European Journal of Medicinal Chemistry 70 (2013) 505–524

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

α- and β-hydrazino acid-based pseudopeptides inhibit the chymotrypsin-like activity of the eukaryotic 20S proteasome

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ARTICLE INFO

Article history:
Received 20 June 2013
Received in revised form
23 September 2013
Accepted 24 September 2013
Available online 17 October 2013

Keywords:
Proteasome inhibitors
Hydrazino acid
Chymotrypsin
Pseudopeptide
Trifluoromethyl
Fluorine

ABSTRACT

We describe the synthesis of a library of new pseudopeptides and their inhibitory activity of the rabbit 20S proteasome chymotrypsin-like (ChT-L) activity. We replaced a natural α-amino acid by an α- or a β-hydrazino acid and obtained inhibitors of proteasome up to a submicromolar range (0.7 μM for molecule **24b**). Structural variations influenced the inhibition of the ChT-L activity. Models of inhibitor/20S proteasome complexes corroborated the inhibition efficacies obtained by kinetic studies.

1. Introduction

The 26S proteasome is a large, multicatalytic threonine protease complex that processively degrades ubiquitinylated proteins to small peptides [1]. The ubiquitin–proteasome pathway plays a central role in the degradation of regulatory proteins that are crucial for many intracellular processes including cell progression, apoptosis and NF-κB activation. The 26S proteasome is composed of a 20S catalytic core particle that is capped at each end by the 19S regulatory complex which is responsible for the recognition, unfolding and translocation of protein substrates into the 20S catalytic core cavity. The eukaryotic 20S proteasome is formed by four stacked rings, and each of the two inner rings is composed of seven different β subunits [2]. Three proteolytic activities are localized in 6 β subunits and are classified as chymotrypsin-like (ChT-L, β5 subunit), trypsin-like (T-L, β2 subunit) and caspase-like or post-acid (PA, β1 subunit) activities since peptide bonds are cleaved on the carboxyl side of hydrophobic, basic and acidic amino acid residues, respectively [3]. The ChT-L activity has been the focus of drug development [4]. Inhibition of ChT-L activity induces cell cycle arrest and selective apoptosis of malignant cells leading to a new category of antineoplastic agents [5]. The dipeptide boronic acid bortezomib (Velcade®) and the epoxyketone carfilzomib (PR-171) [6] have been approved for treating incurable multiple myeloma (both compounds) [7] and mantle lymphoma (bortezomib) [8]. The lactone salinosporamide A (NPI-0052) entered into clinical trials for advanced solid and hematological malignancies [9]. Most natural and synthetic proteasome inhibitors, such as epoxyketones, peptide aldehydes, peptide vinyl sulfones and peptide boronic acids, bear a reactive group that forms a transient or irreversible covalent bond with the catalytic O^{γ} atom of Thr1 of the active sites [10,11]. Although these reactive groups contribute to the inhibitory activity, they can also cause a lack of specificity, excessive reactivity and instability which may increase adverse effects and limit efficacy of proteasome inhibitors *in vivo*. Therefore, non covalent inhibitors

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http://dx.doi.org/10.1016/j.ejmech.2013.09.059

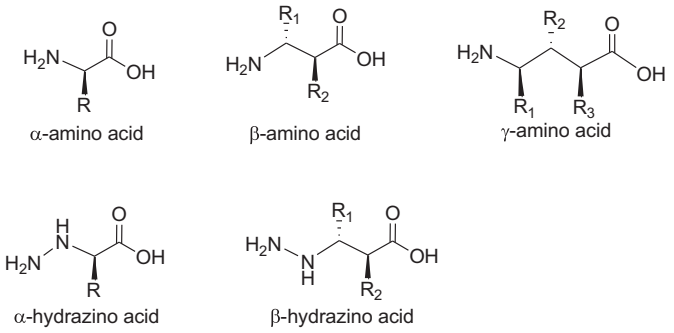


Fig. 1. General structure of amino acids and hydrazino acids. For clarity, each amino acid and hydrazine acid is represented by the residue formula of only one configuration.

that bind reversibly to the active sites may provide an alternative mechanism of inhibition and offer therapeutic advantages. Non covalent inhibitors have been less extensively investigated [12,13]. They include peptidic inhibitors such as ritonavir [14], aminobenzylstatine [15], and 3,4,5-trimethoxy-L-phenylalanine derivatives [16], lipopeptides [17], macrocyclic [18], and linear TMC-95 derivatives [19–22] and sulfonamide compounds [23]. We describe here a new class of non covalent 20S inhibitors based on α- or β-hydrazino acid scaffolds. We postulated that these peptidomimetic elements would mimic the natural α-peptides which are the only elements encountered so far in peptidic proteasome inhibitors. α- and β-hydrazino acid scaffolds are peptidomimetic building blocks that have two nitrogen atoms. They can be considered as analogs of α and β-amino acids, respectively, in which the amine group has been replaced by a hydrazine (Fig. 1). α- and β-hydrazino acid scaffolds can also be considered as analogs of β and γ-amino acids where the C^{β} or C^{γ}-atom is replaced by a nitrogen (Fig. 1) [24]. These structures can mimic the typical secondary structure of native α-peptides, preserving biological activity and enhancing proteolytic stability [25,26] making them useful tools to design new protease inhibitors. Furthermore, the additional nitrogen may allow the formation of additional H-bonds [25,27]. However, to our knowledge, α-hydrazino acid scaffolds have been rarely used in medicinal chemistry and particularly very scarcely introduced within protease inhibitors. α-Hydrazino peptides were reported to inhibit the serine protease leukocyte elastase [25] and retro hydrazino–azapeptoids were recently described as covalent proteasome inhibitors with IC_{50} up to 0.7 μM [28]. Finally, whereas β-amino acids are well documented [29–31] almost nothing is known about the β-hydrazino acid based peptidomimetics.

We previously described preliminary results concerning the first synthesis of CF_3-β-hydrazino acid and the biological evaluation of few compounds based on a central fluorinated β-hydrazino acid scaffold that inhibit the ChT-L activity with IC_{50} values up to 1.6 μM (compounds **1a**, **1b**, **2**, **3a**, **3b**, **4** and **6**, Table 1) [32]. Proteasome inhibition was selective; cytosolic calpain I and lysosomal cathepsin B were not inhibited by compounds **1b**, **3b** and **4** [32]. Using a cell-based chemiluminescent assay, compound **1b** behaved as an inhibitor of the ChT-L activity in human HeLa cells (20% inhibition at 50 μM after 1 h 30 min incubation) [32]. In this present report, we made several changes in the structures of the fluorinated β-hydrazino acid derivatives and we introduced non fluorinated α- and β-hydrazino acid scaffolds. We performed pharmacomodulations around the four moieties of the molecules (Fig. 2) in order to establish structure–activity relationships of this new class of proteasome inhibitors. A library of 40 molecules was designed, synthesized and evaluated on the 20S rabbit proteasome (Fig. 2). At first, we kept in the third moiety the β-hydrazino acid scaffold (C.1. Fig. 2; compounds **5a**, **5b**, **7**, **8**, **9**, **10a**, **10b**, **11a**, **11b**, **12**, **13**, Table 1). We then evaluated the influence of the length of the hydrazino scaffold by replacing the CF_3-β-hydrazino acid scaffold by the

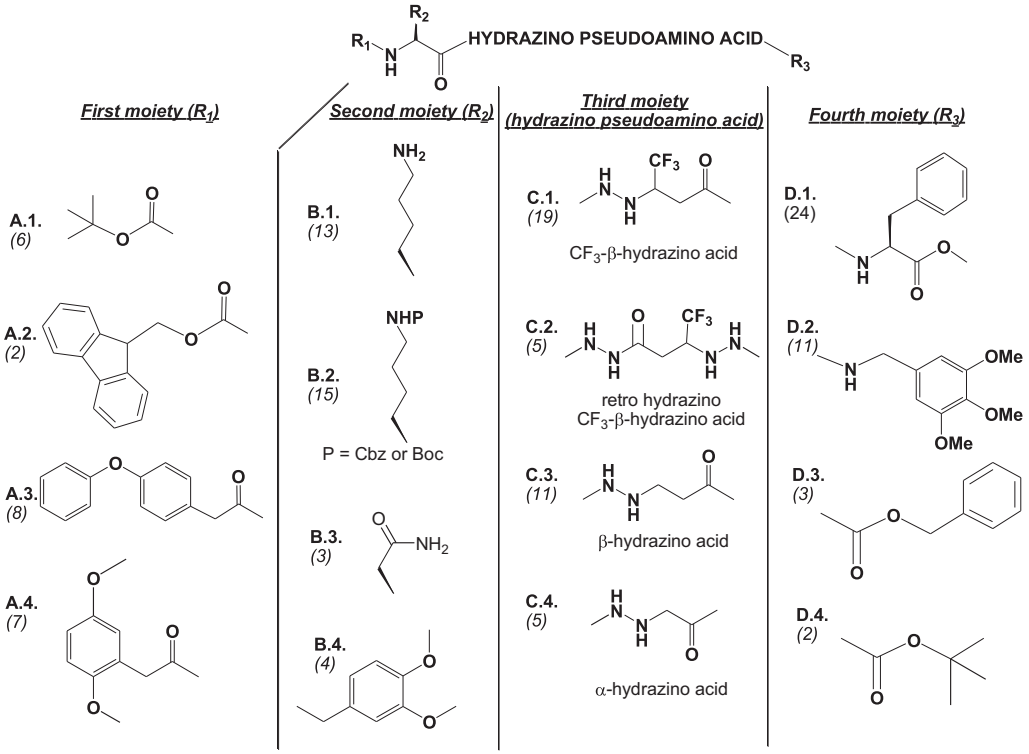


Fig. 2. Schematic representation of hydrazino acid based pseudopeptides. The number of compounds with different A-D groups is indicated in brackets with the numbers of these groups.

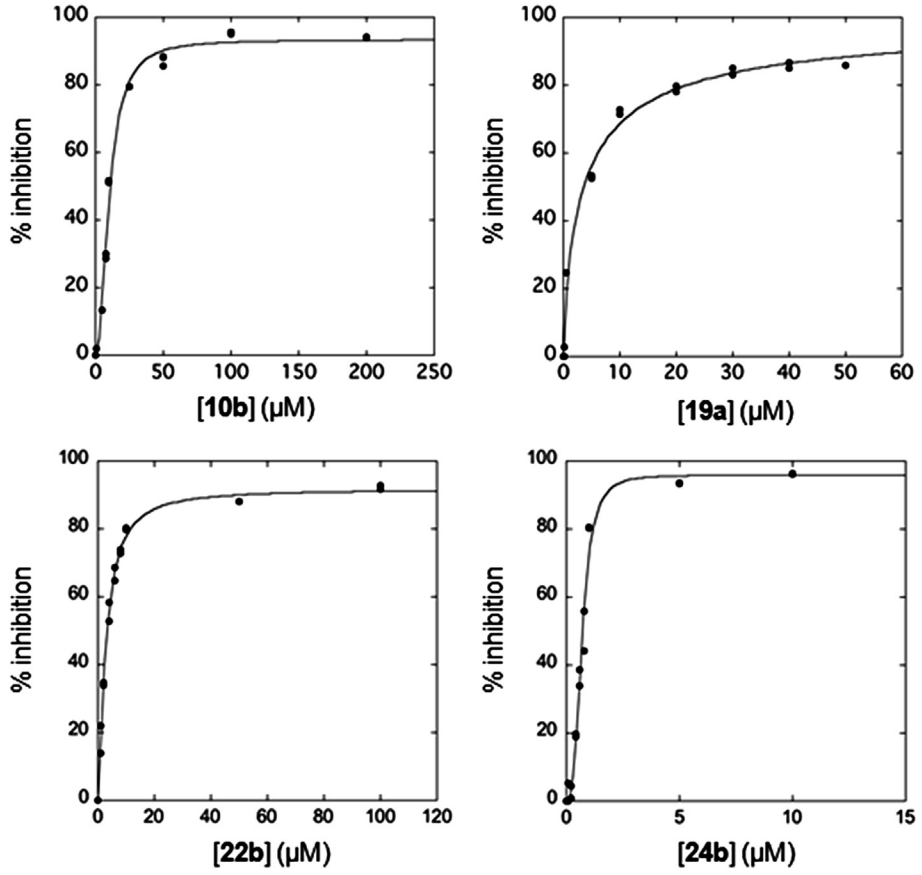


Fig. 3. Inhibition of the ChT-L activity of rabbit 20S proteasome by compounds **10b**, **19a**, **22b** and **24b** at pH 7.5 and 37 °C. The experimental data were fitted to equation 1 or equation 2.

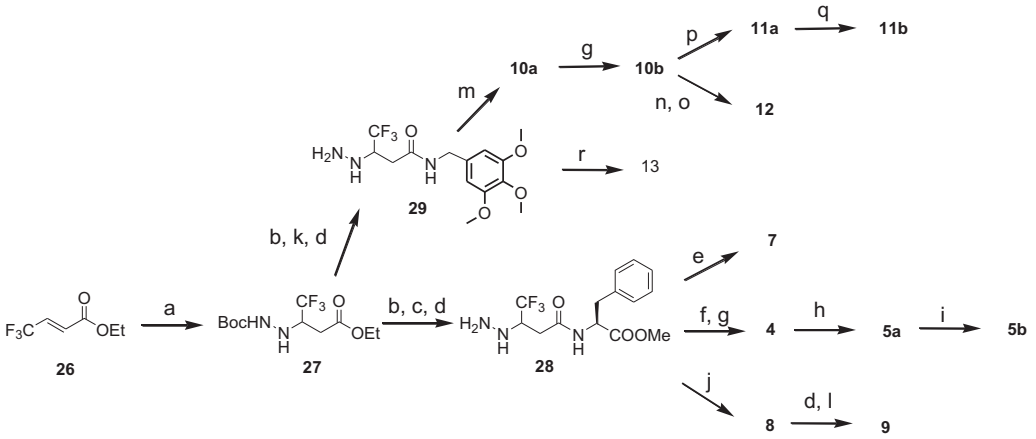
longer retro hydrazino CF_3-β-hydrazino acid (third moiety of the pseudopeptides C.2. Fig. 2; compounds **14a**, **14b**, **15**, **16a**, **16b**, Table 1). We also evaluated the influence on the inhibition of the ChT-L activity, of the trifluoromethyl group on the β-hydrazino acid scaffold when eliminating the fluorinated group on the β-hydrazino acid (C.3. Fig. 2; compounds **17a**, **17b**, **18a**, **18b**, **19a**, **19b**, **20**, **21a**, **21b**, **22a**, **22b**, Table 2). The shorter α-hydrazino acid scaffold (C.4. Fig. 2) was also introduced as the third moiety (compounds **23**, **24a**, **24b**, **25a**, **25b**, Table 3). In parallel, we evaluated the influence of the nature of the amino acid residue (second moiety, Fig. 2): protected or deprotected lysine (B.2. and B.1. respectively), asparagine (B.3.) or 3,4-dimethoxyphenylalanine (B.4.). We also modulated the first part of the molecule (Fig. 2) by introducing the *tert*-butyloxycarbonyl (A.1.), 9-fluorenylmethoxycarbonyl (A.2.), 3-phenoxyphenylacetyl (A.3.) and 3,4-dimethoxyphenylacetyl (A.4.) moieties. In the fourth part (Fig. 2), we kept the phenylalanine residue (D.1.) or replace the amino acid by introducing a 3,4,5-trimethoxybenzylamine moiety (D.2.), a benzyloxycarbonyl (D.3.) or a *tert*-butyloxycarbonyl (D.4.). We proposed a binding mode of this new class of non covalent proteasome inhibitors to ChT-L subunit by molecular modeling studies and we found that the obtained inhibitory activities corroborated the docking calculations performed for inhibitor/proteasome complexes.

2. Results and discussion

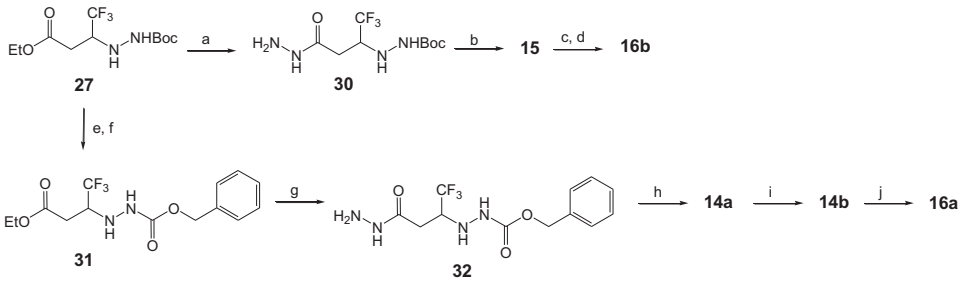
2.1. Chemistry

Compounds **1a**, **1b**, **2**, **3a**, **3b**, **6** were prepared according to our published methods [32]. The synthesis of compounds **4**, **5a**, **5b**, **7**–**9**, **10a**, **10b**, **11a**, **11b**, and **12** is described in Scheme 1. The intermediate **27** was obtained in good yield (94%) by a Michael addition of *tert*-butyl carbazate on **26** [32]. Saponification of the ester **27** followed by peptide coupling with L-phenylalanine methyl ester hydrochloride or 3,4,5-trimethoxybenzylamine using HBTU and HOBT as coupling agent, and the consecutive acidic cleavage of tert-butyl carbamate gave the trifluoroacetic salt of **28** [32] and **29** respectively (91% and 76%). Coupling of **28** with *N*α-Boc-*N*ε-Z-*L*-Lysine followed by acidic cleavage afforded **4** [32], that was subsequently coupled with 2,5-dimethoxyphenylacetic acid to give **5a**. Hydrogenolysis of the benzyl carbamate of **5a** gave **5b** in good yield (82%). The intermediate amine **28** was coupled with 2,5-dimethoxyphenylacetic acid and *N*-Boc-Asparagine to afford **7** and **8** respectively, in good yield (77% and 66%). The amine resulting from the *tert*-butyl carbamate (Boc) cleavage of **8**, was coupled with 2,5-dimethoxyphenylacetic acid to yield **9**. The peptide coupling of **29** with *N*α-Boc-*N*ε-Z-L-Lysine or *N*-Boc-Asparagine afforded **10a** and **13** respectively. The cleavage of Boc of **10a** gave **10b** that was coupled with 2,5-dimethoxyphenylacetic acid to give **11a**. The benzyl carbamate hydrogenolysis of **11a** gave **11b**. Compound **10b** was coupled with 3-phenoxyphenylacetic acid following by hydrogenolysis of the benzyl carbamate to afford **12** as indicated in Scheme 1.

The synthesis of compound **14a**, **14b**, **15**, **16a** and **16b** is described in Scheme 2. Treatment of **27** with hydrazine hydrate afforded the intermediate **30** which was coupled with *N*-Fmoc-3,4-diOMe-Phe-OH in the coupling conditions previously described, to give **15** in satisfactory yield (68%). It is noticed that 2,4,6-collidine was used instead of DIPEA to avoid the cleavage of the Fmoc protecting group. The basic cleavage of the Fmoc group using 10%



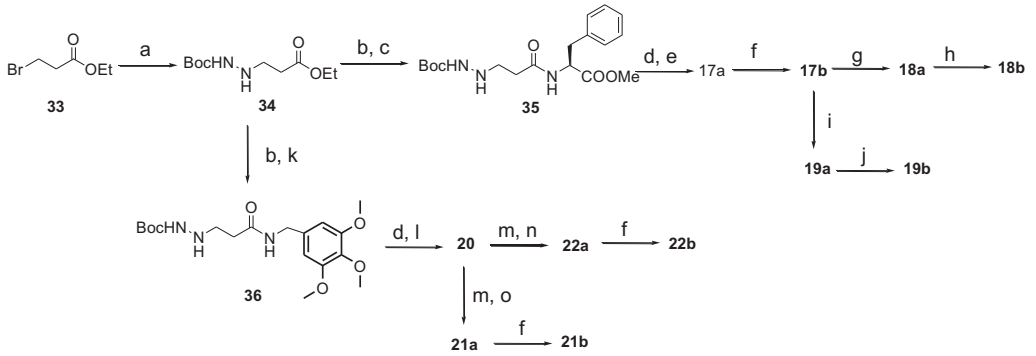
Scheme 1. Synthesis of molecules **4**, **5a**, **5b**, **7**–**9**, **10a**, **10b**, **11a**, **11b**, and **12**. *Reagents and conditions*: a) $NH_2NHBocMeOH$, 70 °C, 94%; b) 2N aq. NaOH, THF/MeOH, rt, 98%; c) L-phenylalanine methyl ester hydrochloride, HBTU, HOBT, DIPEA, DCM/DMF, rt, 91%; d) TFA, DCM, rt, 100%; e) 2,5-dimethoxyphenylacetic acid, HBTU, HOBT, DIPEA, DMF, rt, 77%; f) *N*α-Boc-*N*ε-Z-L-Lysine, HBTU, HOBT, DIPEA, DMF, rt, 57%; g) TFA, DCM, rt, 100%; h) 2,5-dimethoxyphenylacetic acid, HBTU, HOBT, DIPEA, DMF, rt, 84%; i) H_2, 10% Pd/C, rt, DCM/TFA, rt, 82%; j) *N*-Boc-Asparagine, HBTU, HOBT, DIPEA, DMF, rt, 63%; k) 3,4,5-trimethoxybenzylamine, HBTU, HOBT, DIPEA, DMF, rt, 76%; l) 2,5-dimethoxyphenylacetic acid, HBTU, HOBT, DIPEA, DMF, rt, 77%; m) *N*α-Boc-*N*ε-Z-L-Lysine, HBTU, HOBT, DIPEA, DMF, rt, 84%; n) 3-phenoxyphenylacetic acid, HBTU, HOBT, DIPEA, DMF, 24h, 71%; o) H_2, 10% Pd/C, rt, 89%; p) 2,5-dimethoxyphenylacetic acid, HBTU, HOBT, DIPEA, DMF, rt, 77%; q) H_2, 10% Pd/C, rt, 77%; r) *N*-Boc-Asparagine, HBTU, HOBT, DIPEA, DMF, rt, 55%.



