

# Design, synthesis and biological evaluation of new peptide-based ureas and thioureas as potential antagonists of the thrombin receptor PAR1

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## ABSTRACT

By applying a diversity oriented synthesis strategy for the search of new antagonists of the thrombin receptor PAR1, a series of peptide-based ureas and thioureas, including analogues of the PAR1 reference antagonist RWJ-58259, has been designed and synthesized. The general synthetic scheme involves reduction of basic amino acid-derived amino nitriles by hydrogen transfer from hydrazine monohydrate in the presence of Raney Ni, followed by reaction with diverse isocyanates and isothiocyanates, and protecting group removal. All new compounds have been evaluated as inhibitors of human platelet aggregation induced by the PAR1 agonist SFLLRN. Some protected peptide-based ureas displayed significant antagonist activity.

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*Keywords:* PAR1 antagonists; Peptide-derived ureas; Peptide-derived thioureas;  $\alpha$ -Amino nitriles; Platelet antiaggregant activity.

## 1. Introduction

In addition to the key role of thrombin in the blood coagulation cascade [1], this serine protease regulates multiple effects on an increasing variety of cells, such as: platelets [2, 3], endothelial and smooth muscle cells [2, 4], neurons and astrocytes in the nervous system [2, 4-7], immune and inflammatory cells [8, 9], osteoblasts [10], and tumor cells [11-14]. These cellular effects are mainly mediated by the activation of the protease-activated receptor PAR1 [15]. This is a G-protein coupled receptor (GPCR) that is activated by the thrombin-catalyzed cleavage of the N-terminal extracellular domain at the Arg<sup>41</sup>/Ser<sup>42</sup> peptide bond, which

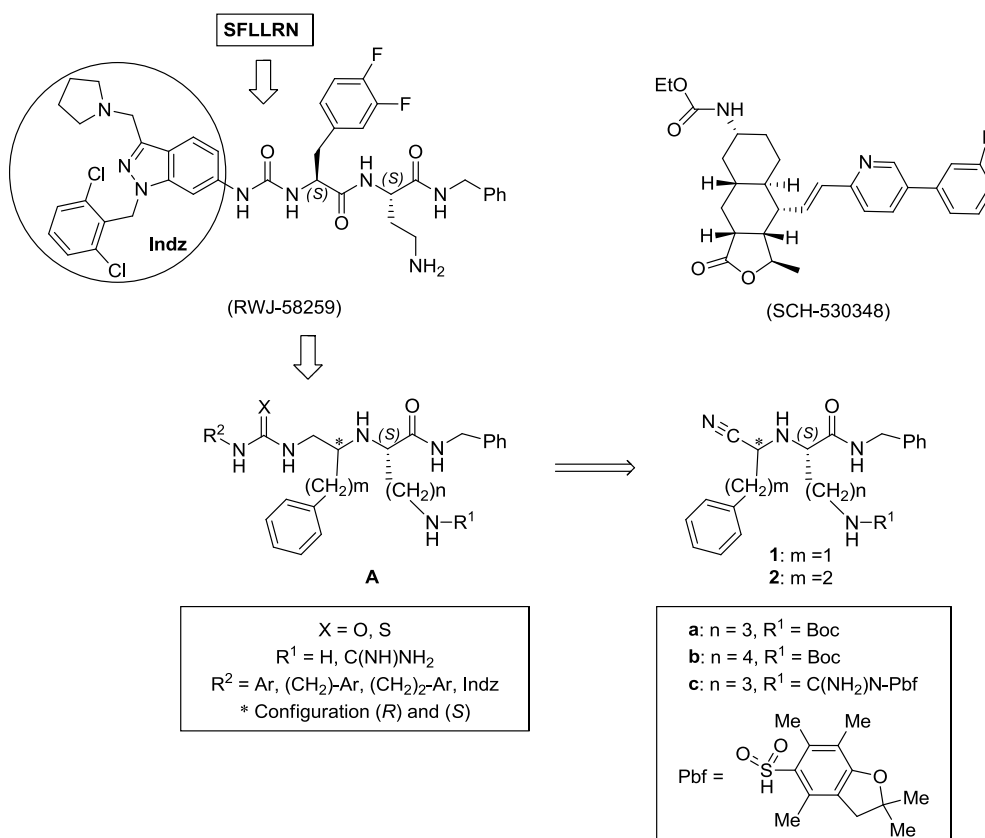
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unveils the recognition sequence SFLLRN that acts as a tethered activation ligand. The conformational changes induced by activation favors the coupling of PAR1 with heterotrimeric G-proteins, which consequently activate complex cellular signaling cascades [16, 17].

Since PAR1 is mainly expressed in platelets, where its activation induces aggregation, it has been proposed that PAR1 antagonists could be good antithrombotic agents without the hemorrhagic drawbacks of thrombin inhibitors. Based on this suggestion, up to now, PAR1 antagonists have been searched almost exclusively in relation to the cardiovascular system [3, 18, 19]. However, numerous studies have shown that PAR1 is overexpressed in invasive and metastatic tumors and that its expression levels directly correlate with the degree of invasiveness of the cancer [20-28]. Based on these facts, this receptor is starting to be also considered a promising target for cancer therapy [15], particularly in the search of angiogenesis inhibitors [29].

The first potent PAR1 antagonists were SFLLRN-based peptidomimetic ureas, represented by the optimized antagonist RWJ-58259 (Figure 1) [19]. This antagonist showed protection against thrombus formation in nonhuman primates, providing the first *in vivo* proof for supporting the potential clinical utility of PAR1 antagonists, although, its low oral bioavailability stopped its clinical development [19]. Later, several laboratories have reported a few series of antagonists obtained from HTS of diverse libraries of non-peptide small molecules, followed by optimization [19, 30]. The most advanced of these antagonists is SCH-530348 (named vorapaxar, Figure 1), derived from the natural product himbacine, which currently is undergoing Phase III clinical trials in patients with acute coronary syndrome and in patients with atherosclerosis [31-33]. Up to now, there is not structural information on the binding sites of these PAR1 antagonists to be used for structure-based design of new antagonists. However, recent mutagenesis studies on thrombin and/or PAR1 [34-42], X-ray of thrombin crystallized with diverse N-terminal fragments of PAR1 [43, 44], and NMR studies on the (Ala<sup>26</sup>-Hse<sup>103</sup>) N-terminal sequence of PAR1 [45] have shown that the first thrombin/PAR1 interaction is produced between the exosite I of thrombin and the hirudin-like sequence of PAR1 (K<sup>51</sup>YEPF<sup>55</sup>), and that this first interaction is essential and determinant for high affinity. It seems that the hydrophobic residues F34, I82, L65 and Y76,



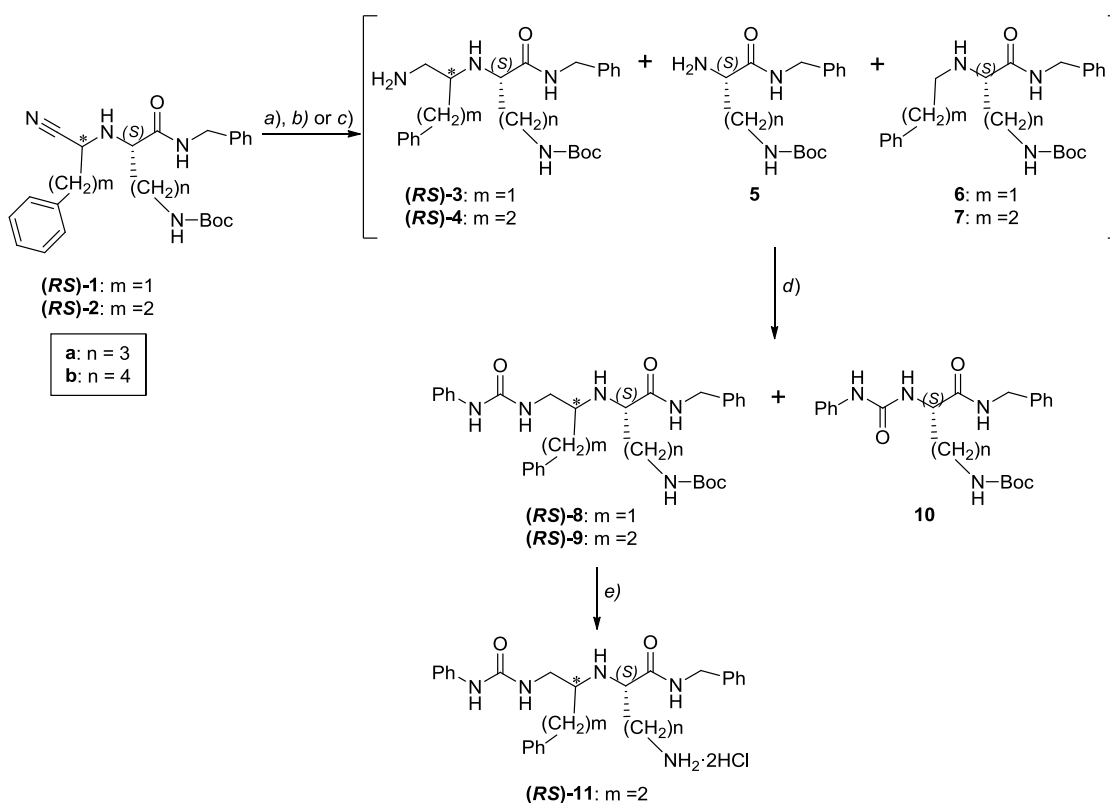
**Fig. 1.** Selected PAR1 antagonists and proposed new structures

and the basic residues R67 and R73, of the exosite I of thrombin are important for high affinity. Taking into account this knowledge, we started a project directed to the search of new PAR1 antagonists based on these hot spots of the exosite I of thrombin for PAR1. As these hot spots are discontinuous and are not localized in a defined secondary structure, we decided to use a diversity oriented synthesis (DOS) strategy for the search of peptidomimetics. For this purpose, we planned the synthesis of diverse small directed libraries of different scaffolds able to display, at least, one or two aromatic groups and one or two basic groups at variable distances and orientations. Among these structures, our first objective was the synthesis of ureas and thioureas of general formula **A**, which could be considered as analogues of the reference antagonist RWJ-58259. To this aim, we devised a synthetic scheme using basic amino acid derived  $\alpha$ -amino nitriles **1** and **2** (Figure 1) as key diversity generation intermediates [46]. Herein, we report the synthesis of ureas and thioureas **A** and their evaluation as human PAR1 antagonists in a platelet aggregation assay.

## 2. Results and discussion

### 2.1. Synthesis

The proposed general synthetic scheme for the preparation of ureas and thioureas **A** involves cyano-reduction of the protected  $\alpha$ -amino nitriles **1** and **2**, followed by reaction of the resulting primary amines with isocyanates or isothiocyanates and subsequent removal of protecting groups. The starting basic amino acid-derived  $\alpha$ -amino nitriles **1** and **2** were obtained by a modified Strecker reaction as (1:1) epimeric mixtures at the cyano-supporting stereocenter, except for the arginine derivatives **1c**, which were obtained in an (*R*)/(*S*) ratio of (1:3) [47]. Only the epimeric mixtures (**RS**)-**1a,b** could be chromatographically resolved into the respective (*R*)- and (*S*)-epimers. Therefore, (**RS**)-**1c** and (**RS**)-**2a,b** were used as such epimeric mixtures through the synthetic pathway.



**Scheme 1.** Reagents and conditions: (a)  $\text{H}_2$ , Pd(C), MeOH, rt. (b)  $\text{H}_2$ , Raney Ni, MeOH, rt. (c)  $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$ , Raney Ni, MeOH or EtOH, T and time as indicated in Table 1. (d) PhNCO,  $\text{CH}_2\text{Cl}_2$ ,  $0^\circ\text{C}$ . (e) 3M HCl, EtOAc, rt.

The epimeric mixture (**RS**)-**1a** was used as a model for optimizing the cyano reduction. Initially, this was attempted by 10% Pd(C) or Raney Ni catalyzed hydrogenation. However, as

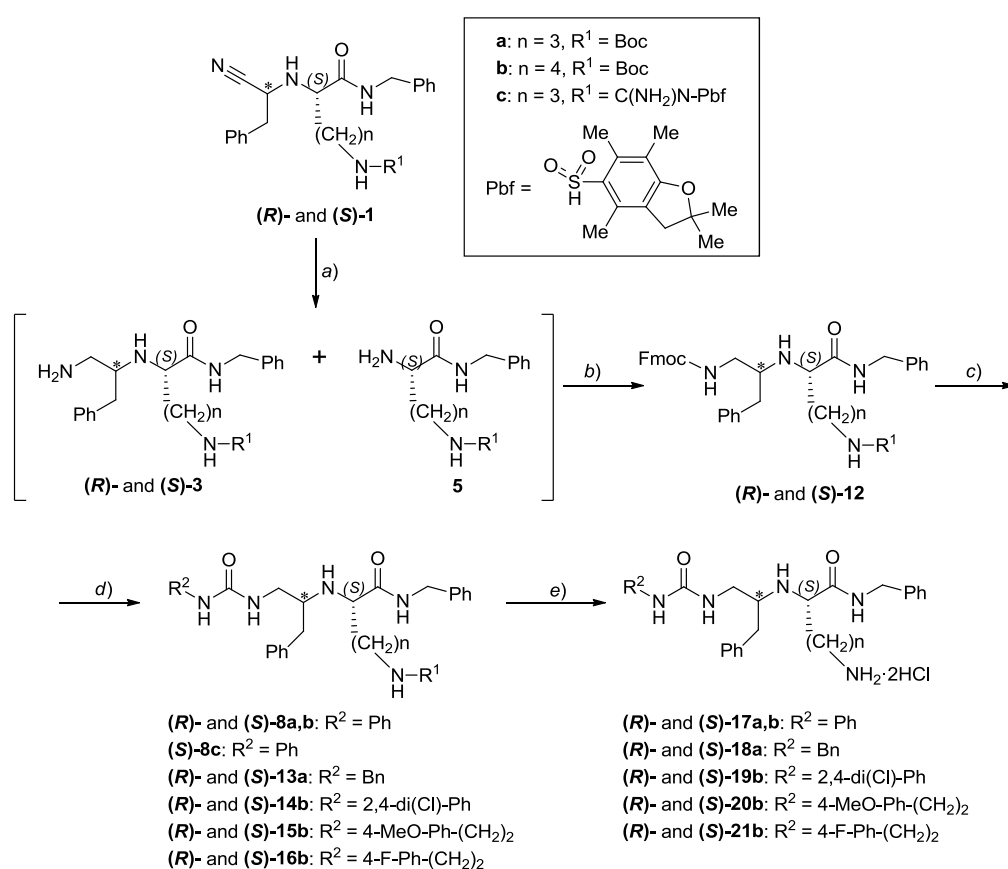
shown in Scheme 1 and Table 1, the HPLC MS analysis of the crude reaction showed that the expected primary amines (**RS**)-**3a** were obtained along with variable percentages of **5a** and **6a**, resulting from retro-Strecker reaction and reduction of the intermediate imine after removal of HCN, respectively. The difficulties in the purification of (**RS**)-**3a** from the crude reaction mixtures moved us to try the reduction by hydrogen transfer, using hydrazine monohydrate as hydrogen source and Raney Ni as catalyst [48]. After the study of reaction conditions (Table 1), the formation of the side product **6a** could be completely avoided, but not that of the retro-Strecker reaction **5a**. This side reaction was minimized to a 5 % by carrying out the reduction under refluxing MeOH for 5 min, using 20 equivalents of hydrazine monohydrate and 100 mg/mmol of Raney Ni (entry 7). These optimized conditions were applied to the reduction of the other starting  $\alpha$ -amino nitriles. It is interesting to point out the importance of the Raney Ni activation, as well as the efficiency of the stirring, to make the reduction as faster as possible, in order to minimize the presence of the amino nitrile within the reaction medium, for minimizing the retro-Strecker reaction. Besides, in the phenylacetaldehyde derivatives **3** ( $m=1$ ), the reduction of the (*R*)-epimer was slower than that of the corresponding (*S*)-epimer and, consequently, its percentage of degradation via retro-Strecker was significantly higher [see for example different yields for reduction of (*R*)- and (*S*) epimers of **1a** and **1b** in Table 2]. In the case of the Arg derivative **3c**, the difference in reactivity between epimers made that the minor (*R*)-epimer were lost in the reduction.

**Table 1**  
Optimization of the reduction conditions for (**RS**)-**1a**

Entry	Catalyst	H <sub>2</sub> source	T (°C)	t (min)	Yield (%) <sup>a</sup>		
					( <b>RS</b> )- <b>3a</b>	<b>5a</b>	<b>6a</b>
1	10 % Pd(C)	H <sub>2</sub>	rt	24 h	80	8	12
2	Raney Ni (100 mg/mmol)	H <sub>2</sub>	rt	24 h	54	6	40
3	Raney Ni (100 mg/mmol)	NH <sub>2</sub> NH <sub>2</sub> ·H <sub>2</sub> O	85	30	37	63	-
4	Raney Ni (100 mg/mmol)	NH <sub>2</sub> NH <sub>2</sub> ·H <sub>2</sub> O	rt	30	67	33	-
5	Raney Ni (200 mg/mmol)	NH <sub>2</sub> NH <sub>2</sub> ·H <sub>2</sub> O	rt	30	73	27	-
6	Raney Ni (100 mg/mmol)	NH <sub>2</sub> NH <sub>2</sub> ·H <sub>2</sub> O	rt	90	92	8	-
7	Raney Ni (100 mg/mmol)	NH <sub>2</sub> NH <sub>2</sub> ·H <sub>2</sub> O	65	5	95	5	-

<sup>a</sup> Determined by HPLC-MS analysis.

Due to the difficulties found in the separation of the primary amines **3** and **4** from the side product of the retro-Strecker reaction **5**, the crude reduction mixtures were initially used without purification for the synthesis of the desired ureas **8** and **9**. However, the difficulties in the purification of these ureas remained, and several column chromatographies were required for their separation from the ureas **10**, byproducts derived from the amino amides **5**, with considerable loss in the yield. Only the epimeric mixture of ornithine-derived ureas (**RS**)-**9a** could be resolved during this chromatographic purification. In view of these results, to avoid the purification difficulties, we decided to isolate the reduction intermediate amines as the corresponding Fmoc-derivatives. This strategy was studied in the phenylacetaldehyde derivatives (**R**)- and (**S**)-**3a,b** and (**RS**)-**3c**. At this point, it is important to note that when the Fmoc-protection was carried out under the standard basic conditions, using Na<sub>2</sub>CO<sub>3</sub> as HCl acceptor in dioxane/water [49], almost complete epimerization of one of the two chiral centers was observed. This epimerization was completely avoided by using propylene oxide as HCl acceptor in CH<sub>2</sub>Cl<sub>2</sub>, to obtain the Fmoc-protected amines (**R**)- and (**S**)-**12a,b** and (**S**)-**12c** in 30-72% overall yield from the amino nitriles (**R**)- and (**S**)-**3a,b** and (**RS**)-**3c** (Scheme 2).



**Scheme 2.** Reagents and conditions: (a) NH<sub>2</sub>NH<sub>2</sub>·H<sub>2</sub>O, Raney Ni, MeOH, 65 °C. (b) Fmoc-Cl, propylene oxide, CH<sub>3</sub>CN, 0°C. (c) Et<sub>2</sub>NH, CH<sub>2</sub>Cl<sub>2</sub>, rt. (d) R<sup>2</sup>NCO, CH<sub>2</sub>Cl<sub>2</sub>, 0°C. (e) 3M HCl, EtOAc, rt.

The Fmoc-removal in **12a-c**, followed by reaction with different aromatic isocyanates (Scheme 2) led to the desired ureas **8** and **13-16** in significantly higher overall yields than by the direct synthesis of the urea after the cyano-reduction (compare yields of methods A and B in Table 2).

