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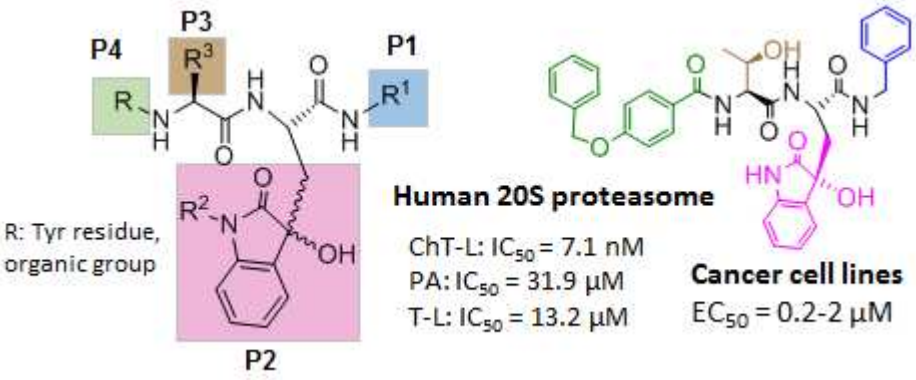

Structure-based design of human immuno- and constitutive proteasomes inhibitors

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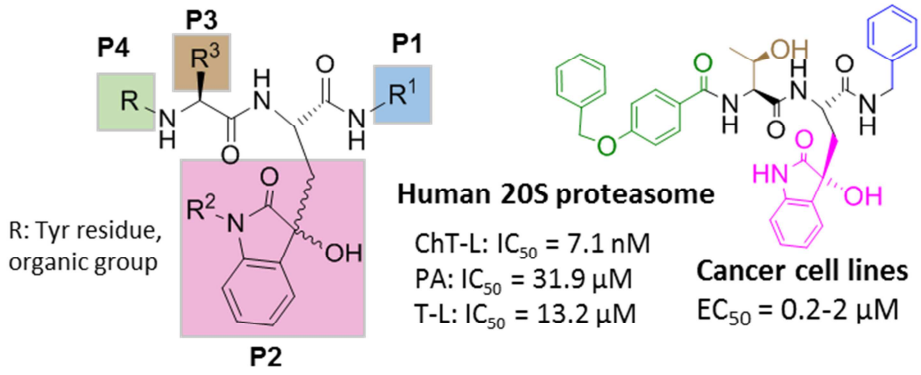
Abstract

Starting from the X-ray structure of our previous tripeptidic linear mimics of TMC-95A in complex with yeast 20S proteasome, we introduced new structural features to induce a differential inhibition between human constitutive and immunoproteasome 20S particles. Libraries of 24 tripeptidic and 6 dipeptidic derivatives were synthesized. The optimized preparation of 3-hydroxyoxindolyl alanine residues from tryptophan and their incorporation in peptides were described. Several potent inhibitors of human constitutive proteasome and immunoproteasome acting at the nanomolar level (IC_{50} = 7.1 nM against the chymotrypsin-like activity for the best inhibitor) were obtained. A cytotoxic effect at the submicromolar level was observed against 6 human cancer cell lines.

Graphical abstract:



Highlights

- X-ray structures drive design of immuno- and constitutive proteasome inhibitors.
- Di- and tripeptide inhibitors are tailored in order to gain subunit selectivity.
- Enantiopure 3-hydroxyoxindolylalaninamide derivatives are efficiently synthesized.
- One, 2 or 3 activities of the proteasomes are inhibited in the nanomolar range.
- The most potent inhibitors are cytotoxic against cancer cell lines.

Keywords

Proteasome inhibitors

Immunoproteasome

Constitutive proteasome

3-Hydroxyoxindolylalanine derivatives

Tryptophan oxidation

1. Introduction

At the end of the ubiquitin-proteasome pathway, the proteasome is a central hub of non lysosomal cellular proteolysis in the cytoplasm as well as in the nucleus of eukaryotic cells [1, 2]. Its inhibition leads to a large variety of cellular responses such a cell cycle arrest and increase of proapoptotic factors and tumor suppressors. The proteasome is an established drug target in the treatment of hematologic malignancies with three approved drugs, bortezomib (Velcade®) [3], carfilzomib (Kyprolis®) [4] and the orally administered ixazomib (Ninlaro®) [5] (Figure 1).

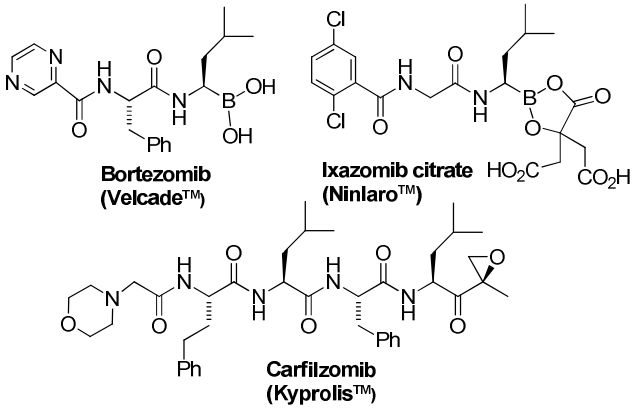


Figure 1: Covalent inhibitors used in cancer therapy.

All these approved molecules target the 20S catalytic core with covalent modification of the Thr1O$^{\gamma}$ group of the catalytic subunits. Moreover, proteasome is a potential target for immune diseases [2], tuberculosis [6] or malaria [7]. Devoid of a reactive function, noncovalent inhibitors have also been developed and have some advantages over covalent ones, such as better tissue penetration, inhibitor stability, lower reactivity and potentially higher selectivity [8]. Several peptides are noncovalent inhibitors [9], such as the natural TMC-95A and its cyclic and linear mimics **1a** and **2a** [10-17], dipeptides (e.g. **3**-**4**) [18-24], and pseudopeptides [25-27] (Figure 2).

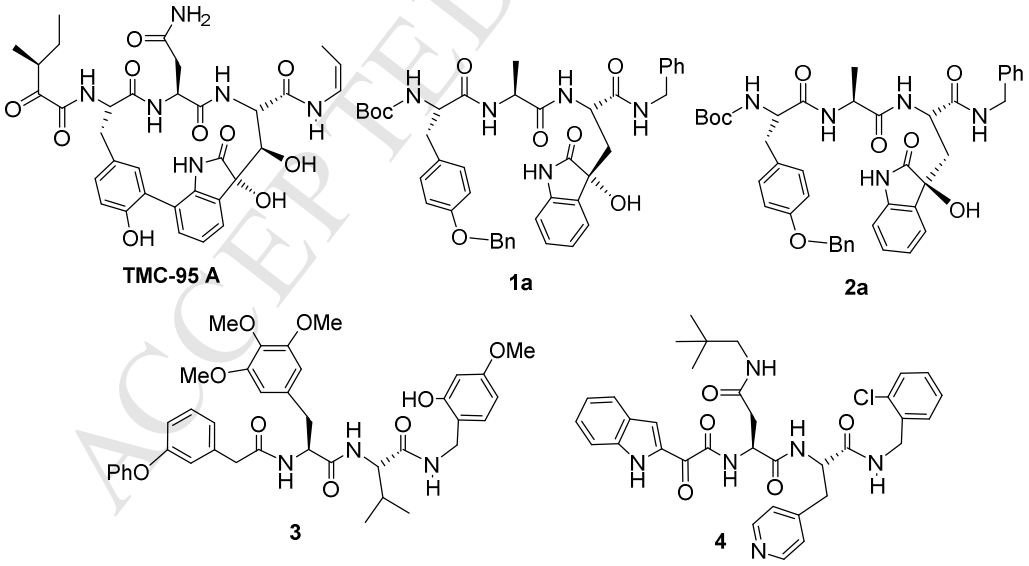


Figure 2: Some noncovalent peptide inhibitors.

Nonpeptidic noncovalent inhibitors have also been described (*e. g.* sulfonamides [28-31], hydroxyurea [32], 1,2,4-oxadiazoles [33], pyrazoles [34], phakellins [35], quinolines [36], psoralene [37]). The three approved covalent drugs inhibit mainly the β5 activity of the

catalytic core of the constitutive proteasome (cCP) but also that of the inducible immunoproteasome (iCP). This partly explain side-effects and resistance observed during cancer treatments [38]. The cCP and iCP catalytic cores are both made of two outer rings of 7 α-subunits and two inner rings of β-subunits. The 2 β1c, 2 β2c and 2 β5c catalytic units of cCP are replaced by the inducible 2 β1i, 2 β2i and 2β3i subunits in iCP. The β1, β2 and β5 catalytic units exhibit caspase-like or post-acid (C-L), trypsin-like (T-L) and chymotrypsin-like (ChT-L) activities, respectively. The catalytic pockets of cCP and iCP show subtle differences that can be exploited to discriminate between the inhibitors targeting proteasomes [39]. Our successes in developing linear mimics of TMC-95A either monovalent **1a** and **2a** [16, 17] or bivalent [40, 41] and their cellular penetration [42] prompted us to pursue their structure-driven development. Based on previous co-crystals of compounds **1a** and **2a** complexed with yeast cCP (Figure 1S, supporting information) [17], and using the reported structural data obtained with mouse constitutive and immunoproteasome 20S particles [39], we decided to explore potential structure-based improvements of the inhibitory effect of tripeptidic (**1**, **2**) and dipeptidic (**5**) linear mimics. The influence on the ChT-L, T-L and C-L activities of both cCP and iCP of the structural variations of residues P1, P2, P3 and P4 present in the starting molecules **1a** and **2a** was systematically explored (Figure 3). We report here the synthesis of molecules **1**, **2** and **5** from the original enantiopure 3-hydroxyoxindolylalaninamide residues **6** and **7**, efficiently prepared from *Z*-tryptophan. After the *in vitro* enzymatic evaluation, the best inhibitors were selected and assayed on seven human tumor cell lines to evaluate their potential as cytotoxic tumor agents.

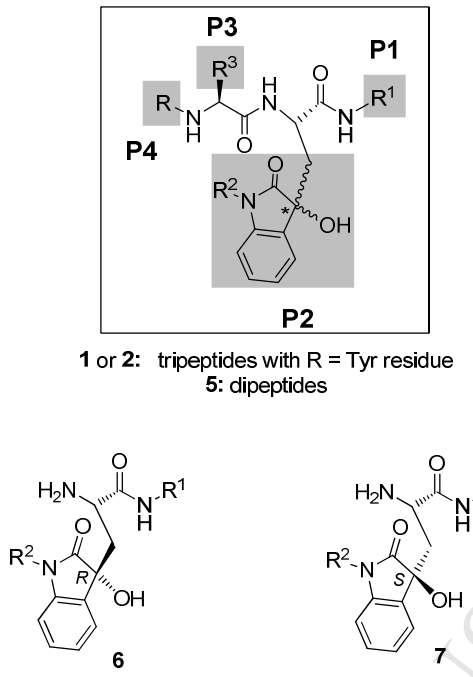


Figure 3: Structures of the studied noncovalent inhibitors **1a-v**, **2a-c** and **5a-f** and their 3-hydroxyoxindolyl alanine chemical precursors **6** and **7**. The occupancy of the S1-S4 binding pockets by P1-P4 groups, respectively, is deduced from co-crystallization of compounds **1a** and **2a** with yeast proteasome [17].

2. Results and discussion

2.1. Structure driven drug-design

The design started from the X-ray structure of epimeric tripeptides **1a** and **2a** in complex with yeast cCP, that showed an antiparallel β-sheet in the substrate binding channel implicating the β5/β6 subunits. Their binding were identical, except for the epimeric oxindole moiety of the side chain in P2 which was rotated (Figure 1S) [17]. The crystallographic knowledge gained from the structures of mouse cCP and iCP and their covalent complexes with the tripeptide epoxy ketone ONX-0914 (PR-957, Figure 2S) was also used [39], as well as the crystallographic structure of human constitutive proteasome [43]. Structural features have been identified in order to potentially target β1i or β5i subunits preferentially to β1c or β5c ones [39]. In the β5i subunit, the subsite S1 appears significantly more spacious than that of β5c, and the subsite S3 is smaller and more hydrophilic. A defined and shallow S2 pocket is also present in β5i. Regarding the β1i subunit, the S1 pocket is small and hydrophobic whereas the corresponding one in β1c accommodates P1 negatively charged residues. Lastly,

the S3 subsite of β1i is smaller and more polar than that of β1c. As P1 and P3 residues play a major role, the main structural variations in **1** were introduced in the R^1 and R^3 groups (Table 1). For R^1, the methylene group mimicking the α-carbon of a decarboxylated peptide was substituted by hydrogen (**1b**, **2b**), aromatic groups with variable bulkiness (**1a**, **1c**, **1f-i**), primary (**1j-m**), secondary (**1n**) and tertiary (**1o-p**) aliphatic groups. The Ala residue (P3) of reference compound **1a**, was replaced by the smaller Gly residue (**1r**), D-Ala (**1q**), the more polar and hydrophilic Asn, Thr or Lys (**1s-v**) residues. Modifications of the oxindole group at P2 (configuration of the C3-carbon: (*R*) for tripeptides **1** or (*S*) for tripeptides **2**, *N*-substitution **1c** and **2c**) were also introduced. The peptide sequence was also shortened by removing the Tyr residue of tripeptides **1** (P4 modulation) in order to explore the activity of dipeptides **5** (Table 2).

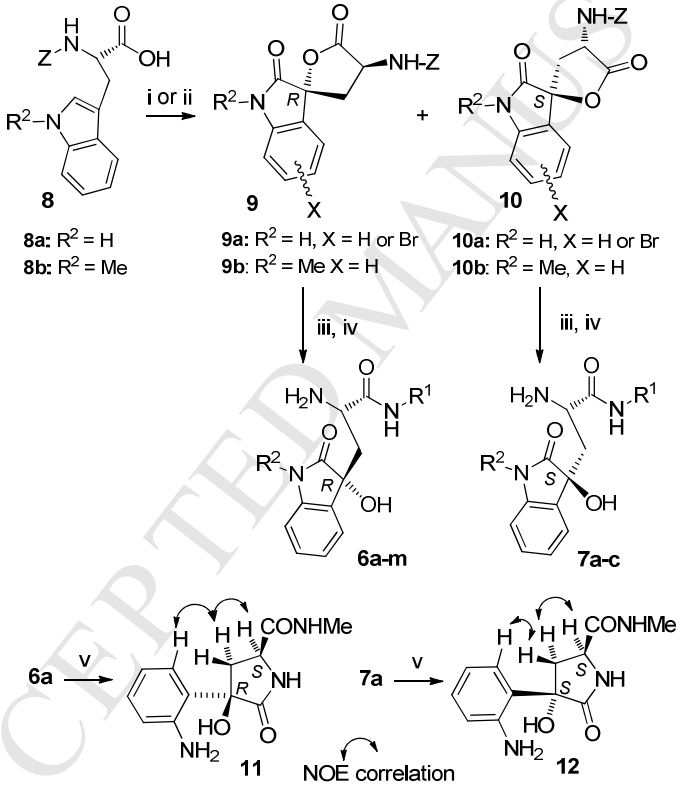
2.2. Chemistry

Molecules **1, 2** and **5** were prepared starting from epimeric 3-hydroxy-2-oxindole derivatives **6** and **7** (Scheme 1). The key intermediates in the preparation of these non-proteogenic amino acids **6** and **7** were respectively the epimeric *N*-Z spirolactones **9** and **10**.

They were obtained in 64-82% total yield by oxidation of Z-L-tryptophan **8** with DMSO in the presence of *t*-BuBr [44, 45], as mixtures with a moderate amount of the corresponding brominated derivative (X = Br), when R^2 = H, indicating that electrophilic bromine was released in the medium. In order to avoid bromination of the aromatic ring, attempts using DMSO activated with methanesulfonic anhydride [46] or oxalyl chloride [47], were performed and led to very low yields (*ca* 10%) in **9a** and **10a** (X = H). As *t*-BuBr could generate hydrogen bromide at 45°C in the presence of DMSO (Scheme 2), the charge-transfer complex Br_2-Me_2S was formed rather than the electrophilic sulfonium [48]. It became the source of electrophilic bromine [48, 49], that reacted with the nucleophilic enanime moiety of the indole ring to give intermediate **A** (Scheme 2). After trapping of the iminium cation by DMSO and bromo-lactonization, intermediate **B** might lead to spiro-oxindole **C**. The more difficult electrophilic bromination of the benzene ring in **C** gave a small amount of **D** (Scheme 2). The oxidative lactonization of indole propanoic acids mediated by oxygen peroxide and catalytic iodide [50] was also applied to *Z*-tryptophan **8a** and afforded the expected pure **9a** (X = H) and **10a** (X = H) in 53% overall yield, which was lower than that obtained using DMSO and *t*-BuBr (*ca* 82%, X = H or Br). We found that the epimeric lactones **9** and **10** could be separated by column chromatography over silica gel on a 12 g scale in respectively 22-37% and 42-45% yields. Opening of the lactone ring in **9** or **10** by

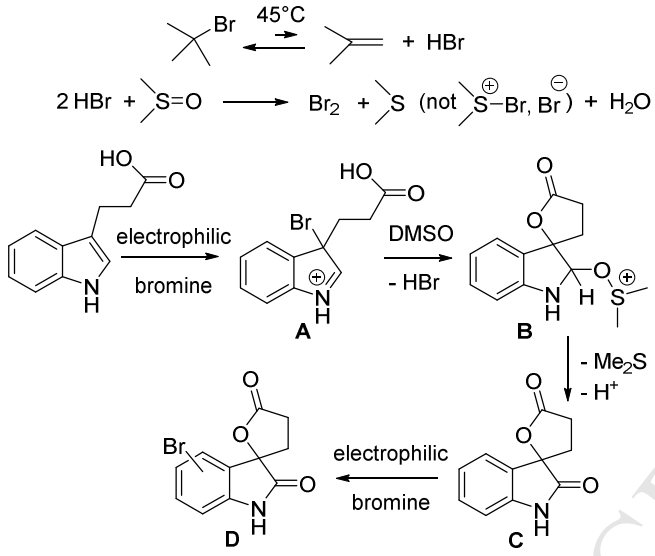
diversely substituted primary amines was easy at room temperature. The presence of the small amount of brominated derivative (X = Br) was not a problem, since further hydrogenation in the presence of palladium on charcoal cleaved the Z group and also the carbon bromine bond. The expected derivatives **6** or **7** were obtained as their stable hydrochloride or TFA salts in 15-39% overall yields from Z-tryptophan **8**. The formation of the rigid lactams **11** and **12** by the slow isomerization of the free amine compounds **6a** and **7a** [51], allowed the configuration determination of the quaternary asymmetric carbon using ^{1}H NMR NOESY experiments in DMSO-D_6 solvent (scheme 1).

Scheme 1: Synthesis of 3-hydroxyoxindolyl alanine precursors **6** and **7** and assignment of the configuration at the created asymmetric carbon in **11** and **12** by ^{1}H NMR NOESY experiment.



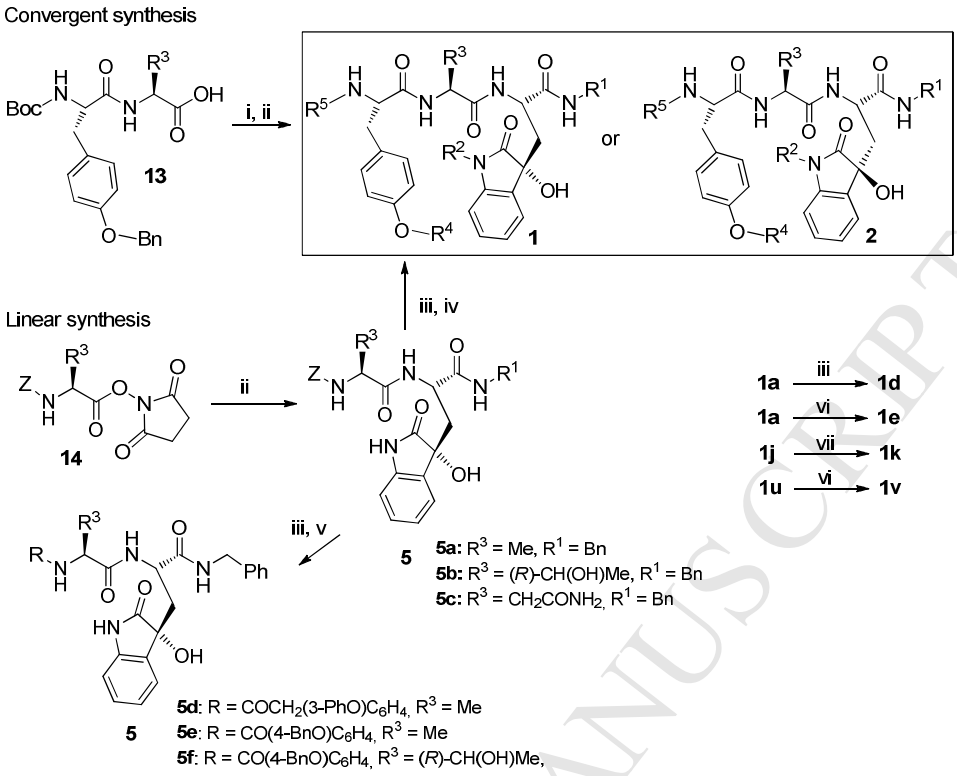
Reagents and conditions: i. DMSO, *t*-BuBr, 45 °C, 24 h, separation of **9** and **10** by chromatography; ii. H_2O_2, catalytic KI (11%), CH_3CN, RT, 18 h, separation of **9a** and **10a** by chromatography. iii. R^1NH_2, THF, RT, 1.5 to 18 h; iv. H_2, Pd/C, MeOH, RT, overnight, then 1 eq. anhydrous HCl or TFA; v. water, 72 h, RT, more than 85% conversion.