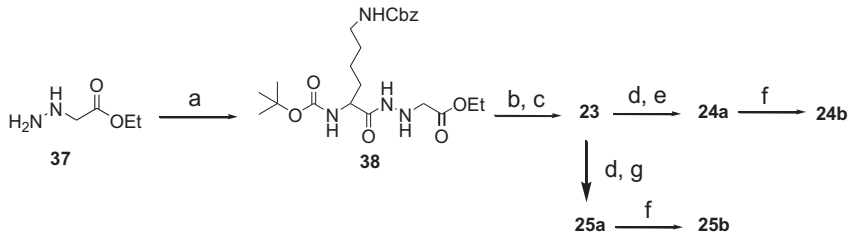
Scheme 2. Synthesis of molecules **14a**, **14b**, **15**, **16a** and **16b**. *Reagents and conditions*: a) $NH_2NH_2.H_2O$, EtOH, 100%; b) N-Fmoc-3,4-diOMe-Phe-OH, HBTU, HOBT, 2,4,6-collidine, DMF, 21 h, rt, 68%; c) 10% piperidine/DMF, 12 h, rt, 88%; d) 3-phenoxyphenylacetic acid, HBTU, HOBT, 2,4,6-collidine, DMF, 24h, 84%; e) TFA, DCM, rt, 100%; f) Cl-Cbz, DIPEA, DCM, 81%; g) $NH_2NH_2.H_2O$, EtOH, reflux, 100%; h) Fmoc-3,4-diOMe-Phe-OH, HBTU, HOBT, 2,4,6-collidine, DMF, 40 h, rt, 67%; i) 10% piperidine/DMF, 12 h, rt, 88%; j) 3-phenoxyphenylacetic acid, HBTU, HOBT, DIPEA, DMF, 24h, 85%.

piperidine in DMF followed by peptide coupling with 3-phenoxyphenylacetic acid afford **16b**. After acidic cleavage of the Boc protecting group of **27**, the amine obtained was protected with benzyl chloroformate to afford **31** in good yield (81%). The next reaction with hydrazine hydrate gave **32** which was coupled with Fmoc-3,4-diOMe-Phe-OH to afford **14a**. Removal of the Fmoc protecting group with 10% piperidine in DMF gave **14b**. Coupling of **14b** with 3-phenoxyphenylacetic acid using HBTU, HOBT, DIPEA in dry DMF, afforded **16a** in good yield (85%) (Scheme 2).

The synthesis of molecules **17a**, **17b**, **18a**, **18b**, **19a**, **19b**, **20**, **21a**, **21b**, 22a and **22b** is described in Scheme 3. The intermediate **34** was obtained in modest yield (43%) by nucleophilic substitution of ethyl bromopropionate **33** by *tert*-butyl carbazate. This modest yield can be explained by the formation of side product resulting from



Scheme 3. Synthesis of molecules **17a**, **17b**, **18a**, **18b**, **19a**, **19b**, **20**, **21a**, **21b**, **22a** and **22b**. Reagents and conditions: a) $BocNHNH_2$, DIPEA, toluene, 80 °C, 43%; b) 2N $NaOH_{aq}$, THF/MeOH, rt, 93%; c) L-phenylalanine methyl ester hydrochloride, HBTU, HOBT, DIPEA, DCM/DMF, rt, 82%; d) TFA, DCM, rt, 100%; e) *N*α-Boc-*N*ε-Z-L-Lysine, HBTU, HOBT, DIPEA, DMF, rt, 62%; f) TFA, DCM, rt, 100%; g) 3-phenoxyphenylacetic acid, HBTU, HOBT, 2,4,6-collidine, DMF, 81%; h) H_2, 10% Pd/C, MeOH, rt, 89%; i) 2,5-dimethoxyphenylacetic acid, HBTU, HOBT, DIPEA, DMF, rt, 37%; j) H_2, 10% Pd/C, MeOH, rt, 81%; k) 3,4,5-trimethoxybenzylamine, HBTU, HOBT, DIPEA, DMF, rt, 52%; l) *N*α-Fmoc-*N*ε-Z-L-Lysine, HBTU, HOBT, 2,4,6-collidine, DMF, rt, 30%; m) 10% piperidine/DMF, rt, 100%; n) 3-phenoxyphenylacetic acid, HBTU, HOBT, DIPEA, DMF, 24h, 52%; o) 2,5-dimethoxyphenylacetic acid, HBTU, HOBT, DIPEA, DMF, rt, 50%.



Scheme 4. Synthesis of molecules **23**, **24a**, **24b**, **25a** and **25b**. Reagents and conditions: a) *N*α-Boc-*N*ε-Z-L-Lysine, HBTU, HOBT, DIPEA, DMF, rt, 76%; b) 2N $NaOH_{aq}$, THF/MeOH, rt, 88%; c) L-phenylalanine methyl ester hydrochloride, HBTU, HOBT, DIPEA, DCM/DMF, rt, 56%; d) TFA, DCM, rt, 100%; e) 3-phenoxyphenylacetic acid, HBTU, HOBT, DIPEA, DMF, 48%; f) H_2, 10% Pd/C, MeOH/DMF, rt, 98%; g) 2,5-dimethoxyphenylacetic acid, HBTU, HOBT, DIPEA, DMF, rt, 55%.

nucleophilic attack of the same nitrogen atom of hydrazine on a second molecule of ethyl bromopropionate **33**. Saponification of **34** and successive coupling with L-phenylalanine methyl ester hydrochloride or 3,4,5-trimethoxybenzylamine, using HBTU, HOBT, DIPEA in dry DMF, gave **35** and **36** respectively. The acidic treatment of **35** followed by peptide coupling with *N*α-Boc-*N*ε-Z-L-Lysine gave **17a** in satisfactory yield (62%). **17b**, obtained after acidic cleavage of *tert*-butyl carbamate of **17a** was coupled with 3-phenoxyphenylacetic acid and 2,5-dimethoxyphenylacetic acid to afford **18a** and **19a** respectively. In both cases, hydrogenolysis of the benzyl carbamate with Pd/C 10% in MeOH provided **18b** and **19b** respectively, in good yield (89% and 81%). **20** was obtained after acidic cleavage of the *tert*-butyl carbamate of **36**, followed by coupling with *N*α-Fmoc-*N*ε-Z-L-Lysine, using HBTU, HOBT, 2,4,6-collidine in dry DMF. Basic cleavage of Fmoc protecting group of the lysine residue and successive coupling with 2,5-dimethoxyphenylacetic acid and 3-phenoxyphenylacetic acid gave **21a** and **22a** respectively. The trifluoroacetic salts **21b** and **22b** were obtained in quantitative yield, by acidic cleavage of *tert*-butyl carbamate of **21a** and **22a** respectively, with TFA in DCM.

The synthesis of molecules **23**, **24a**, **24b**, **25a** and **25b** is outlined in Scheme 4. The intermediate **38** was obtained in good yield (76%) by coupling *N*α-Boc-*N*ε-Z-L-Lysine with ethyl 2-hydrazinylacetate **37**, using HBTU, HOBT DIPEA in dry DMF. Saponification of the ester of **38** and successive coupling with L-phenylalanine methyl ester hydrochloride gave compound **23** in moderate yield (56%). Subsequent Boc cleavage of **23** with TFA in DCM, followed by coupling the trifluoroacetic salt of the amine with 3-phenoxyphenylacetic acid and 2,5-dimethoxyphenylacetic acid yielded **24a** and **25a** respectively (in moderate yield, 48% and 55%). In both cases, the carbamate moiety hydrogenolysis afforded **24b** and **25b** respectively in good yield (Scheme 4).

2.2. Biological results

The inhibition of the ChT-L activity of rabbit 20S proteasome by molecules **1**–**25** was investigated using appropriate fluorogenic substrate Suc-LLVY-AMC (Fig. 3) [17,19]. The aldehyde proteasome inhibitor MG132 (Z-LLL-H) was used as standard [17].

For molecules with a trifluoromethyl-β-hydrazino acid scaffold C.1. as pseudopeptide third moiety (molecules **1**–**13**, Table 1), the results indicated that the nature of the second moiety influenced the inhibitory potency. Replacing the 3,4-dimethoxyphenylalanine (lateral chain B.4.) by the more hydrophilic lysine (lateral chain B.1.) favored the inhibitory potency by a factor of 53 (molecule **1b** compared to molecule **2**). The same tendency was observed for compounds **3b** and **5b** compared to molecule **2** (factors of 2.6 and 14, respectively). Protecting the lysine amino group (B.2.) suppressed the inhibitory activity or decreased it (molecules **1a** *vs*.**1b**, **3a** *vs*. **3b** and **5a** *vs*. **5b**). Replacing the lysine residue (lateral chain B.1.) by an asparagine residue (lateral chain B.3.) was completely deleterious for the inhibitory activity (molecules **8** and **9** compared to molecules **3b** and **5b** respectively). The results showed also that shortening the pseudopeptide by connecting directly the phenoxyphenylacetyl moiety A.3. or the 3,4-dimethoxyphenylacetyl A.4. to the trifluoromethyl-β-hydrazino acid scaffold (no second moiety B) totally suppressed the ChT-L activity inhibition by molecules **6** (compared to **1b** and **2**) and **7** (compared to **5b**). Concerning the first moiety of the trifluoromethyl-β-hydrazino acid based pseudopeptides, the 3-phenoxyphenylacetyl A.3. (**1b**) was slightly more favorable than the 3,4-dimethoxyphenylacetyl A.4. and than the *tert*-butyloxycarbonyl A.1. **1b** *vs*. **3b** (factor of 20); **1b** *vs*. **5b** (factor of 3.7); **12** (IC_{50} of 6.2 μM) *vs*. **13** (no inhibition). Compounds **4** and **10b** devoid of the first moiety A and having the lysine αNH deprotected whereas the εNH was protected by a Cbz group were also good ChT-L activity inhibitors (IC_{50} of 6.0 and 10.1 μM). Inhibition was poorly affected by replacing in the fourth moiety the phenylalanine methyl ester D.1. by the 3,4,5-trimethoxybenzylamine D.2. (molecules **12**/**1b**, **11b**/**5b** and **10b**/**4**).

However, the introduction of the retro hydrazino CF_3-β-hydrazino acid scaffold C.2 totally suppressed the inhibitory activity of molecules **14a**, **14b**, **15**, **16a** and **16b** (both the direction of the trifluomethyl-β-hydrazino acid scaffold and the length of the third moiety were modified).

Similar structure–activity relationships were observed for pseudopeptides displaying the non fluorinated β-hydrazino acid scaffold C.3. within their third moiety (molecules **17**–**22**, Table 2). For comparison with the fluorinated molecules, the favorable lysine residue B.1. was conserved in this new series. Conversely to what was observed with the fluorinated molecules (Table 1), protecting the lysine εNH with a Cbz or a Boc group (B.2.) was not deleterious for the ChT-L inhibitory activity (molecules **18b**, **19b**, **21b** and **22b** compared to molecules **18a**, **19a**, **21a** and **22a**). As previously observed with the fluorinated molecules, compound **17b** devoid of the first moiety of the molecule and whose lysine αNH was deprotected whereas the εNH was protected by a Cbz group, had also a ChT-L inhibition activity (Table 2). In this series of non fluorinated β-hydrazino acid molecules, the 3-phenoxyphenylacetyl moiety A.3. was slightly more favorable than the 3,4-dimethoxyphenylacetyl A.4. (molecules **18b** and **22b** compared to molecule **19b** and **21b** respectively) and the 9-fluorenylmethoxycarbonyl A.2 (molecule **22a** compared to molecule **20**). A more noticeable effect was observed for *tert*-butyloxycarbonyl A.1. (molecule **18a** compared to molecule **17a).** As already observed for the fluorinated molecules, the deprotection of the αNH of the lysine residue (molecule **17b** versus molecule **17a**) whereas the NHε remained protected by a Cbz group led only to a decrease of ChT-L activity inhibition by a factor of 2.6. The inhibitory potency was poorly affected by the nature of fourth moiety D: molecule **22b** (phenylalanine methyl ester moiety D.1.) compared to molecule **18b** (3,4,5-trimethoxybenzylamine D.2.), and molecule **21b** (D.2.) compared to molecule **19b** (D.1.). The comparison of the

Table 1
Chymotrypsin-like (ChT-L) proteasome inhibition potency of molecules including the trifluoromethyl-β-hydrazino acid scaffold in the third part. IC_{50} (μM) or % inhibition at 100 μM of chymotrypsin-like (ChT-L) activity of rabbit 20S proteasome at pH 7.5 and 37 °C. *x*: activation factor.

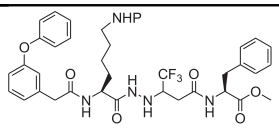
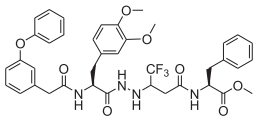
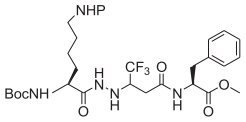
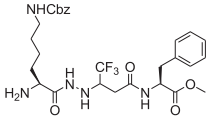
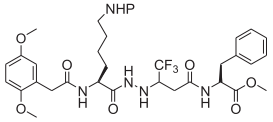
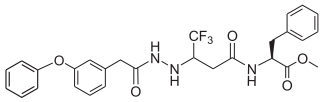
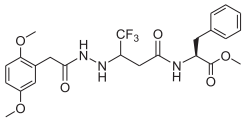
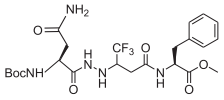
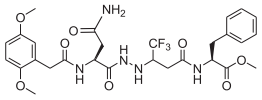
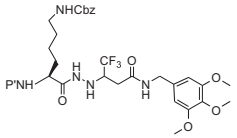
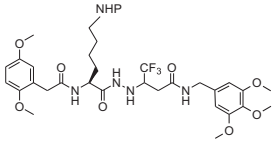
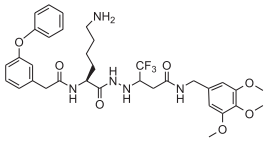
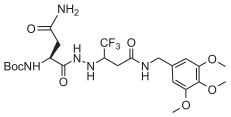
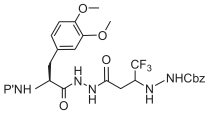
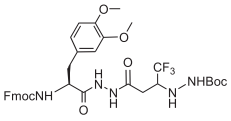
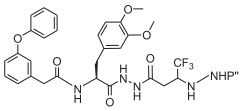
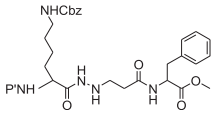
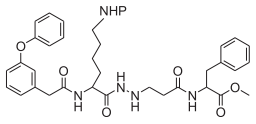
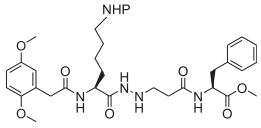
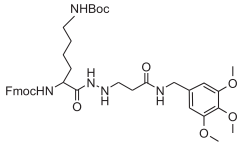
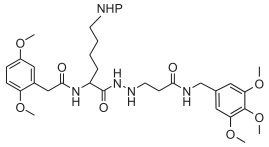
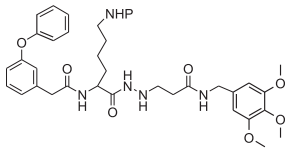
Compound	Structure	ChT-L
1a (P = CBz)		ni
1b (P = H) *racemic*		1.6 ± 0.1
1b[1] (P = H) *Dia 1*[a]		8.4 ± 0.5
1b[2] (P = H) *Dia 2*[a]		5.9 ± 0.1
2		85 ± 15
3a (P = CBz)		ni
3b (P = H)		32 ± 2
4		6.0 ± 0.5
5a (P = CBz)		30%
5b (P = H)		5.9 ± 0.3
6		*x* 2
7		ni
8		30%
9		ni
10a (P′ = Boc)		35%
10b (P′ = H)		10.1 ± 0.4
11a (P = CBz)		ni
11b (P = H)		97 ± 3
12		6.2 ± 0.3

Table 1 (*continued*)

Compound	Structure	ChT-L
13		ni
14a (P′ = Fmoc)		*x* 3.6
14b (P′ = H)		ni
15		ni
16a (P″ = Cbz)		ni
16b (P″ = Boc)		ni

[a] The two diastereoisomers of **1b** (**1b[1]** and **1b[2]**) were separated and evaluated separately.

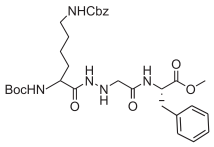
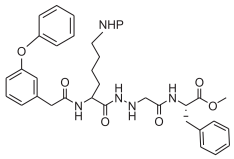
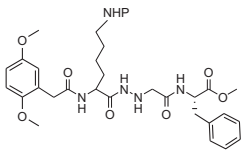
Table 2
Chymotrypsin-like (ChT-L) proteasome inhibition potency of molecules including the β-hydrazino acid scaffold in the third part. IC_{50} (μM) of chymotrypsin-like (ChT-L) activity of rabbit 20S proteasome at pH 7.5 and 37 °C.

Compound	Structure	ChT-L
17a (P′ = Boc)		48.5 ± 2.4
17b (P′ = H)		18.2 ± 0.4
18a (P = CBz)		2.8 ± 0.1
18b (P = H)		1.4 ± 0.1
19a (P = CBz)		3.6 ± 0.3
19b (P = H)		8.8 ± 0.3
20		6.0 ± 0.2
21a (P = Boc)		31.4 ± 0.7
21b (P = H)		11.8 ± 1
22a (P = Boc)		4.7 ± 0.5
22b (P = H)		3.3 ± 0.1

results obtained for both series (Tables 1 and 2) indicated that the presence of the trifluoromethyl group in the β-hydrazino acid scaffold poorly influenced the inhibition of ChT-L activity (compare for example, **1b** to **18b**, **5b** to **19b**, **12** to **22b**, **11b** to **21b**). This poor effect was also in agreement with the similar inhibitory activity observed for compounds **1b[1]** and **1b[2]** (Table 1). The two diastereoisomers of **1b** were separated and evaluated separately demonstrating that the absolute configuration of the carbon bearing the CF_3 group did not influenced significantly the inhibition (IC_{50} of 8.4 μM for **1b[1]** and 5.9 μM for **1b[2]**).

Shorten the third moiety C of the molecules by replacing the β-hydrazino scaffold C.3. by the α-hydrazino acid scaffold C.4. afforded also good ChT-L activity inhibitors with similar or slightly

Table 3
Chymotrypsin-like (ChT-L) proteasome inhibition potency of molecules including the α-hydrazino acid scaffold in the third part. IC_{50} (μM) of chymotrypsin-like (ChT-L) activity of rabbit 20S proteasome at pH 7.5 and 37 °C.

Compound	Structure	ChT-L
23		11.8 ± 1.1
24a (P = CBz)		1.1 ± 0.1
24b (P = H)		0.7 ± 0.03
25a (P = CBz)		7.1 ± 0.2
25b (P = H)		1.0 ± 0.03

increased efficiency (Table 3). Molecule **23** (C.4.; A.1. = Boc), was 4 times more efficient than molecule **17a** (C.3.; A.1. = Boc). Molecules **24a** (IC_{50} = 1.1 μM) and **24b** (IC_{50} = 0.7 μM) bearing the phenoxyphenylacetyl moiety A.4. had quite similar inhibitory activity than that of the corresponding molecules **18a** (IC_{50} = 2.8 μM) and **18b** (IC_{50} = 1.4 μM). Concerning the molecules bearing the dimethoxyphenylacetyl A.4., **25b** (B.1.) was 8.8 times more efficient than **19b** (B.1.) whereas **25a** (B.2. P = Cbz) was two-fold less efficient than **19a** (B.2. P = Cbz). As observed for β-hydrazino acid compounds (C.3.) (Table 2), protecting the lysine εNH with a Cbz group (B.2.) in the α-hydrazino compounds **23**, **24a** and **25a** was not deleterious for the inhibition. Again in this series, the 3-phenoxyphenylacetyl A.3. was more favorable than the 3,4-dimethoxyphenylacetyl A.4. and *tert*-butyloxycarbonyl A.1. in two cases: molecule **24a** versus **25a** (factor of 6.5) and **23** (factor of 10.7) and had no effect for molecule **24b** versus **25b** (factor of 1.4).

