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Research paper

Synthesis and antiproteasomal activity of novel *O*-benzyl salicylamide-based inhibitors built from leucine and phenylalanine

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ABSTRACT

Inhibition of protein degradation is one of strategies for suppression of uncontrolled proliferation of cancer cells. Proteolytic degradation in cells is mainly ensured by proteasome and its inhibition by bortezomib showed benefit in clinical use for the treatment of multiple myeloma. We report here the library of antiproteasomal *O*-benzyl salicylamides built from leucine and phenylalanine. Prepared compounds displayed antiproliferative activity on K562, CEM and U266 cancer cell lines, ranging from high micromolar to submicromolar GI_{50} values. The most potent compounds (series **4** and **6**) were further assayed for their inhibition of chymotrypsin-like protease activity of the 26S proteasome in U266 cells. The majority of compounds inhibited the proteasome in mid-nanomolar concentrations (IC_{50} ranging from 57 to 197 nM) and it correlated with cellular potency. In a cell based assay involving green fluorescence protein (GFP) fused to a short degron that is rapidly degraded by a proteasome the compounds induced accumulation of GFP, visualised and quantified by live-cell imaging. Levels of poly-ubiquitinated proteins in U266 cells treated by compound **4m** were also analyzed by immunoblotting, revealing a typical high molecular mass smear of ubiquitin conjugates. Finally, apoptotic cell death in treated U266 cells was detected biochemically by measuring the activity of caspases 3 and 7 in lysates and by immunoblotting of caspase 7, its substrate poly(ADP-ribose)polymerase, and Mcl-1, which all together showed changes typical for apoptosis. All these observations were in agreement with expected cellular mechanism of action and confirmed proteasome targeting by prepared *O*-benzyl salicylamides.

1. Introduction

Cancer is associated with deregulation or dysfunction of many cellular processes that have been validated successfully as targets of modern therapeutics [1,2]. One of these processes relates to protein stability and degradation. In cells, more than 80% of proteins are degraded by a large protein complex known as a proteasome [3]. Inhibition of its proteolytic function has been shown to affect numerous different processes and pathways, such as cell cycle progression, cell migration, cell adhesion, viability and apoptosis [4], and therefore it offers a promising strategy for cancer treatment.

The proteasome is a multi-subunit protein complex composed of one 20S cylindrical catalytic core and two 19S regulatory subunits [5]. The catalytic part of the proteasome contains several β-type subunits. Three of them, namely β1, β2, and β5 are responsible for the caspase-like, trypsin-like and chymotrypsin-like proteolytic activities of proteasomes, signifying the preference for client peptide. While the chymotrypsin-like activity is responsible for the cleavage of peptide bonds after hydrophobic amino acid residues, the trypsin-like activity causes cleavage after basic residues and the caspase-like activity after acidic residues of amino acids [13].

Proteasomal inhibitors (Fig. 1) can be divided into distinct chemical groups; most of them target the active site by mimicking a substrate and interacting with catalytic Thr1 of the proteasome. While peptide aldehydes (e.g. fellutamide B, MG132) are rapidly oxidised in cells and not so suitable for therapeutic use, peptide boronates (e.g. bortezomib, ixazomib, delanzomib), forming

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pseudo covalent adducts with Thr1, display improved bioavailability and excellent potency towards the proteasome. Next, peptide epoxyketones (e.g. carfilzomib, oprozomib) are characterised by nearly no off-target effects, and the group of peptide vinyl sulphonates by better specificity for trypsin-like active sites. In addition, there are several groups of natural proteasome inhibitors e.g. β-lactones (marizomib) [5–8] and other compounds carrying a non-peptide scaffold [9,10].

Bortezomib (Velcade®, PS-341, Fig. 1) is a dipeptide boronic acid that reversibly binds mainly to the chymotrypsin-like active site of the β5 subunit of the proteasome. It exhibits nanomolar potency; therefore, bortezomib has been approved since 2003 for the treatment of multiple myeloma and mantle cell lymphoma [7,11]. Later, proteasome inhibition by bortezomib has been validated as suitable treatment for other malignancies, but like other drugs, bortezomib has several limitations and produces side-effects in patients [12]. Thus, there is a demand for the development of new generations of proteasome inhibitors.

Inspired by the success of bortezomib (Velcade®), a second generation of promising proteasome inhibitors are currently being evaluated in clinical trials, namely marizomib (salinosporamide A), oprozomib (ONX 0912) or delanzomib (CEP-18770) and recently carfilzomib (PR-171, Kyprolis®) and ixazomib (MLN9708, Ninlaro®) have been approved for the treatment of patients with relapsed or refractory multiple myeloma [13].

Our previous study showed that the 2-hydroxy-*N*-(arylalkyl) benzamides induce apoptosis in cancer cell lines [14]. Their further modification yielded *O*-benzyl salicylamide dipeptides with aliphatic endings, which also displayed moderate activity [15]. Our intention, to prepare compounds based on substituted salicylic acid linked to a short peptide chain by an amidic bond, has remained. We designed, synthesised and fully characterised a new series of compounds, consisting a short dipeptide/tripeptide moiety bonded to *O*-benzyl salicylic acid on the one site of molecule and carrying various functional groups, including aldehyde, that has been found to be highly promising in several recent studies [16,17].

2. Results and discussion

2.1. Chemistry

The synthesis of the starting compound was based on the repetition of a versatile two-step synthetic methodology starting from *O*-benzyl-5-chloro-salicylic acid [18] (Scheme 1). First was the condensation of acid **1** with chosen amino acid methyl ester to produce methyl ester **2**. In comparison with the original approach [15] liberation of free base was not carried out *in situ* but externally. This procedure minimises racemisation during the reaction. The second step consists of basic hydrolysis of the ester **2**, to obtain acid **1** with one amino acid. This reaction cycle can be repeated; in this way esters **2** as well as acids **1** with various numbers of amino acid units were synthesised and characterised (Scheme 1).

Esters **2** and acids **1** are starting materials for further modification. Series of the esters **2** (containing one or two amino acids (described previously) or three amino acids **2a-d)** were synthesised and characterised as original organic compounds. Hydrolysis of prepared esters **2a**–**d** led to appropriate acids **1** (containing one **1a, b** or two **1c**–**h** eventually three **3i, j** amino acids). Both esters and acids are versatile intermediates for further modification and synthesis of targeted compounds. As our initial thoughts were also to prepare compounds with an aldehyde functional moiety (Fig. 1) the first approach to obtain these compounds was partial reduction of esters **2** using DIBAL [19]. The non-selectivity of this process encouraged the use of an alternative methodology. The preparation of targeted aldehydes began from methyl esters **2,** which upon treatment with lithium bis(trimethylsilyl)amide (LiHMDS) and *N,O*-dimethylhydroxylamine hydrochloride were converted to the corresponding *N*-methyl-*O*-methyl amides **3a**–**l** in good to excellent yield [20]. It was also found that alternative synthesis using *i*-PrMgCl was not suitable for our compounds due to slow conversion as well as low selectivity. Problematic separation of desired products was another disadvantage of this technique [21]. *N*-methyl-*O*-methyl amides are generally known compounds and they serve as versatile intermediates [22,23].

Weinreb amides **3a**–**l** were transformed using $LiAlH_4$ to obtain

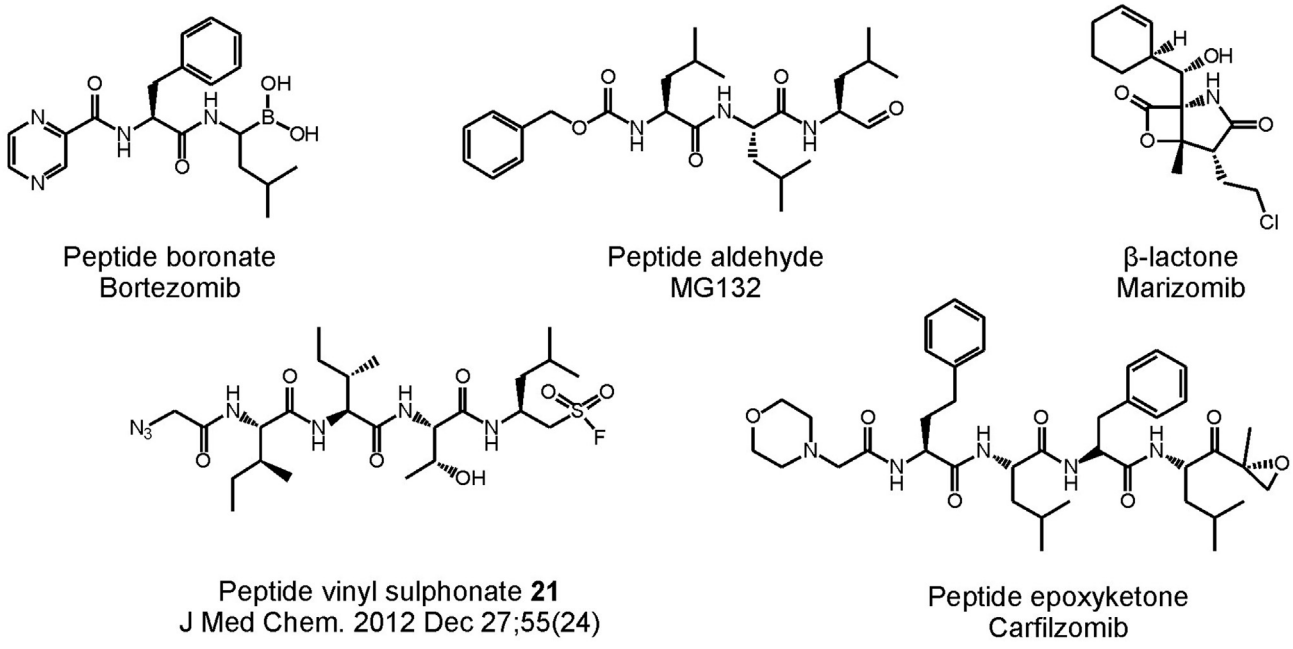
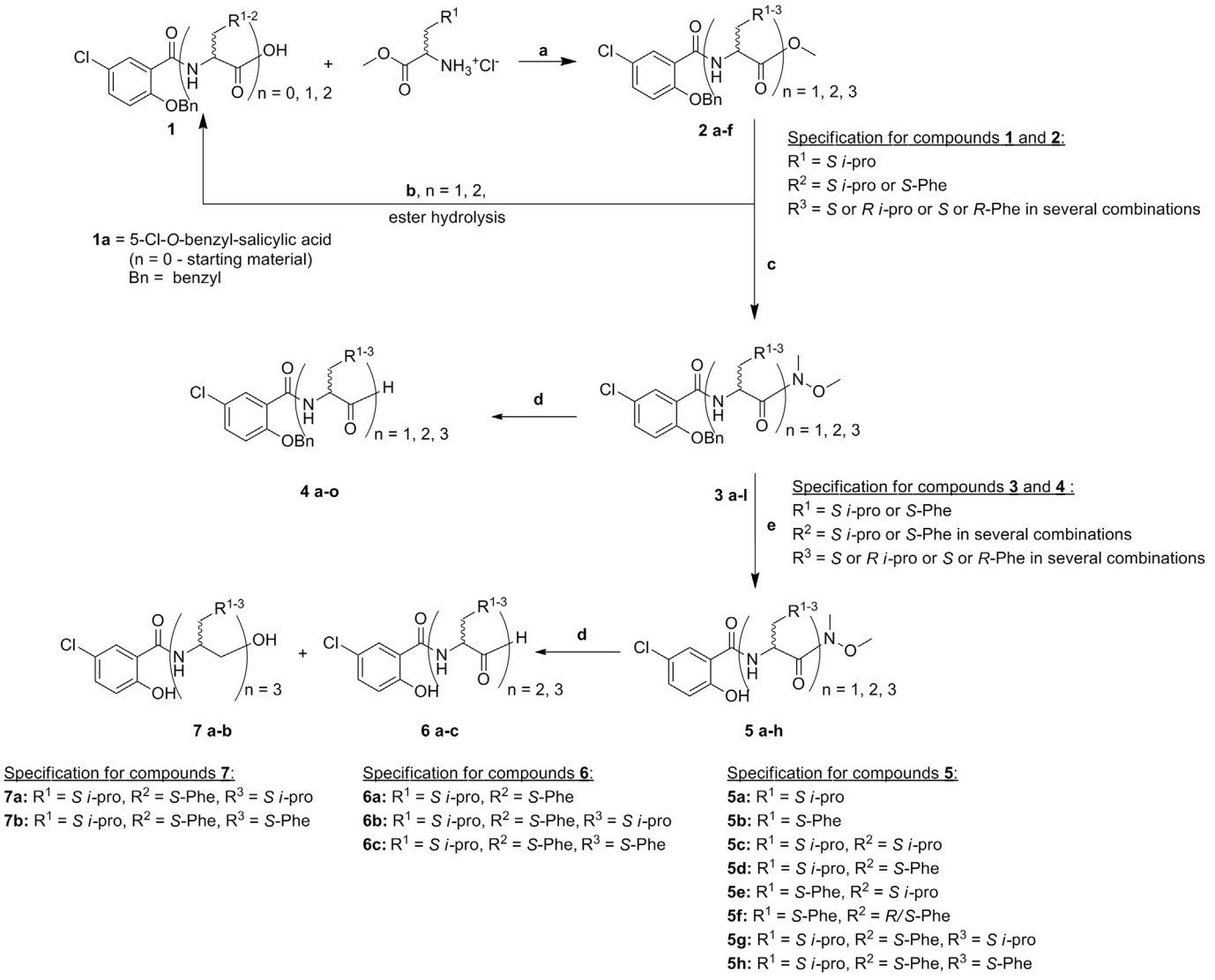


Fig. 1. Example of known inhibitors of proteasome with different reactive groups.



Scheme 1. General reaction scheme for synthesis of targeted compounds. Integrated L- or D-leucine (R^1 = *i*-pro) and L- or D-phenylalanine (R^1 = Ph) in various combinations. Conditions: **a** = EDCI·HCl (0.95 eq), HOBt (1 eq), RT, CH_2Cl_2, 2 h; **b** = LiOH (3 eq), H_2O:1,4-dioxane (vol. 1:1), RT, 2 h; **c** = LiHMDS (1 M in THF, 7 eq), dry THF, CH_3ONHCH_3·HCl (2.5 eq), inert atm., 1 h −20 °C, then 3 h RT; **d** = $LiAlH_4$ (3 eq), dry THF, 3 h −20 °C, then 1 h RT; **e** = 10% Pd/H_2, EtOAc, RT, overnight.

O-benzyl protected aldehydes **4a**–**p** in moderate to good yields. In cases where we wanted to synthesise aldehydes without benzyl group **6**, deprotection of **3** was first performed, and deprotected Weinreb amides **5a**–**h** were transformed to the corresponding aldehydes **6a**–**c** using $LiAlH_4$ solution. Under these reaction conditions two examples of alcohols **7** were also isolated as products of the reduction process (Scheme 1).

An alternative approach to synthesis of **3** was carried out by reaction of the 5-chloro-*O*-benzyl salicylic acid **1** and L-leucine or L-phenylalanine with preformed Weinreb amides of selected amino acids. This approach seems to be more universal than the procedure described in previous paragraphs. However, yields of the prepared compounds and their purity have been in many cases lower than those from the reaction sequence using the sequential building of the target molecules. The final products were structurally identical to compounds obtained by the above-mentioned process. The results of biological testing were also identical. Because of the above disadvantages this procedure has not been applied to the synthesis of other derivatives. The general scheme, experimental procedures and spectral characterization of original intermediates are listed in the supplementary information to this paper.

2.2. Antiproliferative activity of novel salicylamides

In our previous studies [14,15] we described two major groups of substituted salicylamides with dipeptide chains that showed moderate antiproliferative activity against the panel of cancer cell lines. The first study on chloro-substituted salicylamides (alternately 2-hydroxy-*N*-(arylalkyl)benzamides) documented increased cytotoxicity of derivatives bearing halogenated mono-substitution at the terminal end of the molecule. In the following study of *O*-benzyl substituted salicylamides we investigated the presence of further terminal functional groups, namely esters, carboxylic acids or allyl, isobutyl, isopropyl or n-propyl groups. All substitutions mentioned seemed to display mid-micromolar antiproliferative activities.

To follow our recent studies we synthesised and fully characterised a new series of *O*-benzyl salicylamides having dipeptide or tripeptide chain containing optically pure leucines or phenylalanines (or a combination) and terminated with various functional

groups, namely esters (series **2**), Weinreb amides (series **3**) and aldehydes (series **4**). The library was also complemented by a number of unsubstituted derivatives (series **5**–**7**). All novel salicylamides were tested for their antiproliferative properties against various cancer cell lines derived from hematopoietic malignancies (K562, CEM, U266) and the resulting data are presented in Table 1.

Our results show that esters (series **2**) and Weinreb amides (series **3** and **5**) have retained GI_{50} values in mid-micromolar range. While we did not observe significant changes in GI_{50} values with increasing length of peptide chain for all *O*-benzyl derivatives, the antiproliferative activity of unsubstituted Weinreb amides **5g, h** having three integrated amino acids was higher (GI_{50} ranging from 16.4 to 32.9 μM) than for di- or mono-peptide based amides **5a**–**f** (GI_{50} ranging from 48.2 to 100.0 μM). Reasonable cellular activities were observed for diamides bearing terminal aldehyde group (series 4 and 6). All aldehydes having tripeptide chain displayed GI_{50} values reaching mid-nanomolar values in comparison to dipeptide derivatives. This trend was similar for unsubstituted derivatives **6**. The U266 cell line was shown to be the most sensitive to the tested compounds and GI_{50} value determined for the most active derivative **4m** was 0.15 μM.

Results suggested that the length of peptide chain of aldehyde-based salicylamides is important for cellular activity, but we also studied how the type of integrated amino acids affects antiproliferative properties of compounds. Analysis of data for active aldehydes (**4h-o**) on U266 cells showed that salicylamides bearing phenylalanine positioned next to the aldehyde group showed higher GI_{50} than the corresponding compounds having L-leucine at this position (compare $GI_{50\ (U266)}$ for **4n, o** with **4k-m** or **4j** with **4g-i**). The same trend was observed for the pair of unsubstituted salicylamides **6b** and **6c**.

2.3. *In vitro antiproteasomal and apoptotic activities of novel salicylamides*

The structural analogy of prepared salicylamides with known

Table 1
Antiproliferative and antiproteasomal activities of prepared salicylamides.

Series	Cmpd	n	Integrated amino acid/s	GI_{50} (μM)[a]			Proteasomal inhibition IC_{50} (μM)[a]
				K562	CEM	U266	
2	**a**	3	L-Leu-L-Leu-L-Leu	87.5	48.4	84.9	n.t.
2	**b**	3	L-Leu-L-Leu-D-Leu	>100	51.0	82.0	n.t.
2	**c**	3	L-Leu-L-Leu-L-Phe	70.3	31.4	72.2	n.t.
2	**d**	3	L-Leu-L-Phe-L-Leu	58.5	48.5	83.7	n.t.
2	**e**	3	L-Leu-L-Phe-D-Leu	>100	68.2	88.5	n.t.
2	**f**	3	L-Leu-L-Phe-L-Phe	72.0	48.0	82.1	n.t.
3	**a**	1	L-Leu	42.7	45.2	54.1	n.t.
3	**b**	1	L-Phe	50.9	65.2	92.3	n.t.
3	**c**	2	L-Leu-L-Leu	33.2	22.2	35.2	n.t.
3	**d**	2	L-Leu-L-Phe	22.5	19.5	81.9	n.t.
3	**e**	2	L-Phe-D-Leu	25.8	32.2	67.4	n.t.
3	**f**	2	L-Phe-L-Phe	29.6	28.6	72.1	n.t.
3	**g**	3	L-Leu-L-Leu-L-Leu	55.6	30.6	55.2	n.t.
3	**h**	3	L-Leu-L-Leu-D-Leu	34.0	11.5	23.7	n.t.
3	**i**	3	L-Leu-L-Leu-L-Phe	44.2	15.1	47.2	n.t.
3	**j**	3	L-Leu-L-Phe-L-Leu	16.4	19.0	48.6	n.t.
3	**k**	3	L-Leu-L-Phe-D-Leu	28.9	17.6	22.6	n.t.
3	**l**	3	L-Leu-L-Phe-L-Phe	34.7	35.7	56.3	n.t.
4	**a**	1	L-Leu	23.6	24.3	55.8	>1
4	**b**	1	L-Phe	33.3	17.7	63.8	>1
4	**c**	2	L-Leu-DL-Leu	21.0	16.3	37.0	>1
4	**d**	2	L-Leu-L-Phe	16.3	11.0	14.8	>1
4	**e**	2	L-Phe-DL-Leu	17.3	15.1	17.7	>1
4	**f**	2	L-Phe-L-Phe	27.1	15.5	21.7	>1
4	**g**	3	L-Leu-L-Leu-DL-Leu	0.61	0.49	0.29	0.099
4	**h**	3	L-Leu-L-Leu-L-Leu	0.76	0.41	0.20	0.057
4	**i**	3	L-Leu-L-Leu-D-Leu	0.98	0.58	0.38	0.182
4	**j**	3	L-Leu-L-Leu-DL-Phe	1.05	0.79	0.54	0.197
4	**k**	3	L-Leu-L-Phe-L-Leu	1.04	0.43	0.17	0.067
4	**l**	3	L-Leu-L-Phe-D-Leu	0.73	0.40	0.21	0.075
4	**m**	3	L-Leu-L-Phe-DL-Leu	0.57	0.28	0.15	0.090
4	**n**	3	L-Leu-L-Phe-L-Phe	1.45	0.44	0.40	0.110
4	**o**	3	L-Leu-L-Phe-DL-Phe	1.28	0.40	0.46	0.168
5	**a**	1	L-Leu	>100	58.6	>100	n.t.
5	**b**	1	L-Phe	99.1	55.5	72.4	n.t.
5	**c**	2	L-Leu-L-Leu	98.1	63.0	69.0	n.t.
5	**d**	2	L-Leu-L-Phe	93.6	48.2	79.2	n.t.
5	**e**	2	L-Phe-L-Leu	90.9	64.1	71.6	n.t.
5	**f**	2	L-Phe-DL-Phe	79.5	54.2	68.7	n.t.
5	**g**	3	L-Leu-L-Phe-L-Leu	30.9	17.2	32.8	n.t.
5	**h**	3	L-Leu-L-Phe-L-Phe	22.2	16.3	23.1	n.t.
6	**a**	2	L-Leu-L-Phe	57.9	27.7	64.9	>1
6	**b**	3	L-Leu-L-Phe-L-Leu	2.10	0.78	0.67	0.237
6	**c**	3	L-Leu-L-Phe-L-Phe	6.90	2.30	2.23	0.740
7	**a**	3	L-Leu-L-Phe-L-Leu	22.4	8.45	11.8	n.t.
7	**b**	3	L-Leu-L-Phe-L-Phe	27.3	10.8	18.9	n.t.
			Bortezomib	0.007	0.001	0.001	0.004

[a] Tested at least in duplicate; n.t. - not tested.

proteasomal inhibitors (see Fig. 1), together with strong anticancer cytotoxicity *in vitro,* especially of derivatives with terminal aldehyde function (series **4** and **6**), led us to the hypothesis that their mechanism of action may be connected with inhibition of proteasome. All active compounds were therefore subjected to different assays in order to address this hypothesis. Bortezomib and MG132 are known to bind with very high affinity to the β5 site of 20S proteasome with chymotrypsin-like activity. We therefore studied inhibition of the β5 site of the 20S proteasome subunit in U266 cells treated with most potent compounds (compounds **4** and **6**) using a luminescent assay that measured the chymotrypsin-like protease activity (Proteasome-Glo™ assay). Results in Table 1 show that the majority of compounds displayed IC_{50} in mid-nanomolar range (IC_{50} ranging from 57 to 197 nM), that is over 10 times higher than that of bortezomib. Compounds bearing phenylalanine near the terminal aldehyde group (both substituted and unsubstituted) showed higher IC_{50} values than compounds having L-leucine at this position. Importantly, proteasome inhibition correlated strongly with antiproliferative activity of all active compounds (**4g-o**, **6b-c**); linear regression coefficients R^2 of log transformed IC_{50} values equal to 0.716, 0.796 and 0.920 for K562, CEM and U266, respectively.

