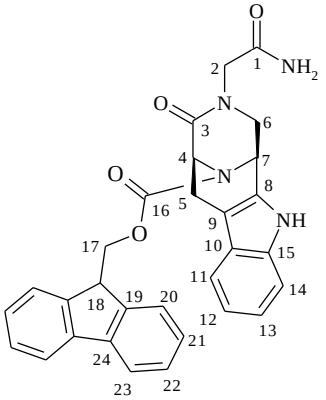


To a stirred solution of **73** (100 mg, 0.48 mmol), Fmoc-Trp-OH (278 mg, 0.65 mmol) and HATU (254.6 mg, 0.67 mmol) in DCM (4 mL), DIPEA (0.2 ml, 1.2 mmol) was added. After stirring for 20 h at room temperature, the solvent was removed. The residue was dissolved in EtOAc (40 mL), washed with $H_2O$ (2x10 mL), brine solution (2x10 mL) and dried over $Na_2SO_4$. Removal of the solvent and column chromatography, using EtOAc as eluent, yielded product **74** (290 mg, 100%) as a yellow solid.

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 8.6 (s, 1H, NH), 7.74 (d, $J$ = 7.5 Hz, 2H, 27-H, 30-H), 7.7 (d, $J$ = 7.7 Hz, 1H, 14-H), 7.53 (d, $J$ = 7.5 Hz, 2H, 24-H, 33-H), 7.38 (t, $J$ = 7.5 Hz, 2H, 26-H, 31-H), 7.33 (m, 1H, 17-H), 7.28 (m, 2H, 25-H, 32-H), 7.17 (m, 1H, 16-H), 7.10 (m, 1H, 15-H), 7.06 (s, 1H, 4-H), 6.2 (br, 1H, NH), 5.85 (d, $J$ = 7.3 Hz, 1H, NH), 5.8 (br, 1H, NH), 5.7 (d, $J$ = 8 Hz, 1H, NH), 5.00 (q, $J$ = 6.6 Hz, 1H, 10-H), 4.5 (t, $J$ = 5 Hz, 1/3 H, 4-H), 4.3 (m, 2H, 21-H), 4.17 (t, $J$ = 5.7 Hz, 2/3H, 4-H), 4.15 (br, 1H, 22-H), 3.97 (q, $J$ = 16 Hz, 2H, 2-H), 3.46-3.38, 3.29-3.25 (m, 4H, 5-H, 7-H), 3.3, 3.17 (m, 4H, 11-H, 3-H), 1.02 (m, 6H, 6-H, 8-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 173.7, 171, 156.1 (C=O), 143.7, 141.2 (C-23), 136.2 (C-18), 127.7 (C-28),

127.2 (C-13), 127.0 (C-25), 125.1 (C-24), 123.7 (C-4), 122.3 (C-16), 14.9 (C-27), 14.7 (C-15), 118.5 (C-14), 109.6 (C-12), 101.2 (C-4), 67.1 (C-21), 63.6, 63.4 (C-7, C-5), 52.1 (C-2), 51.8 (C-3), 51.2 (C-10), 47.2 (C-22), 29.7 (C-11), 15.2, 15.1 (C-6, C-8). **IR** (KBr) ν ($cm^{-1}$): 3421.9 (NH), 1684.9, 1636.6 (CO). **HPLC-ESI-MS**: m/z (%) = 553.2 (100) $[M-C_2H_5O]^+$, 616.3 (7.6) $[M+NH_4]^+$, 621.3 (16) $[M+Na]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{34}H_{38}N_4O_6Na$ $[M+Na]^+$ 621.2684; found 621.2684. $R_f$ = 0.4 (EtOAc).

**9H-Fluoren-9-ylmethyl-(1*S*,12*S*)-14-(carbamoylmethyl)-13-oxo-3,14,16-triazatetracyclo-[10.3.1.$0^{2,10}$.$0^{4,9}$]-hexadeca-2(10),4(9),5,7-tetraene-16-carboxylate (TVC-007, 75)**

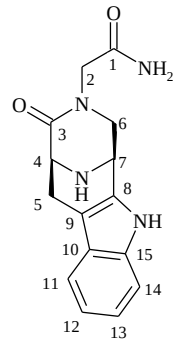



A stirred solution of **74** (120 mg, 0.2 mmol) in $CH_3CN$ (8 mL) at 0 $^oC$ was added dropwise 1N HCl (4 mL). After stirring further for 3 h at 0 $^oC$, EtOAc (20 mL) was added. The organic phase was washed with $H_2O$, saturated $NaHCO_3$, brine solution and dried over $Na_2SO_4$. The solvent was removed and the residue was purified by silica gel column chromatography (EtOAc/EtOH, 10/1) to provide pure product **75** (79 mg, 75%) as a yellow solid.

**$^1$H-NMR** (600 MHz, $CDCl_3$): $\delta$ = 8.4 (s, 1H, NH), 8.03 (s, 1H, NH), 7.78 (d, *J* = 7.2 Hz, 2H, 23-H, 26-H), 7.58 (m, 2H, 20-H, 29-H), 7.45 (d, *J* = 7.8 Hz, 1H, 11-H), 7.32 (overlap, 1H, 14-H), 7.39 (m, 2H, 22-H, 27-H), 7.30 (m, 2H, 21-H, 28-H), 7.18 (m, 1H, 13-H), 7.12 (m, 1H, 12-H), 5.5 (br, 1/2H, 7-H), 5.29 (s, 1H, NH), 5.06 (br, 1/2H, 4-H), 4.99 (br, 1/2H, 7-H), 4.85 (overlap, 1/.2H, 4-H), 4.58 (q, *J* = 5.4 Hz, 2H, 17-H), 4.32 (d, *J* = 16 Hz, 1H, 2-H), 4.26-4.24 (m, 1H, 18-H), 3.92, 3.67 (d, *J* = 12 Hz, 1H, 6-H), 3.48 (d, *J* = 16 Hz, 1H, 2-H), 3.38, 3.22 (d, *J* = 12 Hz, 1H, 6-H), 3.07, 2.96 (m, 2H, 5-H). **$^{13}$C-NMR** (150 MHz, $CDCl_3$): $\delta$ = 170.0, 169.9, 153.9 (CO), 143 (C-4), 141 (C-24), 136.1 (C-15), 129.7 (C-8), 127.9 (C-22), 127.3 (C-21), 126.3 (C-10), 124.7 (C-20), 123.0 (C-13), 120.3 (C-23), 118.8 (C-11), 114.5 (C-12), 111.4 (C-14), 108.8 (C-9), 67.8 (C-17), 54.1 (C-4), 53.4 (C-6), 50.4 (C-2), 47.3 (C-18), 45.96 (C-7), 26.1 (C-5). **IR** (KBr) ν ($cm^{-1}$): 3254.5 (NH), 1683.9, 1639.3 (CO). **HPLC-ESI-MS**: m/z (%) = 507.1 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{30}H_{26}N_4O_4Na$ $[M+Na]^+$ 529.1852; found 529.1806. $R_f$ = 0.11 (EtOAc/EtOH, 10/1).

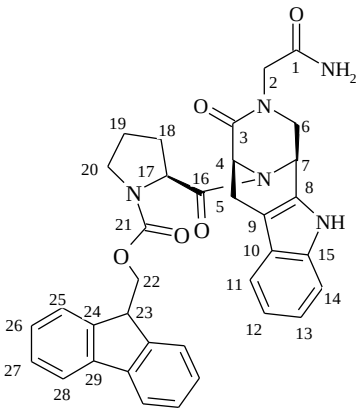
**2-[(1*S*,12*S*)-13-Oxo-3,14,16-triazatetracyclo[10.3.1.$0^{2,10}$.$0^{4,9}$]hexadeca-2(10),4(9),5,7-tetraen-14-yl]-acetamide (TVC-009, 76)**

A stirred solution of **75** (70 mg, 0.14 mmol) in DCM (4 mL) was treated with piperidine (0.14 mL, 1.4 mmol) at room temperature for 18 h. The solvent was evaporated and the crude product was purified by silica gel column chromatography (DCM/MeOH, 5/1) to furnish **76** (30 mg, 76%) as a white solid.



**$^1$H-NMR** (400 MHz, DMSO-$d_6$): $\delta$ = 10.82 (s, 1H, NH), 7.36 (d, $J$ = 7.76 Hz, 1H, 14-H), 7.30 (d, $J$ = 8.02 Hz, 1H, 11-H), 7.04 (dt, $J$ = 7.0 Hz, $J$ = 1.07 Hz, 1H, 11-H), 6.93 (dt, $J$ = 7.86 Hz, $J$ = 0.9 Hz, 1H, 12-H), 4.27 (d, $J$ = 4 Hz, 1H, 7-H), 4.12 (d, $J$ = 16 Hz, 1H, 2-H), 3.94 (dd, $J$ = 11.0 Hz, $J$ = 4.5 Hz, 1H, 6-H), 3.83 (d, $J$ = 5 Hz, 1H, 4-H), 3.24 (d, $J$ = 11.0 Hz, 1H, 6-H), 2.91 (dd, $J$ = 15.0 Hz, $J$ = 6.0 Hz, 1H, 5-H), 2.77 (d, $J$ = 15 Hz, 1H, 5-H). **$^{13}$C-NMR** (100 MHz, DMSO-$d_6$): $\delta$ = 170.9 (1-C), 169.7 (3-C), 135.4 (15-C), 134.5 (8-C), 126.4 (10-C), 120.7 (13-C), 118.4 (12-C), 117.4 (11-C), 111.1 (14-C), 106.5 (9-C), 54.6 (6-C), 53.2 (4-C), 48.9 (2-C), 45.0 (7-C), 26.3 (5-C). **IR** (KBr) ν ($cm^{-1}$): 3382.1 (NH), 1627.4 (CO). **HPLC-ESI-MS**: m/z (%) = 268.1 (100) $[M\text{-}NH_4]^+$, 185.1 $[M+H]^+$, 307.1 $[M+Na]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{15}H_{16}N_4O_2Na$ $[M+Na]^+$ 307.1171; found 307.1165. $R_f$ = 0.15 (MeOH/DCM, 2/5).

**9H-Fluoren-9-ylmethyl-(2*S*)-2-[(1*S*,12*S*)-14-(carbamoylmethyl)-13-oxo-3,14,16-triazatetracyclo-[10.3.1.0$^{2,10}$.0$^{4,9}$]hexadeca-2(10),4(9),5,7-tetraene-16-carbonyl]pyrrolidine-1-carboxylate (TVC-034, 77)**

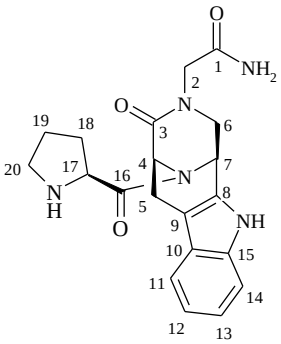


A solution of **76** (250 mg, 0.88 mmol) and Fmoc-L-Pro-OH (385.7 mg, 1.14 mmol) in DMF (10 mL) was added HATU (434.5 mg, 1.14 mmol) and DIPEA (0.44 mL, 3.52 mmol). After stirring at room temperature for 28 h, the reaction mixture was quenched with EtOAc (200 mL), washed with $H_2O$ and brine solution. The crude product was chromatographed eluting EtOAc/EtOH (15/1) to yield the desired product **77** (474 mg, 89% yield) as a yellow solid.

**$^1$H-NMR** (600 MHz, $CDCl_3$): $\delta$ = 9.0 (s, 1H, NH, ), 7.74 (dd, $J$ = 3.3 Hz, $J$ = 7.5 Hz, 2H, 28-H), 7.53 (d, $J$ = 7.5 Hz, 1H, 25-H), 7.49 (d, $J$ = 7.5 Hz, 1H, 25'-H), 7.45 (d, $J$ = 7.9 Hz, 1H, 11-H), 7.39 (q, $J$ = 7 Hz, 2H, 27-H), 7.32-7.29 (m, 3H, 14-H, 26-H), 7.16 (t, $J$ = 7.8 Hz, 1H, 13-H), 7.09 (t, $J$ = 7.4 Hz, 12-H), 6.8 (s, 1H, NH), 5.97 (d, $J$ = 3.8 Hz, 1H, 7-H), 5.29 (overlap, 1H, 4-H), 4.63 (t, $J$ = 6.9 Hz, 1H, 17-H), 4.46 (d, $J$ = 16.9 Hz, 1H, 2-H), 4.27 (m, 1H, 22-H), 4.13 (overlap, 1H, 22-H), 4.13 (br., 1H, 23-H), 4.01 (dd, $J$ = 4.3 Hz, $J$ = 11.8 Hz, 1H, -H), 3.65 (m, 1H, 20-H), 3.6 (m, 1H, 20-H), 3.37 (d, $J$ = 15.1 Hz, 1H, 5-H), 3.28 (d, $J$ = 16.9 Hz, 1H, 2-H), 3.26 (d, $J$ = 11.8 Hz, 1H, 6-H), 3.18 (dd, $J$ = 5.6 Hz, $J$ = 15.6 Hz, 1H, 5-H), 2.20-2.13 (m, 2H, 18-H, 4-

H), 2.10-2.07 (m, 1H, 4-H), 1.97-1.92 (m, 1H, 18-H). **$^{13}$C-NMR** (150 MHz, $CDCl_3$): $\delta$ = 40.6 (1-C), 168.2 (16-C), 162.5 (3-C), 155.2 (21-C), 143.6 (24-C), 141.2 (29-C), 136.3 (15-C), 129.96 (8-C), 127.9 (27-C), 127.2 (26-C), 126.1 (10-C), 125.1 (28-C), 122.7 (13-C), 120.08 (12-C), 120.04 (25-C), 118.4 (11-C), 111.4 (14-C), 108.2 (9-C), 67.7 (22-C), 56.3 (4-C), 55.6 (17-C), 52.4 (6-C), 50.8 (2-C), 47.0 (20-C), 46.9 (23-C), 44.4 (7-C), 30.6 (4-C), 27.1 (5-C), 25.1 (4-C). **IR** (KBr) ν ($cm^{-1}$): 3308.2 (NH), 1662.9 (CO). **HPLC-ESI-MS**: m/z (%) = 604.2 (100) $[M+H]^+$, 587.2 (25) $[M-NH_3]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{35}H_{33}N_5O_5Na$ $[M+Na]^+$ 626.2379; found 626.2374. $R_f$ = 0.37 (AcOEt/EtOH, 6/1).

**2-[(1*S*,12*S*)-13-Oxo-16-[(2*S*)-pyrrolidine-2-carbonyl]-3,14,16-triazatetracyclo[10.3.1.0$^{2,10}$.0$^{4,9}$] hexadeca-2(10),4(9),5,7-tetraen-14-yl]acetamide (TVC-035, 78)**



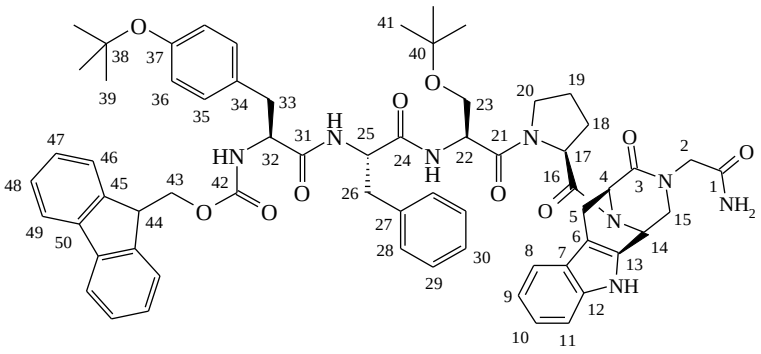
Compound **77** (286 mg, 0.47 mmol) in DCM (15 mL) was treated with piperidine (0.47 mL, 4.74 mmol) at room temperature for 20 h. Afterward, the reaction mixture was concentrated under reduced pressure. The residue was subjected to silica gel column chromatography using EtOH/EtOAc (gradient from 40/60 to 90/10, 2% TEA) to afford **78** (180 mg, 99%) as a solid.

**$^1$H-NMR** (600 MHz, $CD_3OD$): $\delta$ = 7.41 (dd, *J* = 7.9 Hz, 1H, 11-H), 7.35 (dd, *J* = 8.1 Hz, 1H, 14-H), 7.12 (t, *J* = 7.8 Hz, 1H, 13-H), 7.02 (m, 1H, 12-H), 6.0 (d, *J* = 3.9 Hz, 3/5H, 7-H), 5.6 (d, *J* = 3.6 Hz, 2/5H, 7-H), 5.4 (d, *J* = 5.4 Hz, 2/5H, 4-H), 5.1 (s, 3/5H, 4-H), 4.25 (dd, *J* = 16.6 Hz, 1H, 2-H), 4.16 (dd, *J* = 4.2 Hz, 2/5H, 6-H), 4.12 (t, *J* = 7 Hz, 3/5H, 17-H), 4.03 (dd, *J* = 4.3 Hz, 3/5H, 6-H), 3.97 (t, *J* = 6.7 Hz, 2/5H, 17-H), 3.68 (dd, *J* = 16.6 Hz, 1H, 2-H), 3.55 (d, *J* = 11.9 Hz, 2/5H, 6-H), 3.46 (dd, *J* = 11.4 Hz, 3/5H, 6-H), 3.2-3.16 (m, 2H, 5-H), 3.12-3.10 (m, 2/5H, 20-H), 3.04 (m, 3/5H, 20-H), 2.87-2.84 (m, 3/5H, 20-H), 2.80-2.77 (m, 2/5H, 20-H), 2.3 (m, 2/5H, 18-H), 2.2 (m, 3/5H, 18-H), 1.8 (m, 1H, 18-H), 1.9-1.8 (m, 2H, 4-H). **$^{13}$C-NMR** (150 MHz, $CD_3OD$): $\delta$ = 173.1 (1-C), 172.5, 172.4 (3-C), 171.3, 170.8 (16-C), 138.06 (15-C), 131.6, 131.2 (8-C), 127 (10-C), 123.4, 123.2 (13-C), 120.6, 120.5 (12-C), 118.9 (11-C), 112.5 (14-C), 109.2, 108.2 (9-C), 59.2, 58.7 (17-C), 56.4, 53.5 (4-C), 55.4, 54.4 (6-C), 50.7, 50.2 (2-C), 49.2, 45.5 (7-C), 47.9 (20-C), 31.9, 31.7 (18-C), 28.2, 25.9 (5-C), 27.3 (4-C). **IR** (KBr) ν ($cm^{-1}$): 3339.5 (NH), 1669.1 (CO). **HPLC-ESI-MS**: m/z (%) = 383.2 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{20}H_{24}N_5O_3$ $[M+H]^+$ 382.1879; found 382.1874. $R_f$ = 0.2 (EtOH/TEA, 98/2).

**9H-Fluoren-9-ylmethyl-[(1*S*)-1-{[(1*S*)-1-{[(2*S*)-3-(*tert*-butoxy)-1-[(2*S*)-2-[(12*S*)-14-(carbamoyl-methyl)-13-oxo-3,14,16-triazatetracyclo[10.3.1.0$^{2,10}$.0$^{4,9}$]hexadeca-2(10),4(9),5,7-tetraene-16-**

**carbonyl]pyrrolidin-1-yl]-1-oxopropan-2-yl]carbamoyl}-2-phenylethyl]carbamoyl}-2-[4-(*tert*-butoxy)phenyl]ethyl]carbamate (TVC-037)**

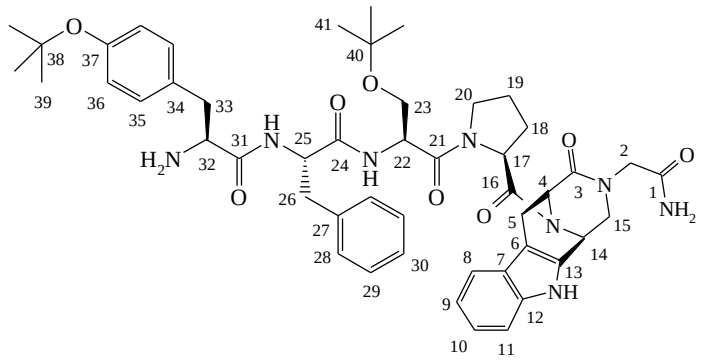
A solution of tripeptide Fmoc-Tyr(*t*Bu)-Phe-Ser(*t*Bu)-OH (170 mg, 0.23 mmol) and **78** (99 mg, 0.26 mmol) in DMF (9 mL) was added HATU (103 mg, 0.27 mmol) and followed by DIPEA (0.11 mL, 0.68 mmol). After stirring at room temperature for 20 h, the reaction mixture was quenched with EtOAc (200 mL), washed with $H_2O$ and brine solution. Flash chromatography (EtOAc/EtOH, 40/1) afforded pure product **TVC-037** (170 mg, 67%) as a white solid.



**$^1$H-NMR** (600 MHz, $CDCl_3$): $\delta$ = 10.4 (s, 1H, NH), 8.26 (d, *J* = 9.5 Hz, 1H, NH), 8.0 (s, 1H, NH), 7.8 (d, *J* = 5.3 Hz, 1H, NH), 7.56 (m, 2H, 49-H), 7.37 (m, 2H, 48-H), 7.31 (t, *J* = 7.5 Hz, 1H), 7.29 (t, *J* = 6.4 Hz, 1H), 7.22 (t, *J* = 7.5 Hz, 1H), 7.17 (t, *J* = 7.4 Hz, 1H, 10-H), 7.09 (m, 3H, 35-H, 9-H), 6.84 (m, 2H, 36-H), 5.36 (m, 1H, 14-H), 5.1, 4.62 (m, 1H, 17-H), 4.83 (d, *J* = 5.0 Hz, 1H, 4-H), 4.46 (dd, *J* = 4.3 Hz, *J* = 12.0 Hz, 1H, 22-H), 4.31 (d, *J* = 16.8 Hz, 1H, 2-H), 4.12 (m, 1H, 43-H), 3.84 (d, *J* = 16.9 Hz, 1H, 2-H), 3.42 (d, *J* = 15.5 Hz, 1H, 5-H), 3.4 (d, *J* = 15.5 Hz, 2H, 23-H), 3.16 (d, *J* = 5.6 Hz, 1H, 5-H), 2.90, 2.51 (m, 2H, 20-H), 2.20, 1.93 (m, 2H, 18-H), 2.06 (overlap, 1H, 19-H), 1.56 (overlap, 1H, 19-H), 1.51 (s, 9H, 39-H), 1.09, (s, 9H, 41-H).**$^{13}$C-NMR** (150 MHz, $CDCl_3$): $\delta$ = 171.4 (31-C), 170.6 (24-C), 170.5 (21-C), 169.9 (16-C), 169.1 (3-C), 167.7 (1-C), 155.4 (42-C), 154.4 (37-C), 143.8, 143.6 (50-C), 141.3, 141.2 (45-C), 137.5 (27-C), 136.6, 136.3, 136.2 (12-C), 131.3 (34-C), 130.3, 130.2 (13-C), 129.9, 129.7 (35-C), 128.5, 128.2 (29-C), 127.7, 127.6 (48-C), 127.1 (28-C), 126.9, 126.7 (47-C), 126.1 (7-C), 125.4 (30-C), 125.0, 124.8 (46-C),124.5, 124.7 (36-C), 122.8, 122.4 (9-C), 120.0 (49-C), 118.4, 118.3 (8-C), 114.7, 114.5 (10-C), 106.5 (6-C), 80.8 (38-C), 78.3(40-C), 67.3, 65.6 (43-C), 47.3, 46.9 (44-C), 61.2, 60.3 (23-C), 57.4, 55.7 (17-C), 54.7 (4-C), 52.8 (22-C, 14-C), 51.9 (2-C), 47.7 (20-C), 30.8 29.7 (18-C), 29.1, 28.8, 27.3, 27.1 (29-C, 41-C), 25.7 (19-C). **IR** (KBr) ν ($cm^{-1}$): 3406.9 (NH), 1660.4 (CO). **HPLC-ESI-MS**: m/z (%) = 1113.5 (100) $[M+H]^+$, 1130.5 (79.9) $[M+NH_4]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{64}H_{72}N_8O_{10}Na$ $[M+Na]^+$ 1135.5269; found 1135.4933. $R_f$ = 0.23 (EtOAc/EtOH, 40/1).

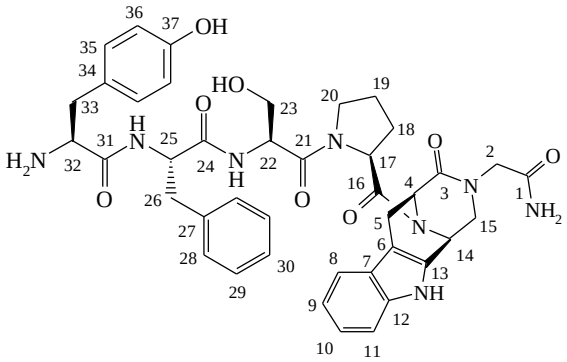
**(2*S*)-2-[(2*S*)-2-Amino-3-[4-(*tert*-butoxy)phenyl]propanamido]-N-[(2*S*)-3-(*tert*-butoxy)-1-[(2*S*)-2-[(1*S*)-14-(carbamoylmethyl)-13-oxo-3,14,16-triazatetracyclo[10.3.1.0$^{2,10}$.0$^{4,9}$] hexadeca-2(10),4(9),5,7-tetraene-16-carbonyl]pyrrolidin-1-yl]-1-oxopropan-2-yl]-3-phenylpropanamide (TVC-041)**

To a solution of **TVC-037** (125 mg, 54 µmol) in DCM (12 mL), piperidine (0.2 mL, 100 µmol) was added and stirred at room temperature for 4 h. After removing the solvent, the crude product was purified on silica gel column chromatography eluting AcOEt/EtOH (10/1) to afford **TVC-041** (75 mg, 75%) as a white solid.



**$^{1}$H-NMR** (600 MHz, $CDCl_3$): $\delta$ = 8.6, 7.97, 7.68 (s, 1H, NH), 7.45 (d, *J* = 7.8 Hz, 1H, 8-H), 7.30 (m, 1H, 11-H), 7.26 (m, 3H, 28-H, 30-H), 7.17 (t, *J* = 7.0 Hz, 1H, 10-H), 7.11 (overlap, 1H, 9-H), 7.08 (overlap, 2H, 29-H), 7.02 (d, *J* = 8.3 Hz, 2H, 35-H), 6.90 (d, *J* = 8.4 Hz, 2H, 36-H), 6.37 (s, NH), 5.89 (d, *J* = 3.8 Hz, 1H, 14-H), 5.37 (d, *J* = 5.3 Hz, 1H, 4-H), 4.8 (d, *J* = 17.3 Hz, 1H, 2-H), 4.76 (m, 1H, 25-H), 4.67 (q, *J* = 6.4 Hz, 1H, 22-H), 4.61 (t, *J* = 7.3 Hz, 1H, 17-H), 4.03-4.0 (m, 1H, 15-H), 3.2 (d, *J* = 11.6 Hz, 1H, 15-H), 3.78 (m, 1H, 20-H), 3.72 (m, 1H, 20-H), 3.63 (dd, *J* = 4 Hz, *J* = 8.6 Hz, 1H, 32-H), 3.49 (m, 1H, 23-H), 3.34 (d, *J* = 15.6 Hz, 1H, 5-H), 3.25 (t, *J* = 8.4 Hz, 1H, 23-H), 3.15 (dd, *J* = 5.6 Hz, *J* = 15.6 Hz, 1H, 5-H), 3.03 (t, *J* = 6.7 Hz, 2H, 26-H), 3.0 (overlap, 1H, 2-H), 2.96 (m, 1H, 5-H), 2.96 (m, 1H, 20-H), 2.46 (dd, *J* = 8.8 Hz, 1H, 33-H), 2.23 (m, 1H, 18-H), 2.09 (m, 2H, 19-H), 1.31 (s, 9H, 39-H), 1.09 (s, 9H, 41-H). **$^{13}$C-NMR** (150 MHz, $CDCl_3$): $\delta$ = 175.3 (24-C), 171.0 (31-H), 170.3 (16-C), 169.8 (3-C), 168.3 (1-C), 154.2 (37-C), 136.4 (27-C), 136.3 (12-C), 131.9 (13-C), 129.9 (7-C), 129.8 (35-C), 129.5 (29-C), 128.6 (28-C), 126.9 (30-C), 126.2 (34-C), 124.2 (36-C), 122.7 (10-C), 120.0 (8-C), 118 (9-C), 111.3 (11-C), 108.9 (6-C), 78.3 (38-C), 73.7 (40-C), 61.3 (23-C), 56.7 (4-C), 55.8 (32-C), 55.7 (17-C), 53.4 (25-C), 52.5 (15-C), 51.7 (22-C), 51.2 (2-C), 47.6 (20-C), 44.5 (14-C), 39.6 (33-C), 37.5 (26-C), 29.8 (18-C), 26.9 (5-C), 25.8 (19-C). **IR** (KBr) ν ($cm^{-1}$): 3410.9 (NH), 1659.4, 1639.3 (CO). **HPLC-ESI-MS**: m/z (%) = 891.4 (100) $[M+H]^+$, 913.4 (45) $[M+Na]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{49}H_{63}N_8O_8$ $[M+H]^+$ 891.4769; found 891.4763. $R_f$= 0.16 (EtOAc/EtOH, 10/1).

**(2*S*)-2-[(2*S*)-2-Amino-3-(4-hydroxyphenyl)propanamido]-N-[(2*S*)-1-[(2*S*)-2-[(1*S*)-14-(carbamoyl-methyl)-13-oxo-3,14,16-triazatetracyclo[10.3.1.0$^{2,10}$.0$^{4,9}$]hexadeca-2(10),4(9),5,7-tetraene-16-carbonyl]pyrrolidin-1-yl]-3-hydroxy-1-oxopropan-2-yl]-3-phenylpropanamide (TVC-044, 79)**



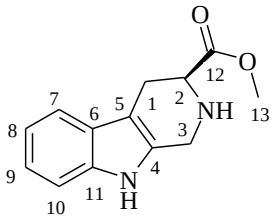
Peptide **TVC-041** (35 mg, 22 µmol) was treated with TFA/TIPS/$H_2O$ (2 mL, 95/2.5/2.5) for 4 h at 0 ºC. After that, the solvent was evaporated under reduced

pressure. The residue was purified by silica gel column chromatography using DCM/MeOH (4/1, 0.1% TEA). The pure product **79** (33.1 mg, 94 %) was dissolved in ACN/$H_2O$ (3 mL, 1/1) and lyophilized to afford white powder.

**$^1$H-NMR** (600 MHz, DMSO-$d_6$): $\delta$ = 11.0 (s, 1H, NH), 8.72 (s, 1H, NH), 8.7 (s, 1H, NH), 7.42 (d, *J* = 7.8 Hz, 1H, 8-H), 7.35 (d, *J* = 8.2 Hz, 1H, 11-H), 7.28 (m, 2H, 29-H), 7.21 (m, 3H, 28-H, 30-H), 7.1 (overlap, 1H, 9-H), 7.05 (d, *J* = 8.4 Hz, 2H, 35-H), 6.99 (t, *J* = 7.6 Hz, 1H, 10-H), 6.7 (d, *J* = 8.4 Hz, 2H, 36-H), 5.76 (d, *J* = 4 Hz, 1H, 14-H), 5.37 (d, *J* = 4.4 Hz, 1H, 4-H), 4.89 (t, *J* = 7.2 Hz, 1H, 17-H), 4.7 (m, 1H, 25-H), 4.58-4.57 (m, 1H, 22-C), 4.3 (d, *J* = 16.8 Hz, 1H, 2-C), 3.9 (br, 1H, 32-H), 3.85 (dd, *J* = 4.3 Hz, *J* = 12 Hz, 1H, 15-H), 3.75 (m, 1H, 20-H), 3.66 (m, 1H, 23-H), 3.46 (m, 1H, 23-H), 3.61 (m, 1H, 20-H), 3.38 (d, *J* = 10.4 Hz, 1H, 15-H), 3.26 (d, *J* = 16.8 Hz, 1H, 2-H), 3.11 (m, 2H, 5-H), 3.07 (d, *J* = 8.3 Hz, 1H, 26-H), 2.97 (m, 1H, 1H, 33-H), 2.79 (m, 1H, 26-H), 2.76 (m, 1H, 1H, 33-H), 2.14 (m, 1H, 18-H), 2.08 (m, 1H, 19-H), 1.89 (m, 1H, 19-H), 1.79 (m, 1H, 18-H). **$^{13}$C-NMR** (150 MHz, DMSO-$d_6$): $\delta$ = 173.1 (24-C), 172.8 (31-C), 172.7 (16-C), 171.0 (21-C), 170.8 (3-C), 169.9 (1-C), 158.3 (37-C), 138.1 (27-C), 138.07 (12-C), 131.7 (34-C), 131.6 (25-C), 130.3 (29-C), 129.7 (28-C), 127.9 (30-C), 127.5 (13-C), 125.8 (7-C), 123.0 (10-C), 120.0 (9-C), 118.9 (8-C), 116.9 (36-C), 112.5 (11-C), 108.6 (6-C), 62.5 (23-C), 57.9 (17-C), 57.6 (4-C), 56.1 (25-C), 55.6 (22-C), 54.9 (32-C), 53.8 (15-C), 51.4 (2-C), 48.9 (20-C), 46.1 (14-C), 38.6 (26-C), 37.8 (33-C), 30.8 (18-C), 28.0 (19-C), 26.5 (5-C). **IR** (KBr) ν ($cm^{-1}$): 3397.4 (NH), 1669.9 (CO). **HPLC-ESI-MS**: m/z (%) = 779.4 (100) $[M+H]^+$, 801.4 (39) $[M+Na]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{41}H_{47}N_8O_8$ $[M+H]^+$ 779.3517; found 779.3511.

### 3.10 Synthesis of hexapeptide TVC-050 (88)

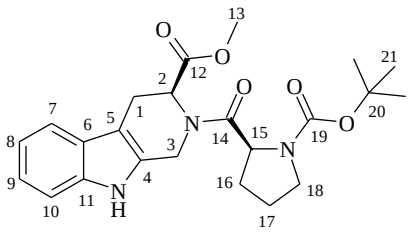
#### (*S*)-Methyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylate (TVC-020, 69)




A solution of L-Trp-OMe*HCl (800 mg, 3.14 mmol) in MeOH/$H_2O$ (10 mL, 10/1) was added dropwise 36% formaldehyde (24 mg, 0.27 mmol) in MeOH (2 mL). After stirring at room temperature for 16 h, the reaction mixture was concentrated under diminished pressure to dryness. The residue was dissolved in $H_2O$ (10 mL) and neutralized with $NaHCO_3$. The aqueous solution was extracted with EtOAc (3x50 mL). The combined organic phases were washed with brine solution (2x20 mL) and dried over $Na_2SO_4$. Removal of the solvent and flash chromatography (EtOAc/EtOH, 20/1) provided **69** (400 mg, 55%) as a white solid.

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 7.98 (s, 1H, NH), 7.5 (d, $J$ = 7.5 Hz, 1H, 7-H), 7.3 (d, $J$ = 7.9 Hz, 1H, 10-H), 7.18 (dt, $J$ = 1.5 Hz, $J$ = 7.1 Hz, 1H, 8-H), 7.12 (dt, $J$ = 0.9 Hz, $J$ = 7.2 Hz, 1H, 9-H), 4.1 (s, 2H, 3-H), 3.83 (overlap, 1H, 2-H), 3.82 (s, 3H, 13-H), 3.14 (dd, $J$ = 4.7 Hz, $J$ = 15.2 Hz, 1H, 1-H), 2.95 (dd, $J$ = 9.6 Hz, $J$ = 15.2 Hz, 1H, 1-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 173.8 (12-C), 135.9 (11-C), 131.9 (4-C), 127.2 (6-C), 121.7 (8-C), 114.5 (9-C), 117.8 (7-C), 110.7 (10-C), 107.3 (5-C), 55.9 (2-C), 52.1 (13-C), 42.1 (3-C), 25.4 (1-C). **IR** (KBr) ν ($cm^{-1}$): 3176.8 (NH), 1733.5 (CO). **HPLC-ESI-MS**: m/z (%) = 231 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{13}H_{15}N_2O_2$ $[M+H]^+$ 231.1134; found 231.1137. $R_f$ = 0.12 (AcOEt/EtOH, 20/1).

**(*S*)-Methyl-2-((*S*)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carbonyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylate (TVC-040, 83)**

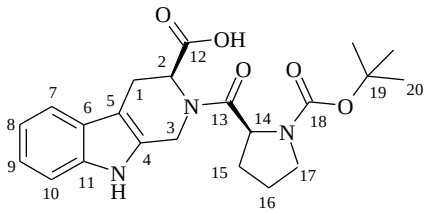


To a solution of **69** (15.0 mg, 0.07 mmol), Boc-L-Pro-OH (16.1 mg, 0.07 mmol) and HATU (30 mg, 0.08 mmol) in DMF (3 mL), DIPEA (0.032 mL, 0.02 mmol) was added. After stirring at room temperature for 24 h, EtOAc (300 mL) was added. The resulting solution was washed with $H_2O$, brine solution and dried over $MgSO_4$. Flash chromatography (eluent: CyH/EtOAc, 1/1) afforded solid product **83** (25 mg, 90%).

**$^1$H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 7.97 (s, 1/2H, NH), 7.42 (t, $J$ = 7.6 Hz, 1H, 7-H), 7.3 (t, $J$ = 8.1 Hz, 1H, 10-H), 7.1-7.0 (m, 1H, 9-H), 7.02-6.9 (m, 1H, 9-H), 5.8 (dd, $J$ = 1.6 Hz, $J$ = 6.0 Hz, 1/2H, 2-H), 5.79 (dd, $J$ = 1.5 Hz, $J$ = 6.0 Hz, 1/2H, 2-H), 4.9 (dd, $J$ = 3.7 Hz, $J$ = 8.6 Hz, 1H, 15-H), 5.2 (d, $J$ = 16.2 Hz, 1/2H, 3-H), 5.0 (d, $J$ = 15.4 Hz, 1/2H, 3-H) 4.75 (d, $J$ = 15.4 Hz, 1H, 3-H), 3.6 (s, 3H, 13-H), 3.56-3.46 (m, 2H, 18-H), 3.53-3.50 (m, 1H, 1-H), 3.1-3.0 (m, 1H, 1-H), 2.43-2.33 (m, 1H, 16-H), 2.05-2.01 (m, 1H, 16-H), 1.99-1.88 (m, 2H, 17-H), 1.4 (s, 5H, 21-H), 1.2 (s, 4H, 21-H). **$^{13}$C-NMR** (100 MHz, $CD_3OD$): $\delta$ = 176.1, 175.4 (12-C), 172.9, 172.3 (14-C), 156.4, 155.8 (4-C), 138.3 (11-C), 127.0 (6-C), 129.0 (4-C), 122.7-122.5 (9-C), 120.1, 120.06 (8-C), 118.6, 118.5 (7-C), 112.0, 111.9 (10-C), 106.6, 105.6 (5-C), 81.4 (20-C), 58.9, 58.7 (15-C), 53.0 (13-C), 52.9 (2-C), 48.1, 47.7 (18-C), 42.5, 42.4 (3-C), 30.8, 29.8 (16-C), 28.7, 28.5 (21-C), 25.0, 24.6 (17-C), 23.7, 23.4 (3-C). **IR** (KBr) ν ($cm^{-1}$): 3440.7 (NH), 1740.9, 1675.3 (CO). **HPLC-ESI-MS**: m/z (%) = 328.2 (100) $[M-Boc]^+$, 428.2 (8) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{23}H_{29}N_3O_5Na$ $[M+Na]^+$ 450.2005; found 450.1990. $R_f$ = 0.26 (EtOAc/CyH, 1/1).