2.3. Molecular modeling

In order to find a plausible explanation for the different activities of these compounds to inhibit ChT-L activity, we engaged their structures into computational docking experiments. To date several theorical modeling studies have been carried out in different laboratories to describe the interaction between structurally diverse inhibitors and 20S proteasome. These studies have been mostly conducted on the basis of crystal structures of ligand and (eukaryotic) yeast proteasome complexes [33–37] even if differences in amino acid sequences of these proteins with that of their homologs in mammals are noticeable. The bovine protein has been less used for docking studies though it exhibits a sequence close to the human one [38–40] (sequence alignments are shown in Fig. 1S in the Supporting information). Our structural analysis and docking studies are therefore based on subunits β5 and β6 (defining the ChT-L active site) from the 2.75 Å resolution crystal structure of bovine proteasome 20S available from the Protein Data Bank [41] (see experimental part for details of the computational procedure).

During analysis of the multiple solutions obtained after a docking run, we estimated the quality of an inhibitor according to its ability to reproduce the previously putative binding mode of non covalent inhibitors based on three criteria. The first one was the tightness of the superimposition of its structure with that of the non covalent pseudopeptidic inhibitor published by Furet et al. (see the structure in Fig. 3S in the Supporting information) [16]. The second criteria was the number of hydrogen bonds contracted with the key residues Thr1, Thr21, Gly23, Gly47, Ala49 and Asp125. Poses maximizing the number of these hydrogen bonds were considered the best. The third criterion was the ability to occupy S1, S3, AS1 and AS2 pockets of proteasome 20S (the accessory pockets AS1 and AS2 are called S4 and S5 in the classical naming scheme for pockets recognizing the corresponding P*n* chains from the substrate).

Before focusing on the compounds synthesized for this study, we tested our docking protocol. We tried to identify a binding mode converging with the result reported by Furet et al. for their non covalent pseudopeptidic inhibitor (see the structure in Fig. 3S in the Supporting information) with the ChT-L active site of their homology model of the human proteasome 20S based on X-ray structure of yeast proteasome [16]. Our first result in this validation phase looked satisfactory overall, but analysis in depth revealed that proper occupation of S1 subpocket was actually lacking in every solution found, which was a major mismatch with the activity criteria we listed. Results recently published by Maréchal et al. [38] demonstrated that the strong non covalent inhibition of the ChT-L activity of human proteasome by 1,2,4-oxadiazole derivatives could also be due to the inhibitor binding within the subsite S5 instead of the S1 one. However, we preferred to start our docking studies with models in which we observed a binding mode within bovine proteasome active site as similar as possible to the one described by Furet et al.

We first evaluated if what we were observing could be explained by an intrinsic different size of S1 pocket in the ChT-L active site in bovine proteasome compared to the one in yeast proteasome (Unno et al. reported differences between yeast and bovine proteasome 20S structure [41]). However, when we superimposed coordinates of the bovine protein structure we chose with those of a comparable structure (without co-crystallized ligands) obtained for yeast proteasome, we observed identical conformation of the S1 pocket.

We therefore decided to carry out a detailed analysis of all the proteasome structures available in the PDB, both proteins devoid of ligand and co-crystals with ligands. This task allowed us to establish a hypothetical mapping of residues defining every subpocket thanks to the probing work performed by the various ligands already cocrystallised (see Table 1S in the Supporting information). More importantly, our structural analysis, and in particular comparison of cocrystals with unbound form of yeast proteasome, revealed a joint movement of side chains of Met45 (directly exposed to the solvent) and Ile35 in order to accommodate “P1” side chain from the ligand upon complexation. We hypothesized that this induction phenomenon could be extrapolated to bovine proteasome, and that the smaller size of S1 pocket in the “apo” form we were using might at the origin of exclusion of bulky substituents, typically benzyl groups in the kind of ligands we are studying (see Fig. 2S in Supporting information). We hypothesized that taking into account adaptability of the size of S1 pocket in the bovine proteasome would be a key element to place ourselves under conditions suitable for *in silico* evaluation of our inhibitors.

Indeed, when we included conformational sampling of the side chains of both aforementioned Met45 and Ile35 residues in our docking protocol, repeating the experiment with the compound described by Furet et al. resulted in solutions where the “P1” side chain of the pseudopeptide was positioned in the S1 pocket of the ChT-L active site, in a similar manner to what was reported by these authors [16]. Moreover, in the best pose, S3 and AS2 pockets were also occupied, and 4 hydrogen bonds were formed with Thr21, Gly47 and Asp125 (see Supporting information, Fig. 4S). With these results, we estimated that the docking procedure had reached the level of reliability required to be used in the simulation of the binding mode of synthesized compounds **1**–**25** to the ChT-L active site of bovine 20S proteasome.

Docking poses retained for compounds that were experimentally good proteasome inhibitors showed a network of hydrogen bond interactions with some or all key residues known to be important for the binding of compounds in the 20S proteasome (Thr1, Thr21, Gly23, Gly47, Ala49 and Asp125) as well as occupation of S1, S3 and AS1 or AS2 pockets. One of the most potent compounds (**24b**) established 6 hydrogen bonds with the protein (contributing to anchor it in the binding site), out of which 3 involved key residues (Fig. 4). First, two NH groups belonging to the phenylalanine and the hydrazide moieties donated hydrogen bonds to the backbone oxygen of Gly47. Then the NH of the second amide group donated another hydrogen bond to the side chain of Asp125. Within the S3 pocket, the NH_3 group of the lysine side chain of compound **24b** shared 2 hydrogen bonds with Ser123 and Ser129 residues sitting at the bottom of the pocket. The phenoxyphenyl oxygen also established a hydrogen bond with Tyr107, delimitating the boundary between AS1 and AS2 pockets. Both S3 and AS1 pocket were filled respectively with the lysine side chain and the phenoxyphenylacetyl moieties. Compound **18b**, which is the β-hydrazino acid homolog of **24b**, showed a similar ChT-L inhibition activity (IC_{50} = 1.4 and 0.7 μM for **18b** and **24b** respectively). Its

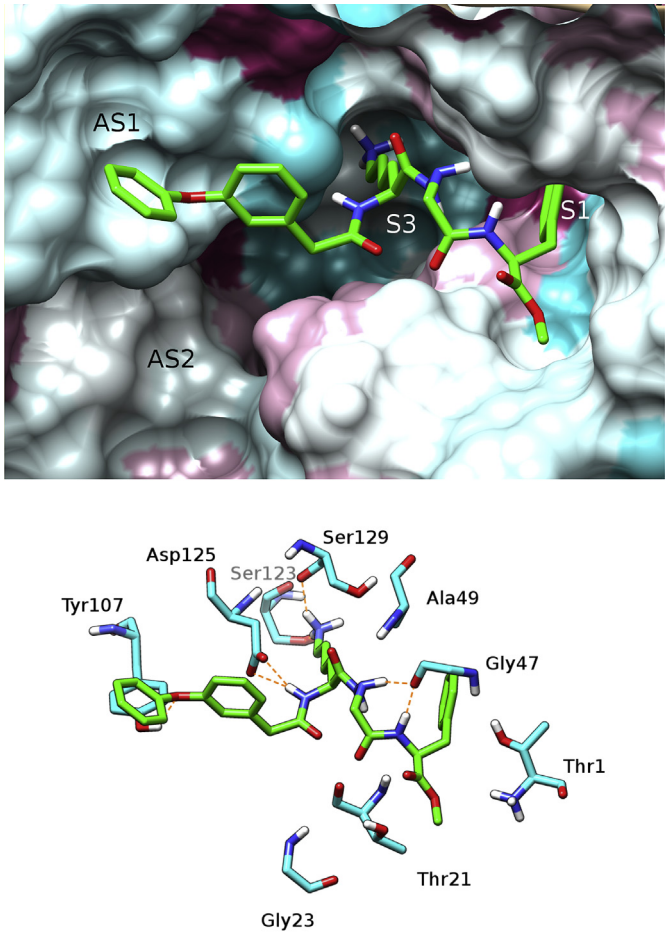


Fig. 4. Docking pose of compound **24b** positioned into the ChT-L binding site pocket of the bovine proteasome X-ray structure. Key residues are shown and hydrogen bonds formed are in yellow dashes. (Color code for SAS: cyan is polar, purple is hydrophobic.) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

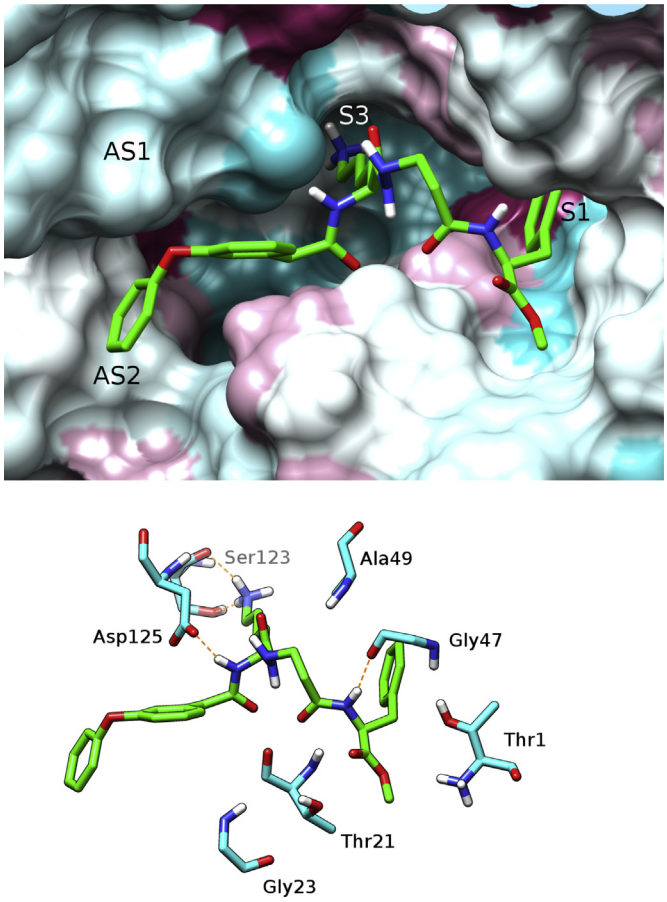


Fig. 5. Docking pose of compound **18b** positioned into the ChT-L binding site pocket of the bovine proteasome X-ray structure. Key residues are shown and hydrogen bonds formed are in orange dashes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

binding mode was also quite similar to that of **24b** (Figs. 5 and 6). **18b** contracted 4 hydrogen bonds in total, out of which two were with key residues Gly47 and Asp125. Additionally, occupation of pocket S3 was very similar (again with two hydrogen bonds donated by the ammonium group of the lysine side chain with Ser123), and AS2 was occupied by the terminal phenyl group (instead of AS1 for **24b**). When superimposed within the active site, compounds **18b** and **24b** were tightly aligned for most of their structure (see Fig. 6), the difference lying mainly in occupation of AS2 instead of AS1 (which, expectedly, is not highly significant in terms of biological activity).

In analogs **18a** and **24a**, protection of the side chain of the lysine moiety by a Cbz group was found to be compatible with S3 pocket occupation, whereas no hydrogen bond could be observed with this part of the molecule (See Figs. 5S and 6S in Supporting informations). The overall β-sheet structure remained intact, with its underpinning network of hydrogen bonds with key residues. In particular, for **18a**, Gly47 exchanged two hydrogen bonds with the NH and CO groups of the phenylalanine moiety, in addition to two hydrogen bonds donated to Thr21 and Asp125 (respectively by one NH group of the hydrazide function and the lysine αNH) (Fig. 5S, Supporting informations). In **24a**, the two amide groups were responsible for the hydrogen bonds, one with Gly47 and Thr21, the other with Asp125 (Fig. 6S, Supporting informations). These observations could explain while both protected and deprotected lysine (NHε-wise) in non-fluorinated α and β-hydrazino acid molecules afforded inhibitors with similar efficiencies.

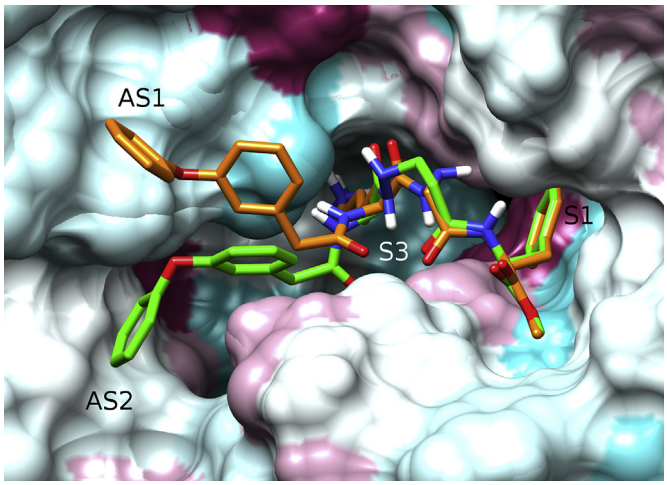


Fig. 6. Superposition of docking poses of compound **24b** (orange) and **18b** (green) positioned into the ChT-L binding site pocket of the bovine proteasome X-ray structure. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In the fluorinated series, remarkably, the two diastereoisomers of mixture **1b** were able to fit nicely into the ChT-L active site with a slightly different binding mode while they showed a similar inhibition activity (IC_{50} = 8.4 and 5.9 M for **1b**[1] and **1b**[2], Table 1). Diastereoisomers SRS and SSS of **1b** presented 7 hydrogen bonds with Thr1, Thr21, Gly47, Phe124 and Asp125 and 5 hydrogen bonds with Gly47, Tyr107, Ser123 and Asp125 respectively. A difference in the orientation of the lysine side chain and the methyl ester moiety was also noted, but activity-critical pockets were occupied in both cases. (see Figs. 7S and 8S in Supporting information).

Furthermore, during our molecular modeling studies, we observed a supplementary and interesting way of binding for compound **17b**, where the protected lysine side chain aligns with the rest of the molecule instead of occupying S3 subsite (see Fig. 9S in Supporting information). The same phenomenon could be observed for both diastereoisomers of compound **4**.

On the contrary, satisfying poses (*ie*. completing the 3 criteria: hydrogen bonding with key residues, superposition with the pseudopeptide of Furet et *al*. [16] and occupation of at least S1 and S3 simultaneously) could not be observed for compounds that were experimentally not able to inhibit 20S proteasome. For example, in the case of compound **16a**, even if the dimethoxyphenylalanine and phenoxyphenylacetyl moieties were accurately orientated in the pockets S3 and AS2 respectively, compound **16a** did not share enough hydrogen bonds with the key residues. The comparison of **16a** with its structurally analog **2** which showed inhibition activity (IC_{50} of 85 μM) is of particular interest. Compound **2** was correctly bound in the active site with 5 hydrogen bonds contracted with the key residues (Thr1, Thr21, Gly47, Asp125). The detailed analysis of docking experiments provided some clues to explain the differences observed between our compounds for their inhibitory activities of 20S proteasome.

3. Conclusions

To our knowledge, α-hydrazino acid scaffolds have only rarely been used in medicinal chemistry and particularly very scarcely introduced in protease inhibitors. Moreover, little was known about the β-hydrazino acid based peptidomimetics. In this work, we confirmed that α- and β-hydrazino acid structures can mimic the typical secondary structure of native α-peptides. As a proof of concept, α- and β-hydrazino acid scaffolds were introduced in a library of 40 pseudopeptides that were tested for their ability to inhibit the 20S proteasome ChT-L activity. The peptidic proteasome inhibitors reported in the literature commonly have borne α-amino acid moieties [12,13]. We described here the easy preparation of pseudopeptides bearing α- and β-hydrazino acid scaffolds that inhibit the ChT-L activity of 20S proteasome up to a submicromolar range (0.7 μM for molecule **24b**). Thus, the ability of preserving biological activity by introducing in peptides, α- and β-hydrazino acid scaffolds is demonstrated. By performing pharmacomodulations around these hydrazino acid scaffolds, we established structure–activity-relationships. We have shown herein the slightly superiority of the phenoxyphenylacetyl moiety in the first part of the pseudopeptides, as previously observed by Furet et al. [16], and also the dramatic superiority of a lysine residue in the second part, and finally the possibility of the nearly complete suppression of the peptidic character of the molecules, by introducing 3,4,5-trimethoxybenzylamine in place of the phenylalanine residue in the fourth part. Finally, we proposed a binding mode of this new class of non covalent proteasome inhibitors to ChT-L subunit by molecular modeling and developed a rational explanation about the different activities of the compounds against the ChT-L activity thanks to the docking calculations.

4. Experimental

4.1. Chemistry

The usual solvents were purchased from commercial sources. Dimethylformamide (DMF) was distilled on $CaSO_4$, tetrahydrofuran (THF) was distilled on sodium/benzophenone, acetonitrile was distilled on $CaCl_2$. TLC was performed on silica gel, 60F-250 (0.26 mm thickness) plates. The plates were visualized with UV light (254 nm) or with a 3.5% solution of phosphomolybdic acid in ethanol or with a solution of ninhydrin in ethanol. Liquid chromatography was performed on Merck 60 silica gel (230–400 mesh). Separation of diastereoisomers of **1b** was performed by HPLC using a WATERS gradient system (pump + controller E600, UV detector PDA 2996, autosampler 717) and a column SUNFIRE (C18, 5 μm, 150 mm × 19 mm). Protected amino acids, *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU), 1-hydroxybenzotriazole (HOBT) and 3-bromo-propionic acid ethyl ester **33** were purchased from commercial sources. 2-[3-(*N*′-{6-Benzyloxycarbonylamino-2-[2-(3-phenoxy-phenyl)acetylamino] hexanoyl}-hydrazino)-4,4,4-trifluoro-butyrylamino]-3-phenyl-propionic acid methyl ester **1a**, 2-[3-(*N*′-{6-Amino-2-[2-(3-phenoxyphenyl)acetylamino]hexanoyl}hydrazino)-4,4,4-trifluoro-butyrylamino]-3-phenyl-propionic acid methyl ester **1b**, 2-[3-(*N*′-{3-(3,4-Dimethoxyphenyl)-2-[2-(3-phenoxyphenyl)acetylamino]propionyl} hydrazino)-4,4,4-trifluorobutyrylamino]-3-phenylpropionic acid methyl ester **2**, 2-{3-[*N*′-(2-Benzyloxycarbonylamino-6-*tert*-butoxycarbonylaminohexanoyl)hydrazino]-4,4,4-trifluorobutyrylamino} -3-phenylpropionic acid methyl ester **3a**, 2-{3-[*N*′-(6-Amino-2-*tert*-butoxycarbonylaminohexanoyl)hydrazino]-4,4,4-trifluorobutyrylamino}-3-phenylpropionic acid methyl ester **3b**, 2-{3-[*N*′-(2-Amino-6-benzyloxycarbonylaminohexanoyl)hydrazino]-4,4,4-trifluoro-butyrylamino}-3-phenylpropionic acid methyl ester **4**, 3-Phenyl-2-(4,4,4-trifluoro-3-{*N*′-[2-(3-phenoxyphenyl)acetyl]hydrazino} butyrylamino) propionic acid methyl ester **6**, Ethyl 4,4,4-trifluorocrotonate **26**, 3-(*N*′-*tert*-butoxycarbonylhydrazino)-4,4,4-trifluorobutyric acid ethyl ester **27**, the trifluoroacetic salt of 3-phenyl-2-(4,4,4-trifluoro-3-hydrazinobutyrylamino) propionic acid methyl ester **28**, were prepared according to published methods [32]. Melting points were determined on a Kofler melting point apparatus. Unless otherwise stated, NMR spectra were performed on a Bruker AVANCE 400 (^{1}H, 400 MHz; ^{13}C, 100 MHz). If stated, NMR spectra were performed on a BRUKER AMX 200 (^{1}H, 200 MHz; ^{13}C, 50 MHz; 188 MHz, ^{19}F) or an Ultrafield AVANCE 300 (^{1}H, 300 MHz; ^{13}C, 75 MHz). Unless otherwise stated, $CDCl_3$ was used as solvent. Chemical shifts δ are in ppm, and the following abbreviations are used : singlet (s), doublet (d), doublet doublet (dd), triplet (t), quintuplet (quint), multiplet (m), broad multiplet (bm), and broad singlet (bs). Mass spectra were obtained using a Bruker Esquire electrospray ionization apparatus at the SAMM (Faculty of Pharmacy at Châtenay–Malabry). Element analyses (C, H, N) were performed on a Perkin–Elmer CHN, Analyser 2400 at the Microanalyses Service of the Faculty of Pharmacy at Châtenay–Malabry (France).