In addition, the effect of compounds **4** and **6** in U266 cells was studied also on the other two subunits of proteasome, namely β1-caspase-like and β2-trypsin-like. Cell-based assays using fluorogenic substrates revealed that the compounds display preference towards the chymotrypsin-like subunit (supplementary Table S1).

Supplementary video related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2017.04.027.

In order to confirm proteasome targeting by an independent method, we set up a cell-based assay involving green fluorescence protein (GFP) fused to a short degron [24] that is rapidly degraded by a proteasome; similar assays were reported recently [25–27]. Accumulation of GFP in cells treated with proteasome inhibitors (corresponding to proteasomal inhibition) was visualised and quantified by live-cell imaging. All results were compared with untreated cells and cells treated with bortezomib (Fig. 2A, B). As shown in Fig. 2A and Fig. S1, the GFP signal in cells emerged approximately 9 h after the compound addition and all tested compounds (**4g, j, k, m-o; 6b, c**) inhibited proteasomal activity in cells in a dose-dependent manner (see also Supplementary Video 1). One of the most potent compounds, **4m,** inhibited the proteasome activity already at 200 nM concentration. The maximum concentration used (6.25 μM) was toxic for cells after 16 h treatment; this was probably the reason why decreased GFP signal was observed (Fig. 2A).

We simultaneously monitored proliferation of treated cells over 72 h (Fig. 2A and Fig. S2). Our results showed that over the period when the inhibition of proteasome was evident, the proliferation was not altered. Compound **4m** (up to 1 μM concentration) did not interfere with proliferation during the first 24 h, while over the following 48 h cells ceased to proliferate and died. 200 nM concentration of **4m** did not influence the viability of treated cells over the full 72 h.

Next, we monitored the levels of polyubiquitinated proteins in treated cells by immunoblotting, using antibody specific towards free ubiquitin and ubiquitin conjugates, forming a typical high molecular mass smear. As shown in Fig. 3, compound **4m** induced accumulation of polyubiquitinated proteins in treated U266 cells and the observed effect displayed a time-dependent tendency, similarly to bortezomib. Besides stabilisation of unspecified proteins, we also observed a dose-dependent increase of $p27^{KIP}$, a short-lived cell cycle regulating protein, known to be degraded by proteasome [28]. In contrast, levels of proteins with slow turnover, such as β-actin and α-tubulin, remained unchanged (Fig. 3).

Finally, we studied the induction of cell death by **4m** in U266 cells. Bortezomib has been shown to promote apoptotic cell death in several cancer cell lines [29,30]. Activation of apoptosis was quantified biochemically by measuring the activity of caspases 3 and 7 in lysates of treated cells; results showed us that compound **4m** can activate apoptotic machinery in a time-dependent manner (Fig. 4). In addition, we analyzed the expression pattern of some apoptosis-relevant proteins. We found that caspase 7 and its substrate poly(ADP-ribose)polymerase (PARP-1) were cleaved, and Mcl-1 was downregulated in cells treated for 16–24 h (Fig. 3). These observations were in agreement with results from previous assays and in all aspects followed the trends observed with proteasomal inhibitor bortezomib.

2.4. Conclusion

A collection of 46 novel *O*-benzyl salicylamides built from leucine and phenylalanine was generated. The most potent compounds having terminal aldehyde group displayed nanomolar inhibitory activities against proteasome. We found a strong correlation between proteasome inhibition and antiproliferative effect in all three cell lines assayed. The most potent compounds demonstrated cellular effects corresponding to proteasome inhibition, including dose-dependent accumulation of polyubiquitinylated proteins and induction of apoptosis in multiple myeloma cells.

3. Experimental part

3.1. Chemistry

All reagents and solvents were purchased from commercial sources (TCI Europe, Sigma-Aldrich, Acros Organics, Fluorochem, Merck, Lach-Ner). Commercial grade reagents were used without further purification. Reactions were monitored by thin layer chromatography plates coated with 0.2 mm silica gel 60 F_{254} (Merck). TLC plates were visualised by the UV irradiation (254 nm). All melting points were determined on a Melting Point B-540 apparatus (Büchi, Switzerland) and are uncorrected. Specific optical rotation was determined on Perkin-Elmer Inc. Model 341 Polarimeter with Na lamp (589 nm) in ethyl acetate or water. The concentration of compound is displayed as g/100 mL. The IR spectra were recorded on a Nicolet 6700 FT-IR spectrometer (Thermo Fisher Scientific, Wal-tham, MA, USA) over the range of 400–4000 cm^{-1} using the ATR technique. The NMR spectra were measured in D_2O or CD_2Cl_2 or $CDCl_3$ or DMSO solutions at ambient temperature on a Bruker Avance™ III 400 spectrometer at frequencies ^1H (400 MHz) and ^{13}C (100.26 MHz) or Bruker Ascend™ 500 spectrometer at frequencies ^1H (500.13 MHz), $^{13}C\{^1H\}$ (125.76 MHz). The chemical shifts, δ, are given in ppm, related to the residual solvent peaks D_2O - 4.79, CD_2Cl_2 - 5.32, $CDCl_3$ - 7.27, DMSO - 2.5, eventually to tetramethylsilane (TMS) as an internal standard. The coupling constants (*J*) are reported in [Hz]. Elemental analyses (C, H, N) were performed on an automatic microanalyser Flash 2000 Organic elemental analyzer. Mass spectrometry with high resolution was determined by "dried droplet" method using MALDI mass spectrometer LTQ Orbitrap XL (Thermo Fisher Scientific) equipped with nitrogen UV laser (337 nm, 60 Hz). Spectra were measured in positive ion mode and in regular mass extent with resolution 100,000 at m/z = 400. 2,5-dihydrobenzoic acid (DBH) was used as the matrix.

3.2. General scheme and procedures for benzyl-protected methyl esters ***2a-f***

Chosen 5-Chloro-*O*-benzyl-salicylic acid-L-leucine-L-

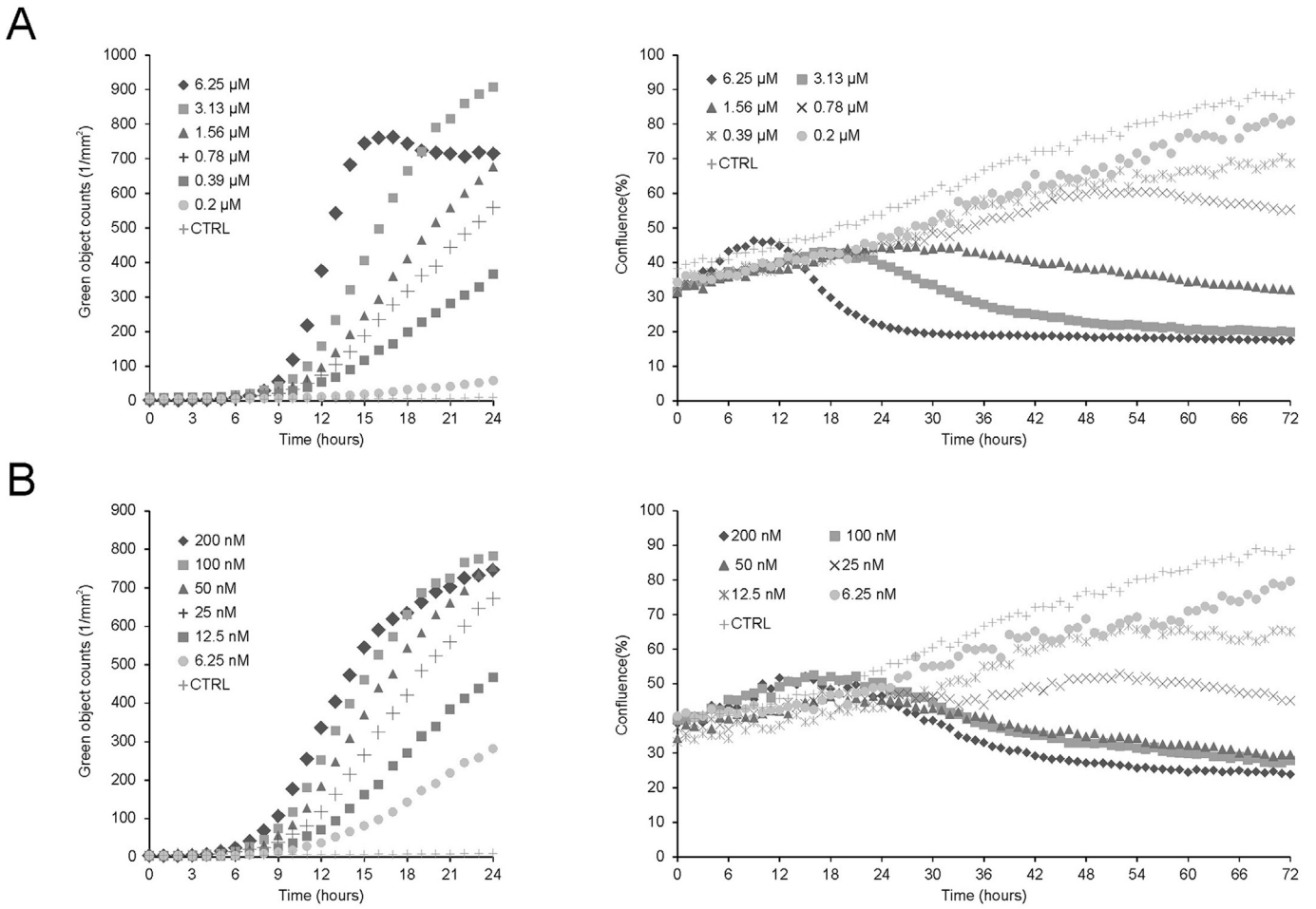


Fig. 2. Inhibition of ubiquitin–proteasome system by salicylamide **4m** (A) and bortezomib as a control (B) in U2OS cells. U2OS cells, stably expressing green fluorescence protein (GFP) fused to a short degron that is rapidly degraded by the proteasome, were treated with different doses of **4m** or bortezomib for 24 h. Inhibition of the proteasome caused dose-dependent accumulation of GFP in treated cells that was acquired with IncuCyte ZOOM® live-cell imaging microscope and expressed as a number of green objects per 1 mm^2 (left panels). Cell proliferation was monitored by analyzing the occupied area (% of confluence) of images over time up to 72 h (right panels).

phenylalanine acid **1** (3.015 g, 6.17 mmol) was dissolved in dichloromethane (100 mL) at ambient temperature. EDCI·HCl (1.183 g, 6.17 mmol) and HOBt (0.833 g, 6.17 mmol) were added. The L-phenylalanine amino acid methylester (0.896 g, 6.17 mmol) was added in the solution in dichloromethane (20 mL). The reaction mixture was stirred at RT for 3 h and then concentrated under reduced pressure. The crude product was dissolved in ethyl acetate (70 mL) and extracted with H_2O (2 × 30 mL), sat. $NaHCO_3$ (2 × 30 mL), 5% citric acid (2 × 30 mL), brine (1 × 30 mL) and H_2O (1 × 30 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography (silica gel 120 g, *n*-hexane/ethyl acetate (2:1)) to afford the product **2f** in yield 70% as white solid. Other derivatives **2a-f** were isolated as white solids in yields 72–94%.

*3.2.1. Characterization of (2S)-methyl 2-((2S)-2-((2S)-2-(2-(benzyloxy)-5-chlorobenzamido)-4-methylpentanamido)-4-methylpentanamido)-4-methylpentanoate **2a***

White solid; yield 89%; mp 139–143 °C; $[\alpha_D^{20}] = -37.3°$ (c 1.00, EtOAc); R_f (hex/EtOAc−1/1) = 0.53. **IR** (ATR):3293, 2957, 2361, 1744, 1638, 1533, 1470, 1272, 1244, 1159, 1009, 809, 697 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 8.16 (1H, d, *J* = 2.8 Hz, N*H*-CH-CHH-CH-$(CH_3)_2$), 8.20 (1H, d, *J* = 6.8 Hz, N*H*-CH-CHH-CH-$(CH_3)_2$), 7.41–7.47 (6H, m, N*H*–CH-CHH-CH-$(CH_3)_2$, Ar-*H*), 7.04 (1H, d, *J* = 8.8 Hz, Ar-*H*), 6.81 (2H, t, *J* = 9.2 Hz, Ar-*H*), 5.20–5.12 (2H, ABq, *J* = 10.4 Hz, O-C*H*$_2$-Ph), 4.60–4.51 (1H, m, NH-C*H*-CHH-CH-$(CH_3)_2$), 4.49–4.35 (2H, m, NH-C*H*-CHH-CH-$(CH_3)_2$, NH-C*H*-CHH-CH-$(CH_3)_2$), 3.72 (3H, s, O-C*H*$_3$), 1.78–1.70 (1H, m, NH-CH-C*H*H-CH-$(CH_3)_2$), 1.67–1.44 (6H, m, NH-CH-C*HH*-C*H*-$(CH_3)_2$, NH-CH-CH*H*-CH-$(CH_3)_2$, NH-CH-CH*H*-C*H*-$(CH_3)_2$), 1.44–1.31 (1H, m, NH-CH-CHH-C*H*-$(CH_3)_2$), 1.21–1.11 (1H, m, NH-CH-C*H*H-CH-$(CH_3)_2$, 0.95 (9H, m, 3xC*H*$_3$), 0.85 (3H, d, *J* = 6.4 Hz, CH_3-CH-C*H*$_3$), 0.79 (3H, d, *J* = 6.4 Hz, C*H*$_3$-CH-CH_3), 0.72 (3H, d, *J* = 6.4 Hz, CH_3-CH-C*H*$_3$). **^{13}C NMR** (100.62 MHz, $CDCl_3$-d_1): δ 173.2, 172.1, 171.7, 164.7, 134.9, 133.1, 132.3, 129.4, 129.3, 128.8, 128.6, 127.3, 122.2, 114.1, 72.1, 52.7, 52.4, 51.9, 50.9, 41.5, 40.3, 39.9, 24.93, 24.87, 23.1, 23.0, 22.05, 22.03. **CHN Analysis:** Calc. for $C_{33}H_{46}ClN_3O_6$ (616.19): C, 64.32; H, 7.52; N, 6.82. Found: C, 64.28 ± 0.02; H, 7.60 ± 0.02; N, 6.78 ± 0.02. **HRMS**: *m*/*z* calc. for $C_{33}H_{46}ClN_3O_6$: 616.31479 $[M+H]^+$; found: 616.31586 $[M+H]^+$.

*3.2.2. Characterization of (2R)-methyl 2-((2S)-2-((2S)-2-(2-(benzyloxy)-5-chlorobenzamido)-4-methylpentanamido)-4-methylpentanamido)-4-methylpentanoate **2b***

White solid; yield 72%; mp 160.4–163.1 °C; $[\alpha_D^{20}] = -16.3°$ (c 1.00, EtOAc); R_f (hex/EtOAc−1/1) = 0.51. **IR** (ATR): 3262, 3077, 2956, 2930, 2870, 1731, 1621, 1541, 1303, 1247, 1216, 1125, 1023, 750, 732, 715, 700, 660 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 8.22 (1H, d, *J* = 7.2 Hz, N*H*-CH-CHH- CH-$(CH_3)_2$), 8.20 (1H, d, *J* = 2.8 Hz, N*H*-CH-

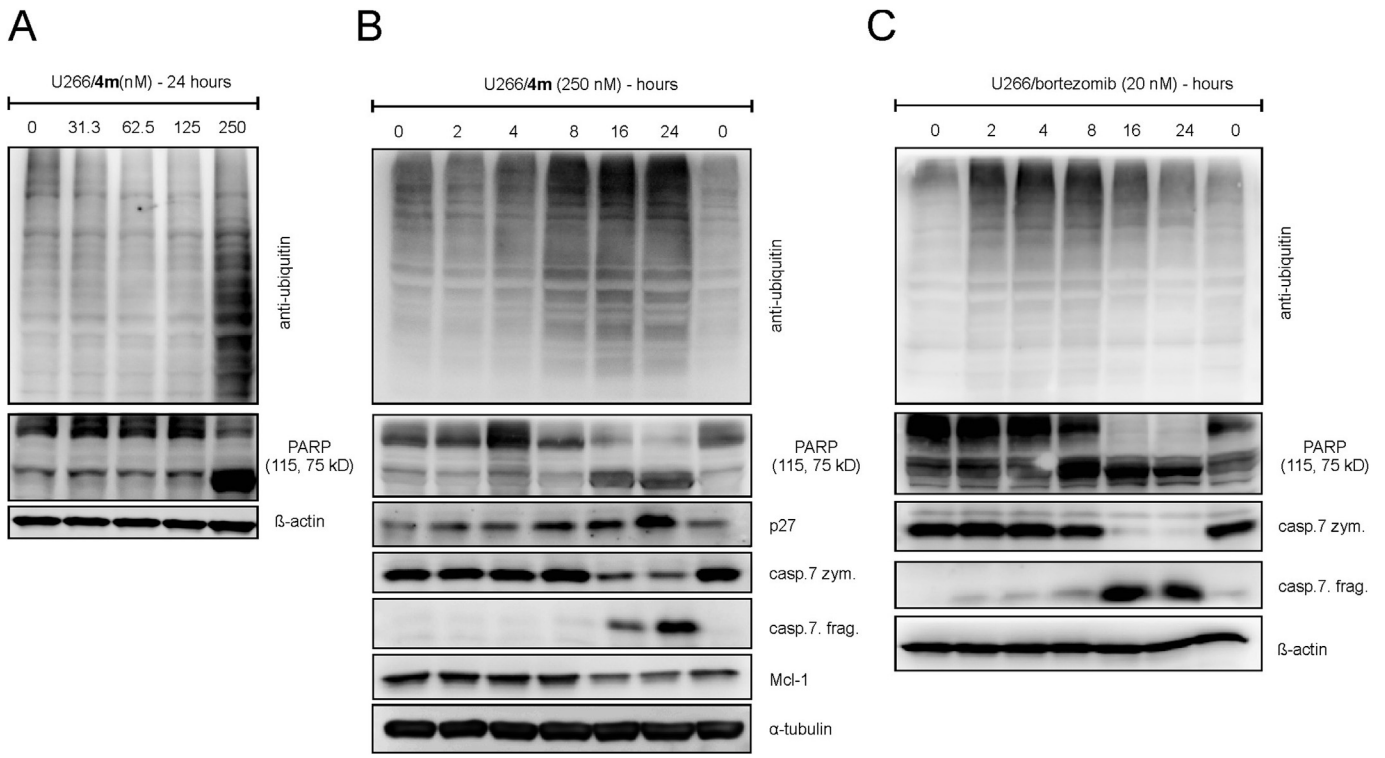


Fig. 3. Immunoblotting analysis of ubiquitinylated proteins and proteins involved in apoptosis in multiple myeloma U266 cells treated by compound **4m** in a dose (A) and a time (B) dependent manner. Bortezomib was used as a positive control (C). β-actin or α-tubulin levels are included as a control for equal loading.

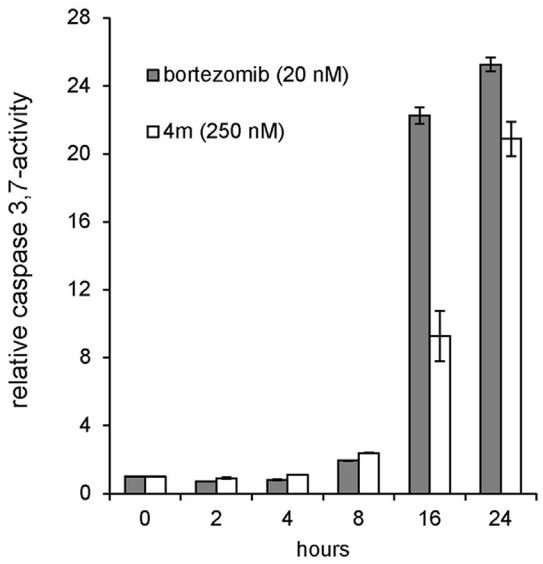


Fig. 4. Relative caspase-3/7 activity in U266 multiple myeloma cells treated with compound **4m** in a time dependent manner. Bortezomib was used as a positive control.