Scheme 2: Proposed mechanism for the oxidative lactonization of indole propanoic acid mediated by DMSO/*t*-BuBr.



A library of peptides **1**, **2** and **5** was then prepared from the 3-hydroxyoxindolyl alanine residues **6** and **7** using peptide conventional liquid-phase synthesis [52] (scheme 3 and tables 1 and 2). As the tertiary alcohol of the side chain in **6** or **7** was not protected, a mild activation of the peptide coupling partner was required and was realized by the pre-formed succinimidyl (Su) esters. In the convergent synthesis, the succinimidyl esters of dipeptides **13** [16] were coupled to the 3-hydroxyoxindolyl alanine residues **6** and **7** to give the expected tripeptides **1a-c, 1f-i, 1o-r, 1u** in 31-76% yields. As some epimerization of activated dipeptide **13** was sometimes observed in large scale experiments, the linear synthesis of tripeptides **1-2** was also performed. *N*-Z-dipeptides **5** were obtained in 56-88% yields from **6** and commercial activated Z-amino acids **14**. Hydrogenolysis of **5** followed by coupling with the tyrosine residue afforded expected tripeptides **1** in 26-64% overall yields from the corresponding **6**. In the case of the reference compound **1a**, the linear synthesis worked better than the convergent one and was applied to a gram scale preparation. Cleavage of protecting groups in tripeptides **1a**, **1u** or **1j** was also realized. Hydrogenolysis of the O-benzyl residue in **1a** led to **1d** in quantitative yield. Acidic cleavage of the Boc residue in **1a** and **1u** to give **1e** and **1v** was complete in less than 30 min at 0°C and should be controlled, since higher temperature or longer reaction time resulted in extensive decomposition of the compounds. Saponification of the methyl ester in **1j** gave quantitatively **1k**. The *N*-terminus groups R encountered in peptide **3** [18] and an isomeric group [19] were also introduced in *N*-capped dipeptides **5d-f** by deprotection of dipeptides **5a** or **5b** followed by coupling with the corresponding activated esters R-OSu.

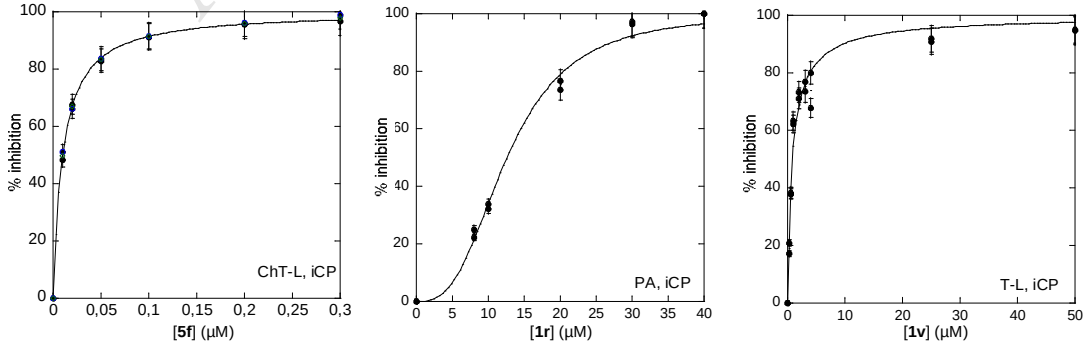
Scheme 3: Synthesis of non-covalent inhibitors **1a-v**, **2a-c** and **5a-g**.



Reagents and conditions: i. DCC, HOSu, DME, overnight; ii. HCl, **6** or HCl, **7**, Et_3N, DMF/CH_2Cl_2, RT, overnight; iii. H_2, Pd/C, MeOH; iv. Boc-Tyr(Bn)-OSu, RT, overnight; v. (3-PhO)$C_6H_4CH_2CO_2Su$ or (4-BnO)-$C_6H_4CO_2Su$; vi. anhydrous HCl, MeOH, 0 °C, 30 min; vii: LiOH, H_2O/THF, then aqueous HCl.

2.2 *In vitro* inhibition studies

The inhibitory activities of compounds **1a-v**, **2a-c** and **5a-f** on the rate of hydrolysis of the β5 substrate Suc-LLVY-AMC and the β2 substrate Boc-LLR-AMC catalyzed by cCP and iCP were determined (Figure 4, Tables 1 and 2). The β1 substrates Z-LLE-βNA and Ac-PAL-AMC were used to detect the activities of cCP and iCP respectively.



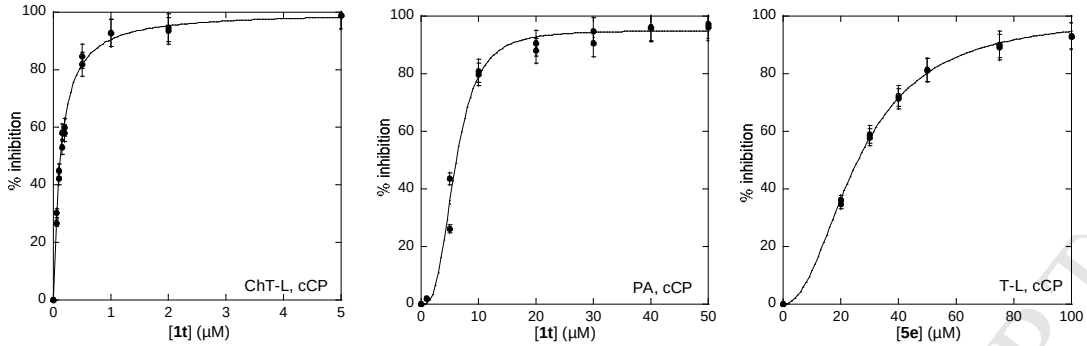


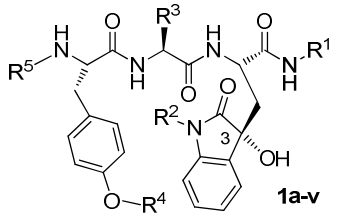
Figure 4: Inhibition profile of the ChT-L, C-L and T-L activities of cCP and iCP by compounds **1r,t,v** or **5e,f** at pH 8 and 37 °C. [cCP] = [iCP] = 0.3 nM; $[Suc\text{-}LLVY\text{-}AMC]_0$ = 20 µM (ChT-L activity); $[Boc\text{-}LLR\text{-}AMC]_0$ = 50 µM (T-L activity); $[Z\text{-}LLE\text{-}\beta NA]_0$ = 50 µM (C-L activity of cCP); $[Ac\text{-}PAL\text{-}AMC]_0$ = 50 µM (C-L activity of iCP). Experimental points were fitted to equation 1 or 2.

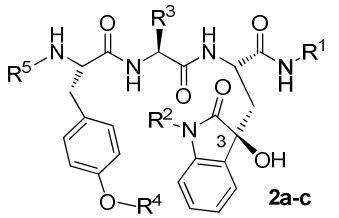
A similar efficiency of the starting compound **1a** was observed against cCP and iCP ChT-L and C-L activities whereas the inhibition of the iCP T-L activity was quasi-abolished. Reduced inhibitory activity was observed when the P1 benzylic group was replaced by the smaller methyl group (**1a**/**1b**) with a larger effect for iCP inhibition. This was expected since based on the mouse crystallographic structures of cCP and iCP [39], β5i seems able to accommodate larger amino acid side chain at position P1 than β5c. The epimerization at carbon 3 (**2a**/**1a** and **2b**/**1b**) resulted in a significantly loss of activity whereas no significant effect was observed for **1c**/**2c** in which P2 substituent R^2 = H present in both **1a** and **2a** was replaced by a methyl group. The replacement of the larger R^5 Boc group (**1a**) by H (compound **1e**) led to a similar decrease effect by a factor ≈ 3 on the ChT-L, C-L and T-L inhibition of cCP and iCP. The removal of the R^4 benzyl group (**1d** *versus* **1a**) was very unfavorable. For further potential optimization, we enlarged side chains at P1 using CH_2(1-Naphthyl) (**1f**), $CH(Ph)_2$ (**1g**), CH_2-(4-PhC_6H_4) (**1h**) and CH_2-(4-*i*PrC_6H_4) (**1i**) to be compared to the side chain Bn (**1a**). Their efficiencies against ChT-L and C-L activities of both proteasomes were quite similar, except for **1f** and **1g** which were more efficient (factors 1.8 to 5.4) on the ChT-L and PA activities of cCP and iCP. Whereas compounds **1g-i** were inefficient to inhibit iCP and cCP T-L activity, **1f** inhibited T-L activities as efficiently than C-L one (IC_{50} of 2.7 µM). The adamantyl compound **1p** was significantly less potent on iCP

ChT-L and C-L activities than on the same cCP activities (factors of 4.7 and 2.2, respectively). The ONX-0914 derivative bearing an adamantyl at P1 failed to inhibit both CPs [53].

Attempts to enhance efficiency against C-L activity by introducing Asp analogs (compounds **1j** and **1k**) were unsuccessful. Conversely to that observed for epoxyketone peptides [53], a cyclohexyl group at the P1 position did not enhance β5i selectivity (compounds **1m** and **1n**). Whereas the efficiency against ChT-L activity of both CPs did not vary compared to that of **1a**, the efficiency against C-L activity was improved (factors of 1.3-3.7) and the effect on the T-L activity was poorer or lost. We investigated the SAR of the P3 residues. The D-Ala residue (**1q**) was less favorable than the L-Ala one (**1a**). The Asn residue also found in the natural product TMC-95A favored inhibition of the ChT-L activity of both CPs (factors of ~ 2). An enhanced ChT-L inhibitory activity was observed when the P3 methyl group (compound **1t**) was replaced by the polar lateral chain of Thr (factors of 28 for cCP and 17 for iCP). The inhibition of the C-L activity of both CPs was poorly affected whereas the iCP T-L activity was noticeable (IC_{50} = 5.8 ± 0.3 µM). Finally, the Lys side chain (compound **1v**) but not its Boc form (compound **1u**) were active against the T-L activity of both CPs at the submicromolar level. The most important improvement was obtained with dipeptides **5**. As for tripeptide derivatives, a favorable effect of the Asn (**5b**) and Thr (**5c**) on the ChT-L activity was observed in comparison with **5a**, factors of 14 for cCP and 9.4 for iCP (compound **5b**) and of 5.8 for cCP and 7 for iCP (compound **5c**). The T-L activity was not affected whereas the C-L one was very poorly inhibited only for compound **5c**. The CO(4-BnO)C_6H_4 *N*-terminal blocking group known to potentially occupy small accessory hydrophobic pockets AS1 and AS2 [18, 25] led to efficient and selective ChT-L inhibition (**5f** and **5e**). This effect was increased by more than one order of magnitude when the R^3 alanine group (**5e**) was replaced with the threonine residue (**5f**). The IC_{50} values of compound **5f** for the ChT-L activity were of 7.1 ± 0.2 nM (cCP) and 10.2 ± 0.1 nM (iCP). Finally, after 15 min treatment, compound **5f** appeared to be more efficient than the covalent inhibitor ONX-0914 against ChT-L activity by a factor of 2 (cCP) and 5 (iCP). This compound is known to act selectively on the ChT-L activity of immunoproteasome [54].

Table 1: Inhibition by tripeptides **1a-v** and **2a-c** of purified human 20S constitutive proteasome (values displayed in normal characters) and human 20S immunoproteasome (values in bold) at pH 8 and 37 °C[a]



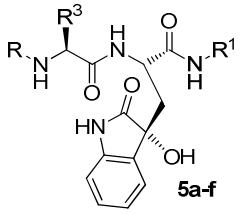


	R^1	R^2	R^3	R^4	R^5	IC_{50} (μM) ChT-L	C-L	T-L
1a[b,c]	Bn	H	Me	Bn	Boc	3.2 ± 0.1	11.5 ± 0.5	7.1 ± 0.2
						2.53 ± 0.07	**9.9 ± 0.3**	**28 ± 2**
1b[c]	Me	H	Me	Bn	Boc	79±2	*20%*	*36%*
						24%	***13%***	**79 ± 3**
1c	Bn	Me	Me	Bn	Boc	5.1 ± 0.1	11.4 ± 0.3	*19%*
						3.93 ± 0.08	**10.32 ± 0.07**	**27%**
2a[c]	Bn	H	Me	Bn	Boc	7.0 ± 0.3	17.2 ± 0.4	ni
						7.3 ± 0.2	**10.6 ± 0.2**	**34 ± 9**
2b[c]	Me	H	Me	Bn	Boc	ni	ni	13%
						8%	***10%***	***19%***
2c	Bn	Me	Me	Bn	Boc	6.8 ± 0.2	13.4 ± 0.5	*56%*
						6.1 ± 0.1	**8.9 ± 0.2**	***18%***
1d[c]	Bn	H	Me	H	Boc	32 ± 2	ni	*19%*
						34 ± 2	***12%***	***10%***
1e[c]	Bn	H	Me	Bn	H	10.7 ± 0.2	30 ± 1	24.8 ± 0.9
						13 ± 1	**21.5 ± 0.6**	**27.2 ± 0.8**
1f	CH_2(1-Naph)	H	Me	Bn	Boc	1.40 ± 0.01	2.14 ± 0.09	2.9 ± 0.1
						1.90 ± 0.03	**2.77 ± 0.08**	**2.7 ± 0.1**
1g	$CH(Ph)_2$	H	Me	Bn	Boc	1.74 ± 0.03	2.2 ± 0.2	*30%*
						2.70 ± 0.06	**4 ± 1**	**ni**
1h	CH_2-(4-PhC_6H_4)	H	Me	Bn	Boc	28.7 ± 0.9	17.6 ± 0.4	ni
						19.7 ± 0.7	**13 ± 1**	**x 1.5**[d]
1i	CH_2-(4-*i*PrC_6H_4)	H	Me	Bn	Boc	10.0 ± 0.4	*37%*	ni
						17.1 ± 0.9	**46 ± 3**	**x 1.3**[d]
1j	$(CH_2)_2CO_2Me$	H	Me	Bn	Boc	64 ± 6	*15%*	*14%*
						31%	***17%***	**106 ± 7**
1k	$(CH_2)_2CO_2H$	H	Me	Bn	Boc	*18%*	*19%*	37.6 ±0.7
						14%	***17%***	**22 ± 2**
1l	$(CH_2)_2Ph$	H	Me	Bn	Boc	6.0 ± 0.1	4.9 ± 0.2	*45%*
						6.5 ± 0.1	**7.31 ± 0.06**	***26%***
1m	$(CH_2)_2$-C_6H_{11}	H	Me	Bn	Boc	2.8 ± 0.1	2.7 ± 0.2	*36%*
						2.7 ± 0.1	**3.5 ± 0.1**	***19%***
1n	CH_2-C_6H_{11}	H	Me	Bn	Boc	4.3 ± 0.1	4.7 ± 0.3	19.3 ±0.4
						5.2 ± 0.1	**4.8 ± 0.2**	**16 ± 1**
1o	CH_2-*t*Bu	H	Me	Bn	Boc	11.3 ± 0.2	15.1 ± 0.3	31 ± 2
						12.6 ± 0.3	**21 ± 1**	**30 ± 3**
1p	CH_2-Ada	H	Me	Bn	Boc	2.13 ± 0.03	2.6 ± 0.2	*19%*
						10.0 ± 0.2	**5.7 ± 0.1**	**ni**
1q	Bn	H	(D)Me	Bn	Boc	9.2 ± 0.3	17.1 ±0.5	18.6 ± 0.2
						13.2 ± 0.3	**12.5± 0.9**	**21.4 ± 0.4**
1r	Bn	H	H	Bn	Boc	6.2 ± 0.1	12.4 ± 0.5	30 ± 1
						7.8 ± 0.2	**12.6 ± 0.5**	**8.9 ± 0.2**
1s	Bn	H	CH_2CONH_2	Bn	Boc	1.48 ± 0.04	20.1 ± 0.8	x 1.4[d]
						1.6 ± 0.1	**16.1 ± 0.9**	**x 1.3**[d]
1t	Bn	H	(*R*)-CH(OH)Me	Bn	Boc	0.125 ± 0.004	6.08 ± 0.09	13.5 ± 0.8
						0.20 ± 0.01	**8.8 ± 0.7**	**5.8 ± 0.3**
1u	Bn	H	$(CH_2)_4NHBoc$	Bn	Boc	22.0 ± 0.4	*15%*	ni
						25.1 ± 0.9	**59 ± 5**	**x 1.3**[d]
1v	Bn	H	$(CH_2)_4NH_2$	Bn	H	2.9 ± 0.1	13.0 ± 0.9	0.52 ± 0.08
						3.8 ± 0.1	**4.3 ± 0.6**	**0.82 ± 0.07**

[a]The inhibition was evaluated after 15 min incubation of the enzyme with the respective compound before adding the appropriate fluorogenic substrate (Suc-LLVY-AMC for ChT-L

activities of cCP and iCP, Z-LLE-βNA for C-L activity of cCP, Ac-PAL-AMC for C-L activity of iCP and Boc-LLR-AMC for T-L activities). ni: no inhibition. IC_{50} values were determined when the % inhibition was higher than 85% at 50 µM. The % inhibition at 50 µM is displayed in italicized text. [b]Published results [42]. [c]Inhibition of the ChT-L activity of yeast proteasome was previously published [17]. [d]Activation factor at 50 µM.

Table 2: Inhibition by dipeptides **5a-f** of purified human 20 S constitutive proteasome (values displayed in normal characters) and human 20S immunoproteasome (values in bold) at pH 8 and 37 °C[a]



5a-f

	R^1	R^3	R	IC_{50} (µM) ChT-L	C-L	T-L
5a	Bn	Me	Z	5.1 ± 0.1	ni	ni
				3.96 ± 0.09	**45 ± 2**	**ni**
5b	Bn	CH_2CONH_2	Z	0.36 ±0.02	ni	ni
				0.42 ± 0.01	**ni**	**ni**
5c	Bn	(*R*)-CH(OH)Me	Z	0.88 ± 0.02	*20%*	ni
				0.572 ± 0.005	**24.6 ± 0.7**	**ni**
5d	Bn	Me	$COCH_2(3\text{-}PhO)C_6H_4$	0.35 ± 0.01	40 ± 1	16.2 ± 0.7
				0.26 ± 0.01	**14 ± 1**	**14.3 ± 0.6**
5e	Bn	Me	$CO(4\text{-}BnO)C_6H_4$	0.113 ± 0.006	35 ± 1	26.0 ± 0.3
				0.170 ± 0.004	**52 ± 2**	**28.3 ± 0.7**
5f	Bn	(*R*)-CH(OH)Me	$CO(4\text{-}BnO)\ C_6H_4$	0.0071 ± 0.0002	31.9 ± 0.6	13.2 ± 0.5
				0.0102 ± 0.0001	**23.8 ± 0.7**	**6.6 ± 0.6**
ONX-0914	-	-	-	0.15 ± 0.02	22 ± 1	4.7 ± 0.6
				0.052 ± 0.007	**0.59 ± 0.03**	**4.85 ± 0.07**

[a]The inhibition was evaluated after 15 min incubation of the enzyme with the respective compound before adding the appropriate fluorogenic substrate (Suc-LLVY-AMC for ChT-L activities of cCP and iCP, Z-LLE-βNA for C-L activity of cCP, Ac-PAL-AMC for C-L activity of iCP and Boc-LLR-AMC for T-L activities). ni: no inhibition. IC_{50} values were determined when the % inhibition was higher than 85% at 50 µM. The % inhibition at 50 µM is displayed in italicized text.

2.4 Tumor cells assays

The cell viability of the best inhibitors was evaluated on several cancer cell lines: hepatocellular carcinoma Huh-7, colorectal adenocarcinoma Caco2, colorectal carcinoma HCT-116, breast carcinoma MDA-MB 231, breast carcinoma MCF7, prostate carcinoma PC3

and lung carcinoid NCI-H727) (Table 3, Figure S3). Human skin fibroblasts were the reference for non-tumor cells. DMSO was used as negative control and the proteasome inhibitor ONX-0914 as positive control. After 48 h treatment with the inhibitors, cells were fixed and the nuclei were stained with Hoechst 33342, and then counted. The survival percentages were calculated as the number of cells after compound treatment over the number of cells after DMSO treatment. According to dose-response curves, the EC_{50} values were determined (Table 3). Dipeptides **5e**,**f** were more cytotoxic than tripeptides **1a**,**s-t**. Dipeptide **5f** displayed toxicities in the sub-micromolar range against 6 over 7 cancer cell lines. Its effect was close to that obtained with ONX-0914 on MDA-MB 231, HCT-116, PC3, NCI-H727 and MCF7. Noticeably, normal fibroblasts were not affected by treatment with our compounds.