**Table 2**

Optimization of the synthesis of ureas **8**, **9** and **13-17**

Amino nitrile	Diamine <sup>a</sup>		Urea <sup>b</sup>		
	N <sup>o</sup>	%	N <sup>o</sup>	A (%) <sup>c</sup>	B (%) <sup>d</sup>
<b>(RS)-1a</b> (1:1) <sup>e</sup>	<b>(RS)-3a</b> (1:3) <sup>e</sup>	74	<b>(RS)-8a</b> (1:3) <sup>e</sup>	36	52
<b>(R)-1a</b>	<b>(R)-3a</b>	55	<b>(R)-8a</b>	-	17
<b>(S)-1a</b>	<b>(S)-3a</b>	92	<b>(S)-8a</b>	-	67
<b>(RS)-1b</b> (1:1) <sup>e</sup>	<b>(RS)-3b</b> (1:1.3) <sup>e</sup>	73	<b>(RS)-8b</b> (1:1.3) <sup>e</sup>	21	44
<b>(R)-1b</b>	<b>(R)-3b</b>	60	<b>(R)-8b</b>	-	44
<b>(S)-1b</b>	<b>(S)-3b</b>	87	<b>(S)-8b</b>	-	57
<b>(RS)-1c</b> (1:3) <sup>e</sup>	<b>(S)-3c</b>	64	<b>(S)-8c</b>	-	25
<b>(R)-1a</b>	<b>(R)-3a</b>	55	<b>(R)-13a</b>	-	25
<b>(S)-1a</b>	<b>(S)-3a</b>	92	<b>(S)-13a</b>	-	51
<b>(R)-1b</b>	<b>(R)-3b</b>	60	<b>(R)-14b</b>	-	40
<b>(S)-1b</b>	<b>(S)-3b</b>	87	<b>(S)-14b</b>	-	52
<b>(R)-1b</b>	<b>(R)-3b</b>	60	<b>(R)-15b</b>	-	42
<b>(S)-1b</b>	<b>(S)-3b</b>	87	<b>(S)-15b</b>	-	61
<b>(R)-1b</b>	<b>(R)-3b</b>	60	<b>(R)-16b</b>	-	40
<b>(S)-1b</b>	<b>(S)-3b</b>	87	<b>(S)-16b</b>	-	62
<b>(RS)-2a</b> (1:1) <sup>e</sup>	<b>(RS)-4a</b> (1:1) <sup>e</sup>	74	<b>(RS)-9a</b> (1:1) <sup>e</sup>	27	-
<b>(RS)-2b</b> (1:1) <sup>e</sup>	<b>(RS)-4b</b> (1:1) <sup>e</sup>	74	<b>(RS)-9b</b> (1:1) <sup>e</sup>	29	-

<sup>a</sup>Yields determined by HPLC-MS analyses.

<sup>b</sup>Isolated yields.

<sup>c</sup>Overall yield of reduction, followed by reaction with the corresponding isocyanate (Method A).

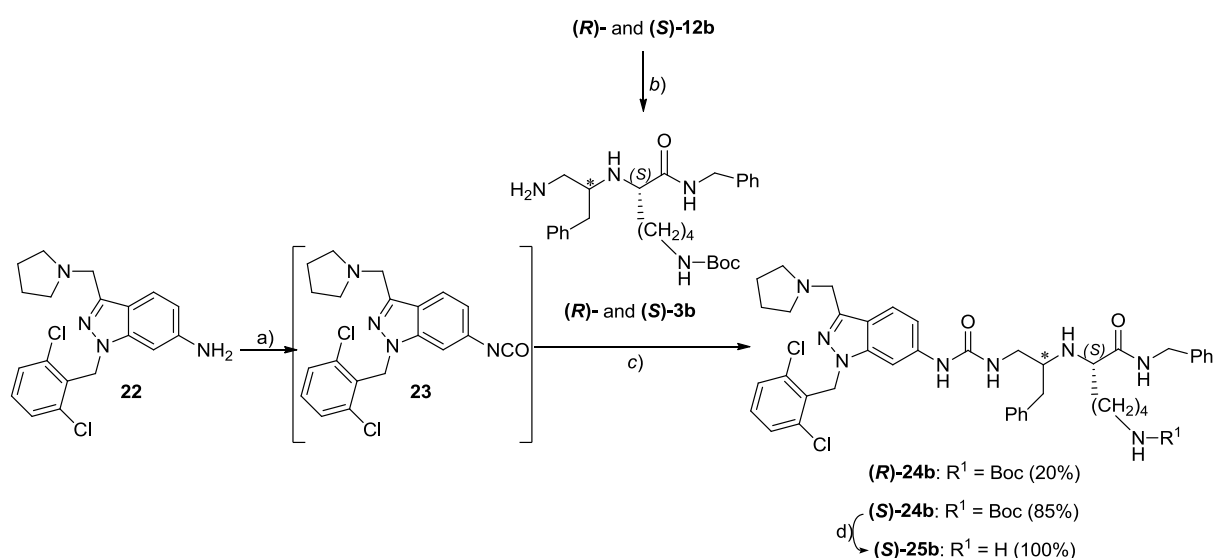
<sup>d</sup>Overall yield of reduction, followed by sequential Fmoc-protection, isolation, Fmoc-deprotection, and reaction with the corresponding isocyanate (Method B).

<sup>e</sup>(*R:S*)-epimer ratio.

Removal of the *N*-Boc protection of the basic side chain in the Orn and Lys derived ureas **8a,b**, **9a,b** and **13a,b-16b** by treatment with 3M HCl solution in EtOAc, yielded the corresponding deprotected compounds **11a,b** and **17a,b-21b** (Schemes 1 and 2). In view of

the poor biological results of these deprotected ornithine and lysine derivatives (see below) and the low overall yield of the arginine-derived urea **8c**, this urea was not deprotected.

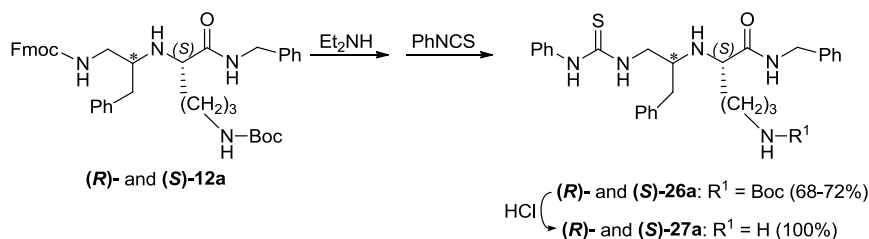
The lysine-derived Fmoc protected diamine **12b** was also used for the synthesis of the PAR1 reference antagonist RWJ-58259 analogue (*S*)-**25b** (Scheme 3). This urea was synthesized by applying our recently reported procedure for the preparation of RWJ-58259 [50]. This procedure involves the *in situ* formation of the indazole-derived isocyanate **23**, by reaction of the required 6-amino-indazole **22** with triphosgene in the presence of propylene oxide as HCl acceptor, followed by reaction with the deprotected diamines (*R*)- and (*S*)-**3b**. Interestingly, as commented for the reduction of the starting amino nitriles, the application of this methodology gave considerably higher yield for (*S*)-**24b** [85 % overall from (*S*)-**12b**] than for its epimer (*R*)-**24b** [20 % overall yield from (*R*)-**12b**]. Due to this low yield, this epimer was reserved for the biological evaluation and was not deprotected.



**Scheme 3.** Reagents and conditions: (a)  $(\text{CCl}_3\text{O})_2\text{CO}$ , propylene oxide, THF, 0 °C . (b)  $\text{Et}_2\text{NH}$ ,  $\text{CH}_2\text{Cl}_2$ , rt. (c) THF, 0 °C. (d) 3M HCl, EtOAc, rt.

The optimized method B of synthesis of urea derivatives **17a** was similarly applied to the synthesis of the thiourea analogues (*R*)- and (*S*)-**27a**, by replacing the phenylisocyanate by phenylisothiocyanate (Scheme 4).





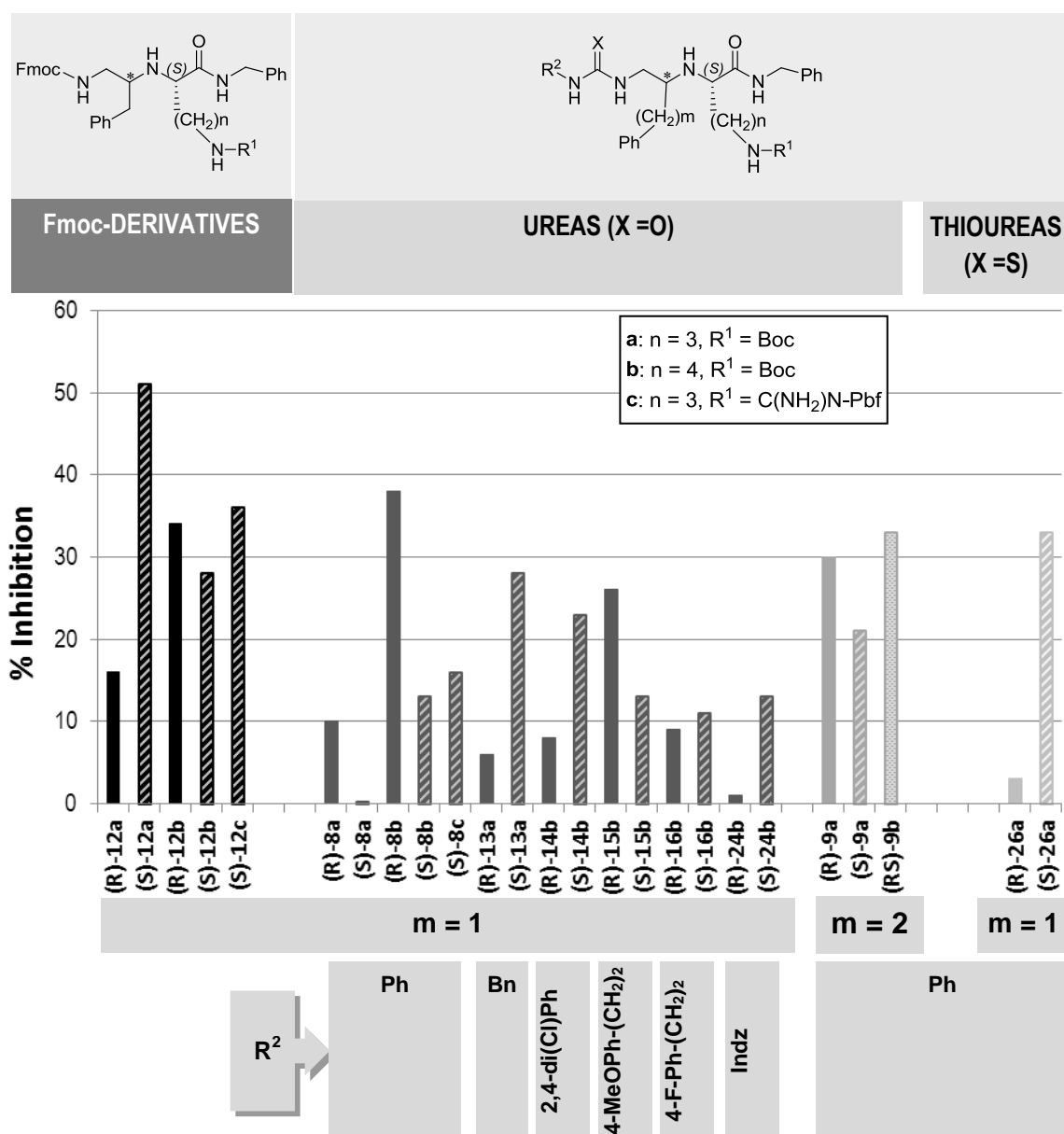
**Scheme 4.** Synthesis of thiourea derivatives

## 2.2. Biological evaluation

Since PAR1 is mainly expressed in platelets, to evaluate the PAR1 antagonist activity, all new compounds were screened as inhibitors of human platelet aggregation induced by a 30  $\mu\text{M}$  concentration of the PAR1 agonist SFLLRN. The antagonist RWJ-58259 was used as a reference. At a 10  $\mu\text{M}$  concentration this antagonist inhibited 98% the platelet aggregation. All compounds were tested at an initial concentration of 0.1 mg/mL ( $\approx$  150  $\mu\text{M}$ ). Unfortunately, none of the deprotected ureas or thioureas inhibited the platelet aggregation. However, as shown in Figure 2, some of the protected ureas, and particularly the Fmoc intermediates **12**, showed significant inhibition. Thus, the Fmoc derivatives (*S*)-**12a** and (*S*)-**12c** inhibited a 51 % and a 36 %, respectively, the platelet aggregation. Among the protected ureas and thioureas, the most potent were the urea (*R*)-**8b** and the thiourea (*S*)-**26a**, which showed 38 and 33 % of inhibition, respectively. Although these results do not allow to establish defined structure-activity relationships, it seems that in the Fmoc derivatives **12** and thioureas **26a**, the (*S*)-epimers showed higher inhibition % than the respective (*R*). However, in the case of the urea derivatives there is not a clear stereochemistry-activity relationship, as the results varied depending on the urea substitution. At this position, the phenyl group was the best (compare ureas **8b** with **14b-16b** and **24b**). Respecting the influence of the basic amino acid moiety, from the comparison of analogue (*S*)-epimers, in the Fmoc-derivatives **12**, the order of activity was Orn(**a**) > Arg(**c**) > Lys(**b**), while in the urea-derivatives **8**, the order was Arg(**c**)  $\geq$  Lys(**b**) > Orn(**a**).

In the structural comparison of the inactive indazole-derived urea (*S*)-**25b** with the family of peptidomimetic urea PAR1 antagonists, to which the reference antagonist RWJ-58259 belongs [51], the main difference is the replacement of the di(F)-Phe residue of RWJ-58259 by the 1-amino-3-phenyl-propan-2-yl moiety in (*S*)-**25b** (see Figure 1). This replacement involves the loss of the carbonyl group of the peptide bond, which could be important for the

interaction with the PAR1 receptor, participating in H-bond formation or for the appropriate orientation of the pharmacophoric groups.



**Fig. 2.** Inhibition (%) of human platelet aggregation induced by a 30  $\mu$ M concentration of SFLLRN

### 3. Conclusion

A series of peptide-based ureas and thioureas, including analogues of the PAR1 reference antagonist RWJ-58259, has been designed and synthesized as potential PAR1 antagonists. A DOS strategy has been applied for the synthesis of these urea derivatives, consisting of

reduction of basic amino acid-derived amino nitriles, followed by reaction with diverse isocyanates or isothiocyanates. To evaluate the PAR1 antagonist activity, all new synthesized compounds have been screened as inhibitors of human platelet aggregation induced by the PAR1 agonist SFLLRN. Although none of the designed peptide-based ureas inhibited aggregation, some of their protected derivatives showed moderate antiaggregant activity. These results could be a good clue for the search of new potent PAR1 antagonists and to explore the thrombin/PAR1 interaction.

## 4. Experimental

### 4.1. General

All reagents were of commercial quality. Solvents were dried and purified by standard methods. Analytical TLC was performed on aluminum sheets coated with a 0.2 mm layer of silica gel 60 F<sub>254</sub>. Silica gel 60 (230-400 mesh) was used for flash chromatography. Analytical RP-HPLC was performed on a Sunfire C<sub>18</sub> (4.6×150 mm, 3.5 μm) column, with a flow rate of 1 mL/min, using a tunable UV detector set at 214 and 254 nm and a (10-100%, 30 min) gradient of CH<sub>3</sub>CN (solvent A) and 0.05 % TFA in H<sub>2</sub>O (solvent B) as mobile phase. HPLC-MS was performed on a Sunfire C<sub>18</sub> (4.6×50 mm, 3.5 μm) column at 30°C, with a flow rate of 1 mL/min. (10-100%, 5 min) Gradient of CH<sub>3</sub>CN with 0.08% of formic acid (solvent A) in 0.1% of formic acid in H<sub>2</sub>O (solvent B) was used as mobile phase. Electrospray in positive mode was used for ionization. Melting points were taken on a Mettler Toledo M170 apparatus and are uncorrected. Elemental analyses were obtained on a CH-O-RAOID apparatus. Optical rotations were determined in a Perkin Elmer 141 polarimeter. NMR spectra were recorded using Varian Inova 300, Varian Inova or Mercury 400, and Varian Unity 500 spectrometers. The NMR spectra assignment was based on COSY, HSQC, and HMBC spectra.

### 4.2. General procedure for the synthesis of basic amino acid-derived ureas. Method A. *Synthesis of ureas (RS)-8a,b and -9a,b, and (R)- and (S)-9a*

Raney Ni (30 mg) and hydrazine monohydrate (0.186 mL, 6 mmol) were added to a solution of the corresponding amino nitrile **(RS)-1a,b** and **(RS)-2a,b** (0.3 mmol) in MeOH (20 mL) and the resulting reaction mixture was refluxed for 5 min. Then, the mixture was filtered through celite, this was washed with MeOH (3×75 mL), and the filtrates were evaporated to dryness. The residue was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (10 mL) and the solution was

cooled at 0°C. The corresponding isocyanate (0.3 mmol) was added to the solution that was stirred at this temperature for 2 h. Then, the solvent was evaporated and the residue was dissolved in EtOAc (50 mL). This solution was successively washed with H<sub>2</sub>O (20 mL) and brine (20 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, and evaporated to dryness. The residue was purified by flash chromatography, using 20-100% EtOAc gradient in hexane as eluant to afford the ureas **(RS)-8a,b** and **(RS)-9a,b**. Only both epimers of the ornithine-derived ureas **(R)-9a** and **(S)-9a** could be separated in this purification. The two epimers of **(RS)-8a,b** were obtained as optical pure compounds from each epimer of the starting amino nitrile **(R)-1a,b** and **(S)-1a,b**, by applying method B described below. The characterization data of **(R)-8a,b** and **(S)-8a,b** are also described below.

#### 4.2.1. N<sub>8</sub>-Boc-N<sub>α</sub>-[(2R)-4-Phenyl-1-(3-phenylureido)butan-2-yl]-ornithine benzyl amide **[(R)-9a]**

Foam (23 mg , 13 %); HPLC *t*<sub>R</sub> 18.54; [α]<sub>D</sub><sup>20</sup> +7.5 (*c* 0.9, MeOH); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.40 (s, 9H, Boc), 1.29-1.81 [m, 6H, 3-H (Bu), γ- and β-H (Orn)], 2.49-2.78 [m, 3H, 2-H and 4-H (Bu)], 2.95-3.43 [m, 4H, 1-H (Bu), α- and δ-H (Orn)], 4.32 [m, 2H, CH<sub>2</sub> (Bn)], 4.92 (bs, 1H, *NH*-Boc), 5.86, and 7.67 (2 bs, 2H, HNCONH), 6.95-7.56 (m, 16 H, Ph and *NH*-Bn); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 28.6 (CH<sub>3</sub>, Boc and CH<sub>2</sub>, C<sub>γ</sub>), 31.6 (CH<sub>2</sub>, C<sub>β</sub>), 32.3 (CH<sub>2</sub>, C<sub>4</sub>), 35.7 (CH<sub>2</sub>, C<sub>3</sub>), 39.5 (CH<sub>2</sub>, C<sub>δ</sub>), 43.1 (CH<sub>2</sub>, C<sub>1</sub>), 43.5 [CH<sub>2</sub>, (Bn)], 57.1 (CH, C<sub>2</sub>), 58.3 (CH, C<sub>α</sub>), 79.8 (C, Boc), 119.8-129.2 (15CH, Ph), 138.5, 139.6 and 141.9 (3C, Ph), 156.2 (HNCONH), 157.1 (CO, Boc), 175.1 (CONH); ES-MS *m/z* [M+1]<sup>+</sup> calcd. for C<sub>34</sub>H<sub>45</sub>N<sub>5</sub>O<sub>4</sub>, 688.35; found 688.17 (100%); Anal. calcd. for C<sub>34</sub>H<sub>45</sub>N<sub>5</sub>O<sub>4</sub>: C, 69.48; H, 7.72; N, 11.92. Found: C, 69.67; H, 7.86; N, 11.78.