CHH-CH-$(CH_3)_2$), 7.54–7.47 (6H, m, NH-CH-CHH-CH-$(CH_3)_2$, Ar-H), 7.12 (1H, d, J = 8.8 Hz, Ar-H), 6.92 (1H, t, J = 8 Hz, Ar-H), 6.49 (1H, t, J = 8.4 Hz, Ar-H), 5.23–5.21 (2H, ABq, J = 8.8 Hz, O-CH_2-Ph), 4.65–4.57 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 4.56–4.48 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 4.45–4.38 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 3.73 (3H, s, O-CH_3), 1.91–1.82 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 1.81–1.68 (3H, m, NH-CH-CHH-CH-$(CH_3)_2$, NH-CH-CHH-CH-$(CH_3)_2$), 1.66–1.50 (3H, m, NH-CH-CHH-CH-$(CH_3)_2$, NH-CH-CHH-CH-$(CH_3)_2$), 1.47–1.35 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 1.26–1.16 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 1.01 (3H, d, J = 6.4 Hz, CH_3-CH-CH_3), 0.99 (3H, d, J = 6.4 Hz, CH_3-CH-CH_3), 0.96 (3H, d, J = 6.4 Hz, CH_3-CH-CH_3), 0.91 (3H, d, J = 6.4 Hz, CH_3-CH-CH_3), 0.83 (3H, d, J = 6.4 Hz, CH_3-CH-CH_3), 0.77 (3H, d, J = 6.4 Hz, CH_3-CH-CH_3). **^{13}C NMR** (100.62 MHz, $CDCl_3$-d_1): δ 173.5, 172.1, 171.9, 165.2, 155.8, 134.8, 133.3, 132.5, 129.6, 129.4, 128.7, 127.3, 122.1, 114.2, 72.2, 53.6, 52.3, 51.7, 51.0, 41.9, 40.3, 40.0, 25.1, 25.0, 24.9, 23.3, 23.1, 23.0, 21.9, 21.75, 21.68. **CHN Analysis:** Calc. for $C_{33}H_{46}ClN_3O_6$ (616.19): C, 64.32; H, 7.52; N, 6.82. Found: C, 64.36 ± 0.01; H 7.53 ± 0.01; N, 6.82 ± 0.01. **HRMS**: m/z calc. for $C_{33}H_{46}ClN_3O_6$: 638.2973 $[M+Na]^+$; found: 638.29778 $[M+Na]^+$.

3.2.3. Characterization of (2S)-methyl 2-((2S)-2-((2S)-2-(2-(benzyloxy)-5-chlorobenzamido)-4-methylpentanamido)-4-methylpentanamido)-3-phenylpropanoate **2c**

White solid; yield 74%; mp 161–163 °C; $[\alpha_D^{20}] = -12.9°$ (c 1.00, EtOAc); R_f (hex/EtOAc–1/1) = 0.51. **IR** (ATR): 3297, 2956, 1742, 1634, 1621, 1541, 1232, 1123, 996, 754, 736, 697 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 8.17 (1H, d, J = 2.8 Hz, NH-CH-CHH-CH-$(CH_3)_2$), 8.13 (1H, d, J = 6.8 Hz, NH-CH-CHH-CH-$(CH_3)_2$), 7.47–7.39 (6H, m, NH-CH-CHH-Ph, Ar-H), 7.30–7.19 (3H, m, Ar-H), 7.13–7.08 (2H, m, Ar-H), 7.04 (1H, d, J = 8.8 Hz, Ar-H), 6.68 (1H, d, J = 7.6 Hz, Ar-H), 6.63 (1H, d, J = 8 Hz, Ar-H), 5.19–5.12 (2H, ABq, J = 10.4 Hz, O-CH_2-Ph), 4.85–4.80 (1H, m, NH-CH-CH_2-Ph), 4.50–4.43 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 4.38–4.30 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 3.70 (3H, s, O-CH_3), 3.14 (1H, dd, J = 6 Hz, J = 14 Hz, NH-CH-CHH-Ph), 3.08 (1H, dd, J = 6 Hz, J = 14 Hz, NH-CH-CHH-Ph), 1.69–1.60 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 1.59–1.32 (4H, m, NH-CH-CHH-CH-$(CH_3)_2$, NH-CH-CHH-CH-$(CH_3)_2$), 1.24–1.13 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$, 0.86 (3H, d, J = 6.4 Hz, CH_3-CH-CH_3), 0.83–0.77 (6H, m, 2xCH_3), 0.72 (3H, d, J = 6.4 Hz, CH_3-CH-CH_3). **^{13}C NMR** (100.62 MHz, $CDCl_3$-d_1): δ 172.1, 171.8, 171.6, 164.7, 155.6, 136.0, 134.9, 133.1, 132.3, 129.44, 129.41, 129.3, 128.7, 128.6, 127.3, 122.3, 114.2, 72.1, 53.4, 52.5,

52.4, 52.0, 40.7, 39.7, 38.0, 24.87, 24.84, 23.1, 22.0, 21.9. **CHN Analysis:** Calc. for $C_{36}H_{44}ClN_3O_6$ (650.20): C, 66.50; H, 6.82; N, 6.46. Found: C, 66.3 ± 0.02; H, 6.83 ± 0.02; N, 6.35 ± 0.02. **HRMS**: *m/z* calc. for $C_{36}H_{44}ClN_3O_6$: 650.29914 $[M+H]^+$; found: 650.30049 $[M+H]^+$.

3.2.4. Characterization of (2S)-methyl 2-((2S)-2-((2S)-2-(2-(benzyloxy)-5-chlorobenzamido)-4-methylpentanamido)-3-phenylpropanamido)-4-methylpentanoate **2d**

White solid; yield 94%; mp 127–130 °C; $[\alpha_D^{20}] = -54.0°$ (c 1.00, EtOAc); R_f (hex/EtOAc–1/1) = 0.42. **IR** (ATR): 3292, 2956, 1744, 1645, 1525, 1272, 1226, 745, 701 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 8.06 (1H, d, *J* = 6.8 Hz, NH-CH-CHH-CH-$(CH_3)_2$), 8.04 (1H, d, *J* = 2.8 Hz, NH-CH-CHH- CH-$(CH_3)_2$), 7.48 (1H, d, *J* = 2.8 Hz, NH-CH-CHH-Ph), 7.46–7.37 (5H, m, Ar-H), 7.13–6.97 (6H, m, Ar-H), 6.75–6.65 (2H, m, Ar-H), 5.17–5.09 (2H, ABq, *J* = 10.4 Hz, O-CH_2-Ph), 4.68–4.62 (1H, m, NH-CH-CH_2-Ph), 4.58–4.51 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 4.38–4.31 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 3.69 (3H, s, O-CH_3), 3.7–3.03 (2H, m, NH-CH-CH_2-Ph), 1.67–1.40 (4H, m, NH-CH-CH_2-CH-$(CH_3)_2$, NH-CH-CH_2-CH-$(CH_3)_2$), 1.31–1.21 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 1.11–1.01 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 0.91 (6H, d, *J* = 6 Hz, 2xCH_3), 0.72 (3H, d, *J* = 6.4 Hz, CH_3-CH-CH_3), 0.65 (3H, d, *J* = 6.4 Hz, CH_3-CH-CH_3). **^{13}C NMR** (100.62 MHz, $CDCl_3$-d_1): δ 173.0, 171.8, 170.8, 164.8, 155.8, 136.7, 134.7, 133.2, 132.4, 129.5, 129.4, 129.3, 128.6, 128.5, 127.2, 126.9, 121.9, 114.1, 72.2, 54.0, 52.9, 52.4, 51.1, 41.2, 39.5, 37.2, 24.85, 24.81, 23.1, 23.0, 21.9, 21.7. **CHN Analysis:** Calc. for $C_{36}H_{44}ClN_3O_6$ (650.20): C, 66.50; H, 6.82; N, 6.46. Found: C, 66.34 ± 0.02; H, 7.01 ± 0.02; N, 6.35 ± 0.02. **HRMS**: *m/z* calc. for $C_{36}H_{44}ClN_3O_6$: 650.29914 $[M+H]^+$; found: 650.30134 $[M+H]^+$.

3.2.5. Characterization of (2R)-methyl 2-((2S)-2-((2S)-2-(2-(benzyloxy)-5-chlorobenzamido)-4-methylpentanamido)-3-phenylpropanamido)-4-methylpentanoate **2e**

White solid; yield 78%; mp 171.4–174.2 °C; $[\alpha_D^{20}] = -28.7°$ (c 1.00, EtOAc); R_f (hex/EtOAc–1/1) = 0.50. **IR** (ATR): 4, 3064, 2955, 2932, 2870, 1744, 1635, 1536, 1482, 1243, 1216, 1125, 1013, 802, 746, 720, 660 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 8.07 (1H, d, *J* = 2.4 Hz, NH-CH-CHH-CH-$(CH_3)_2$), 8.05 (1H, d, *J* = 5.2 Hz, NH-CH-CHH-CH-$(CH_3)_2$), 7.48 (1H, d, *J* = 2.8 Hz, NH-CH-CHH-Ph), 7.46–7.38 (5H, m, Ar-H), 7.15–7.01 (6H, m, Ar-H), 6.79 (1H, d, *J* = 8 Hz, Ar-H), 6.50 (1H, d, *J* = 8 Hz, Ar-H), 5.13–5.12 (2H, ABq, *J* = 8.2 Hz, O-CH_2-Ph), 4.76–4.67 (1H, m, NH-CH-CH_2-Ph), 4.60–4.52 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 4.28–4.21 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 3.69 (3H, s, O-CH_3), 3.16 (1H, dd, *J* = 6.4 Hz, *J* = 14.4 Hz, NH-CH-CHH-Ph), 3.08 (1H, dd, *J* = 7.6 Hz, *J* = 14.4 Hz, NH-CH-CHH-Ph), 1.68–1.56 (3H, m, NH-CH-CH_2-CH-$(CH_3)_2$, NH-CH-CHH-CH-$(CH_3)_2$), 1.39–1.30 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 1.23–1.14 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 1.03–0.96 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 0.95–0.87 (6H, m, 2xCH_3), 0.69 (3H, d, *J* = 6.4 Hz, CH_3-CH-CH_3), 0.63 (3H, d, *J* = 6.4 Hz, CH_3-CH-CH_3). **^{13}C NMR** (100.62 MHz, $CDCl_3$-d_1): δ 173.3, 171.7, 170.9, 165.1, 155.8, 136.8, 134.8, 133.3, 132.6, 129.6, 129.4, 129.2, 128.7, 128.6, 127.3, 126.8, 121.8, 114.0, 72.2, 53.6, 52.3, 51.1, 41.0, 39.7, 37.3, 24.8, 23.1, 22.9, 21.9, 21.6. **CHN Analysis:** Calc. for $C_{36}H_{44}ClN_3O_6$ (650.20): C, 66.5; H, 6.82; N, 6.46. Found: C, 66.67 ± 0.02; H 6.90 ± 0.01; N, 6.45 ± 0.02. **HRMS**: *m/z* calc. for $C_{36}H_{44}ClN_3O_6$: 672.28108 $[M+Na]^+$; found: 672.28256 $[M+Na]^+$.

3.2.6. Characterization of (2S)-methyl 2-((2S)-2-((2S)-2-(2-(benzyloxy)-5-chlorobenzamido)-4-methylpentanamido)-3-phenylpropanamido)-3-phenylpropanoate **2f**

White solid; yield 70%; mp 157–160 °C; $[\alpha_D^{20}] = -30.0°$ (c 1.00, EtOAc); R_f (hex/EtOAc–1/1) = 0.42. **IR** (ATR): 3283, 2957, 1638, 1529, 1216, 743, 702 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 8.19 (1H, d, *J* = 2.8 Hz, NH-CH-CHH-CH-$(CH_3)_2$), 8.08 (1H, d, *J* = 6.8 Hz, NH-CH-CHH-Ph), 7.54 (1H, d, *J* = 2.8 Hz, NH-CH-CHH-Ph), 7.52–7.42 (5H, m, Ar-H), 7.35–7.24 (3H, m, Ar-H), 7.18–7.06 (8H, m, Ar-H), 6.88 (1H, d, *J* = 7.6 Hz, Ar-H), 6.61 (1H, d, *J* = 8 Hz, Ar-H), 5.20–5.14 (2H, ABq, *J* = 10 Hz, O-CH_2-Ph), 4.91–4.83 (1H, m, NH-CH-CH_2-Ph), 4.72–4.64 (1H, m, NH-CH-CH_2-Ph), 4.46–4.39 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 3.74 (3H, s, O-CH_3), 3.20–3.02 (4H, m, NH-CH-CH_2-Ph, NH-CH-CH_2-Ph), 1.52–1.43 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$, 1.41–1.30 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$, 1.17–1.08 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 0.80 (3H, d, *J* = 6.4 Hz, CH_3-CH-CH_3), 0.70 (3H, d, *J* = 6.4 Hz, CH_3-CH-CH_3). **^{13}C NMR** (100.62 MHz, $CDCl_3$-d_1): δ 172.0, 171.6, 170.6, 164.6, 155.7, 136.8, 136.7, 136.1, 134.9, 134.8, 133.1, 132.5, 132.4, 129.5, 129.4, 129.3, 128.7, 128.6, 127.2, 126.8, 122.1, 114.1, 72.1, 54.2, 53.5, 52.5, 52.4, 39.3, 38.0, 37.5, 24.8, 23.0, 21.8. **CHN Analysis:** Calc. for $C_{39}H_{42}ClN_3O_6$ (684.22): C, 68.46; H, 6.19; N. 6.14. Found: C, 68.42 ± 0.02; H, 6.22 ± 0.02; N, 6.15 ± 0.02. **HRMS**: *m/z* calc. for $C_{39}H_{42}ClN_3O_6$: 684.28349 $[M+H]^+$; found: 684.28561 $[M+H]^+$.

3.3. General scheme and procedure for benzyl-protected Weinreb amides **3a-l**

According to [20], the L-phenylalanine methylester derivative (1.034 g, 2.44 mmol) was dissolved in a secured three-neck round-bottom flask in the suspension of *O,N*-dimethylhydroxylamine hydrochloride ($CH_3ONH(CH_3)\cdot HCl$, 0.595 g, 6.1 mmol) in dry tetrahydrofurane (45 mL) under nitrogen atmosphere. The reaction mixture was cooled to −20 °C and LiHMDS (1 M in dry THF, 12.2 mL, 12.2 mmol) was added dropwise via syringe pump over 15 min. The mixture was stirred at −20 °C for 1 h, then slightly heated to −10 °C and stirred for 2 h. The reaction mixture was quenched with saturated solution of NH_4Cl in H_2O (30 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organic phase was washed with H_2O (2 × 20 mL) and dried over Na_2SO_4. The crude product was purified by column chromatography (silica gel 110 g, *n*-hexane/ethyl acetate (1:1)) to afford L-phenylalanine Weinreb amide **3b** in a 69% yield as colourless oil. Other derivatives were isolated in following yields: **3a** in yield 70% as colourless oils, **3c-f** in yields 51–82% as colourless oils and **3g-l** in yields 72–91% as colourless oils or white solids. All derivatives were prepared under similar conditions except for those with three amino acids (**3g-l**). The equivalent of used LiHMDS (1 M in dry THF) was increased from 5 equivalents for compounds with one or two amino acids to 7 equivalents.

3.3.1. Characterization of (2S)-2-benzyloxy-5-chloro-N-(1-(methoxy(methyl)amino)-4-methyl-1-oxopentan-2-yl)benzamide **3a**

Colourless oil; yield 70%; $[\alpha_D^{20}] = -6.5°$ (c 1.00, EtOAc); R_f (hex/EtOAc - 1/1) = 0.47. **IR** (ATR): 3396, 2957, 1651, 1524, 1481, 1467, 1386, 1271, 1226, 1121, 988, 810, 751, 706 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 8.32 (1H, d, *J* = 8 Hz, NH-CH-CHH-CH-$(CH_3)_2$), 8.23 (1H, d, *J* = 2.8 Hz, Ar-H), 7.60–7.52 (2H, m, Ar-H), 7.51–7.40 (4H, m, Ar-H), 7.05 (1H, d, *J* = 8.8 Hz, Ar-H), 5.30–5.10 (3H, m, O-CH_2-Ph, NH-CH-CHH-CH-$(CH_3)_2$), 3.91 (3H, s, O-CH_3), 3.25 (3H, s, N-CH_3), 1.55–1.40 (2H, m, NH-CH-CHH-CH-$(CH_3)_2$, NH-CH-CH_2-CH-$(CH_3)_2$), 1.37–1.26 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$, 0.92 (3H, d, *J* = 6.4 Hz, CH_3-CH-CH_3), 0.79 (3H, d, *J* = 6.4 Hz, CH_3-CH-CH_3). **^{13}C NMR** (100.79 MHz, $CDCl_3$-d_1): δ 173.3, 164.0, 155.7, 135.2, 132.6, 132.3, 129.1, 129.0, 128.5, 127.0, 123.0, 114.3, 71.9, 61.7, 48.5, 41.2, 32.3, 25.0, 23.4, 21.7. **CHN Analysis:** Calc. for $C_{22}H_{27}ClN_2O_4$ (418.91): C, 63.08; H, 6.50; N, 6.69. Found: C, 62.95 ± 0.02; H 6.44 ± 0.02; N, 6.61 ± 0.02. **HRMS**: *m/z* calc. for $C_{22}H_{27}ClN_2O_4$: 441.15516 $[M+Na]^+$; found: 441.15505 $[M+Na]^+$.

3.3.2. Characterization of (2S)-2-benzyloxy-5-chloro-N-(1-(methoxy(methyl)amino)-1-oxo-3-phenylpropan-2-yl)benzamide ***3b***

Colourless oil; yield 69%; $[\alpha_D^{20}] = -10.1^\circ$ (c 1.00, EtOAc); R_f (hex/EtOAc−1/1) = 0.40. **IR** (ATR): 3389, 3030, 2939, 2362, 2340, 1645, 1519, 1480, 1270, 1228, 1121, 989, 810, 749, 699 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 8.53(1H, d, J = 7.2 Hz, N*H*-CH-CHH-Ph), 8.19 (1H, d, J = 2.8 Hz, Ar-*H*), 7.56–7.46 (5H, m, Ar-*H*), 7.46–7.41 (1H, m, Ar-*H*), 7.38 (1H, dd, J = 2.8 Hz, J = 8.8 Hz, Ar-*H*), 7.30–7.20 (2H, m, Ar-*H*), 7.08 (1H, d, J = 8.8 Hz, Ar-*H*), 7.03 (1H, d, J = 6.4 Hz, Ar-*H*), 6.98 (1H, d, J = 8.8 Hz, Ar-*H*), 5.47–5.37 (1H, m, NH-C*H*-CHH-Ph), 5.32–5.15 (2H, m, O-CH_2-Ph), 3.83 (3H, s, O-CH_3), 3.25 (3H, s, N-CH_3), 3.10 (1H, dd, J = 5.6 Hz, J = 13.6 Hz, NH-CH-CH*H*-Ph), 2.81 (1H, dd, J = 8 Hz, J = 13.6 Hz, NH-CH-C*H*H-Ph). **^{13}C NMR** (100.79 MHz, $CDCl_3$-d_1): 163.8, 155.6, 136.8, 135.5, 133.4, 132.6, 132.2, 129.4, 129.3, 129.2, 129.0, 128.5, 128.1, 127.0, 123.0, 114.5, 71.7, 61.7, 51.5, 38.0, 32.3. **CHN Analysis:** Calc. for $C_{25}H_{25}ClN_2O_4$ (452.93): C, 66.29; H, 5.56; N, 6.18. Found: C, 66.21 ± 0.02; H 5.61 ± 0.01; N, 5.97 ± 0.02. **HRMS**: m/z calc. for $C_{25}H_{25}ClN_2O_4$: 475.13951 $[M+Na]^+$; found: 475.14011 $[M+Na]^+$.

3.3.3. Characterization of 2-benzyloxy-5-chloro-N-((2S)-1-(((2S)-1-(methoxy(methyl)amino)-4-methyl-1-oxopentan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)benzamide ***3c***

Colourless oil; yield 51%; $[\alpha_D^{20}] = -20.4^\circ$ (c 1.00, EtOAc); R_f (hex/EtOAc−1/1) = 0.33. **IR** (ATR): 3306, 2958, 2360, 2342, 1643, 1526, 1480, 1272, 1228, 991, 698, 669 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 8.23 (1H, d, J = 2.8 Hz, N*H*-CH-CHH-CH-$(CH_3)_2$), 8.17 (1H, d, J = 7.6 Hz, N*H*-CH-CHH-CH-$(CH_3)_2$), 7.55–7.42 (6H, m, Ar-*H*), 7.06 (1H, d, J = 8.8 Hz, Ar-*H*), 6.75 (1H, d, J = 8.4 Hz, Ar-*H*), 5.23–5.20 (2H, ABq, J = 12.2 Hz, O-CH_2-Ph), 5.08–5.00 (1H, m, NH-C*H*-CHH-CH-$(CH_3)_2$), 4.67–4.57 (1H, m, NH-C*H*-CHH-CH-$(CH_3)_2$), 3.83 (3H, s, O-CH_3), 3.24 (3H, s, N-CH_3), 1.72–1.62 (1H, m, NH-CH-CH*H*-CH-$(CH_3)_2$), 1.62–1.52 (3H, m, 2 x NH-C*H*-CHH-CH-$(CH_3)_2$, NH-CH-CH*H*-CH-$(CH_3)_2$), 1.52–1.42 (1H, m, NH-CH-C*H*H-CH-$(CH_3)_2$), 1.35–1.24(1H, m, NH-CH-C*H*H-CH-$(CH_3)_2$), 0.96 (3H, d, J = 6.4 Hz, C*H*$_3$-CH-CH$_3$), 0.95 (3H, d, J = 6.4 Hz, CH$_3$-CH-C*H*$_3$), 0.87 (3H, d, J = 6.4 Hz, C*H*$_3$-CH-CH$_3$), 0.78 (3H, d, J = 6.4 Hz, CH$_3$-CH-C*H*$_3$). **^{13}C NMR** (100.79 MHz, $CDCl_3$-d_1): δ 172.0, 164.2, 155.6, 135.1, 132.8, 132.4, 129.2, 128.5, 127.1, 122.8, 114.1, 72.0, 61.8, 52.4, 47.9, 41.9, 40.5, 32.4, 24.9, 24.8, 23.5, 23.2, 21.9, 21.8. **CHN Analysis:** Calc. for $C_{28}H_{38}ClN_3O_5$ (532.07): C, 63.21; H, 7.20; N, 7.90. Found: C, 63.24 ± 0.01; H 7.38 ± 0.02; N, 7.89 ± 0.02. **HRMS**: m/z calc. for $C_{28}H_{38}ClN_3O_5$: 554.23922 $[M+Na]^+$; found: 554.24014 $[M+Na]^+$.