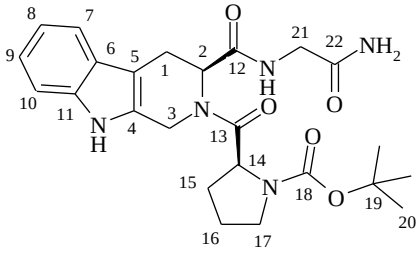
**(*S*)-2-((*S*)-1-(*tert*-Butoxycarbonyl)pyrrolidine-2-carbonyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]-indole-3-carboxylic acid (TVC-043)**

Methyl ester **83** (25 mg, 55 µmol) in THF (6 mL) was treated with 5% LiOH (2 mL) at room temperature for 5 h. Afterward, the reaction mixture was cooled to 0 °C and acidified with 1N HCl to pH≈4. The reaction mixture was extracted with EtOAc (3x50 mL), washed with $H_2O$, brine solution and dried over $MgSO_4$. Removal of the solvent afforded pure product **TVC-043** (23mg, 95%) as a solid.



**$^1$H-NMR** (600 MHz, $CD_3OD$): $\delta$ = 7.42 (t, *J* = 7.9 Hz, 1H, 7-H), 7.3-7.29 (m, 1H, 10-H), 7.08-7.07 (m, 1H, 9-H), 7.02-6.98 (m, 1H, 8-H), 5.8 (d, *J* = 6 Hz, 1/2H, 2-H), 5.76 (d, *J* = 6 Hz, 1/2H, 2-H), 5.1 (d, *J* = 15.3 Hz, 1H, 3-H), 4.76-4.78 (m, 1H, 3-H), 4.9 (dd, *J* = 3.6 Hz, *J* = 8.5 Hz, 1H, 14-H), 3.58-3.54 (m, 2H, 17-H), 3.51-3.44 (m, 1H, 1-H), 3.0-2.97 (m, 1H, 1-H), 2.4-2.3 (m, 1H, 15-H), 2.05-2.03 (m, 1H, 15-H), 1.94-1.89 (m, 2H, 16-H), 1.45 (s, 5H, 20-H), 1.3 (s, 4H, 20-H). **$^{13}$C-NMR** (150 MHz, $CD_3OD$): $\delta$ = 176.0, 175.3 (12-C), 174.8, 174-1 (13-C), 155.8, 156.4, 156.3 (18-C), 138.4, 138.2 (11-C), 129.7 (4-C), 128.1, 127.9 (6-C), 122.4, 122.3 (9-C), 118.4, 118.3 (7-C), 114.8, 114.7 (8-C), 111.7, 111.6 (10-C), 108.8, 106.9, 105.8 (5-C), 81.4, 81.3 (4-C), 59.3, 58.4 (14-C), 52.6, 52.5 (2-C), 47.9, 47.5 (17-C), 42.1, 40.4 (3-C), 30.6, 29.5 (15-C), 28.5, 28.2 (20-C), 24.8, 23.5 (1-C), 24.3, 23.9 (16-C). **IR** (KBr) ν ($cm^{-1}$): 3440.4 (NH), 1726.1, 1676.1 (CO). **HPLC-ESI-MS**: m/z (%) = 414.2 (5) $[M+H]^+$, 314.2 (100) $[M\text{-}Boc]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{22}H_{27}N_3O_5Na$ $[M+Na]^+$ 436.1848; found 436.1867. $R_f$ = 0.31 (DCM/MeOH, 4/1).

**(*S*)-*tert*-Butyl-2-((*S*)-3-((2-amino-2-oxoethyl)carbamoyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-2-carbonyl)pyrrolidine-1-carboxylate (TVC-045, 86)**

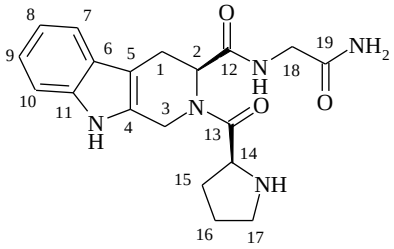


To a solution of acid **TVC-043** (120 mg, 0.29 mmol) and glycinamide hydrochloride (42 mg, 0.38 mmol) in DMF (4 mL), TBTU (112 mg, 0.35 mmol) and DIPEA (0.2 mL, 1.16 mmol) were added. After stirring at room temperature for 20 h, the solvent was removed under reduced pressure. The residue was subjected to silica gel column chromatography, using AcOEt/EtOH (15/1) as eluent, to yield product **86** (120 mg, 88%) as a yellow solid.

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 9.0 (s, 1H, NH), 8.16 (s, 1H, NH), 8.0 (s, 1H, NH), 7.46 (d, *J* = 7.1 Hz, 1H, 7-H), 7.2 (d, *J* = 7.6 Hz, 1H, 10-H), 7.08-7.02 (m, 2H, 9-H, 8-H), 6.3 (s, 1/2H, NH), 5.36 (d, *J* = 17.2 Hz, 1H, 3-H), 4.36 (d, *J* = 17.2 Hz, 1H, 3-H), 5.1 (d, *J* = 5.0 Hz, 1H, 2-H), 4.8-4.77 (m, 1H, 14-H), 3.78-3.72 (m, 2H, 21-H), 3.64-3.62 (m, 1H, 1-H), 3.52-3.50 (m, 2H, 17-H), 2.92-2.90 (m, 1H, 1-H), 2.22-2.4 (m, 1H, 15-H), 2.0 (br., 1H, 15-H), 2.14-2.1 (m, 1H, 16-H), 1.96-1.91 (m, 1H, 16-H), 1.43 (s, 4H, 20-H),

1.37 (s, 5H, 20-H). **$^{13}$C-NMR** (150 MHz, $CDCl_3$): $\delta$ = 173.1 (12-C), 171.7 (22-C), 169.8 (13-C), 155 (18-C), 136.4 (11-C), 128.7 (4-C), 126.5 (6-C), 121.7 (9-C), 118.0 (7-C), 114.4 (8-C), 111.0 (10-C), 105.6 (5-C), 80.8 (4-C), 59.0 (2-C), 56.5 (14-C), 47.0 (17-C), 43.3 (21-C), 39.2 (3-C), 29.9 (15-C), 28.3 (20-C), 24.8 (16-C), 22.7 (1-C). **IR** (KBr) ν ($cm^{-1}$): 3410.0 (NH), 1665.1 (CO). **HPLC-ESI-MS**: m/z (%) = 492.2 (23) $[M+Na]^+$, 470.2 (36) $[M+H]^+$, 370.2 (100) $[M-Boc]^+$ **HR-ESI-MS** (m/z): calcd. for $C_{24}H_{31}N_5O_5Na$ $[M+Na]^+$ 492.2223; found 492.2217. $R_f$= 0.13 (EtOAc/EtOH, 20/1).

**(*S*)-N-(2-Amino-2-oxoethyl)-2-((*S*)-pyrrolidine-2-carbonyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b] indole-3-carboxamide (TVC-047, 87)**



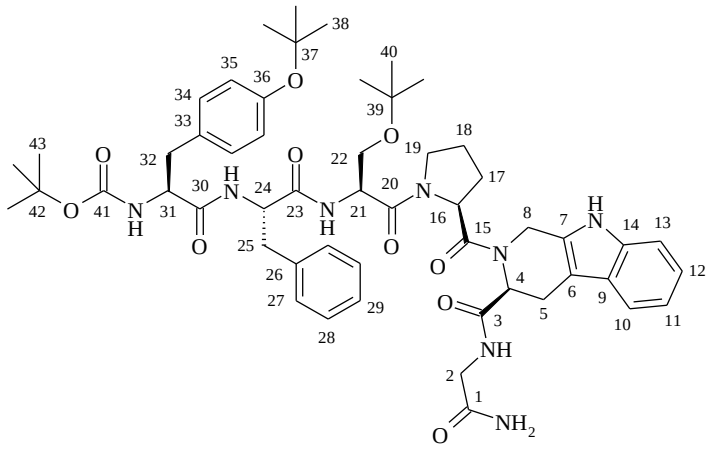

Tripeptide **86** (80 mg, 0.17 mmol) was treated with TFA/TIPS/$H_2O$ (2 mL, 95/2.5/2.5) at 0 ºC for 3 h. Afterward, the solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography using DCM/MeOH (2/1, 1% TEA) to furnish **87** (55 mg, 87%) as a solid.

**$^1$H-NMR** (600 MHz, $CD_3OD$): $\delta$ = 7.44 (d, *J* = 7.9 Hz, 1H, 7-H), 7.26 (d, *J* = 8.1 Hz, 1H, 10-H), 7.06 (q, *J* = 7.3, 1H, 9-H), 7.01-6.99 (m, 1H, 8-H), 5.6 (dd, *J* = 2.2 Hz, *J* = 6.0 Hz, 1/2H, 2-H), 5.2 (d, *J* = 6 Hz, 1/2H, 2-H), 5.26 (d, *J* = 17 Hz, 1/2H, 3-H), 5.0 (d, *J* = 15 Hz, 1/2H, 3-H), 4.8 (overlap, 1/2H, 3-H), 4.46 (d, *J* = 17 Hz, 1/2H, 3-H), 4.2 (t, *J* = 7.1 Hz, 1H, 14-H), 3.82 (d, *J* = 12.7 Hz, 1/2H, 18-H), 3.8 (d, *J* = 12.6 Hz, 1/2H, 18-H), 3.71 (d, *J* = 17 Hz, 1/2H, 18-H), 3.68 (d, *J* = 17 Hz, 1/2H, 18-H), 3.57 (d, *J* = 15.8 Hz, 1/2H, 1-H), 3.4 (d, *J* = 16.5 Hz, 1/2H, 1-H), 3.09-3.06 (m, 1H, 1-H), 3.18-3.15 (m, 1H, 17-H), 2.89-2.84 (m, 1H, 17-H), 2.37-2.32 (m, 1H, 15-H), 1.97-1.95 (m, 1/2H, 15-H), 1.83-1.8 (m, 1/2H, 15-H), 1.91-1.87 (m, 1H, 16-H), 1.83-1.82 (m, 1H, 16-H). **$^{13}$C-NMR** (150 MHz, $CD_3OD$): $\delta$ = 176.1, 175.6 (12-C), 174.1 (13-C), 173.2, 172.4 (4-C), 138.3 (11-C), 130.4 (4-C), 127.9 (6-C), 122.5 (9-C), 118.6 (7-C), 112.1 (10-C), 112.0 (8-C), 106.6 (5-C), 59.7 (14-C), 56.7, 54.7 (2-C), 48.1, 47.9 (17-C), 43.2 (18-C), 42.9, 40.6 (3-C), 31.4, 30.7 (15-C), 27.1, 27.07 (16-C), 24.3, 23.7 (1-C). **IR** (KBr) ν ($cm^{-1}$): 3426.6 (NH), 1674.1 (CO). **HPLC-ESI-MS**: m/z (%) = 370.2 (100) $[M+H]^+$, 392.2 (10) $[M+Na]^+$. **HR-ESI-MS** (m/z): calcd. for $C_4H_{23}N_5O_3Na$ $[M+Na]^+$ 392.1699; found 392.1693. $R_f$=0.21 (DCM/MeOH, 2/1, 1% TEA).

***tert*-Butyl-((*S*)-1-(((*S*)-1-(((*S*)-1-((*S*)-2-((*S*)-3-((2-amino-2-oxoethyl)carbamoyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-2-carbonyl)pyrrolidin-1-yl)-3-(*tert*-butoxy)-1-oxo-propan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-(4-(*tert*-butoxy)phenyl)-1-oxopropan-2-yl)carbamate (TVC-049)**

To a solution of the amine **87** (35 mg, 95 µmol) and tripeptide Boc-Tyr(*t*Bu)-Phe-Ser(*t*Bu)-OH (71 mg, 114 µmol) in DMF (6 mL), HATU (45 mg, 118 µmol) and DIPEA (50 µL, 285 µmol) were added. The

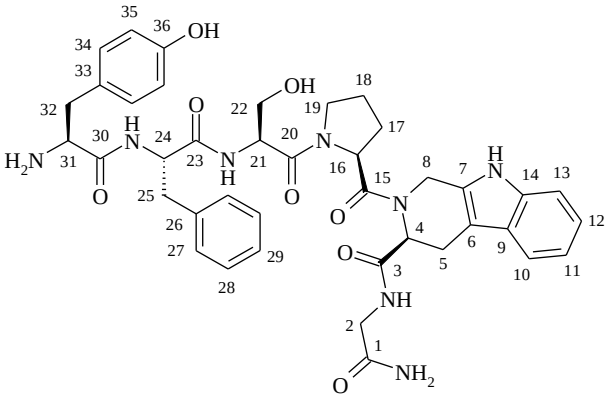
reaction mixture was stirred at room temperature overnight and DMF was removed under reduced pressure. The crude product was purified by silica gel column chromatography (EtOAc/EtOH, 20/1) to afford the desired product **TVC-049** (57 mg, 62%) as a solid.



**$^1$H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 7.44 (m, 1H, 4-H), 7.35-7.25 (m, 5H, 27-H, 28-H, 29-H), 7.27 (m, 1H, 13-H), 7.1 (overlap, 2H, 34-H), 7.05 (overlap. 1H, 10-H), 6.99 (m, 1H, 11-H), 6.87 (dd, *J* = 8.4 Hz, *J* = 11 Hz, 2H, 35-H), 5.63 (m, 1/2H, 4-H), 5.42 (d, *J* = 5.4 Hz, 1/2H, 4-H), 5.34 (d, *J* = 17.2 Hz, 1H, 2-H, 8-H), 5.06 (m, 1H, 26-H), 4.75 (m, 1H, 21-H), 4.68 (br, 1H, 24-H), 4.32 (d, *J* = 17.2 Hz, 1H, 2-H), 4.23 (m, 1H, 31-H), 3.81 (br, 1H, 19-H), 3.78 (br, 1H, 19-H), 3.62 (m, 1H, 22-H), 3.48 (m, 1H, 22-H), 3.42-3.33 (m, 1H, 5-H), 3.06-3.02 (m, 1H), 2.96 (m, 1H, 32-H), 2.95 (m, 1H, 25-H), 2.67 (m, 1H, 25-H), 2.33 (m, 1H, 17-H), 2.4-2.12 (m, 2H, 18-H), 1.94 (m, 1H, 17-H), 1.35, 1.30, 1.29 (27H). **$^{13}$C-NMR** (100 MHz, $CD_3OD$): $\delta$ = 174.7 (23-C), 174.2 (30-C), 174.1 (20-C), 173.9 (3-C), 173.4 (15-C), 172.9 (1-C), 165.0 (41-C), 155.3 (36-C), 130.0 (34-C), 125.2 (33-C), 129.5 (27-C), 129.4 (28-C), 127.7 (26-C), 140.9/ 136.4, 133.7, 130.3/ 130.1, 122.5/122.3, 14.9/120.0, 118.7, 112.0/111.9, 106.4 , 79.5 (37-C), 75.0 (39-C), 74.9 (42-C), 62.6, 62.2 (21-C), 59.4, 58.7 (16-C), 57.2 (31-C), 55.4 (24-C), 57.2, 54.1, 53.6 (21-C), 49.2, 48.7 (19-C), 39.8 (2-C), 38.9 (25-C), 38.5 (32-C), 30.3 (17-C), 26.3, 25.8 (18-C), 23.6, 23.7. **IR** (KBr) ν ($cm^{-1}$): 3421.1 (NH), 1655.3 (CO). **HPLC-ESI-MS**: m/z (%) = 979.6 (100) $[M+H]^+$, 1001.6 (45) $[M+Na]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{53}H_{70}N_8O_{10}Na$ $[M+Na]^+$ 1001.5113; found 1001.5107. $R_f$ = 0.27 (EtOAc/EtOH, 15/1).

**(2*S*)-2-[(2*S*)-2amino-3-(4-hydroxyphenyl)propanamido]-N-[(2*S*)-1-[(2*S*)-2-[(3*S*)-3-[(carbamoyl-methyl)carbamoyl]-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-2-carbonyl]pyrrolidin-1-yl]-3-hydroxy-1-oxopropan-2-yl]-3-phenylpropanamide (TVC-050, 88)**

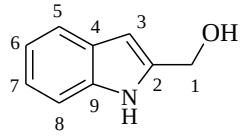
Peptide **TVC-049** (47 mg, 48.0 µmol) was treated with TFA/TIPS/$H_2O$ (2 mL, 95/2.5/2.5) at 0 ºC for 4 h. Afterward, the mixture was concentrated under diminished pressure and subjected to silica gel column chromatography (eluent: DCM/MeOH, 4/1, 0.1% TEA). The product **88** (36 mg, 96%) was dissolved in ACN/$H_2O$ (2 mL, 1/1) and lyophilized to give white powder.



**$^1$H-NMR** (400 MHz, DMSO-$d_6$): $\delta$ = 10.85 (d, *J* = 2.6 Hz, 1H, NH), 8.4 (br, 1/2H, NH), 8.3 (d, *J* = 7.6 Hz, 1/2H, NH), 8.2 (d, *J* = 7.6 Hz, 1H, NH), 7.8 (d, *J* = 5.6 Hz, 1/2H, NH), 7.4 (t, *J* = 7.7 Hz, 1H, 10-H), 7.31 (m, 1H, 13-H), 7.2-7.17 (m, 5H, 27-H, 28-H, 29-H), 7.03 (m, 1H, 12-H), 6.97 (overlap, 1H, 11-H), 6.96 (m, 2H, 34-H), 6.65 (dd, *J* = 4.7, *J* = 8.4 Hz, 2H, 35-H), 5.56 (d, *J* = 6.2 Hz, 1/2H, 4-H), 5.42 (d, *J* = 6.2 Hz, 1/2H, 4-H), 5.2 (d, *J* = 17.2, 1/2H, 8-H), 5.12 (d, *J* = 15.2, 1/2H, 8-H), 5.04 (br, 1H, 16-H), 4.7 (d, *J* = 15.6, 1/2H, 8-H), 4.4 (d, *J* = 17.5, 1/2H, 8-H), 4.65 (m, 1H, 24-H), 4.65 (m, 1H, 21-H), 3.69-3.63 (m, 2H, 19-H), 3.67 (overlap, 1H, 22-H), 3.62 (m, 1H, 2-H), 3.56 (m, 1H, 2-H), 3.52 (m, 1H, 22-H), 3.47 (m, 1H, 32-H), 3.4 (m, 1H, 5-H), 2.82 (m, 1H, 5-H), 3.00-2.95 (m, 1H, 25-H), 2.82 (m, 1H, 25-H), 2.78 (m, 1H, 32-H), 2.26 (m, 1H, 17-H), 2.01 (m, 1H, 18-H), 1.88 (m, 1H, 18-H), 1.77 (m, 1H, 17-H). **$^{13}$C-NMR** (100 MHz, DMSO-$d_6$): $\delta$ = 171.9, 171.4, 170.7, 170.6, 170.5, 170.3, 169.3, 168.5, 169.2, 155.9 (36-C), 137.3 (14-C), 136.1 (33-C), 135.9 (7-C), 134.3, 130.2 (34-C), 129.3 (27-C), 127.8 (28-C), 126.2 (29-C), 120.8, 120.7, 115.0 (35-C), 118.3, 117.5, 110.9, 110.8, 104.7, 104.4, 61.2, 61.0 (22-C), 56.8, 57.4 (16-C), 55.3, 55.20 (31-C), 54.2, 50.9 (4-C), 53.4, 53.3 (21-C), 52.9, 52.8 (24-C), 47.0, 46.2 (19-C), 42.0, 41.8 (2-C), 41.1, 37.8 (8-C), 38.0 (32-C), 37.8 (25-C), 28.6, 27.5 (17-C), 24.8, 24.6 (18-C), 22.5, 22.3 (5-C). **IR** (KBr) $\nu$ ($cm^{-1}$): 3427.7 (NH), 1642.6 (CO). **HPLC-ESI-MS**: m/z (%) = 767.4 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{40}H_{46}N_8O_8Na$ $[M+Na]^+$ 789.3336; found 789.3331.

### 3.11 Preparation of isotryptophan amino acid

**(1H-Indol-2-yl)methanol (TVC-244)**

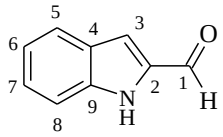


To a suspension of $LiAlH_4$ (61 mg, 1.6 mmol) in dry ether (5 mL) at 0° C under argon atmosphere, a solution of ethyl-indole-2-carboxylate (200 mg, 1.06 mmol) in ether (5 mL) was added slowly. The reaction mixture was stirred at room temperature for 1 h and $H_2O$ (3 mL) was added. The white precipitate was removed by filtration through a Celite pad. The filtrate was dried over $MgSO_4$ and concentrated to afford indole-2-carbinol **TVC-244** (134 mg, 86%) as a solid.

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 7.62 (d, *J* = 8.0 Hz, 1H, 8-H), (d, *J* = 8.0 Hz, 1H, 8-H), 7.32 (d, *J* = 8.0 Hz, 1H, 5-H), 7.22 (t, *J* = 8.0 Hz, 1H, 6-H), 7.14 (t, *J* = 8.0 Hz, 1H, 7-H), 6.42 (m, 1H, 3-H), 4.78 (s, 2H,

1-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 137.5 (9-C), 136.4 (2-C), 128.1 (4-C), 122.1 (6-C), 120.7 (8-C), 119.9 (7-C), 110.9 (5-C), 100.6 (3-C), 58.6 (1-C). **IR** (KBr) ν ($cm^{-1}$): 3256.3, 2843.7. **HPLC-ESI-MS:** m/z (%) = 148 (10) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_9H_8NO$ $[M-H]^+$ 146.0612; found 146.0611. $R_f$ = 0.5 (CyH/EtOAc, 10/1).

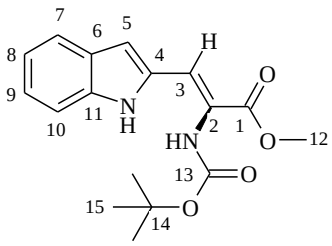
**1H-Indole-2-carbaldehyde (TVC-245, 90)**



To a solution of indole-2-carbinol **TVC-244** (250 mg, 1.7 mmol) in DCM (5 mL) at 0 °C, PCC (732 mg, 3.4 mmol) in DCM (5 mL) was added. The reaction mixture was stirred further for 2 h and *i*PrOH (4 mL) was added. After stirring for 40 min, the solvent was removed and the residue was subjected to silica gel column chromatography using CyH/EtOAc (20/1) to get the 1H-indole-2-carbaldehyde **90** (118 mg, 48%) as a white solid.

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 9.81 (s, 1H, CHO), 7.72 (d, $J$ = 8.0 Hz, 1H, 8-H), 7.47 (d, $J$ = 8.0 Hz, 1H, 5-H), 7.34 (t, $J$ = 8.0 Hz, 1H, 6-H), 7.32 (s, 1H, 3-H), 7.12 (t, $J$ = 8.0 Hz, 1H, 7-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 183.9 (1-C), 140.2 (9-C), 137.8 (2-C), 128.6 (4-C), 126.4 (6-C), 122.6 (8-C), 120.2 (7-C), 114.2 (3-C), 112.1 (5-C). **IR** (KBr) ν ($cm^{-1}$): 1675.9 (CO). **HPLC-ESI-MS:** m/z (%) = 146.1 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_9H_8NO$ $[M+H]^+$ 146.0601; found 146.0600. $R_f$ = 0.51 (CyH/EtOAc, 10/1).

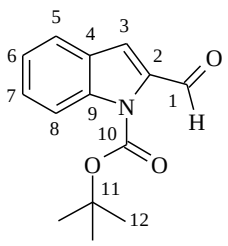
**(*Z*)-Methyl-2-((*tert*-butoxycarbonyl)amino)-3-(1H-indol-2-yl)acrylate (TVC-255, 92)**



A solution of *N*-Boc-phosphonoglycine trimethyl ester (123 mg, 0.41 mmol) in THF (2 mL) was cooled to -78 °C and followed by addition of 1,1,3,3-tetramethylguanidine (53 µL, 0.41 mmol). The reaction mixture was stirred for 5 min at this temperature and the aldehyde **90** (40 mg, 0.28 mmol) in THF was added. After stirring for 1h, the dry-ice bath was removed and the reaction mixture was allowed to stir at room temperature for 3 days. Flash chromatography (CyH/EtOAc, 10/1) afforded **92** (76 mg, 88%) as a white solid.

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 9.17 (s, 1H, NH), 7.64 (d, $J$ = 8.0 Hz, 1H, 7-H), 7.52 (s, 1H, 3-H), 7.37 (d, $J$ = 8.0 Hz, 1H, 10-H), 7.27 (t, $J$ = 8.0 Hz, 1H, 9-H), 7.13 (t, $J$ = 8.0 Hz, 1H, 8-H), 6.85 (s, 1H, 5-H), 3.88 (s, 3H, 12-H), 1.48 (s, 9H, 15-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 165.9 (1-C), 154.8 (13-C), 137.9 (11-C), 132.1 (4-C), 128.4 (2-C), 127.7 (6-C), 124.9 (3-C), 124.7 (9-C), 121.4 (7-C), 120.5 (8-C), 110.6 (5-C), 110.2 (10-C), 81.7 (14-C), 52.6 (12-C), 28.0 (15-C). **IR** (KBr) ν ($cm^{-1}$): 3348.1, 1697.9, 1680.1. **HPLC-ESI-MS:** m/z (%) = 339.2 (40) $[M+Na]^+$, 655.3 (30) $[2M+Na]^+$. **HR-ESI-MS** (m/z): calcd. For $C_{17}H_{20}N_2O_4Na$ $[M+Na]^+$ 339.1315; found 339.1316. $R_f$ = 0.54 (CyH/EtOAc, 4/1).

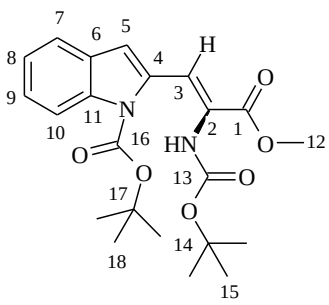
***tert*-Butyl-2-formyl-1H-indole-1-carboxylate (TVC-270, 95)**



Aldehyde **90** (20 mg, 0.14 mmol) in ACN (2 mL) at 0 °C was added $Boc_2O$ (36 mg, 0.15 mmol) and DMAP (3.3 mg, 0.03 mmol). After stirring at room temperature for 4 h, the reaction mixture was quenched with EtOAc, washed with $H_2O$, brine solution and dried over $MgSO_4$. Removal of solvent afforded desired product **95** (33.6 mg, 99%) as a white solid.

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 10.45 (s, 1H, CHO), 8.18 (d, *J* = 8.0 Hz, 1H, 5-H), 7.69 (d, *J* = 8.0 Hz, 1H, 8-H), 7.51 (t, *J* = 8.0 Hz, 1H, 6-H), 7.45 (s, 1H, 3-H), 7.31 (t, *J* = 8.0 Hz, 1H, 7-H), 1.74 (s, 9H, 12-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 184.1 (1-C), 149.8 (10-C), 137.9 (9-C), 137.8 (2-C), 128.3 (7-C), 127.5 (4-C), 123.8 (6-C), 123.3 (8-C), 116.4 (3-C), 116.2 (5-C), 85.0 (11-C), 28.2 (12-C). **IR** (KBr) ν ($cm^{-1}$): 1722.8, 1663.1 (CO). **HPLC-ESI-MS:** m/z (%) = 268.1 (10) $[M+Na]^+$. **HR-ESI-MS** (m/z): calcd. For $C_{14}H_{15}NO_3Na$ $[M+Na]^+$ 268.0944; found 268.0943. $R_f$ = 0.50 (CyH/EtOAc, 8/1).

***tert*-Butyl-2-[(1*Z*)-2-{[(*tert*-butoxy)carbonyl]amino}-3-methoxy-3-oxoprop-1-en-1-yl]-1H-indole-1-carboxylate (TVC-271, 94)**

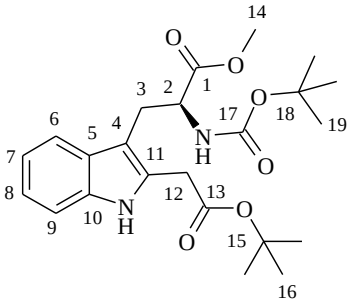


A solution of *N*-Boc-phosphonoglycine trimethyl ester (114.5 mg, ) in THF (2 mL) was cooled to -78 °C and followed by addition of 1,1,3,3-tetramethylguanidine (50 µL). The reaction was stirred for 5 min at this temperature and solution of **90** (63 mg, 0.26 mmol) in THF (2 mL) was added dropwise. After stirring for 1 h, the dry-ice bath was removed and the reaction mixture was allowed to stir at room temperature for 3 days. Flash chromatography (CyH/EtOAc, 5/1) afforded product **94** (86.4 mg, 80%) as a white solid.

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 8.16 (d, *J* = 8.0 Hz, 1H, 7-H), 7.83 (s, 1H, 3-H), 7.56 (d, *J* = 8.0 Hz, 1H, 10-H), 7.32 (t, *J* = 8.0 Hz, 1H, 9-H), 7.26 (t, *J* = 8.0 Hz, 1H, 8-H), 7.0 (s, 1H, 5-H), 6.18 (s, 1H, NH), 3.87 (s, 3H, 12-H), 1.70 (s, 9H, 18-H), 1.43 (s, 9H, 15-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 165.7 (1-C), 152.7 (13-C), 150.1 (16-C), 136.7 (11-C), 133.1 (4-C), 129.0 (2-C), 125.5 (6-C), 124.9 (3-C), 123.2 (9-C), 121.1 (7-C), 121.0 (8-C), 115.7 (10-C), 112.2 (5-C), 84.7 (17-C), 81.1 (14-C), 52.5 (12-C), 28.2 (18-C), 28.1 (15-C). **IR** (KBr) ν ($cm^{-1}$): 1722.8, 1662.3 (CO). **HPLC-ESI-MS:** m/z (%) = 434 (20) $[M+NH_4]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{22}H_{28}N_2O_6Na$ $[M+Na]^+$ 439.1840; found 439.1807. $R_f$ = 0.18 (CyH/EtOAc, 8/1).

### 3.12 Synthesis of Trp-derived tricyclic ester

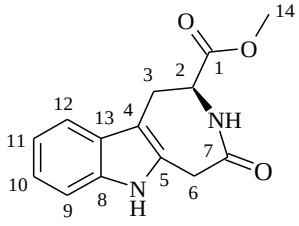
**(*S*)-Methyl-3-(2-(2-(*tert*-butoxy)-2-oxoethyl)-1H-indol-3-yl)-2-((*tert*-butoxycarbonyl)amino) propanoate (TVC-250, 107)**



Lauroyl peroxide (326 mg, 0.82 mmol) in 1,2-dichloroethane (4 mL) was added portionwise to a solution of Boc-Trp-OMe (200 mg, 0.63 mmol) in degassed 1,2-dichloroethane (3 mL) heated at reflux. After refluxing for 6 h, the solvent was removed. Flash chromatography (using gradient of CyH/EtOAc, from 10/1 to 4/1) yielded the desired product **107** (106.4 mg, 40%) as a brown solid.

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 7.56 (d, $J$ = 8.0 Hz, 1H, 9-H), 7.32 (d, $J$ = 8.0 Hz, 1H, 6-H), 7.18 (t, $J$ = 8.0 Hz, 1H, 7-H), 7.11 (t, $J$ = 8.0 Hz, 1H, 8-H), 4.36 (m, 1H, 2-H), 3.78 (s, 2H, 12-H), 3.19 (m, 2H, 3-H), 1.51 (s, 9H, 16-H), 1.42 (s, 9H, 19-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 178.3 (1-C), 170.6 (13-C), 154.3 (17-C), 135.6 (10-C), 129.1 (5-C), 127.7 (11-C), 122.5 (7-C), 120.1 (8-C), 118.5 (9-C), 111.0 (6-C), 107.9 (4-C), 82.6 (15-C), 80.4 (18-C), 56.0 (2-C), 32.9 (12-C), 28.3 (19-C), 27.9 (16-C), 26.8 (3-C). **IR** (KBr) $\nu$ ($cm^{-1}$): 3364.3, 1698.9. **HPLC-ESI-MS:** m/z (%) = 455.3 (10) $[M+Na]^+$, 333.2 (80) $[M-Boc]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{23}H_{32}N_2O_6Na$ $[M+Na]^+$ 455.1556; found 455.2153. $R_f$ = 0.3 (CyH/EtOAc, 6/1).

**(*S*)-Methyl-4-oxo-1,2,3,4,5,6-hexahydroazepino[4,5-b]indole-2-carboxylate (TVC-260, 106)**

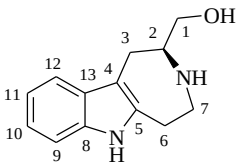


Solution of **107** (15 mg, 34.7 µmol) in TFA/TIPS/$H_2O$ (2 mL, 95/2.5/2.5) was stirred at 0 $^oC$ for 3 h. The reaction mixture was then concentrated under reduced pressure to dryness. The residue was dissolved in DMF (1 mL) and followed by addition of HATU (20.4 mg, 54 µmol) and DIPEA (30 µL, 200 mmol). After stirring at room temperature for 24 h, the solvent was removed. The residue was subjected to flash chromatography using CyH/EtOAc (gradient from 1/4 to 1/1) to afford the pure product **106** (7.2 mg, 57%) as a brown solid.

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 7.34 (d, $J$ = 8.0 Hz, 1H, 9-H), 7.29 (overlap, 1H, 12-H), 7.12 (t, $J$ = 8.0 Hz, 1H, 11-H), 7.03 (d, $J$ = 8.0 Hz, 1H, 9-H), 4.70 (dd, $J$ = 2.6 Hz, $J$ = 12.0 Hz, 1H, 2-H), 4.09 (m, 1H, 6-H), 3.85 (s, 3H, 14-H), 3.52 (d, $J$ = 16.6 Hz, 1H, 6-H), 3.38 (m, 1H, 3-H), 2.99 (m, 1H, 3-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 171.2 (1-C), 134.9 (8-C), 124.4 (5-C), 122.0 (10-C), 119.3 (11-C), 117.3 (12-C),

110.9 (9-C), 106.8 (4-C), 53.6 (2-C), 53.1 (14-C), 34.8 (6-C), 29.4 (3-C). **IR** (KBr) ν ($cm^{-1}$): 3423.5, 2925.8, 1743.3, 1645.6. **HPLC-ESI-MS:** m/z (%) = 259.1 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{14}H_{14}N_2O_3Na$ $[M+Na]^+$ 281.0897; found 281.0897. $R_f$ = 0.35 (EtOAc).

**(2*S*)-1,2,3,4,5,6-hexahydroazepino[4,5-b]indol-2-ylmethanol (TVC-274, 109)**

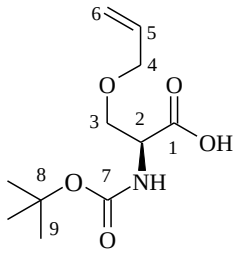



A solution of **106** (30 mg, 0.12 mmol) in dry THF (3 mL) was added dropwise 94% $BH_3.SMe_2$ (38 mg, 45 µL) in THF at 0 °C. The reaction mixture was allowed to warm up to room temperature and stirred for 6h. Methanol (excess) was added to the reaction mixture, stirred for 4 hours and concentrated under reduced pressure. Water (10 mL) was added and the reaction mixture was extracted using EtOAc (3x20 mL). The combined organic phases were dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give the product **109** (21 mg, 86%) as a solid.

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 7.45 (d, *J* = 8.0 Hz, 1H, 12-H), 7.30 (d, *J* = 8.0 Hz, 1H, 9-H), 7.09 (t, *J* = 8.0 Hz, 1H, 10-H), 7.03 (t, *J* = 8.0 Hz, 1H, 11-H), 3.94 (dd, *J* = 2.2 Hz, *J* = 7.5 Hz, 1H, 1-H), 3.73 (m, 1H, 1-H), 3.45 (overlap, 1H, 2-H), 3.32 (m, 2H, 7-H), 3.23 (m, 1H, 3-H), 3.00 (m, 1H, 3-H), 1.62 (m, 2H, 6-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 136.6 (8-C), 134.7 (5-C), 128.1 (13-C), 120.9 (10-C), 118.7 (11-C), 116.9 (12-C), 110.4 (9-C), 107.6 (4-C), 61.8 (1-C), 61.3 (2-C), 44.6 (7-C), 28.7 (6-C), 23.3 (3-C). **IR** (KBr) ν ($cm^{-1}$): **HPLC-ESI-MS:** m/z (%) = 210.1(10) [M+Na]. **HR-ESI-MS** (m/z): calcd. for $C_{13}H_{17}N_2O$ $[M+H]^+$: 217.1335, found: 217.1327. $R_f$ = 0.16 (EtOAc).

### 3.13 Synthesis of macrocyclic peptides 128 and 129

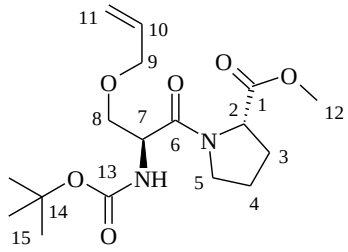
**(2*S*)-2-{[(*tert*-Butoxy)carbonyl]amino}-3-(prop-2-en-1-yloxy)propanoic acid (TVC-143, 117)**




To a stirred suspension of NaH (27 mg, 1.12 mmol) in DMF (0.5 mL) at 0 °C, a solution of Boc-Ser-OH (105 mg, 0.51 mmol) in DMF (1 mL) was added dropwise. After 10 min, allyl bromide (51 µL, 0.59 mmol) was added and the reaction mixture was allowed to warm up to room temperature. After stirring for 20 h, the solvent was removed under reduced pressure. The residue was dissolved with $H_2O$ (10 mL) and extracted with EtOAc (2x20 mL). The aqueous phase was acidified with 1M HCl at 0 °C to pH≈2 and extracted with EtOAc (3x40 mL). The combined organic phases were washed with brine solution and dried over $MgSO_4$. Removal of the solvent yielded oily product **117** (100 mg, 80%).