Table 3. Effect of compounds **1a**,**s**,**t** and **5e**,**f** on the survival of human cancer cells and normal human skin fibroblasts using Hoechst 3342 staining.

	EC_{50} (µM)							
	HuH7	CaCo-2	MDA-MB-231	HCT-116	PC3	NCI-H727	MCF7	Fibroblasts
1a	ne	ne	ne	32	ne	ne	26	ne
1s	ne	ne	ne	ne	ne	ne	ne	ne
1t	ne	32	9	9	10	ne	9	ne
5e	ne	ne	23	10	24	ne	21	ne
5f	ne	5	0.7	0.2	0.4	2	0.7	ne
ONX-0914	0.39	1.3	0.27	0.11	0.33	0.3	0.26	0.16

ne: no effect at 25 µM.

3. Conclusion

We have designed and synthesized 22 new tripeptidic and 6 dipeptidic linear analogs of the natural non covalent inhibitor TMC-95A and analyzed their inhibitory activity against human cCP and iCP. We investigated analogs with various P1 and P3 residues for tripeptides, and various P1, P3 residues and *N*-terminal groups for dipeptides reasoning that the interaction of these residues with the enzyme subsites described in previous studies by us [17] or others [19-21] would contribute to affinity and orientate the inhibitor selectivity towards iCP over cCP. Conversely to that observed with covalent inhibitors such as epoxyketones [53, 55], bulky P1 groups such as naphtyl, 4-isopropyl, 4-biphenyl or cyclohexyl groups did not favor the inhibition of iCP over cCP. The simultaneous introduction of the threonine residue at the P3 position and the CO(4-$BnOC_6H_4$) as *N*-terminal group in dipeptides led to the inhibition of both cCP and iCP at the nanomolar level. Discrepancies between covalent and noncovalent

inhibitors may be correlated to differences in conformational changes upon their binding with the catalytic subsites and the formation of a covalent bond with Thr1O$^{\gamma}$ in the case of covalent inhibitors [56, 57].

4. Experimental section

4.1. Chemistry

General chemistry methods are described in the supporting information.

4.1.1. *Synthesis of 3-hydroxyoxindolyl alanine residues **6** and **7***

4.1.1.1. *General procedure for the preparation of spirolactones **9a** and **10a***

To a solution of Z-L-Trp-OH (12.00 g, 35.5 mmol) in DMSO (26.8 mL, 377 mmol), was added slowly *t*-BuBr (41.5 mL, 357 mmol). The mixture was stirred at 45°C for 20 hours. The excess of *t*-BuBr and DMSO was removed under reduced pressure. After dilution by CH_2Cl_2 (150 mL), the mixture was washed by iced water twice (2 x 20 mL). The organic phase was concentrated *in vacuo* and the residue was purified by chromatography over silica gel (835 g, eluent 5, 10, 15, 20% of AcOEt in CH_2Cl_2) to afford two fractions: the less polar 3(*R*)-spirolactones **9a** as a brown amorphous solid (4.90 g, 70/30 mixture of non brominated (X =H)/ brominated (X = Br) derivatives, *ca* 37% yield) and the more polar 3(*S*)-spirolactones **10a** as a brown amorphous solid (6.00 g, 70/30 mixture of non brominated (X =H)/ brominated (X = Br) derivatives, *ca* 45% yield). An authentic sample of **9a** (X = H) and **10a** (X = H) was obtained by reaction at room temperature of Z-L-Trp-OH (0.364 g, 1.07 mmol) in acetonitrile (6 mL) with potassium iodide (20.3 mmg, 0.12 mmol) and 35 % hydrogene peroxide (0.57 mL, 7.04 mmol). The brownish reaction mixture was stirred overnight at room temperature, and then concentrated *in vacuo*. After chromatography over silica gel (16 g, eluent AcOEt in CH_2Cl_2), spirolactones **9a** (X = H, 57 mg, 15% yield) and **10a** (X = H, 142 mg, 38%) were afforded as solids.

3(R)-spirolactone **9a** (X = H). Yellowish solid. Mp (decomposition) 150 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.49 (dd, *J* = 14.1, 5.4 Hz, 1H), 2.83 (dd, *J* = 14.1, 9.6 Hz, 1H), 5.11 (m, 1H), 5.19 (s, 2H), 6.22 (d, *J* = 9.0 Hz, 1H), 6.93 (d, *J* = 7.5 Hz, 1H), 7.15 (t, *J* = 6.9 Hz, 1H), 7.35-7.40 (m, 7H), 7.94 (br s, 1H). ^{13}C NMR (75.5 MHz, CD_3COCD_3) δ 37.14 (CH_2), 51.00 (CH), 67.31 (CH_2), 81.80 (C), 111.67 (CH), 123.97 (CH), 125.09 (CH), 128.03 (C), 128.78 (CH), 128.82 (CH), 129.24 (CH), 132.05 (CH), 137.82 (C), 142.93 (C), 156.70 (C), 174.47 (C), 175.79 (C). HRMS (ESI) calcd for $C_{19}H_{16}N_2O_5Na$ [$(M+Na)^+$] 375.0953, found 375.0955. R_f (1/4 AcOEt/CH_2Cl_2) 0.45.

3(S)-spirolactone ***10a*** (X = H). White solid. Mp (decomposition) 164 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.76 (m, 1H), 2.92 (m, 1H), 5.00 (m, 1H), 5.14 (s, 1H), 5.68 (d, *J* = 6.0 Hz, 1H), 6.91 (d, *J* = 7.8 Hz, 1H), 7.13 (m, 1H), 7.33-7.55 (m, 7H), 8,31 (br s, 1H). ^{13}C NMR (75.5 MHz, CD_3COCD_3) δ 36.79 (CH_2), 51.12 (CH), 67.21 (CH_2), 80.78 (C), 111.46 (CH), 123.87 (CH), 125.82 (CH), 127.40 (C), 128.74 (CH), 128.79 (CH), 129.24 (CH), 132.16 (CH), 137.75 (C), 143.49 (C), 156.81 (C), 174.50 (C), 176.43 (C). HRMS (ESI) calcd for $C_{19}H_{16}N_2O_5Na$ [$(M+Na)^+$] 375.0953, found 375.0951. R_f (1/4 AcOEt/CH_2Cl_2) 0.3.

4.1.1.2. *Spirolactones* ***9b*** *and* ***10b***

According to the same procedure starting from *Z*-L-methyltryprophan (4.84 g, 13.7 mmol), DMSO (9.9 mL, 140 mmol) and *tert*-butyl bromide (15.6 mL, 139 mmol), the less polar spirolactone **9b** (1.10 g, 22 % yield) and the more polar spirolactone **10b** (2.03 g, 42% yield) were obtained as vitreous orange solids after purification by chromatography over silica gel (260 g, eluent 5, 10, 15, 20% of AcOEt in CH_2Cl_2).

3(R)-spirolactone **9b:** Mp (decomposition) 67 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.43 (dd, *J* = 14.0, 5.0 Hz, 1H), 2.78 (dd, *J* = 14.0, 9.3 Hz, 1H), 3.20 (s, 3H), 5.08 (m, 1H), 5.15 (s, 2H), 6.42 (d, *J* = 9.3 Hz, 1H), 6.89 (d, *J* = 7.8 Hz, 1H), 7.15 (t, *J* = 7.3 Hz, 1H), 7.43-7.47 (m, 7H). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 26.56 (CH_3), 37.35 (CH_2), 50.07 (CH), 67.36 (CH_2), 81.35 (C), 109.23 (CH), 123.96 (CH), 124.30 (CH), 125.44 (C), 128.14 (CH), 128.17 (CH), 128.53 (CH), 131.56 (CH), 135.97 (C), 143.68 (C), 155.97 (C), 173.58 (C), 173.84 (C). HRMS (ESI) calcd for $C_{20}H_{18}N_2O_5Na$ [$(M+Na)^+$] 389.1108, found 389.1107. R_f = 0.49 (AcOEt/CH_2Cl_2 5/95)

3(S)-spirolactone **10b:** Mp (decomposition) 140 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.80 (m, 2H), 3.18 (s, 3H), 4.94-5.03 (m, 1H), 5.14 (s, 2H), 5.65 (d, *J* = 6.1 Hz, 1H), 6.85 (d, *J* = 7.8 Hz, 1H), 7.14 (t, *J* = 7.4 Hz, 1H,), 7.32-7.43 (m, 6H,), 7.52 (d, *J* = 7.2 Hz, 1H). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 26.34 (CH_3), 36.62 (CH_2), 50.65 (CH), 67.30 (CH_2), 81.13 (C), 108.80 (CH), 123.80 (CH), 125.03 (CH), 125.43 (C), 128.15 (CH), 128.30 (CH), 128.55 (CH), 131.44 (CH), 135.77 (C), 144.03 (C), 155.65 (C), 173.70 (C), 173.84 (C). HRMS (ESI) calcd for $C_{20}H_{18}N_2O_5Na$ [$(M+Na)^+$] 389.1108, found 389.1105. R_f = 0.31 (AcOEt/ CH_2Cl_2 5/95)

4.1.1.3. *General procedure for the synthesis of (S)-2-amino-N-methyl-3-((R)-3-hydroxy-2-oxoindolin-3-yl)propanamide* **6a**:

Spirolactone **9a** (200 mg, *ca* 0.53 mmol, based on a 70:30 ratio of H- and Br-oxindole starting material, X = H or Br) was reacted with 2 M $MeNH_2$ in THF (2 mL, 4 mmol) for 3 h at room temperature. Evaporation of the solvent under reduced pressure and chromatography over silica gel (2% MeOH in CH_2Cl_2) afforded a 70:30 mixture of non-brominated and brominated Z-protected **6a** (170 mg, yield *ca* 79%) as a yellow amorphous solid. MPLC purification of a small sample (38 mg) over reversed phase silica (C18, 5 g, 50% MeOH in water) afforded after lyophilization, (*S)-2-benzyloxycarbonylamino-N-methyl-3-((R)-3-hydroxy-2-oxoindolin-3-yl)propanamide* (22.3 mg, ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.36 (broad s, 2H, CH_2β), 1.54 (s, 3H, NCH_3), 4.26 (broad s, 1H, Hα), 4.88 (s, 2H, CH_2Ph), 5.56 (broad s, 1H, OH), 6.27-7.14 (m, 9H, aromatic H), 8.80 (broad s, 1H, NH). HRMS (LSIMS) calcd for $C_{20}H_{22}N_3O_5$ $[M+H]^+$: 384.1559, found 384.1550. R_f (RP18, 50% MeOH in water) 0.18.) and *(S)-2-benzyloxycarbonylamino-N-methyl-3-((R)-5-bromo-3-hydroxy-2-oxoindolin-3-yl)propanamide* (3.5 mg, HRMS (LSIMS) calcd for $C_{20}H_{21}{}^{79}BrN_3O_5$ $[M+H]^+$: 462.0665, found 462.0632. R_f (RP18, 50% MeOH in water) 0.05).

A 70:30 mixture of non-brominated and brominated Z-protected **6a** (132 mg) was placed overnight with 10% Pd on carbon (20 mg) in methanol (2 mL) under H_2 atmosphere. The mixture was filtered through celite and treated with 1 M anhydrous HCl in methanol (0.7 mL, 0.7 mmol). After concentration *in vacuo,* product **6a,** hydrochloride was obtained as a white solid (96.4 mg, 30% from Z-Trp).

^{1}H NMR (300 MHz, D_2O) δ 2.38 (s, 3H, NCH_3), 2.44 (d, *J* = 6.9 Hz, 2H, CH_2β), 4.02 (t, *J* = 6.9 Hz, 1H, Hα), 7.02 (d, *J* = 7.5 Hz, 1H), 7.12 (t, *J* = 7.6 Hz, 1H,), 7.31-7.35 (m, 2H). ^{13}C NMR (75.5 MHz, D_2O) δ 26.6 (NCH_3), 37.4 (CH_2), 50.2 (CHα), 75.1 (C), 111.9 (CH), 124.4 (CH), 125.0 (CH), 129.5 (C), 131.5 (CH), 140.7 (C), 169.3 (C=O), 180.2 (C=O oxindole). HRMS (ESI) calcd for $C_{12}H_{16}N_3O_3$ $[M+H]^+$: 250.1192, found 250.1192.

4.1.1.4. *(S)-2-Amino-N-benzyl-3-((R)-3-hydroxy-2-oxoindolin-3-yl)propanamide* **6b**:

According to the general procedure described above, spirolactone **9a** (314 mg, *ca* 0.83 mmol based on a 70:30 ratio of H- and Br-oxindole starting material) and benzylamine (110 µL, 0.96 mmol) were reacted overnight. Purification by chromatography over silica gel (23.6 g, eluent 40% of AcOEt in CH_2Cl_2 then AcOEt) afforded an orange amorphous solid (419 mg) that was hydrogenated according to the general procedure. Product **6b**, hydrochloride was obtained as a white solid (221 mg, 27% from Z-Trp).

^{1}H NMR (300 MHz, DMSO-d_6) δ 2.08 (dd, *J* = 13.5, 3.0 Hz, 1H, CHβ), 2.26 (dd, *J* = 13.5, 9.0 Hz, 1H, CHβ), 4.15 and 4.27 (ABX system, J_{AB} = 15.0 Hz, J_{AX} = J_{BX} = 5.7 Hz, 2H, NCH_2), 4.41 (t, *J* = 4.5 Hz, 1H, Hα), 6.87 (d, *J* = 6.0 Hz, 1H, H aromatic), 7.02 (td, *J* = 7.5, 1.5 Hz, 1H, H aromatic), 7.2-7.46 (m, 7H, aromatics), 8.08-8.10 (d, *J* = 3.0 Hz, 3H, NH_3^+), 8.83 (t, *J* = 6.0 Hz, 1H, NH), 10.54 (s, 1H, NH oxindole). ^{1}H NMR (300 MHz, D_2O + HCl) δ 2.37 and 2.44 (ABX system, 2H, J_{AX} = 6.7 Hz, J_{BX} = 7 Hz, J_{AB} = 14.7 Hz, CH_2β), 3.96 and 4.11 (AB system, J_{AB} = 14.7 Hz, 2H, CH_2Ph), 4.20 (t, *J* = 6.8 Hz, 1H, CHα), 6.95 (d, *J* = 8.0 Hz, 1H), 7.06-7.15 (m, 3H), 7.23-7.33 (m, 5H). ^{13}C NMR (75.5 MHz, D_2O + HCl) δ 37.5 (CH_2), 44.1 (CH_2), 50.1 (CH), 75.1 (C), 111.9 (CH), 124.4 (CH), 124.9 (CH), 128.1 (CH), 128.3 (CH), 129.4 (CH), 129.9 (C), 131.5 (CH), 137.6 (C), 140.6 (C), 169.0 (C=O), 180.3 (CO oxindole). HRMS (ESI) calcd for $C_{18}H_{19}N_3O_3Na$ $[M + Na]^+$: 348.1324, found 348.1323.

4.1.1.5. *(S)-2-Benzyloxyamino-N-benzyl-3-((R)-3-hydroxy-1-methyl-2-oxoindolin-3-yl) propanamide:*

According to the general procedure described above, spirolactone **9b** (1.60 g, 4.75 mmol) and benzylamine (560 µL, 5.12 mmol) were reacted overnight. The solid was filtered and washed with CH_2Cl_2 to afford *(S)-2-benzyloxyamino-N-benzyl-3-((R)-3-hydroxy-1-methyl-2-oxoindolin-3-yl)propanamide* (*N*-Z-protected **6c)** as a white solid (1.52g, 68%). Mp 156-158 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.25 (dd, *J* = 15.6, 4.5 Hz, 1H), 2.50 (dd, *J* = 15.6, 4.2 Hz, 1H), 3.01 (s, 3H), 4.33 (dd, *J* = 14.8, 5.1 Hz, 1H), 4.48 (dd, *J* = 14.6, 6.3 Hz, 1H), 4.78 (br, 1H), 5.13 (m, 2H), 6.54 (d, *J* = 6.5 Hz, 1H), 6.78 (d, *J* = 7.6 Hz, 1H), 7.10 (t, *J* = 7.5 Hz, 1H), 7.43-7.47 (m, 14H). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 26.09 (CH_3), 42.23 (CH_2), 43.55 (CH_2), 50.13 (CH), 67.35 (CH_2), 74.28 (C), 108.60 (CH), 123.64 (CH), 123.68 (CH), 127.47 (CH), 127.56 (CH), 128.21 (CH), 128.31 (CH), 128.57 (CH), 128.67 (CH), 129.88 (CH), 130.97 (C), 135.89 (C), 137.70 (C), 142.44 (C), 156.66 (C), 171.29 (C), 178.60 (C). HRMS (ESI) calcd for $C_{27}H_{27}N_3O_5Na$ $[(M+Na)^+]$ 496.1843, found 496.1844. R_f (2/3 AcOEt/CH_2Cl_2) 0.31.

4.1.1.6. *(S)-2-Amino-N-benzyl-3-((R)-3-hydroxy-1-methyl-2-oxoindolin-3-yl)propanamide* **6c**: Hydrogenation of (*S*)-2-benzyloxyamino-*N*-benzyl-3-((R)-3-hydroxy-1-methyl-2-oxoindolin-3-yl)propanamide (965 mg, 2.04 mmol) according to the general procedure afforded **6c** as a white solid (691 mg, quantitative, 15% from Z-L-methyl tryptophan). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.88-2.04 (m, 2H), 3.09 (s, 3H), 3.64 (dd, *J* = 10.5, 3.90 Hz, 1H), 4.20 (d, *J* = 4.2 Hz, 2H), 6.95 (d, *J* = 7.5 Hz, 1H), 7.03 (t, *J* = 7.2 Hz, 1H), 7.17-7.32 (m, 1H), 8.38 (br s, 1H)

4.1.1.7. (*S*)-*2-Amino-3-((R)-3-hydroxy-2-oxoindolin-3-yl)-N-(naphthalen-1-ylmethyl) propanamide* **6d:** According to the general procedure described above, spirolactone **9a** (208 mg, *ca* 0.55 mmol based on a 70:30 ratio of H- and Br-oxindole starting material) and naphtylmethylene amine (140 µL, 0.90 mmol) were reacted overnight. Purification by chromatography over silica gel (8.1 g, eluent 10% of AcOEt in CH_2Cl_2 then then 25% AcOEt in CH_2Cl_2) afforded an orange amorphous solid (291 mg) that was hydrogenated according to the general procedure. Product **6d**, hydrochloride was obtained as a white solid (191 mg, 31% from Z-Trp).

^{1}H NMR (300 MHz, CD_3OD) δ 2.14 (dd, *J* = 15.3, 3.0 Hz, 1H, CHβ), 2.63 (dd, *J* = 15.2, 4.2 Hz, 1H, CHβ), 4.75 (m, 3H, Hα, NCH_2), 6.89 (d, *J* = 7.8 Hz, 1H aromatic), 7.00 (td, *J* = 7.5, 0.6 Hz, 1H aromatic), 7.21 (td, *J* = 7.8, 1.0 Hz, 1H aromatic), 7.29-7.43 (m, 5H aromatics) , 7.68-7.78 (m, 2H aromatics), 7.89-7.93 (m, 1H aromatic), 8.42 (br s, NH_3^+, OH) , 8.85 (t, *J* = 5.4 Hz, 1H, NH), 10.31 (s, 1H, NH oxindole). ^{13}C NMR (75.5 MHz, CD_3OD) δ 39.08 (CH_2), 42.48 (CH_2), 50.86 (CH), 75.67 (C), 111.68 (CH), 124.04 (CH), 124.22 (CH), 124.67 (CH), 123.36 (CH), 126.86 (CH), 127.26 (CH), 127.40 (CH), 129.39 (CH), 129.73 (CH), 131.13 (CH), 132.45 (C), 132.70 (C), 134.11 (C), 135.17 (C), 141.86 (C), 170.21 (CO), 180.70 (CO). HRMS (ESI) calcd for $C_{22}H_{21}N_3O_3Na$ $[M + Na]^+$: 398.1475, found 398.1476.

4.1.1.8. (*S)-2-Amino-N-benzhydryl-3-((R)-3-hydroxy-2-oxoindolin-3-yl)propanamide* **6e:** According to the general procedure described above, spirolactone **9a** (233 mg, *ca* 0.62 mmol based on a 70:30 ratio of H- and Br-oxindole starting material) and benzhydrylamine (130 µL, 0.73 mmol) were reacted overnight. Purification by chromatography over silica gel (8 g, eluent 50% of AcOEt in CH_2Cl_2 then 80% AcOEt in CH_2Cl_2) afforded an orange amorphous solid (189 mg) that was hydrogenated according to the general procedure. Product **6e**, hydrochloride was obtained as a white solid (148 mg, 19% from Z-Trp).