4.1.1. Synthesis of the target molecules including the trifluoromethyl-β-hydrazino acid scaffold **1b**, **5a**, **5b**, and **7**–**16b**

4.1.1.1. 2-[3-(N′-{6-Amino-2-[2-(3-phenoxyphenyl)acetylamino]hexanoyl} hydrazino)-4,4,4-trifluoro-butyrylamino]-3-phenyl-propionic acid methyl ester, trifluoroacetic acid salt ***1b***. Compound **1b** has been synthesized as a mixture of 2 diasteroisomers (ratio: 1/1) and described previously.[32] The two diastereoisomers were separated by HPLC (column SUNFIRE, C18, 5 μm, 150 mm × 19 mm; mobile phase: a mixture of A: water (0.1% formic acid) and B: CH_3CN (mixture A/B 73/27 to 68/32 in 15 min); room temperature; flow rate 1 mL/min; detection at 235 nm).

*4.1.1.1.1. Diastereoisomer **1**.* TR = 11.75 min ^{1}H NMR (CD_3OD): δ = 8.44 (br s, 1H), 7.25–7.02 (m, 8H), 6.96–6.72 (m, 6H), 4.62 (m, 1H), 4.17 (m, 1H), 3.62 (m, 1H), 3.58 (s, 3H), 3.39 (s, 2H), 2.92 (dd J = 5.8 and 13.8 Hz, 1H), 2.83 (dd, J = 8.4 and 13.8, 1H), 2.68 (m, 2H), 2.42 (m, 2H), 1.60–1.18 (m, 6H). ^{19}F (188 MHz, CD_3OD): δ = −76.53). Anal. Calcd for $C_{34}H_{41}ClF_3N_5O_6\cdot H_2O$: C, 56.23; H, 5.98; N 9.65; found C, 56.12; H, 5.75; N, 9.45.

*4.1.1.1.2. Diastereoisomer **2**.* TR = 14.13 min ^{1}H NMR (CD_3OD): δ = 8.51 (br s, 1H), 7.37–7.18 (m, 8H), 7.07 (m, 2H), 6.97 (d, J = 7.6 Hz, 3H), 6.84 (dd, J = 8.13 and 1.9 Hz, 1H), 4.74 (dd, J = 8.5 and 5.9, 1H), 4.62 (m, 1H), 3.81 (m, 1H), 3.74 (s, 3H), 3.59 (s, 2H), 3.17 (dd, J = 5.9 and 13.8 Hz, 1H), 3.06 (dd, J = 8.5 and 13.8 Hz, 1H), 3.01 (m, 2H), 2.56 (dd, J = 6.8 Hz, 2H), 1.80–1.65 (m, 4H), 1.35 (m, 2H). ^{19}F (188 MHz, CD_3OD): δ = −76.46. Anal. Calcd for $C_{34}H_{41}ClF_3N_5O_6\cdot H_2O$: C, 56.23; H, 5.98; N 9.65; found C, 56.08; H, 5.90; N, 9.36.

*4.1.1.2. 2-[3-(N′-{6-Benzyloxycarbonylamino-2-[2-(2,5-dimethoxy-phenyl)-acetylamino]-hexanoyl}-hydrazino)-4,4,4-trifluoro-butyrylamino]-3-phenyl-propionic acid methyl ester **5a**.* To a solution of **4** (319 mg, 0.45 mmol, 1.0 eq.) in DMF (2 mL), DIPEA (370 μl, 2.25 mmol, 5.0 eq.) and HOBT (90 mg, 0.67 mmol, 1.5 eq.) were added. Meanwhile a solution of 2,5-dimethoxyphenylacetic acid (132 mg, 0.67 mmol, 1.5 eq.) and HBTU (254 mg, 0.67 mmol, 1.5 eq.) in DMF (2 mL) was stirred for half an hour at room temperature. The two solutions were then combined and the resulting mixture was stirred at room temperature overnight. After concentration under reduced pressure, the resulting yellow oil was taken up in EtOAc (10 mL) and successively washed with 10% aqueous citric acid (2 × 10 mL), 10% aqueous K_2CO_3 (2 × 10 mL) and brine (15 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure to give a slightly yellow solid which after precipitation in a mixture of cyclohexane/EtOAc afforded **5a** as a white solid (298 mg, 0.38 mmol, 84%). mp 140–142 °C; ^{1}H NMR (DMSO-*d*6): δ = 9.53 (t, 1H, J = 5.6 Hz), 8.64 (dd, 1H, J = 7.3 Hz, 8.7 Hz), 7.95 (m, 1H), 7.36–7.20 (m, 10H), 6.87–6.76 (m, 3H), 5.01 (s, 2H), 4.50 (m, 1H), 4.20 (m, 1H) 3.79 (m, 1H), 3.69 (s, 3H), 3.68 (s, 3H), 3.59 (s, 3H), 3.42 (d, 2H, J = 3.2 Hz), 3.06–2.90 (m, 4H), 2.46 (m, 2H), 1.57–1.53 (m, 2H), 1.41–1.36 (m, 2H), 1.28–1.21 (m, 2H); ^{13}C NMR (DMSO-*d*6): δ = δ 171.7, 171.6, 171.4, 169.8, 168.1, 156.0, 152.9, 151.3, 137.2, 137.0, 136.9, 129.0, 128.9, 128.3, 128.2, 127.7, 126.5, 116.7, 111.9, 111.6, 79.4, 78.9, 78.5, 65.1, 55.8, 55.2, 53.7, 53.6, 51.8, 51.0, 50.9, 36.7, 36.6, 32.9, 31.7, 29.0, 22.4; ^{19}F (188 MHz, DMSO-*d*6): δ = −73.74 (d, 0.4F, J = 7.3 Hz) I diastereomer −73.91 (d, 0.6F, J = 7.5 Hz) II diastereomer; IR (cm^{-1}): 3283, 2943, 1739, 1684, 1637, 1531, 1502, 1443, 1360, 1261, 1227, 1207 739; ESI$^+$ MS *m*/*z*: 796 [M + Na]$^+$; Anal. Calcd for $C_{38}H_{46}F_3N_5O_9.0.75\cdot H_2O$: C, 57.95; H, 6.09; N, 8.90; found C, 58.04; H, 6.02; N 9.01.

*4.1.1.3. 2-[3-(N′-{6-Amino-2-[2-(2,5-dimethoxy-phenyl)-acetylamino]-hexanoyl}-hydrazino)-4,4,4-trifluoro-butyrylamino]-3-phenyl-propionic acid methyl ester trifluoroacetic salt **5b**.* To a solution of **5a** (461 mg, 0.60 mmol, 1.0 eq.) in dry methanol (8 mL) was added Pd/C 10% (46 mg). The mixture was stirred overnight under hydrogen atmosphere at room temperature and filtered on a celite pad. The yellowish solid obtained after concentration under reduced pressure was dissolved in DCM/TFA 3:1 (8 mL) and stirred for half an hour. The crude residue obtained after concentration under reduced pressure was dissolved in DCM/Et_2O with just few drops of MeOH and precipitated at −20 °C. The yellowish solid obtained was finally washed with Et_2O to afford **5b** (461 mg, 0.49 mmol, 82%). mp 120–122 °C; ^{1}H NMR (CD_3OD): δ = 7.23–7.17 (m, 5H), 6.86–6.75 (m, 3H), 4.65 (m, 1H), 4.29 (m, 1H), 3.74 (s, 3H), 3.69 (s, 3H), 3.65 (s, 3H), 3.51–3.44 (m, 3H), 3.15–3.08 (m, 1H), 3.00 (m, 1H), 2.88–2.82 (m, 2H), 2.52–2.46 (m, 2H), 1.57–1.53 (m, 2H), 1.41–1.36 (m, 2H), 1.28–1.21 (m, 2H); ^{13}C NMR (CD_3OD): δ = 174.42, 174.41, 155.5, 153.5, 153.4, 152.0, 138.5, 130.6, 129.9, 129.6, 128.3, 126.3, 118.7, 114.3, 114.2, 113.1, 67.3, 60.7, 60.2, 59.9, 59.7, 56.9, 56.4, 56.0, 53.2, 53.1, 40.9, 39.0, 38.7, 34.6, 33.0, 28.4, 23.8, 15.8; ^{19}F (188 MHz,CD_3OD): δ = −76.67 (d, 0.3F, J = 7.3 Hz), −76.62 (d, 0.7F, J = 7.5 Hz), −77.34 (s, 1F); IR (cm^{-1}): 3324, 1639, 1503, 1126, 700; ESI$^+$ MS *m*/*z*: 640 [M + H]$^+$; Anal. Calcd for $C_{32}H_{41}F_6N_5O_9\cdot 0.5\ H_2O$: C, 50.39; H, 5.56; N, 9.18; found C, 50.04; H, 5.37; N, 8.73.

*4.1.1.4. 2-(3-{N′-[2-(2,5-Dimethoxy-phenyl)-acetyl]-hydrazino}-4,4,4-trifluoro-butyrylamino)-3-phenyl-propionic acid methyl ester **7**.* To a solution of the trifluoroacetic salt of **28** [32] (392 mg, 0.88 mmol, 1.0 eq.) in DMF (3 mL), were successively added DIPEA (730 μl, 4.4 mmol, 5.0 eq.) and HOBT (179 mg, 1.32 mmol, 1.5 eq.). Meanwhile a solution of 2,5-dimethoxyphenylacetic acid (259 mg, 1.32 mmol, 1.5 eq.) and HBTU (499 mg, 1.32 mmol, 1.5 eq.) in DMF (3 mL) was stirred for half an hour at room temperature. The solutions were combined and the resulting mixture was stirred at room temperature overnight. After evaporation under reduced pressure, the resulting yellow oil was taken up in EtOAc (10 mL) and successively washed with 10% aqueous citric acid (2 × 10 mL), 10% aqueous K2CO3 (2 × 10 mL) and brine (15 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure to give a slightly yellow solid which was purified by column chromatography (EtOAc) to give **7** as a white solid (347 mg, 0.68 mmol, 77%). mp 100–102 °C; ^{1}H NMR (DMSO-*d*6): δ = 9.37 (d, 1H, J = 5.1 Hz), 8.60 (d, 1H, J = 7.4 Hz), 8.03 (t, 1H, J = 8.8 Hz), 7.34–7.22 (m, 5H), 6.88–6.80 (m, 3H), 4.58–4.50 (m, 2H), 3.68 (s, 3H), 3.67 (s, 3H), 3.58 (s, 3H), 3.40 (s, 2H), 3.05–2.88 (m, 2H), 2.50–2.4 (m, 2H); ^{13}C NMR (DMSO-*d*6): δ = 172.3, 171.6, 168.4, 168.2, 153.7, 151.0, 136.4, 129.2, 129.1, 128.4, 126.9, 123.1, 117.1, 113.4, 11.5, 55.7, 53.8, 53.7, 52.4, 37.3, 37.1, 33.3; ^{19}F (188 MHz, DMSO-*d*6): δ = −74.99 (d, 0.7F, J = 7.3 Hz) I diastereomer −75.37 (d, 0.3F, J = 7.1 Hz) II diastereomer; IR (cm^{-1}): 3301, 1648, 1502, 1229, 1122 698; ESI$^+$ MS *m*/*z*: 534 [M + Na]$^+$; Anal. Calcd for $C_{24}H_{28}F_3N_3O_6$: C, 56.36; H, 5.52; N, 8.22: found C, 56.08; H, 5.43; N, 8.01.

*4.1.1.5. 2-{3-[N′-(2-tert-Butoxycarbonylamino-3-carbamoyl-propionyl)-hydrazino]-4,4,4-trifluoro-butyrylamino}-3-phenyl-propionic acid methyl ester **8**.* To a solution of the trifluoroacetic salt of **28** (163 mg, 0.49 mmol, 1.0 eq.) [32] in DMF (2 mL), DIPEA (400 μl, 2.45 mmol, 5.0 eq.) and HOBT (100 mg, 0.74 mmol, 1.5 eq.) were successively added. Meanwhile a solution of N_α-Boc-Asn (170 mg, 0.74 mmol, 1.5 eq.) and HBTU (280 mg, 0.74 mmol, 1.5 eq.) in DMF (2 mL) was stirred for half an hour at room temperature. The two solutions were then combined and the resulting mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure and the resulting yellow oil was taken up in EtOAc (10 mL) and successively washed with 10% aqueous citric acid (2 × 10 mL), 10% aqueous K_2CO_3 (2 × 10 mL) and brine (15 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure to yield a white solid which was purified by column chromatography (EtOAc:-MeOH 9:1) to give **8** as a white solid (168 mg, 0.30 mmol, 63%). mp 150–152 °C; ^{1}H NMR (DMSO-*d*6): δ = 9.44 (m, 1H), 8.60 (d, 1H, J = 7.6 Hz), 7.35–7.17 (m, 5H), 6.90 (d, 2H, J = 7.6 Hz), 5.43 (m, 1H), 4.49 (m, 1H), 4.19 (m, 1H), 3.74 (m, 1H), 3.59 (s, 3H), 3.05–2.85 (m, 2H), 2.60–2.40 (m, 2H), 2.40–2.31 (m, 2H), 1.37 (s, 1H); ^{13}C NMR (DMSO-*d*6): δ = 171.6, 171.3, 171.1, 171.0, 168.0, 154.9, 137.0, 129.0, 128.2, 126.5, 78.1, 53.7, 51.7, 50.0, 37.1, 36.7, 32.8, 28.1; ^{19}F (188 MHz, DMSO-*d*6): δ = −73.71 (d, 1H, J = 7.4 Hz); IR (cm^{-1}): 3477, 2149, 1639, 1524, 1165; ESI$^+$ MS *m*/*z*: 548 [M + H]$^+$; Anal. Calcd for $C_{23}H_{32}F_3N_5O_7\cdot 0.15\ H_2O$: C, 50.20; H, 5.93; H, 12.74; found C, 49.75; H, 5.75; N, 13.61.

*4.1.1.6. 2-[3-(N′-{3-Carbamoyl-2-[2-(2,5-dimethoxy-phenyl)-acetylamino]-propionyl}-hydrazino)-4,4,4-trifluoro-butyrylamino]-3-phenyl-propionic acid methyl ester **9**.* A solution of **8** (355 mg, 0.64 mmol, 1.0 eq.) in DCM/TFA 2:1 (9 mL) was stirred for 2 h at room temperature. After removal of the solvent under reduced pressure, the excess of trifluoroacetic acid was coevaporated with methanol and the resulting yellowish solid oil precipitated with Et_2O. The resulting white solid was dissolved in DMF (3 mL), then DIPEA (540 μl, 3.25 mmol, 5.0 eq.) and HOBT (129 mg, 0.95 mmol, 1.5 eq.) were successively added. Meanwhile a solution of 2,5-dimethoxyphenylacetic acid (187 mg, 0.95 mmol, 1.5 eq.) and HBTU (361 mg, 0.95 mmol, 1.5 eq.) in DMF (3 mL) was stirred for half an hour at room temperature. The two solutions were combined and the resulting mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure and the resulting yellow oil was taken up in EtOAc (15 mL). The organic layer was successively washed with 10% aqueous citric acid (2 × 10 mL), water (15 mL), 10% aqueous K_2CO_3 (2 × 10 mL) and brine (15 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure to afford a white solid which after washing several times with EtOAc, cyclohexane and Et_2O gave **9** as a white solid: mp 168–170 °C (259 mg, 0.42 mmol, 65%); ^{1}H NMR (DMSO-*d*6): δ = 9.35 (m, 1H), 8.63 (m, 1H), 8.02 (m, 1H), 7.34–7.22 (m, 5H), 6.92–6.82 (m, 3H), 5.42 (bs, 1H), 4.59 (m, 2H), 3.78 (m, 4H), 3.75 (s, 3H), 3.68 (s, 3H), 3.46 (s, 2H), 3.18–3.11 (m, 1H), 3.04–2.97 (m, 1H), 2.52 (m, 4H); ^{13}C NMR (DMSO-*d*6): δ = 171.8, 171.7, 171.1, 171.0, 170.6, 169.8, 168.2,152.9, 151.1, 137.0, 129.0, 128.2, 126.5, 116.6, 112.1, 55.9, 55.3, 53.7, 53.6, 51.7, 48.4, 37.0, 36.7, 32.8; ^{19}F (DMSO-*d*6): δ = −73.72 (d, 0.7F, *J* = 7.4 Hz) I diastereomer −73.85 (d, 0.3, *J* = 7.8 Hz) II diastereomer; IR (cm^{-1}): 3286, 1639, 1537, 1406, 1226, 1124; ESI$^+$ MS *m*/*z*: 626 [M + Na]$^+$; Anal. Calcd for $C_{28}H_{34}F_3N_5O_8$: C, 53.76; H, 5.48; N, 11.19; found C, 53.79; H, 5.30; N, 10.75.

*4.1.1.7. [5-tert-Butoxycarbonylamino-5-(N′-{2,2,2-trifluoro-1-[(3,4,5-trimethoxy-benzylcarbamoyl)-methyl]-ethyl}-hydrazinocarbonyl)-pentyl]-carbamic acid benzyl ester **10a**.* The trifluoroacetic salt of **29** (930 mg, 1.99 mmol, 1.0 eq.) was dissolved in DMF (5 mL), then DIPEA (1.6 mL, 10.0 mmol, 5.0 eq.) and HOBT (323 mg, 2.39 mmol, 1.2 eq.) were successively added. Meanwhile a solution of N_α-Boc-N_ε-Z-Lys (910 mg, 2.39 mmol, 1.2 eq.) and HBTU (906 mg, 2.39 mmol, 1.2 eq.) in DMF (5 mL) was stirred for half an hour at room temperature. The two solutions were then combined and the resulting mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure and the resulting yellow oil was taken up in EtOAc (30 mL). The resulting organic phase was successively washed with 10% aqueous citric acid (2 × 20 mL), 10% aqueous K_2CO_3 (2 × 20 mL) and brine (30 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure to give a slightly yellow solid which was purified by column chromatography (cyclohexane:EtOAc 1:1) to give **10a** as a white solid (1.19 g, 1.67 mmol, 84%): mp 112–114 °C; ^{1}H NMR: δ = 9.48 (d, 1H, *J* = 5.2 Hz), 8.57 (m, 1H), 7.28–7.36 (m, 5H), 7.23 (t, 1H, *J* = 5.3 Hz), 6.85 (m, 1H), 6.58 (s, 2H), 5.54 (m, 1H), 5.00 (s, 2H), 4.20–4.27 (m, 2H), 3.85 (m, 2H), 3.74 (s, 6H), 3.62 (s, 3H), 2.97 (m, 2H), 2.50 (m, 2H), 1.52 (m, 2H), 1.42 (m, 9H), 1.22 (m, 4H); ^{13}C NMR: δ = 167.9, 163.2, 163.0, 155.9, 152.6, 137.1, 136.1, 134.6, 128.2, 127.6, 104.3, 104.3, 77.8, 65.0, 59.8, 55.6, 28.9, 28.0, 22.6; ^{19}F (188 MHz): δ = −74.62 (d, 1F, *J* = 7.5 Hz), −74.89 (d, 1F, *J* = 6.3 Hz); IR (cm^{-1}): 3312, 1637, 1525, 1250, 1126; ESI$^+$ MS *m*/*z*: 714.4 [M + H]$^+$; Anal. Calcd for $C_{33}H_{46}F_3N_5O_9\cdot H_2O$: C, 54.19; H, 6.63; N, 9.58; found C, 54.17; H, 6.19; N, 9.43.

*4.1.1.8. [5-Amino-5-(N′-{2,2,2-trifluoro-1-[(3,4,5-trimethoxy-benzylcarbamoyl)-methyl]-ethyl}-hydrazinocarbonyl)-pentyl]-carbamic acid benzyl ester trifluoroacetic salt **10b**.* A solution of **10a** (741 mg, 1.04 mmol, 1.0 eq.) in DCM/TFA 3:1 (15 mL) was stirred for 2 h at room temperature. After removal of the solvent under reduced pressure, the excess of TFA was coevaporated with methanol. The crude was then precipitated with diethyl ether and washed with cyclohexane (3 × 15 mL) to afford **10b** as a white solid (745 mg, 1.04 mmol, quantitative). mp: 136–138 °C; ^{1}H NMR (DMSO-*d*6): δ = 9.98 (m, 1H), 8.59 (m, 1H), 8.14 (bs, 3H), 8.02 (m, 1H) 7.38–7.29 (m, 5H), 7.21 (m, 1H), 6.57 (s, 2H), 5.00 (s, 2H), 4.20–4.30 (m, 2H), 3.89 (m, 1H), 3.74 (s, 6H), 3.65 (m, 1H), 3.62 (s, 3H), 2.98 (m, 2H), 2.58 (m, 2H), 1.65–1.69 (m, 2H), 1.38–1.42 (m, 2H), 1.25–1.29 (m, 2H); ^{13}C NMR (DMSO-*d*6): δ = 171.0, 169.9, 169.8, 159.0, 138.4, 138.2, 135.8, 129.5, 129.0, 128.8, 106.0, 67.4, 61.1, 60.1, 59.7, 56.6, 53.2, 53.1, 44.5, 41.4, 34.7, 34.6, 32.2, 30.5, 23.0; ^{19}F (188 MHz, DMSO-*d*6): δ = −76.51 (m, 1F), −77.33 (s, 1F); IR (cm^{-1}): 2924, 1667, 1594, 1123; ESI$^+$ MS *m*/*z*: 614 [M + H$^+$]; Anal. Calcd for $C_{30}H_{39}F_6N_5O_9\cdot 1.5\ H_2O$: C, 47.74; H, 5.62; N, 9.28; found C, 47.52; H, 5.04; N, 8.80.