#### 4.2.2. N<sub>8</sub>-Boc-N<sub>α</sub>-[(2S)-4-Phenyl-1-(3-phenylureido)butan-2-yl]-ornithine benzyl amide **[(S)-9a]**

Foam (25 mg , 14 %); HPLC *t*<sub>R</sub> 18.89; [α]<sub>D</sub><sup>20</sup> +5 (*c* 1, MeOH); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.42 (s, 9H, Boc), 1.46-1.80 [m, 6H, 3-H (Bu), γ- and β-H (Orn)], 2.51-2.63 [m, 3H, 2-H and 4-H (Bu)], 2.92-3.31 [m, 4H, 1-H (Bu), α- and δ-H (Orn)], 4.26 [dd, 1H, J = 6 and 15 Hz, CH<sub>2</sub> (Bn)], 4.31 [dd, 1H, J = 6 and 15 Hz, CH<sub>2</sub> (Bn)], 4.73 (bs, 1H, *NH*-Boc), 5.84, and 7.70 (2 bs, 2H, HNCONH), 6.92-7.52 (m, 16 H, Ph and *NH*-Bn); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 28.6 (CH<sub>2</sub>, C<sub>γ</sub>), 28.7 (CH<sub>3</sub>, Boc), 32.2 (CH<sub>2</sub>, C<sub>β</sub>), 32.4 (CH<sub>2</sub>, C<sub>4</sub>), 35.6 (CH<sub>2</sub>,

C<sub>3</sub>), 40.3 (CH<sub>2</sub>, C<sub>δ</sub>), 42.5 (CH<sub>2</sub>, C<sub>1</sub>), 43.4 [CH<sub>2</sub>, (Bn)], 57.4 (CH, C<sub>2</sub>), 59.9 (CH, C<sub>α</sub>), 80.1 (C, Boc), 119.6-129.2 (15CH, Ph), 138.6, 139.6 and 141.9 (3C, Ph), 156.7 (HNCONH), 157.2 (CO, Boc), 174.9 (CONH); ES-MS *m/z* [M+1]<sup>+</sup> calcd. for C<sub>34</sub>H<sub>45</sub>N<sub>5</sub>O<sub>4</sub>, 688.35; found 688.27 (100%); Anal. calcd. for C<sub>34</sub>H<sub>45</sub>N<sub>5</sub>O<sub>4</sub>: C, 69.48; H, 7.72; N, 11.92. Found: C, 69.59; H, 7.82; N, 11.85.

#### 4.2.3. N<sub>ε</sub>-Boc-N<sub>α</sub>-[4-Phenyl-1-(3-phenylureido)butan-2-yl]-lysine benzyl amide [(**RS**)-**9b**]

Foam (52 mg, 29 %); HPLC *t<sub>R</sub>* 18.70 [(**R**)-**9b**] and 19.06 min [(**S**)-**9b**]; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.41 and 1.43 (2s, 9H, Boc), 0.94-1.33 [m, 2H, δ-H (Lys)], 1.50-1.85 [m, 6H, γ- and β-H (Lys), 3-H (Bu)], 2.45-2.74 [m, 3H, 2-H and 4-H (Bu)], 2.90-3.17 [m, 2H, ε-H (Lys)], 3.17-3.27 [m, 1.5H, 1-H (Bu) and α-H (Lys)], 3.38 [m, 0.5H, 1-H (Bu)], 4.36 [m, 2H, CH<sub>2</sub> (Bn)], 4.66 and 4.84 (2bs, 1H, *NH*-Boc), 5.80, 7.84 and 8.10 (3 bs, 2H, HNCONH), 6.86-7.54 (m, 16 H, Ph and *NH*-Bn); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 22.9 (CH<sub>2</sub>, C<sub>γ</sub>), 26.4 and 28.5 (CH<sub>3</sub>, Boc), 29.8 (CH<sub>2</sub>, C<sub>δ</sub>), 32.1 (CH<sub>2</sub>, C<sub>4</sub>), 33.0 (CH<sub>2</sub>, C<sub>β</sub>), 34.8 (CH<sub>2</sub>, C<sub>3</sub>), 40.0 (CH<sub>2</sub>, C<sub>ε</sub>), 42.0 and 43.0 (CH<sub>2</sub>, C<sub>1</sub>), 43.3 [CH<sub>2</sub>, (Bn)], 56.9 and 58.8 (CH, C<sub>2</sub>), 60.1 (CH, C<sub>α</sub>), 80.1 (C, Boc), 119.1-128.9 (15CH, Ph), 139.1 and 141.3 (3C, Ph), 156.2 and 156.7 (2CO, Boc and HNCONH), 174.9 (CONH); ES-MS *m/z* [M+1]<sup>+</sup> calcd. for C<sub>35</sub>H<sub>47</sub>N<sub>5</sub>O<sub>4</sub>, 602.36; found 602.27 (100%); Anal. calcd. for C<sub>35</sub>H<sub>47</sub>N<sub>5</sub>O<sub>4</sub>: C, 69.86; H, 7.87; N, 11.64. Found: C, 69.97; H, 7.84; N, 11.45.

#### 4.3. General procedure for the synthesis of the N-Fmoc-protected amines (**R**)-**12a,b** and (**S**)-**12a-c**

Raney Ni (30 mg) and hydrazine monohydrate (0.186 mL, 6 mmol) were added to a solution of the corresponding amino nitrile (**R**)- and (**S**)-**1a-c** (0.3 mmol) in MeOH (20 mL) and the resulting reaction mixture was refluxed for 5 min. Then, the mixture was filtered through celite, this was washed with MeOH (3×75 mL), and the filtrates were evaporated to dryness. The respective crude reaction mixture of amines (**R**)-**3a,b** and (**S**)-**3a-c** impurified with **5a-c** was dissolved in CH<sub>3</sub>CN (10 mL) and cooled at 0°C. Propylene oxide (56 μL, 8 mmol) and Fmoc-Cl (78 mg, 0.3 mmol) were added. The solution was stirred for 2h. Then, the solvent was evaporated and the residue was dissolved in EtOAc (50 mL). This solution was successively washed with H<sub>2</sub>O (20 mL) and brine (20 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, and evaporated to dryness. The residue was purified by flash chromatography, using 10-40%

EtOAc gradient in hexane as eluant to give the corresponding Fmoc-protected amine (**R**)-**12a,b** and (**S**)-**12a-c**.

4.3.1. *N*<sub>δ</sub>-Boc-*N*<sub>α</sub>-[(2*R*)-3-Phenyl-1-(Fmoc)amino-propan-2-yl]-ornithine benzyl amide [(**R**)-**12a**]

Foam (112 mg, 55%); HPLC *t*<sub>R</sub> 20.56 min; [α]<sub>D</sub><sup>20</sup> +7.1 (*c* 1, MeOH); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.10-1.98 [m, 4H, β-H and γ-H (Orn)], 1.36 (s, 9H, Boc), 2.76-3.14 [m, 5H, 2-H, 3-H (Pr) and δ-H (Orn)], 3.24 [m, 1H, α-H (Orn)], 3.26-3.55 [m, 1H, 1-H (Pr)], 4.19 [t, 1H, *J*=7 Hz, CH (Fmoc)], 4.37 [d, 2H, *J*=7 Hz, CH<sub>2</sub> (Fmoc)], 4.42 [dd, 2H, *J*=5 and 15 Hz, CH<sub>2</sub> (Bn)], 4.71 (bs, 1H, *NH*-Boc), 5.75 (1bs, 1H, *NH*-Fmoc), 7.14-7.76 (m, 19H, aromatics and *NH*-Bn); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 26.0(CH<sub>2</sub>, C<sub>γ</sub>), 28.3 (3CH<sub>3</sub>, Boc), 30.7 (CH<sub>2</sub>, C<sub>β</sub>), 39.1 (CH<sub>2</sub>, C<sub>3</sub>), 39.5 (CH<sub>2</sub>, C<sub>δ</sub>), 43.1 (CH<sub>2</sub>, Bn), 44.6 (CH<sub>2</sub>, C<sub>1</sub>), 47.1 (CH, Fmoc), 59.6 (CH, C<sub>2</sub>), 60.2 (CH, C<sub>α</sub>), 66.5 (CH<sub>2</sub>, Fmoc), 79.2 (C, Boc), 119.9-143.9 (18CH and 6C, aromatics), 156.2 (CO, Boc), 156.8 (CO, Fmoc), 174.2 (CONH); ES-MS *m/z* [M+1]<sup>+</sup> calcd for C<sub>41</sub>H<sub>48</sub>N<sub>4</sub>O<sub>5</sub>, 677.36; found 677.59 (100 %); Anal. calcd. for C<sub>41</sub>H<sub>48</sub>N<sub>4</sub>O<sub>5</sub>: C, 72.76; H, 7.15; N, 8.28. Found: C, 72.93; H, 7.34; N, 8.05.

4.3.2. *N*<sub>δ</sub>-Boc-*N*<sub>α</sub>-[(2*S*)-3-Phenyl-1-(Fmoc)amino-propan-2-yl]-ornithine benzyl amide [(**S**)-**12a**]

Foam (162 mg, 80%); HPLC *t*<sub>R</sub> 21.26 min; [α]<sub>D</sub><sup>20</sup> +15.4 (*c* 1, MeOH); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.41 (s, 9H, Boc), 1.46-1.59 [m, 4H, β-H and γ-H (Orn)], 2.41-2.71 [(m, 2H, 3-H (Pr)], 2.77 [m, 1H, 2-H (Pr)], 2.97-3.16 [m, 2H, δ-H(Orn)], 3.16-3.43 [m, 3H, α-H and 1-H (Orn)], 3.83 [dd, 2H, *J*=7, 15 Hz, CH<sub>2</sub>(Bn)], 4.20 [t, 1H, *J*=6.5 Hz, CH (Fmoc)], 4.35 [dd, 2H, *J*=5, 15 Hz, CH<sub>2</sub> (Bn)], 4.42 [d, 2H, *J*=6.5 Hz, CH<sub>2</sub> (Fmoc)], 4.75 (bs, 1H, *NH*-Boc), 5.32 (1bs, 1H, *NH*-Fmoc), 6.91 (1bs, 1H, *NH*-Bn), 7.04-7.77 (m, 19H, aromatics); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 26.3(CH<sub>2</sub>, C<sub>γ</sub>), 28.4 (3CH<sub>3</sub>, Boc), 30.9 (CH<sub>2</sub>, C<sub>β</sub>), 39.6 (CH<sub>2</sub>, C<sub>3</sub>), 40.1 (CH<sub>2</sub>, C<sub>δ</sub>), 42.7 (CH<sub>2</sub>, Bn), 43.1 (CH<sub>2</sub>, C<sub>1</sub>), 47.2 (CH, Fmoc), 58.7 (CH, C<sub>2</sub>), 59.1 (CH, C<sub>α</sub>), 66.7 (CH<sub>2</sub>, Fmoc), 79.3 (C, Boc), 119.9-143.8 (18CH and 6C, aromatics), 156.3 (CO, Boc), 157.1 (CO, Fmoc), 174.2 (CONH); ES-MS *m/z* [M+1]<sup>+</sup> calcd for C<sub>41</sub>H<sub>48</sub>N<sub>4</sub>O<sub>5</sub>, 677.36; found 677.59 (100 %); Anal. calcd. for C<sub>41</sub>H<sub>48</sub>N<sub>4</sub>O<sub>5</sub>: C, 72.76; H, 7.15; N, 8.28. Found: C, 72.89; H, 7.32; N, 8.12.

4.3.3. *N<sub>ε</sub>-Boc-N<sub>α</sub>-[(2R)-3-Phenyl-1-(Fmoc)amino-propan-2-yl]-lysine benzyl amide [(R)-12b]*

Foam (132 mg, 64%); HPLC  $t_R$  20.68 min;  $[\alpha]_D^{20}$  +8.2 (*c* 0.8, MeOH);  $^1H$  NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  0.91-1.64 [m, 6H,  $\beta$ -H,  $\gamma$ -H and  $\delta$ -H (Lys)], 1.44 (s, 9H, Boc), 2.48-2.83 [m, 3H, 2-H (Pr) and  $\epsilon$ -H (Lys)], 2.88-3.02 [m, 2H, 3-H (Pr)], 3.06-3.19 [m, 3H, 1-H (Pr) and  $\alpha$ -H (Lys)], 4.17 [t, 1H,  $J=7.5$  Hz, CH (Fmoc)], 4.28-4.47 [m, 4H, CH<sub>2</sub> (Fmoc) and CH<sub>2</sub> (Bn)], 4.60 (bs, 1H, *NH*-Boc), 5.18 (1bs, 1H, *NH*-Fmoc), 7.09 (1bs, 1H, *NH*-Bn), 7.11-7.75 (m, 19H, aromatics);  $^{13}C$  NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  22.4 (CH<sub>2</sub>, C <sub>$\gamma$</sub> ), 28.3 (3CH<sub>3</sub>, Boc), 29.6 (CH<sub>2</sub>, C <sub>$\delta$</sub> ), 33.1 (CH<sub>2</sub>, C <sub>$\beta$</sub> ), 38.9 (CH<sub>2</sub>, C<sub>3</sub>), 39.9 (CH<sub>2</sub>, C <sub>$\epsilon$</sub> ), 42.9 (CH<sub>2</sub>, Bn), 44.6 (CH<sub>2</sub>, C<sub>1</sub>), 47.1 (CH, Fmoc), 59.2 (CH, C<sub>2</sub>), 61.1 (CH, C <sub>$\alpha$</sub> ), 66.4 (CH<sub>2</sub>, Fmoc), 78.9 (C, Boc), 119.8-143.8 (18CH and 6C, aromatics), 155.9 (CO, Boc), 156.7 (NHCOO, Fmoc), 174.4 (CONH); ES-MS  $m/z$   $[M+1]^+$  calcd for C<sub>42</sub>H<sub>50</sub>N<sub>4</sub>O<sub>5</sub>, 691.38; found 691.59 (100 %); Anal. calcd. for C<sub>42</sub>H<sub>50</sub>N<sub>4</sub>O<sub>5</sub>: C, 73.02; H, 7.29; N, 8.11. Found: C, 72.89; H, 7.32; N, 8.02.

4.3.4. *N<sub>ε</sub>-Boc-N<sub>α</sub>-[(2S)-3-Phenyl-1-(Fmoc)amino-propan-2-yl]-lysine benzyl amide [(S)-12b]*

Foam (145 mg, 70%); HPLC  $t_R$  21.21 min;  $[\alpha]_D^{20}$  +12.3 (*c* 0.9, MeOH);  $^1H$  NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.16-1.86 [m, 6H,  $\beta$ -H,  $\gamma$ -H and  $\delta$ -H (Lys)], 1.42 (s, 9H, Boc), 2.42-2.68 [m, 2H, 3-H (Pr)], 2.72 [m, 1H, 2-H (Pr)], 2.88-3.44 [m, 4H, 1-H (Pr) and  $\epsilon$ -H(Lys)], 3.22 [m, 1H,  $\alpha$ -H (Lys)], 3.83 [dd, 1H,  $J=7$  and 15 Hz, CH<sub>2</sub> (Bn)], 4.19 [t, 1H,  $J=7.5$  Hz, CH (Fmoc)], 4.35 [dd, 1H,  $J=7$ , 15 Hz, CH<sub>2</sub> (Bn)], 4.41 [d, 2H,  $J=7.5$  Hz, CH<sub>2</sub> (Fmoc)], 4.57 (bs, 1H, *NH*-Boc), 5.19 (1bs, 1H, *NH*-Fmoc), 7.04 (1bs, 1H, *NH*-Bn), 7.07-7.77 (m, 19H, aromatics);  $^{13}C$  NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  22.5(CH<sub>2</sub>, C <sub>$\gamma$</sub> ), 28.4 (3CH<sub>3</sub>, Boc), 29.8 (CH<sub>2</sub>, C <sub>$\delta$</sub> ), 33.5 (CH<sub>2</sub>, C <sub>$\beta$</sub> ), 39.8 (CH<sub>2</sub>, C<sub>3</sub>), 41.5 (CH<sub>2</sub>, C <sub>$\epsilon$</sub> ), 42.7 (CH<sub>2</sub>, Bn), 43.5 (CH<sub>2</sub>, C<sub>1</sub>), 47.2 (CH, Fmoc), 59.3 (CH, C<sub>2</sub>), 60.2 (CH, C <sub>$\alpha$</sub> ), 66.7 (CH<sub>2</sub>, Fmoc), 79.1 (C, Boc), 119.9-143.7 (18CH and 6C, aromatics), 156.2 (CO, Boc), 157.0 (CO, Fmoc), 174.4 (CONH); ES-MS  $m/z$   $[M+1]^+$  calcd for C<sub>42</sub>H<sub>50</sub>N<sub>4</sub>O<sub>5</sub>, 691.38; found 691.59 (100 %)  $[M+1]^+$ ; Anal. calcd. for C<sub>42</sub>H<sub>50</sub>N<sub>4</sub>O<sub>5</sub>: C, 73.02; H, 7.29; N, 8.11. Found: C, 73.14; H, 7.38; N, 7.96.

4.3.5. *N<sub>δ</sub>-Pbf-N<sub>α</sub>-[(2S)-3-Phenyl-1-(Fmoc)amino-propan-2-yl]-arginine benzyl amide [(S)-12c]*

Foam (222 mg, 85 %); HPLC [Sunfire C<sub>18</sub>, 3.9×50 mm, 3.5 μm, (10-100 %, 5 min) gradient of solvent A in solvent B] *t*<sub>R</sub> 4.65 min; [α]<sub>D</sub><sup>20</sup> +6.4 (*c* 1, MeOH); <sup>1</sup>H-RMN (500 MHz, CDCl<sub>3</sub>) δ (ppm): 1.12-1.80 [m, 4H, β-H and γ-H (Arg)], 1.45 [s, 6H, 2 CH<sub>3</sub> (Pbf)], 2.09 [s, 3H, CH<sub>3</sub> (Pbf)], 2.51 [s, 3H, CH<sub>3</sub> (Pbf)], 2.59 [s, 3H, CH<sub>3</sub> (Pbf)], 2.66-2.84 [m, 2H, 1-H (Pr)], 2.73 [m, 1H, 2-H (Pr)], 2.92 [s, 2H, CH<sub>2</sub> (Pbf)], 3.02-3.55 [m, 4H, 1-H (Pr) and δ-H (Arg)], 3.38 [m, 1H, α-H (Arg)], 3.85 [dd, 1H, *J* = 7.5 and 15 Hz, CH<sub>2</sub> (Bn)], 4.17 [t, 1H, *J* = 7 Hz, CH<sub>2</sub> (Fmoc)], 4.29 [dd, 1H, *J* = 7.5 and 15 Hz, CH<sub>2</sub> (Bn)], 4.25-4.53 [m, 2H, CH<sub>2</sub> (Fmoc)], 5.60 (bs, 1H, *NH*-Fmoc), 6.28 (bs, 3H, guanidine NH), 6.97-7.78 (m, 15H, aromatics); <sup>13</sup>C-RMN (125 MHz, CDCl<sub>3</sub>) δ (ppm): 12.9, 18.3, 19.7, 28.9 (5CH<sub>3</sub>, Pbf), 25.7 (CH<sub>2</sub>, C<sub>γ</sub>), 30.1 (CH<sub>2</sub>, C<sub>β</sub>), 31.3 (CH<sub>2</sub>, C<sub>3</sub>), 40.3 (CH<sub>2</sub>, C<sub>δ</sub>), 43.1 (CH<sub>2</sub>, C<sub>1</sub>), 43.6 (CH<sub>2</sub>, Pbf), 47.6 (CH, Fmoc), 59.2 (CH, C<sub>2</sub>), 59.5 (CH, C<sub>α</sub>), 67.1 (CH<sub>2</sub>, Fmoc), 86.77, 117.93, 125.0, 132.6, 133.3 (5C, Pbf), 126.9-139.2 (15CH and 5C, aromatics and Pbf), 156.7 (CO, Fmoc), 157.7-159.2 (C=NH, guanidine), 174.8 (CONH); ES-MS *m/z* [M+1]<sup>+</sup> calcd for C<sub>50</sub>H<sub>58</sub>N<sub>6</sub>O<sub>6</sub>S: 871.77; found 871.77 (100%); Anal. calcd. for C<sub>50</sub>H<sub>58</sub>N<sub>6</sub>O<sub>6</sub>S: C, 68.94; H, 6.71; N, 9.65. Found: C, 69.07; H, 6.82; N, 9.46.