^{1}H NMR (300 MHz, DMSO-d_6) δ 2.07 (dd, *J* = 13.5, 3.0 Hz, 1H, CHβ), 2.26 (dd, *J* = 9.0, 3.0 Hz, 1H, CHβ), 3.97 (s, 1H, OH), 4.22 (ddd, *J* = 18.0, 15.0, 1.5 Hz, 2H, NCH_2), 4.55 (br s, 1H, Hα), 6.07 (d, *J* = 9.0 Hz, 1H, $CH(Ph)_2$), 6.87 (d, *J* = 6.0 Hz, 1H aromatic), 6.93 (m, 1H aromatic), 7.02 (td, *J* = 7.5, 1.0 Hz, 1H aromatic), 7.17-7.37 (m, 11H aromatic) , 8.08 (br s, 3H, NH_3^+) , 9.3 (d, *J* = 9.0 Hz, 1H, NH), 10.58 (s, 1H, NH oxindole). ^{13}C NMR (75.5 MHz, CD_3OD) δ 38.97 (CH_2), 50.86 (CH), 58.61 (CH), 75.66 (C), 111.68 (CH), 124.06 (CH), 124.67 (CH), 128.51 (CH), 128.59 (CH), 129.18 (CH), 129.56 (CH), 129.6 (CH), 129.89 (CH), 131.18 (CH), 132.83 (C), 141.98 (C), 142.32 (C), 142.42 (C), 169.09 (CO), 180.63 (CO). HRMS (ESI) calcd for $C_{24}H_{23}N_3O_3Na$ $[M+Na]^+$: 424.1632, found 424.1630.

4.1.1.9. *(S)-N-([1,1'-Biphenyl]-4-ylmethyl)-2-amino-3-((R)-3-hydroxy-2-oxoindolin-3-yl) propanamide* **6f:**. According to the general procedure described above, spirolactone **9a** (261 mg, *ca* 0.69 mmol based on a 70:30 ratio of H- and Br-oxindole starting material) and 4-phenylbenzylamine (151 mg, 0.82 mmol) were reacted overnight. Purification by chromatography over silica gel (20 g, eluent 30% of AcOEt in CH_2Cl_2) afforded an orange amorphous solid (300 mg) that was hydrogenated according to the general procedure. Product **6f**, trifluoroacetate salt was obtained as a white solid (291 mg, 30% from Z-Trp).

^{1}H NMR (300 MHz, CD_3OD) δ 2.12 (dd, *J* = 15.0, 3.0 Hz, 1H, CHβ), 2.22 (dd, *J* = 15.3, 10.8 Hz, 1H, CHβ), 4.34-4.38 (m, 2H, CH_2), 4.71 (dd, *J* = 10.8, 3.0 Hz, 1H, Hα), 6.93 (d, *J* = 7.8 Hz, 1H aromatic), 7.02 (td, *J* = 7.5, 3.0 Hz, 1H aromatic), 7.22-7.32 (m, 7H aromatics), 7.45-7.49 (m, 4H aromatics), 8.32 (br s, 3H, NH_3^+), 8.79 (t, *J* = 6.0 Hz, 1H, NHCO), 10.21 (s, NH oxindole). ^{13}C NMR (75.5 MHz, CD_3OD) δ 38.98 (CH_2), 44.04 (CH_2), 50.08 (CH), 75.59 (C), 111.66 (CH), 124.03 (CH), 124.72 (CH), 127.77 (CH), 128.04 (CH), 128.28 (CH), 129.14 (CH), 129.76 (CH), 131.14 (CH), 132.70 (C), 138.23 (C), 141.44 (C), 141.78 (C), 141.92 (C), 169.73 (CO), 180.56 (CO). HRMS (ESI) calcd for $C_{24}H_{24}N_3O_3$ $[M+H]^+$: 402.1812, found 402.1802.

4.1.1.10. *(S)-2-Amino-3-((R)-3-hydroxy-2-oxoindolin-3-yl)-N-(4-isopropylbenzyl) propanamide* **6g:**

According to the general procedure described above, spirolactone **9a** (284 mg, *ca* 0.75 mmol based on a 70:30 ratio of H- and Br-oxindole starting material) and 4-(*iso*propyl)benzylamine (132 mg, 0.89 mmol) were reacted overnight. Purification by chromatography over silica gel (11 g, eluent 40% of AcOEt in CH_2Cl_2) afforded an orange amorphous solid (299 mg) that was hydrogenated according to the general procedure. Product **6g**, trifluoroacetate salt was obtained as a white solid (271 mg, 27% from Z-Trp).

^{1}H NMR (300 MHz, CD_3OD) δ 1.15 (s, 3H, CH_3), 1.16 (s, 3H, CH_3), 2.09 (dd, *J* = 15.0, 3.0 Hz, 1H, CHβ), 2.22 (dd, *J* = 15.3, 10.8 Hz, 1H, CHβ), 2.74-2.83 (m, 1H, CH), 4.22-4.33 (m, 2H, CH_2-NHCO), 4.68 (dd, *J* = 8.3, 3.0 Hz, 1H, Hα), 6.92 (d, *J* = 7.5 Hz, 1H aromatic), 7.03 (td, *J* = 7.5, 1.0 Hz, 1H aromatic), 7.08-7.15 (m, 4H aromatics), 7.25 (td, *J* = 7.8, 1.2 Hz, 1H aromatic), 7.31-7.36 (m, 1H, aromatic), 8.30 (br s, 4H, NH_3^+, OH), 8.70 (t, *J* = 6.0 Hz, 1H, NHCO), 10.26 (s, NH oxindole1H). ^{13}C NMR (75.5 MHz, CD_3OD) δ 24.36 (CH_3), 34.93 (CH), 39 (CH_2), 44.2 (CH_2), 50.84 (CH), 75.64 (C), 111.69 (CH), 124.04 (CH), 124.71 (CH), 127.50 (CH), 127.74 (CH), 131.14 (CH), 132.68 (C), 136.45 (C), 141.90 (C), 149.27 (C),

169.65 (CO), 180.61 (CO). HRMS (ESI) calcd for $C_{21}H_{26}N_3O_3$ $[M+H]^+$: 368.1969, found 368.1968.

4.1.1.11. *Methyl 3-((S)-2-amino-3-((R)-3-hydroxy-2-oxoindolin-3-yl)propanamido) propanoate* **6h**:

According to the general procedure described above, spirolactone **9a** (284 mg, *ca* 0.75 mmol based on a 70:30 ratio of H- and Br-oxindole starting material), β-alanine methyl ester, hydrochloride (134 mg, 0.96 mmol) and triethylamine (300 µL, 2.13 mmol) were reacted overnight. Purification by chromatography over silica gel (8 g, eluent 15% of AcOEt in CH_2Cl_2) afforded an orange amorphous solid (213 mg) that was hydrogenated according to the general procedure. Product **6h**, trifluoroacetate salt was obtained as a white solid (191 mg, 21% from Z-Trp).

^{1}H NMR (300 MHz, CD_3OD) δ 2.06 (dd, *J* = 15.3, 3.0 Hz, 1H), 2.45-2.55 (m, 3H), 3.37-3.51 (m, 2H), 3.61 (s, 3H), 4.59 (dd, *J* = 10.8, 3.0 Hz, 1H), 6.94 (d, *J* = 7.5 Hz, 1H), 7.08 (t, *J* = 7.5 Hz, 1H), 7.29 (t, *J* = 7.5 Hz, 1H), 7.36 (d, *J* = 7.5 Hz, 1H). ^{13}C NMR (75.5 MHz, CD_3OD) δ 34.30 (CH_2), 36.59 (CH_2), 39.13 (CH_2), 50.69 (CH), 52.21 (CH_2), 75.60, 111.63 (CH), 124.05 (CH), 124.70 (CH), 131.18 (CH), 132.85 (C), 142.02 (C), 169.95 (C), 173.65 (C), 180.68 (C). HRMS (ESI) calcd for $C_{15}H_{19}N_3O_5Na$ $[M+Na]^+$: 344.1217, found 344.1212.

4.1.1.12. *(S)-2-amino-3-((R)-3-hydroxy-2-oxoindolin-3-yl)-N-phenethylpropanamide* **6i:**

According to the general procedure described above, spirolactone **9a** (260 mg, *ca* 0.7 mmol based on a 70:30 ratio of H- and Br-oxindole starting material) and phenethyl amine (102 µL, 0.81 mmol) were reacted overnight. Purification by chromatography over silica gel (15 g, eluent 2% of MeOH in CH_2Cl_2) afforded an orange amorphous solid (300 mg) that was hydrogenated according to the general procedure. Product **6i**, trifluoroacetate salt was obtained as a white solid (241 mg, 28% yield from Z-Trp).

^{1}H NMR (300 MHz, CD_3OD) δ 1.72 (dd, *J* = 15.5, 3.0 Hz, 1H), 2.30 (dd, *J* = 15.5, 11.4 Hz, 1H), 2.68-2.87 (m, 2H), 3.28-3.37 (m, 1H), 3.50-3.60 (m, 1H), 4.50 (dd, *J* = 11.4, 3.0 Hz, 1H), 6.94 (d, *J* = 7.5 Hz, 1H), 7.05-7.16 (m, 6H), 7.31-7.34 (m, 2H). ^{13}C NMR (75.5 MHz, CD_3OD) δ 34.64 (CH_2), 38.03 (CH_2), 40.44 (CH_2), 49.35 (CH), 74.28 (C), 110.35 (CH), 122.70 (CH), 123.41 (CH), 126.04 (CH), 128.05 (CH), 128.51 (CH), 129.83 (CH), 131.42 (C), 138.64 (C), 140.56 (C), 168.50 (C), 179.27 (C). HRMS (ESI) calcd for $C_{19}H_{21}N_3O_3Na$ $[M+Na]^+$: 362.1475, found 362.1476.

4.1.1.13. *(S)-2-amino-N-(2-cyclohexylethyl)-3-((R)-3-hydroxy-2-oxoindolin-3-yl)propanamide* **6j:**

According to the general procedure described above, spirolactone **9a** (260 mg, *ca* 0.7 mmol based on a 70:30 ratio of H- and Br-oxindole starting material) and 2-cyclohexylethyl amine (103 mg, 0.81 mmol) were reacted overnight. Purification by chromatography over silica gel (15 g, eluent 2% of MeOH in CH_2Cl_2) afforded an orange amorphous solid (272 mg) that was hydrogenated according to the general procedure. Product **6j**, trifluoroacetate salt was obtained as a white solid (208 mg, 24% yield from Z-Trp).

^{1}H NMR (300 MHz, CD_3OD) δ 0.85-0.96 (m, 2H), 1.13-1.41 (m, 6H), 1.68 (m, 5H), 2.06 (dd, *J* = 15.3, 3.3 Hz, 1H), 2.51 (dd, *J* = 15.3, 11.1 Hz, 1H), 3.21 (m, 2H), 4.59 (dd, *J* = 11.1, 3.3 Hz, 1H), 6.95 (d, *J* = 7.8 Hz, 1H), 7.08 (t, *J* = 7.5 Hz, 1H), 7.29 (t, *J* = 7.8 Hz, 1H), 7.38 (d, *J* = 7.5 Hz, 1H). ^{13}C NMR (75.5 MHz, CD_3OD) δ 27.25 (CH_2), 27.54 (CH_2), 34.18 (CH), 36.41 (CH_2), 37.50 (CH_2), 38.50 (CH_2), 39.21 (CH_2), 50.76 (CH), 75.63 (C), 111.64 (CH), 124.02 (CH), 124.68 (CH), 131.15 (CH), 132.81 (C), 142.01 (C), 169.61 (C), 180.63 (C). HRMS (ESI) calcd for $C_{19}H_{27}N_3O_3Na$ $[M+Na]^+$: 368.1945, found 368.1948.

4.1.1.14. *(S)-2-Amino)-N-cyclohexylmethyl-3-((R)-3-hydroxy-2-oxoindolin-3-yl)propanamide* **6k:**

According to the general procedure described above, spirolactone **9a** (151 mg, *ca* 0.40 mmol based on a 70:30 ratio of H- and Br-oxindole starting material) and cyclohexylmethylene amine (113.2 mg, 0.4 mmol) were reacted overnight. Purification by chromatography over silica gel (6 g, eluent 50% of AcOEt in CH_2Cl_2) afforded an orange amorphous solid (163 mg) that was hydrogenated according to the general procedure. Product **6k**, trifluoroacetate salt was obtained as a white solid (121 mg, 26% yield from Z-Trp).

^{1}H NMR (300 MHz, CD_3OD) δ 0.85-0.97 (m, 2H), 1.19-1.26 (m, 3H), 1.46 (br s, 1H), 1.67-1.71 (m, 5H), 2.06 (dd, *J* = 15.3, 3.0 Hz, 1H), 2.52 (dd, *J* = 15.3, 11.1 Hz, 1H), 3.03 (d, *J* = 6.0 Hz, 2H), 4.62 (dd, *J* = 11.1, 2.7 Hz, 2H), 6.95 (d, *J* = 7.8 Hz, 1H), 7.10 (td, *J* = 7.8, 1.0 Hz, 1H), 7.32 (td, *J* = 7.8, 1.5 Hz, 1H), 7.38 (d, *J* = 6.0 Hz, 1H). ^{13}C NMR (75.5 MHz, CD_3OD) δ 26.9 (CH_2), 27.5 (CH_2), 31.9 (CH_2), 32.0 (CH_2), 39.0 (CH), 39.3 (CH_2), 47.0 (CH_2), 50.8 (CH), 75.7 (C), 111.7 (CH), 123.1 (CH), 124.8 (CH), 131.3 (CH), 133.0 (C), 142.2 (C), 169.9 (CO), 180.8 (CO). HRMS (ESI) calcd for $C_{18}H_{26}N_3O_3$ $[M + H]^+$: 332.1969, found 332.1972.

4.1.1.15. *(S)-2-Amino-3-((R)-3-hydroxy-2-oxoindolin-3-yl)-N-neopentylpropanamide* **6l:**

According to the general procedure described above, spirolactone **9a** (250 mg, *ca* 0.66 mmol based on a 70:30 ratio of H- and Br-oxindole starting material) and *tert*-butylmethylene amine (92 μL, 0.78 mmol) were reacted overnight. Purification by chromatography over silica gel (8 g, eluent 20% of AcOEt in CH_2Cl_2) afforded an orange amorphous solid (250 mg) that was hydrogenated according to the general procedure. Product **6l**, hydrochloride was obtained as a white solid (149 mg, 24% from Z-Trp).

^{1}H NMR (300 MHz, DMSO-d_6) δ 0.80 (s, 9H, *t*-Bu), 2.03 (dd, *J* = 14.7, 3.9 Hz, 1H, CHβ), 2.22 (dd, *J* = 14.7, 10.2 Hz, 1H, CHβ), 2.81 and 2.90 (ABX system, 2H, J_{AX} = 6.3 Hz, J_{BX} = 6.3 Hz, J_{AB} = 12.9 Hz, 2H, CH_2-NHCO), 4.36 (dd, *J* = 10.2, 2.1 Hz, 1H, Hα), 6.88 (d, *J* = 7.8 Hz, 1H aromatic), 7.02 (td, *J* = 7.5, 1.0 Hz, 1H aromatic), 7.24-7.38 (m, 2H aromatics), 7.77 (br s, NH_3^+, OH, 4H), 8.23 (t, *J* = 6.0 Hz, 1H, NHCO), 10.56 (s, 1H, NH oxindole). ^{13}C NMR (75.5 MHz, CD_3OD) δ 26.20 (CH_3), 31.68 (C), 38.03 (CH_2), 49.43 (CHα), 50.47 (CH_2), 74.26 (C), 110.24 (CH), 122.70 (CH), 123.25 (CH), 129.81 (CH), 131.56 (C), 140.66 (C), 168.70 (CO), 179.31 (CO). HRMS (ESI) calcd for $C_{16}H_{23}N_3O_3Na$ $[M+Na]^+$: 328.1632, found 328.1632.

4.1.1.16. *(2S)-N-(adamantan-1-yl)methyl)-2-amino-3-((R)-3-hydroxy-2-oxoindolin-3-yl)propanamide* **6m:**

According to the general procedure described above, spirolactone **9a** (208 mg, *ca* 0.55 mmol based on a 70:30 ratio of H- and Br-oxindole starting material) and adamantylmethylene amine (140 μL, 0.90 mmol) were reacted overnight. Purification by chromatography over silica gel (8 g, eluent 15% of AcOEt in CH_2Cl_2) afforded an orange amorphous solid (240 mg) that was hydrogenated according to the general procedure. Product **6m**, trifluoroacetate salt was obtained as a white solid (150 mg, 20% from Z-Trp).

^{1}H NMR (300 MHz, DMSO-d_6) δ 1.37-1.89 (m, 15H, adamantyl), 2.02 (d, *J* = 3.9 Hz, 1H, CHβ), 2.18-2.27 (m, 1H, CHβ), 2.70-2.77 (m, 2H, CH_2-NHCO), 4.38 (br s, 1H, Hα), 6.88 (d, *J* = 7.8 Hz, 1H aromatic), 7.02 (td, *J* = 7.5, 1 Hz, 1H aromatic), 7.24-7.32 (m, 2H aromatics), 8.02 (br s, NH_3^+, OH), 8.16 (t, *J* = 6 Hz, 1H, NH), 10.57 (s, 1H, NH oxindole). ^{13}C NMR (75.5 MHz, CD_3OD) δ 29.61 (CH adamantyl), 35.05 (C), 37.88 (CH_2), 39.36 (CH_2), 41.16 (CH_2), 50.81 (CHα), 52.35 (CH_2), 75.66 (C), 111.70 (CH), 124.06 (CH), 124.65 (CH), 131.16 (CH), 132.85 (C), 141.98 (C), 160.61 (CO), 170.17 (CO), 180.65 (CO). HRMS (ESI) calcd for $C_{22}H_{29}N_3O_3Na$ $[M+Na]^+$: 406.2101, found 406.2105.

4.1.1.17. *(S)-2-Amino-N-methyl-3-((S)-3-hydroxy-2-oxoindolin-3-yl)propanamide* **7a:** According to the general procedure described above, spirolactone **10a** (243 mg, *ca* 0.64 mmol based on a 70:30 ratio of H- and Br-oxindole starting material) and 2 M methylamine in THF (2 mL, 4 mmol) were reacted for 3h. Purification by chromatography over silica gel (8.1 g, eluent 10% of AcOEt in CH_2Cl_2 then then 25% AcOEt in CH_2Cl_2) afforded an orange amorphous solid (189 mg). MPLC purification of a small sample (20 mg) over reversed phase silica (C18, 5 g, 50 % MeOH in water) afforded after lyophilization, (*S*)-2-benzyloxycarbonylamino-*N*-methyl-3-((*S*)-3-hydroxy-2-oxoindolin-3-yl)propanamide (9 mg, ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.32-2.55 (m, 2H, $CH_2\beta$), 4.50 (m, 1H, Hα), 4.88 (broad s, 3H, CH_2Ph + OH), 5.80 (broad, 1H, NHZ), 6.73-7.31 (m, 9H, aromatic H), 9.22 (s, 1H, NH). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 26.2 (NCH_3), 39.8 , 51.2, 66.9 (OCH_2Ph), 75.7 (C_γ), 110.5-140.6 (aromatic C), 155.9 (C=O), 172.4 (C=O), 179.9 (C=O). R_f = 0.27 (RP18, 50 % MeOH in water), HRMS (LSIMS) calcd for $C_{20}H_{22}N_3O_5$ $[M+H]^+$: 384.1559, found 384.1555) and (*S*)-2-benzyloxycarbonylamino-*N*-methyl-3-(5-bromo-(*S*)-3-hydroxy-2-oxoindolin-3-yl)propanamide (5.5 mg, ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.16-2.59 (m, 2H, $CH_2\beta$), 2.72 (s, 3H, NCH_3), 4.48 (broad s, 1H, Hα), 4.90 (s, 2H, CH_2Ph), 5.84 (broad s, 1H, OH), 6.33 (broad , 1H, NH), 6.59-7.60 (m, 8H, aromatic H), 9.20 (broad s, 1H, NH). R_f = 0.13 (RP18, 50 % MeOH in water), HRMS (LSIMS) calcd for $C_{20}H_{21}{}^{79}Br\ N_3O_5$ $[M+H]^+$: 462.0665, found 462.0669).

As above, hydrogenation of the mixture (169 mg) afforded **7a**, HCl salt as a white solid (114 mg, 28 % from Z-Trp).