*4.1.1.9. [5-[2-(2,5-Dimethoxy-phenyl)-acetylamino]-5-(N′-{2,2,2-trifluoro-1-[(3,4,5-trimethoxy-benzylcarbamoyl)-methyl]-ethyl}-hydrazinocarbonyl)-pentyl]-carbamic acid benzyl ester **11a**.* To a solution of **12b** (563 mg, 0.77 mmol, 1.0 eq.) in DMF (4 mL), DIPEA (640 μl, 3.85 mmol, 5.0 eq.) and HOBT (157 mg, 1.16 mmol, 1.5 eq.) were successively added. Meanwhile a solution of 2,5-dimethoxyphenylacetic acid (227 mg, 1.16 mmol, 1.5 eq.) and HBTU (440 mg, 1.16 mmol, 1.5 eq.) in DMF (4 mL) was stirred for half an hour at room temperature. The two solutions were combined and the resulting mixture was stirred at room temperature overnight. After evaporation of the solvent under reduced pressure, the resulting yellow oil was taken up in EtOAc (20 mL) and washed with 10% aqueous citric acid (2 × 15 mL), 10% aqueous K_2CO_3 (2 × 15 mL), brine (20 mL), and distilled water. The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure to give a slightly yellow solid which was purified by precipitation in a hot mixture of methanol/EtOAc to give **11a** as a white solid (434 mg, 0.59 mmol, 77%). mp: 138–140 °C; ^{1}H NMR (DMSO-*d*6): δ = 9.57 (d, 1H, *J* = 5.4 Hz), 8.56 (t, 1H, *J* = 5.8 Hz), 7.95 (d, 1H, *J* = 8.0 Hz), 7.37–7.22 (m, 5H), 7.22 (t, 1H, *J* = 5.4 Hz), 6.86–6.76 (m, 1H), 6.70–6.65 (m, 2H), 6.57 (s, 2H), 5.54 (t, 1H, *J* = 4.4 Hz), 5.00 (s, 2H), 4.24 (m, 3H), 3.86 (m, 1H), 3.73 (s, 6H), 3.67 (s, 6H), 3.62 (s, 3H), 3.40 (m, 2H), 2.99–2.81 (m, 2H), 2.50–2.40 (m, 2H), 1.57–1.49 (m, 2H), 1.46–132 (m, 2H), 1.30–1.20 (m, 2H); ^{13}C NMR (DMSO-*d*6): δ = 171.3, 169.8, 168.0, 156.5, 152.9, 152.7, 151.3, 137.2, 136.3, 134.7, 128.3, 127.9, 127.7, 125.6, 125.5, 116.7, 111.9, 111.6, 104.5, 65.1, 59.9, 55.8, 55.7, 55.2, 51.1, 51.0, 42.4, 36.7, 36.6, 33.2, 31.8, 29.0, 22.4; ^{19}F (188 MHz, DMSO-*d*6): δ = −76.51 (m, 1F), −77.33 (s, 1F); IR (cm^{-1}): 3298, 1633, 1503, 1228, 1126, 698; ESI$^+$ MS *m*/*z*: 793 [M + H$^+$]; Anal. Calcd for $C_{38}H_{48}F_3N_5O_{10}\cdot H_2O$: C, 56.34; H, 6.23; N, 8.65; found C, 56.55; H, 6.38; N, 8.41.

*4.1.1.10. 3-[2-[(2S)-6-Amino-2-[[2-(2,5-dimethoxyphenyl)acetyl]amino]hexanoyl]hydrazino]-4,4,4-trifluoro-N-[(3,4,5-trimethoxyphenyl)methyl]butanamide **11b**.* To a solution of **11a** (119 mg, 0.15 mmol, 1 eq.) in dry DMF/MeOH (5:30 mL, v/v) was added 10% Pd/C (11 mg). The mixture was stirred overnight under hydrogen atmosphere at room temperature. The catalyst was filtrated on a celite pad and the solvent was evaporated under reduced pressure to afford **11b** as a white solid (70 mg, 0.10 mmol, 75%). ^{1}H NMR (300 MHz, DMSO-*d*6): δ = 8.57 (t, 1H, *J* = 5.4 Hz), 7.97 (m, 1H), 6.86 (d, 1H, *J* = 8.7 Hz), 6.76 (m, 2H), 6.57 (s, 2H), 5.54 (d, 1H, *J* = 3.6 Hz), 4.28 (m, 2H), 4.20 (m, 1H), 3.86 (m, 1H), 3.74 (s, 9H), 3.67 (s, 6H), 3.41 (m, 2H), 2.73 (m, 2H), 2.51 (m, 2H), 1.56 (m, 1H), 1.50 (m, 1H), 1.35 (m, 2H), 1.21 (m, 2H). ^{13}C NMR (75 MHz, DMSO-

d6): δ = 171.3, 169.9, 168.1, 152.8, 151.3, 136.3, 134.9, 125.7, 111.9, 111.6, 104.4, 67.1, 59.9, 57.8, 55.7, 51.1, 42.3, 36.7, 33.4, 33.2, 31.9, 31.4, 22.4. ^{19}F (188 MHz, DMSO-*d6*): δ = −71.34 (s), −72.71 (s): 2 diastereoisomers: 75/25. APCI$^+$ MS *m/z*: 658 [M + H]$^+$. Anal. Calcd for $C_{30}H_{42}F_3N_5O_8 \cdot 4\ H_2O$: C, 49.37; H, 6.92; N, 9.60; found: C, 48.83; H, 6.35; N, 9.76.

4.1.1.11. 3-[2-[(2S)-6-Amino-2-[[2-(3-phenoxyphenyl)acetyl]amino] hexanoyl]hydrazino]-4,4,4-trifluoro-N-[(3,4,5-trimethoxyphenyl) methyl]butanamide ***12****.* To a solution of **10b** (376 mg, 0.61 mmol, 1.0 eq.) in dry DMF (10 mL) was successively added 3-phenoxyphenylacetic acid (166 mg, 0.73 mmol, 1.2 eq.), DIPEA (556 mg, 4.30 mmol, 7.0 eq.), HBTU (277 mg, 0.73 mmol, 1.2 eq.), and HOBT (99 mg, 0.73 mmol, 1.2 eq.). The mixture was stirred overnight at room temperature under argon atmosphere. After removal of the solvent under reduced pressure, the residue was taken up in EtOAc (15 mL), washed with 10% aqueous citric acid (2 × 10 mL), water (10 mL), 10% aqueous K_2CO_3 (2 × 10 mL) and brine (10 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure to give a crude product which was purified by column chromatography (EtOAc) to afford the Cbz protected amine (355 mg, 4.30 mmol, 71%) as a white solid. ^{1}H NMR (300 MHz): δ = 8.10 (s, 1H), 7.92 (m, 1H), 7.35–7.18 (m, 6H), 7.02 (m, 2H), 6.92–6.71 (m, 7H), 6.49 (s, 2H), 6.30 (bs, 1H), 5.01 (s, 2H), 4.90 (m, 1H), 4.32 (m, 2H), 4.15 (m, 1H), 3.79 (m, 1H), 3.73 (s, 9H), 3.07 (m, 2H), 2.41 (m, 2H), 2.23 (m, 2H), 1.70–1.41 (m, 2H), 1.32 (m, 2H), 1.15 (m, 2H); ^{13}C NMR (75 MHz): δ = 171.1, 169.7, 168.1, 156.6, 156.4, 152.8, 138.6, 136.3, 136.0, 134.7, 129.9, 129.6, 128.3, 127.7, 124.1, 123.3, 119.2, 118.5, 116.0 (q, *J* = 283.0 Hz), 104.4, 67.1, 66.6, 59.9, 57.5, 55.7, 51.9, 50.9, 42.3, 41.8, 33.3, 32.0, 26.5, 23.1; ^{19}F (188 MHz): δ = −76.51 (m, 1F), −77.33 (s, 1F). To a solution of the Cbz protected amine (183 mg, 0.22 mmol, 1.0 eq.) in dry DMF/MeOH 1/15 (48 mL) was added 10% Pd/C (18 mg). The mixture was stirred overnight under hydrogen atmosphere at room temperature and was filtered on a celite pad. After removal of the solvent under reduced pressure **12** was obtained as a white solid (89%). ^{1}H NMR (DMSO-*d6*): δ = 9.62 (s, 1H), 8.53 (m, 1H), 8.21 (m, 1H), 7.37 (m, 2H), 7.27 (m, 2H), 7.11 (m, 2H), 7.02 (m, 1H), 6.99 (m, 1H), 6.94 (m, 1H), 6,57 (s, 2H), 5,54 (m, 1H), 4.24 (m, 2H), 4.19 (m, 1H), 3.85 (m, 1H), 3.73 (s, 6H), 3.62 (s, 3H), 3.45 (m, 2H), 2.51 (m, 2H), 2.23 (m, 2H), 1.52 (m, 2H), 1.29 (m, 2H), 1.19 (m, 2H); ^{13}C NMR (75 MHz, DMSO-*d6*): δ = 171.1, 169.7, 168.1, 156.6, 156.4, 152.8, 138.6, 136.3, 134.7, 129.9, 129.6, 124.1, 123.3, 119.2, 118.5, 104.4, 115.8 (q, *J* = 283.0 Hz), 59.9, 57.5, 55.7, 51.9, 50.9, 42.3, 41.8, 33.3, 32.0, 26.5, 23.1. ^{19}F (188 MHz, DMSO-*d6*): δ = −71,35 (s, 1F), −72,75 (s, 1F): 2 diastereoisomers: 75/25; ESI$^+$ MS *m/z*: 690 [M + H]$^+$; Anal. Calcd for $C_{34}H_{42}F_3N_5O_7 \cdot 0.75\ H_2O$: C, 58.66; H, 5.97; N, 10.36; found. C, 58.38; H, 6.09; N, 9.40.

4.1.1.12. [2-Carbamoyl-1-(N′-{2,2,2-trifluoro-1-[(3,4,5-trimethoxybenzylcarbamoyl)-methyl]-ethyl}-hydrazinocarbonyl)-ethyl]-carbamic acid tert-butyl ester ***13****.* Compound **13** was synthesized following the same procedure described for **12a** using the trifluoroacetic salt of **29** (548 mg, 1.18 mmol, 1.0 eq.) and N_α-Boc-Asn (328 mg, 1.41 mmol, 1.2 eq.), except that the resulting colorless solid was successively washed with Et_2O, EtOAc and petroleum ether to give **13** as a white solid (367 mg, 0.65 mmol, 55%). mp: 174–176 °C; ^{1}H NMR (DMSO-*d6*): δ = 9.41 (dd, 1H, *J* = 4.9 Hz, *J* = 16.2 Hz), 8.51 (d, 1H, *J* = 7.8 Hz), 7.25 (s, 1H), 6.88 (s, 2H), 6.58 (bs, 2H), 5.53 (m, 1H), 4.24 (m, 3H), 3.83 (m, 1H), 3.74 (s, 6H), 3.62 (s, 3H), 2.50 (m, 2H) 2.39 (m, 2H), 1.36 (s, 9H); ^{13}C NMR (DMSO-*d6*): δ = 171.0, 168.0, 154.9, 152.7, 137.2, 137.0, 135.7, 134.8, 128.3, 125.4, 78.9, 59.9, 57.72 (dd, 1H, *J* = 7.8 Hz, *J* = 27.1 Hz), 53.6, 50.0, 37.6, 33.6, 28.1; ^{19}F (188 MHz, DMSO-*d6*): δ = −73.5 (m); IR (cm^{-1}): 3319, 2361,1637, 1127; ESI$^+$ MS *m/z*: 566 [M + H]$^+$; Anal. Calcd for $C_{23}H_{34}F_3N_5O_8 \cdot 0.5H_2O$: C, 48.12; H, 6.16; N, 12.20; found C, 48.27; H, 5.88; N, 11.86.

4.1.1.13. N′-(3-{N′-[3-(3,4-Dimethoxyphenyl)-2-(9H-fluoren-9-ylmethoxycarbonylamino) propionyl]hydrazino}-3-oxo-1-trifluoromethylpropyl)hydrazinecarboxylic acid benzyl ester ***14a****.* A solution of the **32** (359 mg, 1.12 mmol) and Fmoc-L-3,4-dimethoxyphenylalanine (501 mg, 1.12 mmol) in dry DMF (13 mL) was stirred for 5 min at room temperature. Then were added successively 2,4,6-collidine (447 μL, 3.36 mmol), HOBT (166 mg, 1.23 mmol) and HBTU (467 mg, 1.23 mmol). The mixture was stirred at room temperature under argon atmosphere for 40 h. After removal of the solvent under reduced pressure, the residue was triturated in EtOAc (15 mL) to afford **14a** as a white solid (560 mg, 0.75 mmol, 67%). mp: 202–204 °C; ^{1}H NMR (DMSO-*d6*): δ = 10.23 (bs, 1H), 10.15 (bs, 1H), 8.90 (bs, 1H), 7.90 (d, *J* = 7.4 Hz, 2H), 7.70 (d, *J* = 7.6 Hz, 1H), 7.43–7.26 (m, 11 H), 7.01 (bs, 1H), 6.89–6.80 (m, 2 H), 5.36 (bs, 1H), 5.08 (s, 2H), 4.32 (m, 1H), 4.19–4.10 (m, 3H), 3.93 (m, 1H), 3.72 (s, 3H), 3.68 (s, 3H), 2.99 (m, 1H), 2.76 (m, 1H), 2.52 (m, 2H). ^{13}C NMR (DMSO-d6): δ = 170.6; 170.4, 166.9, 157.0, 155.8, 148.4, 147.4, 143.8; 143.7, 140.6, 136.8, 130.3, 128.4, 127.9, 127.7, 127.6, 127.0, 125.3, 120.1, 113.2, 121.2, 111.6, 65.7, 65.6, 59.7, 55.4, 55.3, 54.9, 46.5, 37.3, 31.5. ^{19}F (188 MHz, DMSO-d6) : δ = −73.6, −73.8 (m). IR (cm^{-1}): 3284, 1696, 1605, 1171, 1124, 692. ESI$^+$ MS *m/z*: 772 [M + Na]$^+$. Anal. Calcd for $C_{38}H_{38}F_3N_5O_8 \cdot 0.4\ H_2O$: C, 60.33; H, 5.18; N 9.26. Found: C, 60.57; H, 4.97; N, 9.01.

4.1.1.14. N′-(3-{N′-[2-Amino-3-(3,4-dimethoxyphenyl)propionyl] hydrazino}-3-oxo-1-trifluoromethyl-propyl)hydrazinecarboxylic acid benzyl ester ***14b****.* To compound **14a** (70 mg, 0.10 mmol) was added a solution of piperidine in DMF (10% v/v, 2 mL). The mixture was stirred at room temperature for 1.30 h. After removing the solvent under reduced pressure, the residue obtained was triturated in Et_2O to yield **14b** as a white powder (37 mg, 0.07 mmol, 78%). mp: 102–104 °C; ^{1}H NMR (300 MHz, DMSO-d6): δ = 8.87 (bs, 1H), 7.40–7.31 (m, 5 H), 6.85–6.70 (m, 3 H), 5.36 (bs, 1H), 5.06 (s, 2H), 3.90 (m, 1H), 3.73 (s, 3H), 3.71 (s, 3H), 3.45 (m, 1H), 2.90 (m, 1H), 2.85 (m, 1H), 2.50 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d6): δ = 172.8, 166.6, 156.9, 148.4, 147.2, 136.7, 130.7, 128.3, 127.8, 127.7, 113.1, 121.3, 111.6, 65.6, 55.0, 54.9, 54.3, 40.8, 31.4. ^{19}F (188 MHz, DMSO-*d6*): δ = −73.7 (d, *J* = 7.3 Hz). IR (cm^{-1}): 3229, 1709, 1602, 1158, 1118, 696. ESI$^+$ MS *m/z*: 550 [M + Na]$^+$. Anal. Calcd for $C_{23}H_{28}F_3N_5O_6$: C, 52.37; H, 5.35; N 13.28. Found: C, 52.40; H, 5.30; N, 13.51.

4.1.1.15. N′-(1-{N′-[3-(3,4-Dimethoxyphenyl)-2-(9H-fluoren-9-ylmethoxycarbonylamino) propionyl]hydrazinocarbonylmethyl}-2,2,2-trifluoroethyl)hydrazinecarboxylic acid tert-butyl ester ***15****.* A solution of **30** (100 mg, 0.35 mmol) and Fmoc-L-3,4-dimethoxyphenylalanine (157 mg, 0.35 mmol) in dry DMF (3 mL) was stirred for 5 min at room temperature. Then 2,4,6-collidine (140 μL, 1.05 mmol), HOBT (53 mg, 0.39 mmol) and HBTU (148 mg, 0.39 mmol) were successively added. The mixture was stirred at room temperature under argon atmosphere for 21 h. After removal of the solvent under reduced pressure, the residue was triturated with EtOAc to give **15** as a white solid (171 mg, 0.24 mmol, 68%). mp: 208–210 °C; ^{1}H NMR (DMSO-d6)): δ = 10.18 (br s, 2H), 8.41 (br s, 1H), 7.81–7.86 (m, 2H), 7.60 (d, *J* = 7.2 Hz, 2H), 7.23–7.40 (m, 5H), 6.98 (d, *J* = 2.8 Hz, 1H), 6.80 (br s, 2H), 5.13 (br s, 1H), 4.28 (m, 1H), 4.10–4.14 (m, 3H), 3.84 (br s, 1H), 3.70 (s, 3H), 3.66 (s, 3H), 2.94 (m, 1H), 2.72 (m, 1H), 2.50 (m, 2H), 1.37 (s, 9H). ^{13}C NMR (DMSO-d6)): δ = 170.8; 170.7, 167.4, 155.7, 148.9, 147.9, 144.1–144.3, 141.1, 130.8, 128.0, 127.5, 125.7, 121.8, 120.5, 113.9, 112.3, 79.3, 66.2, 55.9–56.0, 55.3, 47.0, 31.9, 28.6. ^{19}F (188 MHz, DMSO-d6)): δ = −73.7. IR (cm^{-1}): 3284, 1696, 1159, 1124, 738. ESI$^+$ MS *m/z*:

738 $[M + Na]^+$. Anal. Calcd for $C_{35}H_{40}F_3N_5O_8$: C, 58.73; H, 5.63; N 9.79. Found: C, 58.35; H, 5.55; N, 9.76.

4.1.1.16. N′-[3-(N′-{3-(3,4-Dimethoxyphenyl)-2-[2-(3-phenoxyphenyl) acetylamino] propionyl}hydrazino)-3-oxo-1-trifluoromethyl-propyl]hydrazinecarboxylic acid benzyl ester ***16a***. A solution of **14b** (178 mg, 0.34 mmol) and 3-phenoxyphenylacetic acid (84 mg, 0.37 mmol) in dry DMF (11 mL) was stirred for 5 min at room temperature. Then DIPEA (112 μL, 0.68 mmol, 2.0 eq.), HOBT (50 mg, 0.37 mmol, 1.1 eq.) and HBTU (140 mg, 0.37 mmol, 1.1 eq.) were successively added. The mixture was stirred at room temperature under argon atmosphere for 38 h. After removal of the solvent under reduced pressure, the residue obtained was triturated in EtOAc (20 mL) to yield **16a** as a white solid (213 mg, 0.29 mmol, 85%). mp: 178–180 °C; ^{1}H NMR (DMSO-*d6*): $\delta = 10.22$ (bs, 1H), 10.11 (bs, 1H), 8.85 (bs, 1H), 8.33 (m, 1H), 7.40–7.32 (m, 7H), 7.22 (t, $J = 8.1$ Hz, 1H), 7.14 (t, $J = 7.1$ Hz, 1H), 6.98 (d, $J = 7.9$ Hz, 2H), 6.90–6.71 (m, 6 H), 5.35 (bs, 1H), 5.07 (s, 2H), 4.58 (m, 1H), 3.91 (m, 1H), 3.70 (s, 6H), 3.40 (m, 2H), 2.96 (m, 1H), 2.71 (m, 1H), 2.55 (m, 2H). ^{13}C NMR (100 MHz, DMSO-*d6*): $\delta = 170.2$, 170.0, 169.5, 166.9, 156.9, 156.6, 156.3, 148.3, 147.4, 138.4, 136.8, 130.0, 129.5, 128.3, 127.9, 127.7, 124.0, 123.3, 121.2, 119.4, 118.5, 116.4, 113.1, 111.5, 65.6, 55.4, 55.3, 52.5, 41.7, 37.6, 31.49. ^{19}F (188 MHz, DMSO-*d6*): $\delta = -73.7$ (d, $J = 4.9$ Hz). IR (cm^{-1}): 3271, 1704, 1601, 1161, 1121, 690. ESI$^+$ MS *m/z*: 760 $[M + Na]^+$. Anal. Calcd for $C_{37}H_{38}F_3N_5O_8 \cdot 0.25\ H_2O$: C, 59.91; H, 5.24; N 9.44. Found: C, 59.93; H, 5.32; N, 9.57.