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 7.2 (br, 1H, OH), 5.91 (m, 1H, 5-H), 5.45 (s, 1H, NH), 5.26 (m, 2H, 6-H), 4.48 (br, 1H, 2-H), 4.03 (dt, *J* = 1.5 Hz, *J* = 5.5 Hz, 2H, 4-H), 3.92 (d, *J* = 8.3 Hz, 1H, 3-H), 3.73 (dd, *J* = 3.5 Hz, *J* = 8.3 Hz, 1H, 3-H), 1.47 (s, 9H, 9-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 174.8 (1-C), 155.7 (7-C), 134.1 (5-C), 117.9 (6-C), 80.3 (8-C), 72.3 (4-C), 69.6 (3-C), 53.8 (2-C), 28.7 (9-C). **IR** (KBr) ν ($cm^{-1}$): 2986.9, 1705.9. **HPLC-ESI-MS**: m/z (%) = 268.1 (100) $[M+Na]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{11}H_{19}NO_5Na$ $[M+Na]^+$ 268.1156; found 268.1155. $R_f$ = 0.45 (EtOAc).

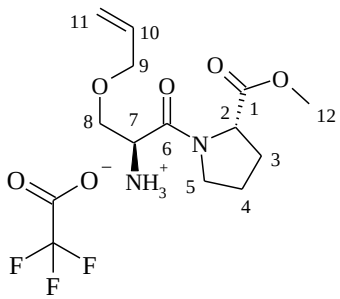
**Methyl-(2*S*)-1-[(2*S*)-2-{[(*tert*-butoxy)carbonyl]amino}-3-(prop-2-en-1-yloxy)propanoyl]-pyrrolidine-2-carboxylate (TVC-145, 118)**




A solution of **117** (162 mg, 0.66 mmol), Pro-OMe*HCl (131.3 mg, 0.79 mmol) and HATU (300 mg, 0.79 mmol) in DMF (4 mL) was added DIPEA (0.5 mL, 4.5 mmol). After stirring at room temperature for 20 h, the solvent was removed under reduced pressure. Flash chromatography (eluent: CyH/EtOAc, 3/1) provided oily product **118** (220 mg, 93%).

**$^1$H-NMR** (600 MHz, $CDCl_3$): $\delta$ = 5.89 (m, 1H, 10-H), 5.29 (d, *J* = 17.2 Hz, 1H, 11-H), 4.67 (d, *J* = 7.1 Hz, 1H, 7-H), 4.53 (dd, *J* = 4.0 Hz, *J* = 8.3 Hz, 1H, 2-H), 4.05 (dd, *J* = 5.0 Hz, *J* = 17.6 Hz, 2H, 8-H), 3.78 (m, 1H, 5-H), 3.73 (m, 1H, 5-H), 3.72 (s, 3H, 12-H), 3.64 (d, *J* = 6.5 Hz, 2H, 8-H), 2.21 (m, 1H, 4-H), 2.05 (m, 1H, 3-H), 1.99 (m, 2H, 3-H, 4-H), 1.43 (s, 9H, 15-H). **$^{13}$C-NMR** (150 MHz, $CDCl_3$): $\delta$ = 172.2 (6-C), 169.4 (1-C), 134.6 (10-C), 116.7 (11-C), 79.7 (14-C), 72.2 (9-C), 70.3 (8-C), 58.8 (2-C), 52.2 (7-C), 51.8 (12-C), 46.7 (5-C), 29.0 (4-C), 28.1 (15-C), 24.7 (3-C). **IR** (KBr) ν ($cm^{-1}$): 2982.3, 1705.7, 1645.9. **HPLC-ESI-MS**: m/z (%) = 357.1 (50) $[M+H]^+$, 257.1 (100) $[M-Boc+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{17}H_{28}N_2O_6Na$ $[M+Na]^+$ 379.1840; found 379.1844. $R_f$ = 0.28 (CyH/EtOAc, 2/1).

**(2*S*)-1-[(2*S*)-2-(Methoxycarbonyl)pyrrolidin-1-yl]-1-oxo-3-(prop-2-en-1-yloxy)propan-2-aminium-trifluoroacetate (TVC-147)**



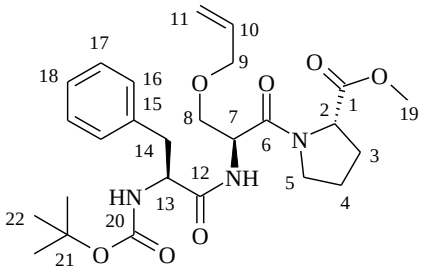

Product **118** (73 mg, 0.2 mmol) was treated with TFA/TIPS/$H_2O$ (2 mL, 95/4/1) at 0 °C for 3 h. Afterward, the reaction mixture was concentrated under diminished pressure to provide **TVC-147** (51.1 mg, 97%) as a TFA salt.

**$^1$H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 5.90 (m, 1H, 10-H), 5.30 (m, 2H, 11-H), 4.55 (dd, *J* = 5.3 Hz, *J* = 8.7 Hz, 1H, 2-H), 4.44 (dd, *J* = 4.0 Hz, *J* = 8.0 Hz,

1H, 7-H), 4.13 (d, *J* = 5.4 Hz, 2H, 9-H), 3.90 (dd, *J*= 4.0 Hz, *J* = 11.0 Hz, 2H, 8-H), 3.75 (m, 1H, 5-H), 3.73 (s, 3H, 12-H), 3.70 (overlap, 1H, 8-H), 3.66 (m, 1H, 5-H), 2.32 (m, 1H, 3-H), 2.06 (m, 2H, 4-H), 2.02 (m, 1H, 3-H). **$^{13}$C-NMR** (100 MHz, $CD_3OD$): $\delta$ = 173.4 (6-C), 166.6 (1-C), 133.2 (10-C), 116.7 (11-C), 72.6 (9-C), 66.4 (8-C), 59.2 (2-C), 52.2 (7-C), 51.3 (12-C), 46.8 (5-C), 28.3 (3-C), 24.6 (4-C). **IR** (KBr) ν ($cm^{-1}$): 2988.2, 1652.7. **HPLC-ESI-MS**: m/z (%) = 257.1 (100) $[M\text{-}CF_3COOH+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{12}H_{20}N_2O_4Na$ $[M+Na]^+$ 279.1340; found 279.1815.

**Methyl-(2*S*)-1-[(2*S*)-2-[(2*S*)-2-{[(*tert*-butoxy)carbonyl]amino}-3-phenyl-propanamido]-3-(prop-2-en-1-yloxy)propanoyl]pyrrolidine-2-carboxylate (TVC-148)**

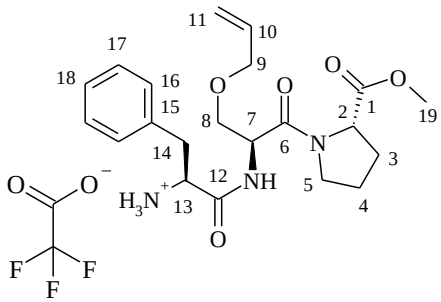
A stirred solution of Boc-Phe-OH (180 mg, 0.68 mmol), TBTU (218 mg, 0.68 mmol) and **TVC-147** (145 mg, 0.57 mmol) in DCM (8 mL) was added dropwise DIPEA (0.38 mL, 2.26 mmol). After stirring at room temperature for 18 h, the solvent was removed. The crude product was purified by silica gel column chromatography using CyH/EtOAc (5/2) as eluent to give product **TVC-148** (264 mg, 93%) as a solid.



**$^1$H-NMR** (600 MHz, $CDCl_3$): $\delta$ = 7.28 (t, *J* = 7.0 Hz, 2H, 17-H), 7.23 (t, *J* = 7.0 Hz, 1H, 18-H), 7.19 (d, *J* = 7.7 Hz, 2H, 16-H), 5.86 (m, 1H, 10-H), 5.29 (d, *J* = 10.0 Hz, 1H, 11-H), 5.20 (d, *J* = 11.0 Hz, 1H, 11-H), 4.91 (d, *J* = 6.5 Hz, 7-H), 4.49 (dd, *J* = 4.3 Hz, *J* = 8.0 Hz, 1H, 2-H), 4.4 (bs, 1H, 13-H), 3.8 (m, 1H, 5-H), 3.72 (s, 3H, 19-H), 3.68 (m, 1H, 5-H), 3.64 (m, 1H, 8-H), 3.58 (m, 1H, 8-H), 3.09 (m, 2H, 14-H), 2.2 (m, 1H, 3-H), 2.0 (overlap, 1H, 3-H, 4-H), 2.06 (m, 1H, 4-H), 1.42 (s, 9H, 22-H). **$^{13}$C-NMR** (150 MHz, $CDCl_3$): $\delta$ = 172.1 (12-C), 170.8 (6-C), 168.5 (1-C), 155.4 (20-C), 134.2 (10-C), 134.0 (15-C), 129.4 (16-C), 128.5 (18-C), 126.9 (17-C), 117.0 (11-C), 80.1 (21-C), 72.1 (9-C), 69.5 (8-C), 58.7 (2-C), 55.5 (13-C), 52.1 (19-C), 50.7 (7-C), 47.2 (5-C), 38.1 (14-C), 28.8 (3-C), 28.0 (22-C), 24.7 (4-C). **IR** (KBr) ν ($cm^{-1}$): 2989.2, 1635.4. **HPLC-ESI-MS**: m/z (%) = 504.3 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{26}H_{37}N_3O_7Na$ $[M+Na]^+$ 526.2524; found 526.2528. $R_f$ = 0.18 (CyH/EtOAc, 5/2).

**(1*S*)-1-{[(2*S*)-1-[(2*S*)-2-(Methoxycarbonyl)pyrrolidin-1-yl]-1-oxo-3-(prop-2-en-1-yloxy)-propan-2-yl]carbamoyl}-2-phenylethan-1-aminium trifluoroacetate (TVC-149, 119)**

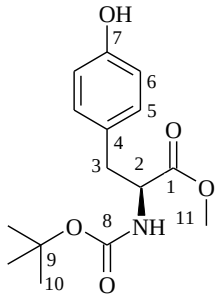
A solution of **TVC-148** (70 mg, 0.14 mmol) in TFA/TIPS/$H_2O$ (2 mL, 95/4/1) was stirred at 0 $^oC$ for 3 h. After that time, the



mixture was concentrated under reduced pressure to afford product **119** (70.4 mg, 98%) as TFA salt.

**$^1$H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 7.37-7.31 (m, 5H, 16-H, 17-H, 18-H), 5.96 (m, 1H, 10-H), 5.34 (d, $J$ = 17.0 Hz, 1H, 11-H), 5.22 (d, $J$ = 10.0 Hz, 1H, 11-H), 4.91 (d, $J$ = 6.5 Hz, 7-H), 4.47 (dd, $J$ = 4.3 Hz, $J$ = 8.0 Hz, 1H, 2-H), 4.22 (bs, 1H, 13-H), 3.79 (m, 2H, 5-H), 3.72 (s, 3H, 19-H), 3.80 (m, 2H, 8-H), 3.28 (m, 1H, 14-H), 3.05 (m, 1H, 14-H), 2.3 (m, 1H, 3-H), 2.03 (overlap, 1H, 3-H, 4-H), 2.02 (m, 1H, 4-H). **$^{13}$C-NMR** (100 MHz, $CD_3OD$): $\delta$ = 170.1 (12-C), 169.8 (6-C), 168.9 (1-C), 136.8 (10-C), 134.7 (15-C), 131.6 (18-C), 131.2 (17-C), 129.9 (16-C), 118.7 (11-C), 74.9 (9-C), 71.2 (8-C), 61.5 (2-C), 56.5 (13-C), 53.8 (19-C, 7-C), 49.6 (5-C), 39.4 (14-C), 30.8 (3-C), 27.3 (4-C). **IR** (KBr) ν ($cm^{-1}$): 2992.5, 1669.5, 1627.1. **HPLC-ESI-MS**: m/z (%) = 404.3 (100) $[M\text{-}CF_3COOH+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{21}H_{29}N_3O_5Na$ $[M+Na]^+$ 426.1999; found 426.1998. $R_f$ = 0.23 (EtOAc/EtOH, 13/1).

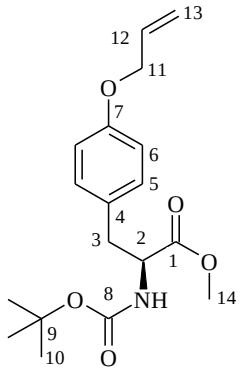
**Methyl-(2*S*)-2-{[(*tert*-butoxy)carbonyl]amino}-3-(4-hydroxyphenyl)propanoate (TVC-136, 121)**




To a cooled suspension of L-Tyr-OMe*HCl (500 mg, 2.16 mmol) in DCM (5 mL) at 0 °C, TEA (0.75 mL, 5.4 mmol) and $Boc_2O$ (495 mg, 2.37 mmol) in DCM (5 mL) were added. The mixture was allowed to warm up to room temperature and stirred overnight. The solvent was removed and the crude product was subjected to silica gel flash chromatography using CyH/EtOAc (3/1) to yield **121** as an oily product (618 mg, 97%).

**$^1$H-NMR** (600 MHz, $CDCl_3$): $\delta$ = 6.99 (d, $J$ = 8.0 Hz, 2H, 5-H), 6.75 (d, $J$ = 8.0 Hz, 2H, 6-H), 4.56 (m, 1H, 2-H), 3.73 (s, 3H, 11-H), 3.05-2.99 (m, 2H, 3-H), 1.44 (s, 9H, 10-H). **$^{13}$C-NMR** (150 MHz, $CDCl_3$): $\delta$ = 172.6 (1-C), 155.3 (8-C), 155.0 (7-C), 127.6 (4-C), 115.4 (6-C), 80.1 (9-C), 54.4 (2-C), 52.3 (11-C), 37.6 (3-C), 28.5 (10-C). **IR** (KBr) ν ($cm^{-1}$): 3353.6, 2975.6, 1681.6. **HPLC-ESI-MS**: m/z (%) = 196.1 (100) $[M\text{-Boc}+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{15}H_{21}NO_5Na$ $[M+Na]^+$ 318.1317; found 318.1318. $R_f$ = 0.37 (CyH/EtOAc, 3/1).

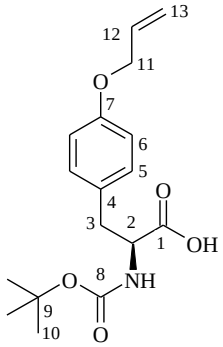
**Methyl-(2*S*)-2-{[(*tert*-butoxy)carbonyl]amino}-3-[4-(prop-2-en-1-yloxy)phenyl]propanoate (TVC-138, 122)**




A suspension solution of **121** (470 mg, 1.59 mmol) and $K_2CO_3$ (484 mg, 3.5 mmol) in DMF (8 mL) was added dropwise allyl bromide (0.28 mL, 3.18 mmol). The reaction mixture was stirred for 20 h at room temperature. The solid was then removed by filtration through a Celite pad and washed with EtOAc. Removal of the solvent to dryness gave **122** (526 mg, 99%) as an oily product.

**$^1$H-NMR** (600 MHz, $CDCl_3$): $\delta$ = 7.0 (d, $J$ = 8.5 Hz, 2H, 5-H), 6.83 (d, $J$ = 8.5 Hz, 2H, 6-H), 6.05 (m, 1H, 11-H), 5.4 (q, 1H, 13-H), 5.26 (q, 1H, 13-H), 4.54 (m, 2H, 11-H), 4.50 (m, 1H, 2-H), 3.70 (s, 3H, 14-H), 3.0 (br, 2H, 3-H), 1.41 (s, 9H, 10-H). **$^{13}$C-NMR** (150 MHz, $CDCl_3$): $\delta$ = 172.0 (1-C), 157.7 (8-C), 133.3 (12-C), 130.0 (5-C), 128.1 (4-C), 117.6 (13-C), 114.8 (6-C), 79.8 (9-C), 69.6 (11-C), 54.5 (2-C), 52.3 (14-C), 37.9 (3-C), 28.5 (10-C). **IR** (KBr) ν ($cm^{-1}$): 2986.9, 1705.9. **HPLC-ESI-MS**: m/z (%) = 358.1 (20) $[M+Na]^+$, 236.1 (100) $[M-Boc+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{18}H_{25}NO_5Na$ $[M+Na]^+$ 358.1630; found 358.1630. $R_f$ = 0.36 (CyH/EtOAc, 5/1).

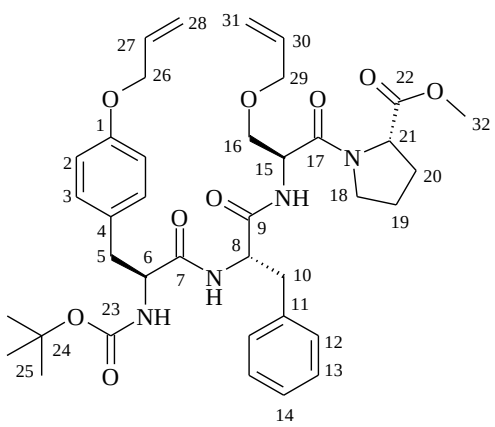
**(2*S*)-2-{[(*tert*-Butoxy)carbonyl]amino}-3-[4-(prop-2-en-1-yloxy)phenyl]propanoic acid (TVC-140, 123)**



Ester **122** (144 mg, 0.43 mmol) in MeOH (2 mL) was treated with 1N NaOH (0.64 mmol) at room temperature for 24 h. Afterward, the solution was acidified with 1N HCl and extracted with EtOAc (3x40 mL). The combined organic phases were washed with brine solution and dried over $MgSO_4$. Removal of the solvent yielded **123** (135.6 mg, 98%) as an oily product.

**$^1$H-NMR** (600 MHz, $CDCl_3$): $\delta$ = 7.1 (d, $J$ = 8.0 Hz, 2H, 5-H), 6.8 (d, $J$ = 8.0 Hz, 2H, 6-H), 6.1 (m, 1H, 12-H), 5.4 (dd, $J$ = 2.0 Hz, $J$ = 17.6 Hz, 2H, 13-H), 4.59 (br, 1H, 2-H), 4.5 (d, $J$ = 5 Hz, 2H, 11-H), 3.1 (m, 1H, 3-H), 3.0 (m, 1H, 3-H), 1.4 (s, 9H, 10-H). **$^{13}$C-NMR** (150 MHz, $CDCl_3$): $\delta$ = 176.4 (1-C), 157.7 (8-C), 155.5 (7-C), 133.2 (12-C), 130.0 (5-C), 128.1 (4-C), 117.4 (13-C), 115.0 (6-C), 80.2 (9-C), 68.7 (11-C), 54.4 (2-C), 36.9 (3-C), 28.2 (10-C). **IR** (KBr) ν ($cm^{-1}$): 2930.8, 1713.4. **HPLC-ESI-MS**: m/z (%) = 344.2 (20) $[M+Na]^+$, 222.2 (100) $[M-Boc+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{17}H_{23}NO_5Na$ $[M+Na]^+$ 344.1474; found 344.1471. $R_f$ = 0.29 (CyH/EtOAc, 6/1).

**Methyl-(2*S*)-1-[(2*S*)-2-[(2*S*)-2-[(2*S*)-2-{[(*tert*-butoxy)carbonyl]amino}-3-[4-(prop-2-en-1-yloxy) phenyl]propanamido]-3-phenylpropanamido]-3-(prop-2-en-1-yloxy)propanoyl]-pyrrolidine-2-carboxylate (TVC-150, 124)**

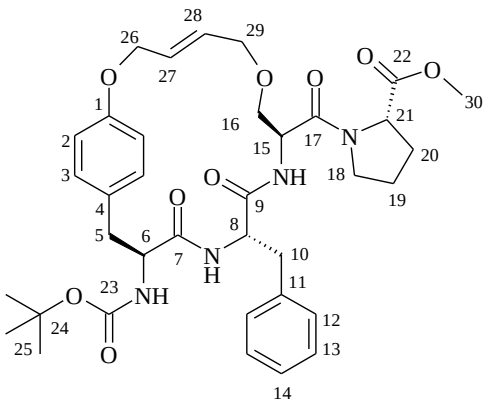


A solution of **119** (140 mg, 0.347 mmol), **123** (134 mg, 0.416 mmol) and TBTU (137 mg, 427 mmol) in DCM (4 mL) was added DIPEA (0.23 mL, 1.39 mmol). After being stirred at room temperature for 20 h, the reaction mixture was concentrated under reduced pressure. The residue was

purified by silica gel column chromatography (CyH/EtOAc, 3/1) to give **124** (236.4 mg, 96%) as a solid.

**$^1$H-NMR** (600 MHz, $CDCl_3$): $\delta$ = 7.24-7.22 (m, 3H, 13-H, 14-H), 7.10 (overlap, 2H, 12-H), 7.09 (d, *J* = 8.2 Hz, 2H, 3-H), 6.84 (d, *J* = 8.2 Hz, 2H, 2-H), 6.06 (m, 1H, 27-H), 5.90 (m, 1H, 30-H), 5.42 (d, *J* = 17.0 Hz, 1H, 28-H), 5.29 (overlap, 28-H, 31-H), 5.18 (d, *J* = 11.0 Hz, 1H, 31-H), 4.83 (q, *J* = 6.5 Hz, 1H, 15-H), 4.65 (q, *J* = 6.5 Hz, 1H, 6-H), 4.52 (m, 2H, 26-H), 4.51 (overlap, 1H, 21-H), 4.27 (bs, 1H, 8-H), 4.0 (m, 2H, 29-H), 3.78 (m, 1H, 18-H), 3.72 (s, 3H, 32-H), 3.67 (m, 1H, 18-H), 3.59 (d, *J* = 6.5 Hz, 2H, 16-H), 3.05 (m, 1H, 5-H), 2.97 (overlap, 3H, 5-H, 10-H), 2.21 (m, 1H, 20-H), 2.08 (m, 1H, 19-H), 2.01 (overlap, 2H, 19-H, 20-H), 1.40 (s, 9H, 25-H). **$^{13}$C-NMR** (150 MHz, $CDCl_3$): $\delta$ = 172.1 (7-C), 171.1 (9-C), 169.9 (17-C), 168.3 (22-C), 157.6 (23-C), 156.1 (1-C), 134.3 (30-C), 134.1 (11-C), 133.3 (27-C), 130.4 (12-C), 128.5 (13-C), 126.8 (14-C), 117.4 (28-C), 116.8 (31-C), 114.8 (2-C), 129.2 (3-C), 79.9 (24-C), 72.2 (29-C), 69.7 (16-C), 68.7 (26-C), 59.0 (21-C), 55.8 (8-C), 54.6 (6-C), 52.1 (32-C), 50.8 (15-C), 47.0 (18-C), 38.2 (5-C), 37.2 (10-C), 29.0 (20-C), 28.3 (25-C), 24.6 (19-C). **IR** (KBr) ν ($cm^{-1}$): 3285.6, 1635.6. **HPLC-ESI-MS**: m/z (%) = 707.4 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{38}H_{50}N_4O_9Na$ $[M+Na]^+$ 729.3470; found 729.3466. $R_f$ = 0.18 (CyH/EtOAc, 3/1).

**Methyl-(2*S*)-1-[(4*E*,9*S*,12*S*,15*S*)-12-benzyl-15-{[(*tert*-butoxy)carbonyl]amino}-11,14-dioxo-2,7-dioxa-10,13-diazabicyclo[15.2.2]henicosa-1(19),4,17,20-tetraene-9-carbonyl]pyrrolidine-2-carboxylate (TVC-151, 125)**

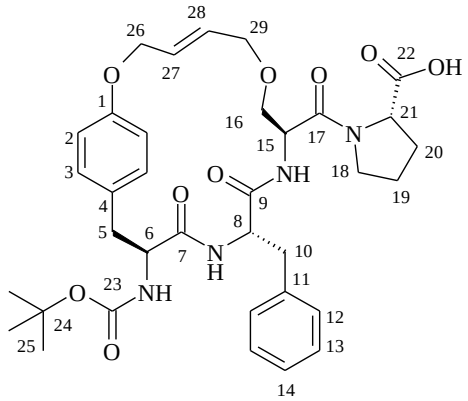


A solution of **124** (163 mg, 0.163 mmol) in degassed (for 40 min with argon) DCM (150 mL) was added Hoveyda-Grubb catalyst 2$^{nd}$ generation (14.5 mg, 10 mol%). The reaction mixture was refluxed for 50 h and the solvent was removed. Flash chromatography using CyH/EtOAc (1/1) as eluent afforded **125** (140 mg, 89%) as a yellow solid product.

**$^1$H-NMR** (600 MHz, $CDCl_3$): $\delta$ = 7.20-7.14 (m, 5H, 12-H, 13-H, 14-H), 7.04 (m, 2H, 3-H), 6.74 (d, *J* = 8.5 Hz, 2H, 2-H), 5.70 (bs, 2H, 27-H, 28-H), 4.78 (t, *J* = 7.1 Hz, 1H, 15-H), 4.62 (bs, 2H, 26-H), 4.45 (t, *J* = 7.1 Hz, 1H, 6-H), 4.41 (m, 1H, 21-H), 4.24 (bs, 1H, 8-H), 4.06 (d, *J* = 12.0 Hz, 1H, 29-H), 3.84 (d, *J* = 11.0 Hz, 1H, 29-H), 3.75 (m, 1H, 18-H), 3.70 (s, 3H, 30-H), 3.51 (m, 1H, 18-H), 3.35 (m, 1H, 16-H), 3.29 (t, *J* = 8.5 Hz, 1H, 16-H), 2.97 (m, 1H, 5-H), 2.92 (m, 2H, 10-H), 2.89 (m, 1H, 5-H), 2.17 (m, 1H, 20-H), 2.03 (m, 1H, 19-H), 1.97 (overlap, 2H, 19-H, 20-H), 1.44 (s, 9H, 25-H). **$^{13}$C-NMR** (150 MHz, $CDCl_3$): $\delta$ = 172.1 (7-C), 169.8 (17-C), 168.5 (22-C), 156.4 (23-C), 156.1 (1-C), 136.1 (11-C), 130.8 (27-C), 130.2 (3-C), 129.6 (28-C), 129.0 (12-C), 128.2 (13-C), 126.7 (14-C), 115.1 (2-C), 79.8 (24-C), 70.1 (29-C), 68.5 (16-C),

66.7 (26-C), 58.8 (21-C), 55.2 (8-C), 54.3 (6-C), 52.3 (30-C), 49.7 (15-C), 46.8 (18-C), 37.7 (10-C, 5-C), 28.8 (20-C), 28.1 (25-C), 24.4 (19-C). **IR** (KBr): ν ($cm^{-1}$) 3286.1, 2971.8, 1635.3. **HPLC-ESI-MS**: m/z (%) = 679.4 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{36}H_{46}N_4O_9Na$ $[M+Na]^+$ 701.3157; found 701.3158. $R_f$ = 0.13 (CyH/EtOAc, 2/3).

**(2*S*)-1-[(4*E*,9*S*,12*S*,15*S*)-12-Benzyl-15-{[(*tert*-butoxy)carbonyl]amino}-11,14-dioxo-2,7-dioxa-10,13-diazabicyclo[15.2.2]henicosa-1(19),4,17,20-tetraene-9-carbonyl]pyrrolidine-2-carboxylic acid (TVC-152, 126)**

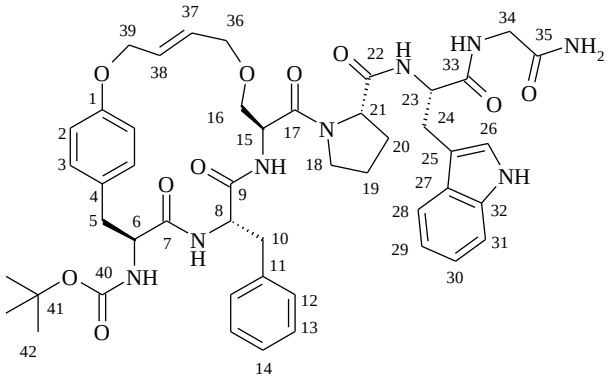


Methyl ester **125** (50 mg, 0.074 mmol) in THF/$H_2O$ (3 mL, 1/1) was added 4% aqueous LiOH (31 mg, 0.74 mmol). The reaction mixture was stirred at room temperature for 18 h, then acidified with 1N HCl at 0 °C to pH≈4 and extracted with AcOEt (3x60 mL). The combined organic phases were washed with brine solution and dried over $MgSO_4$. Removal of the solvent gave **126** (46.5 mg, 98%) as a white solid.

**$^1$H-NMR** (600 MHz, $CD_3OD$): $\delta$ = 7.24-7.19 (m, 5H, 12-H, 13-H, 14-H), 7.07 (d, *J* = 8.2 Hz, 2H, 3-H), 6.80 (d, *J* = 8.2 Hz, 2H, 2-H), 5.85 (m, 1H, 28-H), 5.75 (dt, *J* = 5.0 Hz, *J* = 16.0 Hz, 1H, 27-H), 4.78 (t, *J* = 7.1 Hz, 1H, 15-H), 4.67 (d, *J* = 5.0 Hz, 2H, 26-H), 4.54 (t, *J* = 7.1 Hz, 1H, 6-H), 4.34 (t, *J* = 7.0 Hz, 1H, 21-H), 4.31 (t, *J* = 7.0 Hz, 1H, 8-H), 4.06 (d, *J* = 12.0 Hz, 1H, 29-H), 3.85 (d, *J* = 11.0 Hz, 1H, 29-H), 3.72 (br, 1H, 18-H), 3.70 (s, 3H, 30-H), 3.43 (m, 1H, 18-H), 3.40 (m, 1H, 16-H), 3.06 (t, *J* = 8.5 Hz, 1H, 16-H), 3.04 (m, 1H, 5-H), 2.96-2.90 (m, 2H, 10-H), 2.89 (m, 1H, 5-H), 2.21 (m, 1H, 20-H), 2.02 (overlap, 2H, 19-H, 20-H), 1.93 (m, 1H, 19-H), 1.44 (s, 9H, 25-H). **$^{13}$C-NMR** (150 MHz, $CD_3OD$): $\delta$ = 174.5 (22-C), 172.4 (7-C), 169.5 (17-C), 156.8 (23-C), 154.1 (1-C), 136.6 (11-C), 130.5 (27-C), 129.9 (3-C), 129.8 (28-C), 128.8 (12-C), 127.9 (13-C), 126.1 (14-C), 114.7 (2-C), 79.8 (24-C), 69.9 (29-C), 67.8 (16-C), 66.8 (26-C), 59.0 (21-C), 55.2 (8-C), 53.9 (6-C), 50.0 (15-C), 46.7 (18-C), 37.5 (5-C) 36.9 (10-C), 28.7 (20-C), 27.4 (25-C), 24.1 (19-C). **IR** (KBr) ν ($cm^{-1}$): 3309.7, 1637.9. **HPLC-ESI-MS**: m/z (%) = 665.3 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{35}H_{44}N_4O_9Na$ $[M+Na]^+$ 687.3001; found 687.3001. $R_f$ = 0.41 (EtOAc).

***tert*-Butyl-N-[(4*E*,9*S*,12*S*,15*S*)-12-benzyl-9-[(2S)-2-{[(1S)-1-[(carbamoylmethyl)carbamoyl]-2-(1H-indol-3-yl)ethyl]carbamoyl}pyrrolidine-1-carbonyl]-11,14-dioxo-2,7-dioxa-10,13-diazabicyclo [15.2.2]henicosa-1(19),4,17,20-tetraen-15-yl]carbamate (TVC-153, 127)**

To a solution of **126** (32 mg, 48.14 µmol), **TVC-108** (15 mg, 57.8 µmol) and HATU (22 mg, 57.8 µmol) in DMF (1 mL), DIPEA (40 µL, 241 µmol) was added. The reaction mixture was stirred at room temperature for 18 h and concentrated under reduced pressure to remove DMF. The residue was subjected to silica gel column chromatography eluting EtOAc/EtOH (10/1) to furnish **127** (43.6 mg, 100%) as a solid.

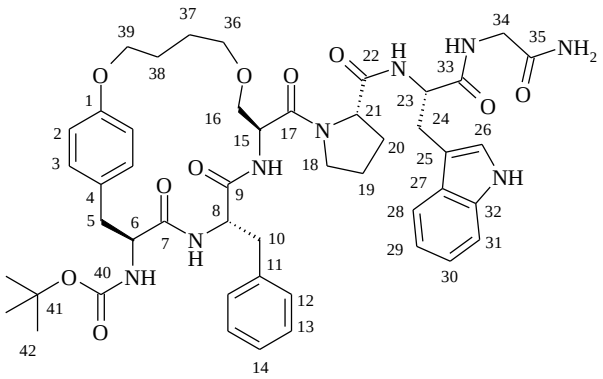


**$^1$H-NMR** (400 MHz, DMSO-$d_6$): $\delta$ = 10.8 (s, 1H, NH), 8.25 (d, *J* = 8.5 Hz, 1H, NH), 8.0 (t, *J* = 6.0 Hz, 1H, NH), 7.95 (d, *J* = 7.0 Hz, 1H, NH), 7.70 (d, *J* = 7.7 Hz, 1H, NH), 7.53 (d, *J* = 8.0 Hz, 1H, 28-H), 7.32 (d, *J* = 8.0 Hz, 1H, 31-H), 7.18-7.12 (m, 5H, 12-H, 13-H, 14-H), 7.14 (overlap, 1H, 26-H), 7.05 (overlap, 1H, 30-H), 7.00 (d, *J* = 8.5 Hz, 2H, 3-H), 6.97 (t, *J* = 7.5 Hz, 1H, 29-H), 6.75 (d, *J* = 8.5 Hz, 2H, 3-H), 5.83 (m, 1H, 38-H), 5.69 (m, 1H, 37-H), 4.68 (overlap, 3H, 15-H, 39-H), 4.50 (m, 1H, 6-H), 4.46 (m, 1H, 23-H), 4.23 (m, 1H, 23-H), 4.17 (q, *J* = 7.0 Hz, 1H, 8-H), 3.99 (dd, *J* = 5.0 Hz, *J* = 11 Hz, 1H, 36-H), 3.75 (m, 1H, 36-H), 3.65 (dd, *J* = 5.0 Hz, *J* = 17.0 Hz, 1H, 34-H), 3.55 (dd, *J* = 5.0 Hz, *J* = 17.0 Hz, 1H, 34-H), 3.50 (m, 1H, 18-H), 3.29 (m, 2H, 16-H), 3.27 (m, 1H, 18-H), 3.19 (m, 1H, 24-H), 2.98 (m, 1H, 24-H), 2.88 (overlap, 3H, 5-H, 10-H), 2.79 (m, 1H, 5-H), 1.90 (m, 1H, 20-H), 1.68 (m, 1H, 20-H), 1.66 (m, 2H, 19-H), 1.41 (s, 9H, 42-H). **$^{13}$C-NMR** (100 MHz, DMSO-$d_6$): $\delta$ = 171.2 (7-C), 170.7 (9-C), 170.1 (33-C), 169.7 (17-C), 169.3 (22-C), 168.4 (35-C), 156.1 (40-C), 154.4 (1-C), 137.1 (4-C), 136.0 (32-C), 131.2 (38-C), 130.9 (37-C), 130.8 (3-C), 129.7 (12-C), 128.2 (13-C), 127.3 (27-C), 126.7 (14-C), 124.0 (26-C), 121.3 (30-C), 118.7 (28-C), 118.6 (29-C), 111.7 (31-C), 115.2 (2-C), 78.2 (41-C), 70.2 (36-C), 68.4 (16-C), 67.3 (39-C), 60.2 (21-C), 54.8 (8-C), 53.7 (6-C), 50.2 (15-C), 47.4 (18-C), 42.5 (34-C), 38.0 (5-C), 36.8 (10-C), 29.2 (20-C), 28.5 (42-C), 24.4 (19-C). **IR** (KBr) ν ($cm^{-1}$): 3433.9, 1653.1. **HPLC-ESI-MS**: m/z (%) = 907.4 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{48}H_{58}N_8O_{10}Na$ $[M+Na]^+$ 929.4168; found 929.4160. $R_f$ = 0.58 (EtOAc/EtOH, 8/1).

### *tert*-Butyl-N-[(9*S*,12*S*,15*S*)-12-benzyl-9-[(2*S*)-2-{[(1*S*)-1-[(carbamoylmethyl)carbamoyl]-2-(1H-indol-3-yl)ethyl]carbamoyl}pyrrolidine-1-carbonyl]-11,14-dioxo-2,7-dioxa-10,13-diazabicyclo [15.2.2]henicosa-1(19),17,20-trie-15-yl]carbamate (TVC-154)

A suspension of **127** (18 mg, 19.85 µmol) and Pd/C (3.6 mg, 20 wt%) in MeOH/EtOAc (6 mL, 2/1) was stirred under a hydrogen atmosphere at room temperature for 20 h. The Pd/C was removed by a filtration

over a Celite pad and washed with EtOAc. Removal of solvent afforded **TVC-154** (18 mg, 99%) as a brown solid.