#### 4.4. General procedure for the synthesis of basic amino acid-derived ureas. Method B. Synthesis of ureas (**R**)-**8a,b**, (**S**)-**8a-c**, (**R**)- and (**S**)-**13a**, and (**R**)- and (**S**)-**14-16b**

Diethyl amine (0.32 mL, 3 mmol) was added to a solution of the corresponding Fmoc-protected amine **12a-c** (0.3 mmol) and the reaction mixture was stirred at rt for 2 h. Then, the solvent was evaporated to dryness and the residue was dissolved in EtOAc (50 mL). The solution was successively washed with H<sub>2</sub>O (20 mL) and brine (20 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, and evaporated to dryness. The residue was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (10 mL), cooled at 0°C. The corresponding isocyanate (0.3 mmol) was added to this solution and the mixture was stirred for 15 min-2 h. Then, the solvent was evaporated and the residue was dissolved in EtOAc (50 mL). This solution was successively washed with H<sub>2</sub>O (20 mL) and brine (20 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, and evaporated to dryness. The residue was purified by flash chromatography, using 20-100% gradient of EtOAc in hexane as eluant to give the corresponding urea (**R**)-**8a,b**, (**S**)-**8a-c**, (**R**)- and (**S**)-**13a**, and (**R**)- and (**S**)-**14-16b**.

##### 4.4.1. N<sub>δ</sub>-Boc-N<sub>α</sub>-[(2R)-3-Phenyl-1-(3-phenylureido)propan-2-yl]-ornithine benzyl amide [(**R**)-**8a**]



Foam (53 mg, 31 %); HPLC  $t_R$  17.45 min;  $[\alpha]_D^{20} + 7.2$  ( $c$  1, MeOH);  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  1.10-2.41 [m, 4H,  $\beta$ -H and  $\gamma$ -H (Orn)], 1.40 (s, 9H, Boc), 2.58-2.76 [m, 2H, 3-H (Pr)], 2.81 [m, 1H, 2-H (Pr)], 2.84-3.02 [m, 5H, 1-H (Pr),  $\alpha$ -H and  $\delta$ -H(Orn)], 4.29 [dd, 1H,  $J=6$  and 15 Hz,  $CH_2$  (Bn)], 4.41 [dd, 1H,  $J=6$  and 15 Hz,  $CH_2$  (Bn)], 4.62 (bs, 1H,  $NH$ -Boc), 6.89-7.42 (m, 16H, aromatics and  $NH$ -Bn), 5.77 and 7.57 (2bs, 2H, NHC ONH);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  26.0 ( $CH_2$ ,  $C_\gamma$ ), 28.4 (3 $CH_3$ , Boc), 30.7 ( $CH_2$ ,  $C_\beta$ ), 39.2 ( $CH_2$ ,  $C_\delta$ ), 39.4 ( $CH_2$ ,  $C_3$ ), 43.2 ( $CH_2$ , Bn), 43.9 ( $CH_2$ ,  $C_1$ ), 60.2 (CH,  $C_\alpha$ ), 60.4 (CH,  $C_2$ ), 79.4 (C, Boc), 119.5-139.2 (15CH and 3 C, aromatics), 156.2 (HNCONH), 156.4 (CO, Boc), 175.0 (CONH); ES-MS  $m/z$   $[M+1]^+$  calcd for  $C_{33}H_{43}N_5O_4$ , 574.33; found 574.27 (100%); Anal. calcd. for  $C_{33}H_{43}N_5O_4$ : C, 69.08; H, 7.55; N, 12.21. Found: C, 69.13; H, 7.62; N, 12.08.

#### 4.4.2. $N_\delta$ -Boc- $N_\alpha$ -[(2S)-3-Phenyl-1-(3-phenylureido)propan-2-yl]-ornithine benzyl amide [(S)-8a]

Foam (144 mg, 84 %); HPLC  $t_R$  18.15 min;  $[\alpha]_D^{20} + 6.7$  ( $c$  1.1, MeOH);  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  1.10-1.74 [m, 4H,  $\beta$ -H and  $\gamma$ -H (Orn)], 1.42 (s, 9H, Boc), 2.40-2.75 [m, 2H, 3-H (Pr)], 2.81-3.14 [m, 2H, 1-H (Pr)], 2.93 [m, 1H, 2-H (Pr)], 3.14- 3.48 [m, 3H,  $\alpha$ -H and  $\delta$ -H (Orn)], 3.70 [dd, 1H,  $J=7$  and 15Hz,  $CH_2$  (Bn)], 4.20 (dd, 1H,  $J=5$  and 15Hz,  $CH_2$  (Bn)], 4.94 (bs, 1H,  $NH$ -Boc), 6.86-7.47 (m, 15H, aromatics), 6.04 and 8.62 (2bs, 2H, NHCONH), 7.75 (bs, 1H,  $NH$ -Bn);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  26.2 ( $CH_2$ ,  $C_\gamma$ ), 28.7 (3 $CH_3$ , Boc), 30.8 ( $CH_2$ ,  $C_\beta$ ), 40.2 ( $CH_2$ ,  $C_\delta$ ), 40.6 ( $CH_2$ ,  $C_3$ ), 42.4 ( $CH_2$ , Bn), 42.9 ( $CH_2$ ,  $C_1$ ), 59.5 (CH,  $C_2$ ), 59.6 (CH,  $C_\alpha$ ), 79.9 (C, Boc), 119.6-139.6 (15CH and 3C, aromatics), 156.8 (HNCONH), 156.9 (CO, Boc), 174.7 (CONH); ES-MS  $m/z$   $[M+1]^+$  calcd for  $C_{33}H_{43}N_5O_4$ , 574.33; found 574.52 (100 %), 699.43 (10%)  $[M+Na]^+$ ; Anal. calcd. for  $C_{33}H_{43}N_5O_4$ : C, 69.08; H, 7.55; N, 12.21. Found: C, 69.17; H, 7.60; N, 12.13.

#### 4.4.3. $N_\epsilon$ -Boc- $N_\alpha$ -[(2R)-3-Phenyl-1-(3-phenylureido)propan-2-yl]-lysine benzyl amide [(R)-8b]

Foam (121 mg, 69%); HPLC  $t_R$  18.29 min;  $[\alpha]_D^{20} + 6.5$  ( $c$  0.6, MeOH);  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  0.73-1.65 [m, 6H,  $\beta$ -H,  $\gamma$ -H and  $\delta$ -H (Lys)], 1.37 (s, 9H, Boc), 2.53-2.87 [m, 3H, 3-H and 2-H (Pr)], 2.89-3.00 [m, 2H, 1-H (Pr)], 3.01-3.42 [m, 3H,  $\alpha$ -H and  $\epsilon$ -H (Lys)], 4.29 [dd, 1H,  $J=6$  and 15 Hz,  $CH_2$  (Bn)], 4.39 [dd, 1H,  $J=6$  and 15 Hz,  $CH_2$  (Bn)], 4.59 (s, 1H,  $NH$ -Boc), 5.51 (bs, 1H, HNCONH), 6.87-7.34 (m, 16H, aromatics and HNCONH), 7.56 (bs, 1H,  $NH$ -Bn);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  22.6 ( $CH_2$ ,  $C_\gamma$ ), 28.4 (3 $CH_3$ , Boc), 29.6

(CH<sub>2</sub>, C<sub>δ</sub>), 33.3 (CH<sub>2</sub>, C<sub>β</sub>), 39.0 (CH<sub>2</sub>, C<sub>3</sub>), 39.9 (CH<sub>2</sub>, C<sub>ε</sub>), 43.1 (CH<sub>2</sub>, Bn), 43.8 (CH<sub>2</sub>, C<sub>1</sub>), 59.1 (CH, C<sub>2</sub>), (61.0 (CH, C<sub>α</sub>), 79.3 (C, Boc), 122.8-138.4 (15CH and 3C, aromatics), 156.0 (HNCONH), 156.2 (CO, Boc), 174.0 (CONH); ES-MS  $m/z$  [M+1]<sup>+</sup> calcd for C<sub>34</sub>H<sub>45</sub>N<sub>5</sub>O<sub>4</sub>, 588.35; found 588.62 (100%); Anal. calcd. for C<sub>34</sub>H<sub>45</sub>N<sub>5</sub>O<sub>4</sub>: C, 69.48; H, 7.72; N, 11.92. Found: C, 69.62; H, 7.89; N, 12.75.

#### 4.4.4. N<sub>ε</sub>-Boc-N<sub>α</sub>-[(2S)-3-Phenyl-1-(3-phenylureido)propan-2-yl]-lysine benzyl amide [(S)-**8b**]

Foam (143 mg, 81%); HPLC  $t_R$  18.49 min;  $[\alpha]_D^{20} + 4.0$  ( $c$  0.9, MeOH); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.00-1.90 [m, 6H,  $\beta$ -H,  $\gamma$ -H and  $\delta$ -H (Lys)], 1.46 (s, 9H, Boc), 2.51-2.68 [m, 2H, 3-H (Pr)], 2.73 [m, 1H, 2-H (Pr)], 2.90-3.18 [m, 2H,  $\epsilon$ -H (Lys)], 3.18-3.50 [m, 3H, 1-H (Pr) and  $\alpha$ -H (Lys)], 3.90 [dd, 1H, J=7 and 15Hz, CH<sub>2</sub> (Bn)], 4.35 [dd, 1H, J=4 and 15Hz, CH<sub>2</sub> (Bn)], 4.88 (bs, 1H, NH-Boc), 6.82-7.20 (m, 16H, aromatics and NH-Bn), 5.98 and 8.09 (2bs, 2H, HNCONH); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  21.1 (CH<sub>2</sub>, C<sub>γ</sub>), 26.3 (3CH<sub>3</sub>, Boc), 27.6 (CH<sub>2</sub>, C<sub>δ</sub>), 27.9 (CH<sub>2</sub>, C<sub>β</sub>), 30.9 (CH<sub>2</sub>, C<sub>3</sub>), 38.1 (CH<sub>2</sub>, C<sub>ε</sub>), 39.8 (CH<sub>2</sub>, C<sub>1</sub>), 40.7 (CH<sub>2</sub>, Bn), 58.0 (CH, C<sub>2</sub>), 58.4 (CH, C<sub>α</sub>), 77.4 (C, Boc), 117.0-137.3 (15CH and 3 C, aromatics), 154.7 (CO, Boc), 154.8 (HNCONH), 172.1 (CONH); ES-MS  $m/z$  [M+1]<sup>+</sup> calcd for C<sub>34</sub>H<sub>45</sub>N<sub>5</sub>O<sub>4</sub>, 588.35; found, 588.55 (100 %); Anal. calcd. for C<sub>34</sub>H<sub>45</sub>N<sub>5</sub>O<sub>4</sub>: C, 69.48; H, 7.72; N, 11.92. Found: C, 69.59; H, 7.84; N, 12.85.

#### 4.4.5. N<sub>ε</sub>-Pbf-N<sub>α</sub>-[(2S)-3-Phenyl-1-(3-phenylureido)propan-2-yl]-arginine benzyl amide [(S)-**8c**]

Foam ( 68 mg, 29 %); HPLC  $t_R$  19.95 min;  $[\alpha]_D^{20} + 2.5$  ( $c$  1, MeOH); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  0.61-1.63 [m, 4H,  $\beta$ - and  $\gamma$ -H (Arg)], 1.38, 2.01, 2.39 and 2.45 [4s, 15H, CH<sub>3</sub> (Pbf)], 2.74 [m, 1H, 2-H (Pr)], 2.85 [s, 2H, CH<sub>2</sub> (Pbf)], 2.92-3.17 [m, 4H, 3-H (Pr) and  $\delta$ -H(Arg)], 3.20-3.42 [m, 2H, 1-H (Pr)], 3.99 [bs, 1H,  $\alpha$ -H (Arg)], 4.17 [d, 1H, J = 6 and 14.5 Hz, CH<sub>2</sub>(Bn)], 4.30 [dd, 1H, J = 4.5 and 14.5 Hz, CH<sub>2</sub>(Bn)], 6.20 [bs, 3H, guanidino], 6.85 (t, 1H, J = 6 Hz, NH-Bn), 7.00-7.34 (m, 15H, aromatics), 7.78 and 8.15 (2bs, 2H, NHCONH); <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  12.9, 18.3, 19.7, 28.9 (CH<sub>3</sub>, Pbf), 25.7 (CH<sub>2</sub>, C<sub>γ</sub>), 30.1 (CH<sub>2</sub>, C<sub>β</sub>), 31.3 (CH<sub>2</sub>, C<sub>3</sub>), 40.3 (CH<sub>2</sub>, C<sub>δ</sub>), 43.6 (CH<sub>2</sub>, C<sub>1</sub>), 43.6 (CH<sub>2</sub>, Pbf), 59.2 (CH, C<sub>2</sub>), 59.5 (CH, C<sub>α</sub>), 86.77, 117.93, 125.0, 132.6, 133.3 (5 C, Pbf), 126.9-139.2 [15 CH and 4C, aromatics and Pbf], 156.4 (HNCONH), 159.2 (guanidino); ES-MS  $m/z$  [M+1]<sup>+</sup> calcd. for C<sub>42</sub>H<sub>53</sub>N<sub>7</sub>O<sub>5</sub>S,

767.38; found, 768.81 (100%). Anal. calcd. for C<sub>42</sub>H<sub>53</sub>N<sub>7</sub>O<sub>5</sub>S: C, 65.69; H, 6.96; N, 12.77; Found: C, 65.82; H, 7.04; N, 12.68.

4.4.6. N<sub>δ</sub>-Boc-N<sub>α</sub>-[(2R)-1-(3-Benzylureido)-3-phenylpropan-2-yl]-ornithine benzyl amide  
[(R)-13a]

Foam (80 mg, 45 %); HPLC *t*<sub>R</sub> 18.47 min; [α]<sub>D</sub><sup>20</sup> +6.5 (*c* 1.1, MeOH); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 1.19-1.69 [m, 4H, β-H and γ-H (Orn)], 1.39 (s, 9H, Boc), 2.61-2.65 [m, 2H, 3-H (Pr)], 2.80 [m, 1H, 2-H (Pr)], 2.87-3.20 [m, 4H, 1-H (Pr), and δ-H(Orn)], 3.21 [m, 1H, α-H (Orn)], 4.27 [m, 2H, CH<sub>2</sub> (Bn-ureido)], 4.32 [dd, 1H, *J* = 6 and 15 Hz, CH<sub>2</sub> (Bn)], 4.40 [dd, 1H, *J* = 6 and 15 Hz, CH<sub>2</sub> (Bn)], 4.58 (bs, 1H, *NH*-Boc), 4.73 and 5.06 (2bs, 2H, NHCONH), 7.07-7.36 (m, 16H, aromatics and *NH*-Bn); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 26.1 (CH<sub>2</sub>, C<sub>γ</sub>), 28.4 (3CH<sub>3</sub>, Boc), 30.6 (CH<sub>2</sub>, C<sub>β</sub>), 39.4 (CH<sub>2</sub>, C<sub>3</sub>), 39.5 (CH<sub>2</sub>, C<sub>δ</sub>), 43.2 (CH<sub>2</sub>, Bn), 43.9 [CH<sub>2</sub>, (Bn-ureido)], 44.4 (CH<sub>2</sub>, C<sub>1</sub>), 59.4 (CH, C<sub>2</sub>), 59.9 (CH, C<sub>α</sub>), 79.4 (C, Boc), 127.2-138.7 (15CH and 3 C, aromatics), 156.2 (CO, Boc), 158.2 (HNCONH), 174.6 (CONH); ES-MS *m/z* [M+1]<sup>+</sup> calcd for C<sub>34</sub>H<sub>45</sub>N<sub>5</sub>O<sub>4</sub>, 588.35; found 588.48 (100%); Anal. calcd. for C<sub>34</sub>H<sub>45</sub>N<sub>5</sub>O<sub>4</sub>: C, 69.48; H, 7.72; N, 11.92. Found: C, 69.61; H, 7.62; N, 12.08.

4.4.7. N<sub>δ</sub>-Boc-N<sub>α</sub>-[(2S)-1-(3-Benzylureido)-3-phenylpropan-2-yl]-ornithine benzyl amide  
[(S)-13a]

Foam (112 mg, 64 %); HPLC *t*<sub>R</sub> 18.86 min; [α]<sub>D</sub><sup>20</sup> + 7.0 (*c* 0.9, MeOH); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.31 (s, 9H, Boc), 1.41-1.85 [m, 4H, β-H and γ-H (Orn)], 2.45-2.81 [m, 2H, 3-H (Pr)], 2.74 [m, 1H, 2-H (Pr)], 2.86-3.34 [m, 4H, 1-H (Pr) and δ-H (Orn)], 3.42 [m, 2H, α-H (Orn)], 3.78 [dd, 1H, *J* = 7 and 15 Hz, CH<sub>2</sub> (Bn)], 4.30 [dd, 1H, *J* = 4 and 15 Hz, CH<sub>2</sub> (Bn)], 4.37 [m, 2H, CH<sub>2</sub> (Bn-ureido)], 4.94 (bs, 1H, *NH*-Boc), 6.86-7.47 (m, 15H, aromatics), 5.81 (1bs, 2H, NHCONH), 6.96 (bs, 1H, *NH*-Bn); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 25.8 (CH<sub>2</sub>, C<sub>γ</sub>), 28.3 (3CH<sub>3</sub>, Boc), 30.5 (CH<sub>2</sub>, C<sub>β</sub>), 39.8 (CH<sub>2</sub>, C<sub>δ</sub>), 40.3 (CH<sub>2</sub>, C<sub>3</sub>), 41.7 (CH<sub>2</sub>, Bn), 42.6 [CH<sub>2</sub>, (Bn-ureido)], 44.3 (CH<sub>2</sub>, C<sub>1</sub>), 58.6 (CH, C<sub>2</sub>), 59.2 (CH, C<sub>α</sub>), 79.7 (C, Boc), 126.3-139.7 (15CH and 3C, aromatics), 156.7 (CO, Boc), 159.2 (HNCONH), 174.4 (CONH); ES-MS *m/z* [M+1]<sup>+</sup> calcd for C<sub>34</sub>H<sub>45</sub>N<sub>5</sub>O<sub>4</sub>, 588.35; found 588.62 (100%); Anal. calcd. for C<sub>34</sub>H<sub>45</sub>N<sub>5</sub>O<sub>4</sub>: C, 69.48; H, 7.72; N, 11.92. Found: C, 69.63; H, 7.84; N, 11.87.