4.1.1.17. N-[1-(N-{3-(3,4-Dimethoxyphenyl)-2-[2-(3-Phenoxyphenyl) acetylamino] propionyl}-hydrazinocarbonylmethyl)-2,2,2-trifluoroethyl]hydrazinecarboxylic acid tert-butyl ester ***16b***. To compound **15** (382 mg, 0.53 mmol) was added a solution of piperidine in DMF (10% v/v, 5 mL). After stirring at room temperature for 1.30 h, the solvent was removed under reduced pressure and the residue obtained was triturated in Et_2O to yield the free amine as a white solid (231 mg, 88%) that was used without further purification. To a solution of the crude amine (76 mg, 0.15 mmol) and 3-phenoxyphenylacetic acid (38 mg, 0.17 mmol) in dry DMF (4 mL) was added, after stirring for 5 min at room temperature, 2,4,6-collidine (60 μL, 0.45 mmol), HOBT (23 mg, 0.17 mmol) and HBTU (64 mg, 0.17 mmol). After stirring at room temperature under argon atmosphere for 24 h, the solvent was removed under reduced pressure, the residue was triturated in EtOAc (15 mL) to afford **16b** as a white solid (88 mg, 0.12 mmol 84%). mp: 184–186 °C; ^{1}H NMR (DMSO-*d6*): $\delta = 10.20$, 10.10 (2s, 2H), 8.41 (s, 1H), 8.30 (m, 1H), 7.35 (t, $J = 7.8$ Hz, 2H), 7.20 (t, $J = 8.3$ Hz, 1H), 7.12 (t, $J = 7.8$ Hz, 1H), 7.00 (d, $J = 7.8$ Hz, 2H), 6.87–6.69 (m, 6H), 5.13 (s, 1H), 4.55 (m, 1H), 3.85 (br s, 1H), 3.67 (s, 6H), 3.40 (m, 2H), 2.93 (m, 1H), 2.70 (m, 1H), 2.49 (m, 2H), 1.38 (s, 9H). ^{13}C NMR (DMSO-d6): $\delta = 170.0$, 170.1, 167.4, 157.1, 156.8, 148.9, 147.9, 138.8, 130.4, 129.9, 123.8, 119.0, 124.5, 121.7, 119.8, 116.8, 113.7, 112.1, 79.3, 55.9, 55.8, 52.9, 42.2, 38.0, 31.9, 28.6. ^{19}F (188 MHz, DMSO-d6): $\delta = -73.7$ (m). IR (cm^{-1}): 3216 (NH), 1699, 1653, 1159, 1121, 691. ESI$^+$ MS *m/z*: 726 $[M + Na]^+$. Anal. Calcd for $C_{34}H_{40}F_3N_5O_8$: C, 57.85; H, 5.73; N 9.95. Found: C, 57.01; H, 5.68; N, 9.86.

4.1.2. Synthesis of the target molecules including the β-hydrazino acid scaffold ***17a–22b***

4.1.2.1. 2-{3-[N′-(6-Benzyloxycarbonylamino-2-tert-butoxycarbonylamino-hexanoyl)-hydrazino]-propionylamino}-3-phenyl-propionic acid methyl ester ***17a***. A solution of **35** (914 mg, 2.41 mmol, 1.0 eq.) in DCM/TFA 3:1 (12 mL) was stirred for 2 h at room temperature. The solvent was evaporated under reduced pressure and the excess of trifluoroacetic acid was coevaporated with methanol. The resulting slightly yellow solid was dissolved in DMF (5 mL), then DIPEA (2.0 mL, 12.0 mmol, 5.0 eq.) and HOBT (390 mg, 2.89 mmol, 1.2 eq.) were successively added. Meanwhile a solution of NαBocNεZLysine (1.10 g, 2.89 mmol, 1.2 eq.) and HBTU (1.09 mg, 2.89 mmol, 1.2 eq.) in DMF (5 mL) was stirred for half an hour at room temperature. The solutions were then combined and the resulting mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure and the resulting yellow oil was taken up in EtOAc (15 mL) and successively washed with 10% aqueous K_2CO3 (2 × 20 mL), brine (30 mL) and water (2 × 20 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure to give a slightly yellow oil which was purified by column chromatography (EtOAc) to give **17a** as a white solid (940 mg, 1.50 mmol, 62%). mp: 106–108 °C; ^{1}H NMR (300 MHz): $\delta = 8.07$ (bs, 1H), 7.20–7.41 (m, 10H), 7.17 (s, 1H), 7.15 (s, 1H), 5.20 (m, 1H), 5.09 (s, 2H), 4.85 (m, 1H), 4.15 (m, 1H), 3.60 (s, 3H), 3.05–3.28 (m, 6H), 2.35 (t, 2H, $J = 6.8$ Hz), 1.31–1.88 (m, 6H), 1.50 (s, 9H); ^{13}C NMR (75 MHz): $\delta = 172.7$, 171.5, 157.8, 156.3, 137.3, 136.6, 136.0, 128.8, 127.7, 126.7, 80.4, 67.5, 66.7, 65.8, 52.9, 52.2, 47.8, 40.2, 38.2, 37.2, 34.4, 32.4, 30.3, 28.0, 23.0; IR (cm^{-1}): 3309, 1686, 1653, 1524, 1248, 1167; ESI$^+$ MS *m/z*: 650 $[M + Na]^+$; Anal. Calcd for $C_{32}H_{45}N_5O_8$ C, 61.23; H, 7.23; N, 11.16; found C, 61.03; H, 7.14; N, 10.94.

4.1.2.2. 2-{3-[N′-(2-Amino-6-benzyloxycarbonylamino-hexanoyl)-hydrazino]-propionylamino}-3-phenyl-propionic acid methyl ester trifluoroacetic salt ***17b***. A solution of **17a** (99 mg, 0.16 mmol, 1.0 eq.) in DCM/TFA 3:1 (4 mL) was stirred for 2 h at room temperature. The solvent was evaporated under reduced pressure and the excess of TFA was coevaporated with methanol. The crude product was then precipitated with diethyl ether and washed with cyclohexane (3 × 15 mL) to afford **17b** as a white solid (101 mg, 0.16 mmol, quantitative). mp: 96–98 °C; ^{1}H NMR (300 MHz, DMSO-*d6*): $\delta = 9.89$ (bs, 1H), 8.41 (s, 1H), 8.20 (s, 3H), 7.95 (s, 1H), 7.17–7.40 (m, 10H), 6.51 (bs, 1H), 5.02 (s, 2H), 4.51 (m, 1H), 3.55 (m, 1H), 3.50 (s, 3H), 3.10–2.82 (m, 6H), 2.23 (t, 2H, $J = 6.8$ Hz), 1.22–1.70 (m, 6H); ^{13}C NMR (DMSO-*d6*, 75 MHz) δ 174.5, 174.1, 169.5, 159.4, 138.8, 138.5, 130.8, 130.2, 129.8; 129.2, 128.3, 67.8, 55.7, 53.6, 53.1, 41.6, 51.6, 38.7, 35.3, 32.5, 30.8, 23.5; IR (cm^{-1}): 2929, 1668, 1531, 1199, 1129; ESI$^+$ MS *m/z*: 528 $[M + H]^+$; Anal. Calcd for $C_{29}H_{38}F_3N_5O_8 \cdot 1.5H_2O$: C, 52.09; H, 6.19; N, 10.48; found C, 52.48; H, 6.28; N, 10.13.

4.1.2.3. 2-[3-(N′-{6-Benzyloxycarbonylamino-2-[2-(4-phenoxyphenyl)-acetylamino]-hexanoyl}-hydrazino)-propionylamino]-3-phenyl-propionic acid methyl ester ***18a***. Compound **18a** was synthesized following the same procedure described for **17a**. Deprotection of **17b** in DCM/TFA gave the trifluoroacetic salt of the amine (491 mg, 0.76 mmol, 1.0 eq.) which was coupled with 2-phenoxyphenylacetic acid to give **18a** (452 mg, 0.61 mmol, 81%) as a white solid. Mp: decomposition at 70–80 °C; ^{1}H NMR (300 MHz): $\delta = 8.45$ (s, 1H), 7.41–7.10 (m, 15H), 7.14 (bs, 1H), 7.01–6.80 (m, 4H), 6.38 (d, 1H, $J = 7.3$ Hz), 5.25 (m, 1H), 5.10 (s, 2H), 4.83 (dd, 1H, $J = 7.5$ and 12.2 Hz), 4.28 (dd, 1H, $J = 7.2$ et 14.0 Hz), 3.79 (s, 3H), 3.52 (s, 2H), 3.16 (m, 1H), 3.13 (m, 2H), 3.03 (m, 1H), 3.02 (m, 2H), 2.22 (m, 2H), 1.75 (m, 1H), 1.55 (m, 1H), 1.46 (m, 2H), 1.24 (m, 2H); ^{13}C NMR (75 MHz): $\delta = 172.9$, 171.8, 171.4, 171.1, 156.6, 153.8, 151.1, 136.6, 136.1, 135.8, 129.1; 128.6, 128.5, 128.0, 127.0, 124.2, 117.2, 113.2, 111.8, 66.6, 56.0, 53.3, 52.5, 51.6, 47.7, 40.5, 37.5, 34.3, 31.1, 29.3, 22.3; IR (cm^{-1}): 3264, 1645, 1529, 1210; ESI$^+$ MS *m/z*: 760 $[M + Na]^+$, 776 $[M + K]^+$; Anal. Calcd for $C_{41}H_{47}N_5O_8 \cdot 0.75\ H_2O$: C, 65.58; H, 6.52; N, 9.53; found C, 65.95; H, 6.40; N, 8.87.

4.1.2.4. Methyl (2S)-2-[3-[2-[(2S)-6-amino-2-[[2-(3-phenoxyphenyl) acetyl]amino] hexanoyl]hydrazino]propanoylamino]-3-phenyl-propanoate ***18b***. To a solution of **18a** (114 mg, 0.15 mmol, 1 eq.) in dry methanol (5 mL) was added 10% Pd/C (11 mg). The mixture was

stirred overnight under hydrogen atmosphere at room temperature and filtered on a celite pad. After the removal of the solvent under reduced pressure, **18b** was obtained as yellow resinous oil (81 mg, 0.13 mmol, 89%). ^{1}H NMR: $\delta = 7.55$ (bs, 1H), 7.23–6.85 (m, 15H), 4.80 (m, 1H), 4.33 (m, 1H), 3.67 (m, 3H), 3.51 (m, 2H), 3.10 (m, 1H), 3.02 (m, 1H), 3.00 (m, 2H), 2.70 (m, 2H), 2.24 (m, 2H), 1.70 (m, 1H), 1.59 (m, 1H), 1.49 (m, 2H), 1.33 (m, 2H). ^{13}C NMR: $\delta = 172.9$, 171.9, 171.2, 171.0, 157.54, 156.9, 136.7, 136.3, 130.0, 129.8, 129.1, 128.5, 127.0, 124.0, 123.4, 119.7, 118.9, 117.4, 53.5, 52.4, 51.8, 47.5, 43.0, 40.5, 37.5, 34.1, 31.7, 30.0, 22.3. ESI$^+$ MS *m/z*: 604 $[M + H]^+$. Anal. Calcd for $C_{33}H_{41}N_5O_6 \cdot 2.5\ H_2O$: C, 61.13; H, 7.17; N, 10.90; found C, 61.36; H, 6.50; N, 10.07.

4.1.2.5. Methyl (2S)-2-[3-[2-[(2S)-6-(benzyloxycarbonylamino)-2-[[2-(2,5-dimethoxyphenyl)acetyl]amino]hexanoyl]hydrazino]propanoylamino]-3-phenyl-propanoate ***19a***. Compound **19a** was synthesized following the same procedure described for **17a**. Deprotection of **17b** in DCM/TFA gave the trifluoroacetic salt of the amine (490 mg, 0.93 mmol, 1 eq.) which was coupled with 2,5-dimethoxyphenylacetic acid (235 mg, 1.2 mmol, 1.2 eq.). Except, the crude product was purified by column chromatography (EtOAc/MeOH, 9:1) to give **19a** as a white solid (247 mg, 0.35 mmol, 37%). mp: 171–173 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.20$–7.33 (m, 10H), 7.14 (d, 1H, $J = 7.3$ Hz), 6.82–6.73 (m, 3H), 6.48 (d, 1H, $J = 7.3$ Hz), 5.10 (s, 2H), 4.83 (dd, 1H, $J = 7.5$ and 12.2 Hz), 4.28 (dd, 1H, $J = 7.2$ and 14.0 Hz), 3.72 (s, 6H), 3.79 (s, 3H), 3.52 (s, 2H), 3.16 (m, 1H), 3.13 (m, 2H), 3.03 (m, 1H), 3.02 (m, 2H), 2.21 (t, 2H, $J = 5.4$ Hz), 1.75 (m, 1H), 1.55 (m, 1H), 1.46 (m, 2H), 1.24 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 172.9$, 171.8, 171.4, 171.1, 156.6, 153.8, 151.1, 136.6, 136.1, 129.1; 128.6, 128.5, 128.0, 127.0, 124.2, 117.2, 113.2, 111.8, 66.6, 56.0, 53.3, 52.5, 51.6, 47.7, 40.5, 38.9, 37.5, 34.3, 31.1, 29.3, 22.3. IR (cm^{-1}): 3283, 1742, 1682, 1638, 1535. APCI$^+$ MS *m/z*: 706 $[M + H]^+$. Anal. Calcd for $C_{37}H_{47}N_5O_9 \cdot 1.5\ H_2O$: C, 60.64; H, 6.89; N, 9.56; found C, 60.48; H, 6; 71; N, 9.31.

4.1.2.6. Methyl (2S)-2-[3-[2-[(2S)-6-amino-2-[[2-(2,5-dimethoxyphenyl)acetyl]amino] hexanoyl]hydrazino]propanoylamino]-3-phenyl-propanoate di(hydrochloride) salt ***19b***. To a solution of **19a** (175 mg, 0.24 mmol, 1 eq.) in dry methanol (5 mL) was added 10% Pd/C (17.5 mg). The mixture was stirred overnight under hydrogen atmosphere at room temperature and filtered on a celite pad. After removal of the solvent under reduced pressure and addition of HCl/MeOH, **19b** was obtained as a white hygroscopic solid (110 mg, 0.17 mmol, 73%). ^{1}H NMR (300 MHz, CD_3OD): $\delta = 7.28$–7.19 (m, 5H), 6.83 (m, 3H), 4.67 (dd, 1H, $J = 5.73$ and 8.71 Hz), 4.34 (dd, 1H, $J = 5.82$ and 8.30), 3.79 (s, 3H), 3.74 (s, 3H), 3.69 (s, 3H), 3.52 (d, 2H, $J = 5.06$ Hz), 3.17 (dd, 1H, $J = 5.63$ and 13.82), 3.01–2.88 (m, 5H), 2.31 (m, 2H), 1.39–1.04 (m, 6H). ^{13}C NMR (75 MHz, CD_3OD): $\delta = 174.7$, 173.4, 172.4, 171.9, 155.0, 153.1, 138.0, 130.3, 129.6, 128.0, 126.0, 118.3, 113.7, 112.7, 68.2, 56.7, 56.2, 55.5, 52.8, 50.0, 40.5, 38.4, 38.2, 32.0, 30.4, 28.0, 23.4. IR (cm^{-1}): 2926, 1682, 1650, 1592. ESI$^+$ MS *m/z*: 572 $[M + H]^+$. Anal. Calcd for $C_{29}H_{43}Cl_2N_5O_7 \cdot 2.5\ H_2O$: C. 50.50, H. 7.03, N. 10.16 found C. 50.58, H. 7.26, N. 9.63.

4.1.2.7. 9H-fluoren-9-ylmethyl N-[(1S)-5-(tert-butoxycarbonylamino)-1-[[[3-oxo-3-[(3,4,5-trimethoxyphenyl)methylamino]propyl] amino] carbamoyl]pentyl]carbamate ***20***. A solution of **36** (1.2 g, 3.28 mmol, 1 eq.) in HCl 4 M in dioxane (25 mL) was stirred for 2 h at room temperature. The solvent was evaporated under reduced pressure to afford the hydrochloride salt of the amine (1.14 g, 3.57 mmol, quantitative) as a colorless oil, which was used without further purification. ^{1}H NMR (300 MHz, DMSO-*d6*): $\delta = 8.51$ (s, 3H), 6.52 (s, 2H), 4.21 (d, 2H, $J = 5.5$ Hz), 3.79 (s, 6H), 3.61 (s, 3H), 3.15 (t, 2H, $J = 6.6$ Hz), 2.60 (t, 2H, $J = 6.6$ Hz). ^{13}C NMR (75 MHz, DMSO-*d6*): $\delta = 172.0$, 158.3, 152.7, 137.1, 134.7, 104.7, 59.9, 55.9, 48.6, 43.8, 34.2. To a solution of the hydrochloride salt of the amine (1.04 g, 3.28 mmol, 1 eq.) in dry DMF (25 mL) was successively added *N*α-Fmoc-*N*ε-Z-L-Lys (1.7 g, 3.68 mmol, 1.1 eq.), collidine (2.5 g, 21.1 mmol, 7 eq.), EDC (694 mg, 3.68 mmol, 1.1 eq.), and HOBT (489 mg, 3.68 mmol, 1.1 eq.). The mixture was stirred overnight at room temperature under argon atmosphere. After removal of the solvent under reduced pressure, the residue was taken up in EtOAc (20 mL) and successively washed with 10% aqueous citric acid (2 × 20 mL), water (20 mL), 10% aqueous K_2CO_3 (2 × 20 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure to give a crude product that was purified by column chromatography (EtOAc) to afford **20** (672 mg, 0.92 mmol, 30%) as a white solid. mp: 140–142 °C; ^{1}H NMR: $\delta = 7.74$ (d, 2H, $J = 7.6$ Hz), 7.55 (d, 2H, $J = 7.5$ Hz), 7.38 (t, 2H, $J = 7.3$ Hz), 7.29 (t, 2H, $J = 7.4$ Hz), 7.13 (t, 1H, $J = 5.3$ Hz), 6.52 (s, 2H), 5.58 (m, 1H), 4.71 (m, 1H), 4.37 (m, 2H), 4.35 (m, 2H), 4.17 (m, 1H), 4.06 (m, 1H), 3.81 (s, 6H), 3.80 (s, 3H), 3.11 (m, 2H), 3.06 (m, 2H), 2.36 (m, 2H), 1.79 (m, 1H), 1.66 (m, 1H), 1.45 (m, 2H), 1.41 (s, 9H), 1.35 (m, 2H). ^{13}C NMR: $\delta = 171.6$, 171.5, 156.3, 153.3, 143.6, 141.3, 137.1, 134.2, 127.8, 127.1, 124.9, 120.0, 104.9, 80.2, 67.0, 60.1, 56.0, 53.6, 48.1, 47.1, 43.7, 39.7, 34.7, 31.6, 29.5, 28.4, 22.4. IR (cm^{-1}): 2926, 1682, 1650, 1592. ESI$^+$ MS *m/z*: 756 $[M + 23]^+$. Anal. Calcd for $C_{39}H_{51}N_5O_9$ C. 62.71, H. 7.10, N. 9.38 found C. 62.86, H. 7.20, N. 9.24.

4.1.2.8. tert-Butyl N-[(5S)-5-[[2-(2,5-dimethoxyphenyl)acetyl]amino]-6-oxo-6-[2-[3-oxo-3-[(3,4,5-trimethoxyphenyl)methylamino] propyl] hydrazino]hexyl] carbamate ***21a***. Compound **21a** was synthesized according to the procedure described for the synthesis of **20a** from **22** (307 mg, 0.42 mmol, 1 eq.), which was successively deprotected and coupled with 2,5-dimethoxyphenylacetic acid (99 mg, 0.50 mmol, 1.2 eq.). **21a** was obtained as a white solid (142 mg, 0.20 mmol, 50%). mp: 138–140 °C; ^{1}H NMR (300 MHz): $\delta = 7.23$ (t, 1H, $J = 5.0$ Hz), 7.18 (m, 1H), 6.79 (m, 1H), 6.78 (m, 1H), 6.77 (m, 1H), 6.54 (s, 2H), 6.41 (d, 1H, $J = 6.9$ Hz), 4.96 (m, 1H), 4.36 (m, 2H), 4.23 (m, 1H), 3.82 (s, 6H), 3.81 (s, 3H), 3.78 (s, 6H), 3.49 (s, 2H), 3.05 (t, 2H, $J = 6.3$ Hz), 3.01 (m, 2H), 2.28 (t, 2H, $J = 6.3$ Hz), 1.78 (m, 1H), 1.55 (m, 1H), 1.42 (s, 9H), 1.41 (m, 2H), 1.25 (m, 2H). ^{13}C NMR (75 MHz): $\delta = 171.7$, 171.6, 171.2, 156.2, 153.9, 153.3, 151.1, 137.1, 134.4, 124.2, 117.4, 113.2, 104.8, 79.2, 60.8, 56.1, 55.7, 51.9, 48.2, 43.7, 40.0, 39.0, 34.6, 31.1, 29.6, 28.4, 22.5. IR (cm^{-1}): 2926, 1680, 1591, 1459, 1225. ESI$^+$ MS *m/z*: 712 $[M + 23]^+$. Anal. Calcd for $C_{34}H_{51}N_5O_{10}$: C. 57.69, H. 7.56, N. 9.90 found C. 57.73, H. 7.17, N. 9.87.