^{1}H NMR (200 MHz, D_2O + HCl) δ 2.39 (ABX system, $1H_A$, J_{AX} = 7 Hz, J_{AB} = 14.7 Hz, $CH_2\beta$), 2.55 (ABX system, $1H_B$, J_{BX} = 7 Hz, J_{AB} = 14.7 Hz, $CH_2\beta$), 2.69 (broad s, 3H, NCH_3), 4.13 (t, 1H, *J* = 7 Hz, Hα), 7.05 (d, 1H, *J* = 7.8 Hz ,H_7), 7.21 (t, 1H, *J* = 7.8 Hz, H_5), 7.33 (t, 1H, *J* = 7.8 Hz, H_6), 7.47 (d, 1H, *J* = 7.8 Hz, H_4). ^{13}C NMR (75.5 MHz, D_2O + HCl) δ 26.5 (Me), 37.7 (CH_2), 50.4 (CHα), 75.1 (C), 112.1 (CH), 124.5 (CH), 124.8 (CH), 129.6 (C), 131.5 (CH), 140.9 (C), 164.4 (C=O), 169.6 (C=O), 180.5 (C=O). HRMS (LSIMS) calcd for $C_{12}H_{16}N_3O_3$ $[M+H]^+$: 250.1192, found 250.1196.

4.1.1.18. *(S)-2-Amino-N-benzyl-3-((S)-3-hydroxy-2-oxoindolin-3-yl)propanamide* **7b:** According to the general procedure described above, spirolactone **10a** (351 mg, *ca* 0.93 mmol based on a 70:30 ratio of H- and Br-oxindole starting material) and benzylamine (120 µL, 1.10 mmol) were reacted overnight. Purification by chromatography over silica gel (8 g,

eluent 15% of AcOEt in CH_2Cl_2) afforded an orange amorphous solid (385 mg) that was hydrogenated according to the general procedure. Product **7b**, HCl salt was obtained as a white solid (290 mg, 39% from Z-Trp).

^{1}H NMR (300 MHz, D_2O) δ 2.32 and 2.46 (ABX system, 2H, J_{AX} = 7.5 Hz, J_{BX} = 6.2 Hz, J_{AB} = 14.9 Hz, CH_2β), 4.23 (t, 1H, *J* = 6.3 Hz, CHα), 4.28 (broad s, 2H, CH_2Ph), 6.93 (d, 1H, *J* = 7.8 Hz), 7.13 (t, 2H, *J* = 6.9 Hz), 7.23-7.42 (m, 6H).^{13}C NMR (75.5 MHz, D_2O) δ 37.8 (CH_2), 44.1 (CH_2), 50.4 (CHα), 75.1 (Cγ), 112.1 (CH), 124.4 (CH), 124.7 (CH), 128.3 (CH), 128.4 (CH), 129.5 (CH), 129.8 (C), 131.5 (CH), 137.7 (C), 140.8 (C), 158.5 (C=O), 169.1 (C=O), 180.6 (C=O). HRMS (ESI) calcd for $C_{18}H_{20}N_3O_3$ $[M+H]^+$: 326.1505, found 326.1512.

4.1.1.19. *(S)-2-Benzyloxyamino-N-benzyl-3-((S)-3-hydroxy-1-methyl-2-oxoindolin-3-yl)propanamide*:

According to the general procedure described above, spirolactone **10b** (1.60 g, 4.75 mmol) and benzylamine (560 µL, 5.12 mmol) were reacted overnight. Purification by chromatography over silica gel (60 g, eluent 10% of AcOEt in CH_2Cl_2) afforded (*S*)-2-benzyloxyamino-*N*-benzyl-3-((*S*)-3-hydroxy-1-methyl-2-oxoindolin-3-yl)propanamide as a beige solid (1.54 g, 69%). Mp 56-59 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.18 (dd, *J* = 14.7, 7.2 Hz, 1H), 2.56 (dd, *J* = 14.8, 6.8 Hz, 1H), 3.11 (s, 3H), 4.40 (m, 2H), 4.69 (m, 1H), 5.01 (m, 2H), 5.90 (br s, 1H), 6.77 (d, *J* = 7.7 Hz, 1H), 7.05 (t, *J* = 7.4 Hz, 1H), 7.17-7.36 (m, 14H). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 26.01 (CH_3), 39.70 (CH_2), 43.40 (CH_2), 51.23 (CH), 66.75 (CH_2), 74.91 (C), 108.47 (CH), 123.23 (CH), 123.77 (CH), 127.11 (CH), 127.40 (CH), 127.86 (CH), 127.93 (CH), 128.28 (CH), 128.39 (CH), 129.46 (CH), 130.49 (C), 135.98 (C), 137.68 (C), 142.43 (C), 155.89 (C), 171.93 (C), 177.47 (C). HRMS (ESI) calcd for $C_{27}H_{27}N_3O_5Na$ $[(M+Na)^+]$ 496.1843, found 496.1842. R_f (2/3 AcOEt/CH_2Cl_2) 0.28.

4.1.1.20. *(S)-2-Amino-N-benzyl-3-((S)-3-hydroxy-1-methyl-2-oxoindolin-3-yl)propanamide* **7c**: Hydrogenation of (*S*)-2-benzyloxyamino-*N*-benzyl-3-((*S*)-3-hydroxy-1-methyl-2-oxoindolin-3-yl)propanamide (972 mg, 2.05 mmol) according to the general procedure afforded **7c** as a white solid (697 mg, quantitative, 29% from Z-L-methyl tryptophan). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.85 (m, 2H), 3.09 (s, 3H), 3.71 (dd, *J* = 8.1, 5.4 Hz, 1H), 4.24 (s, 2H), 6.99 (d, *J* = 7.8 Hz, 1H), 7.05 (t, *J* = 7.5 Hz, 1H), 7.22-7.30 (m, 6H), 7.42 (d, *J* = 7.5 Hz, 1H), 8.42 (br s, 1H).

4.1.2. *Lactams 10 and 11*

Lactam10: ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.06 (dd, *J* = 8.8, 12.0 Hz, 1H, Hβ), 2.60 (d, *J* = 4.6 Hz, 3H, Me), 2.77 (dd, *J* = 12.3, 6.1 Hz, 1H, Hβ), 3.62 (dd, *J* = 8.8, 6.2 Hz, 1H, Hα), 5.25 (s, 2H, NH_2), 6.10 (s, 1H, OH), 6.50 (ddd, *J* = 8.6, 7.3, 1.3 Hz, 1H), 6.69 (dd, *J* = 8.0, 1.2 Hz), 6.82 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.00 (ddd, *J* = 8.7, 7.3, 1.6 Hz, 1H), 8.01 (q, *J* = 4.6 Hz, 1H), 8.31(br s, 1H, NH).

Lactam 11: ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.31 (dd, *J* = 13.5, 5.7 Hz, 1H, Hβ trans to Hα), 2.52 (m, 4H, Hβ cis to Hα and Me), 4.06 (dd, *J* = 7.5, 5.7 Hz, 1H, Hα), 5.05 (s, 2H, NH_2), 6.10 (s, 1H, OH), 6.48 (ddd, *J* = 8.1, 7.3, 1.3 Hz, 1H, H^2), 6.61 (dd, *J* = 8.0, 1.2 Hz, 1H, H^4), 6.94 (ddd, *J* = 8.8, 7.3, 1.6 Hz, 1H, H^3), 7.13 (dd, *J* = 7.7, 1.6 Hz, 1H, H^1), 7.94 (q, *J* = 4.6 Hz, 1H, NH amide), 8.24 (br s, 1H, NH lactam). ^{13}C NMR (DMSO, 100 MHz) δ 25.5 (CH_3), 39.4 (CH_2), 52.64 (CH), 115.2 (CH), 115.8 (CH), 125.2(C), 126.7 (CH), 127.7 (CH), 146.0 (C), 171.9 (CO amide), 176.4 (CO lactam).

4.1.3. *Synthesis of tripeptides* **1-2** *and dipeptides* **5**

4.1.3.1. *General procedure for the convergent synthesis of tripeptide* **1a***:* A mixture of Boc-Tyr(Bn)-Ala-OSu (278.1 mg, 0.515 mmol prepared from **13** (R^3 = Me) [16], DCC and HOSu according to the literature [52]) and oxidized tryptophan **6b**, HCl (152.3 mg, 0.421 mmol) in dry DMF (0.7 mL), triethylamine (60 µl, 0.43 mmol) and dry CH_2Cl_2 (1.3 mL) was stirred at room temperature for two days. After evaporation of the solvent under high vacuum and chromatography of the residue over silica gel (3% MeOH in CH_2Cl_2), tripeptide **1a** was afforded as a white solid (155.7 mg, 49%). Characterization datas of **1a** were previously published [17]. R_f (5/95 MeOH/CH_2Cl_2) 0.49. HPLC (column 1): t_R = 27.2 min, area percent >99% at 254 nm.

4.1.3.2. *General procedure for the linear synthesis of tripeptide* **1a:** To a solution of oxidized tryptophan HCl, **6b** (1.38 g, 3.83 mmol) and commercial Z-Ala-OSu (1.23 g, 3.83 mmol) in dry CH_2Cl_2 (25 mL) was added triethylamine (840 µL, 6 mmol). The mixture was stirred overnight at room temperature. The mixture was washed by water. The aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were dried over sodium sulfate and then concentrated under reduced pressure. After chromatography of the residue over silica gel (60 g, 40% AcOEt in CH_2Cl_2), Z-dipeptide **5a** was afforded as a white solid (1.80 g, 88%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.19 (d, *J* = 7.2 Hz, 3H), 2.18 (dd, *J* = 13.9,

9.0 Hz, 1H), 2.45-2.50 (m, 1H), 3.83-3.90 (m, 1H), 4.11 (dd, *J* = 15.3, 5.5 Hz, 1H), 4.16 (td, *J* = 8.6, 3.6 Hz, 1H), 4.25 (dd, *J* = 15.3, 6.4 Hz, 1H), 4.87 (d, *J* = 12.6 Hz, 1H), 4.98 (d, *J* = 12.6 Hz, 1H), 6.01 (s, 1H), 6.75 (d, *J* = 7.7 Hz, 1H), 6.94 (t, *J* = 7.4 Hz, 1H), 7.16-7.37 (m, 12H), 7.47 (d, *J* = 5.8 Hz, 1H), 7.91-7.96 (m, 2H), 10.08 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.3, 38.6, 42.0, 49.1, 50.9, 65.5, 74.3, 109.5, 121.4, 124.4, 126.5, 126.8, 127.0, 127.1, 127.7, 128.0, 128.2, 128.9, 131.0, 136.7, 139.1, 141.8, 156.2, 171.0, 171.9, 178.9. A mixture of the Z-dipeptide **5a** (1.80 g, 3.40 mmol) and 10 % palladium on charcoal (542 mg) in methanol (20 mL) was stirred overnight at room temperature under H_2 atmosphere. After filtration through celite, the filtrate was concentrated under reduced pressure to afford the deprotected dipeptide as a white solid (1.46 g, quantitative). ^{1}H NMR (300 MHz, CD_3OD+HCl) δ 1.44 (d, *J* = 7.2 Hz, 3H), 2.31 (dd, *J* = 14.4, 7.5 Hz, 1H), 2.50 (dd, *J* = 14.4, 5.1 Hz, 1H), 3.87 (q, *J* = 7.2 Hz, 1H), 4.09 and 4.30 (AB system, J_{AB} = 15.3 Hz, 2H), 4.43 (dd, *J* = 7.5, 5.1 Hz, 1H), 6.91 (d, *J* = 7.5 Hz, 1H), 7.06 (t, *J* = 7.5 Hz, 1H), 7.21-7.37 (m, 7 H). A mixture of the deprotected dipeptide (1.46 g, 3.68 mmol), commercial Boc-Tyr(Bn)-OSu (1.85 g, 3.96 mmol) and triethylamine (690 µL, 4.95 mmol) in dry CH_2Cl_2 (25 mL) was stirred overnight at room temperature. After dilution by CH_2Cl_2, the mixture was washed with water. The aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were dried over sodium sulfate and then concentrated under reduced pressure. After chromatography of the residue over silica gel (88 g, 50% AcOEt in CH_2Cl_2), tripeptide **1a** was afforded as a white solid (2.00 g, 73%). The overall yield from oxidized tryptophan **6b** was 64%. Characterization datas of **1a** were previously published [17].

4.1.3.3. *Tripeptide* **1b:** It was prepared by the convergent synthesis according to the general procedure starting from Boc-Tyr(Bn)-Ala-OSu (159.3 mg, 0.295 mmol) and **6a** (78.1 mg, 0.313 mmol) in DMF (0.3 mL). Tripeptide **1b** was afforded as a white solid (63 mg, 32%). Characterization datas of **1b** were previously published [17].

4.1.3.4. *Tripeptide* **1c:** It was prepared by the convergent synthesis according to the general procedure starting from Boc-Tyr(Bn)-Ala-OSu (1.00 g, 1.85 mmol) and **6c** (0.691 g, 2.04 mmol) in CH_2Cl_2 (20 mL). Tripeptide **1c** was afforded as a white solid (0.789 g, 56%).
^{1}H NMR (300 MHz, DMSO-d_6) δ 1.13 (d, *J* = 7.2 Hz, 3H), 1.27 (s, 9H), 2.20 (m 1H), 2.60 (m, 1H), 2.90 (m, 1H), 2.98 (s, 3H), 3.93 (m, 1H), 4.09-4.30 (m, 4H), 5.05 (s, 2H), 6.07 (s, 1H), 6.85-7.04 (m, 5H), 7.13-7.43 (m, 14H), 7.87-7.94 (m, 3H). ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 18.11 (CH_3), 25.71 (CH_3), 28.11 (CH_3), 36.23 (CH_2), 38.72 (CH_2), 42.12 (CH_2),

48.45 (CH), 49.17 (CH), 55.87 (CH), 69.12 (CH_2), 74.00 (C), 78.13 (C), 108.30 (CH), 114.33 (CH), 122.16 (CH), 123.90 (CH), 126.61 (CH), 126.91 (CH), 127.54 (CH), 127.69 (CH), 128.09 (CH), 128.35 (CH), 129.17 (CH), 130.15 (CH), 130.27 (C), 137.22 (C), 139.16 (C), 143.43 (C), 155.33 (C), 156.85 (C), 170.62 (C), 171.43 (C), 171.63 (C), 176.82 (C). HRMS (ESI) calcd for $C_{43}H_{49}N_5O_8Na$ [$(M+Na)^+$] 786.3473, found 786.3470. R_f (AcOEt) 0.44.

4.1.3.5. *Tripeptide* **1d:** A mixture of tripeptide **1a** (26 mg, 0.035 mmol) and 10% palladium on charcoal (5 mg) in MeOH (1 mL) was hydrogenated at atmospheric pressure overnight. After filtration over a pad of celite, the filtrate was concentrated and the resulting residue was washed with ether to afford **1d** as a white solid (22.7 mg, 99%). Characterization datas of **1d** were previously published [17].

4.1.3.6. *Tripeptide* **1e:** Tripeptide **1a** (34.29 mg, 0.0457 mmol) was dissolved in MeOH (0.1 mL) and treated at 0 °C for 30 min with anhydrous 12 M HCl in MeOH (0.2 mL, 2.4 mmol). The resulting mixture was then concentrated *in vacuo*. Purification by MPLC over RP silica (C18, MeOH/0.005 M aqueous HCl 40/60) afforded tripeptide **1e** (13.0 mg, 41%). Characterization datas of **1e** were previously published [17].

4.1.3.7. *Tripeptide* **1f:** It was prepared by the convergent synthesis according to the general procedure starting from Boc-Tyr(Bn)-Ala-OSu (231 mg, 0.428 mmol) and **6d**, HCl salt (216.5 mg, 0.435 mmol) in CH_2Cl_2 (1.5 mL). Tripeptide **1f** was afforded as a white solid (123.5 mg, 36% yield). It was also prepared by the linear synthesis according to the general procedure. Intermediate Z-dipeptide was obtained as a white solid (214 mg, 77%) from oxidized tryptophan, HCl salt **6d** (236 mg, 0.483 mmol) and Z-Ala-OSu (156 mg, 0.49 mmol).^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22 (d, *J* = 7.2 Hz, 3H), 2.36 (dd, *J* = 16.5, 4.2 Hz, 1H), 2.59 (dd, *J* = 16.5, 6.6 Hz, 1H), 4.21 (q, *J* = 7.2 Hz, 1H), 4.64 (m, 1H), 4.80-5.01 (m, 4H), 5.42 (s, 1H), 5.45 (d, *J* = 7.2 Hz, 1H), 6.59 (d, *J* = 7.8 Hz, 1H), 7.02-7.49 (m, 13H), 7.69-7.94 (m, 5H). Hydrogenation and then coupling with Boc-Tyr(Bn)-OSu (133 mg, 0.290 mmol) afforded tripeptide **1f** as a white solid (142 mg, 48%). The overall yield from oxidized tryptophan **6d** was 37%.

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.14 (d, *J* = 7.0 Hz, 3H, CH_3), 1.29 (s, 9H, Boc), 2.08 (dd, *J* = 14.5, 3.0 Hz, 1H, CHβ), 2.46 (dd, *J* = 14.5, 3.5 Hz, 1H, CHβ), 2.59 (dd, *J* = 14.5, 9.0 Hz, 1H, CHβ), 2.93 (dd, *J* = 15.0, 4.5 Hz, 1H), 4.06-4.13 (m, 2H, NCH_2), 4.23-4.28 (m, 1H, CHα), 4.57 (dd, *J* = 15.0, 5.5 Hz, 1H), 4.71 (dd, *J* = 15.0, 6.0 Hz, 1H), 5.05 (s, 2H, OCH_2),

6.01 (s, 1H, OH), 6.77 (d, *J* = 7.5 Hz, 1H), 6.87-6.95 (m, 4H aromatic), 7.13-7.55 (m, 14H), 7.83 (dd, *J* = 17.0, 7.5 Hz, 1H), 7.92-7.94 (m, 1H), 7.97-8.02 (m, 2H), 10.10 (s, 1H, NH oxindole). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 17.08 (CH_3), 28.73 (CH_3), 30.77 (CH_2), 37.83 (CH_2), 42.25 (CH_2), 51.26 (CH), 51.69 (CH), 57.41 (CH), 70.90 (CH_2), 76.37 (C), 80.89 (C), 114.42 (CH), 115.82 (CH), 123.86 (CH), 124.45 (CH), 125.50 (CH), 126.45 (CH), 126.81 (CH), 126.94 (CH), 127.36 (CH), 128.54 (CH), 128.80 (CH), 129.12 (CH), 129.46 (CH), 129.70 (C), 130.53 (CH), 130.91 (CH), 131.33 (CH), 131.76 (C), 132.92 (C), 134.66 (C), 135.20 (C), 138.79 (C), 142.92 (C), 157.87 (CO), 159.01 (C), 172.99 (CO), 174.67 (CO), 175.16 (CO), 181.50 (CO). HRMS (ESI) calcd for $C_{46}H_{49}N_5O_8Na$ $[M+Na]^+$ 822.3473, found 822.3474. R_f (5/95 MeOH/CH_2Cl_2) 0.21. HPLC (column 1): t_R = 29.9 min, area percent 98% at 254 nm.

4.1.3.8. *Tripeptide* **1g:** It was prepared by the convergent synthesis according to the general procedure starting from Boc-Tyr(Bn)-Ala-OSu (112.4 mg, 0.21 mmol) and **6e**, HCl salt (99.7 mg, 0.19 mmol) in CH_2Cl_2 (0.3 mL). Tripeptide **1g** was afforded as a white solid (67.5 mg, 42% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.16 (d, *J* = 7.0 Hz, 3H, CH_3), 1.29 (s, 9H, Boc), 2.10 (dd, *J* = 15.0, 4.5 Hz, 1H, CHβ), 2.39 (dd, *J* = 15.0, 4.5 Hz, 1H, CHβ), 2.92 (dd, *J* = 10.5, 4.5 Hz, 1H), 4.09-4.15 (m, 3H, NCH_2, CH), 4.24-4.29 (m, 1H, CHα), 5.05 (s, 2H, OCH_2), 6.01 (d, *J* = 8.5 Hz, 1H, $CH(Ph)_2$), 6.05 (s, 1H, OH), 6.76 (d, *J* = 8.0 Hz, 1H aromatic), 6.85-6.93 (m, 4H aromatics), 7.13-7.44 (m, 18H aromatics, 1NH), 7.89 (d, *J* = 7.5 Hz, 1H, NH), 8.00 (d, *J* = 9.0 Hz, 1H, NH), 8.17 (d, *J* = 9.0 Hz, 1H, NH-$CH(Ph)_2$), 10.08 (s, 1H, NH oxindole). NMR signal attribution resulted from HMBC and HSQC experiments. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (d, *J* = 7.2 Hz, 3H, CH_3), 1.37 (s, 9H, Boc), 2.40 (br s, 1H, H^4), 2.54-2.56 (m, 1H, H^4), 2.73 (m, 1H, H^{20}), 2.96 (m, 1H, H^{20}), 4.35 (m, 2H, H^{15}, H^{11}), 4.57 (m, 1H, H^5), 4.98 (s, 2H, H^{22}), 5.04 (m, 1H, H^{16}), 5.38 (br s, 1H, OH), 6.21 (d, *J* = 8.4 Hz, 1H, H^8), 6.72 (d, *J* = 7.8 Hz, 1H aromatic), 6.86 (d, *J* = 8.7 Hz, 1H aromatic), 6.98-7.42 (m, 21H aromatics, NH), 7.68 (d, *J* = 6.0 Hz, 1H, H^7), 7.81 (d, *J* = 7.8 Hz, 1H, H^9), 9.27 (s, 1H, H^1). ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 17.67 (C^{12}), 28.37 (C^{19}), 37.23 (C^{20}), 39.30 (C^4), 49.88 (C^{11}, C^5), 55.85 (C^{15}), 55.17 (C^8), 77.03 (C^{22}), 75.48 (C^3), 80.86 (C^{18}), 110.76 (CH), 115.07 (CH), 123.49 (CH), 124.34 (CH), 127.46 (CH), 127.52 (CH), 127.59 (CH), 127.79 (CH), 128.08 (CH), 128.6 (CH), 128.67 (CH), 128.71 (CH), 129.98 (CH), 130.44 (CH), 137.01 (C^{23}), 140.59 (C), 141.22 (C), 141.48 (C), 156.00 (C^{17}), 157.85 (C^{21}), 170.59 (C^6), 172.42 (C^{14}), 172.67 (C^{10}), 180.78 (C^2).