3.3.4. Characterization of 2-benzyloxy-5-chloro-N-((2S)-1-(((2S)-1-(methoxy(methyl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)benzamide ***3d***

Colourless oil; yield 82%; $[\alpha_D^{20}] = -30.6^\circ$ (c 1.00, EtOAc); R_f (hex/EtOAc−1/1) = 0.38. **IR** (ATR): 3296, 2926, 1643, 1544, 1481, 1247, 1226, 989, 700 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.21 (1H, d, J = 2.8 Hz, N*H*-CH-CHH-CH-$(CH_3)_2$), 8.03 (1H, d, J = 7.2 Hz, N*H*-CH-CHH-Ph), 7.45–7.37 (6H,m, Ar-*H*), 7.15–7.08 (5H, m, Ar-*H*), 7.02 (1H, d, J = 8.8 Hz, Ar-*H*), 6.94 (1H, d, J = 8 Hz, Ar-*H*), 5.23–5.17 (1H, m, NH-C*H*-CHH-Ph), 5.17–5.14 (2H, ABq, J = 10.4 Hz, O-CH_2-Ph), 4.56–4.47 (1H, m, NH-C*H*-CHH-CH-$(CH_3)_2$), 3.69 (3H, s, O-CH_3), 3.18 (3H, s, N-CH_3), 3.12–3.05 (1H, m, NH-CH-C*H*H-Ph), 2.95–2.85 (1H, m, NH-CH-CH*H*-Ph), 1.50–1.41 (1H, m, NH-CH-C*H*H-CH-$(CH_3)_2$), 1.40–1.32 (1H, m, NH-CH-CHH-C*H*-$(CH_3)_2$), 1.21–1.12 (1H, m, NH-CH-C*H*H-CH-$(CH_3)_2$), 0.78 (3H, d, J = 6.4 Hz, C*H*$_3$-CH-CH$_3$), 0.70 (3H, d, J = 6.4 Hz, CH$_3$-CH-C*H*$_3$). **^{13}C NMR** (100.62 MHz, $CDCl_3$): δ 171.6, 164.2, 155.6, 136.5, 135.0, 132.8, 132.5, 129.6, 129.5, 129.3, 128.5, 128.4, 127.1, 126.9, 122.6, 114.1, 72.0 (2xC), 61.8, 52.2, 50.8, 40.0, 38.3, 32.3, 24.8, 23.1, 21.8. **CHN Analysis:** Calc. for $C_{31}H_{36}ClN_3O_5$ (566.09): C, 65.77; H, 6.41; N, 7.42. Found: C, 65.81 ± 0.02; H 6.40 ± 0.01; N, 7.42 ± 0.02. **HRMS**: m/z calc. for $C_{31}H_{36}ClN_3O_5$: 588.22357 $[M+Na]^+$; found: 588.22384 $[M+Na]^+$.

3.3.5. Characterization of 2-benzyloxy-5-chloro-N-((2S)-1-(((2S)-1-(methoxy(methyl)amino)-4-methyl-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)benzamide ***3e***

Colourless oil; yield 74%; $[\alpha_D^{20}] = -15.0^\circ$ (c 1.00, EtOAc); R_f (hex/EtOAc−1/1) = 0.27. **IR** (ATR): 3306, 2958, 2360, 2342, 1690, 1514, 1469, 1271, 1225, 988, 742, 698, 669 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 8.34 (1H, d, J = 7.2 Hz, N*H*-CH-CHH-CH-$(CH_3)_2$), 8.15 (1H, d, J = 2.8 Hz, N*H*-CH-CHH-Ph), 7.45–7.30 (6H, m, Ar-*H*), 7.24–7.13 (3H, m, Ar-*H*), 7.06–7.00 (2H, m, Ar-*H*), 6.91 (1H, d, J = 8.8 Hz, Ar-*H*), 6.65 (1H, d, J = 8.4 Hz, Ar-*H*), 5.17–5.12 (2H, ABq, J = 16.2 Hz, O-CH_2-Ph), 5.07–4.96 (1H, m, NH-C*H*-CHH-Ph), 4.90–4.82 (1H, m, NH-C*H*-CHH-CH-$(CH_3)_2$), 3.77 (3H, s, O-CH_3), 3.20 (3H, s, N-CH_3), 3.11 (1H, dd, J = 8 Hz, J = 14 Hz, NH-CH-CH*H*-Ph), 2.88 (1H, dd, J = 8 Hz, J = 14 Hz, NH-CH-C*H*H-Ph), 1.63–1.52 (1H, m, NH-CH-CH*H*-CH-$(CH_3)_2$), 1.51–1.43 (2H, m, 2xNH-C*H*-CHH-CH-$(CH_3)_2$, NH-CH-C*H*H-CH-$(CH_3)_2$), 0.91 (3H, d, J = 6.4 Hz, C*H*$_3$-CH-CH$_3$), 0.87 (3H, d, J = 6.4 Hz, CH$_3$-CH-C*H*$_3$). **^{13}C NMR** (100.79 MHz, $CDCl_3$-d_1): δ 170.6, 163.9, 155.3, 136.8, 135.2, 132.6, 132.1, 129.2, 129.0, 128.8, 128.4, 127.6, 126.9, 126.7, 122.6, 114.3, 71.5, 61.6, 55.1, 47.7, 41.8, 37.5, 32.1, 24.8, 23.2, 21.8. **CHN Analysis:** Calc. for $C_{31}H_{36}ClN_3O_5$ (566.09): C, 65.77; H, 6.41; N, 7.42. Found: C, 65.75 ± 0.01; H 6.51 ± 0.01; N, 7.36 ± 0.02. **HRMS**: m/z calc. for $C_{31}H_{36}ClN_3O_5$: 588.22357 $[M+Na]^+$; found: 588.22380 $[M+Na]^+$.

3.3.6. Characterization of 2-benzyloxy-5-chloro-N-((2S)-1-(((2S)-1-(methoxy(methyl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)benzamide ***3f***

Colourless oil; yield 54%; $[\alpha_D^{20}] = -26.2^\circ$ (c 1.00, EtOAc); R_f (hex/EtOAc−1/1) = 0.36. **IR** (ATR): 3397, 2360, 2342, 1646, 1521, 1508, 989, 742, 699 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 8.32 (1H, d, J = 7.6 Hz, N*H*-CH-CHH-Ph), 8.22 (1H, d, J = 2.8 Hz, N*H*-CH-CHH-Ph), 7.50–7.35 (6H, m, Ar-*H*), 7.27–7.10 (8H, m, Ar-*H*), 7.06–6.95 (4H, m, Ar-*H*), 5.30–5.21 (1H, m, NH-C*H*-CHH-Ph), 5.18–5.14 (2H, ABq, J = 16.2 Hz, O-CH_2-Ph), 4.92–4.84 (1H, m, NH-C*H*-CHH-Ph), 3.71 (3H, s, O-CH_3), 3.23 (3H, s, N-CH_3), 3.15–3.06 (2H, m, NH-CH-C*H*H-Ph, NH-CH-C*H*H-Ph), 3.00–2.92 (1H, m, NH-CH-CH*H*-Ph), 2.84 (1H, dd, J = 8 Hz, J = 14 Hz, NH-CH-CH*H*-Ph). **^{13}C NMR** (100.79 MHz, $CDCl_3$-d_1): δ 170.6, 164.2, 155.5, 137.0, 136.4, 135.3, 132.8, 132.4, 129.6, 129.2, 129.0, 128.6, 128.4, 127.9, 127.1, 126.9, 126.87, 122.6, 114.5, 71.7 (2xC), 61.8, 55.0, 50.7, 38.4, 37.2, 32.3. **CHN Analysis:** Calc. for $C_{34}H_{34}ClN_3O_5$ (600.10): C, 68.05; H, 5.71; N, 7.00. Found: C, 67.92 ± 0.02; H 5.78 ± 0.01; N, 6.90 ± 0.01. **HRMS**: m/z calc. for $C_{34}H_{34}ClN_3O_5$: 622.20792 $[M+Na]^+$; found: 622.20888 $[M+Na]^+$.

3.3.7. Characterization of N-((5S,8S,11S)-5,8-diisobutyl-3,13-dimethyl-4,7,10-trioxo-2-oxa-3,6,9-triazatetradecan-11-yl)-2-benzyloxy-5-chlorobenzamide ***3g***

Colourless oil; yield 75%; $[\alpha_D^{20}] = -39.7^\circ$ (c 1.00, EtOAc); R_f (hex/EtOAc−1/1) = 0.33. **IR** (ATR): 3293, 2957, 2360, 2342, 1640, 1527, 1468, 1272, 1234, 1122, 990, 809, 749, 696 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 8.16 (1H, d, J = 2.8 Hz, N*H*-CH-CHH-CH-$(CH_3)_2$), 8.12 (1H, d, J = 7.2 Hz, N*H*-CH-CHH-CH-$(CH_3)_2$), 7.48–7.38 (6H, m, N*H*-CH-CHH-CH-$(CH_3)_2$, Ar-*H*), 7.02 (1H, d, J = 8.8 Hz, Ar-*H*), 6.69 (1H, d, J = 8 Hz, Ar-*H*), 6.61 (1H, d, J = 8 Hz, Ar-*H*), 5.16–5.13 (2H, ABq, J = 10.4 Hz, O–CH_2-Ph), 5.30–4.96 (1H, m, NH-C*H*-CHH-CH-$(CH_3)_2$), 4.53–4.46 (1H, m, NH-C*H*-CHH-CH-$(CH_3)_2$), 4.43–4.35 (1H, m, NH-C*H*-CHH-CH-$(CH_3)_2$), 3.78 (3H, s, O-CH_3), 3.18 (3H, s, N-CH_3), 1.70–1.45 (7H, m, 3xNH-CH-CH_2-CH-$(CH_3)_2$, NH-CH-CH$_2$-C*H*-$(CH_3)_2$), 1.45–1.34 (1H, m, NH-CH-CH$_2$-C*H*-$(CH_3)_2$), 1.27–1.15 (1H, m, NH-CH-CH$_2$-C*H*-$(CH_3)_2$), 0.93 (3H, d, J = 6.8 Hz, C*H*$_3$-CH-CH$_3$), 0.91 (3H, d, J = 6.8 Hz, CH$_3$-CH-C*H*$_3$), 0.87 (3H, d, J = 6.4 Hz, C*H*$_3$-CH-

CH_3), 0.83 (3H, d, J = 6.4 Hz, CH_3-CH-C$\underline{H}_3$), 0.80 (3H, d, J = 6.4 Hz, C$\underline{H}_3$-CH-CH_3), 0.73 (3H, d, J = 6.4 Hz, CH_3-CH-C$\underline{H}_3$). **^{13}C NMR** (100.62 MHz, $CDCl_3$-d_1): δ 173.0, 172.0, 171.8, 164.5, 155.6, 134.9, 133.0, 132.3, 129.4, 129.3, 128.6, 127.2, 122.5, 114.2, 72.1, 61.7, 52.4, 51.9, 47.8, 41.9, 40.9, 39.3, 32.3, 24.9, 23.5, 23.2, 23.0, 22.0, 21.8. **CHN Analysis:** Calc. for $C_{34}H_{49}ClN_4O_6$ (645.23): C, 63.29; H, 7.65; N, 8.68. Found: C, 63.02 ± 0.02; H 7.84 ± 0.02; N, 8.58 ± 0.02. **HRMS**: m/z calc. for $C_{34}H_{49}ClN_4O_6$: 667.32328 $[M+Na]^+$; found: 667.32208 $[M+Na]^+$.

3.3.8. Characterization of N-((5R,8S,11S)-5,8-diisobutyl-3,13-dimethyl-4,7,10-trioxo-2-oxa-3,6,9-triazatetradecan-11-yl)-2-benzyloxy-5-chlorobenzamide **3h**

Colourless oil; yield 83%; $[\alpha_D^{20}] = -18.0°$ (c 1.00, EtOAc); R_f (hex/EtOAc−1/1) = 0.29. **IR** (ATR): 3379, 3297, 3067, 2956, 2935, 2870, 1620, 1521, 1467, 1271, 128, 988, 809, 750, 696, 534 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 8.24 (1H, d, J = 2.8 Hz, N$\underline{H}$-CH-CHH-CH-$(CH_3)_2$), 8.20 (1H, d, J = 6 Hz, N$\underline{H}$-CH-CHH-CH-$(CH_3)_2$), 7.55–7.43 (6H, m, N$\underline{H}$-CH-CHH-CH-$(CH_3)_2$, Ar-$\underline{H}$), 7.09 (1H, d, J = 8.8 Hz, Ar-$\underline{H}$), 6.89 (1H, d, J = 8.4 Hz, Ar-$\underline{H}$), 6.78 (1H, d, J = 8 Hz, Ar-$\underline{H}$), 5.22–5.20 (2H, ABq, J = 10.4 Hz, O-C$\underline{H}_2$-Ph), 5.05–4.93 (1H, m, NH-C$\underline{H}$-CHH-CH-$(CH_3)_2$), 4.58–4.50 (1H, m, NH-C$\underline{H}$-CHH-CH-$(CH_3)_2$), 4.50–4.43 (1H, m, NH-C$\underline{H}$-CHH-CH-$(CH_3)_2$), 3.86 (3H, s, O-C$\underline{H}_3$), 3.23 (3H, s, N-C$\underline{H}_3$), 1.85–1.72 (2H, m, 2xNH-CH-CH$\underline{H}$-CH-$(CH_3)_2$), 1.70–1.63 (1H, m, NH-CH-CH$\underline{H}$-CH-$(CH_3)_2$), 1.62–1.51 (4H, m, 3xNH-CH-C$\underline{H}$H-CH-$(CH_3)_2$, NH-CH-CH_2-C$\underline{H}$-$(CH_3)_2$), 1.47–1.37 (1H, m, NH-CH-C$\underline{H}_2$-C$\underline{H}$-$(CH_3)_2$), 1.27–1.18 (1H, m, NH-CH-CH_2-C$\underline{H}$-$(CH_3)_2$), 1.01 (3H, d, J = 6.4 Hz, C$\underline{H}_3$-CH-CH_3), 1.00 (3H, d, J = 6.4 Hz, CH_3-CH-C$\underline{H}_3$), 0.95 (3H, d, J = 6.4 Hz, C$\underline{H}_3$-CH-CH_3), 0.91 (3H, d, J = 6.4 Hz, CH_3-CH-C$\underline{H}_3$), 0.84 (3H, d, J = 6.4 Hz, C$\underline{H}_3$-CH-CH_3), 0.78 (3H, d, J = 6.4 Hz, CH_3-CH-C$\underline{H}_3$). **^{13}C NMR** (100.62 MHz, $CDCl_3$-d_1): δ 173.3, 172.3, 172.1, 165.0, 155.7, 134.9, 133.1, 132.5, 129.4, 129.3, 128.7, 127.2, 122.4, 114.1, 72.2, 61.7, 53.2, 51.8, 48.3, 41.2, 40.9, 40.0, 32.4, 25.1, 25.0, 24.9, 23.5, 23.3, 23.1, 21.8, 21.7. **CHN Analysis:** Calc. for $C_{34}H_{49}ClN_4O_6$ (645.23): C, 63.29; H, 7.65; N, 8.68. Found: C, 63.08 ± 0.02; H 7.74 ± 0.02; N, 8.49 ± 0.02. **HRMS**: m/z calc. for $C_{34}H_{49}ClN_4O_6$: 667.32328 $[M+Na]^+$; found: 667.32406 $[M+Na]^+$.

3.3.9. Characterization of N-((5S,8S,11S)-5-benzyl-8-isobutyl-3,13-dimethyl-4,7,10-trioxo-2-oxa-3,6,9-triazatetradecan-11-yl)-2-benzyloxy-5-chlorobenzamide **3i**

Colourless oil; yield 72%; $[\alpha_D^{20}] = -29.7°$ (c 1.00, EtOAc); R_f (hex/EtOAc−1/1) = 0.36. **IR** (ATR): 3735, 3648, 3628, 3587, 3566, 3300, 2956, 2871, 2360, 2342, 1642, 1525, 1481, 1456, 1272, 1227, 990, 810, 750, 699, 669 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 8.18 (1H, d, J = 2.4 Hz, N$\underline{H}$-CH-CHH-CH-$(CH_3)_2$), 8.15 (1H, d, J = 6 Hz, N$\underline{H}$-CH-CHH-CH-$(CH_3)_2$), 7.48–7.36 (6H, m, N$\underline{H}$-CH-CHH-Ph, Ar-$\underline{H}$), 7.27–7.23 (2H, m, Ar-$\underline{H}$), 7.22–7.17 (1H, m, Ar-$\underline{H}$), 7.16–7.12 (2H,m, Ar-$\underline{H}$), 7.02 (1H, d, J = 7.2 Hz, Ar-$\underline{H}$), 6.95–6.90 (1H, m, Ar-$\underline{H}$), 6.85–6.80 (1H,m, Ar-$\underline{H}$), 5.25–5.19 (1H, m, NH-C$\underline{H}$-CHH-Ph), 5.16–5.13 (2H, ABq, J = 7.6 Hz, O-C$\underline{H}_2$-Ph), 4.56–4.49 (1H, m, NH-C$\underline{H}$-CHH-CH-$(CH_3)_2$), 4.43–4.36 (1H, m, NH-C$\underline{H}$-CHH-CH-$(CH_3)_2$), 3.63 (3H, s, O-C$\underline{H}_3$), 3.15 (3H, s, N-C$\underline{H}_3$), 3.10–3.04 (1H, m, NH-CH-C$\underline{H}$H-Ph), 2.97–2.91 (1H, m, NH-CH- C$\underline{H}$H-Ph), 1.62–1.43 (4H, m, 2 x NH-CH-C$\underline{H}_2$-CH-$(CH_3)_2$, 1.43–1.35 (1H, m, NH-CH-CH_2-C$\underline{H}$-$(CH_3)_2$), 1.27–1.18 (1H, m, NH-CH-CH_2-C$\underline{H}$-$(CH_3)_2$), 0.85 (3H, d, J = 5.2 Hz, C$\underline{H}_3$-CH-CH_3), 0.80 (6H, d, J = 4.8 Hz, C$\underline{H}_3$-CH-C$\underline{H}_3$), 0.73 (3H, d, J = 5.2 Hz, CH_3-CH-C$\underline{H}_3$). **^{13}C NMR** (100.61 MHz, $CDCl_3$-d_1): δ 172.0, 171.5, 164.6, 155.6, 136.4, 134.9, 133.0, 132.4, 132.1, 129.6, 129.4, 129.3, 128.5, 127.2, 127.0, 122.4, 114.2, 72.1, 61.7, 52.3, 52.0, 50.4, 41.1, 39.7, 38.4, 32.3, 24.8, 23.2, 23.1, 22.0, 21.9. **CHN Analysis:** Calc. for $C_{37}H_{47}ClN_4O_6$ (679.25): C, 65.42; H, 6.97; N, 8.25. Found: C, 65.38 ± 0.02; H 6.92 ± 0.01; N, 8.23 ± 0.02. **HRMS**: m/z calc. for $C_{37}H_{47}ClN_4O_6$: 701.30763 $[M+Na]^+$; found: 701.30860 $[M+Na]^+$.

3.3.10. Characterization of N-((5S,8S,11S)-8-benzyl-5-isobutyl-3,13-dimethyl-4,7,10-trioxo-2-oxa-3,6,9-triazatetradecan-11-yl)-2-benzyloxy-5-chlorobenzamide **3j**

White solid; yield 74%; mp 63.3–65.6 °C; $[\alpha_D^{20}] = -43.9°$ (c 1.00, EtOAc); R_f (hex/EtOAc−1/1) = 0.35. **IR** (ATR): 3297, 3067, 2957, 2871, 2362, 1621, 1529, 1500, 1482, 1272, 1234, 1122, 989, 743, 699 cm^{-1}. **^{1}H NMR** (500 MHz, $CDCl_3$-d_1): δ 8.29 (1H, d, J = 3 Hz, N$\underline{H}$-CH-CHH-CH-$(CH_3)_2$), 8.20 (1H, d, J = 6.5 Hz, N$\underline{H}$-CH-CHH-CH-$(CH_3)_2$), 7.67–7.55 (6H, m, N$\underline{H}$-CH-CHH-Ph, Ar-$\underline{H}$), 7.32–7.27 (2H, m, Ar-$\underline{H}$), 7.34–7.17 (4H, m, Ar-$\underline{H}$), 7.06 (1H, d, J = 8 Hz, Ar-$\underline{H}$), 6.84 (1H, d, J = 8.5 Hz, Ar-$\underline{H}$), 5.31–5.29 (2H, ABq, J = 7.6 Hz, O-C$\underline{H}_2$-Ph), 5.21–5.15 (1H, m, NH-C$\underline{H}$-CHH-Ph), 4.88–4.80 (1H, m, NH-C$\underline{H}$-CHH-CH-$(CH_3)_2$), 4.64–4.57 (1H, m, NH-C$\underline{H}$-CHH-CH-$(CH_3)_2$), 3.96 (3H, s, O-C$\underline{H}_3$), 3.37 (3H, s, N-C$\underline{H}_3$), 3.32–3.29 (2H, m, NH-CH-C$\underline{H}_2$-Ph), 1.77–1.58 (4H, m, 2xNH-CH-C$\underline{H}_2$-CH-$(CH_3)_2$, 1.52–1.42 (1H, m, NH-CH-CH_2-C$\underline{H}$-$(CH_3)_2$), 1.29–1.22 (1H, m, NH-CH-CH_2-C$\underline{H}$-$(CH_3)_2$), 1.11 (3H, d, J = 6.5 Hz, CH_3-CH-C$\underline{H}_3$), 1.08 (3H, d, J = 6.5 Hz, CH_3-CH-C$\underline{H}_3$), 0.92 (3H, d, J = 6.5 Hz, CH_3-CH-C$\underline{H}_3$), 0.84 (3H, d, J = 6.5 Hz, CH_3-CH-C$\underline{H}_3$). **^{13}C NMR** (125.77 MHz, $CDCl_3$-d_1): δ 172.6, 171.7, 170.5, 164.4, 155.5, 136.5, 134.5, 132.9, 132.3, 129.3, 129.2, 128.4, 128.3, 127.0, 126.6, 121.8, 113.8, 71.9, 61.6, 54.0, 52.1, 47.7, 41.5, 39.1, 37.3, 32.1, 24.7, 24.6, 23.3, 22.8, 21.6. **CHN Analysis:** Calc. for $C_{37}H_{47}ClN_4O_6$ (679.25): C, 65.42; H, 6.97; N, 8.25. Found: C, 65.60 ± 0.02; H 7.00 ± 0.01; N, 8.21 ± 0.02. **HRMS**: m/z calc. for $C_{37}H_{47}ClN_4O_6$: 701.30763 $[M+Na]^+$; found: 701.30958 $[M+Na]^+$.