4.1.2.9. 3-[2-[(2S)-6-amino-2-[[2-(2,5-dimethoxyphenyl)acetyl] amino]hexanoyl] hydrazino]-N-[(3,4,5-trimethoxyphenyl)methyl] propanamide di(trifluoroacetic) salt ***21b***. A solution of **21a** (93 mg, 0.13 mmol, 1.0 eq.) in DCM/TFA 3:1 (4 mL) was stirred for 2 h at room temperature. The solvent was evaporated under reduced pressure and the excess of TFA was coevaporated with methanol to afford **21b** as a white solid (81 mg, 0.13 mmol, quantitative). ^{1}H NMR (300 MHz, CD_3OD): $\delta = 6.87$ (d, 1H, $J = 8.8$ Hz), 6.80 (s, 1H), 6.79 (m, 1H), 6.62 (s, 2H), 4.32 (m, 1H), 4.31 (m, 2H), 3.82 (s, 6H), 3.77 (s, 3H), 3.73 (s, 6H), 3.60 (s, 2H), 3.44 (m, 2H), 2.91 (m, 2H), 2.68 (m, 2H), 1.88 (m, 1H), 1.78 (m, 1H), 1.68 (m, 2H), 1.44 (m, 2H). ^{13}C NMR (75 MHz, CD_3OD): $\delta = 173.2$, 170.9, 153.7, 153.2, 151.7, 136.9, 134.4, 124.5, 117.0, 112.2, 111.3, 104.8, 59.7, 55.3, 55.2, 54.7, 51.9, 47, 43.0, 39.0, 36.8, 30.4, 30.0, 26.6, 22.3. IR (cm^{-1}): 2926, 1682, 1650, 1592. ESI$^-$ MS *m/z*: 624 $[M + 2H_2O-H]^-$ and 588 $[M - H]^-$. Anal. Calcd for $C_{33}H_{45}F_6N_5O_{12} \cdot 4\ H_2O$: C. 44.54, H. 6.02, N. 7.87 found C. 44.17, H. 5.66, N. 7.37.

4.1.2.10. tert-Butyl N-[(5S)-6-oxo-6-[2-[3-oxo-3-[(3,4,5-trimethoxyphenyl)methylamino] propyl]hydrazino]-5-[[2-(3-phenoxyphenyl)acetyl] amino]hexyl]carbamate ***22a***. To **20** (613 mg, 0.84 mmol, 1 eq.) was added a solution of piperidine in DMF (10% v/

v, 12 mL). The mixture was stirred at room temperature for 2 h. The solvent was evaporated under reduced pressure to afford the free amine (quantitative) as a colorless oil, which was used without further purification. ^{1}H NMR (300 MHz, DMSO-*d6*): δ = 6.74 (m, 1H), 6.58 (s, 2H), 4.98 (m, 1H), 4.30 (d, 2H, J = 5.8 Hz), 4.05 (m, 1H), 3.76 (s, 6H), 3.62 (s, 3H), 3.15 (m, 2H), 3.07 (m, 2H), 2.30 (m, 2H), 1.62–1.28 (m, 6H), 1.38 (s, 9H). ^{13}C NMR (75 MHz, DMSO-*d6*): δ = 169.7, 169.5, 152.7, 146.2, 135.2, 132.1, 104.3, 77.2, 60.0, 55.8, 54.3, 53.6, 47.8, 42.1, 35.0, 34.2, 30.7, 25.7, 24.1. ESI$^-$ MS m/z: 510 [M − H]$^-$. To a solution of the free amine (305 mg, 0.42 mmol, 1 eq.) in dry DMF (10 mL) was added successively 3-phenoxyphenylacetic acid (145 mg, 0.50 mmol, 1.2 eq.), DIPEA (380 mg, 2.94 mmol, 7 eq.), HBTU (191 mg, 0.50 mmol, 1.2 eq.), and HOBT (68 mg, 0.50 mmol, 1.2 eq.). The mixture was stirred overnight at room temperature under argon atmosphere. After removal of the solvent under reduced pressure, the residue was taken up in EtOAc (10 mL) and successively washed with 10% aqueous citric acid (2 × 10 mL), water (10 mL), 10% aqueous K_2CO_3 (2 × 10 mL) and brine (10 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure to yield a crude product which was purified by column chromatography (EtOAc) to afford **22a** (157 mg, 0.21 mmol, 52%) as a white solid. mp: 142°C–144 °C; ^{1}H NMR (300 MHz): δ = 8.31 (t, 1H, J = 5.5 Hz), 8.21 (d, 1H, J = 8.0 Hz), 7.31 (t, 1H, J = 8.2 Hz), 7.25 (m, 1H), 7.17 (t, 1H, J = 5.6 Hz), 7.10 (t, 1H, J = 7.0 Hz), 6.95 (t, 1H, J = 9.0Hz), 6.93 (m, 1H), 6.90 (m, 2H), 6.87 (m, 2H), 6.52 (s, 2H), 6.44 (m, 1H), 4.74 (m, 1H), 4.34 (m, 2H), 4.28 (m, 1H), 3.80 (s, 9H), 3.46 (s, 2H), 3.07 (m, 2H), 3.02 (m, 2H), 2.32 (m, 2H), 1.75 (m, 1H), 1.58 (m, 1H), 1.42 (m, 2H), 1.41 (s, 9H), 1.25 (m, 2H). ^{13}C NMR (75 MHz): δ = 171.6, 171.1, 171.0, 157.7, 156.8, 156.3, 153.3, 153.2, 137.1, 136.4, 134.3, 130.2, 129.8, 123.9, 123.5, 119.5, 117.5, 104.8, 79.2, 60.8, 56.1, 51.8, 48.0, 43.6, 43.1, 36.9, 34.5, 31.2, 29.5, 28.4, 22.5. IR (cm^{-1}): 3585, 2931, 2048, 1638, 1586. ESI$^+$ MS m/z: 744 [M + 23]$^+$. Anal. Calcd for $C_{38}H_{51}N_5O_9$ C. 61.68, H. 7.24, N. 9.47 found C. 61.46, H. 7.18, N. 9.37.

4.1.2.11. 3-[2-[(2S)-6-amino-2-[[2-(3-phenoxyphenyl)acetyl]amino] hexanoyl]hydrazino]-N-[(3,4,5-trimethoxyphenyl)methyl] propanamide hydrochloride salt ***22b***. A solution of **22a** (103 mg, 0.14 mmol, 1 eq.) in HCl 4 M in dioxane (10 mL) was stirred for 2 h at room temperature. The solvent was evaporated under reduced pressure to afford the hydrochloride salt **22b** (100 mg, 0.15 mmol, quantitative) as a colorless oil. ^{1}H NMR (free amine): δ = 7.39 (bs, 1H), 7.30 (m, 2H), 7.22 (t, 1H, J = 8.1 Hz), 7.08 (t, 1H, J = 7.2 Hz), 6.95 (m, 3H), 6.89 (m, 2H), 6.84 (bs, 1H), 6.50 (s, 2H), 4.31 (m, 1H), 4.30 (d, 2H, J = 5.0 Hz), 3.79 (s, 6H), 3.78 (s, 3H), 3.47 (s, 2H), 3.05 (t, 2H, J = 5.5 Hz), 2.61 (t, 2H, J = 6.3 Hz), 2.31 (t, 2H, J = 5.5 Hz), 1.67 (m, 1H), 1.56 (m, 1H), 1.39 (m, 2H), 1.26 (m, 2H). ^{13}C NMR (free amine): δ = 171.7, 171.1, 157.6, 156.8, 153.2, 137.0, 136.6, 134.2, 130.1, 129.9, 129.8, 123.9, 123.5, 119.6, 118.9, 117.3, 104.8, 60.8, 56.0, 51.9, 48.0, 43.6, 43.0, 41.0, 34.5, 31.7, 31.5, 22.4. IR (cm^{-1}): 2926, 1682, 1650, 1592. ESI$^+$ MS m/z: 622 [M + H]$^+$. Anal. Calcd for $C_{33}H_{43}N_5O_7 \cdot 0.75\ H_2O$: C. 62.39, H. 7.08, N. 11.03 found C. 62.69, H. 7.29, N. 10.75 (free amine).

4.1.3. Synthesis of the target molecules including the α-hydrazino acid scaffold ***23–25b***

4.1.3.1. Methyl (2S)-2-[[2-[2-[(2S)-6-(benzyloxycarbonylamino)-2-(tert-butoxycarbonylamino) hexanoyl]hydrazino]acetyl]amino]-3-phenyl-propanoate ***23***. To solution of **38** (2.00 g, 4.16 mmol, 1 eq.) in THF/MeOH (1/1, 30 mL) was added an aqueous 2 N NaOH (4 mL). The reaction was stirred at room temperature over 3 h. The solvent was removed under reduced pressure (without distilling the water) and the remaining solution was brought at pH = 5 by addition of 10% aqueous citric acid. The aqueous phase was extracted with EtOAc (2 × 30 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure to give **23** (1.65 g, 3.60 mmol, 88%) as a white foam which was used in the next step without further purification. ^{1}H NMR (300 MHz): δ = 8.81 (s, 1H), 7.34 (m, 5H), 5.61 (m, 1H), 5.10 (s, 2H), 4.25 (m, 1H), 3.40 (s, 2H), 3.15 (m, 2H), 1.65–1.28 (m, 6H), 1.41 (s, 9H). ^{13}C NMR (75 MHz): δ = 173.8, 172.1, 156.8, 156.0, 136.5, 128.5, 128.0, 80.5, 66.7, 53.2, 52.8, 40.50, 32.0, 29.3, 28.3, 22.5. IR (cm^{-1}): 2951, 1694, 1519, 1367, 1246. APCI$^-$ MS m/z: 451 [M − H]$^-$

To a solution of the carboxylic acid (1.20 g, 2.64 mmol, 1 eq.) in dry DMF (20 mL) was added successively H-Phe-OMe (690 mg, 3.16 mmol, 1.2 eq.), DIPEA (1.36 g, 10.56 mmol, 4 eq.), HBTU (1.20 g, 3.16 mmol, 1.2 éq.), and HOBT (430 mg, 3.16 mmol, 1.2 éq.). The mixture was stirred overnight at room temperature under argon atmosphere. After removal of the solvent under reduced pressure, the residue was taken up in EtOAc (20 mL) and washed with 10% aqueous citric acid (2 × 15 mL), water (15 mL), 10% aqueous K_2CO_3 (2 × 15 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure to give a crude product which was purified by column chromatography (EtOAc) to afford **23** (907 mg, 1.48 mmol, 76%) as a white foam. ^{1}H NMR (300 MHz): δ = 8.08 (m, 1H), 7.37–7.15 (m, 10H), 7.05 (d, 1H, J = 5.6 Hz), 6.99 (d, 1H, J = 5.5 Hz), 5.08 (s, 2H), 4.79 (m, 1H), 3.90 (m, 1H), 3.65 (s, 3H), 3.38 (s, 2H), 3.03 (m, 4H), 1.47 (m, 6H), 1.34 (s, 9H). ^{13}C NMR (75 MHz): δ = 172.4, 172.2, 171.5, 156.7, 155.8, 136.6, 135.9, 129.1, 128.7, 128.0, 128.5, 80.7, 66.6, 53.2, 53.0, 52.4, 37.5, 31.4, 29.3, 28.2, 22.3. IR (cm^{-1}): 3303, 235), 1664, 1517, 1455. APCI$^+$ MS m/z: 614 (M + H)$^+$. Anal. Calcd for $C_{31}H_{43}N_5O_8 \cdot 0.25H_2O$: C, 60.67; H, 7.06; N, 11.41; found: C, 60.25; H, 7.13; N, 10.95.

4.1.3.2. Methyl (2S)-2-[[2-[2-[(2S)-6-(benzyloxycarbonylamino)-2-[[2-(3-phenoxyphenyl) acetyl]amino]hexanoyl]hydrazino]acetyl] amino]-3-phenyl-propanoate ***24a***. A solution of **23** (775 mg, 1.26 mmol, 1 eq.) in DCM/TFA 3/1 (19 mL) was stirred for 2 h at room temperature. The solvent was evaporated under reduced pressure and the excess of TFA was coevaporated with methanol to afford the hydrochloride salt of the amine as a colorless oil, which was used without further purification. ^{1}H NMR (300 MHz, DMSO-*d6*): δ = 9.75 (m, 1H), 8.30 (bs, 3H), 8.08 (m, 1H), 7.05–7.41 (m, 10H), 6.28 (bs, 2H), 5.01 (s, 2H), 4.55 (m, 1H), 3.61 (m, 1H), 3.59 (s, 3H), 3.45 (s, 2H), 2.98 (m, 4H), 1.71–1.18 (m, 6H). ^{13}C NMR (75 MHz, DMSO-*d6*): δ = 171.7, 169.40, 167.8, 156.0, 137.2, 136.9, 129.0, 125.3, 65.1, 53.2, 53.3, 51.8, 37.5, 30.7, 28.8, 21.4. IR (cm^{-1}): 2944, 1664, 1528, 1439, 1157. ESI$^+$ MS m/z: 515 [M + H]$^+$

To a solution of the trifluoroacetic salt of the amine (300 mg, 0.63 mmol, 1 eq.) in dry DMF (10 mL) was successively added 3-phenoxyphenylacetic acid (218 mg, 0.75 mmol, 1.2 éq.), DIPEA (871 mg, 5.30 mmol, 7 eq.), HBTU (287 mg, 0.75 mmol, 1.2 eq.), and HOBT (102 mg, 0.75 mmol, 1.2 eq.). The mixture was stirred overnight at room temperature under argon atmosphere. After removal of the solvent under the reduced pressure, the residue was taken up in EtOAc (10 mL), and washed with 10% aqueous citric acid (2 × 10 mL), water (10 mL), 10% aqueous K_2CO_3 (2 × 10 mL) and brine (10 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated to give a crude product which was purified by column chromatography (EtOAc) to afford the compound **24a** (227 mg, 0.38 mmol, 48%) as a white solid. mp: 156–157 °C; ^{1}H NMR (300 MHz): δ = 8.05 (s, 1H), 7.35 (d, 1H, J = 8.3 Hz), 7.24–6.82 (m, 21H), 6.25 (m, 1H), 5.07 (s, 2H), 4.75 (m, 1H), 4.24 (m, 1H), 3.26 (s, 3H), 3.42 (m, 2H), 3.38 (s, 2H), 3.10 (m, 4H), 1.74 (m, 1H), 1.53 (m, 1H), 1.42 (m, 2H), 1.19 (m, 2H). ^{13}C NMR (75 MHz): δ = 172.7, 171.4, 171.0, 169.9, 157.6, 156.8, 156.7, 136.5, 130.2, 136.0, 130.2, 129.8, 127.2, 123.9, 123.5, 119.6, 119.0, 117.2, 66.6, 54.7, 53.1, 52.5, 51.4, 43.1, 40.4, 37.4, 31.4, 29.3, 22.3. IR (cm^{-1}): 3304, 1690, 1646, 1533, 1487, 1258. APCI$^+$ MS m/z: 724 [M + H]$^+$. Anal. Calcd for $C_{40}H_{45}N_5O_8$: C, 66.37; H, 6.27; N, 9.68; found: C, 66.39; H, 6.37; N, 9.57.

*4.1.3.3. Methyl (2S)-2-[[2-[2-[(2S)-6-amino-2-[[2-(3-phenoxyphenyl)acetyl]amino]hexanoyl] hydrazino]acetyl]amino]-3-phenyl-propanoate hydrochloride salt **24b**.* To a solution of **24a** (114 mg, 0.15 mmol, 1 eq.) in dry MeOH/DMF (4/1, 25 mL) was added Pd/C 10% (11 mg). The reaction mixture was stirred overnight under hydrogen atmosphere at room temperature and filtered on a celite pad. After removal of the solvent under reduced pressure, HCl/MeOH was added and precipitation with Et_2O afforded the hydrochloride salt of **24b** as a colorless solid (81 mg, 0.13 mmol, 98%). mp: 118–120 °C; ^{1}H NMR (DMSO-*d*6): δ = 8.48 (d, 1H, J = 8.0 Hz), 8.35 (d, 1H, J = 7.6 Hz), 8.25 (d, 1H, J = 8.2 Hz), 7.38 (t, 2H, J = 6.7 Hz), 7.28 (m, 4H), 7.21 (m, 2H), 7.13 (t, 1H, J = 7.2 Hz), 7.03 (d, 1H, J = 8.2 Hz), 6.99 (m, 2H), 6.94 (m, 2H), 5.25 (m, 1H), 4.24 (m, 1H), 4.14 (m, 1H), 3.56 (s, 3H), 3.40 (s, 2H), 3.26 (m, 2H), 3.11 (m, 1H), 2.95 (m, 1H), 2.52 (m, 2H), 1.55 (m, 1H), 1.49 (m, 1H), 1.33 (m, 2H), 1.22 (m, 2H). ^{13}C NMR (DMSO-*d*6): δ = 171.7, 171.1, 169.9, 169.7, 156.6, 156.5, 137.0, 135.6, 130.1, 130.0, 129.7, 129.0, 128.3, 126.9, 126.7, 124.2, 123.4, 119.3, 119.2, 118.6, 116.6, 55.2, 53.7, 53.4, 51.9, 41.9, 40.8, 37.0, 32.0, 31.4, 22.6. IR (cm^{-1}): 3514 2926, 2048, 1656, 1485. ESI$^+$ MS *m*/*z*: 590 [M + H]$^+$. Anal. Calcd for $C_{32}H_{40}ClN_5O_6\cdot H_2O$: C. 59.66, H. 6.58, N. 10.87. found C. 59.51, H. 6.57, N. 10.57.

*4.1.3.4. Methyl (2S)-2-[[2-[2-[(2S)-6-(benzyloxycarbonylamino)-2-[[2-(2,5-dimethoxyphenyl) acetyl]amino]hexanoyl]hydrazino]acetyl] amino]-3-phenyl-propanoate **25a**.* Compound **25a** was synthesized according to the procedure described for **24a** from **23** that was successively deprotected and coupled with 2,5-dimethoxy phenylacetic acid (209 mg, 1.07 mmol, 1.2 eq.), except that the crude product was purified by column chromatography (EtOAc/MeOH : 9:1). **25a** was obtained as a white solid (423 mg, 0.61 mmol, 69%). mp: 143–145 °C; ^{1}H NMR: δ = 8.20 (s, 1H), 7.47 (d, 1H, J = 8.3 Hz), 7.24–7.33 (m, 8H), 7.14 (m, 2H), 6.78 (m, 2H), 6.77 (m, 1H), 6.52 (d, J = 7.5 Hz, 1H), 5.12 (m, 1H), 5.07 (s, 2H), 4.83 (m, 1H), 4.63 (m, 1H), 4.24 (m, 1H), 3.78 (s, 3H), 3.72 (s, 3H), 3.69 (s, 3H), 3.49 (m, 2H), 3.38 (s, 2H), 3.15 (m, 2H), 3.10 (m, 2H), 1.74 (m, 1H), 1.53 (m, 1H), 1.42 (m, 2H), 1.19 (m, 2H). ^{13}C NMR: δ = 172.5, 171.4, 169.9, 156.5, 153.7, 151.1, 136.7, 136.0, 129.1, 128.6, 128.5, 128.0, 127.1, 124.3, 117.2, 113.1, 66.5, 56.0, 55.7, 54.8, 53.0, 52.4, 51.4, 40.5, 38.8, 37.4, 31.4, 29.3, 22.3. IR (cm^{-1}): 3285, 1731, 1688, 1649, 1501, 1223. APCI$^+$ MS *m*/*z*: 692 [M + H]$^+$. Anal. calcd for $C_{36}H_{45}N_5O_9$: C, 62.50; H, 6.56; N, 10.12; found: C, 62.45; H, 6.60; N, 10.02.