HRMS (ESI) calcd for $C_{48}H_{51}N_5O_8Na$ $[M+Na]^+$ 848.3630, found 848.3631. R_f (1/9 MeOH/CH_2Cl_2) 0.36. HPLC (column 1): t_R = 31.24 min, area percent 94% at 254 nm.

4.1.3.9. *Tripeptide* **1h:** It was prepared by the linear synthesis according to the general procedure. Intermediate Z-dipeptide was obtained as a white solid (149 mg, 60%) from oxidized tryptophan, TFA salt **6f** (211 mg, 0.41 mmol) and Z-Ala-OSu (156 mg, 0.49 mmol).^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (d, *J* = 6.9 Hz, 3H), 2.32-2.38 (m, 1H), 2.64 (dd, *J* = 15.6, 6.3 Hz, 1H), 3.17 (dd, *J* = 12.9, 6.6 Hz, 1H), 4.28 (t, *J* = 7.2 Hz, 1H), 4.35-4.61 (m, 2H), 4.72-4.78 (m, 1H), 5.06 (br s, 2H), 5.44-5.50 (m, 1H), 6.72 (t, *J* = 8.5 Hz, 1H), 7.06-7.26 (m, 4H), 7.33-7.58 (m, 13H), 7.78-7.88 (m, 2H). Deprotection of the Z group afforded a white solid (117 mg, quantitative).^{1}H NMR (300 MHz, CD_3OD) δ 1.44 (d, *J* = 7.2 Hz, 3H), 2.35 (dd, *J* = 14.5, 6.9 Hz, 1H), 2.53 (dd, *J* = 14.4, 5.1 Hz, 1H), 3.79 (br s, 1H), 4.15-4.45 (m, 3H), 6.92 (d, *J* = 7.5 Hz, 1H), 7.09 (td, *J* = 7.8, 1.0 Hz, 1H), 7.27-7.45 (m, 7H), 7.54-7.60 (m, 4H). Coupling with Boc-Tyr(Bn)-OSu (116 mg, 0.25 mmol) afforded tripeptide **1h** as a white solid (118 mg, 58%). The overall yield from oxidized tryptophan **6f** was 35%.
^{1}H NMR (500 MHz, DMSO-d_6) δ 1.20 (d, *J* = 7.0 Hz, 3H, CH_3), 1.29 (s, 9H, Boc), 2.11 (dd, *J* = 15.0, 10.0 Hz, 1H, CHβ), 2.44 (dd, *J* = 15.0, 5.0 Hz, 1H, CHβ), 2.58-2.61 (m, 1H, CH), 2.95 (dd, *J* = 15.0, 5.0 Hz, 1H), 4.07-4.24 (m, 4H, NCH_2, CH_2), 4.29 (dd, *J* = 15.0, 7.0 Hz, 1H, CHα), 5.04 (s, 2H, OCH_2), 6.02 (s, 1H, OH), 6.77 (d, *J* = 7.5 Hz, 1H aromatic), 6.87-6.95 (m, 4H aromatics), 7.14-7.45 (m, 13H aromatics, 1NH), 7.56 (d, *J* = 7.5 Hz, 2H aromatics), 7.61 (d, *J* = 8.0 Hz, 2H aromatics), 7.84 (d, *J* =8.5 Hz, 1H, 1NH), 7.96 (t, *J* = 5.5 Hz 1H, NH), 8.05 (d, *J* = 7.0 Hz, 1H, NH), 10.11 (s, 1H, NH oxindole).^{13}C NMR (75.5 MHz, DMSO-d_6) δ 17.59 (CH_3), 28.13 (CH_3), 30.38 (CH_2), 36.23 (CH_2), 41.89 (CH_2), 48.98 (CH), 49.10 (CH), 55.78 (CH), 69.11 (CH_2), 74.26 (C), 78.09 (C), 109.66 (CH), 114.29 (CH), 121.48 (CH), 124.45 (CH), 126.45 (CH), 126.53 (CH), 127.25 (CH), 127.59 (CH), 127.72 (CH), 127.93 (CH), 128.37 (CH), 128.86 (CH), 129.98 (CH), 130.16 (CH), 131.02 (C), 132.58 (C), 137.22 (C), 138.53 (C), 138.58 (C), 139.97 (C), 141.76 (C), 155.35 (C), 156.83 (CO), 170.95 (CO), 171.61 (CO), 172.23 (CO), 179.00 (CO). HRMS (ESI) calcd for $C_{48}H_{51}N_5O_8Na$ $[M+Na]^+$ 848.3630, found 848.3632. R_f (1/4 MeOH/CH_2Cl_2) 0.57. HPLC (column 1): t_R = 31.7 min, area percent 98% at 254 nm.

4.1.3.10. *Tripeptide* **1i:** It was prepared by the linear synthesis according to the general procedure. Intermediate Z-dipeptide was obtained as a white solid (249 mg, 78%) from oxidized tryptophan, TFA salt **6g** (267 mg, 0.55 mmol) and Z-Ala-OSu (195 mg, 0.61 mmol).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20 (s, 3H), 1.23 (s, 3H), 1.36 (d, *J* = 7.2 Hz, 3H), 2.31-2.37 (m, 1H), 2.61 (dd, *J* = 15.0, 6.0 Hz, 1H), 2.87 (m, 1H), 4.25-4.45 (m, 2H), 4.70 (br s, 1H), 5.07 (s, 1H), 5.44-5.54 (m, 2H), 6.70 (d, *J* = 6.5 Hz, 1H), 7.06-7.25 (m, 8H), 7.35-7.38 (m, 3H), 7.75 (br d, 2H), 7.87 (br s, 1H). Deprotection of the dipeptide afforded a white solid (188 mg, 98%).^{1}H NMR (300 MHz, CD_3OD) δ 1.22 (br s, 6H), 1.38 (d, *J* = 6.3 Hz, 3H), 2.34-2.46 (m, 1H), 2.52-2.56 (m, 1H), 2.84-2.88 (m, 1H), 3.77 (br s, 1H), 4.10-4.40 (m, 3H), 6.94-7.42 (m, 9H). Coupling with Boc-Tyr(Bn)-OSu (211 mg, 0.45 mmol) afforded tripeptide **1i** as a white solid (285 mg, 83%). The overall yield from oxidized tryptophan **6g** was 63%.

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.14 (s, 3H, CH_3), 1.15 (s, 3H, CH_3), 1.17 (d, *J* = 7.0 Hz, 3H, CH_3), 1.29 (s, 9H, Boc), 2.07 (dd, *J* = 15.0, 8.5 Hz, 1H, CHβ), 2.42 (dd, *J* = 15.0, 5.0 Hz, 1H, CHβ), 2.58-2.62 (m, 1H, CH), 2.79-2.85 (m, 1H, $CH(CH_3)_2$), 2.95 (dd, *J* = 15.0, 5.0 Hz, 1H), 4.07-4.21 (m, 5H, NCH_2,CH_2, CHα), 5.05 (s, 2H, OCH_2), 6.01 (s, 1H, OH), 6.77 (d, *J* = 7.5 Hz, 1H aromatic), 6.89-6.94 (m, 4H aromatics), 7.10-7.44 (m, 12H aromatics, 1NH), 7.80 (d, *J* =8.0 Hz, 1H, 1NH), 7.87 (t, *J* = 6.0 Hz, 1H, NH), 8.03 (d, *J* = 7.0 Hz, 1H, NH), 10.10 (s, 1H, NH oxindole).^{13}C NMR (75.5 MHz, $CDCl_3$) δ 17.64 (CH_3), 22.04 (CH_3), 28.34 (CH_3), 29.76, 33.79 (CH), 37.29 (CH_2), 39.48 (CH_2), 43.33 (CH_2), 49.86 (CH), 55.89 (CH), 69.98 (CH_2), 75.49 (C), 80.67 (C), 110.79 (CH), 115.00 (CH), 123.32 (CH), 124.32 (CH), 126.65 (CH), 127.53 (CH), 127.67 (CH), 128.00 (CH), 128.62 (CH), 129.91 (CH), 130.42 (CH), 130.62 (C), 135.38 (C), 137.04 (C), 140.77 (C), 147.93 (C), 148.03 (C), 156.00 (CO), 157.8 (C), 157.89 (CO), 171.42 (CO), 172.60 (CO), 180.68 (CO). HRMS (ESI) calcd for $C_{45}H_{53}N_5O_8Na$ $[M+Na]^+$ 814.3786, found 814.3782.R_f (1/9 MeOH/CH_2Cl_2) 0.77. HPLC (column 1): t_R = 31.8 min, area percent >99% at 254 nm.

4.1.3.11. *Tripeptide* **1j:** It was prepared by the linear synthesis according to the general procedure. Intermediate Z-dipeptide was obtained as a white solid (80.1 mg, 69%) from oxidized tryptophan, TFA salt **6h** (95.1 mg, 0.218 mmol) and Z-Ala-OSu (79.4 mg, 0.248 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.34 (d, *J* = 6.0 Hz, 3H), 2.46 (m, 4H), 3.39 (m, 2H), 3.62 (s, 3H), 4.25 (m, 1H), 4.45(m, 1H), 5.10 (s, 2H), 5.91 (d, *J* = 8.0 Hz, 1H), 6.64 (d, *J* = 7.8 Hz, 1H), 7.03 (t, *J* = 7.8 Hz, 1H), 7.16 (m, 2H), 7.36-7.38 (m, 6H), 7.78 (d, *J* = 7.8 Hz, 1H), 8.88 (br s, 1H). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 18.15 (CH), 33.56 (CH_2), 35.24 (CH_2), 39.58 (CH_2), 49.51 (CH), 50.86 (CH), 51.81 (CH_3), 67.09 (CH_2), 75.25 (C), 110.60 (CH), 123.31 (CH), 124.22 (CH), 127.94 (CH), 128.21 (CH), 128.57 (CH), 129.88 (CH), 130.25 (CH), 136.13 (C), 140.51 (C), 156.55 (C), 171.40 (C), 172.61 (C), 173.04 (C), 180.41 (C). Deprotection of the dipeptide afforded a white solid (56.9 mg, 95%). Coupling with Boc-

Tyr(Bn)-OSu (75.5 mg, 0.161 mmol) afforded tripeptide **1j** as a white solid (81.2 mg, 75%). The overall yield from oxidized tryptophan **6h** was 49%.

^{1}H NMR (300 MHz, CD_3OD) δ 1.36 (m, 12H), 2.41 (dd, *J* = 9.6, 14.1 Hz, 1H), 2.64-2.78 (m, 4H), 3.09 (dd, *J* = 4.8, 13.8 Hz, 1H), 4.17-4.39 (m, 4H), 4.56 (m, 1H), 5.04 (s, 2H), 6.83-6.91 (m, 2H), 7.01-7.43 (m, 11H). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 17.6 (CH_3), 28.2 (CH_3), 29.7 (CH_2), 33.6 (CH_2), 35.3 (CH_2), 39.6 (CH_2), 49.6 (CH), 49.7 (CH), 51.8 (CH_3), 55.9 (CH), 70.0 (CH_2), 75.3 (C), 80.7 (C), 110.6 (CH), 115.0 (CH), 123.3 (CH), 124.2 (CH), 127.4 (CH), 127.9 (CH), 128.5 (CH), 128.7 (C), 129.8 (CH), 130.4 (CH), 130.5 (C), 136.9 (C), 140.6 (C), 155.9 (C), 157.8 (C), 171.4 (C), 172.3 (C), 172.5 (C), 180.6 (C). HRMS (ESI) calcd for $C_{39}H_{47}N_5O_{10}Na$ [$(M+Na)^+$] 768.3215, found 768.3219. R_f (1/9 MeOH/AcOEt) 0.54. UPLC (column 2): t_R = 17.1 min, area percent >99% at 254 nm.

4.1.3.12. *Tripeptide* **1k:** To a solution of tripeptide **1j** (50.1 mg, 0.0671 mmol) in THF (0.5 ml) was added dropwise a 1 M aqueous LiOH (150 µL, 0.15 mmol). The mixture was stirred at room temperature for 24 h. The solvent was concentrated *in vacuo* and the residue was dissolved in 1 M hydrochloric acid (0.5 mL) and AcOEt (10 mL). The organic phase was dried over sodium sulfate and concentrated *in vacuo* to afford tripeptide **1k** as a white solid (49 mg, quantitative).

^{1}H NMR (300 MHz, DMSO-d_6) δ 1.24 (m, 12H), 2.03 (dd, *J* = 8.6, 14.2 Hz, 1H), 2.28 (m), 2.52 (m), 2.96 (m), 3.16 (m), 3.34 (m, 3H), 2.14 (s, 2H), 5.99 (s, 1H), 6.76 (d, *J* = 7.8 Hz, 1H), 6.90-7.42 (m, 14H), 7.75 (d, *J* = 7.8 Hz, 1H), 8.02 (d, *J* = 7.8 Hz, 1H), 10.08 (s, 1H). HRMS (ESI) calcd for $C_{38}H_{45}N_5O_{10}Na$ [$(M+Na)^+$] 754.3059, found 754.3062. UPLC (column 2): t_R = 17.4 min, area percent 70% at 254 nm.

4.1.3.13. *Tripeptide* **1l:** It was prepared by the linear synthesis according to the general procedure. Intermediate Z-dipeptide was obtained as a white solid (164 mg, 60%) from oxidized tryptophan, TFA salt **6i** (240 mg, 0.53 mmol) and Z-Ala-OSu (160 mg, 0.50 mmol). ^{1}H NMR (300 MHz, CD_3OD) δ 1.36 (d, *J* = 8.7 Hz, 3H), 2.42 (m, 1H), 2.52 (m, 1H), 2.74 (m, 2H), 3.30 (m, 2H), 3.99 (q, *J* = 8.7 Hz, 1H), 4.12 (m, 1H), 5.16 (s, 2H), 6.82 (d, *J* = 7.8 Hz, 1H), 7.07 (t, *J* = 7.8, 1H), 7.23-7.38 (m, 12H). Deprotection of the dipeptide (67.5 mg, 0.124 mmol) afforded a white solid (50.9 mg, quantitative). ^{1}H NMR (300 MHz, CD_3OD) δ 1.22 (d, *J* = 6.9 Hz, 3H), 2.28-2.45 (m, 2H), 2.72 (t, *J* = 7.2 Hz, 2H), 3.40 (m, 1H), 4.11 (dd, *J* = 9.3, 3.6 Hz, 1H), 6.85 (d, *J* = 7.8 Hz, 1H), 7.06 (t, *J* = 7.5 Hz, 1H), 7.15-7.30 (m, 6H), 7.33 (d, *J* =

7.5 Hz, 1H). Coupling with Boc-Tyr(Bn)-OSu (59 mg, 0.124 mmol) afforded tripeptide **1l** as a white solid (65 mg, 68%). The overall yield from oxidized tryptophan **6i** was 41%.

^{1}H NMR (300 MHz, CD_3COCD_3) δ 1.34 (m, 12H), 2.32 (dd, *J* = 8.1 Hz, 14.4 Hz, 1H), 2.64 (dd, *J* = 3.6, 14.4 Hz, 1H), 2.79 (t, *J* = 6.9 Hz, 2H), 2.91 (dd, *J* = 9.3, 13.8 Hz, 1H), 3.18 (dd, *J* = 4.8, 14.1 Hz, 1H), 3.41 and 3.36 (AB system, J_{AB} = 12 Hz, 2H),, 4.28-4.48 (m, 3H), 5.07 (s, 2H), 5.74 (br s, 1H), 6.25 , 6.86-7.02 (m, 4H), 7.14-7.56 (m, 16H), 7.79 (d, *J* = 5.7 Hz, 1H), 9.50 (s, 1H). ^{13}C NMR (75.5 MHz, CD_3COCD_3) δ 17.54 (CH_3), 28.60 (CH_3), 36.13 (CH_2), 37.56 (CH_2), 40.71 (CH_2), 41.70 (CH_2), 50.24 (CH), 50.70 (CH), 57.07 (CH), 70.36 (CH_2), 75.60 (C), 79.83 (C), 110.94 (CH), 115.48 (CH), 123.06 (CH), 125.13 (CH), 126.90 (CH), 128.34 (CH), 128.50 (CH), 129.16 (CH), 129.20 (CH), 129.59 (CH), 130.19 (CH), 130.65 (C), 131.24 (CH), 132.01 (C), 138.47 (C), 140.32 (C), 142.40 (C), 156.76 (C), 158.53 (C), 172.06 (C), 172.68 (C), 173.72 (C), 180.37 (C). HRMS (ESI) calcd for $C_{43}H_{49}N_5O_8Na$ $[M+Na]^+$ 786.3473, found 789.3484. R_f (5/95 MeOH/CH_2Cl_2) 0.31. UPLC (column 2): t_R = 20.9 min, area percent 92% at 254 nm.

4.1.3.14. *Tripeptide* **1m:** It was prepared by the linear synthesis according to the general procedure. Intermediate Z-dipeptide was obtained as a white solid (166 mg, 73%) from oxidized tryptophan, TFA salt **6j** (200 mg, 0.44 mmol) and Z-Ala-OSu (132 mg, 0.41 mmol). ^{1}H NMR (300 MHz, CD_3OD) δ 0.91 (m, 2H), 1.18-1.39 (m, 9H), 1.70 (m, 5H), 2.40 (t, *J* = 12.0 Hz, 1H), 2.62 (d, *J* = 15 Hz, 1H), 3.08-3.15 (m, 2H), 5.15 (s, 2H), 6.83 (d, *J* = 7.8 Hz, 1H), 7.07 (t, *J* = 7.8, 1H), 7.26-7.39 (m, 7H). Deprotection of the dippetide (58.2 mg, 0.105 mmol) afforded a white solid (44.1 mg, 96%). ^{1}H NMR (300 MHz, CD_3OD) δ 0.91 (m, 2H), 1.18-1.35 (m, 9H), 1.70 (m, 5H), 2.36 (dd, *J* = 14.1, 9.3 Hz, 1H), 2.50 (dd, *J* = 14.1, 3.9 Hz, 1H), 3.01-3.17 (m, 2H), 4.13 (dd, *J* = 9.3, 3.9 Hz, 1H), 6.86 (d, *J* = 7.8 Hz, 1H), 7.06 (t, *J* = 7.5 Hz, 1H), 7.26 (t, *J* = 7.8 Hz, 1H), 7.36 (d, *J* = 7.5 Hz, 1H). Coupling with Boc-Tyr(Bn)-OH (40.3 mg, 0.108 mmol) using EDC, HCl (24.5 mg, 0.127 mmol) and HOBt (21.1 mg, 0.137 mmol) afforded tripeptide **1m** as a white solid (45.3 mg, 56%). The overall yield from oxidized tryptophan **6j** was 39%.