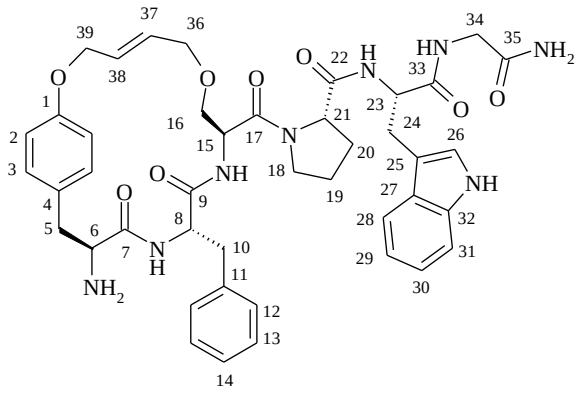


**$^1$H-NMR** (600 MHz, $CD_3OD$): $\delta$ = 7.58 (d, $J$ = 8.6 Hz, 1H, 28-H), 7.36 (d, $J$ = 8.6 Hz, 1H, 31-H), 7.18-7.13 (m, 5H, 12-H, 13-H, 14-H), 7.13 (overlap, 2H, 26-H, 30-H), 7.07 (d, $J$ = 7.5 Hz, 2H, 3-H), 7.04 (t, $J$ = 7.5 Hz, 1H, 29-H), 6.78 (d, $J$ = 7.5 Hz, 2H, 2-H), 4.65 (t, $J$ = 6.5 Hz, 1H, 15-H), 4.56 (t, $J$ = 7.5 Hz, 23-H), 4.35 (m, 1H, 6-H), 4.31 (m, 1H, 21-H), 4.26 (t, $J$ = 6.5 Hz, 1H, 8-H), 4.1 (m, 2H, 39-H), 3.87 (d, $J$ = 17.0 Hz, 1H, 34-H), 3.69 (d, $J$ = 17.0 Hz, 1H, 34-H), 3.58 (m, 1H, 18-H), 3.50 (m, 1H, 16-H), 3.43 (m, 2H, 16-H, 18-H), 3.39 (m, 2H, 36-H), 3.37 (m, 1H, 24-H), 3.25 (m, 1H, 24-H), 3.04 (m, 1H, 5-H), 2.93 (m, 1H, 10-H), 2.82 (m, 1H, 5-H), 2.80 (m, 1H, 10-H), 2.07 (m, 1H, 20-H), 1.78 (m, 3H, 20-H, 19-H), 1.74 (m, 2H, 38-H), 1.62 (m, 2H, 37-H), 1.43 (s, 9H, 42-H). **$^{13}$C-NMR** (150 MHz, $CD_3OD$): $\delta$ = 172.3 (7-C), 171.5 (9-C), 170.2 (22-C), 169.5 (33-C), 168.4 (35-C), 157.6 (40-C), 156.8 (1-C), 136.7 (32-C), 129.7 (3-C), 127.6 (12-C), 127.5 (13-C), 127.2 (27-C), 126.2 (14-C), 123.0 (26-C), 121.2 (30-C), 118.5 (29-C), 117.8 (28-C), 114.2 (2-C), 110.0 (31-C), 106.8 (25-C), 79.8 (41-C), 70.6 (16-C), 68.9 (36-C), 66.8 (39-C), 60.5 (21-C), 56.1 (8-C), 54.7 (23-C), 53.5 (6-C), 51.3 (15-C), 47.2 (18-C), 42.0 (34-C), 37.7 (5-C), 37.1 (10-C), 28.6 (20-C), 28.3 (42-C), 26.2 (24-C), 25.3 (38-C), 24.6 (37-C), 24.4 (19-C). **IR** (KBr) ν ($cm^{-1}$): 3447.9, 1654.5. **HPLC-ESI-MS**: m/z (%) = 909.4 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{48}H_{60}N_8O_{10}Na$ $[M+Na]^+$ 931.4325; found 931.4328. $R_f$ = 0.37 (DCM/MeOH, 15/1).

**General procedure for *N*-Boc deprotection**

Macrocyclic peptides were treated with TFA/TIPS/$H_2O$ (2 mL, 95/4/1) at 0 $^oC$ for 4 h. The reaction mixtures were then concentrated under reduced pressure to dryness. The crude products were subjected to silica gel column chromatography using DCM/MeOH (6/1, 0.1% TEA) to provide pure products, which were dissolved in ACN/$H_2O$ (3 mL, 1/1) and lyophilized to obtain white powders.

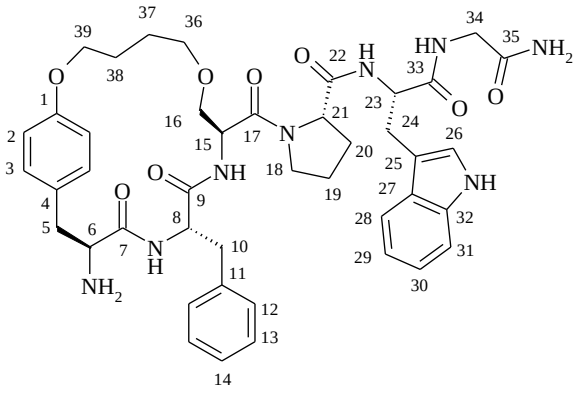
**(2*S*)-2-{[(2*S*)-1-[(4*E*,9*S*,12*S*,15*S*)-15-Amino-12-benzyl-11,14-dioxo-2,7-dioxa-10,13-diaza-bicyclo [15.2.2]heicosa-1(19),4,17,20-tetraene-9-carbonyl]pyrrolidin-2-yl]formamido}-N-(carbamoyl methyl)-3-(1H-indol-3-yl)propanamide (TVC-156, 128)**



Starting material **127** (24 mg, 26.46 µmol) gave product **128** (20 mg, 94%).

**$^1$H-NMR** (600 MHz, $CD_3OD$): $\delta$ = 7.65 (d, $J$ = 8.0 Hz, 1H, 28-H),7.36 (d, $J$ = 8.0 Hz, 1H, 31-H), 7.24-7.19 (m, 5H, 12-H, 13-H, 14-H), 7.14 (s, 1H, 26-H), 7.13 (overlap, 1H, 30-H), 7.11 (d, $J$ = 8.4 Hz, 2H, 3-H), 7.05 (t, $J$ = 7.5 Hz, 1H, 29-H), 6.86 (d, $J$ = 8.4 Hz, 2H, 2-H), 5.84 (br, 1H, 37-H), 5.71 (br, 1H, 38-H), 4.71 (bs, 2H, 39-H), 4.63 (t, $J$ = 7.5 Hz, 1H, 23-H), 4.24 (m, 1H, 21-H), 4.60 (t, $J$ = 8.0 Hz, 1H, 6-H), 4.02 (d, $J$ = 11.0 Hz, 1H, 36-H), 3.84 (d, $J$ = 17.0 Hz, 1H, 34-H), 3.73 (d, $J$ = 17.0 Hz, 1H, 34-H), 3.66 (t, $J$ = 11.0 Hz, 1H, 36-H), 3.57 (m, 1H, 18-H), 3.35 (overlap, 2H, 18-H, 24-H), 3.31 (m, 1H, 10-H), 3.14 (m, 1H, 24-H), 3.06 (d, $J$ = 7.5 Hz, 2H, 5-H), 3.04 (m, 1H, 10-H), 2.03 (m, 1H, 20-H), 1.78 (m, 1H, 19-H), 1.72 (m, 1H, 20-H), 1.64 (m, 1H, 19-H). **$^{13}$C-NMR** (150 MHz, $CD_3OD$): $\delta$ = 174.2 (9-C), 174.0 (7-C), 173.9 (22-C), 171.9 (17-C), 171.1 (33-C), 168.5 (35-C), 159.3 (1-C), 138.0 (11-C), 137.7 (32-C), 132.3 (38-C), 130.4 (3-C), 130.0 (37-C), 129.1 (12-C), 128.2 (12-C), 126.5 (13-C), 126.0 (27-C), 123.2 (26-C), 121.1 (30-C), 118.5 (29-C), 117.6 (28-C), 111.0 (31-C), 106.5 (25-C), 70.2 (36-C), 67.5 (16-C), 66.9 (39-C), 60.5 (21-C), 54.7 (6-C), 54.3 (23-C), 53.2 (8-C), 49.5 (15-C), 47.6 (18-C), 41.9 (34-C), 37.3 (5-C), 35.2 (35.2), 28.6 (20-C), 26.5 (24-C), 24.6 (19-C). **HPLC-ESI-MS**: m/z (%) = 708.4 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{43}H_{51}N_8O_8$ $[M+H]^+$ 807.3824; found 807.3853.

**(2*S*)-2-{[(2*S*)-1-[(9*S*,12*S*,15*S*)-15-Amino-12-benzyl-11,14-dioxo-2,7-dioxa-10,13-diaza-bicyclo[15.2.2]henicosa-1(19),17,20-triene-9-carbonyl]-pyrrolidin-2-yl]formamido}-N-(carbamoylmethyl)-3-(1H-indol-3-yl)propanamide (TVC-157, 129)**



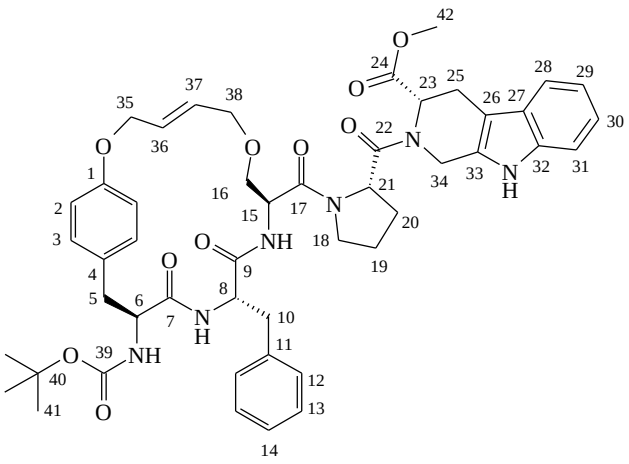
Starting material **TVC-154** (18 mg, 26.46 µmol) afforded product **129** (15 mg, 94%).

**$^1$H-NMR** (600 MHz, $CD_3OD$): $\delta$ = 7.58 (d, $J$ = 8.6 Hz, 1H, 28-H), 7.36 (d, $J$ = 8.6 Hz, 1H, 31-H), 7.18-7.10 (m, 5H, 12-H, 13-H, 14-H), 7.13 (overlap, 2H, 26-H, 30-H), 7.08 (overlap, 2H, 3-H), 7.04 (t, $J$ = 7.5 Hz, 1H, 29-H), 6.84 (d, $J$ = 7.5 Hz, 2H, 2-H), 4.64 (t, $J$ = 6.5 Hz, 1H, 15-H), 4.55 (t, $J$ = 7.5 Hz, 23-H), 4.44 (t, $J$ = 6.5 Hz, 1H, 8-H), 4.29 (m, 1H, 21-H), 4.15 (m, 1H, 6-H), 4.11 (m, 2H, 36-H), 3.87 (d, $J$ = 17.0 Hz, 1H, 34-H), 3.68 (d, $J$ = 17.0 Hz, 1H, 34-H), 3.55 (m, 1H, 18-H), 3.45 (m, 2H, 18-H), 3.49 (m, 2H, 39-H), 3.40

(m, 2H, 16-H), 3.35 (m, 1H, 24-H), 3.25 (m, 1H, 24-H), 3.22 (m, 1H, 5-H), 2.98 (m, 2H, 10-H), 2.94 (m, 1H, 5-H), 2.07 (m, 1H, 20-H), 1.78 (m, 3H, 20-H, 19-H), 1.74 (m, 2H, 38-H), 1.62 (m, 2H, 37-H). **$^{13}$C-NMR** (150 MHz, $CD_3OD$): $\delta$ = 171.5 (9-C), 170.3 (7-C), 170.0 (22-C), 169.2 (33-C), 168.9 (35-C), 155.8 (1-C), 136.2 (32-C), 130.1 (3-C), 129.2 (13-C), 127.7 (12-C), 127.5 (27-C), 126.5 (14-C), 123.0 (26-C), 121.3 (30-C), 118.4 (29-C), 117.9 (28-C), 114.9 (2-C), 110.0 (31-C), 106.6 (25-C), 70.5 (36-C), 69.0 (16-C), 66.7 (39-C), 60.5 (21-C), 54.7 (23-C), 53.9 (8-C), 53.5 (6-C), 51.2 (15-C), 47.4 (18-C), 41.9 (34-C), 38.0 (10-C), 35.9 (5-C), 28.7 (20-C), 26.6 (24-C), 25.2 (38-C), 24.5 (37-C), 24.5 (19-C). **HPLC-ESI-MS**: m/z (%) = 809.4 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{43}H_{52}N_8O_8Na$ $[M+Na]^+$ 831.3800; found 831.3819.

### 3.14 Hybrid synthesis

**Methyl-(3*S*)-2-[(2*S*)-1-[(4*E*,9*S*,12*S*,15*S*)-12-benzyl-15-{[(*tert*-butoxy)carbonyl]amino}-11,14-dioxo-2,7-dioxa-10,13-diazabicyclo[15.2.2]henicosa-1(19),4,17,20-tetraene-9-carbonyl]pyrrolidine-2-carbonyl]-1,2,3,4,9-pentahydropyrido[3,4-b]indole-3-carboxylate (TVC-189, 130)**

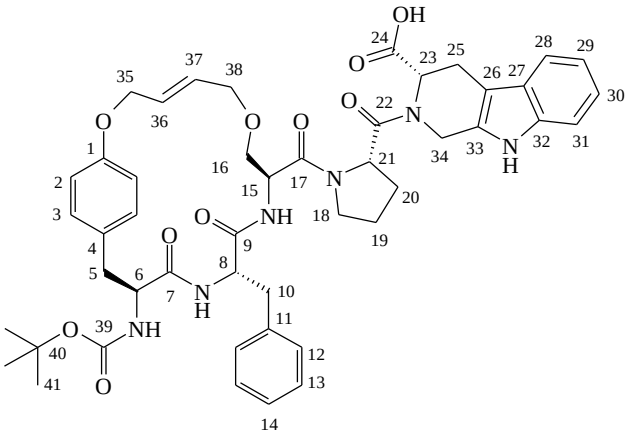


To a solution of **126** (40 mg, 60.17 µmol), **69** (18 mg, 78.23 µmol) in DMF (1.5 mL), HATU (29.7 mg, 78.23 µmol) and DIPEA (50 µL, 241 µmol) were added. After stirring at room temperature for 18 h, the solvent was removed and the crude residue was purified by silica gel column chromatography using EtOAc as eluent to afford product **130** (41 mg, 78%).

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 7.37 (d, *J* = 8.2 Hz, 1H, 28-H), 7.27 (d, *J* = 8.2 Hz, 1H, 31-H), 7.22-7.13 (m, 5H, 12-H, 13-H, 14-H), 7.11 (overlap, 1H, 30-H), 7.05 (overlap, 1H, 29-H), 6.98 (d, *J* = 8.0 Hz, 2H, 3-H), 6.68 (d, *J* = 8.0 Hz, 2H, 2-H), 5.66 (m, 1H, 36-H), 5.56 (m, 1H, 37-H), 5.05 (d, *J* = 17.1 Hz, 1H, 34-H), 5.02 (m, 1H, 21-H), 4.81 (t, *J* = 7.0 Hz, 1H, 15-H), 4.76 (overlap, 1H, 23-H), 4.72 (d, *J* = 14.2 Hz, 1H, 34-H), 4.60 (m, 2H, 35-H), 4.42 (t, *J* = 6.5 Hz, 1H, 8-H), 4.21 (bs, 1H, 6-H), 4.05 (m, 1H, 38-H), 3.84 (m, 1H, 38-H), 3.74 (br, 1H, 18-H), 3.60 (m, 1H, 18-H), 3.54 (s, 3H, 42-H), 3.38 (m, 1H, 16-H), 3.32 (m, 1H, 16-H), 3.30 (overlap, 1H, 25-H), 2.93 (m, 2H, 10-H), 2.90 (overlap, 1H, 5-H), 2.80 (m, 1H, 5-H), 2.73 (m, 1H, 25-H), 2.27 (m, 1H, 20-H), 2.11 (m, 1H, 20-H), 1.97 (m, 2H, 19-H), 1.44 (s, 9H, 41-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 172.3 (7-C), 171.5 (9-C), 170.1 (17-C), 169.6 (22-C), 168.5

(24-C), 156.3 (39-C), 155.2 (1-C), 136.5 (32-C), 135.9 (33-C), 130.7 (36-C), 130.0 (3-C), 129.5 (37-C), 129.3 (12-C), 128.5 (13-C), 126.9 (14-C), 121.8 (30-C), 119.4 (29-C), 118.0 (28-C), 115.4 (2-C), 111.1 (31-C), 105.6 (26-C), 70.5 (38-C), 68.0 (16-C), 66.8 (35-C), 57.7 (21-C), 55.5 (6-C), 54.1 (8-C), 52.3 (42-C), 50.7 (15-C), 50.2 (23-C), 47.4 (18-C), 42.2 (34-C), 37.8 (5-C), 37.6 (10-C), 28.3 (41-C), 27.9 (20-C), 24.3 (19-C), 22.1 (25-C). **IR** (KBr) ν ($cm^{-1}$): 3421.3, 1653.1. **HPLC-ESI-MS**: m/z (%) = 877.4 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{48}H_{56}N_6O_{10}Na$ $[M+Na]^+$ 899.3950; found 899.3950. $R_f$ = 0.21 (EtOAc).

**(3*S*)-2-[(2*S*)-1-[(4*E*,9*S*,12*S*,15*S*)-12-Benzyl-15-{[(*tert*-butoxy)carbonyl]amino}-11,14-dioxo-2,7-dioxa-10,13-diazabicyclo[15.2.2]henicosa-1(19),4,17,20-tetraene-9-carbonyl]pyrrolidine-2-carbonyl]-1,2,3,4,9-pentahydropyrido[3,4-b]indole-3-carboxylic acid (TVC-191, 131)**

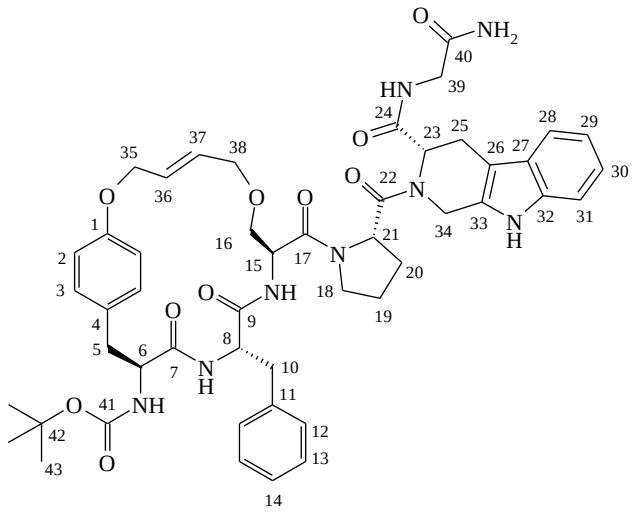


Methyl ester **130** (34 mg, 38.7 µmol) in THF (1 mL) was treated with 4% aqueous LiOH (0.4 mL) at room temperature for 5 h. The reaction mixture was then acidified with 1N HCl at 0 °C to pH≈4 and extracted with EtOAc (3x40 mL). The combined organic phases were washed with $H_2O$, brine solution and dried over $Na_2SO_4$. Removal of the solvent yielded **131** (30.5 mg, 91%) as a solid.

**$^1$H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 7.44 (d, $J$ = 8.2 Hz, 1H, 28-H), 7.27 (d, $J$ = 8.2 Hz, 1H, 31-H), 7.25-7.17 (m, 5H, 12-H, 13-H, 14-H), 7.14 (overlap, 1H, 30-H), 7.09 (overlap, 1H, 29-H), 7.06 (d, $J$ = 8.0 Hz, 2H, 3-H), 6.78 (d, $J$ = 8.0 Hz, 2H, 2-H), 5.76 (m, 2H, 36-H, 37-H), 5.15 (m, 1H, 34-H), 5.04 (m, 1H, 21-H), 4.84 (overlap, 1H, 15-H), 4.63 (overlap, 1H, 23-H), 4.82 (overlap, 1H, 34-H), 4.66 (br, 2H, 35-H), 4.56 (t, $J$ = 6.5 Hz, 1H, 8-H), 4.31 (m, 1H, 6-H), 4.10 (m, 1H, 38-H), 3.81 (m, 1H, 38-H), 3.76 (br, 1H, 18-H), 3.63 (m, 1H, 18-H), 3.41 (m, 1H, 16-H), 3.35 (m, 1H, 16-H), 3.36 (overlap, 1H, 25-H), 2.98 (m, 2H, 10-H), 2.90 (overlap, 1H, 5-H), 2.84 (m, 1H, 5-H), 2.78 (m, 1H, 25-H), 2.31 (m, 1H, 20-H), 2.21 (m, 1H, 20-H), 1.97 (m, 2H, 19-H), 1.44 (s, 9H, 41-H). **$^{13}$C-NMR** (100 MHz, $CD_3OD$): $\delta$ = 171.3 (7-C), 171.0 (9-C), 170.6 (17-C), 156.7 (39-C), 154.9 (1-C), 136.7 (32-C), 135.2 (33-C), 130.8 (3-C), 130.3 (36-C, 37-C), 129.6 (12-C), 128.5 (13-C), 127.6 (14-C), 122.5 (30-C), 119.8 (29-C), 118.4 (28-C), 116.2 (2-C), 111.7 (31-C), 106.6 (26-C), 70.0 (38-C), 66.8 (35-C), 66.7 (16-C), 57.7 (21-C), 55.3 (6-C), 54.9 (8-C), 50.3 (15-C), 49.8 (23-C), 47.6 (18-C), 41.2 (34-C), 37.4 (5-C), 37.2 (10-C), 28.6 (41-C), 28.2 (20-C), 24.4 (19-C), 22.6 (25-C). **IR** (KBr) ν ($cm^{-1}$): 3426.6, 1716.3, 1646.8. **HPLC-ESI-MS**: m/z (%) = 863.4

$[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{47}H_{54}N_6O_{10}Na$ $[M+Na]^+$ 885.3794; found 885.3793. $R_f$ = 0.12 (EtOAc/EtOH, 7/1).

***tert*-Butyl-N-[(4*E*,9*S*,12*S*,15*S*)-12-benzyl-9-[(2*S*)-2-[(3*S*)-3-[(carbamoylmethyl)carba-moyl]-1,2,3,4,9-pentahydropyrido[3,4-b]indole-2-carbonyl]pyrrolidine-1-carbonyl]-11,14-dioxo-2,7-dioxa-10,13-diazabicyclo[15.2.2]henicosa-1(19),4,17,20-tetraen-15- yl]carbamate (TVC-193)**

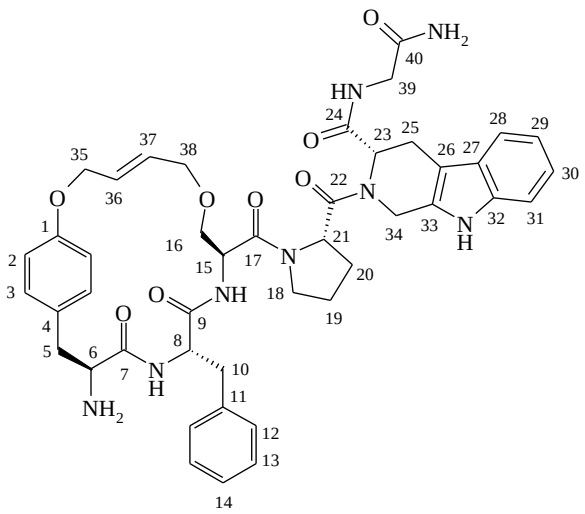


A stirred solution of **131** (28 mg, 32.45 µmol), HCl*H-Gly-NH2 (4.7 mg, 42.2 µmol) and TBTU (12.5 mg, 38.9 µmol) in DMF (2 mL) was added DIPEA (0.03 mL, 0.23 mmol). The reaction was stirred at room temperature for 20 h and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography using EtOAc/EtOH (7/1) to obtain **TVC-193** (23 mg, 77%) as a solid product.

**$^1$H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 7.46 (d, $J$ = 8.0 Hz, 1H, 28-H), 7.29 (d, $J$ = 8.0 Hz, 1H, 31-H), 7.26-7.17 (m, 5H, 12-H, 13-H, 14-H), 7.07 (overlap, 1H, 30-H, 3-H), 7.01 (overlap, 1H, 29-H), 6.78 (d, $J$ = 8.0 Hz, 2H, 2-H), 5.76 (m, 2H, 36-H, 37-H), 5.11 (m, 1H, 34-H), 5.01 (m, 1H, 21-H), 4.81 (overlap, 1H, 15-H), 4.88 (overlap, 1H, 34-H), 4.82 (overlap, 1H, 23-H), 4.67 (br, 2H, 35-H), 4.54 (m, 1H, 6-H), 4.30 (m, 1H, 8-H), 3.87 (m, 2H, 38-H, 39-H), 3.71 (m, 2H, 38-H, 39-H), 3.73 (br, 1H, 18-H), 3.61 (m, 1H, 18-H), 3.41 (m, 2H, 16-H), 3.40 (overlap, 1H, 25-H), 3.01 (m, 1H, 25-H), 2.99 (m, 2H, 10-H), 2.91 (overlap, 2H, 5-H), 2.33 (m, 1H, 20-H), 1.92 (m, 1H, 20-H), 1.99-1.92 (m, 2H, 19-H), 1.46 (s, 9H, 41-H). **$^{13}$C-NMR** (100 MHz, $CD_3OD$): $\delta$ = 174.1 (9-C), 173.3 (7-C), 172.1 (24-C), 171.6 (17-C), 170.9 (22-C), 169.8 (40-C), 158.1 (41-C), 157.2 (1-C), 138.9 (32-C), 138.2 (33-C), 130.0 (3-C), 130.2 (36-C, 37-C), 129.0 (12-C), 128.0 (13-C), 126.4 (14-C), 121.1 (30-C), 118.5 (29-C), 117.4 (28-C), 114.9 (2-C), 110.7 (31-C), 106.4 (26-C), 70.0 (38-C), 66.9 (35-C), 67.5 (16-C), 57.8 (21-C), 55.6 (8-C), 54.2 (6-C), 50.3 (15-C), 49.2 (23-C), 47.0 (18-C), 42.2 (39-C), 41.9 (34-C), 37.3 (5-C), 37.1 (10-C), 28.6 (43-C), 28.9 (20-C), 24.9 (19-C), 22.4 (25-C). **IR** (KBr) ν ($cm^{-1}$): 3421.0, 2932.8, 1662.5. **HPLC-ESI-MS**: m/z (%) = 919.4 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{49}H_{58}N_8O_{10}Na$ $[M+Na]^+$ 941.4168; found 941.4167. $R_f$ = 0.28 (EtOAc/EtOH, 7/1).

**2-{[(3*S*)-2-[(2*S*)-1-[(4*E*,9*S*,12*S*,15*S*)-15-Amino-12-benzyl-11,14-dioxo-2,7-dioxa-10,13-diazabicyclo-[15.2.2]henicosa-1(19),4,17,20-tetraene-9-carbonyl]pyrrolidine-2-carbonyl]-1,2,3,4,9-pentahydro-pyrido[3,4-b]indol-3-yl]formamido}acetamide (TVC-199, 132)**

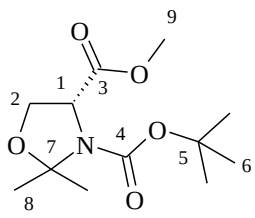
Cyclic peptide **TVC-193** (30 mg, 30 µmol) was treated with TFA/TIPS/$H_2O$ (2 mL, 95/4/1) at 0 $^oC$ for 3 h. The solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography eluting DCM/MeOH (8/1, 0.1% TEA) to afford pure product **132** (23 mg, 86%), which was dissolved in ACN/$H_2O$ (2 mL, 1/1) and lyophilized to afford white powder.



**$^1$H-NMR** (600 MHz, $CD_3OD$): $\delta$ = 7.47 (d, $J$ = 8.0 Hz, 1/2H, 28-H), 7.43 (d, $J$ = 8.0 Hz, 1/2H, 28-H), 7.29 (overlap, 1H, 31-H), 7.26-7.18 (m, 5H, 12-H, 13-H, 14-H), 7.08 (overlap, 1H, 30-H, 3-H), 7.00 (overlap, 1H, 29-H), 6.82 (d, $J$ = 8.0 Hz, 2H, 2-H), 5.77 (m, 1H, 36-H), 5.73 (m, 1H, 37-H), 5.41 (m, 1H, 34-H), 4.97 (m, 1H, 21-H), 4.85 (overlap, 1H, 15-H), 4.52 (m, 1H, 34-H), 4.71 (overlap, 1H, 23-H), 4.69 (br, 2H, 35-H), 4.62 (m, 1H, 8-H), 4.11 (m, 1H, 38-H), 3.94 (m, 2H, 6-H, 39-H), 3.87 (m, 2H, 38-H, 39-H), 3.79 (m, 2H, 38-H, 39-H), 3.74 (m, 1H, 18-H), 3.69 (overlap, 1H, 25-H), 3.65 (m, 1H, 18-H), 3.58 (m, 1H, 25-H), 3.38 (m, 2H, 16-H), 2.99 (m, 2H, 10-H), 2.91 (overlap, 2H, 5-H), 2.34 (m, 1H, 20-H), 2.13 (m, 1H, 19-H), 1.94 (m, 1H, 20-H), 1.94 (m, 1H, 20-H). **$^{13}$C-NMR** (150 MHz, $CD_3OD$): $\delta$ = 174.2 (9-C), 173.7 (7-C), 173.2 (24-C), 172.9 (17-C), 170.9 (22-C), 169.8 (40-C), 156.0 (1-C), 138.4 (32-C), 137.4 (33-C), 132.0 (3-C), 131.1 (37-C), 129.8 (36-C), 129.0 (12-C), 128.0 (13-C), 126.3 (14-C), 121.1 (30-C), 118.6 (29-C), 117.2 (28-C), 115.1 (2-C), 110.6 (31-C), 105.0 (26-C), 69.9 (38-C), 67.4 (16-C), 67.1 (35-C), 57.4 (21-C), 54.6 (23-C), 54.4 (8-C), 53.9 (6-C), 49.0 (15-C), 47.0 (18-C), 42.0 (39-C), 39.0 (34-C), 37.5 (10-C), 36.8 (5-C), 28.6 (20-C), 24.7 (19-C), 22.2 (25-C). **HPLC-ESI-MS**: m/z (%) = 819.4 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{44}H_{51}N_8O_8$ $[M+H]^+$ 819.3824; found 819.3830.

### 3.15 Macrocylic ether synthesis

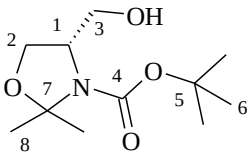
**(*R*)-3-*tert*-Butyl-4-methyl-2,2-dimethyloxazolidine-3,4-dicarboxylate (TVC-261, 151)**



To a solution of methyl-(2*R*)-2-{[(*tert*-butoxy)carbonyl]amino}-3-hydroxy-propanoate (1.5 g, 6.84 mmol) in acetone (10 mL) at 0 °C, 2,2-dimethoxypropane (4.2 mL, 30 mmol) was added and followed by addition of $BF_3.Et_2O$ (49 mg, 0.34 mmol). The reaction mixture was allowed to stir for 2 h at room temperature and concentrated to dryness. The residue was dissolved in EtOAc (150 mL), washed with saturated $NaHCO_3$ and dried over $MgSO_4$. Removal of the solvent yielded product **TVC-261** (1.76 mg, 98%) as a yellow oil.

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 4.49 (d, *J* = 6.6 Hz, 1/2H, 1-H), 4.38 (d, *J* = 6.6 Hz, 1/2H, 1-H), 4.14 (m, 1H, 2-H), 4.05 (t, *J* = 8.5 Hz, 1H, 2-H), 3.76 (s, 3H, 9-H), 1.68 (s, 3H, 8-H), 1.54 (s, 3H, 8-H), 1.43 (s, 9H, 6-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 171.6 (3-C), 152.0 (4-C), 95.0 (7-C), 80.8 (5-C), 65.9 (2-C), 59.3 (1-C), 52.3 (9-C), 28.3 (6-C), 24.8 (8-C). **IR** (neat) ν ($cm^{-1}$): 1754.9, 1702.8. **HPLC-ESI-MS:** m/z (%) = 282.1 (10) $[M+Na]^+$, 541.3 (15) $[2M+Na]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{12}H_{21}NO_5Na$ $[M+Na]^+$ 282.1312; found 282.1308. $R_f$ = 0.36 (CyH/EtOAc, 8/1).

**(*S*)-*tert*-Butyl-4-(hydroxymethyl)-2,2-dimethyloxazolidine-3-carboxylate (TVC-233, 152)**

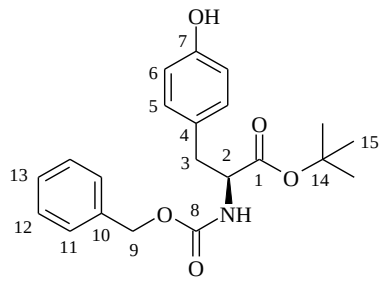


To a suspension solution of LAH (111 mg, 2.9 mmol) in dry THF (5 mL) at 0 °C, a solution of **151** (0.5 g, 1.93 mmol) in THF (8 mL) was added. The reaction mixture was allowed to warm up to room temperature and stirred for 20 min. Afterward, the mixture was cooled with an ice-water bath while 10% NaOH (3 mL) was added. After stirring further for 1 h at room temperature, the white precipitate was removed by filtration over a Celite pad and washed with EtOAc. The filtrate was concentrated under reduced pressure to obtain alcohol **152** (445 mg, 99%) as a yellow oil.

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 4.10 (m, 1H, 1-H), 4.01 (m, 1H, 2-H), 3.76 (m, 2H, 2-H, 3-H), 3.63 (m, 1H, 3-H), 1.56 (s, 3H, 8-H), 1.49 (overlap, 12H, 8-H, 6-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 154.3 (4-C), 94.1 (7-C), 81.1 (5-C), 65.33 (2-C, 3-C), 59.7 (1-C), 28.5 (6-C), 26.9 (8-C). **IR** (neat) ν ($cm^{-1}$): 3443.2, 1695.1. **HPLC-ESI-MS:** m/z (%) = 254.2 (10) $[M+Na]^+$, 485.4 (13) $[2M+Na]^+$. **HR-ESI-MS** (m/z): calcd. for $C_9H_{17}NO_3Na$ $[M+Na]^+$ 254.1163; found 254.1163. $[\alpha]_D^{20}$ = -22.8 (c 1.5, MeOH). $R_f$ = 0.4 (CyH/EtOAc, 3/1).

**(*S*)-*tert*-Butyl-2-(((benzyloxy)carbonyl)amino)-3-(4-hydroxyphenyl)propanoate (TVC-197)**

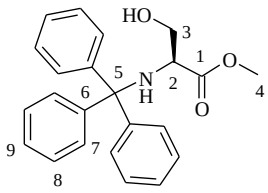
A solution of (*S*)-tyrosine-*tert*-butyl ester (200 mg, 0.84 mmol) in a two-phase mixture of water (10 mL) and ether (8 mL) at room temperature was treated with $Na_2CO_3$ (268 mg, 2.53 mmol) and benzyl chloroformate (151.4 mg, 0.84 mmol). The resulting solution was stirred at room temperature for 3 h and



the ether layer was separated. The aqueous phase was extracted with ether (2x50 mL). The combined organic phases were washed with brine solution and dried over $MgSO_4$. Removal of the solvent and silica gel column chromatography using CyH/EtOAc (10/1) provided **TVC-197** (290 mg, 93%) as a colourless oil.

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 7.36-7.32 (m, 5H, 11-H, 12-H, 13-H), 7.02 (d, $J$ = 8.5 Hz, 2H, 5-H), 6.70 (d, $J$ = 8.5 Hz, 2H, 6-H), 5.11 (d, $J$ = 5.0 Hz, 2H, 9-H), 4.50 (d, $J$ = 8.5 Hz, 1H, 2-H), 3.05 (t, $J$ = 6.0 Hz, 2H, 3-H). 1.44 (s, 9H, 15-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 170.1 (1-C), 155.7 (8-C), 130.8 (5-C), 128.6 (11-C, 12-C, 13-C), 115.2 (6-C), 82.4 (14-C), 67.1 (9-C), 55.3 (2-C), 37.6 (3-C), 28.1 (15-C). **IR** (neat) ν ($cm^{-1}$): 3334.3, 1691.3. **HPLC-ESI-MS:** m/z (%) = 372.1 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): Calcd for $C_{21}H_{25}NO_5Na$ $[M+Na]^+$ 394.1624; found 394.1625. $R_f$ = 0.34 (CyH/EtOAc, 5/1).

**Methyl-(2*S*)-3-hydroxy-2-[(triphenylmethyl)amino]propanoate (TVC-200, 141)**

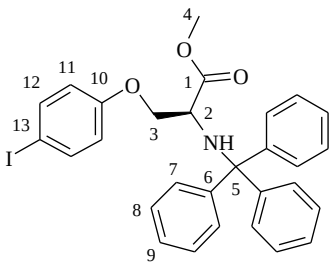


To a cooled solution of L-serine methyl ester hydrochloride (300 mg, 1.93 mmol) and $Et_3N$ (0.54 mL, 3.86 mmol) in DCM (8 mL), a solution of TrCl (564.4 mg, 2.02 mmol) in DCM (2 mL) was added dropwise. The resulting mixture was allowed to stir at room temperature for 18 h and then successively quenched with DCM (300 mL). The mixture was washed with brine solution and dried over $MgSO_4$. Removal of the solvent and flash chromatography (CyH/EtOAc, 3/1) afforded **141** (480.0 mg, 69%) as a white solid.

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 7.52 (d, $J$ = 8.0 Hz, 6H, 7-H), 7.30 (t, $J$ = 8.0 Hz, 6H, 8-H), 7.21 (m, 3H, 9-H), 3.75 (m, 1H, 3-H), 3.59 (m, 2H, 2-H, 3-H), 3.32 (s, 3H, 4-H), 2.33 (br, 1H, OH). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 173.9 (1-C), 145.6 (6-C), 128.9 (7-C), 128.1 (8-C), 126.7 (9-C), 70.9 (5-C), 65.0 (3-C), 58.0 (2-C), 52.2 (4-C). **IR** (KBr) ν ($cm^{-1}$): 3455.2, 1702.6. **HPLC-ESI-MS:** m/z (%) = 384.2 (10) $[M+Na]^+$, 745.2 (10) $[2M+Na]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{23}H_{23}NO_3Na$ $[M+Na]^+$ 384.1570; found 384.1576. $R_f$ = 0.19 (CyH/EtOAc, 2/1).

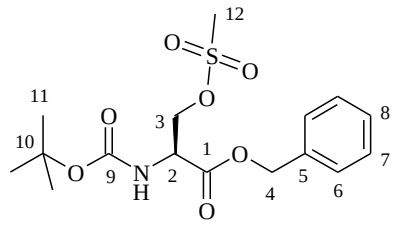
**Methyl-(2*S*)-3-(4-iodophenoxy)-2-[(triphenylmethyl)amino]propanoate (TVC-203, 144)**

A stirred solution of **141** (30 mg, 83 µmol), 4-iodophenol (27.4 mg, 125 µmol) and $PPh_3$ (32 mg, 124.5 µmol) in dry benzene (1 mL) at 0 $^oC$ was added DEAD (22 mg, 124.5 µmol) in benzene under argon atmosphere. The reaction was kept at room temperature overnight. Removal of the solvent and flash chromatography (CyH/EtOAc, 10/1) afforded product **144** (39 mg, 83%) as a white solid.