3.3.11. Characterization of N-((5R,8S,11S)-8-benzyl-5-isobutyl-3,13-dimethyl-4,7,10-trioxo-2-oxa-3,6,9-triazatetradecan-11-yl)-2-benzyloxy-5-chlorobenzamide **3k**

Colourless oil; yield 91%; $[\alpha_D^{20}] = -27.0°$ (c 1.00, EtOAc); R_f (hex/EtOAc−1/1) = 0.35. **IR** (ATR): 3377, 3293, 3064, 2955, 2933, 2869, 1630, 1519, 1481, 1467, 1385, 1270, 1226, 987, 808, 744, 697, 534 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 8.23 (1H, d, J = 2.8 Hz, N$\underline{H}$-CH-CHH-CH-$(CH_3)_2$), 8.09 (1H, d, J = 5.6 Hz, N$\underline{H}$-CH-CHH-CH-$(CH_3)_2$), 7.53–7.43 (6H, m, N$\underline{H}$-CH-CHH-Ph, Ar-$\underline{H}$), 7.24–7.11 (5H, m, Ar-$\underline{H}$), 7.09 (1H, d, J = 8.8 Hz, Ar-$\underline{H}$), 6.92 (1H, d, J = 8 Hz, Ar-$\underline{H}$), 6.77 (1H, d, J = 8 Hz, Ar-$\underline{H}$), 5.18–5.17 (2H, ABq, J = 8.8 Hz, O-C$\underline{H}_2$-Ph), 5.02–4.92 (1H, m, NH-C$\underline{H}$-CHH-Ph), 4.84–4.75 (1H, m, NH-C$\underline{H}$-CHH-CH-$(CH_3)_2$), 4.44–4.36 (1H, m, NH-C$\underline{H}$-CHH-CH-$(CH_3)_2$), 3.87 (3H, s, O-C$\underline{H}_3$), 3.35–3.20 (4H, m, N-C$\underline{H}_3$, NH-CH-CH$\underline{H}$-Ph), 3.01 (1H, dd, J = 8.4 Hz, J = 14 Hz, NH-CH-C$\underline{H}$H-Ph), 1.68–1.45 (3H, m, NH-CH-C$\underline{H}_2$-CH-$(CH_3)_2$, NH-CH-CH$\underline{H}$-CH-$(CH_3)_2$), 1.44–1.35 (1H, m, NH-CH-C$\underline{H}$H-CH-$(CH_3)_2$), 1.34–1.25 (1H, m, NH-CH-CH_2-C$\underline{H}$-$(CH_3)_2$), 1.11–1.01 (1H, m, NH-CH-CH_2-C$\underline{H}$-$(CH_3)_2$), 0.98 (3H, d, J = 6.4 Hz, CH_3-CH-C$\underline{H}_3$), 0.97 (3H, d, J = 6.4 Hz, C$\underline{H}_3$-CH-CH_3), 0.77 (3H, d, J = 6.4 Hz, CH_3-CH-C$\underline{H}_3$), 0.70 (3H, d, J = 6.4 Hz, C$\underline{H}_3$-CH-CH_3). **^{13}C NMR** (100.79 MHz, $CDCl_3$-d_1): δ 173.1, 171.9, 171.1, 164.9, 155.7, 137.0, 134.8, 133.1, 132.7, 129.4, 129.32, 129.27, 128.64, 128.59, 127.2, 126.8, 122.2, 114.0, 72.1, 61.7, 54.1, 53.0, 48.4, 41.2, 39.7, 38.0, 32.4, 24.83, 24.78, 23.5, 23.0, 21.8, 21.7. **CHN Analysis:** Calc. for $C_{37}H_{47}ClN_4O_6$ (679.25): C, 65.42; H, 6.97; N, 8.25. Found: C, 65.63 ± 0.02; H 7.01 ± 0.01; N, 8.17 ± 0.02. **HRMS**: m/z calc. for $C_{37}H_{47}ClN_4O_6$: 701.30763 $[M+Na]^+$; found: 701.30889 $[M+Na]^+$.

3.3.12. Characterization of N-((5S,8S,11S)-5,8-dibenzyl-3,13-dimethyl-4,7,10-trioxo-2-oxa-3,6,9-triazatetradecan-11-yl)-2-benzyloxy-5-chlorobenzamide **3l**

White solid; yield 74%; mp 72.8–76.5 °C; $[\alpha_D^{20}] = -35.1°$ (c 1.00, EtOAc); R_f (hex/EtOAc−1/1) = 0.38. **IR** (ATR): 3307, 3064, 3031, 2955, 2360, 2342, 1645, 1522, 1272, 1230, 989, 810, 744, 699 cm^{-1}. **^{1}H NMR** (500 MHz, $CDCl_3$-d_1): δ 8.36 (1H, d, J = 2.5 Hz, N$\underline{H}$-CH-CHH-CH-$(CH_3)_2$), 8.19 (1H, d, J = 7 Hz, N$\underline{H}$-CH-CHH-Ph), 7.67–7.55 (6H, m, N$\underline{H}$-CH-CHH-Ph, Ar-$\underline{H}$), 7.47–7.37 (3H, m, Ar-$\underline{H}$), 7.32–7.25 (4H, m, Ar-$\underline{H}$), 7.24–7.20 (4H, m, Ar-$\underline{H}$), 7.07 (1H, d, J = 7.5 Hz, Ar-$\underline{H}$), 6.84 (1H, d, J = 8 Hz, Ar-$\underline{H}$), 5.43–5.35 (1H, m, NH-C$\underline{H}$-CHH-Ph),

5.31–5.29 (2H, ABq, J = 7.6 Hz, O-CH_2-Ph), 4.83–4.77 (1H, m, NH-CH-CHH-Ph), 4.65–4.57 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 3.83 (3H, s, O-CH_3), 3.34 (3H, s, N-CH_3), 3.28–3.22 (2H, m, NH-CH-CH_2-Ph), 3.16–3.05 (2H, m, NH-CH-CH_2-Ph), 1.65–1.57 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$, 1.54–1.43 (1H, m, NH-CH-CH_2-CH-$(CH_3)_2$), 1.32–1.25 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$, 0.94 (3H, d, J = 6.5 Hz, CH_3-CH-CH_3), 0.86 (3H, d, J = 6.5 Hz, CH_3-CH-CH_3). **^{13}C NMR** (125.78 MHz, $CDCl_3$-d_1): δ 171.9, 171.2, 170.2, 164.4, 155.5, 136.5, 136.3, 134.6, 132.9, 132.4, 129.4, 129.24, 129.20, 129.16, 128.4, 128.3, 127.0, 126.9, 126.6, 122.0, 113.9, 71.9, 61.6, 54.1, 52.0, 50.3, 39.0, 38.2, 37.6, 32.0, 24.6, 22.9, 21.6. **CHN Analysis:** Calc. for $C_{40}H_{45}ClN_4O_6$ (713.26): C, 67.21; H, 6.36; N, 7.86. Found: C, 67.21 ± 0.01; H 6.31 ± 0.01; N, 7.86 ± 0.02. **HRMS**: m/z calc. for $C_{40}H_{45}ClN_4O_6$: 735.29198 $[M+Na]^+$; found: 735.29319 $[M+Na]^+$.

3.4. General scheme and procedure for benzyl-protected aldehydes **4a-o**

Reduction of Weinreb amides with one amino acid to aldehyde was done according to similar reaction described in Ref. [19]. Solution of L-phenylalanine Weinreb amide (0.6 g, 1.42 mmol) in dichlormethane (30 mL) was cooled to −78 °C and DIBAL (0.89M in THF, 3.19 mL, 2.84 mmol) was added dropwise over 15 min. The reaction was stirred for 1 h at this temperature and then quenched with saturated solution of NH_4Cl in H_2O (20 mL) and slowly heated to ambient temperature. The crude product was extracted with dichlormethane (3 × 20 mL). The combined organic phase was washed with H_2O (2 × 20 mL) and dried over Na_2SO_4. The crude product was purified by column chromatography (silica gel 40 g, *n*-hexane/ethyl acetate (3:1)) to afford aldehyde **4b** in a 78% yield as colourless oil for L-phenylalanine. L-leucine derivative **4a** was obtained in a 46% yield as a colourless oil.

Reduction of Weinreb amide with two amino acids to aldehyde was done according to similar reaction described in Ref. [31]: L-Phenylalanine-L-phenylalanine Weinreb amide (0.256 g, 0.427 mmol) was dissolved in a secured three-neck round-bottom flask in dry tetrahydrofurane (30 mL) under nitrogen atmosphere and cooled to −20 °C. Via syringe the suspension of $LiAlH_4$ (0.0225 g, 0.593 mmol) in dry tetrahydrofurane (10 mL) was added dropwise and stirred for 1 h. The reaction was diluted with ethyl acetate (30 mL) and washed with 0.1 M HCl (3 × 15 mL). The water phase was washed with ethyl acetate (3 × 15 mL). Organic phases were combined and washed with H_2O (3 × 15 mL) and dried over Na_2SO_4. The crude product was purified by column chromatography (silica gel 30 g, *n*-hexane/ethyl acetate (1:1)) to afford aldehydes **4c-f** in yield 55–97% as colourless oil or white solid.

Procedure for Weinreb amides with three amino acids follows the same condition as above mentioned for derivatives with two amino acids, however the procedure was slightly altered in several aspects. Reaction temperature was increased from −20 °C to −8 °C and reaction time extended to 5 h. The equivalent of added $LiAlH_4$ was increased from 1.5 to 3. Also $LiAlH_4$ was not added via syringe as a suspension but as a solid under the inflow of nitrogen in three portions over the period of 2 h. In other aspect as work up the procedure stays unchanged. The aldehydes **4g-o** were obtained in yields 20–75% as colourless oils or white solids.

3.4.1. Characterization of (2S)-2-benzyloxy-5-chloro-N-(4-methyl-1-oxopentan-2-yl)benzamide **4a**

Colorless oil; yield 46%; R_f (hex/EtOAc–3/1) = 0.31. **IR** (ATR): 3371, 2960, 1725, 1640, 1527, 1481, 1273, 1220, 990, 824, 744, 719 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 9.51 (1H, s, CHO), 8.30–8.15 (2H, m, NH-CH-CHH-Ph, Ar-H), 7.48–7.37 (6H, m, Ar-H), 7.04 (1H, d, J = 8.8 Hz, Ar-H), 5.17–5.15 (2H, ABq, J = 8.4 Hz, O-CH_2-Ph), 4.58–4.50 (1H, m, NH-CH-CHH-Ph), 1.56–1.47 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 1.46–1.37 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 1.17–1.07 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 0.82 (3H, d, J = 6.4 Hz, CH_3-CH-CH_3), 0.74 (3H, d, J = 6.4 Hz, CH_3-CH-CH_3). **^{13}C NMR** (100.79 MHz, $CDCl_3$-d_1): δ 199.8, 164.1, 155.6, 134.8, 132.8, 132.2, 129.2, 129.1, 128.5, 127.1, 122.2, 114.0, 72.0, 57.7, 37.3, 24.7, 23.1, 21.7. **CHN Analysis:** Calc. for $C_{20}H_{22}ClNO_3$ (359.85): C, 66.75; H, 6.16; N, 3.89. Found: C, 66.52 ± 0.02; H 6.21 ± 0.01; N, 3.81 ± 0.01. **HRMS**: m/z calc. for $C_{20}H_{22}ClNO_3$: 360.13610 $[M+H]^+$; found: 360.13638 $[M+Na]^+$.

3.4.2. Characterization of ((2S)-2-benzyloxy-5-chloro-N-(1-oxo-3-phenylpropan-2-yl)benzamide **4b**

Colourless oil; yield 78%; $[\alpha_D^{20}]$ = −1.5° (c 1.00, EtOAc); R_f (hex/EtOAc–3/1) = 0.15. **IR** (ATR): 3376, 3063, 2924, 1730, 1645, 1520, 1478, 1270, 1224, 1124, 992, 808, 741 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 9.53 (1H, s, CHO), 8.34 (1H, d, J = 6 Hz, NH-CH-CHH-Ph), 8.11 (1H, d, J = 2.8 Hz, Ar-H), 7.35–7.25 (4H, m, Ar-H), 7.20–7.10 (5H, m, Ar-H), 6.98–6.93 (2H, m, Ar-H), 6.87 (1H, d, J = 8.8 Hz, Ar-H), 5.04–5.01 (2H, ABq, J = 10.6 Hz, O-CH_2-Ph), 4.72 (1H, q, J = 6.4 Hz, NH-CH-CHH-Ph), 3.07 (1H, dd, J = 6.4 Hz, J = 14.4 Hz, NH-CH-CHH-Ph), 2.92 (1H, dd, J = 6.4 Hz, J = 14.4 Hz, NH-CH-CHH-Ph). **^{13}C NMR** (100.79 MHz, $CDCl_3$-d_1): δ 198.8, 164.0, 155.5, 135.9, 135.0, 132.8, 132.1, 129.2, 129.0, 128.8, 128.7, 127.8, 127.1, 127.0, 122.2, 114.4, 71.7, 60.5, 34.8. **CHN Analysis:** Calc. for $C_{23}H_{20}ClNO_3$ (393.86): C, 70.14; H, 5.12; N, 3.56. Found: C, 69.89 ± 0.03; H 5.17 ± 0.02; N, 3.52 ± 0.03. **HRMS**: m/z calc. for $C_{23}H_{20}ClNO_3$: 394.12045 $[M+H]^+$; 416.10239 $[M+Na]^+$; found: 394.12090 $[M+H]^+$; 416.10286 $[M+Na]^+$.

3.4.3. Characterization of 2-benzyloxy-5-chloro-N-(2S)-4-methyl-1-((4-methyl-1-oxopentan-2-yl)amino)-1-oxopentan-2-yl) benzamide **4c**

Product is a mixture of two diastereomers, A and B. Colourless oil; yield 88%; $[\alpha_D^{20}]$ = −9.3° (c 0.667, EtOAc); R_f (hex/EtOAc–1/1) = 0.51. **IR** (ATR): 3380, 3312, 2955, 2930, 2870, 1638, 1505, 1480, 1466, 1295, 1227, 1121, 995, 809, 744, 696 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 9.60–9.54 (1H, m, (CHO)$_{A+B}$), 8.28–8.15 (2H, m, (2 x NH-CH-CHH-CH-$(CH_3)_2)_{A+B}$), 7.50–7.40 (6H, m, (Ar-H)$_{A+B}$), 7.15–7.06 (1H, m, (Ar-H)$_{A+B}$), 7.04–6.92 (1H, m, (Ar-H)$_{A+B}$), 5.30–5.13 (2H, m, (O-CH_2-Ph)$_{A+B}$), 4.69–4.59 (1H, m, (NH-CH-CHH-CH-$(CH_3)_2)_{A+B}$), 4.52–4.41 (1H, m, (NH-CH-CHH-CH-$(CH_3)_2)_{A+B}$), 1.83–1.67 (2H, m, (2 x NH-CH-CHH-CH-$(CH_3)_2)_{A+B}$), 1.66–1.56 (1H, m, (NH-CH-CHH-CH-$(CH_3)_2)_{A+B}$), 1.56–1.41 (2H, m, (2 x NH-CH-CHH-CH-$(CH_3)_2)_{A+B}$), 1.32–1.21 (1H, m, (NH-CH-CHH-CH-$(CH_3)_2)_{A+B}$), 1.06–0.91 (6H, m, (CH_3-CH-CH_3)$_{A+B}$), 0.90–0.83 (3H, m, (CH_3-CH-CH_3)$_{A+B}$), 0.83–0.75 (3H, m, (CH_3-CH-CH_3)$_{A+B}$). **^{13}C NMR** (100.79 MHz, $CDCl_3$-d_1): δ 199.8, 172.47, 172.35, 164.68, 164.58, 155.68, 155.64, 134.86, 133.07, 133.04, 132.34, 132.28, 131.25, 130.50, 129.66, 129.38, 129.33, 128.57, 128.52, 127.28, 122.40, 114.25, 114.20, 72.18, 72.12, 57.51, 57.47, 52.20, 42.29, 39.88, 39.77, 37.85, 24.96, 24.90, 24.88, 24.85, 23.24, 23.19, 22.98, 22.04, 21.96. **HRMS**: m/z calc. for $C_{26}H_{33}ClN_2O_4$: 473.22016 $[M+H]^+$; 495.20211 $[M+Na]^+$; found: 473.22068 $[M+H]^+$; 495.20256 $[M+Na]^+$.

3.4.4. Characterization of 2-benzyloxy-5-chloro-N-((2S)-4-methyl-1-oxo-1-(((2S)-1-oxo-3-phenylpropan-2-yl)amino)pentan-2-yl) benzamide **4d**

White solid; yield 97%; mp 161–165 °C; $[\alpha_D^{20}]$ = −51.8° (c 0.4, EtOAc); R_f (hex/EtOAc - 1/1) = 0.36. **IR** (ATR): 3356, 3068, 2965, 2828, 1732, 1688, 1656, 1644, 1530, 1514, 1278, 1242, 1219, 987, 757, 741, 715, 700 cm^{-1}. **^{1}H NMR** (400 MHz, CD_2Cl_2-d_2): δ 9.64 (1H, s, CHO), 8.19 (1H, d, J = 2.8 Hz, NH-CH-CHH-CH-$(CH_3)_2$), 8.08 (1H, d, J = 7.2 Hz, NH-CH-CHH-Ph), 7.56 (1H, dd, J = 2.8 Hz, J = 8.8 Hz, Ar-H), 7.53–7.46 (5H, m, Ar-H), 7.25–7.13 (6H, m, Ar-H), 6.97 (1H, d, J = 4.8 Hz, Ar-H), 5.22–5.21 (2H, ABq, J = 1.6 Hz, O-CH_2-Ph), 4.60–4.50 (2H, m, NH-CH-CH_2-Ph, NH-CH-CHH-CH-$(CH_3)_2$), 3.23

(1H, dd, J = 5.6 Hz, J = 14 Hz, NH-CH-CHH-Ph), 3.03 (1H, dd, J = 5.6 Hz, J = 14 Hz, NH-CH-CHH-Ph), 1.56–1.49 (1H, m, NH-CH-CH_2-CH-$(CH_3)_2$), 1.25–1.15 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 1.11–1.01 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 0.85 (3H, d, J = 6.4 Hz, CH_3-CH-CH_3), 0.79 (3H, d, J = 6.4 Hz, CH_3-CH-CH_3). **^{13}C NMR** (100.62 MHz, CD_2Cl_2-d_2): δ 199.2, 172.1, 164.3, 155.8, 136.3, 135.1, 133.0, 132.1, 129.4, 129.3, 129.2, 128.6, 128.6, 126.9, 126.8, 122.4, 114.3, 72.1, 59.9, 52.0, 38.9, 34.8, 24.7, 22.8, 21.6. **CHN Analysis:** Calc. for $C_{29}H_{31}ClN_2O_4$ (507.02): C, 68.70; H, 6.16; N, 5.53. Found: C, 68.53 ± 0.02; H 6.41 ± 0.01; N, 5.48 ± 0.02. **HRMS**: m/z calc. for $C_{29}H_{31}ClN_2O_4$: 507.20451 $[M+H]^+$; 529.18646 $[M+Na]^+$; found: 507.20525 $[M+H]^+$; 529.18719 $[M+Na]^+$.

3.4.5. Characterization of 2-benzyloxy-5-chloro-N-((2S)-1-((4-methyl-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl) benzamide **4e**

Product is a mixture of two diastereomers, A and B. Colourless oil; yield 84%; $[\alpha_D^{20}]$ = −5.2° (c 0.667, EtOAc); R_f (hex/EtOAc−1/1) = 0.51. **IR** (ATR): 3297, 2957, 2360, 2342, 1734, 1636, 1539, 1271, 1003, 810, 747, 698 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 9.45–9.35 (1H, m, (CHO)$_{A+B}$), 8.44–8.40 (1H, m, (NH-CH-CHH-CH-$(CH_3)_2)_{A+B}$), 8.13 (1H, m, (NH-CH-CHH-Ph)$_{A+B}$), 7.48–7.33 (6H, m, (Ar-H)$_{A+B}$), 7.23–7.14 (3H, m, (Ar-H)$_{A+B}$), 7.08–7.00 (2H, m, (Ar-H)$_{A+B}$), 6.98–6.92 (1H, m, (Ar-H)$_{A+B}$), 6.82–6.67 (1H, m, (Ar-H)$_{A+B}$), 5.24–5.07 (2H, m, (O-CH_2-Ph)$_{A+B}$), 4.93–4.84 (1H, m, (NH-CH-CHH-CH-$(CH_3)_2)_{A+B}$), 4.38–4.24 (1H, m, (NH-CH-CHH- Ph)$_{A+B}$), 3.07–2.95 (1H, m, (NH-CH-CHH-Ph)$_{A+B}$), 2.90–2.82 (1H, m, (NH-CH-CHH-Ph)$_{A+B}$), 1.63–1.50 (1H, m, (NH-CH-CHH-CH-$(CH_3)_2)_{A+B}$), 1.50–1.40 (1H, m, (NH-CH-CHH-CH-$(CH_3)_2)_{A+B}$), 1.40–1.29 (1H, m, (NH-CH-CHH-CH-$(CH_3)_2)_{A+B}$), 0.90–0.80 (6H, m, (CH_3-CH-CH_3)$_{A+B}$). **^{13}C NMR** (100.79 MHz, $CDCl_3$-d_1): δ 199.90, 199.82, 136.80, 136.78, 135.17, 135.14, 133.02, 132.20, 132.18, 129.34, 129.30, 129.27, 129.18, 129.16, 128.76, 128.12, 128.05, 127.11, 127.08, 122.47, 122.44, 114.57, 114.55, 71.90, 71.85, 57.52, 57.41, 55.30, 55.18, 37.71, 37.69, 37.63, 37.58, 24.78, 24.65, 23.19, 23.15, 22.02, 21.97, 14.39. **CHN Analysis:** Calc. for $C_{29}H_{31}ClN_2O_4$ (507.02): C, 68.70; H, 6.16; N, 5.53. Found: C, 68.60 ± 0.02; H 6.39 ± 0.02; N, 5.31 ± 0.02. **HRMS**: m/z calc. for $C_{29}H_{31}ClN_2O_4$: 507.20451 $[M+H]^+$; 529.18646 $[M+Na]^+$; found: 507.20490 $[M+H]^+$; 529.18675 $[M+Na]^+$.