^{1}H NMR (300 MHz, CD_3COCD_3) δ 0.90 (m, 3H), 1.12-1.42 (m, 17H), 1.60-1.76 (m, 5H), 2.19 (dd, *J* = 7.8 Hz, 14.7 Hz, 1H), 2.66 (dd, *J* = 4.8 Hz, 14.7 Hz, 1H), 2.91 (dd, *J* = 9.0 Hz, 14.1 Hz, 1H), 3.12-3.26 (m, 3H), 4.17-4.46 (m, 3H), 5.09 (s, 2H), 5.77 (s, 1H), 6.16 (d, *J* = 6.9 Hz, 1H), 6.85 (d, *J* = 7.8 Hz, 1H), 6.94 (d, *J* = 8.7 Hz, 2H), 7.00 (t, *J* = 7.5 Hz, 1H), 7.12-7.49 (m, 11H), 7.66 (d, *J* = 5.7 Hz, 1H), 9.29 (s, 1H). ^{13}C NMR (75.5 MHz, CD_3COCD_3) δ 17.49 (CH_3), 26.93 (CH_2), 27.26 (CH_2), 28.61 (CH_3), 33.77 (CH_2), 33.91 (CH_2), 35.74 (CH),

37.48 (CH_2), 37.89 (CH_2), 40.93 (CH_2), 50.32 (CH), 51.02 (CH), 57.34 (CH), 70.39 (CH_2), 75.66 (C), 79.90 (C), 110.93 (CH), 115.51 (CH), 123.02 (CH), 125.15 (CH), 128.36 (CH), 128.53 (CH), 129.22 (CH), 130.14 (CH), 130.56 (C), 131.23 (CH), 132.23 (C), 138.50 (C), 142.54 (C), 156.85 (C), 158.59 (C), 172.07 (C), 172.64 (C), 173.84 (C), 180.44 (C). HRMS (ESI) calcd for $C_{43}H_{55}N_5O_8Na$ $[M+Na]^+$ 792.3943, found 792.3944. R_f (1/9 MeOH/CH_2Cl_2) 0.50. UPLC (column 2): t_R = 23.9 min, area percent 99% at 254 nm.

4.1.3.15. *Tripeptide* **1n:** It was prepared by the linear synthesis according to the general procedure. Intermediate Z-dipeptide was obtained as a white solid (88 mg, 63%) from oxidized tryptophan, TFA salt **6k** (116 mg, 0.26 mmol) and Z-Ala-OSu (83 mg, 0.26 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88-0.92 (m, 3H), 1.17-1.20 (m, 1H), 1.42 (d, *J* = 8.7 Hz, 3H), 1.60-1.69 (m, 8H), 2.28-2.32 (m, 1H), 2.58 (dd, *J* = 15.0, 4.8 Hz, 1H), 3.03-3.07 (m, 2H), 4.29-4.34 (m, 1H), 4.71 (br s, 1H), 5.16 (s, 2H), 5.42 (br s, 1H), 6.74 (d, *J* = 7.8 Hz, 1H), 6.89 (m, 1H), 7.10 (td, *J* = 7.8, 1.0 Hz, 1H), 7.23 (d, *J* = 6.0 Hz, 1H), 7.36-7.38 (m, 6H), 7.83 (br s, 1H). Deprotection of the dippetide afforded a white solid (62 mg, 97%). ^{1}H NMR (300 MHz, CD_3OD) δ 0.88-0.92 (m, 3H), 1.13-1.17 (m, 2H), 1.40 (d, *J* = 6.0 Hz, 3H), 1.64-1.68 (m, 8H), 2.35-2.44 (m, 2H), 3.03 (br s, 2H), 3.69 (br s, 1H), 4.81 (br s, 1H), 6.85-7.10 (m, 3H), 7.23 (d, *J* = 8.1 Hz, 1H), 7.35 (d, *J* = 6.9 Hz, 1H), 8.62 (br s, 1H). Coupling with Boc-Tyr(Bn)-OSu (73 mg, 0.16 mmol) afforded tripeptide **1n** as a white solid (65 mg, 57%). The overall yield from oxidized tryptophan **6k** was 35%.

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89-0.94 (m, 3H), 1.18-1.23 (m, 1H), 1.40 (s, 9H), 1.68-1.73 (m, 9H), 2.46 (br s, 2H), 2.98-3.12 (m, 4H), 4.40-4.47 (m, 2H), 4.61-4.63 (m, 1H), 4.98 (br s, 1H), 5.04 (s, 2H), 5.79 (br s, 1H), 6.79-6.93 (m, 5H), 7.05-7.15 (m, 3H), 7.32 (td, *J* = 7.8, 1.2 Hz, 1H), 7.33-7.44 (m, 6H), 7.79 (d, *J* = 6.0 Hz, 1H), 8.40 (br s, 1H). ^{13}C NMR (75.5 MHz, CD_3OD) δ 15.84 (CH_3), 25.61 (CH_2), 26.14 (CH_2), 27.41 (CH_3), 30.50 (CH_2), 36.53 (CH_2), 37.47 (CH), 38.40 (CH_2), 45.61 (CH_2), 49.78 (CH), 50.23 (CH), 56.38 (CH), 69.62 (CH_2), 75.00 (C), 79.64 (C), 110.09 (CH), 114.61 (CH), 122.49 (CH), 124.11 (CH), 127.15 (CH), 127.44 (CH), 128.10 (CH), 129.29 (C), 129.54 (CH), 130.02 (CH), 130.35 (C), 137.38 (C), 141.48 (C), 156.63 (C),157.73 (C), 171.71 (C), 173.09 (C), 173.75 (C), 180.14 (C). HRMS (ESI) calcd for $C_{42}H_{53}N_5O_8Na$ $[M+Na]^+$ 778.3786, found 778.3787. R_f (1/9 MeOH/CH_2Cl_2) 0.48. UPLC (column 2): t_R = 22.4 min, area percent 92% at 254 nm.

4.1.3.16. *Tripeptide* **1o:** It was prepared by the convergent synthesis according to the general procedure starting from Boc-Tyr(Bn)-Ala-OSu (228 mg, 0.42 mmol) and **6l**, HCl salt (149

mg, 0.44 mmol) in CH_2Cl_2 (1.5 mL). Tripeptide **1o** was afforded as a white solid (224 mg, 73% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 0.78 (s, 9H, *t*-Bu), 1.20 (d, *J* = 7.0 Hz, 3H, CH_3), 1.30 (s, 9H, Boc), 2.04 (dd, *J* = 14.5, 4.0 Hz, 1H, CHβ), 2.35 (dd, *J* = 14.0, 4.0 Hz, 1H, CHβ), 2.66-2.84 (m, 3H, CH_2, CH), 2.95 (dd, *J* = 15.0, 4.0 Hz, 1H, CH), 4.09-4.21 (m, 3H, NCH_2, CHα), 5.05 (s, 2H, OCH_2), 6.04 (s, 1H, OH), 6.77 (d, *J* = 7.5 Hz, 1H aromatic), 6.90-6.96 (m, 4H aromatics), 7.13-7.44 (m, 8H aromatics, 2NH), 7.83 (d, *J* = 8.5 Hz, 1H, NH), 7.99 (d, *J* = 8.0 Hz, 1H, NH), 10.08 (s, 1H, NH oxindole). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 18.14 (CH_3), 27.28 (CH_3), 28.38 (CH_3), 32.18 (C), 37.36 (CH_2), 39.71 (CH_2), 49.57 (CH), 50.01 (CH), 50.87 (CH_2), 56.04 (CH), 70.06 (CH_2), 75.46 (C), 80.88 (C), 110.82 (CH), 115.12 (CH), 123.44 (CH), 124.32 (CH), 127.53 (C), 127.57 (CH), 128.06 (CH), 128.67 (CH), 129.95 (CH), 130.50 (CH), 130.70 (C), 137.05 (C), 140.76 (C), 156.05 (CO), 157.9 (C), 171.59 (CO), 172.22 (CO), 172.60 (CO), 180.92 (CO). HRMS (ESI) calcd for $C_{40}H_{51}N_5O_8Na$ $[M+Na]^+$ 752.3629, found 752.3631. R_f (1/9 MeOH/CH_2Cl_2) 0.37. HPLC (column 1): t_R = 28.9 min, area percent >99% at 254 nm.

4.1.3.17. *Tripeptide* **1p:** It was prepared by the convergent synthesis according to the general procedure starting from Boc-Tyr(Bn)-Ala-OSu (162 mg, 0.300 mmol) and **6m,** TFA salt (151 mg, 0.303 mmol) in CH_2Cl_2 (1.5 mL). Tripeptide **1p** was afforded as a white solid (140 mg, 58% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.21 (d, *J* = 7.0 Hz, 3H, CH_3), 1.30 (s, 9H, Boc), 1.36-1.88 (m, 15H, adamantyl), 2.04 (dd, *J* = 14.0, 5.0 Hz, 1H, CHβ), 2.36 (dd, *J* = 14.0, 5.0 Hz, 1H, CHβ), 2.63-2.74 (m, 3H, CH_2, CH), 2.96 (dd, *J* = 13.5, 4.5 Hz, 1H, CH), 4.09-4.19 (m, 3H, NCH_2, CHα), 5.05 (s, 2H, OCH_2), 6.03 (s, 1H, OH), 6.76 (d, *J* = 8.0 Hz, 1H aromatic), 6.91-6.95 (m, 4H aromatics), 7.14-7.44 (m, 8H aromatics, 2NH), 7.82 (d, *J* = 9.5 Hz, 1H, NH), 8.00 (d, *J* = 8.0 Hz, 1H, NH), 10.06 (s, 1H, NH oxindole). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 18.14 (CH_3), 28.26 (CH_3), 28.38 (CH_3), 33.94 (C), 36.96 (CH_2), 37.33 (CH_2), 39.80 (CH_2), 40.11 (CH_2), 49.58 (CH), 49.94 (CH), 51.26 (CH_2), 56.03 (CH), 70.04 (CH_2), 75.43 (C), 80.90 (C), 110.77 (CH), 115.11 (CH), 123.45 (CH), 124.35 (CH), 127.53 (C), 127.58 (CH), 128.07 (CH), 128.67 (CH), 129.93 (CH), 130.48 (CH), 130.75 (C), 137.02 (C), 140.71 (C), 156.05 (CO), 157.91 (C), 171.65 (CO), 172.22 (CO), 172.52 (CO), 180.94 (CO). HRMS (ESI) calcd for $C_{46}H_{57}N_5O_8Na$ $[M+Na]^+$ 830.4099, found 830.4101. R_f (1/9 MeOH/CH_2Cl_2) 0.51. HPLC (column 1): t_R = 34.5 min, area percent >99% at 254 nm.

4.1.3.18. *Tripeptide* **1q:** It was prepared by the convergent synthesis according to the general procedure starting from Boc-Tyr(Bn)-D-Ala-OSu (77.9 mg, 0.144 mmol) and **6b** (38.4 mg, 0.118 mmol) in DMF (0.2 mL) and CH_2Cl_2 (0.4 mL). Tripeptide **1q** was afforded as a white solid (67.4 mg, 76%).

^{1}H NMR (300 MHz, DMSO-d_6) δ 1.16 (d, J = 7 Hz, 3H, CH_3), 1.27 (s, 9H, Boc), 2.07 (dd, J = 10 Hz, J = 13.9 Hz, 1H), 2.5-2.8 (m, 3H), 4.06-4.27 (m, 5H), 5.05 (s, 2H), 6.02 (s, 1H, OH), 6.75 (d, J = 7.6 Hz, 1H), 6.87-7.00 (m, 4H), 7.09-7.43 (m, 14H), 8.08 (m, 3H), 10.10 (s, 1H, NH oxindole). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 17.6 (CH_3), 28.2 (CH_3), 38.0 (CH_2), 39.0 (CH_2), 43.5 (NCH_2Ph), 49.7 (CH), 50.2 (CH), 55.5 (CH), 69.9 (CH_2), 75.3 (C), 80.4 (C), 110.6 (CH), 114.9 (CH), 123.3 (CH), 124.2 (CH), 127.3 (CH), 127.4 (CH), 127.5 (CH), 127.9 (CH), 128.5 (CH), 128.7 (C), 129.8 (CH), 130.3 (CH), 130.8 (C), 136.9 (C), 137.9 (C), 140.4 (C), 155.7 (C), 157.7 (C), 171.5 (C), 171.9 (C), 173.1 (C), 180.6 (C). HRMS (ESI) calcd for $C_{42}H_{47}N_5O_8Na$ [$(M+Na)^+$] 772.3322, found 772.3328. R_f (1/9 MeOH/CH_2Cl_2) 0.40. HPLC (column 2): t_R = 20.4 min, area percent >99% at 254 nm.

4.1.3.19. *Tripeptide* **1r:** It was prepared by the convergent synthesis according to the general procedure starting from Boc-Tyr(Bn)-Gly-OH [16] (34.9 mg, 0.0813 mmol), EDC, HCl (19.5 mg, 0.101 mmol), HOBt (13.6 mg, 0.089 mmol), Et_3N (15 µL, 0.10 mmol) and **6b,** TFA salt (41.8 mg, 0.0855 mmol) in CH_2Cl_2 (1.4 mL). Tripeptide **1r** was afforded as a white solid (35.9 mg, 55%).

^{1}H NMR (300 MHz, CD_3OD) δ 1.35 (s, 9H), 2.40 (dd, J = 14.1, 8.4 Hz, 1H), 2.65-2.79 (m, 2H), 3.05 (dd, J = 14.1, 8.4 Hz, 1H), 3.72 and 3.81 (AB system, J_{AB} = 16.5 Hz, 2H), 4.21-4.41 (m, 4H), 5.04 (s, 2H), 6.85-6.93 (m, 3H), 7.05-7.41 (m, 15H). ^{13}C NMR (75.5 MHz, CD_3OD) δ 27.34 (CH_3), 36.69 (CH_2), 38.71 (CH_2), 42.60 (CH_2), 42.82 (CH_2), 49.78 (CH), 56.32 (CH), 69.59 (CH_2), 74.99 (C), 79.47 (C), 110.12 (CH), 114.53 (CH), 122.45 (CH), 124.03 (CH), 126.69 (CH), 127.03 (CH), 127.11 (CH), 129.41 (C), 127.41 (CH), 128.05 (CH), 129.56 (CH), 129.99 (CH), 130.25 (C), 137.42 (C), 138.26 (C), 141.52 (C), 156.46 (C), 157.68 (C), 169.90, (C), 171.82 (C), 173.92 (C), 180.20 (C). HRMS (ESI) calcd for $C_{41}H_{45}N_5O_8Na$ $[M+Na]^+$ 758.3160, found 758.3165. R_f (AcOEt) 0.38. UPLC (column 2): t_R = 19.9 min, area percent 92% at 254 nm.

4.1.3.20. *Tripeptide* **1s:** It was prepared by the linear synthesis according to the general procedure. Intermediate Z-dipeptide **5c** was obtained as a white solid (79.6 mg, 56%) by coupling of oxidized tryptophan, TFA salt **6b** (121 mg, 0.248 mmol) and Z-Asn-OH (71.62

mg, 0.269 mmol) in the presence of EDC (59.7 mg, 0.311 mmol), HOBt (46.5 mg, 0.304 mmol) and Et_3N (50 µL, 0.355 mmol) [52]. ^{1}H NMR (300 MHz, CD_3OD) δ 2.39 (dd, *J* = 14.1, 8.4 Hz, 1H), 2.61-2.80 (m, 3H), 3.74 (br s, 1H), 4.23-4.36 (m, 3H), 6.89 (d, *J* = 8.1 Hz, 1H), 7.06 (t, *J* = 8.1 Hz, 1H), 7.19-7.29 (m, 6H), 7.38 (d, *J* = 8.1 Hz, 1H). ^{13}C NMR (75.5 MHz, DMSO-d_6) δ: 37.51, 42.67, 49.78, 52.23, 65.36, 66.08, 74.72, 110.09, 121.98, 124.94, 127.01, 127.37, 128.16, 128.52, 128.79, 129.37, 131.63, 137.28, 139.71, 142.10, 156.39, 171.01, 171.64, 172.26, 178.50. Deprotection of **5c** (61.5 mg, 0.107 mmol) afforded a white solid (47.1 mg, 100%). Coupling with Boc-Tyr(Bn)-OSu (55.25 mg, 0.118 mmol) afforded tripeptide **1s** as a white solid (48.0 mg, 56%). The overall yield from oxidized tryptophan **6b** was 31%.

^{1}H NMR (300 MHz, CD_3OD) δ 1.35 (m, 9H), 2.40 (dd, *J* = 14.2, 9.6 Hz, 1H), 2.64-2.84 (m, 4H), 3.09 (dd, *J* = 14.2, 5.0 Hz, 1H), 4.13-4.39 (m, 4H), 4.57 (t, *J* = 6.0 Hz), 5.05 (s, 2H), 6.84 (d, *J* = 7.7 Hz, 1H), 6.90 (d, *J* = 8.7 Hz, 2H), 7.04 (t, *J* = 7.7 Hz, 1H), 7.14-7.45 (m, 14H). ^{13}C NMR (75.5 MHz, CD_3OD) δ 28.71 (CH_3), 37.26 (CH_2), 37.75 (CH_2), 39.54 (CH_2),44.19 (CH_2), 51.58 (CH), 51.87 (CH), 57.98 (CH), 71.02 (CH_2), 76.44 (C), 81.02 (C), 111.53 (CH), 115.97 (CH), 123.87 (CH), 125.42 (CH), 128.02 (CH), 128.52 (CH), 128.53 (CH), 128.81 (CH), 129.39 (CH), 129.47 (CH), 130.91 (CH), 131.40 (CH), 131.76 (C), 139.82 (C), 139.77 (C), 142.87 (C), 159.09 (C), 172.53 (C), 173.16 (C), 174.66 (C), 174.88 (C), 179.64 (C), 181.53 (C). HRMS (ESI) calcd for $C_{43}H_{48}N_6O_9Na$ $[M+Na]^+$ 815.3375, found 815.3383. R_f (1/9 MeOH/AcOEt) 0.47. UPLC (column 2): t_R = 19.0 min, area percent >99% at 254 nm.

4.1.3.21. *Tripeptide* **1t:** It was prepared by the linear synthesis according to the general procedure. Intermediate Z-dipeptide **5b** was obtained as a white solid (54.0 mg, 85%) by coupling of oxidized tryptophan, TFA salt **6b** (50.0 mg, 0.114 mmol) and commercial Z-L-Thr-OSu (41.8 mg, 0.119 mmol). ^{1}H NMR (300 MHz, DMSO) δ 0.99 (d, *J* = 6.3 Hz, 3H), 2.05 (dd, *J* = 14.7, 8.7 Hz, 1H), 2.44 (dd, *J* = 14.7, 3.9 Hz, 1H), 3.87-3.98 (m, 2H), 4.09-4.23 (m, 2H), 4.29-4.35 (m, 1H), 4.94-5.08 (m, 3H), 6.02 (s, 1H), 6.74 (d, *J* = 7.5 Hz, 1H), 6.87-6.94 (m, 2H), 7.14-7.37 (m, 12H), 7.97 (d, *J* = 8.1 Hz, 1H), 8.05 (t, *J* = 6.0 Hz, 1H), 10.12 (s, 1H). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 18.50 (CH_3), 38.93 (CH_2), 43.65 (CH_2), 50.10 (CH), 60.19 (CH), 67.32 (CH), 67.41 (CH_2), 75.78 (C), 110.88 (CH), 123.44 (CH), 124.22 (CH), 127.38 (CH), 127.75 (CH), 128.07 (CH), 128.33 (CH), 128.61 (CH), 128.65 (CH), 129.95 (CH), 130.58 (C), 136.08 (C), 137.88 (C), 140.46 (C), 157.11 (C), 170.92 (C), 171.34 (C), 180.53 (C). Deprotection of **5b** (48.0 mg, 0.0856 mmol) afforded a white solid (37 mg, 100%). ^{1}H NMR (300 MHz, CD_3OD) δ 1.18 (d, *J* = 6.3 Hz, 3H), 2.34 (dd, *J* = 14.1, 6.9 Hz,

1H), 2.54 (dd, *J* = 14.1, 5.7 Hz, 1H), 3.62 (d, *J* = 6.3 Hz, 1H), 4.03 (q, *J* = 6.3 Hz, 1H), 4.08 and 4.29 (AB system, J_{AB} = 15.0 Hz, 2H), 4.47 (dd, *J* = 6.9, 5.7 Hz, 1H), 6.91 (d, *J* = 7.8 Hz, 1H), 7.08 (t, *J* = 7.5 Hz, 1H), 7.20-7.40 (m, 7H). Coupling with Boc-Tyr(Bn)-OSu (40.6 mg, 0.0866 mmol) afforded tripeptide **1t** as a white solid (21 mg, 31%). The overall yield from oxidized tryptophan **6b** was 26%.

^{1}H NMR (300 MHz, DMSO) δ 0.98 (d, *J* = 6.3 Hz, 3H), 1.31 (s, 9H), 2.04 (dd, *J* = 14.7, 9.6 Hz, 1H), 2.38 (m, 1H), 2.64 (m, 1H), 2.95 (dd, *J* = 15.3, 3.9 Hz, 1H), 3.98-4.38 (m, 5H), 5.06 (s, 2H), 5.11 (d, *J* = 6.3 Hz, 1H), 6.04 (s, 1H), 6.76 (d, *J* = 7.8 Hz, 1H), 6.89-7.45 (m, 15H), 7.05-7.41 (m, 17H), 7.65 (d, *J* = 7.8 Hz, 1H), 7.88 (d, *J* = 8.4 Hz, 1H), 8.12 (m, 1H), 10.17 (s, 1H). ^{13}C NMR (75.5 MHz, CD_3COCD_3) δ 20.11(CH_3), 28.57 (CH_3), 36.85 (CH_2), 40.43 (CH_2), 43.56 (CH_2), 50.09 (CH), 57.67, 60.11, 67.04, 70. 42 (CH_2), 75.93 (C), 80.35 (C), 110.97 (CH), 115.57 (CH), 123.24 (CH), 125.17 (CH), 127.62 (CH), 128.25 (CH), 128.36 (CH), 128.55 (CH), 129.07 (CH), 129.25 (CH), 130.33 (C), 131.20 (CH), 132.05 (CH), 138.54 (C), 140.04 (C), 142.49 (C), 157.16 (C), 158.65 (C), 170.80 (C), 171.86 (C), 173.85 (C), 180.39 (C), 209.96 (C). HRMS (ESI) calcd for $C_{43}H_{49}N_5O_9Na$ $[M+Na]^+$ 802.3422, found 802.3429. R_f (AcOEt) 0.19. UPLC (column 2): t_R = 19.9 min, area percent 96% at 254 nm.