**$^{1}$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 7.56 (d, $J$ = 9.3 Hz, 2H, 12-H), 7.45 (d, $J$ = 7.8 Hz, 6H, 7-H), 7.30 (t, $J$ = 7.8 Hz, 6H, 8-H), 7.22 (t, $J$ = 7.8 Hz, 3H, 9-H), 6.68 (d, $J$ = 9.3 Hz, 2H, 11-H), 4.23 (dd, $J$ = 5.0 Hz, $J$ = 9.4 Hz, 1H, 3-H), 3.99 (dd, $J$ = 5.0 Hz, $J$ = 9.4 Hz, 1H, 3-H), 3.75 (t, $J$ = 6.0 Hz, 1H, 2-H), 3.27 (s, 3H, 4-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 173.3 (1-C), 158.4 (10-C), 145.6 (6-C), 138.3 (12-C), 129.0 (7-C), 127.9 (8-C), 126.6 (9-C), 117.1 (11-C), 83.2 (13-C), 70.9 (5-C), 70.6 (3-C), 56.0 (2-C), 51.9 (4-C). **IR** (KBr) ν ($cm^{-1}$): 3447.5, 1735.0. **HPLC-ESI-MS:** 438.1 (10) $[M-I]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{29}H_{26}NO_3Na$ $[M+Na]^+$ 586.0856; found 586.0850. $R_f$ = 0.18 (CyH/EtOAc, 20/1).

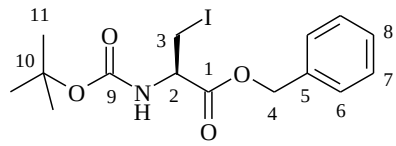
**Benzyl-(2*S*)-2-{[(*tert*-butoxy)carbonyl]amino}-3-(methanesulfonyloxy)propanoate (TVC-209)**



Mesyl chloride (0.02 mL,0.25 mmol) was added dropwise to a cooled solution of BocSerOBn (50 mg, 0.17 mmol) and triethylamine (0.035 mL, 0.25 mmol) in $CH_2Cl_2$ (1.5mL). The reaction mixture was stirred at 0 °C for 30 min and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using CyH/EtOAc (3/1) to obtain the pure product **TVC-209** (62.5 mg, 98%).

**$^{1}$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 7.39 (m, 5H, 6-H, 7-H, 8-H), 5.21 (q, $J$ = 11.0 Hz, 2H, 4-H), 4.65 (bs, 1H, 2-H), 4.62 (m, 1H, 3-H), 4.52 (m, 1H, 3-H), 2.90 (s, 3H, 12-H), 1.47 (s, 9H, 11-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 168.5 (1-C), 155.0 (9-C), 134.8 (5-C), 128.6 (6, 7, 8-C) 68.2 (3-C), 67.9 (4-C), 53.2 (2-C), 37.3 (12-C), 28.4 (11-C). **IR** (KBr) ν = 1745.6. **HPLC-ESI-MS:** 374.1 $[M+H]^+$.**HR-ESI-MS** (m/z): calcd. for C16H23NO7SNa $[M+Na]^+$ 396.1087; found 396.1090. $R_f$ = 0.20 (CyH/EtOAc, 20/1).

**Benzyl-(2*R*)-2-{[(*tert*-butoxy)carbonyl]amino}-3-iodopropanoate (TVC-208, 146)**

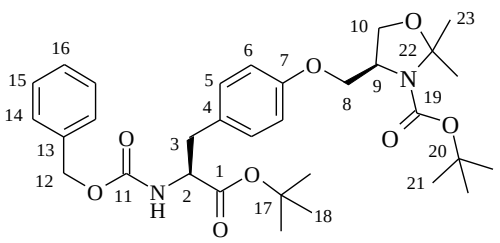


A solution of sodium iodide (60 mg, 0.4 mmol) in acetone (1 mL) was added dropwise to a solution of the mesylate **TVC-209** (50 mg, 0.13 mmol) in acetone (1 mL). The resulting yellow solution was stirred overnight at room temperature. The reaction mixture was filtered over Celite pad and the filtrate was concentrated under reduced pressure to yield product **146** (48.4 mg, 89%) as a yellow oil .

**$^{1}$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 7.39 (m, 5H, 6-H, 7-H, 8-H), 5.38 (d, $J$ = 7.2 Hz, 1H, NH), 5.23 (d, $J$ = 6.0 Hz, 2H, 4-H), 4.57 (d, $J$ = 7.2 Hz, 1H, 2-H), 3.60 (m, 2H, 3-H), 1.45 (s, 9H, 11-H). **$^{13}$C-NMR** (100

MHz, $CDCl_3$): $\delta$ = 169.2 (1-C), 154.8 (9-C), 134.9 (5-C), 128.8 (6,7,8-C), 80.5 (10-C), 68.0 (4-C), 62.6 (3-C), 54.2 (2-C), 28.3 (11-C). **IR** (KBr) ν: 1733.7. **HPLC-ESI-MS:** 406.1 (10) $[M+H]^+$, 428 (80) $[M+Na]^+$. $R_f$ = 0.16 (CyH/EtOAc, 20/1).

**(*S*)-*tert*-Butyl-4-((4-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-(*tert*-butoxy)-3-oxopropyl)phenoxy) methyl)-2,2-dimethyloxazolidine-3-carboxylate (TVC-254, 153)**

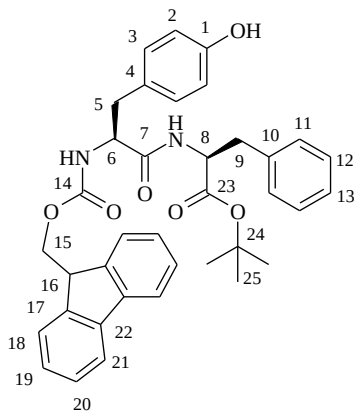


To a cooled solution of **TVC-197** (53 mg, 142.7 µmol), **152** (22.0 mg, 95.1 µmol) and $PPh_3$ (37.4 mg,142.7 µmol) in dry toluene (1.5 mL) under argon atmosphere, a solution of DEAD (25.6 mg, 142.7 µmol) in toluene (0.5 mL) was added dropwise. The resulting solution was then heated at 90 °C for 48 h. The solvent was removed under reduced pressure and the residue was subjected to silica gel column chromatography using CyH/EtOAc (4/1) to provide **153** (36 mg, 65%) as a yellow solid product.

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 7.38-7.34 (m, 5H, 14-H, 15-H, 16-H), 7.05 (d, *J* = 8.0 Hz, 2H, 5-H), 6.86 (m, 2H, 6-H), 5.23 (s, 1H, NH), 5.11 (d, *J* = 4.5 Hz, 2H, 12-H), 4.5 (d, *J* = 6.0 Hz, 1H, 2-H), 4.29 (br, 1/2H, 9-H), 4.17 (br, 1/2H, 9-H), 4.10 (overlap, 1H, 10-H), 4.08 (overlap, 1H, 8-H), 4.01 (m, 1H, 10-H), 3.84 (m, 1H, 8-H), 3.04 (bs, 2H, 3-H), 1.61 (s, 3H, 8-H), 1.51 (overlap, 12H, 8-H, 18-H), 1.43 (s, 9H, 21-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 170.6 (1-C), 157.5 (11-C), 155.6 (19-C), 152.3 (7- C), 136.4 (13-C), 130.5 (5-C), 128.3 (14-C, 15-C, 16-C), 128.0 (4-C), 114.5 (6-C), 94.0 (22-C), 82.2 (17-C), 80.4 (20-C), 66.8 (12-C), 66.5 (8-C), 65.3 (10-C), 55.9 (9-C), 55.2 (2-C), 37.6 (3-C), 28.3 (18-C), 27.9 (21-C), 27.0, 23.1 (23-C). **IR** (KBr) ν ($cm^{-1}$): 3434.1, 2978.7, 1701.0, 1245.5. **HPLC-ESI-MS:** m/z (%) = 585.3 (10) $[M+H]^+$, 602.3 (100) $[M+NH_4]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{32}H_{44}N_2O_8Na$ $[M+Na]^+$ 607.2990; found 607.2990. $R_f$ = 0.31 (CyH/EtOAc, 4/1).

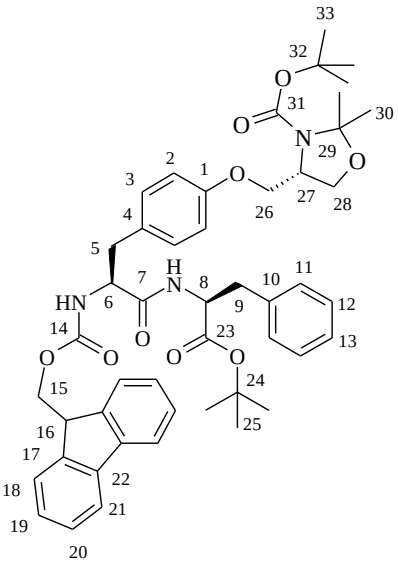
***tert*-Butyl-(2*S*)-2-[(2*S*)-2-{[(9H-fluoren-9-ylmethoxy)carbonyl]amino}-3-(4-hydroxyphenyl)-propanamido]-3-phenylpropanoate (TVC-277, 157)**

A solution of Fmoc-Tyr-OH (600 mg, 1.49 mmol), Phe-O*t*Bu (422 mg, 1.64 mmol) and HATU (685 mg, 1.80 mmol) in DMF (4 mL) was added DIPEA (1 mL, 5.9 mmol). After being stirred at room temperature for 18 h, the solvent was removed under reduced pressure. Flash chromatography (CyH/EtOAc, 4/1) provided **157** (760 mg, 84%) as a white solid.



**$^{1}$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 7.78 (d, *J* = 7.5 Hz, 2H, 18-H), 7.55 (t, *J* = 7.5 Hz, 2H, 21-H), 7.42 (t, *J* = 7.5 Hz, 2H, 19-H), 7.32 (m, 2H, 20-H), 7.21 (m, 3H, 12-H, 13-H), 7.06 (d, *J* = 7.0 Hz, 2H, 11-H), 7.02 (overlap, 2H, 3-H), 6.73 (d, *J* = 8.0 Hz, 2H, 2-H), 6.32 (s, 1H, NH), 5.76 (s, 1H, NH), 5.34 (br, 1H, OH), 4.67 (d, *J* = 7.5 Hz, 1H, 6-H), 4.45 (m, 1H, 15-H), 4.38 (overlap, 1H, 8-H), 4.33 (m, 1H, 15-H), 4.20 (t, *J* = 7.5 Hz, 1H, 16-H), 3.04 (d, *J* = 5.8 Hz, 2H, 5-H), 2.98 (br, 2H, 9-H), 1.38 (s, 9H, 25-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 170.3 (23-C), 155.5 (14-C), 155.0 (1-C), 143.7 (17-C), 141.8 (22-C), 135.8 (10-C), 130.6 (3-C), 129.5 (11-C), 128.4 (12-C), 127.7 (19-C), 127.2 (20-C), 127.1 (13-C), 125.1 (21-C), 119.9 (18-C), 115.6 (2-C), 67.2 (15-C), 56.2 (8-C), 53.8 (6-C), 46.9 (16-C), 38.0 (5-C), 37.6 (9-C), 28.0 (25-C). **IR** (KBr) ν ($cm^{-1}$): 3342.6, 2977.6, 1725.3, 1699.0, 1660.5. **HPLC-ESI-MS:** m/z (%) = 607.3 (60) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{37}H_{37}N_2O_6Na$ $[M+Na]^+$ 629.2621; found 629.2622. $R_f$ = 0.29 (CyH/EtOAc, 2/1).

***tert*-Butyl-(4*S*)-4-{4-[(2*S*)-2-{[(2*S*)-1-(*tert*-butoxy)-1-oxo-3-phenylpropan-2-yl]carbamoyl}-2-{[(9H-fluoren-9-ylmethoxy)carbonyl]amino}ethyl]phenoxymethyl}-2,2-dimethyl-1,3-oxazolidine-3-carboxylate (TVC-278, 158)**



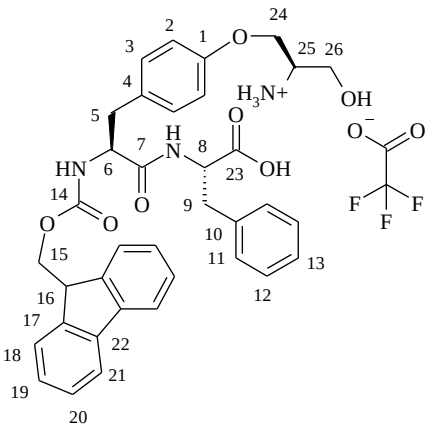
To a cooled solution of **157** (330 mg, 543.9 µmol), **152** (163 mg, 707.1 µmol) and $PPh_3$ (214 mg, 820 µmol) in dry toluene (8 mL) under argon atmosphere, DEAD (0.13 mL, 816 µmol) was added. The reaction mixture was refluxed for 48 h and concentrated under reduced pressure to dryness. The crude product was purified by silica gel column chromatography (CyH/EtOAc, 8/1) to give **158** (239 mg, 54%) as a yellow solid product.

**$^{1}$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 7.69 (d, *J* = 7.5 Hz, 2H, 18-H), 7.46 (t, *J* = 7.5 Hz, 2H, 21-H), 7.33 (t, *J* = 7.5 Hz, 2H, 19-H), 7.23 (t, *J* = 7.5 Hz, 20-H), 7.11-7.09 (m, 3H, 12-H, 13-H), 7.0 (overlap, 2H, 3-H), 6.97 (overlap, 2H, 11-H), 6.77 (t, *J* = 8.0 Hz, 2H, 2-H), 6.17 (s, 1H, NH), 5.18 (s, 1H, NH), 4.57 (q, *J* = 7.2 Hz, 1H, 6-H), 4.35 (m, 1H, 15-H), 4.29 (m, 1/2H, 27-H), 4.24 (m, 1H, 15-H), 4.19 (m, 1/2H, 27-H), 4.12 (overlap, 1H, 8-H), 4.11 (br, 2H, 28-H), 4.07 (overlap, 1H, 16-H), 3.97 (br, 1H, 26-H), 3.89 (br, 1H, 26-H), 2.95 (d, *J* = 5.8 Hz, 4H, 5-H, 9-H), 1.53, 1.41 (s, 6H, 30-H), 1.40 (s, 9H, 25-H), 1.30 (s, 9H, 33-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 170. 1 (23-C), 169.9 (7-C), 143.7 (17-C), 141.3 (22-C), 135.8 (10-C), 130.5 (3-C), 129.4

(11-C), 128.4 (12-C), 127.7 (4-C), 127.0 (19-C), 126.9 (13-C), 126.2 (20-C), 125.0 (21-C), 119.9 (18-C), 114.8 (2-C), 94.1 (29-C), 82.4 (24-C), 80.5 (32-C), 67.0 (15-C), 65.2 (26-C), 61.8 (28-C), 56.0 (27-C), 55.7 (16-C), 53.6 (6-C), 37.9 (5-C, 9-C), 28.3 (25-C), 27.8 (33-C), 27.1, 24.1 (30-C). **IR** (KBr) ν ($cm^{-1}$): 3319.2, 1701.0,1256.8. **HPLC-ESI-MS:** m/z (%) = 820.4 (15) $[M+H]^+$, 837.4 (30) $[M+NH_4]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{48}H_{57}N_3O_9Na$ $[M+Na]^+$ 842.3989; found 842.3987. $R_f$ = 0.24 (CyH/EtOAc, 6/1).

**(2*S*)-2-[(2*S*)-2-{[(9H-Fluoren-9-ylmethoxy)carbonyl]amino}-3-{4-[(2*R*)-3-hydroxy-2-methylpropoxy]phenyl}propanamido]-3-phenylpropanoic acid trifluoroacetate (TVC-279, 159)**

Compound **158** (150 mg, 0.18 mmol) in TFA/TIPS/$H_2O$ (2 mL, 95/2.5/2.5) was stirred at 0 °C for 3 h. The reaction mixture was concentrated under reduced pressure to obtain product **159** (134.8 mg, 100%) as a TFA salt.

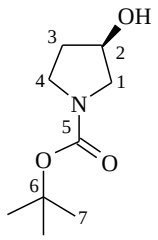


**$^1$H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 7.80 (d, *J* = 7.3 Hz, 2H, 18-H), 7.59 (t, *J* = 7.3 Hz, 2H, 21-H), 7.41 (t, *J* = 7.3 Hz, 2H, 19-H), 7.31 (t, *J* = 7.3 Hz, 2H, 20-H), 7.19 (d, *J* = 8.9 Hz, 2H, 3-H), 6.88 (d, *J* = 8.9 Hz, 2H, 2-H), 4.69 (q, *J* = 5.5 Hz, 1H, 6-H), 4.36 (m, 1H, 8-H), 4.28 (m, 1H, 15-H), 4.19 (overlap, 1H, 15-H), 4.11 (overlap, 3H, 16-H, 24-H), 3.79 (m, 1H, 26-H), 3.68 (q, *J* = 6.0 Hz, 1H, 26-H), 3.58 (m, 1/2H, 25-H), 3.51 (m, 1/2H, 25-H), 3.23 (q, *J* = 5.5 Hz, 1H, 5-H), 3.03 (overlap, 2H, 5-H, 9-H), 2.77 (q, *J* = 5.5 Hz, 1H, 9-H). **$^{13}$C-NMR** (100 MHz, $CD_3OD$): $\delta$ = 172.9 (7-C), 172.4 (23-C), 156.9 (14-C), 151.7 (1-C), 143.9 (17-C), 141.2 (22-C), 138.1 (10-C), 130.2 (3-C), 128.9 (11-C), 128.1 (12-C), 127.4 (19-C), 126.8 (20-C), 126.5 (13-C), 124.8 (21-C), 119.6 (18-C), 114.3 (2-C), 66.8 (15-C), 65.1 (24-C), 58.9 (26-C), 56.3 (8-C), 53.5 (6-C), 52.4, 51.3 (25-C), 46.8 (16-C), 36.9 (5-C), 36.7 (9-C). **IR** (KBr) ν ($cm^{-1}$): 3424.7, 2944.6, 1674.6, 1203.7. **HPLC-ESI-MS:** m/z (%) = 624.3 (100) $[M+H-CF_3COOH]^+$, **HR-ESI-MS** (m/z): calcd. for $C_{36}H_{38}N_3O_7$ $[M+H]^+$ 624.2704; found 624.2704.

### 3.16 Synthesis of β-proline amino acid

**(*R*)-*tert*-Butyl-3-hydroxypyrrolidine-1-carboxylate (TVC-238, 162)**

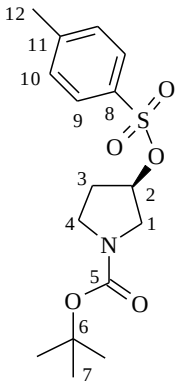
A suspension of 4-(*R*)-hydroxy-(L)-proline (2 g, 15.25 mmol) and 2-cyclohexen-1-one (0,2 mL, 2.08 mmol) in cyclohexanol (10 mL) was heated at 160°C for 6 h, until all the solid was dissolved. The resulting red solution was cooled to room temperature and an aqueous solution of acetic acid (20%, 10 mL) and toluene (10 mL) were added. The aqueous layer was washed twice with toluene (2x10 mL), then



solid $K_2CO_3$ (6 g) was added until pH≈9 and followed by addition of $Boc_2O$ (3.33 g, 15.3 mmol) in THF (20 mL). After stirring at room temperature for 20 h, the reaction mixture was extracted twice with EtOAc (2x100 mL). The combined organic layers were concentrated and ethanol (40 mL) was added. The solution was heated under reflux for 20 h and the solvent was evaporated. Flash chromatography using (CyH/EtOAc, 1/1) gave the desired product **162** (2.4 g, 84%) as a brown solid.

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 4.45 (m, 1H, 2-H), 3.48 (overlap, 3H, 4-H, 1-H), 3.37 (m, 1H, 1-H), 1.93 (m, 2H, 3-H), 1.47 (s, 9H, 7-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 154.7 (5-C), 79.3 (6-C), 71.0 (2-C), 54.2 (1-C), 43.7 (4-C), 33.7 (3-C), 28.4 (7-C). **IR** (neat) ν ($cm^{-1}$): 3396.0, 1652.7. **HPLC-ESI-MS:** m/z (%) = 210.1 (10) $[M+Na]^+$. **HR-ESI-MS** (m/z): calcd. for $C_9H_{17}NO_3Na$ $[M+Na]^+$ 210.1101; found 210.1101. $[\alpha]_D^{20}$ = -22.8 (c 1.5, MeOH). $R_f$ = 0.21 (CyH/EtOAc, 1/1).

**(*R*)-*tert*-Butyl-3-((methylsulfonyl)oxy)pyrrolidine-1-carboxylate (TVC-241)**

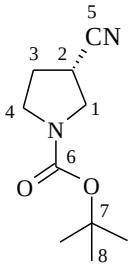


To a solution of **162** (150 mg, 0.8 mmol) and TEA (122 µL, 0.88 mmol) in DCM (15 mL) at 0 °C, TsCl (275 mg, 1.44 mmol) in DCM (10 mL) was added dropwise. After stirring at 0 °C for 2.h, the mixture was allowed to warm up to room temperature and further stirred for 18 h. Removal of the solvent and column chromatography on silica gel using EtOAc/CyH (10/1) as eluent yielded product **TVC-241** (170 mg, 62%) as a colourless oil.

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 7.80 (d, *J* = 8.3 Hz, 2H, 9-H), 7.36 (d, *J* = 8.3 Hz, 2H, 10-H), 5.06 (bs, 1H, 2-H), 3.47 (m, 4H, 1-H, 4-H), 2.48 (s, 3H, 12-H), 2.21 (m, 1H, 3-H), 2.05 (m, 1H, 3-H), 1.45 (s, 9H, 7-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 154.1 (5-C), 145.0 (8-C), 130.1 (10-C), 127.7 (9-C), 80.9 (2-C), 79.5 (6-C), 52.3 (4-C), 43.8 (1-C), 31.7 (3-C), 28.7 (7-C), 21.8 (12-C). **IR** (neat) ν ($cm^{-1}$): 1691.3. **HPLC-ESI-MS:** m/z (%) = 359.2 (16) $[M+NH_4]^+$, 705.2 (8) $[2M+Na]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{16}H_{23}N_2O_5Na$ $[M+Na]^+$ 364.1505; found 364.1188. $R_f$ = 0.5 (CyH/EtOAc, 1/1).

**(*S*)-*tert*-Butyl-3-cyanopyrrolidine-1-carboxylate** (**TVC-242, 163**)

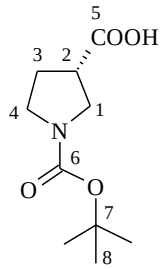
A suspension of tosylate **TVC-241** (910 mg, 2.67 mmol) and NaCN (288 mg, 5.86 mmol) in dry DMSO (10 mL) was warmed up to 80 °C for 21 h. Afterward, a saturated aqueous $NaHCO_3$ was added and the mixture was extracted with EtOAc (3x100 mL). The combined organic layers were washed with $H_2O$, brine solution and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the crude material was



purified by silica gel column chromatography eluting CyH/EtOAc (3/1) to provide the desired cyanate **163** (334.4 mg, 64%). as a yellow oil.

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 3.67 (m, 1H, 1-H), 3.59 (m, 1H, 1-H), 3.55 (m, 1H, 4-H), 3.45 (m, 1H, 4-H), 3.1 (m, 1H, 2-H), 2.25 (m, 2H, 3-H), 1.47 (s, 9H, 8-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 153.8 (6-C), 119.9 (5-C), 80.1 (7-C), 48.7 (1-C), 44.4 (4-C), 29.7 (3-C), 28.6 (2-C), 28.3 (8-C). **IR** (neat) ν ($cm^{-1}$): 1695.1 (CO). **HPLC-ESI-MS:** m/z (%) = 214.1 (100) $[M+NH_4]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{10}H_{16}N_2O_2Na$ $[M+Na]^+$ 219.1092; found: 219.1104. $[\alpha]_D^{20}$= + 25.6 (c 1.4, MeOH). $R_f$ = 0.27 (CyH/EtOAc, 3/1).

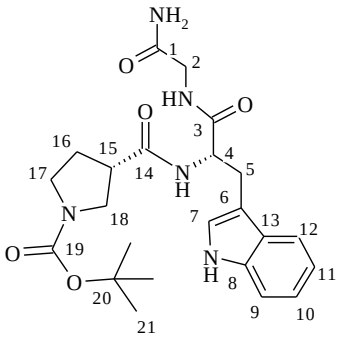
**(*S*)-1-(*tert*-Butoxycarbonyl)pyrrolidine-3-carboxylic acid (TVC-247, 164)**



Cyanate **163** (44 mg, 22 mmol) in 35% HCl (3 mL) was refluxed for 5 h. The reaction mixture was concentrated to dryness. The residue was dissolved in $H_2O$ (1 mL) and followed by addition of $NaHCO_3$ (55.4 mg, 0.66 mmol) and $Boc_2O$ (53 mg, 0.24 mmol) in THF (2 mL). After stirring at room temperature for 18 h, the reaction mixture was extracted with EtOAc (2x100 mL). The combined organic layers were washed with brine solution and dried over $MgSO_4$. Removal of the solvent afforded desired acid **164** (46.3 mg, 98%) as white powder.

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 3.59 (m, 2H, 4-H), 3.50 (m, 1H, 1-H), 3.37 (m, 1H, 1-H), 3.08 (br, 1H, 2-H), 2.15 (m, 2H, 3-H), 1.46 (s, 9H, 8-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 177.7 (5-C), 154.7 (6-C), 47.9 (4-C), 45.2 (1-C), 42.9 (2-C), 28.5 (3-C), 28.4 (8-C). **IR** (neat) ν ($cm^{-1}$): 3196.3, 1739.5, 1664.3. **HPLC-ESI-MS:** m/z (%) = 238.1 (100) $[M+Na]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{10}H_{17}NO_4Na$ $[M+Na]^+$ 238.1050; found 238.1050. $[\alpha]_D^{20}$ = + 19.2 (c 0.27, MeOH). $R_f$ = 0.14 (CyH/EtOAc, 3/2).

**(*S*)-*tert*-Butyl-3-(((*S*)-1-((2-amino-2-oxoethyl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)-carbamoyl)pyrrolidine-1-carboxylate (TVC-252, 165)**

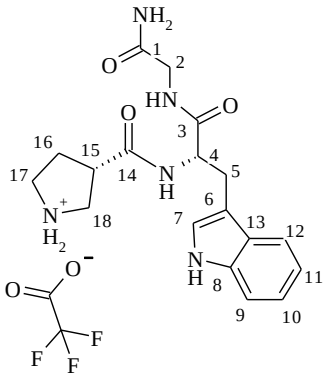


To a solution of (*S*)-1-(*tert*-butoxycarbonyl)pyrrolidine-3-carboxylic acid **164** (33 mg, 0.15 mmol) and TFA*H-Trp-Gly-$NH_2$ (68.8 mg, 0.18 mmol) in DMF (2 mL), HATU (64 mg, 0.17 mmol) and DIPEA (91 µL, 0.61 mmol) were added. The reaction mixture was stirred at room temperature for 24 h and quenched with EtOAc (100 mL). The resulting solution was washed with $H_2O$ (2x10 mL), brine solution (20 mL) and dried over $Na_2SO_4$. Removal of the solvent and flash chromatography using

EtOAc/EtOH (20/1) gave the desired product **165** (53.3 mg, 76%) as a solid.

**$^1$H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 7.53 (d, $J$ = 7.6 Hz, 1H, 12-H), 7.31 (d, $J$ = 8.6 Hz, 1H, 9-H), 7.10 (t, $J$ = 7.0 Hz, 1H, 10-H), 7.03 (overlap, 2H, 7-H, 11-H), 4.58 (bs, 1H, 4-H), 3.79 (d, $J$ = 17.0 Hz, 1H, 2-H), 3.63 (d, $J$ = 17.0 Hz, 1H, 2-H), 3.43 (br, 1H, 18-H), 3.35 (br, 1H, 17-H), 3.30 (overlap, 1H, 18-H), 3.20 (overlap, 1H, 5-H), 3.18 (m, 1H, 17-H), 3.12 (overlap, 1H, 5-H), 2.82 (bs, 1H, 15-H), 1.93-1.89 (m, 2H, 16-H), 1.38 (s, 9H, 21-H). **$^{13}$C-NMR** (100 MHz, $CD_3OD$): $\delta$ = 140.1 (8-C), 127.2 (11-C), 125.8 (10-C), 123.1 (7-C), 122.1 (12-C), 115.3 (9-C), 82.2 (20-C), 58.7 (4-C), 51.8 (18-C), 49.6 (17-C), 48.0 (15-C), 45.8 (2-C), 32.3 (16-C), 32.2 (21-C), 31.1 (5-C). **IR** (KBr) ν ($cm^{-1}$): 3397.9, 1670.6. **HPLC-ESI-MS**: m/z(%) = 458 (32) $[M+H]^+$**,** 915 (30) $[2M+H]^+$. **HR-ESI-MS**: Calcd for $C_{23}H_{31}N_5O_5Na$ $[M+Na]^+$ 480.2223; found 480.2213. $R_f$ = 0.25 (EtOAc/EtOH, 10/1).

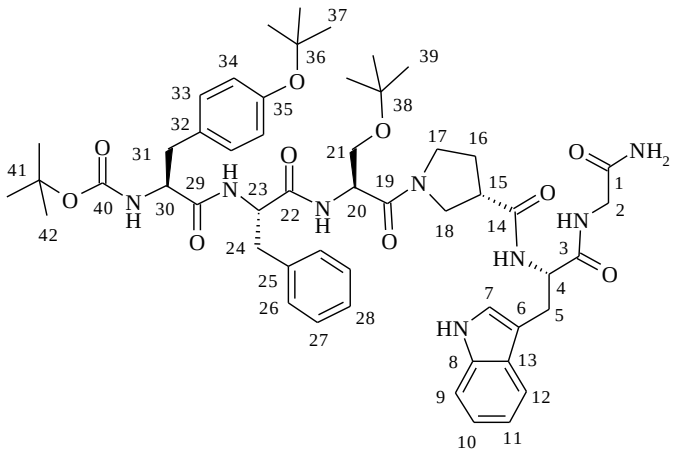
**(*S*)-3-(((*S*)-1-((2-Amino-2-oxoethyl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)carbamoyl)-pyrrolidin-1-ium triflouroacetate (TVC-264)**



Tripeptide **165** (45 mg, 0.1 mmol) was treated with TFA/TIPS/$H_2O$ (2 mL, 95/2.5/2.5) at 0 $^oC$ for 3 h. The reaction mixture was concentrated to dryness to obtain **TVC-264** (46 mg, 100%) as a TFA salt.

**$^1$H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 7.63 (dd, $J$ = 3.2 Hz, $J$ = 7.8 Hz, 1H, 12-H), 7.37 (d, $J$ = 8.0 Hz, 1H, 9-H), 7.16 (s, 1H, 7-H), 7.13 (d, $J$ = 7.0 Hz, 1H, 10-H), 7.05 (t, $J$ = 7.8 Hz, 1H, 11-H), 4.72 (t, $J$ = 7.2 Hz, 1/2H, 4-H), 4.62 (t, $J$ = 7.2 Hz, 1/2H, 4-H), 3. 9 (dd, $J$ = 4.2 Hz, $J$ = 17.0 Hz, 1H, 2-H), 3.70 (dd, $J$ = 3.2 Hz, $J$ = 17.0 Hz, 1H, 2-H), 3.37 (overlap, 1H, 5-H), 3.33 (overlap, 1H, 17-H), 3.32 (overlap, 1H, 18-H), 3.25 (overlap, 1H, 18-H), 3.20 (overlap, 1H, 15-H), 3.18 (m, 2H, 17-H, 5-H), 2.28 (m, 1H, 18-H), 2.18 (m, 2H, 16-H). **$^{13}$C-NMR** (100 MHz, $CD_3OD$): $\delta$ = 176.9 (14-C), 176.8 (3-C), 140.5 (8-C), 127.2 (7-C), 125.1 (10-C), 122.8 (11-C), 121.9 (12-C), 114.9 (9-C), 58.9 (4-C), 51.2 (18-C), 48.8 (17-C), 46.1 (15-C), 45.2 (2-C), 32.4 (16-C), 31.0 (5-C). **IR** (KBr) ν ($cm^{-1}$): 3420.3, 1670.5. **HPLC-ESI-MS**: m/z (%) = 358 (100) $[M+H]^+$**,** 715 (30) $[2M+H]^+$. **HR-ESI-MS**: Calcd for $C_{23}H_{31}N_5O_5Na$ $[M+Na]^+$ 380.1699; found 380.1692.

***tert*-Butyl-((*S*)-1-(((*S*)-1-(((*S*)-1-((*S*)-3-(((*S*)-1-((2-amino-2-oxoethyl)amino)-3-(1H-indol-3-yl)-1-oxo-propan-2-yl)carbamoyl)pyrrolidin-1-yl)-3-(*tert*-butoxy)-1-oxopropan-2-yl)amino)-1-oxo-3-phenyl-propan-2-yl)amino)-3-(4-(*tert*-butoxy)phenyl)-1-oxopropan-2-yl)carbamate (TVC-266)**

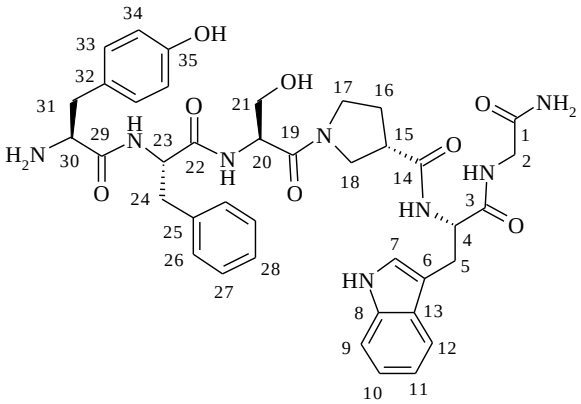


To a solution of **TVC-264** (40 mg, 85 µmol) and tripeptide Boc-Tyr(*t*Bu)-Phe-Ser(*t*Bu)-OH (69.2 mg, 110.3 µmol) in DMF (2 mL), HATU (48.4 mg, 127 µmol) and DIPEA (57 µL, 339 µmol) were added. After being stirred for 18 h at room temperature, the solvent was removed under reduced pressure. The residue was subjected to silica gel column chromatography using EtOAc/EtOH (6/1) to afford peptide **TVC-266** (71.2 mg, 87%) as a white solid.

**$^1$H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 7.68 (m, 1H, 12-H), 7.35 (m, 1H, 9-H), 7.26-7.20 (m, 5H, 26-H, 27-H, 28-H), 7.13 (m, 1H, 10-H), 7.12 (overlap, 3H, 7-H), 7.18 (overlap, 1H, 34-H), 7.05 (m, 1H, 11-H), 6.90 (d, *J* = 7.4 Hz, 2H, 34-H), 4.68 (m, 1H, 23-H), 4.66 (m, 1H, 20-H), 4.61 (m, 1H, 4-H), 4.26 (m, 1H, 30-H), 3.87 (overlap, 1H, 2-H), 3.68 (overlap, 1H, 2-H), 3.58 (overlap, 1H, 21-H), 3.51 (overlap, 1H, 18-H), 3.51 (overlap, 1H, 21-H), 3.62 (overlap, 1H, 17-H), 3.60 (m, 1H, 18-H), 3.54 (overlap, 1H, 17-H), 3.43 (m, 1H, 5-H), 3.18 (overlap, 1H, 5-H), 3.08 (m, 1H, 24-H), 2.99 (overlap, 1H, 31-H), 3.05-3.00 (overlap, 1H, 15-H), 2.94 (overlap, 1H, 24-H), 2.70 (overlap, 1H, 31-H), 2.16 (m, 1H, 16-H), 1.99 (m, 1H, 16-H), 1.36 (s, 9H, 37-H), 1.34 (s, 9H, 39-H), 1.16 (s, 9H, 42-H). **$^{13}$C-NMR** (100 MHz, $CD_3OD$): $\delta$ = 175.6 (19-C), 174.7 (22-C), 174.6 (29-C), 174.0 (14-C), 172.7 (3-C), 170.6 (1-C), 155.2 (40-C), 142.8 (35-C), 133.7 (8-C), 129.5 (34-C), 129.1 (27-C), 128.7 (32-C),128.2 (26-C), 127.9 (13-C), 126.5 (28-C), 123.7 (33-C), 121.2 (7-C), 123.1 (10-C), 118.6 (11-C), 118.0 (12-C), 111.1 (9-C), 107 (6-C), 83.6 (36-C), 82.7 (38-C), 80.7 (41-C), 61.7 (21-C), 56.1 (30-C), 54.8 (4-C), 54.2 (23-C), 51.2 (20-C), 48.4 (18-C), 45.9 (17-C), 43.7 (15-C), 41.9 (2-C), 37.5 (24-C), 37.2 (31-C), 29.2 (37-C), 29.1 (16-C), 28.8 (39-C), 27.6 (42-C), 26.9 (5-C). **IR** (neat) ν ($cm^{-1}$): 3278.4, 1657.8. **HPLC-ESI-MS**: m/z (%) = 967.5 (100) $[M+H]^+$. **HR-ESI-MS**: Calcd for $C_{52}H_{71}N_8O_{10}$ $[M+H]^+$ 967.5293; found 967.5288. $R_f$ = 0.34 (EtOAc/EtOH, 5/1).

**(2*S*)-2-[(2*S*)-2-Amino-3-(4-hydroxyphenyl)propanamido]-N-[(2*S*)-1-[(3*S*)-3-{[(1*S*)-1-[(carbamoylmethyl)carbamoyl]-2-(1H-indol-3-yl)ethyl]carbamoyl}pyrrolidin-1-yl]-3-hydroxy-1-oxopropan-2-yl]-3-phenylpropanamide (TVC-268, 166)**

Hexapeptide **TVC-266** (29 mg, 20 µmol) in TFA/TIPS/$H_2O$ (2 mL, 95/2.5/2.5) was stirred at 0 °C for 4 h. The mixture was concentrated to dryness. The residue was chromatographed using DCM/MeOH (6/1,



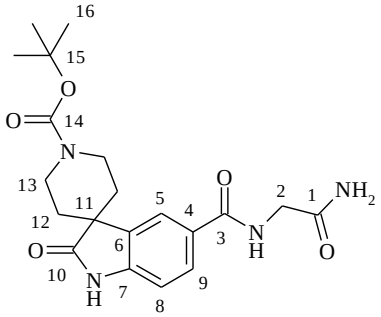
0.1% TEA) as eluent to provide pure product (13.5 mg, 86%), which was dissolved in ACN/$H_2O$ (3 mL, 2/1) and lyophilized to obtain white powder.