3.4.6. Characterization of 2-benzyloxy-5-chloro-N-((2S)-1-oxo-1-(((2S)-1-oxo-3-phenylpropan-2-yl)amino)-3-phenylpropan-2-yl) benzamide **4f**

White solid; yield 55%; mp 152–154 °C; $[\alpha_D^{20}]$ = −54.8° (c 0.25, EtOAc); R_f (hex/EtOAc - 2/3) = 0.58. **IR** (ATR): 3290, 1738, 1633, 1535, 1243, 1004, 756, 698 cm^{-1}. **^{1}H NMR** (500 MHz, $CDCl_3$): δ 9.49 (1H, s, CHO), 8.28 (1H, d, J = 5.6 Hz, NH-CH-CHH-Ph), 8.09 (1H, d, J = 2.4 Hz, NH-CH-CHH-Ph), 7.45–7.36 (4H,m, Ar-H), 7.34–7.28 (2H, m, Ar-H), 7.22–7.15 (3H, m, Ar-H), 7.13–7.07 (3H, m, Ar-H), 7.06–7.00 (2H, m, Ar-H), 6.99–6.92 (3H, m, Ar-H), 6.84 (1H, d, J = 5.2 Hz, Ar-H), 5.10–5.08 (2H, ABq, J = 8.2 Hz, O-CH_2-Ph), 4.81 (1H, q, J = 5.4 Hz, NH-CH-CHH-Ph), 4.52 (1H, q, J = 5.4 Hz, NH-CH-CHH-Ph), 3.09 (1H, dd, J = 6 Hz, J = 11.2 Hz, NH-CH-CHH-Ph), 3.01 (1H, dd, J = 6 Hz, J = 11.6 Hz, NH-CH-CHH-Ph), 2.96 (1H, dd, J = 6 Hz, J = 11.6 Hz, NH-CH-CHH-Ph), 2.77 (1H, dd, J = 6 Hz, J = 11.2 Hz, NH-CH-CHH-Ph). **^{13}C NMR** (125.78 MHz, $CDCl_3$): δ 198.9, 171.2, 164.2, 155.4, 136.6, 135.6, 134.9, 132.9, 132.2, 129.2, 129.12, 129.09, 129.09, 129.07, 128.63, 128.60, 127.9, 126.94, 129.91, 122.0, 114.2, 71.7, 59.8, 54.7, 36.8, 34.9. **CHNAnalysis:** Calc. for $C_{32}H_{29}ClN_2O_4$ (541.04): C, 71.04; H, 5.40; N, 5.18. Found: C, 71.02 ± 0.02; H 5.42 ± 0.01; N, 5.18 ± 0.01. **HRMS**: m/z calc. for $C_{32}H_{29}ClN_2O_4$: 563.17081 $[M+Na]^+$; found: 563.17087 $[M+Na]^+$.

3.4.7. Characterization of 2-benzyloxy-5-chloro-N-((2S)-4-methyl-1-(((2S)-4-methyl-1-((4-methyl-1-oxopentan-2-yl)amino)-1-oxopentan-2-yl)amino)-1-oxopentan-2-yl)benzamide **4g**

Product is a mixture of two diastereomers, A and B. White solid; yield 65%; mp 62.6–66.2 °C; $[\alpha_D^{20}]$ = −11.9° (c 1.00, EtOAc); R_f (hex/EtOAc−1/1) = 0.53. **IR** (ATR): 3289, 3069, 2957, 2871, 1733, 1635, 1537, 1483, 1468, 1272, 1245, 1123, 1007, 809, 752, 697 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 9.53–9.48 (1H, m, (CHO)$_{A+B}$), 8.25–8.05 (2H, m, 2 x (NH-CH-CHH-CH-$(CH_3)_2)_{A+B}$), 7.55–7.35 (6H, m, (NH-CH-CHH-CH-$(CH_3)_2)_{A+B}$, (Ar-H)$_{A+B}$), 7.1–7.05 (1H, m, (Ar-H)$_{A+B}$), 7.05–6.90 (1H, m, (Ar-H)$_{A+B}$), 6.60 (1H, m, (Ar-H)$_{A+B}$), 5.24–5.06 (2H, m, (O-CH_2-Ph)$_{A+B}$), 4.50–4.20 (3H, m, 3 x (NH-CH-CH_2-CH-$(CH_3)_2)_{A+B}$), 1.82–1.10 (9H, m 3 x (NH-CH-CH_2-CH-$(CH_3)_2)_{A+B}$), 1.00–0.80 (12H, m, 4 x (C$H_3)_{A+B}$), 0.73–0.72 (3H, m, (C$H_3)_{A+B}$), 0.71–0.70 (3H, m, (C$H_3)_{A+B}$). **^{13}C NMR** (100.62 MHz, $CDCl_3$-d_1): δ 200.35, 200.03, 172.47, 172.37, 172.23, 165.28, 165.00, 155.79, 155.75, 134.77, 133.43, 133.37, 132.4, 132.26, 132.07, 129.60, 129.55, 129.01, 128.88, 127.38, 127.35, 121.98, 121.91, 114.2, 72.26, 72.21, 57.52, 57.40, 53.64, 53.15, 52.03, 51.8, 40.13, 39.97, 39.91, 37.91, 37.44, 29.88, 25.17, 24.96, 24.89, 24.80, 23.31, 23.25, 23.16, 23.02, 22.99, 21.94, 21.88, 21.83, 21.74, 21.71. **CHN Analysis:** Calc. for $C_{32}H_{44}ClN_3O_5$ (586.16): C, 65.57; H, 7.57; N, 7.17. Found: C, 65.54 ± 0.01; H 7.79 ± 0.01; N, 7.24 ± 0.01. **HRMS**: m/z calc. for $C_{32}H_{44}ClN_3O_5$: 608.28617 $[M+Na]^+$; found: 608.25668 $[M+Na]^+$.

3.4.8. Characterization of 2-benzyloxy-5-chloro-N-((2S)-4-methyl-1-(((2S)-4-methyl-1-(((2S)-4-methyl-1-oxopentan-2-yl)amino)-1-oxopentan-2-yl)amino)-1-oxopentan-2-yl)benzamide **4h**

White solid; yield 75%; mp 60.6–65.2 °C; $[\alpha_D^{20}]$ = −22.9° (c 1.00, EtOAc); R_f (hex/EtOAc−1/1) = 0.53. **IR** (ATR): 3289, 3069, 2957, 2871, 1733, 1635, 1537, 1483, 1468, 1272, 1245, 1123, 1007, 809, 752, 697 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 9.54 (1H, s, CHO), 8.24 (1H, d, J = 5.6 Hz, NH-CH-CHH-CH-$(CH_3)_2$), 8.19 (1H, d, J = 2.8 Hz, NH-CH-CHH-CH-$(CH_3)_2$), 7.58–7.40 (6H, m, NH-CH-CHH-CH-$(CH_3)_2$, Ar-H), 7.13 (1H, d, J = 8.8 Hz, Ar-H), 7.10–7.04 (1H, m, Ar-H), 6.55 (1H, d, J = 7.6 Hz, Ar-H), 5.24–5.21 (2H, ABq, J = 9.2 Hz, O-CH_2-Ph), 4.55–4.38 (3H, m, 3 x NH-CH-CHH-CH-$(CH_3)_2$), 1.90–1.80 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 1.80–1.70 (2H, m, NH-CH-CH_2-CH-$(CH_3)_2$), 1.70–1.50 (4H, m, 3 x NH-CH-CHH-CH-$(CH_3)_2$, NH-CH-CH_2-CH-$(CH_3)_2$), 1.48–1.35 (1H, m, NH-CH-CH_2-CH-$(CH_3)_2$), 1.26–1.16 (1H, m, NH-CH-CH_2-CH-$(CH_3)_2$), 1.08–0.95 (9H, m, 3 x CH_3), 0.92 (3H, d, J = 6.4 Hz, CH_3), 0.84 (3H, d, J = 6.4 Hz, CH_3), 0.78 (3H, d, J = 6.4 Hz, CH_3). **^{13}C NMR** (100.62 MHz, $CDCl_3$-d_1): δ 200.3, 172.4, 165.0, 155.7, 134.8, 133.4, 132.3, 129.6, 129.5, 129.4, 128.8, 128.7, 127.4, 122.0, 114.2, 72.2, 57.4, 53.2, 52.0, 40.1, 39.9, 37.7, 37.4, 29.9, 25.2, 25.1, 24.9, 24.8, 23.3, 23.2, 23.0, 21.9. **CHN Analysis:** Calc. for $C_{32}H_{44}ClN_3O_5$ (586.16): C, 65.57; H, 7.57; N, 7.17. Found: C, 65.52 ± 0.01; H 7.69 ± 0.02; N, 7.14 ± 0.02. **HRMS**: m/z calc. for $C_{32}H_{44}ClN_3O_5$: 608.28617 $[M+Na]^+$; found: 608.25662 $[M+Na]^+$.

3.4.9. Characterization of 2-benzyloxy-5-chloro-N-((2S)-4-methyl-1-(((2S)-4-methyl-1-(((2R)-4-methyl-1-oxopentan-2-yl)amino)-1-oxopentan-2-yl)amino)-1-oxopentan-2-yl)benzamide **4i**

Colourless oil; yield 52%; $[\alpha_D^{20}]$ = −13.5° (c 1.00, EtOAc); R_f (hex/EtOAc−2/1) = 0.51. **IR** (ATR): 3280, 3067, 3036, 2955, 2930, 2870, 1734, 1625, 1526, 1499, 1481, 1467, 1385, 1270, 1226, 1122, 1001, 808, 745, 696 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 9.54 (1H, s, CHO), 8.25 (1H, d, J = 5.2 Hz, NH-CH-CHH-CH-$(CH_3)_2$), 8.15 (1H, d, J = 2.8 Hz, NH-CH-CHH-CH-$(CH_3)_2$), 7.58–7.40 (5H, m, NH-CH-CHH-CH-$(CH_3)_2$, Ar-H), 7.13 (1H, d, J = 8.8 Hz, Ar-H), 7.07 (1H, d, J = 7.6 Hz, Ar-H), 6.55 (1H, d, J = 8.4 Hz, Ar-H), 5.23–5.21 (2H, ABq, J = 9.2 Hz, O-CH_2-Ph), 4.57–4.45 (2H, m, 2 x NH-CH-CHH-CH-$(CH_3)_2$), 4.44–4.36 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 1.92–1.69 (3H, m, 3 x NH-CH-CHH-CH-$(CH_3)_2$), 1.65–1.50 (4H, m, 3 x NH-CH-CHH-CH-$(CH_3)_2$ NH-CH-CH_2-CH-$(CH_3)_2$), 1.43–1.35 (1H, m, NH-CH-CH_2-

C*H*-(CH_3)$_2$), 1.25–1.15 (1H, m, NH-CH-CH_2-C*H*-(CH_3)$_2$), 1.27–1.18 (1H, m, NH-CH-CH_2-C*H*-(CH_3)$_2$), 1.03 (3H, d, *J* = 6.4 Hz, C*H*$_3$-CH-CH_3), 1.00 (3H, d, *J* = 6.4 Hz, CH_3-CH-C*H*$_3$), 0.97 (3H, d, *J* = 6.4 Hz, C*H*$_3$-CH-CH_3), 0.92 (3H, d, *J* = 6.4 Hz, CH_3-CH-C*H*$_3$), 0.83 (3H, d, *J* = 6.4 Hz, C*H*$_3$-CH-CH_3), 0.77 (3H, d, *J* = 6.4 Hz, CH_3-CH-C*H*$_3$). **^{13}C NMR** (100.62 MHz, $CDCl_3$-d_1): δ 200.0, 172.5, 172.2, 165.3, 155.8, 134.8, 133.4, 132.3, 129.6, 129.4, 128.7, 127.4, 121.9, 114.2, 72.3, 57.5, 53.6, 51.8, 40.2, 40.0, 37.4, 25.2, 25.0, 24.9, 23.3, 23.2, 23.0, 21.9, 21.74, 21.70. **CHN Analysis:** Calc. for $C_{32}H_{44}ClN_3O_5$ (586.16): C, 65.57; H, 7.57; N, 7.17. Found: C, 65.46 ± 0.02; H 7.69 ± 0.02; N, 7.14 ± 0.02. **HRMS**: *m*/*z* calc. for $C_{32}H_{44}ClN_3O_5$: 608.28617 $[M+Na]^+$; 586.30423 $[M+H]^+$; found: 608.287235 $[M+Na]^+$; 586.30546 $[M+H]^+$.

3.4.10. Characterization of 2-benzyloxy-5-chloro-N-((S)-4-methyl-1-(((S)-4-methyl-1-oxo-1-((1-oxo-3-phenylpropan-2-yl)amino) pentan-2-yl)amino)-1-oxopentan-2-yl)benzamide ***4j***

Product is a mixture of two diastereomers, A and B. White solid; yield 69%; mp 141.3–151.7 °C; $[\alpha_D^{20}]$ = −13.9° (c 1.00, EtOAc) R_f (hex/EtOAc−1/1) = 0.35. **IR** (ATR):3292, 2957, 2360, 2341, 1652, 1646, 1539, 1522, 1272, 1232, 748, 699 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 9.60–9.55 (1H, m, (C*H*O)$_{A+B}$), 8.70–8.08 (2H, m, 2 x (N*H*)$_{A+B}$), 7.50–7.39 (6H, m, (N*H*, Ar-*H*)$_{A+B}$), 7.30–7.12 (5H, m, (Ar-*H*)$_{A+B}$), 7.08–6.92 (2H, m, (Ar-*H*)$_{A+B}$), 6.70–6.58 (1H, m, (Ar-*H*)$_{A+B}$), 5.20–5.19 (2H, m, (O-C*H*$_2$-Ph)$_{A+B}$), 4.67–4.58 (1H, m, (NH-C*H*)$_{A+B}$), 4.45–4.32 (2H, m, 2 x (NH-C*H*)$_{A+B}$), 3.24–3.03 (2H, m, (NH-CH-C*H*$_2$-Ph)$_{A+B}$), 1.75–1.61 (1H, m, (NH-CH*H*-CH-(CH_3)$_2$)$_{A+B}$), 1.55–1.30 (4H, m, (NH-C*HH*-CH-(CH_3)$_2$)$_{A+B}$, (NH-C*H*H-C*H*-(CH_3)$_2$)$_{A+B}$), 1.23–1.11 (1H, m, (NH-CHH-CH-C*H*-(CH_3)$_2$)$_{A+B}$), 1.00–0.65 (12H, m, 4 x (CH_3)$_{A+B}$). **^{13}C NMR** (100.79 MHz, $CDCl_3$-d_1): δ 199.21, 199.16, 172.36, 172.26, 172.20, 165.03, 164.92, 155.74, 155.70, 136.19, 136.10, 134.82, 133.29, 132.31, 132.26, 129.5, 129.47, 129.43, 129.35, 128.87, 128.67, 128.62, 127.31, 127.17, 122.10, 122.07, 114.20, 72.19, 60.02, 59.75, 53.12, 52.81, 52.12, 51.81, 40.44, 39.86, 39.66, 35.14, 35.08, 25.15, 25.00, 24.99, 24.87, 23.11, 23.03, 21.89, 21.83. **CHN Analysis:** Calc. for $C_{35}H_{42}ClN_3O_5$ (620.18): C, 67.78; H, 6.83; N, 6.78. Found: C, 67.57 ± 0.01; H 6.91 ± 0.01; N, 6.69 ± 0.01. **HRMS**: *m*/*z* calc. for $C_{35}H_{42}ClN_3O_5$: 642.27052 $[M+Na]^+$; found: 642.27215 $[M+Na]^+$.

3.4.11. Characterization of 2-benzyloxy-5-chloro-N-((2S)-4-methyl-1-(((2S)-1-(((2S)-4-methyl-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxopentan-2-yl)benzamide ***4k***

Colourless oil; yield 20%; $[\alpha_D^{20}]$ = −50.7° (c 1.00, EtOAc); R_f (hex/EtOAc−1/1) = 0.50. **IR** (ATR): 3276, 3066, 2957, 2871, 1734, 1635, 1598, 1541, 1271, 1246, 1124, 1008, 811, 750, 697 cm^{-1}. **^{1}H NMR** (400 MHz, CD_2Cl_2-d_2): δ 9.50 (1H, s, C*H*O), 8.22 (1H, d, *J* = 4.8 Hz, N*H*-CH-CHH-CH-(CH_3)$_2$), 7.99 (1H, d, *J* = 2.8 Hz, N*H*-CH-CHH-CH-(CH_3)$_2$), 7.65–7.48 (6H, m, N*H*-CH-CHH-Ph, Ar-*H*), 7.25–6.95 (7H, m, Ar-*H*), 6.49 (1H, d, *J* = 8 Hz, Ar-*H*), 5.28–5.25 (2H, ABq, *J* = 10.4 Hz, O-C*H*$_2$-Ph), 4.73–4.63 (1H, m, NH-C*H*-CHH-Ph), 4.40–4.32 (1H, m, NH-C*H*-CHH-CH-(CH_3)$_2$), 4.30–4.20 (1H, m, NH-C*H*-CHH-CH-(CH_3)$_2$), 3.27–3.10 (2H, m, NH-CH-C*H*$_2$-Ph), 1.70–1.52 (3H, m, NH-CH-CH_2-C*H*-(CH_3)$_2$, NH-CH-C*H*$_2$-CH-(CH_3)$_2$), 1.50–1.40 (1H, m, NH-CH-C-H*H*-CH-(CH_3)$_2$), 1.35–1.25 (1H, m, NH-CH-C-*H*H-CH-(CH_3)$_2$), 1.20–1.11 (1H, m, NH-CH-CH_2-C*H*-(CH_3)$_2$), 1.03 (3H, d, *J* = 6 Hz, CH_3-CH-C*H*$_3$), 1.00 (3H, d, *J* = 6 Hz, C*H*$_3$-CH-CH_3), 0.79 (3H, d, *J* = 6.4 Hz, CH_3-CH-C*H*$_3$), 0.74 (3H, d, *J* = 6.4 Hz, C*H*$_3$-CH-CH_3). **^{13}C NMR** (100.79 MHz, CD_2Cl_2-d_2): δ 200.8, 171.5, 171.4, 165.1, 156.0, 136.5, 134.9, 133.5, 132.0, 129.6, 129.4, 129.1, 128.8, 128.6, 127.0, 125.6, 121.5, 114.3, 72.3, 57.5, 53.8, 39.6, 37.3, 36.8, 30.3, 24.8, 24.5, 23.2, 22.7, 21.4, 21.3. **CHN Analysis:** Calc. for $C_{35}H_{42}ClN_3O_5$ (620.17): C, 67.78; H, 6.83; N, 6.78. Found: C, 67.59 ± 0.01; H 6.89 ± 0.02; N, 6.69 ± 0.02. **HRMS**: *m*/*z* calc. for $C_{35}H_{42}ClN_3O_5$: 642.27052 $[M+Na]^+$; found: 642.27185 $[M+Na]^+$.

3.4.12. Characterization of 2-benzyloxy-5-chloro-N-((2S)-4-methyl-1-(((2S)-1-(((2R)-4-methyl-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxopentan-2-yl)benzamide ***4l***

White solid; yield 46%; mp 147.5–151 °C; $[\alpha_D^{20}]$ = −28.9° (c 1.00, EtOAc); R_f (hex/EtOAc−1/1) = 0.46. **IR** (ATR): 3279, 3065, 3033, 2956, 2929, 2869, 1733, 1635, 1531, 1498, 1482, 1466, 1384, 1270, 1243, 1006, 808, 746, 696 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 9.52 (1H, s, C*H*O), 8.16 (1H, d, *J* = 4.8 Hz, N*H*-CH-CHH-CH-(CH_3)$_2$), 8.02 (1H, d, *J* = 2.8 Hz, N*H*-CH-CHH-CH-(CH_3)$_2$), 7.58–7.438 (6H, m, N*H*-CH-CHH-Ph, Ar-*H*), 7.27–7.22 (1H, m, Ar-*H*), 7.20–7.11 (4H, m, Ar-*H*), 7.10–7.05 (1H, m, Ar-*H*), 7.04–7.00 (1H, m, Ar-*H*), 6.56 (1H, d, *J* = 8.4 Hz, Ar-*H*), 5.22–5.19 (2H, ABq, *J* = 12.6 Hz, O-C*H*$_2$-Ph), 4.81–4.72 (1H, m, NH-C*H*-CHH-Ph), 4.49–4.42 (1H, m, NH-C*H*-CHH-CH-(CH_3)$_2$), 4.32–4.25 (1H, m, NH-C*H*-CHH-CH-(CH_3)$_2$), 3.27–3.10 (2H, m, NH-CH-C*H*$_2$-Ph), 1.76–1.65 (2H, m, 2 x NH-CH-C*H*H-CH-(CH_3)$_2$), 1.65–1.53 (1H, m, NH-CH-CH*H*-CH-(CH_3)$_2$), 1.48–1.39 (1H, m, NH-CH-CH*H*-CH-(CH_3)$_2$), 1.30–1.20 (1H, m, NH-CH-CH_2-C*H*-(CH_3)$_2$), 1.13–1.03 (1H, m, NH-CH-CH_2-C*H*-(CH_3)$_2$), 1.03–0.97 (6H, m, 2 x C*H*$_3$), 0.75 (3H, d, *J* = 6.4 Hz, CH_3-CH-C*H*$_3$), 0.69 (3H, d, *J* = 6.4 Hz, C*H*$_3$-CH-CH_3). **^{13}C NMR** (100.79 MHz, $CDCl_3$-d_1): δ 199.8, 171.7, 171.4, 165.2, 155.8, 136.5, 134.7, 133.4, 132.5, 129.7, 129.4, 129.2, 128.8, 128.7, 127.4, 127.0, 121.5, 114.0, 72.3, 57.6, 53.7, 53.7, 39.7, 37.4, 37.1, 24.9, 24.7, 23.3, 23.0, 21.8, 21.6. **CHN Analysis:** Calc. for $C_{35}H_{42}ClN_3O_5$ (620.17): C, 67.78; H, 6.83; N, 6.78. Found: C, 67.78 ± 0.02; H 6.87 ± 0.01; N, 6.71 ± 0.02. **HRMS**: *m*/*z* calc. for $C_{35}H_{42}ClN_3O_5$: 620.28858 $[M+H]^+$; found: 620.28986 $[M+H]^+$.