4.1.3.22. *Tripeptide* **1u:** It was prepared by the convergent synthesis according to the general procedure starting from Boc-Tyr(Bn)-Lys(Boc)-OSu [16] (236.7 mg, 0.340 mmol) and oxidized tryptophan **6b** (118.0 mg, 0.363 mmol) in DMF (0.7 mL) and CH_2Cl_2 (1.3 mL). A first crop of **1u** precipitated in CH_2Cl_2 as a white solid (54.0 mg, 18%) before chromatography. A second fraction was afforded as a white solid after chromatography (39.6 mg, 13% ; 31% overall yield).

^{1}H NMR (300 MHz, DMSO-d_6) δ 1.05-1.57 (m, 24H), 2.01 (dd, *J* = 8.3, 14.1 Hz, 1H), 2.39 (dd, *J* = 14.1, 4.3 Hz, 1H), 2.67 (m, 1H), 2.88 (m, 3H), 4.08-4.35 (m, 5H), 5.05 (s, 2H), 6.02 (s, 1H, OH), 6.74-6.94 (m, 6H), 7.16-7.45 (m, 14H), 7.93 (m, 3H), 10.12 (s, 1H, NH). ^{13}C NMR (125.5 MHz, CD_3OD) δ 24.3(CH_2), 28.8 (CH_3), 28.9 (CH_3), 30.5 (CH_2), 32.0 (CH_2), 37.9 (CH_2), 39.7 (CH_2), 41.1 (CH_2), 44.1 (CH_2), 51.3 (CH), 55.6 (CH), 57.6 (CH), 71.0 (CH_2), 76.4 (C), 79.9 (C), 80.9 (C), 111.5 (CH), 116.0 (CH), 123.9 (CH), 125.6 (CH), 128.2 (CH), 128.6 (CH), 128.7 (CH), 128.9 (CH), 129.5 (CH), 130.8 (C), 131.0 (CH), 131.4 (CH), 131.9 (C), 138.9 (C), 139.7 (C), 142.9 (C), 158.0 (C), 158.6 (C), 159.1 (C), 173.2 (C), 173.9 (C), 175.3 (C), 181.6 (C). HRMS (ESI) calcd for $C_{50}H_{62}N_6O_{10}Na$ $[(M+Na)^+]$ 929.4420, found 929.4424. R_f (1/9 MeOH/CH_2Cl_2) 0.50. UPLC (column 2): t_R = 22.5 min, area percent >99% at 254 nm.

4.1.3.23. *Tripeptide* **1v:** Tripeptide **1u** (43.33 mg, 0.0478 mmol) was dissolved in MeOH (0.1 mL) and treated with anhydrous 6 M HCl in MeOH (0.15 mL, 0.9 mmol) at 0 °C for 10 min and then 10 min at room temperature. The resulting mixture was then concentrated *in vacuo* to afford tripeptide **1v** (37 mg, quantitative).

^{1}H NMR (500 MHz, D_2O) δ 1.05 (m, 1H, CH Lys), 1.12 (m, 1H, CH Lys), 1.43 (m, 2H, CH_2 Lys), 1.53 (m, 2H, CH_2 Lys), 2.39 (dd, *J* = 6.5 Hz, *J* = 13.8 Hz, 1H, CH oxidized Trp), 2.58 (dd, *J* = 7.4 Hz, *J* = 13.8 Hz, 1H, CH oxidized Trp), 2.64 (m, 2H, CH_2N Lys), 3.03 (dd, *J* = 2.3 Hz, *J* = 7.1 Hz, 2H, CH_2 Tyr), 3.80 (d, *J* = 15.4 Hz, 1H, COCHN), 3.97 (m, 2H, COCHN and CHα oxidized Trp), 4.12 (m, 2H, CHα Lys and Tyr), 5.03 (s, 2H, OCH_2Ph), 6.90 (m, 3H, CH ar), 7.07 (m, 5H, CH ar), 7.23-7.37 (m, 10H, CH ar). ^{13}C NMR (125.5 MHz, D_2O) δ 21.8 (CH_2), 26.2 (CH_2), 30.5 (CH_2), 35.9 (CH_2), 37.3 (CH_2), 39.0 (CH_2), 43.0 (CH_2), 50.1 (CH), 53.1 (CH), 54.1 (CH), 70.3 (CH_2), 75.0 (C), 123.6 (CH), 124.7 (CH), 126.4 (CH), 126.9 (CH), 127.3 (CH), 128.1 (CH), 128.3 (CH), 128.4 (CH), 128.6 (CH), 128.8 (C), 128.9 (CH), 130.7 (CH), 130.8 (C), 136.4 (C), 137.5 (C), 140.6 (C), 157.5 (C), 168.7 (C), 171.5 (C), 171.8 (C), 180.0 (C). HRMS (ESI) calcd for $C_{40}H_{47}N_6O_6$ [$(M+H)^+$] 707.3552, found 707.3556. UPLC (column 2): t_R = 10.9 min, area percent 89% at 254 nm.

4.1.3.24. *Tripeptide* **2a:** It was prepared by the convergent synthesis according to the general procedure starting from Boc-Tyr(Bn)-Ala-OSu (105.6 mg, 0.195 mmol) and oxidized tryptophan **7b** (65.4 mg, 0.201 mmol) in DMF (0.3 mL) and CH_2Cl_2 (0.5 mL). Tripeptide **2a** was afforded as a white solid (76.13 mg, 52%). Characterization datas of **2a** were previously published [17].

4.1.3.25. *Tripeptide* **2b:** It was prepared by the convergent synthesis according to the general procedure starting from Boc-Tyr(Bn)-Ala-OSu (115 mg, 0.213 mmol) and **7a** (66.1 mg, 0.265 mmol, containing 10% **6a**). Tripeptide **2b** was afforded as a white solid (59.5 mg, 42%) which contained 10% **1b**. Characterization datas of **2b** were previously published [17].

4.1.3.26. *Tripeptide* **2c:** It was prepared by the convergent synthesis according to the general procedure starting from Boc-Tyr(Bn)-Ala-OSu (1.00 g, 1.85 mmol) and **7c** (0.690 g, 2.03 mmol) in CH_2Cl_2 (20 mL). Tripeptide **2c** was afforded as a white solid (0.890 g, 63%).

^{1}H NMR (300 MHz, DMSO-d_6) δ 1.07 (d, *J* = 7.2 Hz, 3H), 1.29 (s, 9H), 2.15-2.32 (m, 2H), 2.61 (dd, *J* = 13.8, 10.8 Hz, 1H), 2.96 (dd, *J* = 13.8, 3.9 Hz, 1H), 3.03 (s, 3H), 3.81 (q, *J* = 6.6 Hz, 1H), 4.07-4.29 (m, 4H), 5.05 (s, 2H), 6.15 (s, 1H), 6.82-7.01 (m, 4H), 7.14-7.44 (m, 15H), 7.76-7.81 (m, 2H), 8.05 (br s 1H). ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 18.07 (CH_3), 25.76 (CH_3), 28.10 (CH_3), 36.29 (CH_2), 38.65 (CH_2), 42.13 (CH_2), 48.29 (CH), 49.50 (CH), 55.82 (CH), 69.11 (CH_2), 74.31 (C), 78.12 (C), 108.37 (CH), 114.33 (CH), 122.05 (CH), 123.86 (CH), 126.62 (CH), 126.94 (CH), 127.52 (CH), 127.68 (CH), 128.10 (CH), 128.34 (CH), 129.03 (CH), 130.13 (CH), 130.34 (C), 130.74 (C), 137.22 (C), 139.15 (C), 142.92 (C), 155.36 (C), 156.84 (C), 170.76 (C), 171.15 (C), 171.29 (C), 176.67 (C). HRMS (ESI) calcd for $C_{43}H_{49}N_5O_8Na$ [$(M+Na)^+$] 786.3473, found 786.3472. R_f (AcOEt) 0.33.

4.1.3.27. *Dipeptide* **5d:** A mixture of the deprotected dipeptide obtained from **5a** (59.24 mg, 0.149 mmol), 3-PhO-C_6H_4-CH_2-CO_2Su (55.46 mg, 0.1739 mmol) and triethylamine (26 µL, 0.18 mmol) in dry CH_2Cl_2 (1.5 mL) was stirred overnight at room temperature. After dilution by CH_2Cl_2, the mixture was washed with water. The aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were dried over sodium sulfate and then concentrated under reduced pressure. After chromatography of the residue over silica gel (15 g, 5% MeOH in CH_2Cl_2), dipeptide **5d** was afforded as a white solid (20.0 mg, 23%).
^{1}H NMR (300 MHz, DMSO-d_6) δ 1.18 (d, *J* = 7.1 Hz, 3H, CH_3), 2.11 (dd, *J* = 14.1, 9.2 Hz, 1H, CHβ), 2.44 (m, 1H, CHβ), 3.43 and 3.50 (AB system, J_{AB} = 14.6 Hz, 2H), 3.96-4.17 (m, 4H), 6.01 (s, 1H, OH), 6.72 (d, *J* = 7.6 Hz, 1H), 6.83-7.02 (m, 6H), 7.10-7.44 (m, 11H), 7.80-7.86 (m, 2H), 8.31 (d, *J* = 6.0 Hz, 1H, NH), 10.06 (s, 1H, NH oxindole).^{13}C NMR (75.5 MHz, CD_3COCD_3) δ 17.41 (CH_3), 40.44 (CH_2), 43.08 (CH_2), 43.57 (CH_2), 50.53 (CH), 51.14 (CH), 75.69 (C), 110.85 (CH), 117.76 (CH), 119.63 (CH), 120.68 (CH), 123.03 (CH), 124.14 (CH), 125.08 (CH), 125.22 (CH), 127.56 (CH), 128.19 (CH), 129.06 (CH), 130.26 (CH), 130.60 (CH), 130.74 (CH), 132.00 (C), 138.87 (C), 140.18 (C), 142.69 (C), 158.14 (C), 172.07 (C), 172.42 (C), 172.95 (C), 180.30 (C). HRMS (ESI) calcd for $C_{35}H_{34}N_4O_6Na$ [$(M+Na)^+$] 629.2370, found 629.2373. R_f (1/9 MeOH/ CH_2Cl_2) 0.63. UPLC (column 2): t_R = 16.0 min, area percent >99% at 254 nm.

4.1.3.28. *Dipeptide* **5e:** A mixture of the deprotected dipeptide obtained from **5a** (45.85 mg, 0.115 mmol), 4-BnO-C_6H_4-CO_2Su (42.96 mg, 0.139 mmol) and triethylamine (20 µL, 0.14 mmol) in dry CH_2Cl_2 (1 mL) was stirred overnight at room temperature. After dilution by CH_2Cl_2, the mixture was washed with water. The aqueous phase was extracted three times

with CH_2Cl_2. The combined organic phases were dried over sodium sulfate and then concentrated under reduced pressure. After chromatography of the residue over silica gel (12 g, 5% MeOH in CH_2Cl_2), dipeptide **5e** was afforded as a white solid (21.7 mg, 31%).

^{1}H NMR (300 MHz, DMSO-d_6) δ 1.32 (d, *J* = 7.2 Hz, 3H, CH_3), 2.16 (dd, *J* = 13.8, 9.3 Hz, 1H, CHβ), 2.50 (1H, CHβ), 4.09-4.30 (m, 4H, NCH_2, CHα), 5.19 (s, 2H, OCH_2), 5.98 (s, 1H, OH), 6.76 (d, *J* = 7.8 Hz, 1H), 6.95 (t, *J* = 7.5 Hz, 1H), 7.04 (d, *J* = 8.9 Hz, 2H), 7.16-7.48 (m, 12H), 7.83 (d, *J* =8.9 Hz, 2H), 7.89 (d, *J* =8.4 Hz, 1H), 7.97 (t, *J* = 6.0 Hz, 1H, NH), 8.33 (d, *J* = 5.8 Hz, 1H, NH), 10.10 (s, 1H, NH oxindole).^{13}C NMR (75.5 MHz, CD_3COCD_3) δ 17.36 (CH_3), 40.16 (CH_2), 43.59 (CH_2), 50.42 (CH), 51.87 (CH), 70.59 (CH_2), 75.76 (C), 110.83 (CH), 115.25 (CH), 123.09 (CH), 125.18 (CH), 127.14 (C), 127.61 (CH), 128.29 (CH), 128.45 (CH), 128.79 (CH), 129.09 (CH), 129.37 (CH), 130.27 (CH), 130.42 (CH), 132.01 (C), 137.95 (C), 140.13 (C), 142.63 (C), 162.46 (C), 168.40 (C), 172.27 (C), 173.29 (C), 180.30 (C). HRMS (ESI) calcd for $C_{35}H_{34}N_4O_6Na$ [$(M+Na)^+$] 629.2370, found 629.2377. R_f (1/9 MeOH/CH_2Cl_2) 0.66. UPLC (column 2): t_R = 15.7 min, area percent 98% at 254 nm.

4.1.3.29. *Dipeptide* **5f:** A mixture of the deprotected dipeptide obtained from **5b** (57 mg, 0.133 mmol), 4-BnO-C_6H_4-CO_2H (33 mg, 0.14 mmol), EDC, HCl (35 mg, 0.18 mmol), HOBt, H_2O (25 mg, 0.16 mmol) and triethylamine (45 µL, 0.33 mmol) in a mixture of dry CH_2Cl_2 (0.4 mL) and DMF (0.2 mL) was stirred overnight at room temperature. After dilution by CH_2Cl_2, the mixture was washed with water. The aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were dried over sodium sulfate and then concentrated under reduced pressure. After chromatography of the residue over silica gel (5 g, AcOEt), dipeptide **5f** was afforded as a white solid (60.9 mg, 72%).

^{1}H NMR (300 MHz, DMSO-d_6) δ 1.07 (d, *J* = 6.3 Hz, 3H), 2.10 (dd, *J* = 13.8, 9.0 Hz, 1H), 2.47 (m, 1H), 4.09-4.32 (m, 5H), 5.05 (d, *J* = 6.3 Hz, 1H), 5.20 (s, 2H), 6.03 (s, 1H), 6.76 (d, *J* = 7.8 Hz, 1H), 6.93 (t, *J* = 7.5 Hz, 1H), 7.09 (d, *J* = 8.7 Hz, 2H), 7.17-7.49 (m, 12H), 7.78 (d, *J* = 7.8 Hz, 1H), 7.84 (d, *J* = 8.7 Hz, 2H), 8.04 (d, *J* =8.4 Hz, 1H), 10.13 (s, 1H). ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 19.76 (CH_3), 38.41 (CH_2), 42.22 (CH_2), 49.13 (CH), 59.46 (CH), 66.53 (CH), 69.34 (CH_2), 74.34 (C), 109.68 (CH), 114.37 (CH), 121.54 (CH), 124.49 (CH), 126.39 (C), 126.63 (CH), 126.98 (CH), 127.73 (CH), 127.94 (CH), 128.15 (CH), 128.47 (CH), 129.04 (CH), 129.36 (CH), 131.17 (C), 136.69 (C), 139.12 (C), 141.74 (C), 160.83 (C), 166.27 (C), 169.71 (C), 171.02 (C), 179.09 (C). HRMS (ESI) calcd for $C_{36}H_{36}N_4O_7Na$ [$(M+Na)^+$] 659.2476, found 659.2478. R_f (AcOEt) 0.20. UPLC (column 2): t_R = 15.7 min, area percent 99% at 254 nm.

4.2. Enzyme studies

Purified human 20S constitutive proteasome cCP and 20S immunoproteasome iCP were obtained from Boston Biochem (Bio-Techne, Lille, France). The fluorogenic substrates Suc-LLVY-AMC (ChT-L activity, cCP and iCP), Z-LLE-*β*NA (C-L activity, cCP) and Boc-LRR-AMC (T-L activity, cCP and iCP) were obtained from Bachem (Bubendorf, Switzerland) and Ac-PAL-AMC (C-L activity, iCP) from Boston Biochem (Bio-Techne, Lille, France).

Proteasome activities were determined by monitoring the hydrolysis of the appropriate substrate (λ_{exc} = 360 nm and λ_{em} = 460 nm for AMC substrates; λ_{exc} = 340 nm and λ_{em} = 404 nm for the βNA substrate). The enzyme-catalyzed fluorescence emission was followed for 45 min at 37°C in microplates using the FLUOstar Optima (BMG Labtech). The enzymes were either untreated (controls) or treated by various concentrations of the tested compounds. Before use, substrates and compounds were previously dissolved in DMSO. The percentage of this co-solvent was maintained equal to 2% (v/v) in all experiments. In addition to DMSO, the buffers (pH 8) used to measure ChT-L and C-L activities contained 20 mM Tris-HCl, 10% glycerol, 0.01% (w/v) SDS, whereas SDS was replaced with 0.005% Triton X-100 (w/v) in the buffer used for T-L activity. The enzyme concentrations were 0.3 nM in both cases; the substrate concentrations were 20 µM for Suc-LLVY-AMC (ChT-L activity of cCP and iCP), 50 µM for Z-LLE-*β*NA (C-L activity of cCP), 50 µM for Ac-PAL-AMC (C-L activity of iCP) and 50 µM for Boc-LRR-AMC (T-L activity of cCP and iCP). To determine the IC_{50} values (inhibitor concentration giving 50% inhibition) the enzyme (cCP or iCP) and inhibitors (0.001-50 µM) were incubated for 15 min before the measurement of the remaining activity. They were obtained by fitting the experimental data to eq 1 or eq 2 (n: Hill number), with V_0 being the initial rate of the control and *Vi* the initial rate in the presence of the tested compound at the concentration [I].

$$\%Inhibition = 100 \times \left(1 - \frac{V_i}{V_0}\right) = \frac{100 \times [I]}{IC_{50} + [I]} \quad (eq\ 1)$$

$$\%Inhibition = \frac{100 \times [I]^n}{IC_{50}^n + [I]^n} \quad (eq\ 2)$$

4.3. Cellular assays

Skin normal fibroblastic cells were purchased from Lonza (Basel, Switzerland), HuH7, Caco-2, MDA-MB-231, HCT116, PC3, HaCaT and NCI-H727 cancer cell lines were obtained from the ECACC collection (Porton, UK). Cells were grown according to ECACC recommendations in DMEM for HuH7, MDA-MB-231, HaCaT and fibroblast, in EMEM for CaCo-2, in McCoy's for HCT116 and in RPMI for PC3, NCI-H727 at 37°C and 5% CO_2. All culture media were added with 10% of FBS (fetal bovine serum), 1% of penicillin-streptomycin and 2 mM glutamine. The proteasome inhibitor ONX-0914 was obtained from Boston Biochem.

Proteasome inhibitors were solubilized in DMSO at a concentration of 10 mM (stock solutions) and diluted in culture medium to the desired final concentrations. The dose effect on cytotoxicity assays (IC_{50} determination) were performed by increasing concentrations of each chemical (final well concentrations: 0.1 μM – 0.3 μM – 0.9 μM – 3 μM – 9 μM – 25 μM). Cells were plated in 96 wells (4000 cells/well). Twenty-four hours after seeding, cells were exposed to chemicals. After 48 h of treatment, cells were washed in PBS and fixed in cooled 90% ethanol/5% acetic acid for 20 min. Then, the nuclei were stained with Hoechst 33342 (B2261 Sigma). Image acquisition and analysis were performed using a Cellomics ArrayScan VTI/HCS Reader (ThermoScientific). The survival percentages were calculated as the number of cells after proteasome inhibitor treatment over the number of cells after DMSO treatment. The EC_{50} values (inhibitor concentrations leading to 50% survival) were determined from dose-response curves.

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Conflict of interest

All authors declare no conflict of interest.

Appendix. Supplementary data

Supplementary data (Figure S1, Figure S2, representative dose-response curves for tumor cell essays, general methods for chemistry, copies of ^{1}H and ^{13}C NMR spectra) related to this article can be found at https:

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Highlights

- X-ray structures drive design of immuno- and constitutive proteasome inhibitors.
- Di- and tripeptide inhibitors are tailored in order to gain subunit selectivity.
- Enantiopure 3-hydroxyoxindolylalaninamide derivatives are efficiently synthesized.
- One, 2 or 3 activities of the proteasomes are inhibited in the nanomolar range.
- The most potent inhibitors are cytotoxic against cancer cell lines.