**$^1$H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 7.60 (m, 1H, 12-H), 7.36 (t, $J$ = 7.5 Hz, 1H, 9-H), 7.29-7.22 (m, 5H, 26-H, 27-H, 28-H), 7.14 (m, 1H, 10-H), 7.11 (overlap, 3H, 7-H, 34-H), 7.04 (t, $J$ = 7.4 Hz, 1H, 11-H), 6.78 (d, $J$ = 7.4 Hz, 2H, 34-H), 4.70 (m, 1H, 23-H), 4.69 (m, 1H, 20-H), 4.61 (m, 1H, 4-H), 4.02 (m, 1H, 30-H), 3.89 (overlap, 1H, 2-H), 3.76 (overlap, 1H, 21-H), 3.71 (overlap, 1H, 18-H), 3.69 (overlap, 1H, 21-H), 3.67 (overlap, 1H, 17-H), 3.65 (overlap, 1H, 2-H), 3.58 (m, 1H, 18-H), 3.56 (overlap, 1H, 17-H), 3.35 (m, 1H, 5-H), 3.18 (overlap, 1H, 5-H), 3.18 (overlap, 1H, 31-H), 3.15 (m, 1H, 24-H), 3.10 (overlap, 1H, 15-H), 2.96 (overlap, 1H, 24-H), 2.93 (overlap, 1H, 31-H), 2.16 (m, 1H, 16-H), 2.03 (m, 1H, 16-H). **$^{13}$C-NMR** (100 MHz, $CD_3OD$): $\delta$ = 175.7 (19-C), 174.9 (22-C), 172.9 (29-C), 170.3 (14-C), 169.8 (3-C), 162 (1-C), 158.3 (35-C), 142.8 (25-C), 138.0 (8-C), 130.4 (34-C), 128.8 (27-C), 128.7 (32-C),128.3 (26-C), 126.5 (28-C), 127.9 (13-C), 123.3 (10-C), 121.2 (7-C), 115.5 (33-C), 118.5(11-C), 118.2 (12-C), 111.0 (9-C), 61.2 (21-C), 54.9 (4-C), 54.5 (23-C), 54.3 (30-C), 53.1 (20-C), 48.4 (18-C), 45.9 (17-C), 43.7 (15-C), 41.8 (2-C), 37.5 (24-C), 36.3 (31-C), 29.3 (16-C), 26.8 (5-C). **HPLC-ESI-MS:** m/z (%) = 755.4 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{39}H_{47}N_8O_8$ $[M+H]^+$ 755.3511; found 755.3509.

### 3.17 Spirolactam-Scan

#### 3.17.1 Spirolactamscan of Trp-Pro

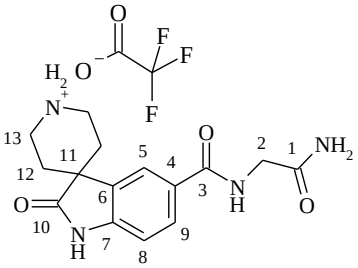
***tert*-Butyl-5-[(carbamoylmethyl)carbamoyl]-2-oxo-1,2-dihydrospiro[indole-3,4'-piperidine]-1'-carboxylate (TVC-159, 168)**



To a solution of 1'-[(*tert*-butoxy)carbonyl]-2-oxo-1,2-dihydro-spiro[indole- 3,4'-piperidine]-5-carboxylic acid **167** (200 mg, 0.58 mmol), glycinamide hydrochloride (83 mg, 0.75 mmol) and TBTU (222.5 mg, 0.69 mmol) in DMF (4 mL), DIPEA (0.38 mL, 2.31 mmol) was added. The reaction mixture was stirred at room temperature for 18 h and quenched with EtOAc (200 mL). The resulting solution was washed with $H_2O$, brine solution and dried over $MgSO_4$. Removal of the solvent gave the desired product **168** (217 mg, 94%) as a white solid.

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 7.96 (s, 1H, NH), 7.79 (m, 1H, 5-H),7.73 (dd, $J$ = 2.0 Hz, $J$ = 8.0 Hz, 1H, 8-H), 6.9 (d, $J$ = 8.0 Hz, 1H, 9-H), 4.05 (s, 2H, 2-H), 3.77 (br, 4H, 13-H), 1.89 (m, 4H, 12-H), 1.47 (s, 9H, 16-H). **$^{13}$C-NMR** (400 MHz, $CDCl_3$): $\delta$ = 181.9 (10-C), 172.1 (3-C), 167.7 (1-C), 155.0 (14-C), 143.7 (7-C), 134.4 (6-C), 127.8 (8-C), 122.8 (5-C), 109.4 (9-C), 79.9 (15-C), 45.4 (11-C), 42.4 (2-C), 38.7 (13-C), 32.3 (12-C), 28.3 (16-C). **IR** (KBr) ν ($cm^{-1}$): 3338.1, 1666.4. **HPLC-ESI-MS:** m/z (%) = 403.2 (15) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{20}H_{26}N_4O_5Na$ $[M+Na]^+$ 425.1801; found 425.1796. $R_f$ = 0.35 (EtOAc/EtOH, 6/1).

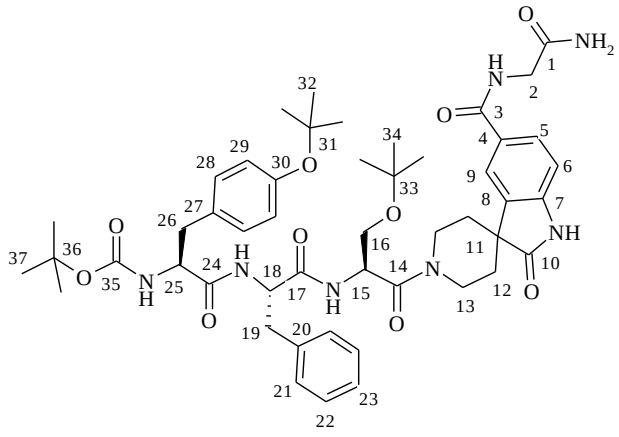
**5-[(Carbamoylmethyl)carbamoyl]-2-oxo-1,2-dihydrospiro[indole-3,4'-piperidi]-1'-ium-trifluoro-acetate (TVC-160, 169)**



Compound **168** (60 mg, 26.46 µmol) was treated with TFA/TIPS/$H_2O$ (2 mL, 95/4/1) at 0 °C for 4 h. After that, the solvent was removed to dryness to afford product **169** (60.9 mg, 98%) as a TFA salt.

**$^1$H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 7.88 (d, $J$ = 1.7 Hz, 1H, 5-H), 7.86 (dd, $J$ = 1.7 Hz, $J$ = 8.0 Hz, 1H, 9-H), 7.02 (d, $J$ = 8.0 Hz, 1H, 8-H), 4.06 (s, 2H, 2-H), 3.83 (m, 2H, 13-H), 3.45 (m, 2H, 13-H), 2.2-2.1 (m, 4H, 12-H). **$^{13}$C-NMR** (400 MHz, $CD_3OD$): $\delta$ = 182.0 (10-C), 174.5 (3-C), 169.0 (1-C), 128.2 (9-C), 122.3 (5-C), 109.2 (8-C), 44.4 (11-C), 42.1 (2-C), 38.8 (13-C), 28.9 (12-C). **IR** (KBr) ν ($cm^{-1}$): 3420.1, 1683.4. **HPLC-ESI-MS:** m/z (%) = 303.2 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{15}H_{19}N_4O_3$ $[M+H]^+$ 303.1457; found 303.1452. $R_f$ = 0.36 (EtOAc/EtOH, 6/1).

***tert*-Butyl-N-[(1*S*)-1-{[(1*S*)-1-{[(2*S*)-3-(*tert*-butoxy)-1-{5-[(carbamoylmethyl)carbamoyl]-2-oxo-1,2-dihydrospiro[indole-3,4'-piperidine]-1'-yl}-1-oxopropan-2-yl]carbamoyl}-2-phenylethyl]carbamo-yl}-2-[4-(tert-butoxy)phenyl]ethyl]carbamate (TVC-169, 170)**

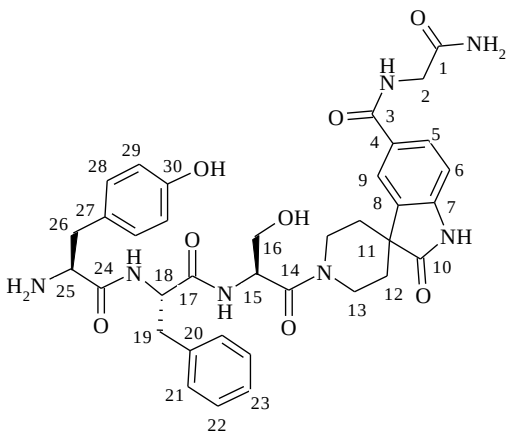


To a stirred solution of dipeptide **169** (40 mg, 96.1 µmol) and tripeptide Boc-Tyr(*t*Bu)-Phe-Ser(*t*Bu)-OH (60.3 mg, 96.1 µmol) in DMF (2 mL), HATU (40.2 mg, 106 µmol) and DIPEA (71 µL, 480 µmol) were added. The reaction mixture was stirred at room temperature for 20 h. Removal of the solvent and flash chromatography (eluent:

EtOAc/EtOH, 7/1) provided product **170** (60 mg, 69%) as a solid.

**$^{1}$H-NMR** (600 MHz, $CD_3OD$): $\delta$ = 7.97 (s, 1H, 9-H), 7.86 (t, *J* = 8.3 Hz, 1H, 5-H), 7.3-7.2 (m, 5H, 21-H, 22-H, 23-H), 7.13 (t, *J* = 7.7 Hz, 2H, 28-H), 7.02 (d, *J* = 8.3 Hz, 1H, 6-H), 6.89 (t, *J* = 7.7 Hz, 2H, 29-H), 5.03 (t, *J* = 7.4 Hz, 1H, 15-H), 4.73 (t, *J* = 7.5 Hz, 1H, 18-H), 4.32 (bs, 1H, 25-H), 4.12 (m, 2H, 13-H), 4.07-4.02 (overlap, 2H, 2-H), 3.93 (m, 2H, 13-H), 3.60 (m, 1H, 16-H), 3.57 (m, 1H, 16-H), 3.15 (m, 1H, 19-H), 3.01 (overlap, 1H, 26-H), 3.00 (overlap, 1H, 19-H), 2.76 (m, 1H, 26-H), 1.94 (m, 2H, 12-H), 1.85 (br, 2H, 12-H), 1.36 (s, 9H, 32-H), 1.30 (s, 9H, 34-H), 1.22 (9H, 37-H). **$^{13}$C-NMR** (150 MHz, $CD_3OD$): $\delta$ = 183.5 (10-C), 174.5 (14-C), 172.7 117-C), 171.0 (24-C), 169.9 (1-C), 167.4 (3-C), 157.5 (35-C), 155.2 (30-C), 145.5 (7-C), 138.1 (8-C), 135.6 (20-C), 133.7 (4-C), 130.9 (28-C), 130.4 (22-C), 129.4 (21-C), 128.9 (5-C), 127.6 (23-C), 125.1 (29-C), 123.9 (9-C), 110.5 (6-C), 80.6 (31-C), 79.4 (33-C), 74.8 (36-C), 63.4 (16-C), 57.4 (11-C), 57.3 (25-C), 55.3 (18-C), 50.6 (15-C), 42.5 (2-C), 39.6 (19-C), 38.8 (13-C), 38.5 (26-C), 33.5 (12-C), 29.1 (34-C), 28.6 (32-C), 27.8 (37-C). **IR**(KBr) ν ($cm^{-1}$): 3405.5, 1662.7. **HPLC-ESI-MS:** m/z (%) = 912.5 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{49}H_{65}N_7O_{10}Na$ $[M+Na]^+$ 934.4690; found 934.4685. $R_f$ = 0.39 (EtOAc/EtOH, 7/1).

**(2*S*)-2-[(2*S*)-2-Amino-3-(4-hydroxyphenyl)propanamido]-N-[(2*S*)-1-{5-[(carbamoylmethyl)-carbamoyl]-2-oxo-1,2-dihydrospiro[indole-3,4'-piperidine]-1'-yl}-3-hydroxy-1-oxopropan-2-yl]-3-phenylpropanamide (TVC-174, 171)**



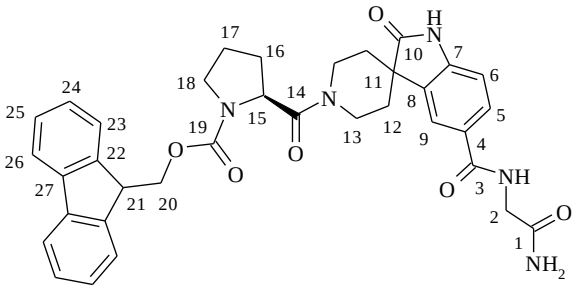
Peptide **170** (36 mg, 39.5 µmol) was treated with TFA/TIPS/$H_2O$ (2 mL, 95/4/1) at 0 $^{o}$C for 5 h. The mixture was then concentrated under reduced pressure to dryness. The crude product was chromatographed using DCM/MeOH (5/1, 0.1% TEA) to yield **171** (24 mg, 88%), which was dissolved in ACN/$H_2O$ (2 mL, 1/1) and lyophilized to obtain white powder.

**$^{1}$H-NMR** (600 MHz, $CD_3OD$): $\delta$ = 7.93 (d, *J* = 3.0 Hz, 1H, 9-H), 7.85 (dd, *J* = 1.5 Hz, *J* = 8.2 Hz, 1H, 5-H), 7.3-7.2 (m, 5H, 21-H, 22-H, 23-H), 7.04 (overlap, 2H, 28-H), 7.01 (overlap, 1H, 6-H), 6.75 (t, *J* = 8.7 Hz, 2H, 29-H), 5.02 (t, *J* = 6.0 Hz, 1H, 15-H), 4.73 (m, 1H, 18-H), 4.14 (m ,1H, 13-H), 4.04 (overlap, 1H, 2-H), 3.95 (overlap, 1H, 2-H), 3.91 (m, 1H, 13-H), 3.83 (m, 1H, 16-H), 3.76 (m, 1H, 16-H), 3.72 (m, 1H, 25-H), 3.14 (m, 1H, 19-H), 2.99 (m, 1H, 19-H), 2.98 (m, 1H, 26-H), 2.73 (m, 1H, 26-H), 2.03 (m ,1H, 12-H), 1.86 (m, 1H, 12-H). **$^{13}$C-NMR** (150 MHz, $CD_3OD$): $\delta$ = 183.7 (10-C), 174.6 (14-C), 174.3 (17-C), 172.9 (24-C), 170.6 (3-C), 170.0 (1-C), 157.6 (30-C), 145.6 (7-C), 138.1 (8-C), 135.6 (20-C), 130.4 (27-C),

130.1 (28-C), 129.5 (4-C), 128.9 (22-C), 128.1 (21-C), 128.0 (5-C), 126.5 (23-C), 122.5 (9-C), 115.1 (29-C), 108.9 (6-C), 61.7 (16-C), 55.8 (11-C), 55.3 (25-C), 54.5 (18-C), 50.8 (15-C), 40.9 (2-C), 38.4 (26-C), 37.6 (19-C), 37.5 (13-C), 32.4 (12-C). **HPLC-ESI-MS:** m/z (%) = 700.3 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{36}H_{42}N_7O_8$ $[M+H]^+$ 700.3088; found 700.3089.

### 3.17.2 Spirolactamscan of Trp

**9H-Fluoren-9-ylmethyl-(2S)-2-({5-[(carbamoylmethyl)carbamoyl]-2-oxo-1,2-dihydro-spiro[indole-3,4'- piperidine]-1'-yl}carbonyl)pyrrolidine-1-carboxylate (TVC-162, 172)**

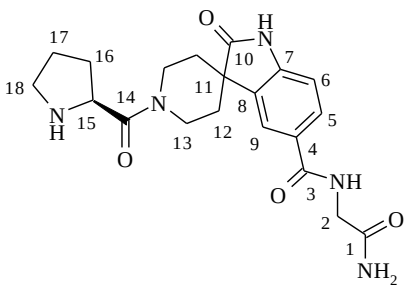


To a stirred solution of **169** (121 mg, 0.29 mmol) and Fmoc-Pro-OH (95.1 mg, 0.28 mmol) in DMF (3 mL), HATU (121.6 mg, 0.32 mmol) and DIPEA (0.17 mL, 1.16 mmol) were added dropwise. After stirring at room temperature for 20 h, the solvent was removed. Flash chromatography (EtOAc/EtOH, 6/1) afforded **172** (151 mg, 86%) as a white solid product.

**$^1$H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 7.87 (overlap, 1H, 9-H), 7.85 (overlap, 1H, 6-H), 7.83 (m, 2H, 25-H), 7.62 (d, $J$ = 7.8 Hz, 2H, 26-H), 7.42 (m, 2H, 24-H), 7.35 (m, 2H, 23-H), 7.01 (d, $J$ = 8.5 Hz, 1H, 5-H), 4.84 (overlap, 1/2H, 15-H), 4.6 (d, $J$ = 5.0 Hz, 1/2H, 15-H), 4.5 (m, 1H, 20-H), 4.4 (m, 1H, 20-H), 4.3 (t, $J$ = 6.2 Hz, 1/2H, 21-H), 4.2 (d, $J$ = 7.0 Hz, 1/2H, 21-H), 4.09 (m, 2H, 13-H), 4.08 (overlap, 1H, 2-H), 3.95 (overlap, 1H, 2-H), 3.78 (overlap, 2H, 13-H), 3.63 (m, 1H, 18-H), 3.53 (m, 1H, 18-H), 2.3 (m, 2H, 16-H), 1.94 (m, 2H, 17-H), 1.93 (overlap, 2H, 12-H), 1.82 (m, 2H, 12-H). **$^{13}$C-NMR** (100 MHz, $CD_3OD$): $\delta$ = 180.2 (10-C), 172.7 (3-C), 169.8 (14-C), 167.3 (1-C), 156.3 (19-C),128.1 (9-C), 127.5 (24-C), 126.9 (23-C), 124.6 (26-C), 119.7 (6-C), 119.6 (25-C), 109.2 (5-C), 67.0 (20-C), 57.1 (15-C), 47.3 (21-C), 46.6 (18-C), 40.5 (2-C), 37.3 (13-C), 32.2 (12-C), 30.3 (16-C), 29.7 (17-C). **IR** (KBr) ν ($cm^{-1}$): 3420.7, 1683.4. **HPLC-ESI-MS:** m/z (%) = 622.2 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{35}H_{35}N_5O_6Na$ $[M+Na]^+$ 644.2481; found 644.2480. $R_f$ = 0.26 (EtOAc/EtOH, 6/1).

**2-{2-Oxo-1'-[(2*S*)-pyrrolidine-2-carbonyl]-1,2-dihydrospiro[indole-3,4'-piperidine]-5-yl-formamido}acetamide (TVC-167, 174)**

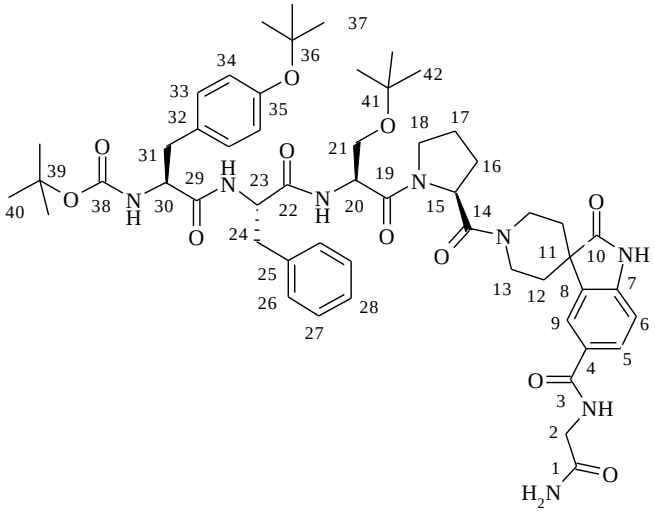
Peptide **172** (38 mg, 0.06 mmol ) in DCM (5 mL) was treated with piperidine (60 µL, 0.60 mmol) at room temperature for 1 h. The reaction mixture was concentrated and subjected to silica gel column



chromatography using DCM/MeOH (5/1, 1% TEA). The pure product **174** (23.6 mg, 97%) was obtained as a white solid.

**$^1$H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 7.97 (dd, $J$ = 1.3 Hz, $J$ = 12.0 Hz, 1H, 9-H), 7.87 (m, 1H, 6-H), 7.04 (dd, $J$ = 1.3 Hz, $J$ = 12.0 Hz, 1H, 5-H), 4.15 (m, 2H, 13-H), 4.06 (overlap, 2H, 2-H), 4.05 (overlap, 1H, 18-H), 3.99 (m, 2H, 13-H), 3.88 (m, 1H, 18-H), 2.44 (m, 1H, 16-H), 2.01 (m, 1H, 17-H), 1.99 (m, 2H, 12-H), 1.92 (m, 1H, 17-H), 1.91 (m, 2H, 12-H), 1.90 (m, 1H, 16-H). **$^{13}$C-NMR** (100 MHz, $CD_3OD$): $\delta$ = 183.4 (10-C), 174.4 (14-C), 170.9 (1-C), 169.9 (3-C), 145.6 (7-C), 135.4 (8-C), 126.8 (6-C), 121.0 (9-C), 107.8 (5-C), 56.5 (15-C), 53.7 (11-C), 40.6 (2-C), 38.8 (18-C), 36.1 (13-C), 30.7 (12-C), 28.2 (16-C), 23.3 (17-C). **IR** (KBr) ν ($cm^{-1}$): 3422.5, 1701.2, 1647.3. **HPLC-ESI-MS:** m/z (%) = 400.2 (100) $[M+H]^+$, 799.0 $[2M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{20}H_{26}N_5O_4$ $[M+H]^+$ 400.1979; found 400.1979. $R_f$ = 0.24 (DCM/MeOH, 3/1).

***tert*-Butyl-N-[(1*S*)-1-{[(1*S*)-1-{[(2*S*)-3-(*tert*-butoxy)-1-[(2*S*)-2-({5-[(carbamoylmethyl)carba-moyl]-2-oxo-1,2-dihydrospiro[indole-3,4'-piperidine]-1'-yl}carbonyl)pyrrolidin-1-yl]-1-oxopropan-2-yl]-carbamoyl}-2-phenylethyl]carbamoyl}-2-[4-(*tert*-butoxy)phenyl]ethyl]-carbamate (TVC-170, 175)**

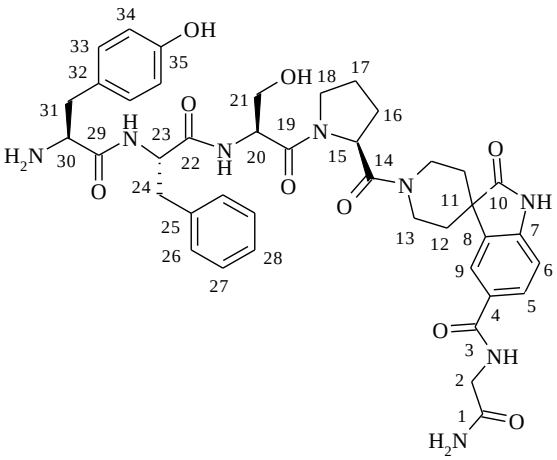


A stirred solution of amine **174** (25 mg, 62.6 µmol), tripeptide Boc-Tyr(*t*Bu)-Phe-Ser(*t*Bu)-OH (29.3 mg, 62.6 µmol) and HATU (26.2 mg, 68.8 µmol) in DMF (2 mL) was added DIPEA (46 µL, 313 µmol). The reaction mixture was stirred at room temperature for 20 h and concentrated to dryness. The crude product was purified by silica gel column chromatography (EtOAc/EtOH, 6/1) to afford **175** (36.2 mg, 57%) as a yellow solid product.

**$^1$H-NMR** (600 MHz, $CD_3OD$): $\delta$ = 8.03 (s, 1H, 9-H), 7.86 (dd, $J$ = 1.5 Hz, $J$ = 8.4 Hz, 1H, 6-H), 7.25-7.16 (m, 5H, 26-H, 27-H, 28-H), 7.06 (overlap, 1H, 33-H), 6.86 (d, $J$ = 8.4 Hz, 1H, 34-H), 7.02 (t, $J$ = 8.5 Hz, 1H, 5-H), 4.94 (m, 1H, 15-H), 4.74 (m, 1H, 20-H), 4.44 (bs, 1H, 30-H), 4.27 (m, 1H, 23-H), 4.08 (m, 2H, 13-H), 4.06 (overlap, 1H, 18-H), 3.94 (m, 2H, 13-H), 3.85 (overlap, 1H 2-H), 3.78 (overlap, 2H, 2-H, 21-H), 3.58 (m, 1H, 21-H), 3.54 (m, 1H, 18-H), 3.0 (m, 1H, 24-H), 2.96 (m, 1H, 31-H), 2.71 (m, 1H, 31-H), 2.69 (m, 1H, 24-H), 2.32 (m, 1H, 16-H), 2.15 (m, 1H, 17-H), 2.04 (m, 1H, 17-H), 1.98 (m, 2H, 12-H), 1.91 (m, 1H, 16-H), 1.75 (m, 2H, 12-H), 1.36 (s,

9H, 37-H), 1.31 (s, 9H, 42-H), 1.24 (s, 9H, 40-H). **$^{13}$C-NMR** (150 MHz, $CD_3OD$): $\delta$ = 183.2 (10-C), 175.8 (3-C), 174.4 (19-C), 174.1 (14-C), 172.8 (1-C), 170.2 (22-C), 169.2 (29-C), 157.6 (38-C), 145.5 (35-C), 143.3 (7-C), 135.8 (8-C), 133.7 (25-C), 130.5 (4-C), 129.7 (32-C), 129.4 (33-C), 129.3 (26-C), 128.1 (6-C), 127.9 (27-C), 126.2 (28-C), 123.7 (34-C), 122.3 (9-C), 108.9 (5-C), 79.5 (36-C), 75.4 (41-C), 74.8 (39-C), 61.5 (21-C), 56.8 (15-C), 55.9 (23-C), 55.8 (11-C, 30-C), 54.0 (20-C), 47.2 (18-C), 42.3 (2-C), 37.5 (24-C), 37.4 (31-C), 37.2 (13-C), 31.8 (12-C), 28.7 (16-C), 27.7 (42-C), 27.2 (37-C), 26.3 (40-C), 24.4 (17-C). **IR** (KBr) ν ($cm^{-1}$): 3420.6, 1654.7. **HPLC-ESI-MS:** m/z (%) = 1009.5 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{54}H_{72}N_8O_{11}Na$ $[M+Na]^+$ 1031.5213; found 1031.5192. $R_f$ = 0.38 (EtOAc/EtOH, 5/1).

**(2*S*)-2-[(2*S*)-2-Amino-3-(4-hydroxyphenyl)propanamido]-N-[(2*S*)-1-[(2*S*)-2-({5-[(carbamo-ylmethyl) carbamoyl]-2-oxo-1,2-dihydrospiro[indole-3,4'-piperidine]-1'-yl}carbonyl)-pyrrolidin-1-yl]-3-hydroxy-1-oxopropan-2-yl]-3-phenylpropanamide (TVC-175, 176)**



Hexapeptide **175** (26 mg, 25.8 µmol) was treated with TFA/TIPS/$H_2O$ (2 mL, 95/4/1) at 0 $^oC$ for 4 h. The reaction mixture was concentrated under diminished pressure to dryness and subjected to silica gel column chromatography using (DCM/MeOH, 5/1, 0.1% TEA). The pure product **176** (18 mg, 89%) was dissolved in ACN/$H_2O$ (2 mL, 1/1) and lyophilized to obtain white powder.

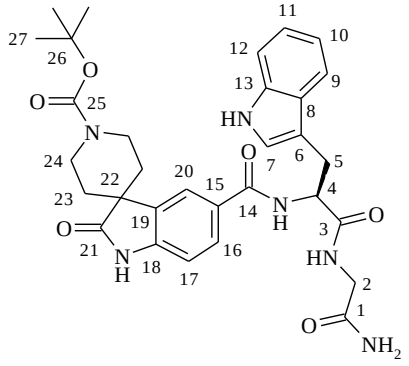
**$^1$H-NMR** (600 MHz, $CD_3OD$): $\delta$ = 8.02 (s, 1H, 9-H), 7.86 (m, 1H, 6-H), 7.25 (m, 4H, 26-H, 27-H), 7.19 (m, 1H, 28-H), 7.01 (overlap, 3H, 5-H, 33-H), 6.72 (d, *J* = 8.0 Hz, 2H, 34-H), 4.98 (dd, *J* = 5.2 Hz, *J* = 8.2 Hz), 4.93 (dd, *J* = 5.2 Hz, *J* = 8.2 Hz), 4.82 (m, 1H, 20-H), 4.80 (m, 1H, 23-H), 4.13 (bs, 1H, 2-H), 3.97 (m, 1H, 2-H), 3.92 (m, 1H, 30-H), 3.88 (m, 1H, 18-H), 3.80 (overlap, 1H, 18-H), 3.87-3.80 (overlap, 2H, 21-H), 3.75 (m, 2H, 12-H), 3.63 (m, 2H, 12-H), 3.18 (m, 1H, 24-H), 3.10 (m, 1H, 31-H), 3.00 (m, 1H, 24-H), 2.86 (m, 1H, 31-H), 2.35-2.38 (m, 1H, 16-H), 2.13 (overlap, 1H, 17-H), 2.04 (m, 1H, 17-H), 1.99 (m, 2H, 12-H), 1.98 (m, 1H, 16-H), 1.74 (m, 2H, 12-H). **$^{13}$C-NMR** (150 MHz, $CD_3OD$): $\delta$ = 182.5 (10-C), 173.1 (14-C), 171.5 (19-C), 171.4 (22-C), 170.8 (29-C), 168.8 (1-C), 168.4 (3-C), 156.5 (35-C), 144.1 (7-C), 136.6 (8-C), 134.3 (25-C), 133.9 (32-C), 130.1 (33-C), 128.9 (4-C), 128.8 (27-C), 128.1 (6-C, 26-C), 126.4 (28-C), 122.4 (9-C), 115.2 (34-C), 109.0 (5-C), 61.7 (21-C), 57.3 (15-C), 54.9 (23-C), 54.8 (11-C), 54.4 (30-C), 53.4 (20-C), 47.2 (18-C), 46.6 (13-C), 40.8 (2-C), 37.4 (24-C), 37.1 (31-C), 31.9 (12-C),

28.7 (16-C), 24.5 (17-C). **HPLC-ESI-MS:** m/z (%) = 797.4 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{41}H_{49}N_8O_9$ $[M+H]^+$ 797.3617; found 797.3618.

### 3.17.3 Spirolactamscan of Pro

**_tert_-Butyl-5-{[(1*S*)-1-[(carbamoylmethyl)carbamoyl]-2-(1H-indol-3-yl)ethyl]carbamoyl}-2-oxo-1,2-dihydrospiro[indole-3,4'-piperidine]-1'-carboxylate (TVC-161, 177)**

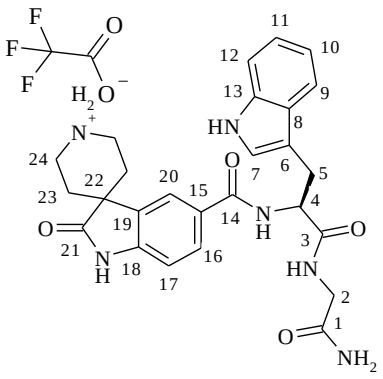
To a stirred solution of dipeptide TFA*H-Trp-Gly-$NH_2$ (128 mg, 0.34 mmol) and 1'-[(*tert*-butoxy) carbonyl]-2-oxo-1,2-dihydrospiro[indole-3,4'- piperidine]-5-carboxylic acid **167** (106 mg, 0.31 mmol) in DMF (6 mL), TBTU (109 mg, 0.34 mmol) and DIPEA (0.23 mL, 1.37 mmol) were added. After stirring for 20 h, the solvent was removed and flash chromatography (EtOAc/EtOH, 5/1) provided solid product **177** (168.7 mg, 84%).



**$^1$H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 7.7 (dd, $J$ = 2.0 Hz, $J$ = 8.0 Hz, 1H, 16-H), 7.65 (d, $J$ = 8.7 Hz, 1H, 9-H), 7.6 (d, $J$ = 2.0 Hz, 1H, 20-H), 7.35 (d, $J$ = 8.7 Hz, 1H, 12-H), 7.2 (s, 1H, 7-H), 7.12 (t, $J$ = 6.9 Hz, 1H, 11-H), 7.02 (t, $J$ = 6.9 Hz, 1H, 10-H), 6.9 (d, $J$ = 8.0 Hz, 1H, 17-H), 3.9 (d, $J$ = 17.0 Hz, 1H, 2-H), 3.8 (br, 4H, 24-H), 3.7 (d, $J$ = 17.0 Hz, 1H, 2-H), 3.4 (m, 2H, 5-H), 1.75 (m, 4H, 23-H), 1.54 (s, 9H, 27-H). **$^{13}$C-NMR** (100 MHz, $CD_3OD$): $\delta$ = 183.5 (21-C), 174.9 (3-C), 174.3 (14-C), 170.0 (1-C), 156.0 (25-C), 145.6 (18-C), 138.0 (13-C), 135.6 (19-C), 128.2 (16-C), 127.3 (6-C), 123.4 (7-C), 122.2 (20-C), 121.0 (11-C), 118.6 (10-C), 117.7 (9-C), 110.9 (12-C), 110.5 (17-C), 119.2 (8-C), 81.0 (26-C), 55.6 (4-C), 41.7 (2-C), 38.9 (24-C), 31.8 (23-C), 27.2 (27-C), 26.6 (5-C). **IR** (KBr) ν ($cm^{-1}$): 3336.7, 1670.3. **HPLC-ESI-MS:** m/z (%) = 589.3 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{31}H_{36}N_6O_6Na$ $[M+Na]^+$ 611.2591; found 611.2589. $R_f$ = 0.45 (EtOAc/EtOH, 6/1).

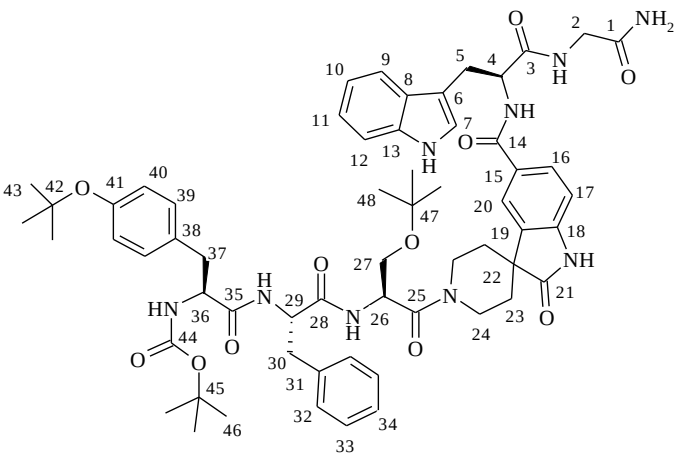
**5-{[(1*S*)-1-[(Carbamoylmethyl)carbamoyl]-2-(1H-indol-3-yl)ethyl]carbamoyl}-2-oxo-1,2-dihydro-spiro[indole-3,4'-piperidin]-1'-ium trifluoroacetate (TVC-165, 178)**

Peptide **177** (130 mg, 220.8 µmol) was treated with TFA/TIPS/$H_2O$ (2 mL, 95/4/1) at 0 °C for 4 h. The solvent was evaporated under reduced pressure to give product **178** (126 mg, 95%) as a TFA salt.



**$^1$H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 7.73 (d, *J* = 2.0 Hz, 1H, 20-H), 7.69 (dd, *J* = 2.0 Hz, *J* = 8.0 Hz, 1H, 16-H), 7.65 (d, *J* = 8.0 Hz, 1H, 9-H), 7.34 (d, *J* = 8.7 Hz, 1H, 12-H), 7.19 (s, 1H, 7-H), 7.09 (t, *J* = 7.6 Hz, 1H, 11-H), 7.01 (t, *J* = 7.6 Hz, 1H, 10-H), 6.94 (d, *J* = 8.0 Hz, 1H, 17-H), 4.85 (t, *J* = 7.5 Hz, 1H, 4-H), 3.94 (d, *J* = 17.5 Hz, 1H, 2-H), 3.82 (m, 2H, 24-H), 3.71 (d, *J* = 17.5 Hz, 1H, 2-H), 3.44 (bs, 1H, 5-H), 3.42 (m, 2H, 24-H), 3.34 (bs, 1H, 5-H), 2.17 (m, 2H, 23-H), 2.01 (m, 2H, 23-H). **$^{13}$C-NMR** (100 MHz, $CD_3OD$): $\delta$ = 129.9 (15-C), 129.3 (8-C), 128.4 (6-C), 128.6 (16-C), 123.3 (7-C), 122.1 (20-C), 121.0 (11-C), 118.4 (10-C), 118.0 (9-C), 111.0 (12-C), 109.2 (17-C), 55.6 (4-C), 41.6 (2-C), 38.9 (24-C), 28.8 (23-C), 27.2 (5-C). **IR** (KBr) ν ($cm^{-1}$): 3408.9, 1672.2. **HPLC-ESI-MS:** m/z (%) = 489.2 (100) $[M\text{-}CF_3COOH+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{26}H_{28}N_6O_4Na$ $[M+Na]^+$ 511.2063; found 511.2063.

***tert*-Butyl-N-[(1*S*)-1-{[(1*S*)-1-{[(2*S*)-3-(*tert*-butoxy)-1-(5-{[(1*S*)-1-[(carbamoylmethyl)carba-moyl]-2-(1H-indol-3-yl)ethyl]carbamoyl}-2-oxo-1,2-dihydrospiro[indole-3,4'-piperidine]-1'-yl)-1-oxopropan-2-yl]carbamoyl}-2-phenylethyl]carbamoyl}-2-[4-(*tert*-butoxy)phenyl]-ethyl]carbamate (TVC-168, 179)**

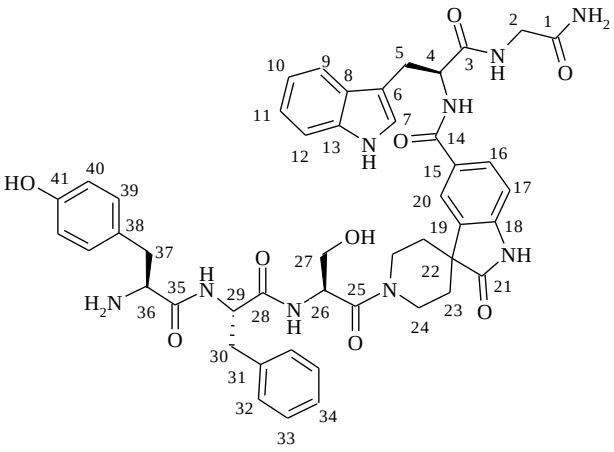


A solution of **178** (27 mg, 44.8 µmol), tripeptide Boc-Tyr(*t*Bu)-Phe-Ser(*t*Bu)-OH (28.2 mg, 44.8 µmol) and HATU (18.7 mg, 49.3 µmol) in DMF (2 mL) was added DIPEA (33 µL, 224 µmol). After being stirred at room temperature for 20 h, the solvent was removed. The residue was purified by silica gel column chromatography (EtOAc/EtOH, 5/1) to obtain **179** (32 mg, 65%) as a solid product.