4.4.8. N<sub>ε</sub>-Boc-N<sub>α</sub>-[(2R)-1-(3-(2,4-Dichlorophenyl)ureido)-3-phenylpropan-2-yl]-lysine  
benzyl amide [(R)-14b]

Foam (125 mg, 63%); HPLC  $t_R$  20.05 min;  $[\alpha]_D^{20} +7.5$  ( $c$  1, MeOH);  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  0.73-1.65 [m, 6H,  $\beta$ -H,  $\gamma$ -H and  $\delta$ -H (Lys)], 1.42 (s, 9H, Boc), 2.63 [dd, 1H,  $J$ = 6 and 13.5 Hz, 3-H (Pr)], 2.72 [dd, 1H,  $J$ = 6 and 13.5 Hz, 3-H (Pr)], 2.83-3.14 [m, 5H, 1-H (Pr), 2-H (Pr), and  $\epsilon$ -H (Lys)], 3.32 [m, 1H,  $\alpha$ -H (Lys)], 4.30 [dd, 1H,  $J$ = 6 and 15 Hz,  $CH_2$  (Bn)], 4.40 [dd, 1H,  $J$ = 6 and 15 Hz,  $CH_2$  (Bn)], 4.55 (s, 1H,  $NH$ -Boc), 5.96 (bs, 2H, HNCONH), 7.04-7.31 (m, 13H, aromatics), 7.33 (bs, 1H,  $NH$ -Bn);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  22.5 ( $CH_2$ ,  $C_\gamma$ ), 28.4 (3 $CH_3$ , Boc), 29.6 ( $CH_2$ ,  $C_\delta$ ), 33.6 ( $CH_2$ ,  $C_\beta$ ), 39.2 ( $CH_2$ ,  $C_3$ ), 39.8 ( $CH_2$ ,  $C_\epsilon$ ), 43.2 ( $CH_2$ , Bn), 43.9 ( $CH_2$ ,  $C_1$ ), 58.9 (CH,  $C_2$ ), 60.8 (CH,  $C_\alpha$ ), 79.4 (C, Boc), 121.5-138.2 (13CH and 5C, aromatics), 154.9 (HNCONH), 156.3 (CO, Boc), 175.0 (CONH); ES-MS  $m/z$   $[M+1]^+$  calcd for  $C_{34}H_{43}Cl_2N_5O_4$ , 656.27; found, 656.66 (100%), 658.62 (70%)  $[M+3]^+$ , 660.65 (10%)  $[M+5]^+$ ; Anal. calcd. for  $C_{34}H_{43}Cl_2N_5O_4$ : C, 62.19; H, 6.60; N, 10.67. Found: C, 62.32; H, 6.75; N, 10.48.

4.4.9.  $N_\epsilon$ -Boc- $N_\alpha$ -[(2S)-1-(3-(2,4-Dichlorophenyl)ureido)-3-phenylpropan-2-yl]-lysine benzyl amide [(S)-14b]

Foam (147 mg, 74%); HPLC  $t_R$  20.03 min;  $[\alpha]_D^{20} +3.0$  ( $c$  0.8, MeOH);  $^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$  0.86-2.19 [m, 6H,  $\beta$ -H,  $\gamma$ -H and  $\delta$ -H (Lys)], 1.45 (s, 9H, Boc), 2.66-2.91 [m, 3H, 2-H and 3-H (Pr)], 3.00-3.54 [m, 5H, 1-H (Pr),  $\alpha$ -H and  $\epsilon$ -H (Lys)], 4.00 [dd, 1H,  $J$ = 6.5 and 15 Hz,  $CH_2$  (Bn)], 4.37 [dd, 1H,  $J$ = 6.5 and 15 Hz,  $CH_2$  (Bn)], 4.86 (s, 1H,  $NH$ -Boc), 6.33 (bs, 2H, HNCONH), 6.90-7.58 (m, 13H, aromatics), 7.70 (bs, 1H,  $NH$ -Bn);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  20.8 ( $CH_2$ ,  $C_\gamma$ ), 28.5 (3 $CH_3$ , Boc), 29.7 ( $CH_2$ ,  $C_\beta$  and  $C_\delta$ ), 36.6 ( $CH_2$ ,  $C_3$ ), 39.7 ( $CH_2$ ,  $C_\epsilon$ ), 40.0 ( $CH_2$ , Bn), 42.9 ( $CH_2$ ,  $C_1$ ), 60.2 (CH,  $C_2$ ), 60.4 (CH,  $C_\alpha$ ), 79.9 (C, Boc), 121.4-138.5 (13CH and 5C, aromatics), 154.7 (CO, Boc), 157.2 (HNCONH), 170.2 (CONH); ES-MS  $m/z$   $[M+1]^+$  calcd for  $C_{34}H_{43}Cl_2N_5O_4$ , 656.27; found, 656.46 (100%), 658.48 (80%)  $[M+3]^+$ , 660.49 (15%)  $[M+5]^+$ ; Anal. calcd. for  $C_{34}H_{43}Cl_2N_5O_4$ : C, 62.19; H, 6.60; N, 10.67. Found: C, 62.29; H, 6.71; N, 10.55.

4.4.10.  $N_\epsilon$ -Boc- $N_\alpha$ -[(2R)-1-(3-(4-Methoxyphenethyl)ureido)-3-phenylpropan-2-yl]-lysine benzyl amide [(R)-15b]

Foam (127 mg, 66%); HPLC  $t_R$  18.29 min;  $[\alpha]_D^{20} +8.5$  ( $c$  1.1, MeOH);  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  0.78-1.71 [m, 6H,  $\beta$ -H,  $\gamma$ -H and  $\delta$ -H (Lys)], 1.42 (s, 9H, Boc), 2.66 [t, 2H,  $J$ = 7 Hz, 2-H (ethyl)], 2.66-2.82 [m, 3H, 2-H and 3-H (Pr)], 2.91-3.18 [m, 5H, 1-H (Pr),  $\alpha$ -H and  $\epsilon$ -H (Lys)], 3.28 [dd, 2H,  $J$ = 7 and 13 Hz, 1-H (ethyl)], 3.76 (s, 3H, OMe), 4.32 [dd, 1H,  $J$ = 6

and 14.5 Hz, CH<sub>2</sub> (Bn)], 4.41 [dd, , 1H, J= 6 and 14.5 Hz, CH<sub>2</sub> (Bn)], 4.55 (bs, 1H, *NH*-Boc), 4.70 (bs, 2H, HNCONH), 7.04-7.33 (m, 14H, aromatics), 7.57 (bs, 1H, *NH*-Bn); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 22.6 (CH<sub>2</sub>, C<sub>γ</sub>), 28.4 (3CH<sub>3</sub>, Boc), 29.7 (CH<sub>2</sub>, C<sub>δ</sub>), 30.0 (CH<sub>2</sub>, C<sub>β</sub>), 35.4 (CH<sub>2</sub>, C<sub>3</sub>), 35.5 [CH<sub>2</sub>, C<sub>2</sub> (ethyl)], 38.9 [CH<sub>2</sub>, C<sub>ε</sub> and C<sub>1</sub> (ethyl)], 43.1 (CH<sub>2</sub>, Bn), 43.9 (CH<sub>2</sub>, C<sub>1</sub>), 55.2 (CH<sub>3</sub>, OMe), 59.5 (CH, C<sub>2</sub>), 60.9 (CH, C<sub>α</sub>), 79.1 (C, Boc), 113.9-138.5 (14CH and 4C, aromatics), 156.0 (CO, Boc), 158.3 (HNCONH), 174.6 (CONH); ES-MS *m/z* [M+1]<sup>+</sup> calcd for C<sub>37</sub>H<sub>51</sub>N<sub>5</sub>O<sub>5</sub>, 646.39; found, 646.72 (100 %); Anal. calcd. for C<sub>37</sub>H<sub>51</sub>N<sub>5</sub>O<sub>5</sub>: C, 68.81; H, 7.96; N, 10.84. Found: C, 68.96; H, 8.07; N, 10.48.

4.4.11. N<sub>ε</sub>-Boc-N<sub>α</sub>-[(2S)-1-(3-(4-Methoxyphenethyl)ureido)-3-phenylpropan-2-yl]-lysine  
benzyl amide [(S)-15b]

Foam (143 mg, 87%); HPLC *t<sub>R</sub>* 18.43 min; [α]<sub>D</sub><sup>20</sup> +5.9 (*c* 1, MeOH); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 1.09-2.23 [m, 6H, β-H, γ-H and δ-H (Lys)], 1.43 (s, 9H, Boc), 2.60-2.69 [m, 2H, 3-H (Pr)], 2.65-2.72 [m, 2H, 2-H (Pr)], 2.76 [t, 2H, J= 7 Hz, 2-H (ethyl)], 2.99-3.17 [m, 4H, 1-H (Pr) and ε-H (Lys)], 3.29-3.50 [m, 1H, α-H (Lys)], 3.40 [dd, 2H, J= 7 and 13 Hz, 1-H (ethyl)], 3.80 (s, 3H, OMe), 3.91 [dd, 1H, J= 7 and 15 Hz, CH<sub>2</sub> (Bn)], 4.35 [dd, , 1H, J= 7 and 15 Hz, CH<sub>2</sub> (Bn)], 4.85 (bs, 1H, *NH*-Boc), 5.15 and 5.29 (2bs, 2H, HNCONH), 6.75-7.44 (m, 15H, aromatics and *NH*-Bn); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 23.6 (CH<sub>2</sub>, C<sub>γ</sub>), 28.8 (3CH<sub>3</sub>, Boc), 30.1 (CH<sub>2</sub>, C<sub>δ</sub>), 30.5 (CH<sub>2</sub>, C<sub>β</sub>), 33.5 (CH<sub>2</sub>, C<sub>3</sub>), 35.5 [CH<sub>2</sub>, C<sub>2</sub> (ethyl)], 40.5 (CH<sub>2</sub>, C<sub>ε</sub>), 40.8 [CH<sub>2</sub>, C<sub>1</sub> (ethyl)], 42.2 (CH<sub>2</sub>, Bn), 43.1 (CH<sub>2</sub>, C<sub>1</sub>), 55.6 (CH<sub>3</sub>, OMe), 60.6 (CH, C<sub>2</sub>), 60.8 (CH, C<sub>α</sub>), 79.9 (C, Boc), 114.3-139.5 (14CH and 4C, aromatics), 157.1 (CO, Boc), 159.5 (HNCONH), 174.9 (CONH); ES-MS *m/z* [M+1]<sup>+</sup> calcd for C<sub>37</sub>H<sub>51</sub>N<sub>5</sub>O<sub>5</sub>, 646.39; found, 646.52 (100 %); Anal. calcd. for C<sub>37</sub>H<sub>51</sub>N<sub>5</sub>O<sub>5</sub>: C, 68.81; H, 7.96; N, 10.84. Found: C, 68.93; H, 8.01; N, 10.69.

4.4.12. N<sub>ε</sub>-Boc-N<sub>α</sub>-[(2R)-1-(3-(4-Fluorophenethyl)ureido)-3-phenylpropan-2-yl]-lysine  
benzyl amide [(R)-16b]

Foam (118 mg, 63%); HPLC *t<sub>R</sub>* 18.47 min; [α]<sub>D</sub><sup>20</sup> +7.6 (*c* 1, MeOH); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.81-2.11 [m, 6H, β-H, γ-H and δ-H (Lys)], 1.46 (s, 9H, Boc), 2.56-2.89 [m, 3H, 2-H and 3-H (Pr)], 2.77 [t, 2H, J= 7 Hz, 2-H (ethyl)], 2.93-3.28 [m, 5H, 1-H (Pr), α-H and ε-H (Lys)], 3.32 [dd, 2H, J= 7 and 13 Hz, 1-H (ethyl)], 4.41 [d, 2H, J= 7 Hz, CH<sub>2</sub> (Bn)], 4.58 (bs, 1H, *NH*-Boc), 4.80 (bs, 2H, HNCONH), 6.89-7.48 (m, 14H, aromatics), 7.52 (bs, 1H, *NH*-Bn); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 22.3 (CH<sub>2</sub>, C<sub>γ</sub>), 28.0 (3CH<sub>3</sub>, Boc), 29.3 (CH<sub>2</sub>, C<sub>δ</sub>), 30.5

(CH<sub>2</sub>, C<sub>β</sub>), 32.9 [CH<sub>2</sub>, C<sub>2</sub> (ethyl)], 35.2 (CH<sub>2</sub>, C<sub>3</sub>), 38.9 (CH<sub>2</sub>, C<sub>ε</sub>), 41.2 [CH<sub>2</sub>, C<sub>1</sub> (ethyl)], 42.7 (CH<sub>2</sub>, Bn), 43.8 (CH<sub>2</sub>, C<sub>1</sub>), 59.1 (CH, C<sub>2</sub>), 60.7 (CH, C<sub>ω</sub>), 79.8 (C, Boc), 114.7-159.5 (14CH and 4C, aromatics), 155.7 (CO, Boc), 157.9 (HNCONH), 174.4 (CONH); ES-MS *m/z* [M+1]<sup>+</sup> calcd for C<sub>36</sub>H<sub>48</sub>FN<sub>5</sub>O<sub>4</sub>, 634.37; found, 634.68 (100 %); Anal. calcd. for C<sub>36</sub>H<sub>48</sub>FN<sub>5</sub>O<sub>4</sub>: C, 68.22; H, 7.63; N, 11.05. Found: C, 68.38; H, 7.75; N, 10.89.

4.4.13. N<sub>ε</sub>-Boc-N<sub>α</sub>-[(2S)-1-(3-(4-Fluorophenethyl)ureido)-3-phenyl]propan-2-yl]-lysine  
benzyl amide [(S)-16b]

Foam (168 mg, 89%); HPLC *t<sub>R</sub>* 18.57 min; [α]<sub>D</sub><sup>20</sup> +3.5 (*c* 1.1, MeOH); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.77-2.23 [m, 6H, β-H, γ-H and δ-H (Lys)], 1.45 (s, 9H, Boc), 2.58-2.73 [m, 3H, 2-H and 3-H (Pr)], 2.73 [t, 2H, J= 7 Hz, 2-H (ethyl)], 2.94-3.28 [m, 4H, 1-H (Pr) and ε-H (Lys)], 3.28-3.37 [m, 1H, α-H (Lys)], 3.40 [dd, 2H, J= 7 and 13 Hz, 1-H (ethyl)], 3.89 [dd, J= 7 and 15 Hz, CH<sub>2</sub> (Bn)], 4.34 [dd, J= 7 and 15 Hz, CH<sub>2</sub> (Bn)], 4.88 (bs, 1H, NH-Boc), 5.26 and 5.41 (2bs, 2H, HNCONH), 6.88-7.60 (m, 15H, aromatics and NH-Bn); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 23.6 (CH<sub>2</sub>, C<sub>γ</sub>), 28.8 (3CH<sub>3</sub>, Boc), 30.1 (CH<sub>2</sub>, C<sub>δ</sub>), 30.5 (CH<sub>2</sub>, C<sub>β</sub>), 33.6 [CH<sub>2</sub>, C<sub>2</sub> (ethyl)], 36.2 (CH<sub>2</sub>, C<sub>3</sub>), 40.5 (CH<sub>2</sub>, C<sub>ε</sub>), 40.9 [CH<sub>2</sub>, C<sub>1</sub> (ethyl)], 42.0 (CH<sub>2</sub>, Bn), 43.1 (CH<sub>2</sub>, C<sub>1</sub>), 60.6 (CH, C<sub>2</sub>), 60.8 (CH, C<sub>ω</sub>), 79.8 (C, Boc), 115.5-139.6 (14CH and 4C, aromatics), 157.1 (CO, Boc), 160.3 (HNCONH), 175.0 (CONH); ES-MS *m/z* [M+1]<sup>+</sup> calcd for C<sub>36</sub>H<sub>48</sub>FN<sub>5</sub>O<sub>4</sub>, 634.37; found, 634.43 (100 %); Anal. calcd. for C<sub>36</sub>H<sub>48</sub>FN<sub>5</sub>O<sub>4</sub>: C, 68.22; H, 7.63; N, 11.05. Found: C, 68.36; H, 7.78; N, 10.95.

4.5. General procedure for the N-Boc-deprotection of protected ureas (RS)-9b, (R)- and (S)-8a,b, (R)-, (S)-9a and -13a, (R)- and (S)-14-16b. Synthesis of urea hydrochlorides (R)- and (S)-11a, (RS)-11b, (R)- and (S)-17a,b, (R)- and (S)-18a, (R)- and (S)-19-21b

The corresponding protected urea (0.10 mmol) was dissolved in a 3M solution of HCl in EtOAc (2 mL) and stirred at rt for 2 h. Then, the solvent was evaporated under reduced pressure, the residue was dissolved in H<sub>2</sub>O (3 mL) and the solution was lyophilized.

4.5.1. N<sub>α</sub>-[(2R)-4-Phenyl-1-(3-phenylureido)butan-2-yl]-ornithine benzyl amide  
dihydrochloride [(R)-11a]

Amorphous solid (56 mg, 100 %); [α]<sub>D</sub><sup>20</sup> + 6.9 (*c* 0.9, MeOH); <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 1.51-2.06 [m, 6H, 3-H (Bu), γ- and β-H (Orn)], 2.64-2.94 [m, 4H, 4-H (Bu) and δ-H (Orn)], 3.08 [m, 1H, 2-H (Bu)], 3.22-3.45 [m, 2H, 1-H (Bu)], 4.09 [m, 1H, α-H (Orn)],

4.25 [dd, 1H, J = 5 and 15 Hz, CH<sub>2</sub> (Bn)], 4.40 [dd, 1H, J = 4 and 15 Hz, CH<sub>2</sub> (Bn)], 6.74 and 6.91 (2 bs, 2H, HNCONH), 6.97-7.47 (m, 15H, Ph), 7.95 [bs, 3H, δ-NH<sub>3</sub><sup>+</sup> (Orn)], 9.11 and 9.43 [2bs, 2H, α-NH<sub>2</sub><sup>+</sup> (Orn)], 9.33 (bs, 1H, NH-Bn); <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>) δ 22.7 (CH<sub>2</sub>, C<sub>γ</sub>), 27.2 (CH<sub>2</sub>, C<sub>β</sub>), 28.1 (CH<sub>2</sub>, C<sub>4</sub>), 29.4 (CH<sub>2</sub>, C<sub>3</sub>), 31.0 (CH<sub>2</sub>, C<sub>δ</sub>), 38.1 [CH<sub>2</sub>, (Bn)], 42.6 (CH<sub>2</sub>, C<sub>1</sub>), 56.6 (CH, C<sub>2</sub>), 57.8 (CH, C<sub>α</sub>), 118.7-140.7 (15CH, and 3C, Ph), 156.5 (HNCONH), 166.9 (CONH); ES-MS *m/z* [M+1]<sup>+</sup> calcd. for C<sub>29</sub>H<sub>37</sub>N<sub>5</sub>O<sub>2</sub>, 488.29; found, 488.50 (100%).

4.5.2. N<sub>α</sub>-[*(2S)*-4-Phenyl-1-(3-phenylureido)butan-2-yl]-ornithine benzyl amide dihydrochloride [(*S*)-**11a**]

Amorphous solid (55 mg, 100 %); [α]<sub>D</sub><sup>20</sup> + 11.3 (*c* 1, MeOH); <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 1.61-2.09 [m, 6H, 3-H (Bu), γ- and β-H (Orn)], 2.55-2.96 [m, 4H, 4-H (Bu) and δ-H (Orn)], 3.05 [m, 1H, 2-H (Bu)], 3.35-3.71 [m, 2H, 1-H (Bu)], 4.25 [m, 1H, α-H (Orn)], 4.33 [d, 2H, J = 5, CH<sub>2</sub> (Bn)], 6.92 and 7.32 (2 bs, 2H, HNCONH), 7.12-7.64 (m, 15H, Ph), 8.07 [bs, 3H, δ-NH<sub>3</sub><sup>+</sup> (Orn)], 9.11 and 9.43 [2bs, 2H, α-NH<sub>2</sub><sup>+</sup> (Orn)], 9.19 (bs, 1H, NH-Bn); <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>) δ 22.4 (CH<sub>2</sub>, C<sub>γ</sub>), 27.1 (2CH<sub>2</sub>, C<sub>4</sub> and C<sub>β</sub>), 30.5 (CH<sub>2</sub>, C<sub>3</sub>), 30.9 (CH<sub>2</sub>, C<sub>δ</sub>), 37.8 [CH<sub>2</sub>, (Bn)], 42.4 (CH<sub>2</sub>, C<sub>1</sub>), 57.2 (CH, C<sub>2</sub>), 57.5 (CH, C<sub>α</sub>), 117.7-139.9 (15CH, and 3C, Ph), 156.0 (HNCONH), 166.5 (CONH); ES-MS *m/z* [M+1]<sup>+</sup> calcd. for C<sub>29</sub>H<sub>37</sub>N<sub>5</sub>O<sub>2</sub>, 488.29; found, 488.50 (100%).