3.4.13. Characterization of 2-benzyloxy-5-chloro-N-((2S)-4-methyl-1-(((2S)-1-((4-methyl-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxopentan-2-yl)benzamide ***4m***

Product is a mixture of two diastereomers, A and B. Colourless oil; yield 30%; $[\alpha_D^{20}]$ = −25.2° (c 1.00, EtOAc); R_f (hex/EtOAc−1/1) = 0.50. **IR** (ATR): 3276, 3066, 2957, 2871, 1734, 1635, 1598, 1541, 1271, 1246, 1124, 1008, 811, 750, 697 cm^{-1}. **^{1}H NMR** (400 MHz, CD_2Cl_2-d_2): δ 9.51–9.49 (1H, m, (C*H*O)$_{A+B}$), 8.25–8.17 (1H, m, (N*H*-CH-CHH-CH-(CH_3)$_2$)$_{A+B}$), 8.05–7.95 (1H, m, (N*H*-CH-CHH-CH-(CH_3)$_2$)$_{A+B}$), 7.65–7.44 (6H, m, (N*H*-CH-CHH-Ph)$_{A+B}$), (Ar-*H*)$_{A+B}$), 7.25–6.98 (7H, m, (Ar-*H*)$_{A+B}$), 6.61–6.47 (1H, m, (Ar-*H*)$_{A+B}$), 5.31–5.17 (2H, m, (O-C*H*$_2$-Ph)$_{A+B}$), 4.75–4.62 (1H, m, (NH-C*H*-CH_2-Ph)$_{A+B}$), 4.43–4.20 (2H, m, 2 x NH-C*H*-CH_2-CH-(CH_3)$_2$)$_{A+B}$), 3.28–3.09 (2H, m, (NH-CH-C*H*$_2$-Ph)$_{A+B}$), 1.76–1.11 (6H, m, 2 x (NH-CH-C*H*$_2$-C*H*-(CH_3)$_{A+B}$), 1.05–0.66 (12H, m, 4 x (C*H*$_3$)$_{A+B}$). **^{13}C NMR** (100.79 MHz, CD_2Cl_2-d_2): δ 200.84, 200.21, 171.58, 171.51, 171.40, 171.36, 165.16, 165.11, 156.02, 136.73, 136.50, 134.95, 133.44, 133.41, 132.06, 132.01, 129.36, 129.33, 129.18, 129.15, 128.85, 128.84, 121.63, 114.32, 72.28, 72.26, 57.61, 57.46, 53.69, 39.74, 37.28, 37.23, 37.10, 36.89, 24.85, 24.80, 24.60, 24.47, 23.19, 23.07, 22.75, 22.71, 22.26, 21.56, 21.48, 21.36, 21.31. **CHN Analysis:** Calc. for $C_{35}H_{42}ClN_3O_5$ (620.17): C, 67.78; H, 6.83; N, 6.78. Found: C, 67.69 ± 0.02; H 6.99 ± 0.02; N, 6.89 ± 0.01. **HRMS**: *m*/*z* calc. for $C_{35}H_{42}ClN_3O_5$: 642.27052 $[M+Na]^+$; found: 642.27105 $[M+Na]^+$.

3.4.14. Characterization of 2-benzyloxy-5-chloro-N-((2S)-4-methyl-1-oxo-1-(((2S)-1-oxo-1-(((2S)-1-oxo-3-phenylpropan-2-yl) amino)-3-phenylpropan-2-yl)amino)pentan-2-yl)benzamide ***4n***

Colourless oil; yield 21%; $[\alpha_D^{20}]$ = −45.6° (c 0.125, EtOAc); R_f (hex/EtOAc−1/1) = 0.33. **IR** (ATR): 3259, 3063, 2926, 2360, 2341, 1726, 1636, 1534, 1498, 1489, 1454, 1276, 1257, 803, 739, 728, 701 cm^{-1}. **^{1}H NMR** (400 MHz, CD_2Cl_2-d_2): δ 9.59 (1H, s, C*H*O), 8.14 (1H, d, *J* = 5.6 Hz, N*H*-CH-CHH-Ph), 8.11 (1H, d, *J* = 2.8 Hz, N*H*-CH-CHH-CH-(CH_3)$_2$), 7.59 (1H, dd, *J* = 2.8 Hz, *J* = 8.8 Hz, Ar-*H*), 7.57–7.48 (7H, m, N*H*-CH-CHH-Ph, Ar-*H*), 7.40–7.25 (5H, m, Ar-*H*), 7.24–7.15 (3H, m, Ar-*H*), 7.07–6.97 (2H, m, Ar-*H*), 6.64 (1H, d, *J* = 6.2 Hz, Ar-*H*), 5.24–5.22 (2H, ABq, *J* = 10.4 Hz, O-C*H*$_2$-Ph), 4.71–4.58 (2H, m, 2 x NH-C*H*-CHH-Ph), 4.35–4.25 (1H, m, NH-C*H*-

CHH-CH-$(CH_3)_2$), 3.30–3.20 (1H, m, NH-CH-CH*H*-Ph), 3.20–3.10 (1H, m, NH-CH-C*H*H-Ph), 3.10–3.00 (2H, m, NH-CH-C*H*$_2$-Ph), 1.48–1.35 (1H, m, NH-CH-CH*H*-CH-$(CH_3)_2$), 1.35–1.25 (1H, m, NH-CH-C*H*H-CH-$(CH_3)_2$), 1.180–1.05 (1H, m, NH-CH-CH_2-C*H*-$(CH_3)_2$), 0.80 (3H, d, $J = 6.4$ Hz, CH_3-CH-C*H*$_3$), 0.74 (3H, d, $J = 6.4$ Hz, C*H*$_3$-CH-CH_3). **^{13}C NMR** (100.79 MHz, CD_2Cl_2-d_2): δ 199.2, 171.3, 171.0, 164.9, 156.0, 136.5, 135.0, 133.0, 132.1, 129.4, 129.3, 129.2, 128.7, 128.6, 127.0, 126.9, 125.6, 121.8, 121.7, 114.3, 72.2, 59.9, 53.9, 53.1, 39.4, 37.1, 34.8, 30.3, 24.8, 22.8, 21.4. **CHN Analysis:** Calc. for $C_{38}H_{40}ClN_4O_5$ (654.19): C, 69.77; H, 6.16; N, 6.42. Found: C, 69.82 ± 0.02; H 6.30 ± 0.02; N, 6.37 ± 0.02. **HRMS**: *m/z* calc. for $C_{38}H_{40}ClN_3O_5$: 676.25487 $[M+Na]^+$; found: 676.25641 $[M+Na]^+$.

3.4.15. Characterization of 2-benzyloxy-5-chloro-N-((2S)-4-methyl-1-oxo-1-(((2S)-1-oxo-1-((1-oxo-3-phenylpropan-2-yl)amino)-3-phenylpropan-2-yl)amino)pentan-2-yl)benzamide **4o**

Product is a mixture of two diastereomers, A and B. Colourless oil; yield 24%; $[\alpha_D^{20}] = -22.5°$ (c 0.125, EtOAc); R_f (hex/EtOAc–1/1) = 0.33. **IR** (ATR): 3259, 3063, 2926, 2360, 2341, 1726, 1636, 1534, 1498, 1489, 1454, 1276, 1257, 803, 739, 728, 701 cm^{-1}. **^{1}H NMR** (400 MHz, CD_2Cl_2-d_2): δ 9.61–9.55 (1H, s, (C*H*O)$_{A+B}$), 8.16–8.06 (2H, m, 2 x (N*H*-CH)$_{A+B}$), 7.62–7.57 (1H, m, (N*H*-CH)$_{A+B}$), 7.56–7.48 (6H, m, (Ar-*H*)$_{A+B}$), 7.40–7.05 (11H, m, (Ar-*H*)$_{A+B}$), 6.75–6.62 (1H, m, (Ar-*H*)$_{A+B}$), 5.29–5.160 (2H, m, (O-C*H*$_2$-Ph)$_{A+B}$), 4.74–4.65 (1H, m, (NH-C*H*)$_{A+B}$), 4.64–4.56 (1H, m, (NH-C*H*)$_{A+B}$), 4.37–4.23 (1H, m, (NH-C*H*)$_{A+B}$), 3.30–3.00 (4H, m, 2 x (NH-CH-C*H*$_2$-Ph)$_{A+B}$), 1.49–1.05 (3H, m, NH-CH-C*H*$_2$-C*H*-$(CH_3)_2)_{A+B}$), 0.84–0.65 (6H, m, 2 x $(CH_3)_{A+B}$). **^{13}C NMR** (100.79 MHz, CD_2Cl_2-d_2): δ 199.2, 171.3, 171.0, 164.9, 156.0, 136.5, 135.0, 133.0, 132.1, 129.4, 129.3, 129.2, 128.7, 128.6, 127.0, 126.9, 125.6, 121.8, 121.7, 114.3, 72.2, 59.9, 53.9, 53.1, 39.4, 37.1, 34.8, 30.3, 24.8, 22.8, 21.4. **CHN Analysis:** Calc. for $C_{38}H_{40}ClN_3O_5$ (654.19): C, 69.77; H, 6.16; N, 6.42. Found: C, 69.80 ± 0.02; H 6.20 ± 0.01; N, 6.31 ± 0.02. **HRMS**: *m/z* calc. for $C_{38}H_{40}ClN_4O_5$: 676.25487 $[M+Na]^+$; found: 676.25649 $[M+Na]^+$.

3.5. General scheme and procedure for deprotected Weinreb amides **5a-h**

Modified method of [32] was used: Benzyl-protected L-phenylalanine Weinreb amide (0.6 g, 1.32 mmol) was dissolved in ethyl acetate (100 mL). After addition of 10% Pd/C (0.1 g) the hydrogen atmosphere was introduced to the sealed reaction mixture and the reaction mixture was stirred at ambient temperature for 5 h. Pd/C was separated by filtration. Volume of the solution was reduced under reduced pressure. The residue p urified via column chromatography (silica gel 110 g, *n*-hexane/ethyl acetate (2:1)) to afford debenzylated L-phenylalanine Weinreb amide **5b** in a 54% yield as a white solid. Other derivatives were isolated in following yields: **5a** in yield 54% as white solid, **5c-f** in yields 32–78% as white solids and **5g,h** in yields 77–93% as white solids. All derivatives were prepared under similar conditions except for those with two and three amino acids (**5c-h**). Instead of purification via column chromatography they were purified via crystallization.

3.5.1. Characterization of (2S)-5-chloro-2-hydroxy-N-(1-(methoxy(methyl)amino)-4-methyl-1-oxopentan-2-yl)benzamide **5a**

Colourless solid; yield 45%; mp 108.6–112.7 °C; $[\alpha_D^{20}] = +28.6°$ (c 1.00, EtOAc); R_f (hex/EtOAc–1/1) = 0.45. **IR** (ATR):3279, 3073, 2959, 2871, 2360, 2342, 1639, 1592, 1551, 1477, 1366, 1296, 1232, 820, 719 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 12.26 (1H, brs, Ar-O*H*), 8.31 (1H, d, $J = 8$ Hz, N*H*-CH-CHH-CH-$(CH_3)_2$), 7.55 (1H, d, $J = 2.4$ Hz, Ar-*H*), 7.26 (1H, dd, $J = 2.4$ Hz, $J = 8.8$ Hz, Ar-*H*), 6.74 (1H, d, $J = 8.8$ Hz, Ar-*H*), 5.35–5.26 (1H, m, NH-C*H*-CHH-CH-$(CH_3)_2$), 4.00 (3H, s, O-C*H*$_3$), 3.37 (3H, s, N-C*H*$_3$), 1.91–1.79 (2H, m, NH-CH-CH*H*-CH-$(CH_3)_2$, NH-CH-CH_2-C*H*-$(CH_3)_2$), 1.67–1.56 (1H, m, NH-CH-C*H*H-CH-$(CH_3)_2$, 1.10–0.98 (6H, m, C*H*$_3$-CH-C*H*$_3$). **^{13}C NMR** (100.79 MHz, $CDCl_3$-d_1): δ 173.6, 169.2, 160.1, 134.0, 126.1, 123.3, 119.7, 114.8, 61.9, 48.2, 40.9, 32.4, 25.1, 23.5, 21.6. **CHN Analysis:** Calc. for $C_{15}H_{21}ClN_2O_4$ (328.79): C, 54.79; H, 6.44; N, 8.52. Found: C, 54.82 ± 0.02; H 6.52 ± 0.01; N, 8.57 ± 0.02. **HRMS**: *m/z* calc. for $C_{15}H_{21}ClN_2O_4$: 351.10821 $[M+Na]^+$; found: 351.10863 $[M+Na]^+$.

3.5.2. Characterization of (2S)-5-chloro-2-hydroxy-N-(1-(methoxy(methyl)amino)-1-oxo-3-phenylpropan-2-yl)benzamide **5b**

White solid; yield 54%; mp 106–109 °C; $[\alpha_D^{20}] = -31.8°$ (c 1.00, EtOAc); R_f (hex/EtOAc–1/1) = 0.50. **IR** (ATR): 3448, 3294, 3065, 2942, 1630, 1588, 1558, 1541, 1486, 1474, 1365, 1296, 1232, 984, 821 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ 12.10 (1H, brs, Ar-O*H*), 9.10 (1H, d, $J = 8$ Hz, N*H*-CH-CHH-Ph), 8.03–7.98 (1H, m, Ar-*H*), 7.43 (1H, dd, $J = 2.4$ Hz, $J = 8.8$ Hz, Ar-*H*), 7.34–7.163 (5H, m, Ar-*H*), 6.93 (1H, d, $J = 8.8$ Hz, Ar-*H*), 5.22–5.10 (1H, m, NH-C*H*-CHH-Ph), 3.77 (3H, s, O-C*H*$_3$), 3.14 (3H, s, N-C*H*$_3$), 3.10–2.94 (2H, m, NH-CH-C*H*$_2$-Ph. **^{13}C NMR** (100.79 MHz, DMSO-d_6): δ 167.0, 160.2, 158.3, 138.0, 134.1, 134.0, 129.7, 129.0, 128.8, 128.2, 127.3, 123.2, 119.8, 117.8, 52.0, 37.0. **CHN Analysis:** Calc. for $C_{18}H_{19}ClN_2O_4$ (362.80): C, 59.59; H, 5.28; N, 7.72. Found: C, 59.20 ± 0.02; H 5.20 ± 0.02; N, 7.40 ± 0.02. **HRMS**: *m/z* calc. for $C_{18}H_{19}ClN_2O_4$: 385.09256 $[M+Na]^+$; found: 385.09324 $[M+Na]^+$.

3.5.3. Characterization of 5-chloro-2-hydroxy-N-((2S)-1-(((2S)-1-(methoxy(methyl)amino)-4-methyl-1-oxopentan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)benzamide **5c**

White solid; yield 78%; mp 244.5–248.1 °C; $[\alpha_D^{20}] = -4°$ (c 0.5, EtOAc); R_f (hex/EtOAc–1/1) = 0.56. **IR** (ATR): 3335, 3245, 3076, 2958, 2360, 1636, 1590, 1549, 1474, 1370, 1233, 903, 826, 758, 719, 699 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 12.33 (1H, brs, Ar-O*H*), 7.89 (1H, d, $J = 6$ Hz, N*H*-CH-CHH-CH-$(CH_3)_2$), 7.57 (1H, d, $J = 2.4$ Hz, N*H*-CH-CHH-CH-$(CH_3)_2$), 7.32 (1H, dd, $J = 2$ Hz, $J = 8.4$ Hz, Ar-*H*), 6.94 (1H, d, $J = 7.6$ Hz, Ar-*H*), 6.86 (1H, d, $J = 8.8$ Hz, Ar-*H*), 5.71–5.60 (1H, m, NH-C*H*-CHH-CH-$(CH_3)_2$), 4.85–5.75 (1H, m, NH-C*H*-CHH-CH-$(CH_3)_2$), 3.87 (3H, s, O-C*H*$_3$), 3.30 (3H, s, N-C*H*$_3$), 1.82–1.70 (3H, m, NH-CH-CH*H*-CH-$(CH_3)_2$, NH-CH-C*H*H-CH-$(CH_3)_2$, NH-CH-CH_2-C*H*-$(CH_3)_2$), 1.70–1.58 (3H, m, NH-CH-C*H*H-CH-$(CH_3)_2$, NH-CH-CH*H*-CH-$(CH_3)_2$, NH-CH-CH_2-C*H*-$(CH_3)_2$), 1.07–0.90 (12H, m, 4 x C*H*$_3$). **^{13}C NMR** (100.79 MHz, $CDCl_3$-d_1): δ 173.0, 172.7, 168.8, 160.1, 134.1, 126.0, 123.5, 119.9, 115.1, 61.8, 52.0, 48.3, 41.9, 41.8, 32.5, 25.0, 24.9, 23.5, 23.2, 21.9, 21.6. **CHN Analysis:** Calc. for $C_{21}H_{32}ClN_3O_5$ (441.95): C, 57.07; H, 7.30; N, 9.51. Found: C, 57.35 ± 0.03; H 7.50 ± 0.02; N, 9.42 ± 0.03. **HRMS**: *m/z* calc. for $C_{21}H_{32}ClN_3O_5$: 464.19227 $[M+Na]^+$; found: 464.19309 $[M+Na]^+$.

3.5.4. Characterization of 5-chloro-2-hydroxy-N-((2S)-1-(((2S)-1-(methoxy(methyl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)benzamide **5d**

White solid; yield 78%; mp 222–226 °C; $[\alpha_D^{20}] = -15.3°$ (c 1.00, EtOAc); R_f (hex/EtOAc–1/1) = 0.33. **IR** (ATR):3337, 3251, 3071, 2947, 2360, 2341, 2360, 2341, 1638, 1590, 1552, 1479, 1368, 1261, 1231, 903, 829, 716 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 12.33 (1H, brs, Ar-O*H*), 7.60 (1H, d, $J = 8$ Hz, N*H*-CH-CHH-CH-$(CH_3)_2$), 7.55 (1H, d, $J = 2$ Hz, N*H*-CH-CHH-Ph), 7.37–7.32 (1H, m, Ar-*H*), 7.32–7.17 (5H, m, Ar-*H*), 7.08 (1H, d, $J = 8.4$ Hz, Ar-*H*), 6.91 (1H, d, $J = 8.8$ Hz, Ar-*H*), 5.41–5.33 (1H, m, NH-C*H*-CHH-CH-$(CH_3)_2$), 4.80–4.70 (1H, m, NH-C*H*-CHH-Ph), 3.77 (3H, s, O-C*H*$_3$), 3.29 (3H, s, N-C*H*$_3$), 3.22 (1H, dd, $J = 5.6$ Hz, $J = 13.6$ Hz, NH-CH-CH*H*-Ph), 3.05 (1H, dd, $J = 5.6$ Hz, $J = 13.6$ Hz, NH-CH-C*H*H-Ph), 1.79–1.66 (2H, m, NH-CH-CH*H*-CH-$(CH_3)_2$, NH-CH-CH_2-C*H*-$(CH_3)_2$), 1.65–1.55 (1H, m, NH-CH-C*H*H-CH-$(CH_3)_2$), 0.98–0.89 (6H, m, C*H*$_3$-CH-C*H*$_3$). **^{13}C NMR** (100.79 MHz, $CDCl_3$-d_1): δ 172.1, 170.1, 168.9, 160.3, 136.8, 134.2, 129.7, 128.6, 127.2,

125.9, 123.1, 120.0, 114.8, 61.8, 52.0, 50.9, 45.1, 43.2, 32.3, 24.9, 23.2, 21.9. **CHN Analysis:** Calc. for $C_{24}H_{30}ClN_3O_5$ (475.97): C, 60.56; H, 6.35; N, 8.83. Found: C, 60.79 ± 0.01; H 6.39 ± 0.02; N, 8.83 ± 0.03. **HRMS**: *m/z* calc. for $C_{24}H_{30}ClN_3O_5$: 498.17662 $[M+Na]^+$; found: 498.17737 $[M+Na]^+$.

3.5.5. Characterization of 5-chloro-2-hydroxy-N-((2S)-1-(((2S)-1-(methoxy(methyl)amino)-4-methyl-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)benzamide **5e**

White solid; yield 32%; mp 221.7–224.4 °C; $[\alpha_D^{20}] = -42.3°$ (c 1.00, EtOAc); R_f (hex/EtOAc–1/1) = 0.43. **IR** (ATR):3345, 3253, 3069, 2970, 2359, 2341, 1616, 1596, 1552, 1477, 1224, 827, 820, 743, 717, 698 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 12.20 (1H, brs, Ar-OH), 7.96 (1H, d, *J* = 7.6 Hz, NH-CH-CHH-CH-$(CH_3)_2$), 7.51 (1H, d, *J* = 2.4 Hz, NH-CH-CHH-Ph), 7.33–7.21 (6H, m, Ar-H), 7.19 (1H, d, *J* = 8.8 Hz, Ar-H), 6.84 (1H, d, *J* = 8.8 Hz, Ar-H), 5.25–5.15 (1H, m, NH-CH-CHH-Ph), 5.06–4.99 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 3.81 (3H, s, O-CH_3), 3.31 (3H, s, N-CH_3), 3.20 (2H, d, *J* = 6.4 Hz, NH-CH-CH_2-Ph), 1.75–1.65 (1H, m, NH-CH-CH_2-CH-$(CH_3)_2$), 1.65–1.55 (2H, m, NH-CH- CH_2-CH-$(CH_3)_2$, 1.00 (3H, d, *J* = 6.4 Hz, CH_3-CH-CH_3), 0.92 (3H, d, *J* = 6.4 Hz, CH_3-CH-CH_3). **^{13}C NMR** (100.79 MHz, $CDCl_3$-d_1): δ 173.1, 171.3, 168.6, 160.1, 136.5, 134.2, 129.5, 128.7, 127.3, 126.0, 123.5, 119.9, 115.1, 61.9, 54.6, 48.2, 42.0, 38.7, 32.5, 25.0, 23.4, 21.8. **CHN Analysis:** Calc. for $C_{24}H_{30}ClN_3O_5$ (475.97): C, 60.56; H, 6.35; N, 8.83. Found: C, 60.79 ± 0.01; H 6.43 ± 0.01; N, 8.79 ± 0.01. **HRMS**: *m/z* calc. for $C_{24}H_{30}ClN_3O_5$: 498.17662 $[M+Na]^+$; found: 498.17758 $[M+Na]^+$.

3.5.6. Characterization of 5-chloro-2-hydroxy-N-((2S)-1-((1-(methoxy(methyl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)benzamide **5f**

Product is a mixture of two diastereomers, A and B. White solid; yield 42%; mp 197.8–200.2 °C; $[\alpha_D^{20}] = -51.0°$ (c 0.4, EtOAc); R_f (hex/EtOAc–1/1) = 0.22. **IR** (ATR): 3305, 3067, 2939, 2352, 2341, 1657, 1633, 1592, 1547, 1493, 1227, 823, 748, 720, 699, 669 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 12.19–12.16 (1H, m, (Ar-OH)$_{A+B}$), 7.62–7.52 (1H, m, (NH-CH-CHH-Ph)$_{A+B}$), 7.48–7.38 (1H, m, (NH-CH-CHH-Ph)$_{A+B}$), 7.40–7.18 (9H, m, (Ar-H)$_{A+B}$), 7.18–7.09 (2H, m, (Ar-H)$_{A+B}$), 6.95–6.82 (2H, m, (Ar-H)$_{A+B}$), 5.37–5.25 (1H, m, (NH-CH-CHH-Ph)$_{A+B}$), 4.98–4.33 (1H, m, (NH-CH-CHH-Ph)$_{A+B}$), 3.75–3.60 (3H, m, (N-CH_3)$_{A+B}$), 3.32–3.25 (3H, m, (O-CH_3)$_{A+B}$), 3.24–2.90 (4H, m, 2 x (NH-CH-CH_2-Ph)$_{A+B}$). **^{13}C NMR** (100.79 MHz, $CDCl_3$-d_1): δ 170.9, 170.5, 168.6, 160.2, 136.4, 136.0, 134.4, 129.6, 129.5, 128.8, 128.6, 127.4, 127.3, 125.8, 123.5, 120.1, 115.0, 61.8, 54.6, 50.9, 38.5, 38.4, 32.4. **CHN Analysis:** Calc. for $C_{27}H_{28}ClN_3O_5$ (509.98): C, 63.59; H, 5.53; N, 8.24. Found: C, 63.58 ± 0.01; H 5.55 ± 0.01; N, 8.24 ± 0.01. **HRMS**: *m/z* calc. for $C_{27}H_{28}ClN_3O_5$: 532.16097 $[M+Na]^+$; found: 532.16205 $[M+Na]^+$.