**$^1$H-NMR** (600 MHz, $CD_3OD$): $\delta$ = 7.73 (d, *J* = 8.5 Hz, 1H, 16-H), 7.67 (s, 1H, 20-H), 7.63 (d, *J* = 8.0 Hz, 1H, 9-H), 7.35 (t, *J* = 8.0 Hz, 1H, 12-H), 7.25-7.22 (m, 5H, 32-H, 33-H, 34-H), 7.20 (s, 1H, 7-H), 7.10 (overlap, 1H, 11-H), 7.08 (overlap, 2H, 39-H), 7.02 (overlap, 1H, 10-H), 6.96 (br, 1H, 17-H), 6.88 (m, 2H, 40-H), 5.06 (br, 1H, 25-H), 4.81 (overlap, 1H, 4-H), 4.79 (br, 1H,36-H), 4.30 (s, 1H, 29-H), 3.96 (m, 2H, 24-H), 3.90 (overlap, 1H, 2-H), 3.87 (m, 2H, 24-H), 3.73 (overlap, 1H, 2-H), 3.44 (m, 1H, 5-H), 3.32 (overlap, 1H, 5-H), 3.16 (br, 1H, 37-H), 2.97 (m, 1H, 37-H), 2.95 (m, 1H, 30-H), 2.69 (br, 1H, 30-

H), 1.93 (m, 2H, 23-H), 1.78 (m, 2H, 23-H), 1.29 (s, 9H, 43-H), 1.28 (s, 9H, 48-H), 1.21 (s, 9H, 46-H). **$^{13}$C-NMR** (150 MHz, $CD_3OD$): $\delta$ = 183.6 (21-C), 176.1 (25-C), 175.0 (3-C), 174.4 (14-C), 173.5 (1-C), 172.9 (35-C), 171.1 (28-C), 155.2 (44-C), 145.6 (41-C), 142.6 (18-C), 138.1 (19-C), 135.4 (13-C), 133.7 (31-C), 130.4 (15-C), 129.3 (39-C), 129.2 (32-C), 129.0 (38-C), 128.2 (16-C), 127.9 (33-C), 127.8 (8-C), 123.5 (40-C), 123.2 (7-C), 122.5 (20-C), 121.0 (11-C), 118.6 (10-C), 117.8 (9-C), 111.0 (12-C), 108.9 (17-C), 108.2 (6-C), 80.7 (42-C), 79.5 (47-C), 74.9 (45-C), 61.9 (27-C), 55.9 (29-C), 55.5 (4-C), 55.4 (22-C), 54.1 (36-C), 49.5 (26-C), 41.7 (2-C), 37.8 (24-C), 37.4 (37-C), 37.1 (30-C), 32.4 (23-C), 27.3 (43-C), 27.2 (48-C), 26.8 (5-C), 26.3 (46-C). **IR** (KBr) ν ($cm^{-1}$): 3446.2, 1653.1. **HPLC-ESI-MS:** m/z (%) = 1098.6 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{60}H_{75}N_9O_{11}Na$ $[M+Na]^+$ 1120.5478; found 1120.5482. $R_f$ = 0.37 (EtOAc/EtOH, 7/1).

**(2*S*)-2-[(2*S*)-2-Amino-3-(4-hydroxyphenyl)propanamido]-N-[(2*S*)-1-(5-{[(1*S*)-1-[(carbamo-ylmethyl) carbamoyl]-2-(1H-indol-3-yl)ethyl]carbamoyl}-2-oxo-1,2-dihydrospiro[indole-3,4'-piperidine]-1'-yl) -3-hydroxy-1-oxopropan-2-yl]-3-phenylpropanamide (TVC-176, 180)**



Hexapeptide **179** (21 mg, 18.2 µmol) was treated with TFA/TIPS/$H_2O$ (2 mL, 95/4/1) at 0 °C for 6 h. After that, the reaction mixture was concentrated and purified by silica gel column chromatography (DCM/MeOH, 5/1, 0.1% TEA) to afford pure product **180** (10 mg, 62%).

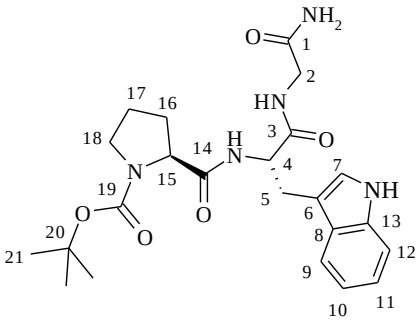
**$^1$H-NMR** (600 MHz, $CD_3OD$): $\delta$ = 7.71 (t, $J$ = 9.0 Hz, 1H, 17-H), 7.69 (overlap, 1H, 20-H), 7.65 (m, 1H, 9-H), 7.35 (d, $J$ = 7.5 Hz, 1H, 12-H), 7.30-7.25 (m, 5H, 32-H, 33-H, 34-H), 7.19 (s, 1H, 7-H), 7.12 (t, $J$ = 7.7 Hz, 1H, 11-H), 7.08 (d, $J$ = 7.6 Hz, 2H, 39-H), 7.01 (overlap, 1H, 10-H), 6.96 (t, $J$ = 7.0 Hz, 1H, 16-H), 6.77 (d, $J$ = 7.6 Hz, 2H, 40-H), 5.04 (m, 1H, 26-H), 4.78 (overlap, 1H, 4-H), 4.72 (m, 1H, 29-H), 3.97 (overlap, 2H, 24-H), 3.91 (m, 1H, 2-H), 3.86 (overlap, 2H, 24-H), 3.84 (overlap, 1H, 36-H), 3.80-3.78 (m, 2H, 27-H), 3.71 (m, 1H, 2-H), 3.44 (m, 1H, 5-H), 3.32 (m, 1H, 5-H), 3.15 (m, 1H, 30-H), 3.05 (m, 1H, 37-H), 2.99 (m, 1H, 30-H), 2.78 (m, 1H, 37-H), 1.80-1.79 (m, 4H, 23-H). **$^{13}$C-NMR** (150 MHz, $CD_3OD$): $\delta$ = 183.5 (21-C), 174.9 (25-C), 174.5 (28-C), 172.6 (14-C), 172.4 (35-C), 170.2 (1-C), 167.6 (3-C), 157.9 (41-C), 145.6 (18-C), 138.1 (19-C), 138.0 (13-C), 135.4 (31-C), 130.2 (39-C), 129.6 (15-C, 38-C), 128.9 (33-C), 128.3 (32-C), 128.1 (17-C), 127.9 (8-C), 126.6 (34-C), 123.2 (7-C), 121.0 (11-C), 118.4 (10-C), 117.9 (9-C), 115.2 (40-C), 111.2 (12-C), 110.5 (6-C), 109.0 (16-C), 61.7 (27-C), 55.9 (22-C), 55.6 (4-C), 54.8 (36-C), 54.6 (29-C), 51.0 (26-C),

41.9 (2-C), 41.2 (24-C), 37.5 (30-C), 37.3 (37-C), 31.9 (23-C), 26.8 (5-C). **HPLC-ESI-MS:** m/z (%) = 886.4 (50) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. For $C_{47}H_{51}N_9O_9Na$ $[M+Na]^+$ 908.3702; found 908.3706.

### 3.17.4 Spirolactamscan of Phe

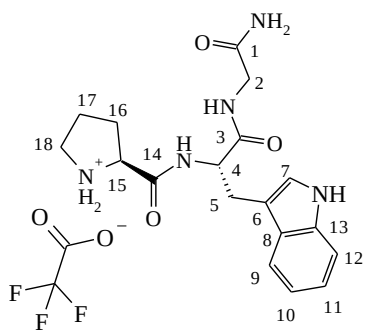
**_tert_-Butyl-(2*S*)-2-({1-[(carbamoylmethyl)carbamoyl]-2-(1H-indol-3-yl)ethyl}carbamoyl)-pyrrolidine -1-carboxylate (TVC-163, 182)**

A solution of dipeptide TFA*H-Trp-Gly-$NH_2$ (300 mg, 0.80 mmol), Boc-Pro-OH (190 mg, 0.88 mmol) and TBTU (309 mg, 0.96 mmol) in DMF (6 mL) was added dropwise DIPEA (0.6 mL). The reaction mixture was then stirred at room temperature for 20 h. Removal of the solvent to dryness and flash chromatography (EtOAc/EtOH, 10/1) afforded solid product **182** (338 mg, 92%).



**$^1$H-NMR** (600 MHz, $CD_3OD$): $\delta$ = 7.6 (dd, $J$ = 1.8 Hz, $J$ = 8.8 Hz, 1H, 9-H), 7.36 (t, $J$ = 8.8 Hz, 1H, 12-H), 7.17 (s, 1H, 7-H), 7.13 (overlap, 1H, 11-H), 7.05 (t, $J$ = 7.5 Hz, 1H, 10-H), 4.6 (t, $J$ = 7.5 Hz, 1H, 4-H), 4.16-4.10 (m, 1H, 15-H), 3.87 (d, $J$ = 17.0 Hz, 1H, 2-H), 3.65 (d, $J$ = 17.0 Hz, 1H, 2-H), 3.34 (br, 2H, 18-H), 3.3 (m, 1H, 5-H), 3.12 (m, 1H, 5-H), 2.18 (m, 1H, 16-H), 2.08 (m, 1H, 16-H), 1.75 (br, 2H, 17-H), 1.38 (s, 9H, 21-H). **$^{13}$C-NMR** (150 MHz, $CD_3OD$): $\delta$ = 150.7 (19-C), 123.3 (7-C), 121.0 (11-C), 118.3 (10-C), 117.6 (9-C), 111.1 (12-C), 60.5 (15-C), 54.6 (4-C), 46.7 (18C), 41.8 (2-C), 30.8 (16-C), 27.5 (21-C), 27.2 (5-C), 23.5 (17-C). **IR** (KBr) ν ($cm^{-1}$): 3419.6, 1669.8. **HPLC-ESI-MS:** m/z (%) = 458.2 (95) $[M+H]^+$, 358.2 (100) $[M-Boc+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{23}H_{31}N_5O_5Na$ $[M+Na]^+$ 480.2217; found 480.2209. $R_f$ = 0.17 (EtOAc/EtOH, 6/1).

**(2*S*)-2-{[(1*S*)-1-[(Carbamoylmethyl)carbamoyl]-2-(1H-indol-3-yl)ethyl]carbamoyl}-pyrrolidin-1-ium trifluoroacetate (TVC-164, 183)**

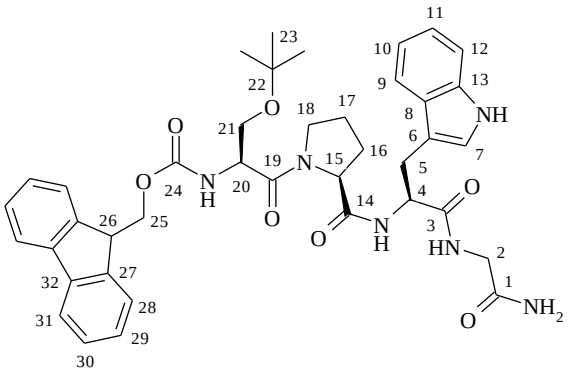


Tripeptide **182** (300 mg, 0.66 mmol) in TFA/TIPS/$H_2O$ (2 mL, 95/4/1) was stirred at 0 $^oC$ for 4 h. After that time, the solvent was removed under reduced pressure to dryness to provide product **183** (290 mg, 96%) as a TFA salt.

**$^1$H-NMR** (600 MHz, $CD_3OD$): $\delta$ = 7.6 (d, $J$ = 7.5 Hz, 1H, 9-H), 7.36 (d, $J$ = 8.3 Hz, 1H, 12-H), 7.17 (s, 1H, 7-H), 7.11 (t, $J$ = 7.5 Hz, 1H, 11-H), 7.04 (t, $J$ = 7.5 Hz, 1H, 10-H), 4.66 (t, $J$ = 7.8 Hz, 1H, 4-H), 4.25 (t, $J$ =

7.8 Hz, 1H, 15-H), 3.87 (d, *J* = 17.2 Hz, 1H, 2-H), 3.65 (d, *J* = 17.2 Hz, 1H, 2-H), 3.38 (m, 2H, 18-H), 3.34 (m, 1H, 5-H), 3.22 (m, 1H, 5-H), 2.40 (m, 1H, 16-H), 2.09 (m, 1H, 16-H), 2.08 (overlap, 1H, 17-H), 2.01 (br, 1H, 17-H). **$^{13}$C-NMR** (150 MHz, $CD_3OD$): $\delta$ = 176.3 (14-C), 174.5 (3-C), 174.3 (1-C), 138.4 (13-C), 128.6 (8-C), 124.8 (6-C), 123.0 (7-C), 121.1 (11-C), 118.3 (10-C), 117.8 (9-C), 111.0 (12-C), 59.5 (15-C), 55.2 (4-C), 45.8 (18-C), 41.9 (2-C), 29.2 (16-C), 26.9 (5-C), 23.6 (17-C). **IR** (KBr) ν ($cm^{-1}$): 3420.8, 2919.2, 1653.1. **HPLC-ESI-MS:** m/z (%) = 358.2 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{18}H_{23}N_5O_3Na$: 380.1699; found 380.1693. $R_f$ = 0.12 (DCM/MeOH, 5/1).

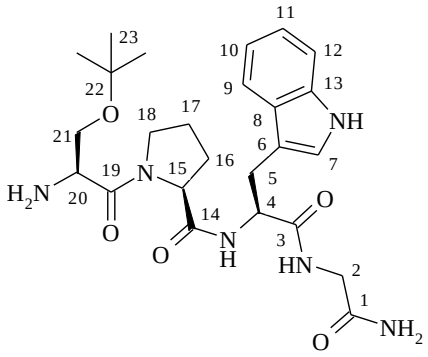
**9H-Fluoren-9-yl-methyl-N-[(2*S*)-3-(*tert*-butoxy)-1-[(2*S*)-2-{[(1*S*)-1-[(carbamoylmethyl)car-bamoyl]-2-(1H-indol-3-yl)ethyl]carbamoyl}pyrrolidin-1-yl]-1-oxopropan-2-yl]carbamate (TVC-166, 184)**



To a stirred solution of **183** (120 mg, 0.25 mmol), Fmoc-Ser(*t*Bu)-OH (88 mg, 0.23 mmol) and HATU (97 mg, 0.25 mmol) in DMF (2 mL), DIPEA (0.26 mL, 1.53 mmol) was added. The reaction mixture was then stirred at room temperature for 20 h and concentrated. The residue was purified by silica gel column chromatography (EtOAc/EtOH, 10/1) to afford **184** (150 mg, 83%) as a solid product.

**$^1$H-NMR** (600 MHz, $CD_3OD$): $\delta$ = 7.80 (m, 2H, 32-H), 7.60 (m, 2H, 28-H), 7.59 (overlap, 1H, 9-H), 7.40 (m, 2H, 30-H), 7.35 (overlap, 1H, 12-H), 7.32 (m, 2H, 29-H), 7.14 (s, 1H, 7-H), 7.11 (t, *J* = 7.5 Hz, 1H, 11-H), 7.03 (t, *J* = 7.5 Hz, 1H, 10-H), 4.58 (m, 1H, 4-H), 4.47 (m, 1H, 20-H), 4.41 (m, 2H, 25-H), 4.36 (m, 1H, 15-H), 4.25 (m, 1H, 26-H), 3.87 (d, *J* = 17.0 Hz, 1H, 2-H), 3.75 (m, 1H, 18-H), 3.71 (d, *J* = 17.0 Hz, 1H, 2-H), 3.62 (m, 1H, 21-H), 3.52 (m, 1H, 21-H), 3.49 (m, 1H, 18-H), 3.39 (dd, *J* = 5.6 Hz, *J*= 13.5 Hz, 1H, 5-H), 3.26 (dd, *J* = 5.6 Hz, *J*= 13.5 Hz, 1H, 5-H), 2.07 (m, 1H, 17-H), 2.05 (m, 1H, 16-H), 1.92 (m, 1H, 16-H), 1.76 (m, 1H, 17-H), 1.17 (s, 9H, 23-H). **$^{13}$C-NMR** (150 MHz, $CD_3OD$): $\delta$ = 174.4 (19-C), 174.1 (3-C), 173.7 (14-C), 172.5 (1-C), 158.0 (24-C), 145.2 (27-C), 142.5 (32-C), 138.1 (13-C), 128.5 (8-C), 127.3 (29-C), 126.7 (30-C), 124.7 (28-C), 124.6 (12-C), 122.9 (7-C), 121.1 (11-C), 119.5 (31-C), 118.4 (10-C), 117.7 (9-C), 111.1 (6-C), 74.9 (22-C), 66.7 (25-C), 61.8 (21-C), 60.5 (15-C), 54.5 (4-C), 53.2 (20-C), 47.2 (18-C), 47.0 (26-C), 41.9 (2-C), 30.7 (16-C), 28.4 (17-C), 26.3 (5-C), 26.1 (23-C). **IR** (KBr) ν ($cm^{-1}$): 3429.8, 2973.8, 1662.6. **HPLC-ESI-MS:** m/z (%) = 723.3 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{40}H_{46}N_6O_7Na$ $[M+Na]^+$ 745.3320; found 745.3321. $R_f$ = 0.45 (EtOAc/EtOH, 7/1).

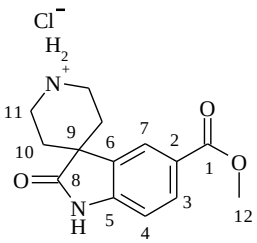
**(2*S*)-2-{[(2*S*)-1-[(2*S*)-2-Amino-3-(*tert*-butoxy)propanoyl]pyrrolidin-2-yl]formamido}-N-(carbamoyl-methyl)-3-(1H-indol-3-yl)propanamide (TVC-171, 185)**



A solution of **184** (150 mg, 0.02 mmol) in DCM (5 mL) was treated with piperidine (0.02 ml, 021 mmol) at room temperature for 20 min. Afterward, the solvent was removed and the crude product was subjected to silica gel column chromatography eluting DCM/MeOH (4/1) to obtain peptide **185** (96 mg, 92%).

**$^1$H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 7.6 (d, $J$ = 8.0 Hz, 1H, 9-H), 7.36 (d, $J$ = 7.2 Hz, 1H, 12-H), 1.17 (s, 1H, 7-H), 7.11 (t, $J$ = 7.0 Hz, 1H, 11-H), 7.04 (t, $J$ = 7.0 Hz, 10-H), 4.77 (m, 1H, 20-H), 4.53 (m, 1H, 4-H), 4.44 (m, 1H, 15-H), 3.9 (d, $J$ = 17.0 Hz, 1H, 2-H), 3.72-3.68 (m, 2H, 18-H), 3.66 (d, $J$ = 17.0 Hz, 1H, 2-H), 2.09 (m, 1H, 16-H), 2.08 (m, 1H, 17-H), 1.91 (m, 1H, 17-H), 1.90 (m, 1H, 16-H), 1.22 (s, 9H, 23-H). **$^{13}$C-NMR** (100 MHz, $CD_3OD$): $\delta$ = 174.6 (19-C), 174.5 (14-C), 174.4 (3-C), 174.3 (1-C), 128.7 (8-C), 123.3 (7-C), 121.2 (11-C), 118.3 (10-C), 117.9 (9-C), 110.9 (12-C), 112.4 (6-C), 75.1 (22-C), 60.3 (15-C), 54.9 (4-C), 54.4 (20-C), 47.1 (18-C), 41.8 (2-C), 31.1 (16-C), 28.6 (17-C), 26.7 (5-C), 26.3 (23-C). **IR** (KBr) ν ($cm^{-1}$): 3365.7, 1662.2. **HPLC-ESI-MS:** m/z (%) = 501.2 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{25}H_{36}N_6O_5Na$: 523.2639; found 523.2648. $R_f$ = 0.14 (DCM/MeOH, 5/1).

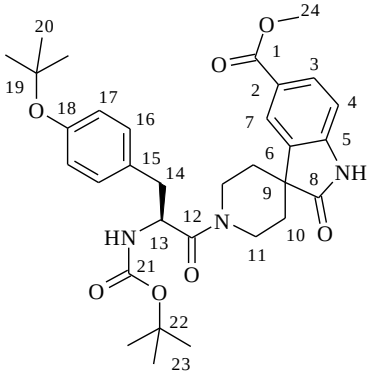
**5-(Methoxycarbonyl)-2-oxo-1,2-dihydrospiro[indole-3,4'-piperidin]-1'-ium chloride (TVC-181, 188)**



To a solution of 1'-[(*tert*-butoxy)carbonyl]-2-oxo-1,2-dihydrospiro[indole-3,4'-piperidine]-5-carboxylic acid **167** (200 mg, 0.58 mmol) in MeOH (5 mL) at 0 $^o$C, $SOCl_2$ (50 µL, 0.64 mmol) was added dropwise. The reaction mixture was allowed to warm up to room temperature and stirred for 20 h. After that the solvent was removed to dryness. The residue was dissolved in MeOH/TBME (5.5 mL, 1/10) and collected. The ester **188** (151 mg, 88%) was obtained as a white solid.

**$^1$H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 8.01 (m, 1H, 7-H), 7.99 (br, 1H, 4-H), 7.03 (d, $J$ = 8.0 Hz, 1H, 3-H), 3.9 (s, 3H, 12-H), 3.85 (dt, $J$ = 3.5 Hz, $J$ = 12.0 Hz, 2H, 11-H), 3.44 (dt, $J$ = 3.5 Hz, $J$ = 12.0 Hz, 2H, 11-H), 2.27 (m, 2H, 10-H), 2.06 (br, 2H, 10-H). **$^{13}$C-NMR** (100 MHz, $CD_3OD$): $\delta$ = 168.2 (1-C), 146.9 (5-C), 134.5 (6-C), 131.1 (7-C), 126.2 (2-C), 123.7 (4-C), 109.5 (3-C), 52.6 (9-C), 44.2 (12-C), 40.5 (11-C), 30.5 (10-C). **IR** (KBr) ν ($cm^{-1}$): 3429.1, 1707.3. **HPLC-ESI-MS:** m/z (%) = 261.1 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{14}H_{17}N_2O_3$ $[M+H]^+$ 261.1234; found 261.1234.

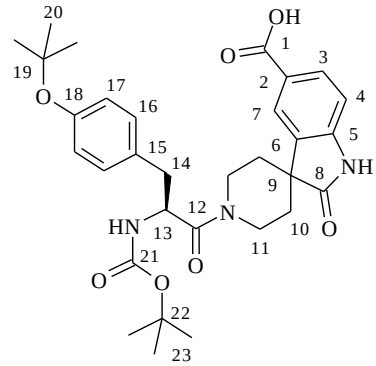
**Methyl-1'-[(2*S*)-2-{[(*tert*-butoxy)carbonyl]amino}-3-[4-(*tert*-butoxy)phenyl]propanoyl]-2-oxo-1,2-dihydrospiro[indole-3,4'-piperidine]-5-carboxylate (TVC-183, 189)**



To a solution of amine **188** (50 mg, 0.17 mmol), Boc-Tyr(*t*Bu)-OH (65.4 mg, 0.19 mmol) and HATU (77 mg, 0.21 mmol) in DMF (2 mL), DIPEA (0.12 mL, 0.84 mmol) was added. After being stirred at room temperature for 20 h, the solvent was removed and flash chromatography (eluent: EtOAc/CyH, 1/1) yielded **189** (72 mg, 74%) as a white solid product.

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 7.98 (d, $J$ = 7.8 Hz, 1H, 4-H), 7.83 (d, $J$ = 7.8 Hz, 1H, 7-H), 7.12 (d, $J$ = 8.8 Hz, 2H, 17-H), 6.94 (overlap, 3H, 3-H, 17-H), 4.9 (br, 1H, 13-H), 3.95 (s, 3H, 24-H), 3.01 (m, 1H, 14-H), 2.96 (m, 1H, 14-H), 1.45 (s, 9H, 20-H), 1.34 (s, 9H, 23-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 170.5 (12-C), 166.5 (1-C), 154.5 (18-C), 154.1 (18-C), 144.0 (5-C), 133.7 (6-C), 131.4 (15-C), 130.9 (4-C), 130.0 (16-C), 124.7 (2-C), 124.4 (7-C), 124.2 (17-C), 109.4 (3-C), 79.6 (19-C), 78.4 (22-C), 53.4 (9-C), 52.0 (24-C), 51.2 (13-C), 41.0 (11-C), 39.7 (14-C), 32.4 (10-C), 28.7 (23-C), 28.1 (20-C). **IR** (KBr) ν ($cm^{-1}$): 3428.3, 2977.2, 1714.8, 1621.7. **HPLC-ESI-MS:** m/z (%) = 580.3 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{32}H_{41}N_3O_7Na$ $[M+Na]^+$ 602.2837; found 602.2839. $R_f$ = 0.28 (CyH/EtOAc, 1/1).

**1'-[(2*S*)-2-{[(*tert*-Butoxy)carbonyl]amino}-3-[4-(*tert*-butoxy)phenyl]propanoyl]-2-oxo-1,2-dihydro-spiro[indole-3,4'-piperidine]-5-carboxylic acid (TVC-185, 190)**



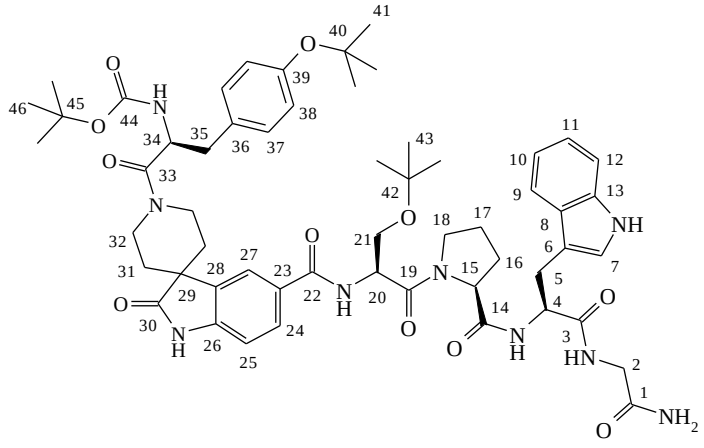
Methyl ester **189** (50 mg, 90 µmol ) in MeOH (2 mL) was treated with 1N NaOH (5.2 mg, 130 µmol) at room temperature for 24 h. The resulting solution was acidified with 1N HCl to pH≈4 at 0 °C and extracted with EtOAc (3x60 mL). The combined organic phases were washed $H_2O$, brine solution and dried over $MgSO_4$. Removal of the solvent gave acid **TVC-185** (46 mg, 94%) as a white solid.

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 9.0 (s, 1H, NH), 8.05 (d, $J$ = 8.8 Hz, 1H, 4-H), 7.97 (s, 1H, 7-H), 7.14 (d, $J$ = 8.8 Hz, 2H, 16-H), 6.98 (overlap, 1H, 3-H), 6.94 (d, $J$ = 8.8 Hz, 2H, 17-H), 5.72 (s, 1H, NH), 4.95 (br, 1H, 13-H), 3.75 (br, 2H, 11-H), 3.63 (br, 2H, 11-H), 3.04 (m, 1H, 14-H), 2.95 (m, 1H, 14-H), 1.99 (br, 2H, 10-H), 1.81 (br, 2H, 10-H), 1.43 (s, 9H, 20-H), 1.34 (s, 9H, 23-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$): $\delta$ = 182.3 (8-C), 170.1 (12-C), 169.7 (1-C), 155.3 (21-C), 154.2 (18-C), 144.5 (5-C), 140.0 (6-C), 131.5 (4-C), 130.1 (15-C), 129.9

(16-C), 125.2 (7-C), 124.3 (17-C), 124.2 (2-C), 109.5 (3-C), 79.8 (19-C), 78.5 (22-C), 58.7 (9-C), 51.2 (13-C), 41.1 (11-C), 39.5 (14-C), 32.4 (10-C), 28.7 (23-C), 28.2 (20-C). **IR** (KBr) ν ($cm^{-1}$): 3421.8, 2976.8, 1710.3, 1619.2. **HPLC-ESI-MS:** m/z (%) = 566.3 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{31}H_{39}N_3O_7Na$ $[M+Na]^+$ 588.2680; found 588.2681. $R_f$ = 0.30 (EtOAc).

**_tert_-Butyl-N-[(2*S*)-1-(5-{[(2*S*)-3-(_tert_-butoxy)-1-[(2*S*)-2-{[(1*S*)-1-[(carbamoylmethyl)carba-moyl]-2-(1H-indol-3-yl)ethyl]carbamoyl}pyrrolidin-1-yl]-1-oxopropan-2-yl]carbamoyl}-2-oxo-1,2-dihydro-spiro[indole-3,4'-piperidine]-1'-yl)-3-[4-(_tert_-butoxy)phenyl]-1-oxopropan-2-yl]carbamate (TVC-179, 191)**

A solution of **190** (25 mg, 44.2 µmol), tripeptide **185** (26.5 mg, 53.0 µmol) and HATU (20.2 mg, 53.0 µmol) in DMF (3 mL) was added DIPEA (33 µL, 221 µmol). After stirring at room temperature for 20 h, the solvent was removed under reduced pressure. Flash chromatography eluting EtOAc/EtOH (6/1) provided peptide **191** (24 mg, 52%) as a solid.

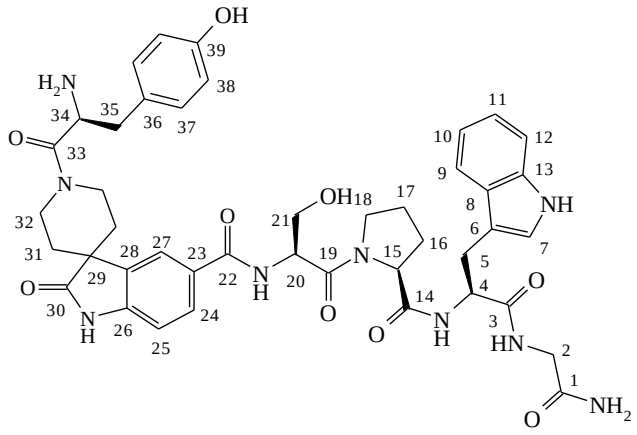


**$^1$H-NMR** (600 MHz, $CD_3OD$): $\delta$ = 7.86 (t, $J$ = 7.2 Hz, 1H, 25-H), 7.6 (t, $J$ = 7.7 Hz, 1H, 9-H), 7.33 (bs, 1H, 12-H), 7.18 (overlap, 1H, 7-H), 7.16 (overlap, 2H, 37-H), 7.09 (overlap, 1H, 11-H), 7.01 (overlap, 1H, 24-H), 6.99 (overlap, 1H, 10-H), 6.91 (br, 2H, 38-H), 5.03 (t, $J$ = 8.3 Hz, 1H, 15-H), 4.85 (overlap, 1H, 20-H), 4.78 (m, 1H, 34-H), 4.57 (m, 1H, 4-H), 3.9 (m, 1H, 2-H), 3.84 (m, 2H, 32-H), 3.80 (m, 1H, 21-H), 3.73 (overlap, 1H, 32-H), 3.71 (m, 2H, 2-H, 21-H), 3.39 (overlap, 1H, 5-H), 3.23 (m, 1H, 5-H), 2.97 (br, 1H, 35-H), 2.89 (m, 1H, 35-H), 2.06 (m, 1H, 16-H), 1.93 (m, 1H, 16-H), 1.86 (m, 2H, 31-H), 1.85 (overlap, 2H, 17-H), 1.77 (m, 2H, 31-H), 1.39 (s, 9H, 41-H), 1.34 (s, 9H, 43-H), 1.22 (s, 9H, 46-H). **$^{13}$C-NMR** (150 MHz, $CD_3OD$): $\delta$ = 183.2 (30-C), 175.2 (19-C), 174.7 (33-C), 174.1 (22-C), 173.6 (14-C), 172.4 (1-C), 169.9 (3-C), 157.1 (44-C), 155.4 (39-C), 145.8 (26-C), 138.1 (28-C), 135.6 (13-C), 129.8 (23-C), 129.6 (37-C), 128.6 (8-C), 128.4 (25-C), 123.6 (38-C), 122.9 (7-C), 122.3 (27-C), 121.1 (11-C), 118.7 (10-C), 117.8 (9-C), 110.9 (12-C), 110.6 (6-C), 109.1 (24-C), 79.5 (40-C), 75.1 (42-C), 74.9 (45-C), 62.1 (21-C), 60.5 (15-C), 54.8 (4-C), 54.0 (29-C), 52.6 (34-C), 51.7 (20-C), 47.5 (18-C), 41.9 (2-C), 40.8 (32-C), 37.7 (35-C), 31.9 (31-C), 30.1 (16-C), 27.7 (43-C), 27.3 (41-C), 26.6 (5-C), 26.2 (46-C), 24.6 (17-C). **IR** (KBr) ν ($cm^{-1}$): 3415.1, 2975.4, 1636.8. **HPLC-ESI-MS:** m/z (%) = 1048.5 (100)

$[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{56}H_{73}N_9O_{11}Na$ $[M+Na]^+$ 1070.5317; found 1070.5322. $R_f$ = 0.27 (EtOAc/EtOH, 6/1).

**1'-[(2*S*)-2-Amino-3-(4-hydroxyphenyl)propanoyl]-N-[(2*S*)-1-[(2*S*)-2-{[(1*S*)-1-[(carbamoylmethyl)-carbamoyl]-2-(1H-indol-3-yl)ethyl]carbamoyl}pyrrolidin-1-yl]-3-hydroxy-1-oxo-propan-2-yl]-2-oxo-1,2-dihydrospiro[indole-3,4'-piperidine]-5-carboxamide (TVC-187, 192)**

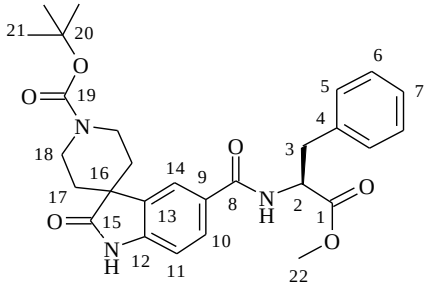
Peptide **191** (13 mg, 12.4 µmol) was dissolved in TFA/TIPS/$H_2O$ (1 mL, 95/4/1) and stirred at 0 °C for 4 h. The reaction mixture was concentrated under reduced pressure to dryness. The residue was subjected to silica gel column chromatography eluting DCM/MeOH (4/1, 0.1% TEA). The white power product **192** (10 mg, 96%) was obtained from lyophilization in ACN/$H_2O$ (3 mL, 2/1).



**$^1$H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 7.84 (s, 1H, 27-H), 7.79 (dd, *J* = 2.0 Hz, *J* = 7.8 Hz, 1H, 25-H), 7.60 (d, *J* = 8.0 Hz, 1H, 9-H), 7.56 (s, 1H, 7-H), 7.36 (d, *J* = 8.0 Hz, 1H, 12-H), 7.17 (d, *J* = 8.0 Hz, 2H, 37-H), 7.11 (overlap, 1H, 11-H), 7.03 (overlap, 1H, 10-H), 6.96 (overlap, 1H, 24-H), 6.95 (d, *J* = 8.0 Hz, 2H, 38-H), 5.08 (t, *J* = 7.2 Hz, 1H, 20-H), 4.66 (dd, *J* = 5.1 Hz, *J* = 10.1 Hz, 1H, 4-H), 4.43 (dd, *J* = 5.0 Hz, *J* = 8.2 Hz, 1H, 15-H), 4.35 (m, 1H, 34-H), 4.03 (m, 1H, 21-H), 3.94 (d, *J* = 17.0 Hz, 2H, 2-H, 21-H), 3.92 (m, 1H, 18-H), 3.74 (d, *J* = 17.0 Hz, 1H, 2-H), 3.72 (m, 3H, 18-H, 32-H), 3.44 (m, 2H, 32-H), 3.42 (dd, *J* = 5.1 Hz, *J* = 14.5 Hz, 1H, 5-H), 3.1 (m, 1H, 5-H), 3.01 (m, 1H, 35-H), 2.87 (m, 1H, 35-H), 2.03 (m, 1H, 16-H), 1.81 (m, 1H, 17-H), 1.63-1.56 (m, 4H, 31-H), 1.54 (m, 1H, 17-H), 1.51 (m, 1H, 16-H). **$^{13}$C-NMR** (100 MHz, $CD_3OD$): $\delta$ = 183.3 (30-C), 174.3 (33-C), 174.2 (3-C), 173.4 (14-C), 172.7 (22-C), 170.1 (1-C), 157.7 (39-C), 145.7 (26-C), 138.0 (28-C), 135.3 (13-C), 132.0 (36-C), 130.7 (37-C), 129.7 (23-C), 128.8 (8-C), 128.6 (25-C), 123.0 (11-C), 122.4 (27-C), 122.0 (7-C), 118.5 (10-C), 117.9 (9-C), 115.8 (38-C), 112.4 (6-C), 111.1 (12-C), 109.2 (24-C), 61.8 (21-C), 61.0 (15-C), 54.6 (4-C), 54.3 (29-C), 53.6 (20-C), 50.9 (34-C), 47.6 (18-C), 41.9 (2-C), 40.6 (32-C), 39.9 (35-C), 31.6 (31-C), 28.6 (16-C), 26.4 (5-C), 23.9 (17-C). **HPLC-ESI-MS:** m/z (%) = 836.4 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{43}H_{49}N_9O_9Na$ $[M+Na]^+$ 858.3549; found 858.3546.

### 3.17.5 Spirolactamscan of Tyr

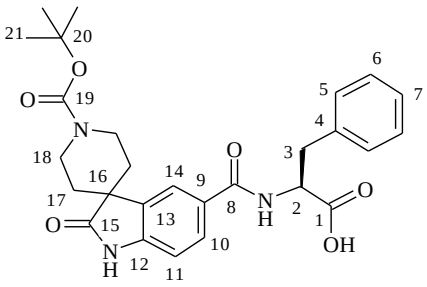
***tert*-Butyl-5-{[(2*S*)-1-methoxy-1-oxo-3-phenylpropan-2-yl]carbamoyl}-2-oxo-1,2-dihydro-spiro-[indole-3,4'-piperidine]-1'-carboxylate (TVC-182, 193)**



To a stirred solution of phenylalanine methylester (137 mg, 0.64 mmol), 1'-[(*tert*-butoxy)carbonyl]-2-oxo-1,2-dihydrospiro[indole-3,4'-piperidine]-5-carboxylic acid **167** (200 mg, 0.58 mmol) and TBTU (204 mg, 0.64 mmol) in DMF (4 mL), DIPEA (0.38 mL, 2.3 mmol) was added dropwise. After stirring for 20 h at room temperature, the solvent was removed and crude product was chromatographed (EtOAc/CyH, 3/2) to provide **193** (286 mg, 98%) as a white solid.