4.5.3. N<sub>α</sub>-[4-Phenyl-1-(3-phenylureido)butan-2-yl]-lysine benzyl amide dihydrochloride [(*RS*)-**11b**]

Amorphous solid (57 mg, 29 %); HPLC *t*<sub>R</sub> 13.26 [(*R*)-**9b**] and 13.45 min [(*S*)-**9b**]; <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 1.23-1.40 [m, 2H, γ-H (Lys)], 1.43-1.62 [m, 2H, β-H (Lys)], 1.70-1.96 [m, 4H, δ-H (Lys) and 3-H (Bu)], 2.58-2.78 [m, 4H, 4-H (Bu) and ε-H (Lys)], 2.91-3.06 [m, 1H, 2-H (Bu)], and α-H (Lys)], 3.38-3.65 [m, 2H, 1-H (Bu)], 4.03 and 4.16 [2m, 1H, α-H (Lys)], 4.26 [dd, 0.5H, J= 7 and 15 Hz, CH<sub>2</sub> (Bn)], 4.30 [d, 1H, J = 5 Hz, CH<sub>2</sub> (Bn)], 4.35 [dd, 0.5H, J= 7 and 15 Hz, CH<sub>2</sub> (Bn)], 6.77 (bs, 2H, HNCONH), 7.10-7.43 (m, 15 H, Ph), 7.92 [bs, 3H, δ-NH<sub>3</sub><sup>+</sup> (Lys)], 8.93, 8.77, 9.33 and 9.46 [4bs, 2H, α-NH<sub>2</sub><sup>+</sup> (Lys)], 9.32 (bs, 1H, NH-Bn); <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>) δ 22.9 (CH<sub>2</sub>, C<sub>γ</sub>), 29.8 (CH<sub>2</sub>, C<sub>δ</sub>), 32.1 (CH<sub>2</sub>, C<sub>4</sub>), 33.0 (CH<sub>2</sub>, C<sub>β</sub>), 34.8 (CH<sub>2</sub>, C<sub>3</sub>), 40.0 (CH<sub>2</sub>, C<sub>ε</sub>), 42.0 and 43.0 (CH<sub>2</sub>, C<sub>1</sub>), 43.3 [CH<sub>2</sub>, (Bn)], 56.9 and 58.8 (CH, C<sub>2</sub>), 60.1 (CH, C<sub>α</sub>), 119.1-141.3 (15CH, and 3C, Ph), 156.2 and 156.7

(HNCONH), 174.9 (CONH); ES-MS  $m/z$   $[M+1]^+$  calcd. for  $C_{30}H_{39}N_5O_2$ , 502.31; found, 502.50 (100%).

4.5.4.  $N_{\alpha}$ -[(2R)-3-Phenyl-1-(3-phenylureido)propan-2-yl]-ornithine benzyl amide dihydrochloride [(R)-17a]

Amorphous solid (54 mg, 100 %); HPLC  $t_R$  12.87 min;  $[\alpha]_D^{20} + 5.5$  ( $c$  1.3, MeOH);  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  1.56-1.79 [m, 2H,  $\gamma$ -H (Orn)], 1.80-2.07 [m, 2H,  $\beta$ -H (Orn)], 2.69-3.31 [m, 7H, 1-H, 2-H, 3-H (Pr) and  $\delta$ -H(Orn)], 4.25 [dd, 1H,  $J=6$  and 15 Hz,  $CH_2$  (Bn)], 4.33 [m, 2H,  $\alpha$ -H (Orn)], 4.50 [dd, 1H,  $J=6$  and 15 Hz,  $CH_2$  (Bn)], 6.89 (bs, 2H, NHCONH), 6.79-7.48 (m, 15H, aromatics), 8.08 [bs, 3H,  $\delta$ - $NH_3^+$  (Orn)], 9.15 and 9.78 [2bs, 2H,  $\alpha$ - $NH_2^+$  (Orn)], 9.65 (bs, 1H,  $NH$ -Bn);  $^{13}C$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  22.1 ( $CH_2$ ,  $C_{\gamma}$ ), 27.1 ( $CH_2$ ,  $C_{\beta}$ ), 28.2 ( $CH_2$ ,  $C_3$ ), 33.5 ( $CH_2$ ,  $C_{\delta}$ ), 38.0 ( $CH_2$ , Bn), 42.6 ( $CH_2$ ,  $C_1$ ), 56.7 (CH,  $C_2$ ), 59.5 (CH,  $C_{\alpha}$ ), 118.1-148.0 (15CH and 3 C, aromatics), 156.2 (HNCONH), 167.0 (CONH); ES-MS  $m/z$   $[M+1]^+$  calcd for  $C_{28}H_{35}N_5O_2$ , 474.28; found, 574.27 (100%).

4.5.5.  $N_{\alpha}$ -[(2S)-3-Phenyl-1-(3-phenylureido)propan-2-yl]-ornithine benzyl amide dihydrochloride [(S)-17a]

Amorphous solid (55 mg, 100 %); HPLC  $t_R$  12.89 min;  $[\alpha]_D^{20} + 26.6$  ( $c$  0.9, MeOH);  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  1.67-1.78 [m, 2H,  $\gamma$ -H (Orn)], 1.82-2.06 [m, 2H,  $\beta$ -H (Orn)], 2.74-2.96 [m, 3H, 3-H (Pr) and  $\delta$ -H (Orn)], 2.99-3.18 [m, 1H, 3-H (Pr)], 3.11 [m, 1H, 1-H (Pr)], 3.23 [m, 1H, 2-H (Pr)], 3.53 [m, 1H, 1-H (Pr)], 4.27-4.44 [m, 1H,  $\alpha$ -H (Orn)], 4.35 [d, 2H,  $J=6.5$  Hz,  $CH_2$  (Bn)], 6.86-7.45 (m, 15H, aromatics), 6.94 and 7.10 (2bs, 2H, NHCONH), 8.12 [bs, 3H,  $\delta$ - $NH_3^+$  (Orn)], 9.41 and 9.50 [2bs, 2H,  $\alpha$ - $NH_2^+$  (Orn)], 9.64 (bs, 1H,  $NH$ -Bn);  $^{13}C$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  22.7 ( $CH_2$ ,  $C_{\gamma}$ ), 27.3 ( $CH_2$ ,  $C_{\beta}$ ), 34.9 ( $CH_2$ ,  $C_3$ ), 37.7 ( $CH_2$ ,  $C_{\delta}$ ), 38.8 ( $CH_2$ , Bn), 42.7 ( $CH_2$ ,  $C_1$ ), 57.7 (CH,  $C_2$ ), 59.0 (CH,  $C_{\alpha}$ ), 117.8-140.2 (15CH and 3 C, aromatics), 156.2 (HNCONH), 167.2 (CONH); ES-MS  $m/z$   $[M+1]^+$  ES-MS  $m/z$   $[M+1]^+$  calcd for  $C_{28}H_{35}N_5O_2$ , 474.28; found, 574.52 (100%).

4.5.6.  $N_{\alpha}$ -[(2R)-3-Phenyl-1-(3-phenylureido)propan-2-yl]-lysine benzyl amide dihydrochloride [(R)-17b]

Amorphous solid (56 mg, 100%); HPLC  $t_R$  12.78 min;  $[\alpha]_D^{20} + 16.5$  ( $c$  1.6, MeOH);  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  1.25-1.48 [m, 2H,  $\gamma$ -H (Lys)], 1.50-1.70 [m, 2H,  $\delta$ -H (Lys)], 1.72-2.08 [m, 2H,  $\beta$ -H (Lys)], 2.72 [m, 2H,  $\epsilon$ -H (Lys)], 2.77-2.92 [m, 1H, 3-H (Pr)], 3.08-3.34



[m, 3H, 1-H and 3-H (Pr)], 3.24 [m, 1H, 2-H (Pr)], 4.20 [m, 1H,  $\alpha$ -H (Lys)], 4.28 [dd, 1H, J= 6 and 15 Hz, CH<sub>2</sub> (Bn)], 4.45 [dd, 1H, J= 5 and 15 Hz, CH<sub>2</sub> (Bn)], 6.89 (bs, 2H, HNCONH), 7.09-7.49 (m, 15H, aromatics), 8.01 [bs, 3H,  $\delta$ -NH<sub>3</sub><sup>+</sup> (Lys)], 9.01 and 9.77 [2bs, 2H,  $\alpha$ -NH<sub>2</sub><sup>+</sup> (Lys)], 9.47 (bs, 1H, NH-Bn); <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>)  $\delta$  22.0 (CH<sub>2</sub>, C <sub>$\gamma$</sub> ), 27.1 (CH<sub>2</sub>, C <sub>$\delta$</sub> ), 30.2 (CH<sub>2</sub>, C <sub>$\beta$</sub> ), 34.3 (CH<sub>2</sub>, C<sub>3</sub>), 38.9 (CH<sub>2</sub>, C <sub>$\epsilon$</sub> ), 38.9 (CH<sub>2</sub>, Bn), 43.3 (CH<sub>2</sub>, C<sub>1</sub>), 57.9 (CH, C<sub>2</sub>), 60.3 (CH, C <sub>$\alpha$</sub> ), 118.9-140.7 (15CH and 3C, aromatics), 157.3 (HNCONH), 167.8 (CONH); ES-MS  $m/z$  [M+1]<sup>+</sup> calcd for C<sub>29</sub>H<sub>37</sub>N<sub>5</sub>O<sub>2</sub>, 488.29; found, 488.50 (100%).

4.5.7. N <sub>$\alpha$</sub> -[(2S)-3-Phenyl-1-(3-phenylureido)propan-2-yl]-lysine benzyl amide dihydrochloride [(S)-17b]

Amorphous solid (57 mg, 100 %); HPLC  $t_R$  13.26 min; [ $\alpha$ ]<sub>D</sub><sup>20</sup> +19.3 (*c* 1.5, MeOH); <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  1.28-1.49 [m, 2H,  $\gamma$ -H (Lys)], 1.51-1.69 [m, 2H,  $\delta$ -H (Lys)], 1.74-2.01 [m, 2H,  $\beta$ -H (Lys)], 2.69 [m, 2H,  $\epsilon$ -H (Lys)], 2.78-2.94 [m, 1H, 3-H (Pr)], 2.99-3.18 [m, 2H, 1-H and 3-H (Pr)], 3.23 [m, 1H, 2-H (Pr)], 3.51-3.60 [m, 1H, 1-H (Pr)], 4.23-4.48 [m, 1H,  $\alpha$ -H (Lys)], 4.33 [d, 2H, J= 5 Hz, CH<sub>2</sub> (Bn)], 6.80-7.48 [m, 18H, aromatics and  $\delta$ -NH<sub>3</sub><sup>+</sup> (Lys)], 6.89 and 7.15 (2bs, 2H, HNCONH), 9.09 and 9.51 [2bs, 2H,  $\alpha$ -NH<sub>2</sub><sup>+</sup> (Lys)], 9.51 (bs, 1H, NH-Bn); <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>)  $\delta$  22.1 (CH<sub>2</sub>, C <sub>$\gamma$</sub> ), 27.0 (CH<sub>2</sub>, C <sub>$\delta$</sub> ), 30.4 (CH<sub>2</sub>, C <sub>$\beta$</sub> ), 35.7 (CH<sub>2</sub>, C<sub>3</sub>), 38.5 (CH<sub>2</sub>, C <sub>$\epsilon$</sub> ), 38.9 (CH<sub>2</sub>, Bn), 43.3 (CH<sub>2</sub>, C<sub>1</sub>), 58.9 (CH, C<sub>2</sub>), 59.8 (CH, C <sub>$\alpha$</sub> ), 118.5-140.9 (15CH and 3C, aromatics), 157.0 (HNCONH), 168.0 (CONH); ES-MS  $m/z$  [M+1]<sup>+</sup> calcd for C<sub>29</sub>H<sub>37</sub>N<sub>5</sub>O<sub>2</sub>, 488.29; found, 488.50 (100%).

4.5.8. N <sub>$\alpha$</sub> -[(2R)-1-(3-Benzylureido)-3-phenylpropan-2-yl]-ornithine benzyl amide dihydrochloride [(R)-18a]

Amorphous solid (56 mg, 100 %); HPLC  $t_R$  12.87 min; [ $\alpha$ ]<sub>D</sub><sup>20</sup> +5.2 (*c* 1, MeOH); <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>)  $\delta$  1.56-1.82 [m, 2H,  $\gamma$ -H (Orn)], 1.90-2.10 [m, 2H,  $\beta$ -H (Orn)], 2.74-3.02 [m, 4H, 3-H (Pr) and  $\delta$ -H (Orn)], 3.23 [m, 1H, 2-H (Pr)], 3.09-3.22 [m, 2H, 1-H (Pr)], 4.30 [m, 2H, CH<sub>2</sub> (Bn-ureido)], 4.32-4.38 [m, 1H,  $\alpha$ -H (Orn)], 4.35 [dd, 1H, J= 6.5 and 15 Hz, CH<sub>2</sub> (Bn)], 4.60 [dd, 1H, J=5 and 15 Hz, CH<sub>2</sub> (Bn)], 6.72 and 7.00 (2bs, 2H, HNCONH), 7.21-7.65 (m, 15H, aromatics), 8.08 [bs, 3H,  $\delta$ -NH<sub>3</sub><sup>+</sup> (Orn)], 9.56 and 9.81 [2bs, 2H,  $\alpha$ -NH<sub>2</sub><sup>+</sup> (Orn)], 9.56 (bs, 1H, NH-Bn); <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>)  $\delta$  22.7 (CH<sub>2</sub>, C <sub>$\gamma$</sub> ), 27.2 (CH<sub>2</sub>, C <sub>$\beta$</sub> ), 28.9 (CH<sub>2</sub>, C<sub>3</sub>), 33.5 (CH<sub>2</sub>, C <sub>$\delta$</sub> ), 38.1 (CH<sub>2</sub>, Bn), 42.6 [CH<sub>2</sub>, (Bn-ureido)], 43.1 (CH<sub>2</sub>, C<sub>1</sub>), 56.7 (CH, C<sub>2</sub>), 60.4 (CH, C <sub>$\alpha$</sub> ), 126.9-140.2 (15CH and 3C, aromatics), 159.7 (HNCONH), 166.9 (CONH); ES-MS  $m/z$  [M+1]<sup>+</sup> calcd for C<sub>29</sub>H<sub>37</sub>N<sub>5</sub>O<sub>2</sub>, 488.29; found, 488.52 (100%).

4.5.9.  $N_{\alpha}$ -[(2S)-1-(3-Benzylureido)-3-phenylpropan-2-yl]-ornithine benzyl amide dihydrochloride [(S)-18a]

Amorphous solid (55 mg, 100 %); HPLC  $t_R$  12.95 min;  $[\alpha]_D^{20} +2.1$  ( $c$  1.5, MeOH);  $^1H$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  1.58-1.74 [m, 2H,  $\gamma$ -H (Orn)], 1.80-1.99 [m, 2H,  $\beta$ -H (Orn)], 2.74-3.10 [m, 5H, 1-H, 3-H (Pr) and  $\delta$ -H (Orn)], 3.18 [m, 1H, 2-H (Pr)], 3.38-3.46 [m, 1H, 1-H (Pr)], 4.20 [dd, 1H,  $J=6$  and 15 Hz,  $CH_2$  (Bn-ureido)], 4.28 [d, 2H,  $J=6$   $CH_2$  (Bn)], 4.32 [m, 1H,  $\alpha$ -H (Orn)], 4.41 [dd, 1H,  $J=6$  and 15 Hz,  $CH_2$  (Bn-ureido)], 4.60 [dd, 1H,  $J=5$  and 15 Hz,  $CH_2$  (Bn)], 6.94 and 7.11 (2bs, 2H, NHCONH), 7.18-7.30 (m, 15H, aromatics), 8.09 [bs, 3H,  $\delta$ - $NH_3^+$  (Orn)], 9.19 and 9.86 [2bs, 2H,  $\alpha$ - $NH_2^+$  (Orn)], 9.54 (bs, 1H, NH-Bn);  $^{13}C$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  23.3 ( $CH_2$ ,  $C_{\gamma}$ ), 27.9 ( $CH_2$ ,  $C_{\beta}$ ), 28.8 ( $CH_2$ ,  $C_3$ ), 35.4 ( $CH_2$ ,  $C_{\delta}$ ), 38.6 ( $CH_2$ , Bn), 43.4 [ $CH_2$ , (Bn-ureido)], 43.7 ( $CH_2$ ,  $C_1$ ), 58.5 (CH,  $C_2$ ), 60.6 (CH,  $C_{\alpha}$ ), 127.4-140.9 (15CH and 3C, aromatics), 160.5 (HNCONH), 167.9 (CONH); ES-MS  $m/z$   $[M+1]^+$  calcd for  $C_{29}H_{37}N_5O_2$ , 488.29; found, 488.38 (100%).

4.5.10.  $N_{\alpha}$ -[(2R)-1-(3-(2,4-Dichlorophenyl)ureido)-3-phenylpropan-2-yl]-lysine benzyl amide dihydrochloride [(R)-19b]

Amorphous solid (62 mg, 100 %); HPLC  $t_R$  14.85 min;  $[\alpha]_D^{20} + 9.8$  ( $c$  0.4, MeOH);  $^1H$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  1.37-1.49 [m, 2H,  $\gamma$ -H (Lys)], 1.53-1.71 [m, 2H,  $\delta$ -H (Lys)], 1.82-2.05 [m, 2H,  $\beta$ -H (Lys)], 2.80 [m, 2H,  $\epsilon$ -H (Lys)], 2.91 [m, 1H, 3-H (Pr)], 3.23 [m, 1H, 2-H (Pr)], 3.27-3.40 [m, 3H, 1-H and 3-H (Pr)], 3.26 [m, 1H,  $\alpha$ -H (Lys)], 3.26 [dd, 1H,  $J=6$  and 15 Hz,  $CH_2$  (Bn)], 4.52 [dd, 1H,  $J=5$  and 15 Hz,  $CH_2$  (Bn)], 7.18-7.44 (m, 13H, aromatics), 7.59 (bs, 2H, HNCONH), 7.94 [bs, 3H,  $\delta$ - $NH_3^+$  (Lys)], 8.99 and 9.67 [2bs, 2H,  $\alpha$ - $NH_2^+$  (Lys)], 9.43 (bs, 1H, NH-Bn);  $^{13}C$  NMR (125 MHz, DMSO- $d_6$ )  $\delta$  21.3 ( $CH_2$ ,  $C_{\gamma}$ ), 26.5 ( $CH_2$ ,  $C_{\delta}$ ), 29.4 ( $CH_2$ ,  $C_{\beta}$ ), 32.0 ( $CH_2$ ,  $C_3$ ), 37.5 ( $CH_2$ ,  $C_{\epsilon}$ ), 38.3 ( $CH_2$ , Bn), 42.6 ( $CH_2$ ,  $C_1$ ), 57.2 (CH,  $C_2$ ), 58.8 (CH,  $C_{\alpha}$ ), 116.9-140.2 (13CH and 5C, aromatics), 155.9 (HNCONH), 167.1 (CONH); ES-MS  $m/z$   $[M+1]^+$  calcd for  $C_{29}H_{35}Cl_2N_5O_2$ , 556.22; found, 556.63 (100%), 558.52 (64%)  $[M+3]^+$ , 560.42 (10%)  $[M+5]^+$ .