3.5.7. Characterization of N-((5S,8S,11S)-8-benzyl-5-isobutyl-3,13-dimethyl-4,7,10-trioxo-2-oxa-3,6,9-triazatetradecan-11-yl)-5-chloro-2-hydroxybenzamide **5g**

White solid; yield 77%; mp 130.4–134.6 °C; $[\alpha_D^{20}] = -31.5°$ (c 1.00, EtOAc); R_f (hex/EtOAc–1/1) = 0.41. **IR** (ATR): 3298, 3067, 2957, 2870, 2361, 2341, 1637, 1592, 1538, 1474, 1367, 1290, 1220, 824, 699 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 12.45 (1H, brs, Ar-OH), 8.08 (1H, d, *J* = 8 Hz, NH-CH-CHH-CH-$(CH_3)_2$), 7.95 (1H, d, *J* = 8 Hz, NH-CH-CHH-CH-$(CH_3)_2$), 7.69 (1H, d, *J* = 2 Hz, NH-CH-CHH-Ph), 7.39 (1H, dd, *J* = 2.5 Hz, *J* = 8.8 Hz, Ar-H), 7.07–6.97 (5H, m, Ar-H), 6.97–6.91 (1H, m, Ar-H), 6.88 (1H, d, *J* = 8.4 Hz, Ar-H), 5.30–5.20 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 5.20–5.10 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 4.84–4.72 (1H, m, NH-CH-CHH-Ph), 3.81 (3H, s, O-CH_3), 3.28 (3H, s, N-CH_3), 2.99 (2H, d, *J* = 7.2 Hz, NH-CH-CH_2-Ph), 1.82–1.58 (4H, m, NH-CH-CHH-CH-$(CH_3)_2$, NH-CH-CHH-CH-$(CH_3)_2$, 2 x NH-CH-CH_2-CH-$(CH_3)_2$), 1.58–1.48 (2H, m, NH-CH-CHH-CH-$(CH_3)_2$, NH-CH-CHH-CH-$(CH_3)_2$), 0.97 (9H, d, *J* = 6.4 Hz, 3 x CH_3), 0.92 (3H, d, *J* = 6.4 Hz, CH_3). **^{13}C NMR** (100.79 MHz, $CDCl_3$-d_1): δ 172.7, 171.7, 170.9, 169.0, 160.3, 136.9, 134.2, 129.5, 128.3, 127.0, 126.7, 123.3, 119.9, 115.6, 61.9, 54.1, 52.0, 47.3, 42.4, 41.2, 39.3, 32.5, 25.03, 25.01, 23.4, 23.0, 22.4, 22.2. **CHN Analysis:** Calc. for $C_{30}H_{41}ClN_4O_6$ (589.12): C, 61.16; H, 7.01; N, 9.51. Found: C, 60.96 ± 0.02; H 6.82 ± 0.02; N, 9.80 ± 0.02. **HRMS**: *m/z* calc. for $C_{30}H_{41}ClN_4O_6$: 611.26068 $[M+Na]^+$; found: 611.26186 $[M+Na]^+$.

3.5.8. Characterization of N-((5S,8S,11S)-5,8-dibenzyl-3,13-dimethyl-4,7,10-trioxo-2-oxa-3,6,9-triazatetradecan-11-yl)-5-chloro-2-hydroxybenzamide **5h**

White solid; yield 93%; mp 130–133.7 °C; $[\alpha_D^{20}] = -20.0°$ (c 1.00, EtOAc); R_f (hex/EtOAc–1/1) = 0.37. **IR** (ATR):3291, 3066, 2957, 2361, 2343, 1619, 1591, 1535, 1493, 1478, 1290, 1222, 824, 743, 699 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 12.50 (1H, brs, Ar-OH), 8.18–8.02 (2H, m, NH-CH-CHH-CH-$(CH_3)_2$, NH-CH-CHH-Ph), 7.62 (1H, d, *J* = 2.4 Hz, NH-CH-CHH-Ph), 7.33 (1H, dd, *J* = 2.5 Hz, *J* = 8.8 Hz, Ar-H), 7.26–7.05 (6H, m, Ar-H), 7.04–6.82 (6H, m, Ar-H), 5.35–5.22 (2H, m, 2 x NH-CH-CHH-Ph), 4.83–4.73 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 3.55 (3H, s, O-CH_3), 3.14 (3H, s, N-CH_3), 3.10–2.85 (4H, m, 2 x NH-CH-CH_2-Ph), 1.78–1.65 (2H, m, NH-CH-CHH-CH-$(CH_3)_2$, NH-CH-CH_2-CH-$(CH_3)_2$), 1.61–1.50 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 0.96–0.85 (6H, m, 2 x CH_3). **^{13}C NMR** (100.79 MHz, $CDCl_3$-d_1): δ 171.9, 171.2, 170.9, 169.2, 160.5, 136.3, 136.1, 134.2, 129.6, 129.5, 128.5, 128.3, 127.1, 127.0, 126.6, 123.2, 120.0, 115.7, 61.8, 54.1, 51.9, 50.2, 41.1, 39.8, 39.2, 32.5, 25.1, 23.3, 22.1. **CHN Analysis:** Calc. for $C_{33}H_{39}ClN_4O_6$ (623.14): C, 63.61; H, 6.31; N, 8.99. Found: C, 63.81 ± 0.01; H 6.49 ± 0.01; N, 8.75 ± 0.02. **HRMS**: *m/z* calc. for $C_{33}H_{39}ClN_4O_6$: 645.24503 $[M+Na]^+$; found: 645.24582 $[M+Na]^+$.

3.6. General scheme and procedure for deprotected aldehydes **6a-c** and alcohols **7a,b**

Reduction of Weinreb amide to aldehyde was done according to similar reaction described in Ref. [31]. L-leucine-L-phenylalanine Weinreb amide **5d** (0.166 g, 0.349 mmol) was dissolved in a secured three-neck round-bottom flask in dry tetrahydrofurane (40 mL) under nitrogen atmosphere and cooled to −5 °C. $LiAlH_4$ (0.039 g, 1.03 mmol) was added in two portions (0.026 g at the beginning and 0.013 g after 45 min) under the inflow of nitrogen. The reaction was stirred for the total of 90 min, then quenched with saturated solution of NH_4Cl in H_2O (20 mL) and 10% HCl (3 mL) and extracted with ethyl acetate (3 × 40 mL). The combined organic phase was washed with H_2O (2 × 20 mL) and dried over Na_2SO_4. The product **6a** was isolated by reducing the volume of solvent and drying on rotary vacuum evaporator in 83% yield as colourless oil.

Derivatives with three amino acids undergo the reaction under same conditions except for the reaction period, which was extended to 1 h. From the reaction mixture aldehydes **6b,c** in yields between 30 and 48% were isolated as white solid or colourless oil. Also corresponding alcohols **7a,b** in yields between 40 and 44% were isolated.

3.6.1. Characterization of 5-Chloro-2-hydroxy-N-((2S)-4-methyl-1-oxo-1-(((2S)-1-oxo-3-phenylpropan-2-yl)amino)pentan-2-yl) benzamide **6a**

Colourless oil; yield 83%; $[\alpha_D^{20}] = -25.8°$ (c 1.00, EtOAc); R_f (hex/EtOAc–1/1) = 0.38. **IR** (ATR): 3302, 3066, 2958, 2928, 1736, 1640, 1593, 1536, 1485, 1366, 1290, 1255, 1228, 1114, 823, 750, 721, 701 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$-d_1): δ 12.11 (1H, s, Ar-OH), 9.72 (1H, s, CHO), 7.62 (1H, d, *J* = 8 Hz, NH-CH-CHH-CH-$(CH_3)_2$), 7.52 (1H, d, *J* = 2.8 Hz, NH-CH-CHH-Ph), 7.33–7.18 (6H, m, Ar-H), 6.90 (1H, d, *J* = 7.2 Hz, Ar-H), 6.70 (1H, d, *J* = 6.8 Hz, Ar-H), 4.84 (1H, q, *J* = 6.4 Hz, NH-CH-CHH-Ph), 4.74–4.63 (1H, m, NH-CH-CHH-CH-

$(CH_3)_2$), 3.33–3.15 (2H, m, NH-CH-CH_2-Ph), 1.85–1.68 (3H, m, NH-CH-CH_2-CH-(CH_3), NH-CH-CH_2-CH-(CH_3), 1.05–0.80 (6H, m, CH_3-CH-CH_3). **^{13}C NMR** (100.79 MHz, CD_2Cl_2-d_2): δ 198.2, 172.7, 169.1, 160.1, 135.1, 134.5, 129.5, 129.0, 127.5, 125.9, 123.6, 120.1, 114.8, 60.1, 52.0, 40.9, 35.2, 25.0, 23.1, 22.1. **CHN Analysis:** Calc. for $C_{22}H_{25}ClN_2O_4$ (416.90): C, 63.38; H, 6.04; N, 6.72. Found: C, 63.68 ± 0.02; H 7.15 ± 0.02; N, 5.82 ± 0.02. **HRMS**: *m/z* calc. for $C_{22}H_{25}ClN_2O_4$: 439.13951 $[M+Na]^+$; found: 439.14021 $[M+Na]^+$.

3.6.2. Characterization of N-((2S)-4-methyl-1-(((2S)-1-(((2S)-4-methyl-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxopentan-2-yl-5-chloro-2-hydroxybenzamide **6b**

White solid; yield 48%; mp 145–148.2 °C; $[\alpha_D^{20}] = -44.3°$ (c 1.00, EtOAc) R_f (hex/EtOAc–1/1) = 0.47. **IR** (ATR): 3307, 3066, 2958, 2871, 2360, 2342, 1734, 1637, 1596, 1533, 1496, 1489, 1368, 1287, 1227, 1114, 824, 746, 700, 669 cm^{-1}. **^{1}H NMR** (400 MHz, CD_2Cl_2-d_2): δ 12.01 (1H, s, Ar-OH), 9.44 (1H, s, CHO), 7.61 (1H, d, *J* = 8 Hz, NH-CH-CHH-Ph), 7.44 (1H, d, *J* = 2.8 Hz, NH-CH-CHH-CH-$(CH_3)_2$), 7.42 (1H, d, *J* = 2.8 Hz, NH-CH-CHH-CH-$(CH_3)_2$), 7.35–7.19 (5H, m, Ar-H), 7.12–7.03 (1H, m, Ar-H), 6.99 (1H, d, *J* = 8.8 Hz, Ar-H), 6.75–6.65 (1H, m, Ar-H), 4.88 (1H, q, *J* = 7.2 Hz, NH-CH-CHH-Ph), 4.80–4.72 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 4.47–4.39 (1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 3.25–3.12 (2H, m, NH-CH-CH_2-Ph), 1.80–1.60 (5H, m, 2 x NH-CH-CH_2-CH-$(CH_3)_2$, NH-CH-CH_2-CH-$(CH_3)_2$), 1.45–1.30 (1H, m, NH-CH-CH_2-CH-$(CH_3)_2$), 1.00–0.87 (12H, m, 4 x CH_3). **^{13}C NMR** (100.79 MHz, CD_2Cl_2-d_2): δ 199.5, 171.9, 171.1, 168.8, 159.9, 136.3, 134.4, 129.4, 128.8, 127.2, 126.3, 123.6, 120.0, 115.3, 57.5, 54.6, 52.1, 41.1, 38.3, 37.7, 25.0, 24.7, 22.9, 22.8, 21.9, 21.7. **CHN Analysis:** Calc. for $C_{28}H_{36}ClN_3O_5$ (530.06): C, 63.45; H, 6.85; N, 7.93. Found: C, 63.31 ± 0.02; H 6.86 ± 0.01; N, 7.89 ± 0.01. **HRMS**: *m/z* calc. for $C_{28}H_{36}ClN_3O_5$: 552.22357 $[M+Na]^+$; found: 552.22415 $[M+Na]^+$.

3.6.3. Characterization of N-((2S)-4-methyl-1-oxo-1-(((2S)-1-oxo-1-(((2S)-1-oxo-3-phenylpropan-2-yl)amino)-3-phenylpropan-2-yl)amino)pentan-2-yl)-5-chloro-2-hydroxybenzamide **6c**

Colourless oil; yield 30%; R_f (hex/EtOAc–1/1) = 0.38. **IR** (ATR): 3289, 3065, 2960, 2925, 1633, 1593, 1537, 1494, 1258, 1081, 1017, 797, 708, 697, 649 cm^{-1}. **^{1}H NMR** (400 MHz, CD_2Cl_2-d_2): δ 12.08 (1H, s, Ar-OH), 9.52 (1H, s, CHO), 7.65–7.58 (2H, m, 2 x NH), 7.43–7.10 (12H, m, NH, Ar-H), 6.99–6.93 (1H, m, Ar-H), 6.85–6.70 (1H, m, Ar-H), 4.95–4.83 (1H, m, NH-CH), 4.82–4.70 (1H, m, NH-CH), 4.70–4.60 (1H, m, NH-CH), 3.2–3.00 (4H, m, 2 x NH-CH-CH_2-Ph), 1.79–1.70 (3H, m, NH-CH-CH_2-CH-$(CH_3)_2$), 1.00–0.88 (6H, m, 2 x CH_3). **^{13}C NMR** (100.79 MHz, CD_2Cl_2-d_2): δ 198.5, 172.3, 171.0, 168.9, 159.9, 136.2, 135.8, 134.3, 129.45, 129.35, 128.9, 128.8, 127.3, 126.4, 123.5, 119.9, 60.0, 54.7, 52.1, 41.0, 38.6, 35.1, 25.0, 22.9, 21.8. **CHN Analysis:** Calc. for $C_{31}H_{34}ClN_3O_5$ (564.07): C, 66.01; H, 6.08; N, 7.45. Found: C, 65.97 ± 0.02; H 6.17 ± 0.01; N, 7.29 ± 0.01. **HRMS**: *m/z* calc. for $C_{31}H_{34}ClN_3O_5$: 586.20792 $[M+Na]^+$; found: 586.20897 $[M+Na]^+$.

3.6.4. Characterization of N-((2S)-1-(((2S)-1-(((2S)-1-hydroxy-4-methylpentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)-5-chloro-2-hydroxybenzamide **7a**

Colourless oil; yield 44%; R_f (hex/EtOAc–1/1) = 0.18. **IR** (ATR): 3307, 3066, 2958, 2871, 2360, 2341, 1734, 1636, 1595, 1540, 1533, 1496, 1473, 1457, 1367, 1288, 1230, 1114, 1068, 1031, 903, 823, 745, 700, 669 cm^{-1}. **^{1}H NMR** (500 MHz, $CDCl_3$-d_1): δ 12.85 (1H, brs, Ar-OH), 9.38 (1H, brs, NH), 7.44 (1H, brs, NH), 7.42 (1H, brs, NH), 7.50–7.23 (6H, m, Ar-H), 7.23–7.00 (2H, m, Ar-H), 5.40–5.20 (1H, m, NH-CH), 5.20–5.00 (1H, m, NH-CH), 4.00–3.80 (1H, m, NH-CH), 3.39 (2H, brs, CH_2-OH), 3.24 (1H, brs, CH_2-OH), 1.90–1.30 (8H, m, 2 x CH_2-CH-$(CH_3)_2$, CH_2-Ph), 1.10–0.60 (12H, m, 4 x CH_3). **^{13}C NMR** (125.78 MHz, $CDCl_3$-d_1): δ 172.5, 172.2, 171.1, 168.2, 166.1, 135.6, 134.1, 133.5, 129.4, 128.8, 127.5, 126.9, 124.4, 64.4, 61.6, 51.1, 47.5, 42.3, 39.3, 31.4, 30.2, 29.7, 24.6, 23.3, 22.9, 22.4. **CHN Analysis:** Calc. for $C_{28}H_{38}ClN_3O_5$ (532.07): C, 63.21; H, 7.20; N, 7.90. Found: C, 63.32 ± 0.02; H 7.29 ± 0.02; N, 7.82 ± 0.02. **HRMS**: *m/z* calc. for $C_{28}H_{38}ClN_3O_5$: 532.25728 $[M+H]^+$; found: 532.25776 $[M+H]^+$.

3.6.5. Characterization of N-((2S)-1-(((2S)-1-(((2S)-1-hydroxy-3-phenylpropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)-5-chloro-2-hydroxybenzamide **7b**

Colourless oil; yield 40%; R_f (hex/EtOAc–1/1) = 0.20. **IR** (ATR): 3304, 3066, 2958, 2361, 2339, 1726, 1640, 1597, 1546, 1536, 1494, 1288, 1257, 1228, 1113, 1034, 903, 823, 744, 700, 669 cm^{-1}. **^{1}H NMR** (400 MHz, CD_2Cl_2-d_2): δ 12.10 (1H, s, Ar-OH), 7.80 (1H, d, *J* = 6 Hz, NH), 7.66 (1H, d, *J* = 2.4 Hz, NH), 7.47–7.35 (2H, m, NH, Ar-H), 7.35–7.15 (10H, m, Ar-H), 7.00 (1H, d, *J* = 8.8 Hz, Ar-H), 6.68 (1H, d, *J* = 7.2 Hz, Ar-H), 4.90–4.70 (2H, m, 2 x NH-CH), 4.20–4.10 (1H, m, NH-CH), 3.50–3.40 (2H, m, CH_2-OH), 3.20–3.05 (2H, m, NH-CH-CH_2-Ph), 3.85–3.70 (2H, m, NH-CH-CH_2-Ph), 2.31 (1H, brs, CH_2-OH), 1.80–1.60 (2H, m, NH-CH-CHH-CH-$(CH_3)_2$), 1.40–1.30(1H, m, NH-CH-CHH-CH-$(CH_3)_2$), 1.00–0.85 (6H, m, 2 x CH_3). **^{13}C NMR** (100.79 MHz, $CDCl_3$-d_1): δ 173.5, 171.0, 168.8, 159.6, 137.5, 136.3, 135.9, 134.4, 129.5, 129.4, 129.3, 129.0, 128.9, 128.8, 126.8, 126.2, 123.9, 120.1, 63.0, 59.9, 55.4, 53.1, 52.2, 41.3, 38.8, 36.9, 35.3, 29.9, 25.0, 23.3, 22.1. **CHN Analysis:** Calc. for $C_{31}H_{36}ClN_3O_5$ (566.09): C, 65.77; H, 6.41; N, 7.42. Found: C, 64.49 ± 0.02; H 6.45 ± 0.02; N, 6.62 ± 0.02. **HRMS**: *m/z* calc. for $C_{31}H_{36}ClN_3O_5$: 588.22357 $[M+Na]^+$; found: 588.22440 $[M+Na]^+$.

3.7. Antiproliferative assays

Cancer cell lines of different histological origin were cultivated according to the manufacturer's instructions. The cells were assayed with compounds using three-fold dilutions in triplicate. Treatment lasted for 72 h, followed by addition of Calcein AM solution, and measurement of the fluorescence of live cells at 485/538 nm (ex/em) with a Fluoroskan Ascent microplate reader (Labsystems). The GI_{50} value, the drug concentration lethal to 50% of the tumour cells, was calculated from the obtained dose response curves.

3.8. Proteasome-Glo™ chymotrypsin-like cell based assay

Multiple myeloma U266 cells were plated at the appropriate density (20,000 cells/well) in 96-well LumiNunc™ plates and treated with different concentrations of tested compounds for 1 h at 37 °C in duplicate. The activity of the 26S proteasome was measured immediately by hydrolysis of the β5 (chymotrypsin-like) substrate Suc-LLVY-aminoluciferin in the presence of luciferase using the Proteasome-Glo™ assay reagents according to the manufacturer's instructions (Promega). Luminescence was measured using a TECAN Infinite® M200 Multi-mode Microplate Reader. Proteasome activity was normalised against activity of untreated cells and the IC_{50} values were calculated from the obtained dose response curves.

3.9. Time lapse microscopy

For time lapse microscopy, cells were plated at the appropriate density (10,000 cells/well) in 96- plates and treated with different concentrations of tested compounds for 72 h. Time lapse images and videos were captured by IncuCyte ZOOM® (Essen BioScience). An acquisition of images (1 image per hour) in a green channel and further an integrated software analysis was used for quantification of cells expressing GFP. Cell proliferation was monitored by analysing the occupied area (% of confluence) of cell images over time with confluence image mask (Gold) using IncuCyte™ cell

proliferation assay.

3.10. Caspase activity assay

The cells were homogenised in an extraction buffer (10 mM KCl, 5 mM HEPES, 1 mM EDTA, 1 mM EGTA, 0.2% CHAPS, inhibitors of proteases, pH 7.4) on ice for 20 min. The homogenates were clarified by centrifugation at 10,000 × g for 30 min at 4 °C, and proteins quantified and diluted to equal concentrations. Lysates were then incubated for the indicated time with 100 μM Ac-DEVD-AMC (Enzo Life Sciences) as a substrate of caspases- 3,7 in the assay buffer (25 mM PIPES, 2 mM EGTA, 2 mM $MgCl_2$, 5 mM DTT, pH 7.3). The fluorescence of the product was measured using a Fluoroskan Ascent microplate reader (Labsystems) at 355/460 nm (ex/em) and data normalised against an untreated control.

3.11. Immunoblotting and antibodies

Briefly, cellular lysates were prepared by harvesting cells in Laemmli sample buffer. Proteins were separated on SDS-polyacrylamide gels and electroblotted onto nitrocellulose membranes. After blocking, the membranes were incubated with specific primary antibodies overnight, washed and then incubated with peroxidase-conjugated secondary antibodies. Finally, peroxidase activity was detected using Pierce™ ECL western blotting substrates and a LAS-4000 CCD camera (Fujifilm).

Specific antibodies were purchased from Sigma-Aldrich (anti-α-tubulin, clone DM1A; peroxidase-labelled secondary antibodies), Cell Signaling (anti-PARP, clone 46D11; anti-caspase-7; anti-caspase-3, clone 3G2; anti-Mcl-1, clone D35A5), Santa Cruz Biotechnology (anti-β-actin antibody, clone C4; anti-p27, clone F-8) or Dako Cytomation (anti-ubiquitin).

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2017.04.027.

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