**$^1$H-NMR** (600 MHz, $CDCl_3$): $\delta$ = 7.70 (s, 1H, 14-H), 7.57 (d, $J$ = 8.0 Hz, 1H, 11-H), 7.33-7.26 (m, 3H, 6-H, 7-H), 7.16 (m, 2H, 5-H), 6.95 (d, $J$ = 8.0 Hz, 1H, 10-H), 5.07 (q, $J$ = 7.4 Hz, 1H, 2-H), 3.83 (m, 4H, 18-H), 3.78 (s, 3H, 22-H), 3.27 (m, 2H, 3-H), 1.85 (m, 4H, 17-H), 1.53 (s, 9H, 21-H). **$^{13}$C-NMR** (150 MHz, $CDCl_3$): $\delta$ = 181.8 (15-C), 172.3 (8-C), 166.6 (1-C), 154.9 (19-C), 143.4 (12-C), 135.9 (4-C), 134.6 (13-C), 129.4 (5-C), 128.8 (6-C), 128.1 (9-C), 127.3 (11-C), 127.1 (7-C), 122.8 (14-C), 109.5 (10-C), 79.8 (20-C), 53.5 (2-C), 52.6 (22-C), 45.4 (16-C), 38.9 (18-C), 37.7 (3-C), 32.2 (17-C), 28.3 (21-C). **IR** (KBr) ν ($cm^{-1}$): 3406.2, 2937.5, 1717.4. **HPLC-ESI-MS:** m/z (%) = 508.2 (10) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. For $C_{28}H_{33}N_3O_6Na$ $[M+Na]^+$ 530.2265; found 530.2262. $R_f$ = 0.24 (CyH/EtOAc, 2/3).

**(2*S*)-2-{1'-[(*tert*-Butoxy)carbonyl]-2-oxo-1,2-dihydrospiro[indole-3,4'-piperidine]-5-yl-formamido}-3-phenylpropanoic acid (TVC-184, 194)**



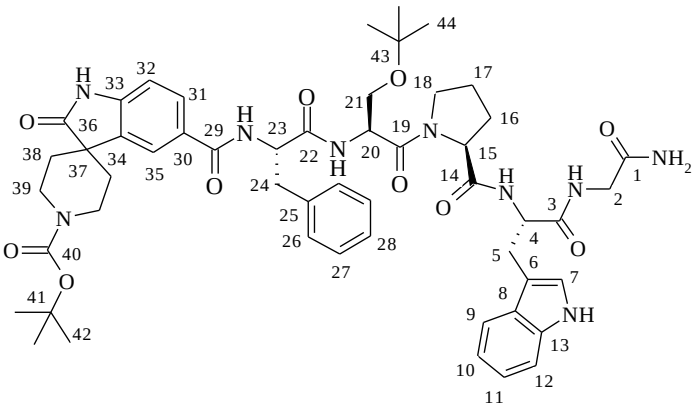
Methyl ester **193** (63 mg, 0.12 mmol) in MeOH (2 mL) was treated with 1N NaOH (7.4 mg, 0.19 mmol) at room temperature for 24 h. After that time the reaction mixture was acidified with 1N HCl at 0 $^o$C to pH≈4. The resulting solution was extracted with EtOAc (3x60 mL). The combined organic phases were washed with brine solution and dried over $MgSO_4$. Removal of the solvent gave product **194** (57 mg, 93%) as a yellow solid.

**H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 7.71 (s, 1H, 14-H), 7.69 (d, $J$ = 8.0 Hz, 1H, 11-H), 7.32-7.26 (m, 3H, 6-H, 7-H), 7.21 (m, 2H, 5-H), 6.96 (d, $J$ = 8.0 Hz, 1H, 10-H), 4.87 (overlap, 1H, 2-H), 3.88 (m, 2H, 18-H), 3.76 (m, 2H, 18-H), 3.36 (d, $J$ = 9.9 Hz, 1H, 3-H), 3.14 (d, $J$ = 9.9 Hz, 1H, 3-H), 1.84-1.75 (br, 4H, 17-H), 1.54 (s, 9H, 21-H). **$^{13}$C-NMR** (100 MHz, $CD_3OD$): $\delta$ = 182.6 (15-C), 174.0 (8-C), 168.9 (1-C),

119-C), 55.7 ( 144.5 (12-C), 137.9 (13-C), 134.6 (4-C), 129.3 (9-C), 128.8 (6-C), 128.1 (5-C, 11-C), 126.3 (7-C), 122.5 (14-C), 109.2 (10-C), 80.3 (20-C), 60.5 (16-C), 54.5 (2-C), 38.7 (18-C), 36.7 (3-C), 32.1 (17-C), 27.8 (21-C). **IR** (KBr) ν ($cm^{-1}$): 3377.9, 2831.9, 1717.8, 1642.2. **HPLC-ESI-MS:** m/z (%) = 494.2 (10) $[M+H]^+$, 394.2 (30) $[M-Boc+H]^+$. **HR-ESI-MS** (m/z): calcd. For $C_{27}H_{31}N_3O_6Na$ $[M+Na]^+$ 516.2105; found 516.2101. $R_f$ = 0.26 (EtOAc/EtOH, 7/1).

***Tert*-Butyl-5-{[(1*S*)-1-{[(2*S*)-3-(*tert*-butoxy)-1-[(2*S*)-2-{[(1*S*)-1-[(carbamoylmethyl)carbamo-yl]-2-(1H-indol-3-yl)ethyl]carbamoyl}pyrrolidin-1-yl]-1-oxopropan-2-yl]carbamoyl}-2-phenylethyl] carbamoyl}-2-oxo-1,2-dihydrospiro[indole-3,4'-piperidine]-1'-carboxylate (TVC-180, 195)**

To a stirred solution of **194** (35.5 mg, 71.9 µmol), **185** (30 mg, 60 µmol) and TBTU (23 mg, 72 µmol) in DMF (2 mL), DIPEA (0.04 mL, 0.24 mmol) was added. After stirring for 20 h, the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography using EtOAc/EtOH (6/1) to afford the desired product **195** (36 mg, 62%) as a white solid.

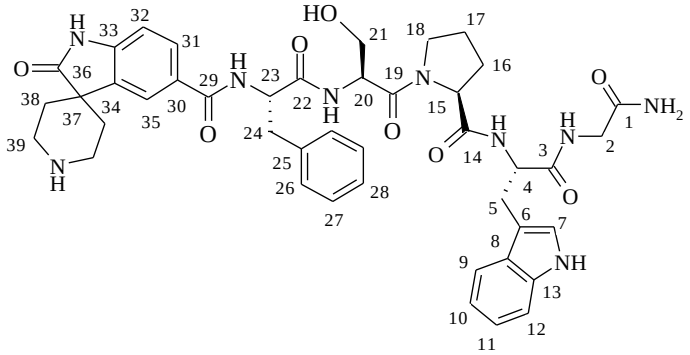


**$^1$H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 7.89 (d, $J$ = 8.3 Hz, 1H, 9-H), 7.76 (d, $J$ = 8.3 Hz, 1H, 12-H), 7.72 (m, 1H, 35-H), 7.70 (m, 1H, 32-H), 7.57 (t, $J$ = 6.8 Hz, 1H, 11-H), 7.51 (t, $J$ = 6.8 Hz, 1H, 10-H), 7.33-7.23 (m, 5H, 26-H, 27-H, 28-H), 7.23 (overlap, 1H, 7-H), 6.91 (m, 1H, 31-H), 4.91 (overlap, 1H, 23-H), 4.65 (m, 1H, 20-H), 4.55 (m, 1H, 4-H), 4.36 (m, 1H, 15-H), 3.92 (overlap, 1H, 2-H), 3.76 (overlap, 1H, 2-H), 3.70 (overlap, 2H, 39-H), 3.67 (m, 1H, 18-H), 3.66 (m, 1H, 21-H), 3.65 (m, 2H, 39-H), 3.61 (m, 1H, 18-H), 3.57 (m, 1H, 21-H), 3.33 (overlap, 1H, 5-H), 3.29 (m, 1H, 24-H), 3.26 (overlap, 1H, 5-H), 3.11 (m, 1H, 24-H), 2.04 (overlap, 2H, 16-H, 17-H), 1.91 (m, 1H, 16-H), 1.83 (m, 2H, 38-H), 1.81 (m, 1H, 17-H), 1.68 (m, 2H, 38-H), 1.53 (s, 9H, 44-H), 1.15 (s, 9H, 42-H). **$^{13}$C-NMR** (100 MHz, $CD_3OD$): $\delta$ = 183.5 (36-C), 174.4 (19-C), 173.9 (22-C), 173.7 (14-C) 173.0 (29-C),, 171.5 (1-C), 169.8 (3-C), 156.6 (40-C), 145.5 (33-C), 138.8 (34-C), 137.9 (13-C), 135.6 (25-C), 130.4 (30-C), 129.1 (27-C), 128.2 (32-C), 128.1 (26-C), 127.8 (8-C), 126.9 (11-C), 126.4 (28-C), 125.8 (10-C), 122.6 (35-C), 117.0 (9-C), 111.2 (6-C), 110.4 (12-C), 109.2 (31-C), 81.3 (43-C), 74.9 (41-C), 61.5 (21-C), 60.6 (15-C), 55.0 (4-C), 54.7 (23-C), 54.1 (37-C), 52.2 (20-C), 47.4 (18-C), 42.0 (2-C), 41.8 (39-C), 37.1 (24-C), 31.7 (38-C), 30.8 (16-C), 28.4 (17-C), 27.3 (44-C), 26.5 (5-C), 26.3 (42-C). **IR**

(KBr) ν ($cm^{-1}$): 3420.0, 1653.3. **HPLC-ESI-MS:** m/z (%) = 976.4 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{52}H_{65}N_9O_{10}Na$ $[M+Na]^+$ 998.4747; found 998.4816. $R_f$ = 0.21 (EtOAc/EtOH, 5/1).

**(2*S*)-N-[(2*S*)-1-[(2*S*)-2-{[(1*S*)-1-[(Carbamoylmethyl)carbamoyl]-2-(1H-indol-3-yl)ethyl]carbamoyl}-pyrrolidin-1-yl]-3-hydroxy-1-oxopropan-2-yl]-2-{2-oxo-1,2-dihydrospiro-[indole-3,4'-piperidine]-5-ylformamido}-3-phenylpropanamide (TVC-186, 196)**

Peptide **195** (21 mg, 18.2 µmol) was treated with TFA/TIPS/$H_2O$ (2 mL, 95/4/1) at 0 °C for 6 h. The solvent was removed under reduced pressure. The crude product was subjected to silica gel column chromatography (eluent: DCM/MeOH, 6/1, 0.1% TEA) to furnish product **196** (12 mg, 71%), which was lyophilized in ACN/$H_2O$ (2 mL, 1/1) to afford white powder.



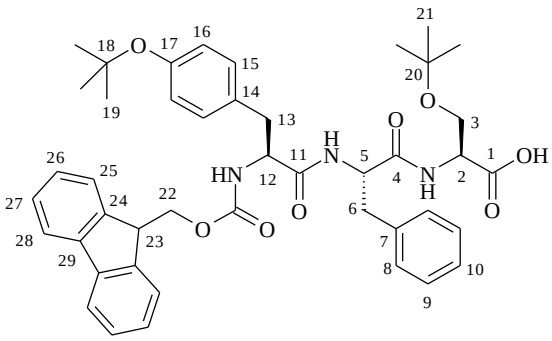
**$^1$H-NMR** (400 MHz, $CD_3OD$): $\delta$ = 7.80 (s, 1H, 35-H), 7.69 (d, $J$ = 9.2 Hz, 1H, 32-H), 7.59 (d, $J$ = 8.0 Hz, 1H, 9-H), 7.35 (d, $J$ = 8.0 Hz, 1H, 12-H), 7.30-7.22 (m, 4H, 26-H, 27-H), 7.19 (m, 1H, 28-H), 7.11 (t, $J$ = 7.5 Hz, 1H, 11-H), 7.03 (t, $J$ = 7.5 Hz, 1H, 10-H), 6.95 (d, $J$ = 9.2 Hz, 1H, 31-H), 4.88 (overlap, 1H, 23-H), 4.82 (overlap, 1H, 20-H), 4.64 (q, $J$ = 5.3 Hz, 1H, 4-H), 4.30 (d, $J$ = 7.5 Hz, 1H, 15-H), 3.97 (d, $J$ = 17.0 Hz, 1H, 2-H), 3.91 (m, 1H, 21-H), 3.82 (m, 1H, 21-H), 3.74 (d, $J$ = 17.0 Hz, 1H, 2-H), 3.71 (m, 1H, 18-H), 3.61 (m, 1H, 18-H), 3.57 (overlap, 2H, 39-H), 3.43 (d, $J$ = 5.3 Hz, 1H, 5-H), 3.40 (d, $J$ = 5.3 Hz, 1H, 5-H), 3.27 (overlap, 1H, 24-H), 3.23 (m, 2H, 39-H), 3.09 (m, 1H, 24-H), 2.04 (m, 1H, 16-H), 2.01-1.96 (m, 4H, 38-H), 1.76 (m, 1H, 17-H), 1.52 (m, 1H, 17-H), 1.44 (m, 1H, 16-H). **$^{13}$C-NMR** (100 MHz, $CD_3OD$): $\delta$ = 183.1 (36-C), 174.2 (3-C), 173.7 (22-C), 172.5 (14-C), 172.3 (19-C), 169.8 (1-C), 169.7 (29-C), 145.6 (33-C), 138.6 (34-C), 138.0 (13-C), 135.4 (25-C), 131.5 (26-C), 130.5 (27-C), 129.1 (30-C), 128.8 (28-C), 127.8 (8-C), 125.7 (11-C), 124.9 (35-C), 123.7 (7-C), 121.0 (10-C), 120.2 (9-C), 113.4 (12-C), 110.5 (6-C), 64.3 (21-C), 63.3 (15-C), 57.6 (23-C), 56.6 (4-C), 55.1 (20-C), 54.1 (37-C), 49.9 (18-C), 44.3 (2-C), 42.0 (39-C), 39.7 (24-C), 33.0 (38-C), 31.0 (16-C), 29.1 (5-C), 26.4 (17-C). **HPLC-ESI-MS:** m/z (%) = 820.4 (100) $[M+H]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{43}H_{49}N_9O_8Na$ $[M+Na]^+$ 842.3596; found 842.3619.

### 3.18 Synthesis of *C*-terminal hydrazide helicokinin analogue TVC-031 (198)

**General procedure for solid phase peptide synthesis**

In a vessel reactor, 2-chlorotritylchloride resin (498 mg, 0.8 mmol/g) was swollen in DMF (4mL) for 30 min. After removing the solvent, the resin was treated with the first amino acid (5.0 equiv.) and DIPEA (10 equiv.). After shaking for 3 h, the excess of reagents was removed by filtration and the resin was washed with DMF (2x5 mL). The coupling steps were repeated once. The unreacted chloride atoms were neutralized by treatment with dry MeOH/DIPEA/DCM (7 mL, 1/1/5) for 30 min. Afterward, the resin was washed with DMF (3x5 mL), MeOH (3x5 mL), DCM (3x5 mL) and followed by removal of Fmoc group (30% piperidine in DMF, 2x4 mL, 2x15 min). The resin was again washed with DMF(3x5 mL), MeOH (3x5 mL), DCM (3x5 mL). Subsequent amino acids were introduced by double couplings for each step (2x8 h) using Fmoc-amino acids (2.5 equiv.), TBTU (2.5 equiv.) and DIPEA (5.0 equiv.) in DMF (8.0 mL). After each coupling- and Fmoc-cleavage step, the resin was washed with DMF (3x5 mL), MeOH (3x5 mL), DCM (3x5 mL). Coupling- and Fmoc-cleavage steps were confirmed by chloroanil-test in the presence of acetaldehyde. The side chain-protected acid products were obtained by treatments of the resin in AcOH/TFE/DCM (2x7 mL, 1/1/3) for 2 h, filtered and washed with DCM (2x5 mL). The filtrate was concentrated under reduced pressure to obtain pure product, which was lyophilized in ACN/$H_2O$ (1/1, 6 mL) to afford white powder.

**(2*S*)-3-(*tert*-Butoxy)-2-[(2*S*)-2-[(2*S*)-3-[4-(*tert*-butoxy)phenyl]-2-{[(9H-fluoren-9-yl-methoxy) carbonyl]amino}propanamido]-3-phenylpropanamido]propanoic acid (TVC-027)**



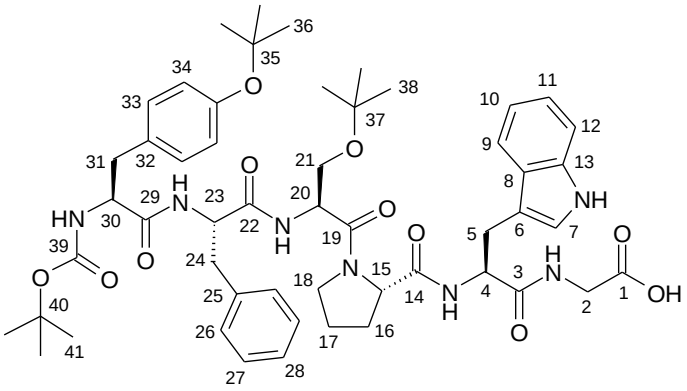
2-Chlorotrityl resin (800 mg, 0.8 mmol/g) afforded **TVC-027** (750 mg, purity 96%, HPLC).

**$^1$H-NMR** (400 MHz, $CDCl_3$): $\delta$ = 7.7 (d, $J$ = 7.5 Hz, 2H, 28-H), 7.5 (d, $J$ = 7.3 Hz, 2H, 25-H), 7.4 (t, $J$ = 7.4 Hz, 2H, 27-H), 7.3 (t, $J$ = 7.4 Hz, 2H, 26-H), 7.20 (t, $J$ = 7.1 Hz, 2H, 9-H), 7.16-7.14 (m, 3H, 8-H, 10-H), 7.02 (d, $J$ = 6.7 Hz, 2H, 15-H), 6.8 (d, $J$ = 8.3 Hz, 2H, 16-H), 6.57 (d, $J$ = 7.3 Hz, 1H, NH), 5.3 (d, $J$ = 7.9 Hz, 1H, NH), 4.66 (q, $J$ = 6.8 Hz, 1H, 5-H), 4.55 (br, 1H, 2-H), 4.43 (br, 1H, 12-H), 4.39 (dd, $J$ = 7 Hz, 1H, 22-H), 4.27 (t, $J$ = 6.7 Hz, 1H, 22-H), 4.15 (t, $J$ = 7 Hz, 1H, 23-H), 3.81-3.78 (m, 1H, 3-H), 3.5 (br, 1H, 3-H), 3.04-3.02 (m, 2H, 6-H), 2.98-2.96 (m, 2H, 13-H), 1.28 (s, 9H, 21-H), 1.12 (s, 9H, 4-H). **$^{13}$C-NMR** (100 MHz, $CDCl_3$):$\delta$ = 172.4, 170.9, 170.6, 154.4 (CO), 154.4 (17-C), 143.7 (24-C), 141.3 (29-C), 136.1 (7-C), 130.9 (14-C), 129.8 (15-C), 129.4 (9-C), 128.6 (8-C), 127.7 (27-C), 127.1 (26-C), 125.09 (10-C), 125.04 (25-C), 124.3 (16-C), 14.9 (28-C), 78.4 (18-C), 74.3 (20-C), 67.2 (22-C), 61.07 (3-C), 55.9 (12-C), 54.5 (5-C), 52.9 (2-C), 47.06 (23-C), 38.5 (6-C), 37.7 (13-C), 28.8 (4-C), 27.2 (21-C). **IR** (KBr) ν ($cm^{-1}$): 3304.9 (NH), 1649.7 (CO). **HPLC-ESI-MS**: m/z (%)

= 750.4 (100) $[M+H]^+$, 772.4 (15) $[M+Na]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{44}H_{51}N_3O_8Na$ $[M+Na]^+$ 772.3574; found 772.3568. $R_f$ = 0.4 (DCM/MeOH, 10/1).

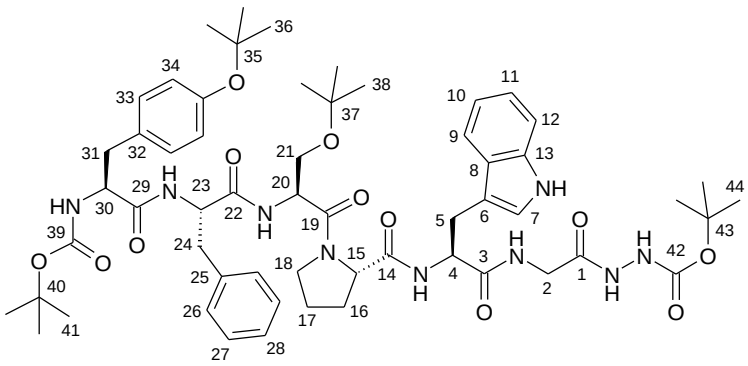
**2-((*S*)-2-((*S*)-1-((6*S*,9*S*,12*S*)-9-Benzyl-6-(4-(*tert*-butoxy)benzyl)-12-(*tert*-butoxymethyl)-2,2-dimethyl-4,7,10-trioxo-3-oxa-5,8,11-triazatridecan-13-oyl)pyrrolidine-2-carboxamido)-3-(1H-indol-3-yl)-propan-amido)acetic acid (TVC-015, 197)**

2-Chlorotritylchloride resin (498 mg, 0.8 mmol/g) afforded acid **197** (340 mg, purity: 98%, HPLC).



**$^1$H-NMR** (400 MHz, $CD_3CN$): $\delta$ = 9.2 (br, 1H, NH), 7.6 (d, $J$ = 7.9 Hz, 1H, 9-H), 7.37 (d, $J$ = 8.1 Hz, 1H, 12-H), 7.39-7.21 (m, 3H, 27-H, 28-H), 7.17 (m, 1H, 11-H), 7.11-7.04 (m, 3H, 26-H, 7-H), 7.04 (m, 1H, 10-H), 5.56 (m, 1H, 4-H), 4.70-4.56 (br, 3H, 20-H, 23-H, 30-H), 4.22 (t, $J$ = 7.4 Hz, 1H, 15-H), 3.93 (d, $J$ = 5.8 Hz, 1/2 H, 2-H), 3.9 (d, $J$ = 6.6 Hz, 1/2 H, 2-H), 3.8 (d, $J$ = 5.5 Hz, 1H, 2-H), 3.8 (overlap, 1H, 24-H), 3.61-3.56 (m, 2H, 18-H), 3.56 (m, 1H, 21-H), 3.49 (m, 1H, 21-H), 3.45 (m, 1H, 24-H), 3.34 (m, 1H, 31-H), 3.05 (m, 1H, 31-H), 2.92, 2.66 (m, 2H, 5-H), 2.01 (m, 1H, 16-H), 1.78-1.72 (m, 2H, 17-H), 1.59 (m, 1H, 16-H), 1.28, 1.10, 1.07 (s, 27H, 41-H, 38-H, 36-H). **$^{13}$C-NMR** (100 MHz, $CD_3CN$): $\delta$ = 172.7 (29-C), 172.4 (22-C), 171.6 (3-C), 171.5 (19-C), 171.4 (14-C), 171.3 (1-C), 155.2 (39-C), 133.0 (27-C), 130.5 (26-C), 124.6 (28-C), 137.9 (33-C), 130.2 (32-C), 127.4 (34-C), 137.3 (13-C), 128.5 (8-C), 124.2 (12-C), 122.3 (7-C), 114.8 (9-C), 112.2 (10-C), 111.5 (11-C), 78.8 (35-C), 74.7 (37-C), 74.4 (40-C), 63.2, (21-C), 62.1 (15-C), 55.6 (23-C), 54.6 (20-C), 54.4 (30-C), 53.4 (4-C), 48.6 (18-C), 41.9 (2-C), 38.3 (24-C), 37.7 (31-C), 29.3 (16-C), 28.9 (36-C, 38-C), 27.9 (5-C), 27.4 (41-C), 25.5 (17-C). **IR** (KBr) $\nu$ ($cm^{-1}$): 3315.8 (NH), 1654.4 (CO). **HPLC-ESI-MS**: m/z (%) = 968.6 (100) $[M+H]^+$, 986.6 (57.3) $[M+NH_4]^+$, 991.6 (16.3) $[M+Na]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{52}H_{69}N_7O_{11}Na$ $[M+Na]^+$ 990.4953; found 990.4947. $R_f$ = 0.13 (DCM/MeOH, 5/1).

***tert*-Butyl-2-(2-((*S*)-2-((*S*)-1-((6*S*,9*S*,12*S*)-9-benzyl-6-(4-(*tert*-butoxy)benzyl)-12-(*tert*-butoxy-methyl)-2,2-dimethyl-4,7,10-trioxo-3-oxa-5,8,11-triazatridecan-13-oyl)pyrrolidine-2-carboxamido)-3-(1H-indol-3-yl)propanamido)acetyl)hydrazinecarboxylate (TVC-016)**

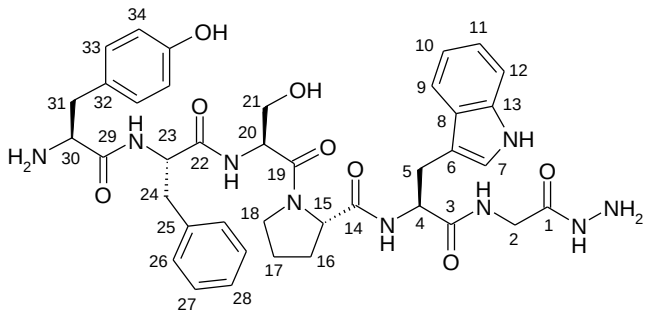


A stirred solution of **197** (200 mg, 0.21 mmol), *tert*-butyl carbazate (35.5 mg, 0.27 mmol) in DMF (8 mL) was added TBTU (86.2 mg, 0.27 mmol), followed by addition of DIPEA (0.1 mL, 0.62 mmol). The reaction mixture was stirred at room temperature for 22 h and quenched with EtOAc (120 mL). The organic layer was washed with $H_2O$ (3x15 mL), brine solution (30 mL) and dried over $Na_2SO_4$. Removal of the solvent afforded **TVC-016** (220 mg, 98%) as a solid product.

**$^1$H-NMR** (600 MHz, $CDCl_3$): $\delta$ = 9.2, 8.9, 8.7, 7.8, 7.4, 6.7 (s, NH), 7.6 (t, *J* = 7.5 Hz, 1H, 9-H), 7.31 (m, 1H, 12-H), 7.22-7.17 (m, 3H, 7-H, 27-H), 7.11 (t, *J* = 11 Hz, 11-H), 7.08 (m, 1H, 10-H), 7.06-7.04 (m, 3H, 26-H, 28-H), 7.03 (overlap, 2H, 33-H), 6.9 (d, *J* = 8.0 Hz, 2H, 34-H), 4.85 (m, 2/5 H, 15-H), 4.7 (m, 1/2H, 4-H), 4.67 (m, 1/2H, 23-H), 4.62 (m, 1/2H, 4-H), 4.60 (br, 1H, 20-H), 4.26 (m, 1/2H, 30-H), 4.04 (m, 1/2H, 23-H), 4.02 (m, 3/5 H, 15-H), 4.18 (br, 1H, 2-H), 3.89 (m, 1/2H, 2-H), 3.56 (dd, *J* = 4.5 Hz, *J* = 9.3 Hz, 1H, 21-H), 3.4 (m, 1H, 21-H), 3.38 (m, 1H, 5-H), 3.31 (m, 1H, 5-H), 2.1 (m, 16-H), 3.09 (m, 2H, 24-H), 2.94 (m, 1H, 31-H), 2.85 (m, 1H, 31-H), 1.42, 1.31, 1.04 (36H, 44-H, 41-H, 38-H, 36-H). **$^{13}$C-NMR** (150 MHz, $CDCl_3$): $\delta$ = 172.6 (29-C), 172.1 (22-C), 171.8 (3-C), 171.7 (1-C), 171.3 (14-C), 170.8 (19-C), 169.7 (42-C), 155.6 (39-C), 129.6 (33-C), 124.3 (34-C), 136.2 (25-C), 129.2 (27-C), 128.6 (26-C), 127.0 (28-C), 136.1 (13-C), 127.7 (8-C), 123.9 (7-C), 122.0/121.8 (10-C), 114.6/114.3 (11-C), 118.5 (9-C), 62.5, 61.7 (21-C), 61.5, 61.0 (15-C), 55.9 (30-C), 54.8 (23-C), 54.5 (20-C), 53.9 (4-C), 47.9 (18-C), 42.1 (2-C), 37.2 (24-C), 36.9 (31-C), 31.4 (16-C), 28.8 (36-C), 28.2 (38-C), 27.2 (44-C, 41-C), 27.2, 27.1 (5-C), 25.2 (17-C). **IR** (KBr) ν ($cm^{-1}$): 3299.9 (NH), 1655.1 (CO). **HPLC-ESI-MS:** m/z (%) = 1082.6 (65) $[M+H]^+$, 1099.6 (100) $[M+NH_4]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{57}H_{79}N_9O_{12}Na$ $[M+Na]^+$ 1104.5746; found 1104.5740. $R_f$ = 0.43 (DCM/MeOH, 15/1).

**(*S*)-1-((*S*)-2-((*S*)-2-((*S*)-2-Amino-3-(4-hydroxyphenyl)propanamido)-3-phenylpropanamido)-3-hydroxy-propanoyl)-N-((*S*)-1-((2-hydrazinyl-2-oxoethyl)amino)-3-(1H-indol-3-yl)-1-oxo-propan-2-yl)pyrrolidine-2-carboxamide (TVC-031, 198)**

Compound **TVC-016** (200 mg, 0.18 mmol) was treated with TFA/TIPS/$H_2O$ (5 mL, 95/2.5/2.5) at 0 $^oC$ for 2 h. Afterward, the reaction mixture was concentrated under reduced pressure and purified on silica gel column chromatography. The product **198** (135 mg, 94%) was lyophilized in ACN/$H_2O$ (3 mL, 2/1) to yield white powder.



**$^{1}$H-NMR** (600 MHz, DMSO-$d_6$): $\delta$ = 10.8 (br, 1H, NH), 9.26 (s, 1H, NH), 8.48 (d, $J$ = 7 Hz, 1H, NH), 8.4 (br, 1H, NH), 7.58 (m, 1H, 9-H), 7.31 (m, 1H, 12-H), 7.23-7.21 (m, 2H, 27-H), 7.18-7.14 (m, 3H, 26-H, 28-H), 7.12 (br, 1H, 7-H), 7.05 (m, 1H, 11-H), 6.99 (overlap, 1H, 10-H), 6.99 (m, 2H, 33-H), 6.67 (m, 2H, 34-H), 4.73 (m, 2/5H, 20-H), 4.67 (m, 3/5H, 20-H), 4.66 (m, 1/2H, 23-H), 4.57 (m, 1/2 H, 4-H), 4.52 (m, 1/2H, 23-H), 4.46 (m, 1/2H, 4-H), 4.3 (m, 1/2H, 15-H), 4.2 (m, 1/2H, 15-H), 3.95 (dd, $J$ = 6.3 Hz, $J$ = 16.0 Hz, 1/2H, 2-H), 3.75 (m, 1/2H, 2-H), 3.74 (m, 1/2H, 30-H), 3.72 (m, 1H, 21-H), 3.68 (m, 1H, 21-H), 3.66 (m, 1/2H, 30-H), 3.67 (m, 1/2H, 2-H), 3.64 (m, 1H, 18-H), 3.61 (m, 1/2H, 2-H), 3.59 (m, 1H, 18-H), 3.21 (m, 1H, 5-H), 2.99 (m, 1H, 23-H), 2.89 (m, 1H, 5-H), 2.89 (m, 1H, 31-H), 2.82 (m, 1H, 24-H), 2.62 (m, 1H, 31-H), 1.92 (m, 1H, 16-H), 1.66 (m, 1H, 17-H), 1.56 (m, 1H, 17-H), 1.35 (m, 1H, 16-H). **$^{13}$C-NMR** (150 MHz, DMSO-$d_6$): $\delta$ = 171.3 (22-C), 170.6 (29-C), 170.5 (3-C), 170.4 (19-C), 170.3 (14-C), 169.7 (1-C), 156.2/156.1 (35-C), 135.9 (32-C), 130.3 (33-C), 115.1 (34-C), 137.3 (25-C), 129.2 (27-C), 127.9 (26-C), 126.2 (28-C), 137.2 (13-C), 127.2 (8-C), 123.4/123.3 (7-C), 120.8 (9-C), 118.23/118.2 (11-C), 118.0/118.1 (10-C), 110.1 (12-C), 61.8, 61.5 (21-C), 60.2, 59.9 (15-C), 54.4, 54.1 (30-C), 53.9, 53.8 (4-C), 53.4, 53.2 (23-C), 52.4, 52.1 (20-C), 47.1, 46.9 (18-C), 40.9 (2-C), 37.6 (24-C), 37.4 (31-C), 28.7, 28.6 (16-C), 27.1 (5-C), 23.9 (17-C). **IR** (KBr): $\nu$ ($cm^{-1}$): 3407.9 (NH), 1676.2 (CO). **HPLC-ESI-MS**: m/z (%) = 770.3 (100) $[M+H]^+$, 792.3 $[M+Na]^+$. **HR-ESI-MS** (m/z): calcd. for $C_{39}H_{48}N_9O_8$ $[M+H]^+$ 770.3626; found 770.3620.

### 3.19 Chemical shift of the membrane-bound peptides

**Chemical shifts of peptide TVC-135 (34a) in DPC micelles**

| AA | NH | $H_\alpha$ | $H_\beta$ | Other protons |
|---|---|---|---|---|
| Tyr[1] | - | 3.96 | 2.88 | δ: 6.89, ε: 6.71 |
| Phe[2] | / | 4.47 | 2.96, 3.05 | δ: 7.21, ε: /, ζ: / |
| Apy[4] | 8.15 | 4.06 | 1.57, 2.02 | γ: 4.23 |
| Trp[5] | 8.58 | 4.71 | 3.12, 3.30 | δ1: 7.22, ε1: / , ζ2: 7.40, η2: 7.00, ζ3: 6.95, ε3: 7.55 |
| Gly[6] | 8.53 | 3.81 | -- | -- |

**Chemical shifts of peptide TVC-134 (34b) in DPC micelles**

| AA | NH | $H_\alpha$ | $H_\beta$ | Other protons |
|---|---|---|---|---|
| Tyr[1] | - | 4.01 | 2.95 | δ: 6.98, ε: 6.74 |
| Phe[2] | / | 4.40 | 3.02 | δ: 7.21, ε: /, ζ: / |
| Apy[4] | 8.07 | 3.97 | 1.60, 1.82 | γ: 4.05 |
| Trp[5] | 8.15 | 4.68 | 3.16, 3.38 | δ1: 7.21, ε1: / , ζ2: 7.37, η2: 7.02, ζ3: 7.00, ε3: 7.56 |
| Gly[6] | 8.45 | 3.75, 3.88 | -- | -- |

**Chemical shifts of peptide TVC-119 (35a) in DPC micelles**

| AA | NH | $H_\alpha$ | $H_\beta$ | Other protons |
|---|---|---|---|---|
| Tyr[1] | - | 3.99 | 2.88 | δ: 6.92, ε: 6.76 |
| Phe[2] | 7.33 | 4.55 | 3.07, 3.23 | δ: 7.25, ε: /, ζ: / |
| Ser[3] | 8.35 | 4.34 | 3.22, 3.79 | -- |
| Apy[4] | 8.08 | 4.18 | 1.82, 2.25 | γ: 4.33 |
| Trp[5] | 8.65 | 4.75 | 3.16, 3.34 | δ1: 7.24, ε1: 10.48 , ζ2: 7.38, η2: /, ζ3: /, ε3: 7.57 |
| Gly[6] | 8.49 | 3.89 | -- | -- |

**Chemical shifts of peptideTVC-117 (35b) in DPC micelles**

| AA | NH | $H_\alpha$ | $H_\beta$ | Other protons |
|---|---|---|---|---|
| Tyr[1] | - | 3.95 | 2.87, 2.92 | δ: 6.88, ε: 6.74 |
| Phe[2] | 7.35 | 4.55 | 3.04, 3.24 | δ: 7.27, ε: /, ζ: / |
| Ser[3] | 8.40 | 4.35 | 3.79 | -- |
| Apy[4] | 8.05 | 3.88 | 1.96, 2.21 | γ: 4.21 |
| Trp[5] | 8.20 | 4.73 | 3.20, 3.38 | δ1: 7.23, ε1: 10.41 , ζ2: 7.41, η2: /, ζ3: /, ε3: 7.58 |
| Gly[6] | 8.49 | 3.80, 3.90 | -- | -- |

**Chemical shifts of peptide 47 in DPC micelles**

| AA | NH | $H_\alpha$ | $H_\beta$ | Other protons |
|---|---|---|---|---|
| Tyr[1] | - | 4.04 | 2.97 | δ: 7.04 , ε: 6.81 |
| Phe[2] | 8.22 | 4.53 | 3.04, 2.95 | δ: 7.17, ε: /, ζ: / |
| Ser[3] | 8.22 | 4.80 | 3.65, 3.70 | -- |
| Oxopiperazine[4] | / | 4.42 | 1.36 | γ: 0.55, 2x$CH_2$: 3.24, 3.41, 3.59, 3.89 |
| Trp[5] | / | 5.57 | 3.12, 3.46 | δ1: 7.30, ε1: 10.62 , ζ2: 7.42, η2: /, ζ3: /, ε3: 7.57 |
| Gly[6] | 8.47 | 3.88 | -- | -- |

**Chemical shifts of peptide TVC-044 (79) in DPC micelles**

| AA | NH | $H_\alpha$ | $H_\beta$ | Other protons |
|---|---|---|---|---|
| Tyr[1] | -- | 4.20 | 3.10 | δ: 7.11, ε:6.83 |
| Phe[2] | 8.54 | 4.59 | 3.09, 3.00 | δ: 7.26, ε: /, ζ: / |
| Ser[3] | 8.22 | 4.64 | 3.52, 3.73 | -- |
| Pro[4] | -- | 4.59 | 2.04 | γ: 1.85, δ 3.62 |
| Trp[5] | -- | 5.20 | 3.31, 3.13 | ε1: 11.14, ζ2: 7.51, η2: 7.15, ζ3: 6.99, ε3: 7.34 |
| Gly[6] | -- | 4.33, 3.63 | -- | -- |
| $NH_2$ | 7.41 | -- | -- | -- |
| others | -- | -- | -- | $CH_2$: 4.04, 3.59, CH: 5.97 |

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