4.5.11.  $N_{\alpha}$ -[(2S)-1-(3-(2,4-Dichlorophenyl)ureido)-3-phenylpropan-2-yl]-lysine benzyl amide dihydrochloride [(S)-19b]

Amorphous solid (63 mg, 100 %); HPLC  $t_R$  14.09 min;  $[\alpha]_D^{20} + 12.3$  ( $c$  1.1, MeOH);  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  1.17-1.42 [m, 2H,  $\gamma$ -H (Lys)], 1.46-1.681 [m, 2H,  $\delta$ -H (Lys)],

1.68-1.98 [m, 2H,  $\beta$ -H (Lys)], 2.61-2.95 [m, 2H, 3-H (Pr)], 2.71 [m, 2H,  $\epsilon$ -H (Lys)], 2.96-3.19 [m, 2H, 1-H (Pr)], 3.23 [m, 1H, 2-H (Pr)], 4.3 [m, 1H,  $\alpha$ -H (Lys)], 4.36 [d, 2H,  $J=5$  Hz,  $\text{CH}_2$  (Bn)], 7.11-7.39 (m, 15H, aromatics and HNCONH), 7.92 [bs, 3H,  $\delta$ - $\text{NH}_3^+$  (Lys)], 9.16 [bs, 2H,  $\alpha$ - $\text{NH}_2^+$  (Lys)], 9.41 (bs, 1H, *NH*-Bn);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  21.9 ( $\text{CH}_2$ ,  $\text{C}_\gamma$ ), 26.9 ( $\text{CH}_2$ ,  $\text{C}_\delta$ ), 29.5 ( $\text{CH}_2$ ,  $\text{C}_\beta$ ), 35.0 ( $\text{CH}_2$ ,  $\text{C}_3$ ), 38.3 ( $\text{CH}_2$ , Bn), 38.8 ( $\text{CH}_2$ ,  $\text{C}_\epsilon$ ), 43.1 ( $\text{CH}_2$ ,  $\text{C}_1$ ), 58.5 (CH,  $\text{C}_2$ ), 59.0 (CH,  $\text{C}_\alpha$ ), 116.5-141.5 (13CH and 5C, aromatics), 156.2 (HNCONH), 167.9 (CONH); ES-MS  $m/z$   $[\text{M}+1]^+$  calcd for  $\text{C}_{29}\text{H}_{35}\text{Cl}_2\text{N}_5\text{O}_2$ , 556.22; found, 556.63 (100%), 558.52 (64%)  $[\text{M}+3]^+$ , 560.42 (10%)  $[\text{M}+5]^+$ .

4.5.12.  $\text{N}_\alpha$ -[*(2R)*-1-(3-(4-Methoxyphenethyl)ureido)-3-phenylpropan-2-yl]-lysine benzyl amide dihydrochloride [(**R**)-**20b**]

Amorphous solid (61 mg, 100 %); HPLC  $t_R$  13.46 min;  $[\alpha]_D^{20} +15.6$  ( $c$  0.8, MeOH);  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  1.27-1.39 [m, 2H,  $\gamma$ -H (Lys)], 1.39-1.64 [m, 2H,  $\delta$ -H (Lys)], 1.74-1.95 [m, 2H,  $\beta$ -H (Lys)], 2.68-2.79 [m, 1H, 3-H (Pr)], 2.63 [t, 2H,  $J=7$  Hz, 2-H (ethyl)], 2.71 [m, 2H,  $\epsilon$ -H (Lys)], 3.03-3.25 [m, 6H, 1-H, 2-H, 3-H (Pr) and 1-H (ethyl)], 3.69 (s, 3H, OMe), 4.09 [m, 1H,  $\alpha$ -H (Lys)], 4.28 [dd, 1H,  $J=6$  and 15 Hz,  $\text{CH}_2$  (Bn)], 4.47 [dd, 1H,  $J=5$  and 15 Hz,  $\text{CH}_2$  (Bn)], 6.37 and 6.50 (2bs, 2H, HNCONH), 6.69-7.38 (m, 14H, aromatics), 7.80 [bs, 3H,  $\delta$ - $\text{NH}_3^+$  (Lys)], 9.25 [bs, 2H,  $\alpha$ - $\text{NH}_2^+$  (Lys)], 9.45 (bs, 1H, *NH*-Bn);  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ )  $\delta$  21.6 ( $\text{CH}_2$ ,  $\text{C}_\gamma$ ), 27.2 ( $\text{CH}_2$ ,  $\text{C}_\delta$ ), 30.5 ( $\text{CH}_2$ ,  $\text{C}_\beta$ ), 34.3 ( $\text{CH}_2$ ,  $\text{C}_3$ ), 35.4 [ $\text{CH}_2$ ,  $\text{C}_2$  (ethyl)], 38.0 [ $\text{CH}_2$ ,  $\text{C}_\epsilon$  and  $\text{C}_1$  (ethyl)], 38.0 ( $\text{CH}_2$ ,  $\text{C}_\epsilon$ ), 38.8 ( $\text{CH}_2$ , Bn), 41.9 ( $\text{CH}_2$ ,  $\text{C}_1$ ), 55.4 (CH,  $\text{C}_2$ ), 55.4 ( $\text{CH}_3$ , OMe), 56.8 (CH,  $\text{C}_\alpha$ ), 114.0-158.0 (14CH and 4C, aromatics), 161.5 (HNCONH), 167.9 (CONH); ES-MS  $m/z$   $[\text{M}+1]^+$  calcd for  $\text{C}_{32}\text{H}_{43}\text{N}_5\text{O}_3$ , 546.34; found, 546.62(100%).

4.5.13.  $\text{N}_\alpha$ -[*(2S)*-1-(3-(4-Methoxyphenethyl)ureido)-3-phenylpropan-2-yl]-lysine benzyl amide dihydrochloride [(**S**)-**20b**]

Amorphous solid (62 mg, 100 %); HPLC  $t_R$  13.52 min;  $[\alpha]_D^{20} +19.7$  ( $c$  0.7, MeOH);  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  1.35 [m, 2H,  $\gamma$ -H (Lys)], 1.594 [m, 2H,  $\delta$ -H (Lys)], 1.83 [m, 2H,  $\beta$ -H (Lys)], 2.62 [m, 2H,  $\epsilon$ -H (Lys)], 2.71 [m, 2H, 2-H (ethyl)], 2.96 [m, 1H, 3-H (Pr)], 3.03 [m, 3H, 1-H and 3-H (Pr)], 3.15 [d, 2H,  $J=7$  Hz, 1-H (ethyl)], 3.20 [m, 1H, 2-H (Pr)], 3.69 (s, 3H, OMe), 4.21 [m, 1H,  $\alpha$ -H (Lys)], 4.36 [m, 2H,  $\text{CH}_2$  (Bn)], 6.57 and 6.76 (2bs, 2H, HNCONH), 7.14-7.3 (m, 14H, aromatics), 8.03 [bs, 3H,  $\delta$ - $\text{NH}_3^+$  (Lys)], 9.00 and 9.92 [2bs, 2H,  $\alpha$ - $\text{NH}_2^+$  (Lys)], 9.45 (bs, 1H, *NH*-Bn);  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ )  $\delta$  21.3 ( $\text{CH}_2$ ,  $\text{C}_\gamma$ ),

26.3 (CH<sub>2</sub>, C<sub>δ</sub>), 29.6 (CH<sub>2</sub>, C<sub>β</sub>), 34.7 [CH<sub>2</sub>, C<sub>2</sub> (ethyl)], 34.9 (CH<sub>2</sub>, C<sub>3</sub>), 38.1 [CH<sub>2</sub>, C<sub>1</sub> (ethyl)], 38.7 (CH<sub>2</sub>, C<sub>ε</sub>), 40.1 (CH<sub>2</sub>, Bn), 42.6 (CH<sub>2</sub>, C<sub>1</sub>), 54.9 (CH<sub>3</sub>, OMe), 58.4 (CH, C<sub>2</sub>), 60.0 (CH, C<sub>α</sub>), 113.8-157.6 (14CH and 4C, aromatics), 159.8 (HNCONH), 167.4 (CONH); ES-MS *m/z* [M+1]<sup>+</sup> calcd for C<sub>32</sub>H<sub>43</sub>N<sub>5</sub>O<sub>3</sub>, 546.34; found, 546.62(100%).

4.5.14. N<sub>α</sub>-[(2R)-1-(3-(4-Fluorophenethyl)ureido)-3-phenyl]propan-2-yl]-lysine benzyl amide dihydrochloride [(R)-21b]

Amorphous solid (60 mg, 100 %); HPLC *t<sub>R</sub>* 13.65 min; [α]<sub>D</sub><sup>20</sup> +15.2 (*c* 0.8, MeOH); <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 1.30-1.41 [m, 2H, γ-H (Lys)], 1.50-1.67 [m, 2H, δ-H (Lys)], 1.75-1.98 [m, 2H, β-H (Lys)], 2.68 [t, 2H, J= 7 Hz, 2-H (ethyl)], 2.71 [m, 2H, ε -H (Lys)], 2.78 [m, 1H, 3-H (Pr)], 3.04 [m, 1H, 2-H (Pr)], 3.08-3.26 [m, 5H, 1-H, 3-H (Pr), and 1-H (ethyl)], 4.21 [m, 1H, α-H (Lys)], 4.28 [dd, 1H, J= 6.5 and 15 Hz, CH<sub>2</sub> (Bn)], 4.46 [dd, 1H, J= 5 and 15 Hz, CH<sub>2</sub> (Bn)], 6.43 and 6.59 (2bs, 2H, HNCONH), 7.02-7.36 (m, 14H, aromatics), 7.95 [bs, 3H, δ-NH<sub>3</sub><sup>+</sup> (Lys)], 9.42 and 9.84 [2bs, 2H, α-NH<sub>2</sub><sup>+</sup> (Lys)], 9.48 (bs, 1H, NH-Bn); <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>) δ 21.6 (CH<sub>2</sub>, C<sub>γ</sub>), 26.8 (CH<sub>2</sub>, C<sub>δ</sub>), 30.0 (CH<sub>2</sub>, C<sub>β</sub>), 33.9 (CH<sub>2</sub>, C<sub>3</sub>), 35.3 [CH<sub>2</sub>, C<sub>2</sub> (ethyl)], 38.2 [CH<sub>2</sub>, C<sub>1</sub> (ethyl)], 38.7 (CH<sub>2</sub>, C<sub>ε</sub>), 41.7 (CH<sub>2</sub>, Bn), 42.9 (CH<sub>2</sub>, C<sub>1</sub>), 57.6 (CH, C<sub>2</sub>), 61.2 (CH, C<sub>α</sub>), 115.2-160.1 (14CH and 4C, aromatics), 162.2 (HNCONH), 167.5 (CONH); ES-MS *m/z* [M+1]<sup>+</sup> calcd for C<sub>31</sub>H<sub>40</sub>FN<sub>5</sub>O<sub>2</sub>, 534.32; found, 534.65 (100 %).

4.5.15. N<sub>α</sub>-[(2S)-1-(3-(4-Fluorophenethyl)ureido)-3-phenyl]propan-2-yl]-lysine benzyl amide dihydrochloride [(S)-21b]

Amorphous solid (59 mg, 100 %); HPLC *t<sub>R</sub>* 13.69 min; [α]<sub>D</sub><sup>20</sup> +21.5 (*c* 1.3, MeOH); <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 1.36 [m, 2H, γ-H (Lys)], 1.59 [m, 2H, δ-H (Lys)], 1.84 [m, 2H, β-H (Lys)], 2.68 [t, 2H, J= 7 Hz, 2-H (ethyl)], 2.72 [m, 2H, ε -H (Lys)], 2.78 [m, 1H, 3-H (Pr)], 2.97 [m, 1H, 1-H (Pr)], 3.04 [m, 1H, 3-H (Pr)], 3.15 [m, 1H, 2-H (Pr)], 3.18 [m, 1H, 1-H (Pr)], 3.22 [t, 2H, J= 7 Hz, 1-H (ethyl)], 4.23 [m, 1H, α-H (Lys)], 4.36 [d, 2H, J= 6 Hz, CH<sub>2</sub> (Bn)], 6.63 and 6.82 (2bs, 2H, HNCONH), 7.10-7.34 (m, 14H, aromatics), 8.07 [bs, 3H, δ-NH<sub>3</sub><sup>+</sup> (Lys)], 9.02 and 9.91 [2bs, 2H, α-NH<sub>2</sub><sup>+</sup> (Lys)], 9.48 (bs, 1H, NH-Bn); <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>) δ 20.1 (CH<sub>2</sub>, C<sub>γ</sub>), 26.7 (CH<sub>2</sub>, C<sub>δ</sub>), 31.8.0 (CH<sub>2</sub>, C<sub>β</sub>), 35.2 (CH<sub>2</sub>, C<sub>3</sub>), 35.5 [CH<sub>2</sub>, C<sub>2</sub> (ethyl)], 38.7 [CH<sub>2</sub>, C<sub>1</sub> (ethyl)], 39.1 (CH<sub>2</sub>, C<sub>ε</sub>), 40.6 (CH<sub>2</sub>, Bn), 43.0 (CH<sub>2</sub>, C<sub>1</sub>), 58.9 (CH, C<sub>2</sub>), 60.4 (CH, C<sub>α</sub>), 115.4-160.3 (14CH and 4C, aromatics), 162.2 (HNCONH), 167.9 (CONH); ES-MS *m/z* [M+1]<sup>+</sup> calcd for C<sub>31</sub>H<sub>40</sub>FN<sub>5</sub>O<sub>2</sub>, 534.32; found, 534.65 (100 %).

#### 4.6. Synthesis of the indazole-containing protected ureas (**R**)- and (**S**)-**24b**

Diethyl amine (0.22 mL, 2.1 mmol) was added to a solution of the corresponding Fmoc-protected amine (**R**)- and (**S**)-**12b** (0.21 mmol) and the reaction mixture was stirred at rt for 2 h. Then, the solvent was evaporated to dryness and the residue was dissolved in EtOAc (50 mL). The solution was successively washed with H<sub>2</sub>O (20 mL) and brine (20 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, and evaporated to dryness to give the deprotected amines (**R**)- and (**S**)- **3b**, which were reserved. Apart, propylene oxide (118  $\mu$ L, 1.68 mmol) was added to a 0 °C cooled solution of 1-(2,6-dichlorobenzyl)-3-(pyrrolidin-1-ylmethyl)-1*H*-indazole-6-amine [50] (**22**) (79 mg, 0.21 mmol) in dry THF (5 mL). Then, a solution of bis(trichloromethyl)carbonate (20 mg, 0.07 mmol) in dry THF (1mL) was added dropwise and stirring was maintained at 0 °C for 15 min. Afterwards, the mixture was added dropwise to a 0 °C cooled solution of (**R**)- and (**S**)- **3b** (98 mg, 0.21 mmol) in dry THF (5 mL) and stirred for 1 h. Then, the reaction mixture was diluted with EtOAc (50 mL), washed successively with H<sub>2</sub>O (20 mL) and brine (20 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, and evaporated to dryness. The residue was purified by reversed phase chromatography, using 10-100% CH<sub>3</sub>CN gradient in 0.05 % TFA solution in H<sub>2</sub>O as mobile phase.

##### 4.6.1. N<sub>ε</sub>-Boc-N<sub>α</sub>-[(2*R*)-1-(3-(1-(2,6-dichlorobenzyl)-3-(pyrrolidin-1-ylmethyl)-1*H*-indazol-6-yl)ureido)-3-phenylpropan-2-yl]-lysine benzyl amide [(**R**)-**24b**]

Foam (36 mg, 20 %); HPLC  $t_R$  16.33 min; HPLC-MS  $t_R$  3.26 min;  $[\alpha]_D^{20}$  +5.6 (*c* 1, MeOH); <sup>1</sup>H-RMN (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.02-1.46 [m, 4H,  $\gamma$ - and  $\delta$ -H (Lys)], 1.31 (s, 9H, Boc), 1.69, 1.85, 3.02, 3.45 (4m, 8H, pyrrolidine), 1.75-1.94 [m, 2H,  $\beta$ -H (Lys)], 2.74-2.92 [m, 2H,  $\epsilon$ -H (Lys)], 2.74-3.07 [m, 2H, 3-H (Pr)], 3.27 [m, 1H, 2-H (Pr)], 3.21-3.43 [m, 2H, 1-H (Pr)], 3.93 [bs, 1H,  $\alpha$ -H (Lys)], 4.29 [d, *J* = 7 Hz, 2H, CH<sub>2</sub> (Bn)], 4.36 (s, 2H, CH<sub>2</sub>-pyrrolidine), 4.64 (s, 1H, NH-Boc), 5.52 (s, 2H, CH<sub>2</sub>-diClPh), 6.94 [d, *J* = 7 Hz, 1H, 5-H (indazole)], 7.36-7.00 (m, 13H, Ph), 7.41 [d, *J* = 7 Hz, 1H, 4-H (indazole)], 7.87 [s, 1H, 7-H (indazole)], 8.12 (s, 1H, NH-Bn), 8.72 and 11.8 (2bs, 2H, HNCONH); <sup>13</sup>C-RMN (100 MHz, CDCl<sub>3</sub>) 21.7 (CH<sub>2</sub>, C <sub>$\gamma$</sub> ), 23.4 (CH<sub>2</sub>, pyrrolidine), 28.3 (CH<sub>3</sub>, Boc), 29.1 (CH<sub>2</sub>, C <sub>$\delta$</sub> ), 29.5 (CH<sub>2</sub>, C <sub>$\beta$</sub> ), 34.9 (CH<sub>2</sub>, C<sub>3</sub>), 40.8 (CH<sub>2</sub>, C<sub>1</sub>), 43.8 (CH<sub>2</sub>, Bn), 47.7 (CH<sub>2</sub>-diClPh), 48.1, 52.3 (CH<sub>2</sub>, pyrrolidine and CH<sub>2</sub>-pyrrolidine), 59.3 (CH, C <sub>$\alpha$</sub> ), 60.2 (CH, C<sub>2</sub>), 79.5 (C, Boc), 98.8 [C<sub>7</sub> (indazole)], 116.3 [C<sub>5</sub> (indazole)], 119.2 [C<sub>4</sub> (indazole)], 127.6-129.2 (CH, Ph), 130.1 (C, Ph), 131.2 (C, diClPh), 133.7 [C<sub>5</sub> (indazole)], 136.8 (C, Bn), 137.3 (2C, diClPh), 138.8 [C<sub>6</sub>

(indazole)], 141.6 [ $C_{7a}$  (indazole)], 157.4 2 (HNCONH), 162.2 (CO, Boc), 167.2 (CONH); ES-MS  $m/z$   $[M+1]^+$  calcd. for  $C_{47}H_{58}Cl_2N_8O_4$ , 869.40; found, 435.29 (100%)  $[(M+2)/2]^+$ , 869.49 (6%)  $[M+1]^+$ . Anal. calcd. for  $C_{47}H_{58}Cl_2N_8O_4$ : C, 64.89; H, 6.72; N, 12.88; Found: C, 64.95; H, 6.82; N, 12.95.