*4.1.3.5. Methyl (2S)-2-[[2-[2-[(2S)-6-amino-2-[[2-(2,5-dimethoxyphenyl)acetyl] amino]hexanoyl]hydrazino]acetyl]amino]-3-phenyl-propanoate **25b**.* To a solution of **25a** (100 mg, 0.14 mmol, 1 eq.) in dry MeOH/DMF (4/1 v/v 25 mL) was added 10% Pd/C (11 mg). The mixture was stirred overnight under hydrogen atmosphere at room temperature and filtered on a celite pad. After removal of the solvent under reduced pressure **25b** was obtained as a white solid (79 mg, 0.13 mmol, quantitative). mp: 84–86 °C; ^{1}H NMR (DMSO-*d*6): δ = 8.48 (d, 1H, J = 7.4 Hz), 8.09 (d, 1H, J = 8 Hz), 7.96 (d, 1H, J = 8.0 Hz), 7.28–7.21 (m, 5H), 6.86 (d, 1H, J = 8.5 Hz), 6.75 (d, 2H, J = 4.4 Hz), 5.25 (m, 1H), 4.48 (dd, 1H, J = 7.4 and 14.2 Hz), 4.16 (m, 1H), 3.69 (s, 3H), 3.67 (s, 3H), 3.57 (s, 3H), 3.42 (s, 2H), 3.26 (m, 2H), 3.04 (m, 1H), 2.92 (m, 1H), 2.53 (m, 2H), 1.59 (m, 1H), 1.49 (m, 1H), 1.34 (m, 2H), 1.25 (m, 2H). ^{13}C NMR (DMSO-*d*6): δ = 171.7, 171.2, 169.8, 169.7, 152.9, 151.3, 137.0, 130.0, 129.0, 128.3, 126.6125.7, 116.8, 111.9, 111.6, 55.9, 55.3, 53.7, 53.4, 51.8, 51.2, 41.0, 36.9, 36.7, 31.9, 31.8, 22.5. IR (cm^{-1}): 2932, 1647, 1500, 1224. ESI$^+$ MS *m*/*z*: 558 [M + H]$^+$. Anal. Calcd for $C_{28}H_{39}N_5O_7\cdot 0.75H_2O$ C. 58.91, H. 7.17, N. 12.27 found C. 58.87, H. 6.79, N. 12.36.

*4.1.4. Synthesis of the intermediates **29**–**36** and **38***

*4.1.4.1. N′-{2,2,2-Trifluoro-1-[(3,4,5-trimethoxy-benzylcarbamoyl)-methyl]-ethyl}-hydrazine carboxylic acid tert-butyl ester **29**.* To a solution of **27** (1.34 g, 4.92 mmol, 1.0 eq.) and HBTU (2.23 g, 5.90 mmol, 1.2 eq.) in DMF (10 mL) were successively added HOBT (798 mg, 5.90 mmol, 1.2 eq.), DIPEA (1.65 mL, 9.84 mmol, 2.0 eq.) and phenylalanine hydrochloride. The reaction was performed under argon atmosphere at room temperature overnight. The solvent was evaporated over reduced pressure. The product was taken up in EtOAc (25 mL), and the organic layer was successively washed with 10% aqueous citric acid (2 × 15 mL), water (30 mL), 10% aqueous K_2CO_3 (2 × 15 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated in vacuo to give a slightly yellow solid which was purified by column chromatography (EtOAc:cyclohexane 1:1) to give the protected compound as a white solid (1.70 g, 3.76 mmol, 76%): mp 138–140 °C; ^{1}H NMR δ = 7.90 (bs, 1H), 6.64 (s, 2H), 6.29 (s, 1H), 4.45 (m, 2H), 4.34 (m, 1H), 3.89 (s, 6H), 3.86 (s, 3H), 3.79 (m, 2H), 2.57 (s, 1H), 1.67 (s, 9H); ^{13}C δ = 168.2,156.7, 153.2, 137.1, 105.1, 81.5, 60.8, 59.0, 56.1, 44.0, 33.3, 28.2; ^{19}F (188 MHz): δ = −75.09 (d, 1H, J = 7.0 Hz); IR (cm^{-1}): 3300, 1652, 1506, 1234, 1123; ESI$^+$ MS *m*/*z*: 474.2 [M + Na]$^+$; Anal. Calcd for $C_{19}H_{28}F_3N_3O_6\cdot H_2O$: C, 48.61; H, 6.45; N, 8.95; found C, 48.40; H, 5.88; N, 8.49. A solution of the Boc-protected compound (926 mg, 1.99 mmol, 1.0 eq.) in DCM/TFA 3:1 (12 mL) was stirred for 2 h at room temperature. The solvent was evaporated under reduced pressure to give the trifluoroacetic salt of **29** in quantitative yield, as a slightly yellow solid and that was used without further purification.

*4.1.4.2. N′-(2,2,2-Trifluoro-1-hydrazinocarbonylmethylethyl)hydrazine carboxylic acid tert-butyl ester **30**.* To a solution of **27** (2.0 g, 6.7 mmol) in ethanol (50 mL) was added hydrazine monohydrate (3.34 g, 66.7 mmol, 10.0 eq.). After stirring at room temperature overnight, the solvent was removed under reduced pressure, to afford **30** as a white solid (2.11 g, quantitative yield). ^{1}H NMR (CD_3OD): δ = 3.60 (m, 1H), 2.32 (m, 2H), 1.32 (s, 9H); ^{13}C NMR (CD_3OD): δ = 173.6, 129.4 (q, J = 286.3 Hz), 85.0, 62.5 (q, J = 27.7 Hz), 35.4, 32.0. ^{19}F (CD_3OD): δ = −75.1 (d, J = 7.1 Hz).

*4.1.4.3. 3-(N′-Benzyloxycarbonylhydrazino)-4,4,4-trifluorobutyric acid ethyl ester **31**.* Compound **27** (200 mg, 0.66 mmol) was dissolved in dry CH_2Cl_2 (2.5 mL) and trifluoroacetic acid (2.5 mL, 33.0 mmol, 50.0 eq.) was added dropwise at room temperature. The reaction mixture was stirred at room temperature for 2 h. Solvent was evaporated under reduced pressure to yield light brown solid oil (228 mg, with excess of TFA). Azeotropic removal of excess TFA with toluene gave the deprotected product that was used without any further purification in the course of the synthesis. This compound (207 mg, 0.66 mmol) was dissolved in CH_2Cl_2 (6 mL) and benzylchloroformiate (104 μL, 0.73 mmol, 1.1 eq.) was added dropwise at 0 °C. After was added DIPEA (287 μL, 1.65 mmol, 2.5 eq.) and the reaction mixture was stirred at room temperature for 21 h. After removal of the solvent under reduced pressure, the residue was dissolved in EtOAc (9 mL). The organic phase was successively washed with 10% aqueous citric acid (9 mL), water (9 mL), 10% aqueous K_2CO_3 (9 mL) and brine (9 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated. A purification by chromatography on silica gel, eluting with EtOAc/cyclohexane (5/5), afforded **31** as a colorless oil (168 mg, 0.5 mmol, 76%). ^{1}H NMR (200 MHz): δ = 7.42–7.28 (m, 5 H), 6.29 (br s, 1H), 5.11 (s, 2H), 4.20 (q, J = 7.2 Hz, 2H), 3.89 (m, 1H), 2.58 (m, 2H), 1.22 (t, J = 7.1 Hz, 3H). ^{13}C NMR (75 MHz): δ = 169.7, 157.2, 135.7, 128.6, 128.4, 128.2, 125.2 (q, J = 279.8 Hz), 67.5, 61.5, 58.2 (q, J = 27.8 Hz), 32.1, 14.0. ^{19}F (188 MHz):

$\delta = -75.5$ (d, $J = 7.2$ Hz). IR (cm^{-1}): 3331, 1722, 1159, 1128, 699. ESI^+ MS m/z: 357 $[M + Na]^+$

4.1.4.4. N′-(2,2,2-Trifluoro-1-hydrazinocarbonylmethylethyl)hydrazinecarboxylic acid benzyl ester ***32****.* To a solution of **31** (148 mg, 0.44 mmol) in EtOH (3 mL) was added hydrazine monohydrate (214 μL, 4.4 mmol) and the reaction mixture was stirred at room temperature for 23 h. After removing the solvent under reduced pressure, **32** was obtained as a white solid (140 mg, 0.44 mmol, quantitative yield). mp: 146–148 °C). ^1H NMR (200 MHz, CD_3OD): $\delta = 7.33–7.23$ (m, 5 H), 5.09 (s, 2H), 3.80 (m, 1H), 2.48 (dd, $J = 4.8$ and 15.0 Hz, 1H), 2.39 (m, 1H). ^{13}C NMR (75 MHz, CD_3OD): $\delta = 170.9$, 159.6, 138.0, 129.5, 129.1, 129.0, 123.3 (q, $J = 279.8$ Hz), 67.9, 59.5 (q, $J = 27.8$ Hz), 32.6. ^{19}F (188 MHz, CD_3OD): $\delta = -76.9$ (d, $J = 7.3$ Hz). IR (cm^{-1}): 3307, 1705, 1654, 1174, 1120, 696. ESI^+ MS m/z: 343 $[M + Na]^+$. Anal. Calcd for $C_{12}H_{15}F_3N_4O_3 \cdot 0.4H_2O$: C, 44.03; H, 4.88; N 17.12. Found: C, 43.93; H, 4.54; N, 17.57.

4.1.4.5. 3-(N′-tert-Butoxycarbonyl-hydrazino)-propionic acid ethyl ester ***34****.* A solution of 3-bromo-propionic acid ethyl ester **33** (2 mL, 15.7 mmol, 1.0 eq.), DIPEA (2.6 mL, 15.7 mmol, 1.0 eq.) and Boc-hydrazine (3.1 g, 3.1 g, 23.55 mmol, 1.5) in toluene was heated at 80 °C for 4 days. After removal of the solvent under reduced pressure, the crude product was purified by column chromatography (cyclohexane:EtOAc 6:4) to give **34** as a slightly yellow oil (1.58 g, 6.80 mmol, 43%). ^1H NMR: $\delta = 6.26$ (bs, 1H), 4.08 (q, 2H, $J = 7.1$ Hz), 3.06 (t, 2H, $J = 6.6$ Hz), 2.41 (t, 2H, $J = 6.6$ Hz), 1.39 (s, 9H), 1.19 (t, 3H, $J = 7.1$ Hz); ^{13}C NMR: $\delta = 172.2$, 156.7, 80.3, 60.3, 47.3, 33.0, 28.2, 14.0; IR (cm^{-1}): 2980, 1714, 1252, 1151, 1025; ESI^+ MS m/z: 233 $[M + H^+]$; Anal. Calcd for $C_{10}H_{20}N_2O_4$: C, 51.71; H, 8.68; N, 12.06; found C, 51.61; H, 8.25; N, 11.58.

4.1.4.6. 2-[3-(N′-tert-Butoxycarbonyl-hydrazino)-propionylamino]-3-phenyl-propionic acid methyl ester ***35****.* An aqueous solution of NaOH 2 N (4.8 mL, 9.6 mmol, 1.1 eq.) was added to a solution of **34** (2.00 g, 8.6 mmol, 1.0 eq.) in THF/MeOH (10/10 mL, v/v). The reaction was stirred at room temperature over 3 h. After removing the solvent under reduced pressure without distilling the water, the remaining solution was brought at pH = 5 by addition of aqueous solution of 1 N HCl. The aqueous phase was extracted with EtOAc (3 × 20 mL). Then, the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure to give the 3-(*N′-tert*-Butoxycarbonyl-hydrazino)-propionic acid as a white solid (1.64 g, 8.0 mmol, 93%) which was used in the next step without further purification. mp: 112–114 °C; ^1H NMR (DMSO-*d*6): $\delta = 8.17$ (m, 2H), 3.30 (s, 1H), 2.87 (t, 2H, $J = 7.0$ Hz), 2.30 (t, 2H, $J = 7.0$ Hz), 1.39 (s, 9H); ^{13}C NMR (DMSO-*d*6): $\delta = 173.2$, 156.3, 78.2, 46.7, 32.5, 28.0; IR (cm^{-1}): 3350, 2981, 1679, 1437, 1156, 860; ESI^+ MS m/z: 227 $[M + H]^+$, 431.1 $[2M + Na]^+$; Anal. Calcd for $C_8H_{16}N_2O_4$: C 47.05; H, 7.90; N, 13.72; found C, 47.43; H, 7.90; N, 13.33. A solution of the acid compound (0.819 g, 4.0 mmol, 1.0 eq.) and HBTU (1.52 g, 4.0 mmol, 1.0 eq.) in DMF (10 mL) was stirred for 30 min, then were successively added HOBT (650 mg, 4.8 mmol, 1.2 eq.), DIPEA (1.4 mL, 8.0 mmol, 2.0 eq.) and phenylalanine hydroxychloride (1.04 g, 4.8 mmol, 1.2 eq.). The reaction was performed under argon atmosphere a room temperature overnight. After removal of the solvent under reduced pressure, the crude product was taken up in EtOAc (15 mL) and successively washed with 10% aqueous K_2CO_3 (2 × 15 mL), brine (20 mL) and water (2 × 15 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure to give a slightly yellow solid which was purified by column chromatography (EtOAc) to give **35** as a white solid (1.26 g, 3.3 mmol, 82%). mp; 86–88 °C; ^1H NMR (300 MHz, DMSO-*d*6): $\delta = 8.45$ (s, 1H), 8.30 (s, 1H), 7.28–7.13 (m, 5H), 6.10 (bs, 1H), 4.85 (dd, 1H, $J = 6.9$ and 13.1 Hz), 3.73 (s, 3H), 3.11 (dd, 1H, $J = 6.9$ and 13.1 Hz), 3.09–2.99 (m, 3H), 2.34 (t, 2H, $J = 5.4$ Hz), 1.45 (s, 9H); ^{13}C NMR (75 MHz): $\delta = 172.0$, 171.1, 137.2, 129.2, 129.0, 128.1, 126.5, 78.3, 53.4, 51.7, 47.4, 36.7, 33.8, 28.1; IR (cm^{-1}): 2935, 1728, 1529, 1156, 837; ESI^+ MS m/z: 388.3 $[M + Na]^+$; Anal. Calcd for $C_{18}H_{27}N_3O_5$: C, 59.16; H, 7.45; N, 11.50; found C, 59.20; H, 7.20; N, 11.05.

4.1.4.7. tert-Butyl N-[[3-oxo-3-[(3,4,5-trimethoxyphenyl)methylamino]propyl]amino] carbamate ***36****.* Compound **34** was saponified to give the 3-(*N′-tert*-butoxycarbonyl-hydrazino)-propionic acid according the same procedure described for the synthesis of compound **35**. To a solution of 3-(*N′-tert*-butoxycarbonyl-hydrazino)-propionic acid (1.3 g, 6.38 mmol, 1 eq.) in dry DMF (30 mL) was successively added 3,4,5-trimethoxybenzylamine (1.5 g, 7.65 mmol, 1.2 eq.), DIPEA (3.3 g, 25.5 mmol, 7 eq.), HBTU (2.9 g, 7.65 mmol, 1.2 eq.), and HOBT (1.0 g, 7.65 mmol, 1.2 eq.). The mixture was stirred overnight at room temperature under argon atmosphere. After removal of the solvent under reduced pressure, the residue was taken up in EtOAc (20 mL) and successively washed with 10% aqueous citric acid (2 × 20 mL), water (20 mL), 10% aqueous K_2CO_3 (2 × 20 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure to give a crude product which was purified by column chromatography (EtOAc) to afford **36** (1.26 g, 3.28 mmol, 52%) as a white foam. ^1H NMR (300 MHz): $\delta = 7.18$ (bs, 1H), 6.51 (s, 2H), 6.45 (bs, 1H, NH), 6.01 (s, 1H), 4.31 (d, 2H, $J = 5.7$ Hz), 3.81 (s, 6H), 3.80 (s, 3H), 3.06 (t, 2H, $J = 6.1$ Hz), 2.35 (t, 2H, $J = 6.1$ Hz), 1.45 (s, 9H). ^{13}C NMR (75 MHz): $\delta = 171.7$, 157.3, 153.3, 136.9, 134.3, 104.8, 80.8, 60.8, 56.1, 48.5, 43.6, 34.4, 28.6. IR (cm^{-1}): 3303, 2352, 1647, 1592, 1457. ESI^+ MS m/z: 406 $[M + Na]^+$

4.1.4.8. Ethyl 2-[2-[6-(benzyloxycarbonylamino)-2-(tert-butoxycarbonylamino)hexanoyl] hydrazino]acetate ***38****.* To a solution of ethyl 2-hydrazinylacetate hydrochloride **37** (1.0 g, 6.47 mmol, 1 eq.) in dry DMF (20 mL) was successively added N_α-Boc-N_ε-Z-Lys (2.95 g, 7.76 mmol, 1.2 eq.), DIPEA (5.8 g, 4.3 mmol, 7 eq.), HBTU (2.95 g, 7.76 mmol, 1.2 eq.), and HOBt (1.04 g, 7.76 mmol, 1.2 eq.). The mixture was stirred overnight at room temperature under argon atmosphere. After removal of the solvent under reduced pressure, the residue was taken up in EtOAc (20 mL) and washed successively with 10% aqueous citric acid (2 × 15 mL), water (15 mL), 10% aqueous K_2CO_3 (2 × 15 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product obtained was purified by column chromatography (EtOAc/cyclohexane, 8:2) to give **38** (2.35 g, 4.9 mmol, 76%) as a white foam. ^1H NMR (300 MHz): $\delta = 8.30$ (m, 1H), 8.16 (s, 1H), 7.27 (m, 5H), 5.25 (m, 1H), 5.01 (s, 2H), 4.12 (q, 2H, $J = 7.1$ Hz), 3.90 (m, 1H), 3.56 (s, 2H), 3.10 (m, 2H), 1.47 (m, 6H), 1.34 (s, 9H), 1.18 (t, 3H, $J = 7.1$ Hz). ^{13}C NMR (75 MHz): $\delta = 172.2$, 171.5, 156.7, 155.8, 136.6, 128.5, 128.0, 80.3, 66.6, 53.2, 40.3, 31.4, 29.3, 28.2, 22.3, 15.26. IR (cm^{-1}): 3290, 1684, 1519, 1367, 1245. ESI^+ MS m/z: 503 $[M + Na]^+$

4.2. Enzymatic studies

ChT-L activity was determined by monitoring the hydrolysis of Suc-LLVY-AMC, for 45 min at 37 °C in the absence (control) or presence of test compounds (0.1–200 μM). The buffer (pH 7.5) was 20 mM Tris, 1 mM DTT, 10% glycerol, 0.02% (w/v). The final concentration of rabbit 20S proteasome and Suc-LLVY-AMC were 0.3 nM and 50 nM respectively. Substrate and inhibitors were previously dissolved in DMSO leading to the final and constant DMSO concentration of 2% (v/v) in the enzymatic assays. The IC_{50} values (inhibitor concentrations giving 50% inhibition) were obtained by plotting the percent inhibition against inhibitor

concentration to equation 1: % inhibition = $100[I]/(IC_{50} + [I])$, or equation 2: % inhibition = $100[I]^{nH}/(IC_{50}^{nH} + [I]^{nH})$ where n_H is the Hill number. The K_m value of the fluorogenic substrate Suc-LLVY-AMC in our experimental conditions was 30 ± 5 μM.

4.3. Molecular modeling

Coordinates of "apo" bovine proteasome were retrieved from the X-ray structure available in the PDB [42] (accession code 1IRU, resolution 2.75 Å) [41]. Hydrogen atoms were added and only chains L and M defining the binding site at their interface were retained.

Initial coordinates for relevant stereoisomers of the ligands were generated using CORINA v3.44 software [43]. Ligands were then docked individually using GOLD v5.1 software [44] with default parameters (but using of GoldScore scoring function) and full torsional freedom for the side chains of Met45 and Ile35 (belonging to the S1 subpocket) was allowed.

During docking calculations, the scoring function was successively configured to bias conformational space exploration towards the solutions exhibiting hydrogen bonds with the maximum number of key residues from one of the two following subsets: Thr21, Ala49 and Asp125 or Gly47, Ala49 and Asp125 (40 solutions were calculated for each case.)

Solutions were analyzed and cherry-picked using Hermes v1.5 (companion program of GOLD) on the basis of criteria detailed in the main discussion of this article. Geometry of retained poses was slightly optimized using Protein Preparation Wizard module [45] from the Schrödinger 2011 suite with default parameters (including OPLS2005 force field) [46] before final analysis and depiction with UCSF Chimera v1.7 [47].

Sequences were retrieved from UniProt database [48] and alignments were performed with ClustalX v2.1 [49].

Acknowledgments

Claire Troufflard is gratefully acknowledged for NMR experiments. We thank the European Community for the financial support for AB and LF (Marie Curie Early Stage training Fellowship of the European Community's Sixth Framework Programme: contract MESTCT-2004-515968). We thank also the Ministère de la Recherche et des Technologies (MRT) for a financial support of MK and XM and the 'Association Française contre les Myopathies' (AFM) for a grant n° 14999 (MRR).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.09.059.

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