4.6.2.  $N_\epsilon$ -Boc- $N_\alpha$ -[(2S)-1-(3-(1-(2,6-dichlorobenzyl)-3-(pyrrolidin-1-ylmethyl)-1H-indazol-6-yl)ureido)-3-phenylpropan-2-yl]-lysine benzyl amide [(S)-**24b**]

Foam (155 mg, 85%); HPLC  $t_R$  16.51 min; HPLC-MS  $t_R$  3.26 min;  $[\alpha]_D^{20}$  +8.4 (c 1, MeOH);  $^1H$ -RMN (500 MHz,  $CDCl_3$ )  $\delta$  1.08-1.42 [m, 4H,  $\gamma$ - and  $\delta$ -H (Lys)], 1.29 (s, 9H, Boc), 1.70, 1.87, 2.98, 3.45 (4m, 8H, pyrrolidine), 1.76-1.83 [m, 2H,  $\beta$ -H (Lys)], 2.72-2.86 [m, 2H,  $\epsilon$ -H (Lys)], 2.74-3.07 [m, 2H, 3-H (Pr)], 3.23 [m, 1H, 2-H (Pr)], 3.18-3.31 [m, 1H, 1-H (Pr)], 3.55 [m, 1H, 1-H (Pr)], 4.25 [bs, 1H,  $\alpha$ -H (Lys)], 4.30 [dd, J = 5 and 15 Hz, 2H,  $CH_2$  (Bn)], 4.36 (s, 2H,  $CH_2$ -pyrrolidine), 4.70 (s, 1H,  $NH$ -Boc), 5.50 (s, 2H,  $CH_2$ -diClPh), 6.99 [d, J = 7 Hz, 1H, 5-H (indazole)], 7.09-7.34 (m, 13H, Ph), 7.43 [d, J = 7 Hz, 1H, 4-H (indazole)], 7.80 [s, 1H, 7-H (indazole)], 9.18 (s, 1H,  $NH$ -Bn), 8.92 and 11.78 (2bs, 2H, HNCONH);  $^{13}C$ -RMN (125 MHz,  $CDCl_3$ )  $\delta$  21.9 ( $CH_2$ ,  $C_\gamma$ ), 23.6 ( $CH_2$ , pyrrolidine), 28.5 ( $CH_3$ , Boc), 29.3 ( $CH_2$ ,  $C_\delta$ ), 30.7 ( $CH_2$ ,  $C_\beta$ ), 30.7 ( $CH_2$ ,  $C_\beta$ ), 36.6 ( $CH_2$ ,  $C_3$ ), 39.7 ( $CH_2$ ,  $C_\epsilon$ ), 40.3 ( $CH_2$ ,  $C_1$ ), 44.2 ( $CH_2$ , Bn), 48.0 ( $CH_2$ -diClPh), 48.6 ( $CH_2$ -pyrrolidine), 52.8 ( $CH_2$ , pyrrolidine), 60.6 (CH,  $C_\alpha$ ), 61.5 (CH,  $C_2$ ), 79.2 (C, Boc), 98.8 [ $C_7$  (indazole)], 116.4 [ $C_5$  (indazole)], 119.3 [ $C_4$  (indazole)], 119.8 [ $C_{3a}$  (indazole)], 167.6 (CONH), 127.8-129.5 (CH, Ph), 130.5 (C, Ph), 131.6 (C, diClPh), 133.9 [ $C_5$  (indazole)], 134.3 (C, Bn), 137.0 (2C, diClPh), 138.8 [ $C_6$  (indazole)], 141.7 [ $C_{7a}$  (indazole)], 158.5 (C, HNCONH), 162.1 (CO, Boc), 167.6 (CONH); ES-MS  $m/z$   $[M+1]^+$  calcd. for  $C_{47}H_{58}Cl_2N_8O_4$ , 869.40; found, 435.29 (100%)  $[(M+2)/2]^+$ , 869.49 (5%)  $[M+1]^+$ ; Anal. calcd. for  $C_{47}H_{58}Cl_2N_8O_4$ : C, 64.89; H, 6.72; N, 12.88; Found: C, 65.02; H, 6.85; N, 12.92.

4.6.3.  $N$ -Boc deprotection of (S)-**24b**. Synthesis of  $N_\alpha$ -[(2S)-1-(3-(1-(2,6-dichlorobenzyl)-3-(pyrrolidin-1-ylmethyl)-1H-indazol-6-yl)ureido)-3-phenylpropan-2-yl]-lysine benzyl amide trihydrochloride [(S)-**25b**]

The protected urea (S)-**24b** (130 mg, 0.15 mmol) was dissolved in a 3M solution of HCl in EtOAc (2 mL) and stirred at rt for 2 h. Then, the solvent was evaporated under reduced pressure, the residue was dissolved in  $H_2O$  (3 mL) and the solution was lyophilized to give the urea (S)-**25b** (131 mg, 100%). HPLC  $t_R$  13.11 min; HPLC-MS  $t_R$  2.73 min;  $[\alpha]_D^{20}$  +1.6 (c

1.2, MeOH);  $^1\text{H}$ -RMN (500 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 1.32 [m, 2H,  $\gamma$ -H (Lys)], 1.53 [m, 2H,  $\delta$ -H (Lys)], 1.74 [m, 2H,  $\beta$ -H (Lys)], 1.77-1.95 [m, 4H, 2CH<sub>2</sub> (pyrrolidine)], 2.66 [m, 2H,  $\epsilon$ -H (Lys)], 2.84 [dd, 1H, J = 10 and 13 Hz, 3-H (Pr)], 3.04 [m, 4H, 2CH<sub>2</sub> (pyrrolidine)], 3.08-3.16 [m, 1H, 3-H (Pr)], 3.12 [m, 1H, 2-H (Pr)], 3.19-3.59 [m, 2H, 1-H (Pr)], 4.14 [m, 1H,  $\alpha$ -H (Lys)], 4.32 [t, 2H, J = 7 Hz, CH<sub>2</sub> (Bn)], 4.54 (s, 2H, CH<sub>2</sub>-pyrrolidine), 5.57 (s, 2H, CH<sub>2</sub>-diClPh), 7.02-7.95 (m, 16H, aromatics), 8.01 and 9.66 (2s, 2H, HNCONH), 9.08 (s, 1H, NH-Bn), 9.43 and 10.58 [2bs, 2H,  $\alpha$ -NH<sub>2</sub><sup>+</sup> (Lys)].  $^{13}\text{C}$ -RMN (125 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 21.4 (CH<sub>2</sub>, C <sub>$\gamma$</sub> ), 22.6 (2CH<sub>2</sub>, pyrrolidine), 26.4 (CH<sub>2</sub>, C <sub>$\delta$</sub> ), 29.7 (CH<sub>2</sub>, C <sub>$\beta$</sub> ), 35.1 (CH<sub>2</sub>, C<sub>3</sub>), 38.2 (CH<sub>2</sub>, C <sub>$\epsilon$</sub> ), 38.3 (CH<sub>2</sub>, C<sub>1</sub>), 42.6 (CH<sub>2</sub>, Bn), 47.4 (CH<sub>2</sub>, diClPh), 47.7 (CH<sub>2</sub>, CH<sub>2</sub>-pyrrolidine), 52.7 (2CH<sub>2</sub>, pyrrolidine), 58.2 (CH, C <sub>$\alpha$</sub> ), 58.8 (CH, C<sub>2</sub>), 98.4 [C<sub>7</sub> (indazole)], 114.6 [C<sub>5</sub> (indazole)], 117.8 [C<sub>4</sub> (indazole)], 120.6 [C<sub>3a</sub> (indazole)], 127.1-131.4 (CH, aromatics), 135.5 (C, Bn), 136.1 (C, diClPh), 136.2 (C, Ph), 138.2 [C<sub>5</sub> (indazole)], 138.4 (2C, diClPh), 139.7 [C<sub>6</sub> (indazole)], 141.3 [C<sub>7a</sub> (indazole)], 156.1 (HNCONH), 167.3 (CONH); ES-MS  $m/z$  [M+1]<sup>+</sup> calcd for C<sub>42</sub>H<sub>50</sub>Cl<sub>2</sub>N<sub>8</sub>O<sub>2</sub>, 769.34; found, 385.28 (60%) [(M+2)/2]<sup>+</sup>, 769.37 (6%) [M+1]<sup>+</sup>.

#### 4.7. General procedure for the synthesis of thioureas (**R**)- and (**S**)-**26a**

Diethyl amine (0.32 mL, 3 mmol) was added to a solution of the corresponding Fmoc-protected amine (**R**)- and (**S**)-**12a** (0.3 mmol) and the reaction mixture was stirred at rt for 2 h. Then, the solvent was evaporated to dryness and the residue was dissolved in EtOAc (50 mL). The solution was successively washed with H<sub>2</sub>O (20 mL) and brine (20 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, and evaporated to dryness to give the deprotected amines (**R**)- and (**S**)- **3a**. These amines were dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (10 mL) and the solution was cooled at 0°C. Phenyl isothiocyanate (40.5 mg, 0.3 mmol) was added and the mixture was stirred for 2 h. Then, the solvent was evaporated and the residue was dissolved in EtOAc (50 mL). This solution was successively washed with H<sub>2</sub>O (20 mL) and brine (20 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, and evaporated to dryness. The residue was purified by flash chromatography, using 20-100% EtOAc gradient in hexane as eluant to give the corresponding thiourea (**R**)- and (**S**)-**26a**.

##### 4.7.1. N <sub>$\delta$</sub> -Boc-N <sub>$\alpha$</sub> -[(2R)-3-Phenyl-1-(3-phenylthioureido)propan-2-yl]-ornithine benzyl amide [(**R**)-**26a**]

Foam (121 mg, 68%); HPLC  $t_R$  18.42 min;  $[\alpha]_D^{20}$  +3.0 ( $c$  1, MeOH);  $^1\text{H}$  NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  1.38 (m, 9H, Boc), 1.10-1.65 [m, 4H,  $\beta$ -H and  $\gamma$ -H (Orn)], 2.71-2.91 [m, 3H, 3-H

(Pr) and  $\delta$ -H (Orn)], 2.97 [dd, 1H,  $J = 7.5$  and  $13\text{Hz}$ , 3-H (Pr)], 3.09-3.30 [m, 2H,  $\alpha$ -H (Orn) and 2-H (Pr)], 3.63 [d, 2H,  $J = 5\text{Hz}$ , 1-H (Pr)], 4.21 [dd,  $J = 6.5$  and  $15\text{Hz}$ , 1H,  $\text{CH}_2$  (Bn)], 4.43 [dd, 1H,  $J = 5$  and  $15\text{Hz}$ ,  $\text{CH}_2$  (Bn)], 4.53 (s, 1H,  $\text{NH-Boc}$ ), 6.79 and 7.85 (2s, 2H, HnCSNH), 7.04-7.63 (m, 16H, aromatics and  $\text{NH-Bn}$ );  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  25.9 ( $\text{CH}_2$ ,  $\text{C}_\gamma$ ), 28.3 ( $\text{CH}_3$ , Boc), 30.5 ( $\text{CH}_2$ ,  $\text{C}_\beta$ ), 39.4 ( $\text{CH}_2$ ,  $\text{C}_\delta$ ), 43.2 ( $\text{CH}_2$ , Bn), 58.1 ( $\text{CH}$ ,  $\text{C}_\alpha$ ), 59.7 ( $\text{CH}$ ,  $\text{C}_2$ ), 79.3 (C, Boc), 125.0-138.3 (15 CH and 3 C, aromatics), 156.3 (CO, Boc), 174.9 (CONH), 181.2 (CS); ES-MS  $m/z$   $[\text{M}+1]^+$  calcd. for  $\text{C}_{33}\text{H}_{44}\text{N}_5\text{O}_3\text{S}$ , 590.31; found, 590.51 (100 %). Anal. calcd. for  $\text{C}_{33}\text{H}_{43}\text{N}_5\text{O}_3\text{S}$ : C, 67.20; H, 7.35; N, 11.87; Found: C, 67.24; H, 7.55 ; N, 11.93.

#### 4.7.2. $\text{N}_\delta$ -Boc- $\text{N}_\alpha$ -[(2S)-3-Phenyl-1-(3-phenylthioureido)propan-2-yl]-ornithine benzyl amide [(S)-26a]

Foam (127 mg, 72%); HPLC  $t_R$  18.67 min;  $[\alpha]_D^{20} +7.5$  ( $c$  1.1, MeOH);  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  1.38 (s, 9H, Boc), 1.23-1.90 [m, 4H,  $\beta$ -H and  $\gamma$ -H (Orn)], 2.87-3.17 [m, 6H, 1- and 3-H (Pr), and  $\delta$ -H (Orn)], 3.40 [m, 1H, 2-H (Pr)], 3.62 [m, 1H,  $\alpha$ -H (Orn)], 4.10 [dd,  $J = 7$  and  $15\text{Hz}$ , 1H,  $\text{CH}_2$  (Bn)], 4.38 [dd,  $J = 5$  and  $15\text{Hz}$ , 1H,  $\text{CH}_2$  (Bn)], 4.71 (s, 1H,  $\text{NH-Boc}$ ), 6.69 and 8.05 (2s, 2H, HnCSNH), 7.02-7.54 (m, 16H, aromatics and  $\text{NH-Bn}$ );  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  26.2 ( $\text{CH}_2$ ,  $\text{C}_\gamma$ ), 28.4 ( $\text{CH}_3$ , Boc), 29.3 ( $\text{CH}_2$ ,  $\text{C}_\beta$ ), 39.5 ( $\text{CH}_2$ ,  $\text{C}_\delta$ ), 40.5 ( $\text{CH}_2$ ,  $\text{C}_3$ ), 43.1 ( $\text{CH}_2$ , Bn), 58.3 ( $\text{CH}$ ,  $\text{C}_\alpha$ ), 59.3 ( $\text{CH}$ ,  $\text{C}_2$ ), 79.5 (C, Boc), 124.5-138.4 (15CH and 3C, aromatics), 156.4 (CO, Boc), 173.4 (CONH), 181.3 (CS); ES-MS  $m/z$   $[\text{M}+1]^+$  calcd. for  $\text{C}_{33}\text{H}_{43}\text{N}_5\text{O}_3\text{S}$ , 590.31; found, 590.58 (100%). Anal. calcd. for  $\text{C}_{33}\text{H}_{43}\text{N}_5\text{O}_3\text{S}$ : C, 67.20; H, 7.35; N, 11.87; Found: C, 67.15; H, 7.45; N, 11.86.

#### 4.8. $\text{N-Boc-deprotection of (R)- and (S)-26a}$ . Synthesis of the thiourea hydrochlorides (R)- and (S)-27a

It was carried out by applying the above described methodology for  $\text{N-Boc}$  removal in the urea analogues.

##### 4.8.1. $\text{N}_\alpha$ -[(2R)-3-Phenyl-1-(3-phenylthioureido)propan-2-yl]-ornithine benzyl amide dihydrochloride [(R)-27a]

Amorphous solid (56 mg, 100%); HPLC  $t_R$  13.29 min;  $[\alpha]_D^{20} +10.7$  ( $c$  1, MeOH);  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO-d}_6$ )  $\delta$  1.50-1.76 [m, 2H,  $\gamma$ -H (Orn)], 1.78-2.06 [m, 2H,  $\beta$ -H (Orn)], 2.72-3.27 [m, 4H, 3-H (Pr) and  $\delta$ -H (Orn)], 3.46-3.66 [m, 2H, 1-H (Pr)], 3.80 [m, 1H, 2-H (Pr)],



4.25 [dd, 1H, J=6 and 15 Hz, CH<sub>2</sub>(Bn)], 4.37 [m, 1H, α-H (Orn)], 4.53 [dd, 1H, J = 5 and 15 Hz, CH<sub>2</sub>(Bn)], 7.10 (s, 1H, HNCSNH), 6.93-7.55(m, 16H, aromatics and HNCSNH), 8.05 [bs, 3H, δ-NH<sub>3</sub><sup>+</sup> (Orn)], 8.75 and 10.22 [2bs, 2H, α-NH<sub>2</sub><sup>+</sup> (Orn)], 9.41(s, 1H, NH-Bn); <sup>13</sup>C NMR (125 MHz, DMSO-d<sub>6</sub>) δ 22.8 (CH<sub>2</sub>, C<sub>γ</sub>), 27.1 (CH<sub>2</sub>, C<sub>β</sub>), 28.2 (CH<sub>2</sub>, C<sub>3</sub>), 34.1 (CH<sub>2</sub>, C<sub>δ</sub>), 38.1 (CH<sub>2</sub>, Bn), 42.7 (CH<sub>2</sub>, C<sub>1</sub>), 57.0 (CH, C<sub>2</sub>), 58.3 (CH, C<sub>α</sub>), 117.7-139.9 (15CH and 3C, aromatics), 166.9 (CONH), 181.0 (CS); ES-MS *m/z* [M+1]<sup>+</sup> calcd. for C<sub>28</sub>H<sub>35</sub>N<sub>5</sub>OS, 490.26; found 246.13(100%) [(M+2)/2]<sup>+</sup>, 490.48 (15%) [M+1]<sup>+</sup>.

#### 4.8.2. N<sub>α</sub>-[*(2S)*-3-Phenyl-1-(3-phenylthioureido)propan-2-yl]-ornithine benzyl amide dihydrochloride [(*S*)-**27a**]

Amorphous solid (54 mg, 100%); HPLC *t<sub>R</sub>* 13.40 min; [α]<sub>D</sub><sup>20</sup> +2.9 (*c* 0.7, MeOH); <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 1.51-2.06 [m, 4H, β- and γ-H (Orn)], 2.73-3.22 [m, 5H, 2- and 3-H (Pr), and δ-H (Orn)], 3.52-3.60 [m, 2H, 1-H (Pr)], 4.29 [m, 1H, α-H (Orn)], 4.40 [d, 2H, J = 6 Hz, CH<sub>2</sub> (Bn)], 7.10 (s, 1H, HNCSNH), 6.69-7.71 (m, 15H, aromatics and HNCSNH), 7.94 [bs, 3H, δ-NH<sub>3</sub><sup>+</sup> (Orn)], 9.42 (s, 1H, NH-Bn), 9.19 and 9.54 [2bs, 2H, α-NH<sub>2</sub><sup>+</sup> (Orn)]; <sup>13</sup>C NMR (125 MHz, DMSO-d<sub>6</sub>) δ 22.7 (CH<sub>2</sub>, C<sub>γ</sub>), 27.1 (CH<sub>2</sub>, C<sub>β</sub>), 31.6 (CH<sub>2</sub>, C<sub>3</sub>), 38.3 (CH<sub>2</sub>, C<sub>δ</sub>), 38.9 (CH<sub>2</sub>, Bn), 42.8 (CH<sub>2</sub>, C<sub>1</sub>), 57.0 (CH, C<sub>2</sub>), 58.8 (CH, C<sub>α</sub>), 123.4-138.7 (15CH and 3C, aromatics), 166.9 (CONH), 181.7 (CS); ES-MS *m/z* [M+1]<sup>+</sup> calcd. for C<sub>28</sub>H<sub>35</sub>N<sub>5</sub>OS, 490.26; found 246.13 (100%) [(M+2)/2]<sup>+</sup>, 490.48 (15%) [M+1]<sup>+</sup>.

#### 4.9. Platelet aggregation inhibition assay

Whole blood was obtained from human volunteers who were not taking any platelet altering drugs for two weeks prior to donation. Blood was collected by venous puncture into 2.7 mL vacutainer tubes containing 3.2% buffered sodium citrate. Blood was centrifuged at 250×g for 7 min to obtain platelet rich plasma (PRP). After removal of PRP, the blood was re-centrifuged at 900×g for 10 min to obtain platelet poor plasma (PPP). The PPP was used as a reference in the optical aggregation and as a diluent to achieve a final platelet concentration of 200,000 platelet/μL in PRP. Tests were performed in an optical aggregometer (Chrono-Log Model 440 Four Channel). Briefly, a 0.5 mL sample of diluted PRP was added to a glass cuvette and incubated with either vehicle (DMSO solution) or tested compound, at a 0.1 mg/mL concentration, for 5 min at 37 °C. At the beginning of each experiment, aggregation response to SFLLRN (30μM) was evaluated and the maximum aggregation value at the end of 5 min was recorded. Aggregation response to SFLLRN plus compound was recorded and

compared to control (SFLLRN/vehicle) to determine the % of inhibition. Each compound was tested twice and the results are the mean of the two assays.

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