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Development of Novel Peptidomimetics Containing a Vinyl Sulfone Moiety as Proteasome Inhibitors

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Proteasome inhibition is a topic of great interest in anticancer research. The proteolytic activity of this multicatalytic complex relies on three subunits, β1, β2 and β5, containing a caspase-like, a trypsin-like and a chymotrypsin-like active site, respectively. Several studies have demonstrated that, of the three activities, the chymotrypsin-like activity was the most necessary for cell viability and protein processing. Thus, most efforts towards the development of proteasome inhibitors have focused on the selective inhibition of the β5 subunit active site. Herein, we report the design and synthesis of a series of conformationally constrained tripeptidyl vinyl sulfones were determined to be good inhibitors of the chymotrypsin-like activity of proteasome, with K_i values in the sub-micromolar to micromolar range. These compounds were also tested against bovine pancreatic α-chymotrypsin and human cathepsin B and L, revealing a good selectivity for the target enzyme over these related enzymes.

Introduction

The ubiquitin/proteasome system is the major nonlysosomal proteolytic system for the degradation of abnormal or damaged proteins, as well as proteins that are no longer required.[1] Defects in the ubiquitin/proteasome pathway (UPP) can lead to uncontrolled cell proliferation and tumor development. For these reasons, UPP inhibition has become a new and significant strategy for the drug development of cancer therapeutics.

In order to be recognized by the proteolytic system and targeted for degradation, eukaryotic proteins are firstly marked by addition of ubiquitin chains. Once tagged, these polyubiquitin proteins are degraded by 26S proteasome into ubiquitin and short peptides, 3–25 amino acids in length, that are further processed into recyclable amino acids.[2] The 26S proteasome is composed of a 20S catalytic core capped by two 19S regulatory complexes. The 19S caps contain a lid- and base-like structure. The lid component is responsible for recognition of polyubiquitinated substrates and deubiquitinating activity, which allows recycling of ubiquitin moieties. The base component consists of six ATPases required for the unfolding of substrates and the opening of the narrow entry pore of the 20S proteasome.[3]

The 20S proteasome is a barrel-like structure with twofold symmetry composed of four stacked rings of seven subunits each, enclosing a central chamber. Seven different but related α1–α7 subunits form the two outer rings, whereas the two inner rings are composed of seven different β1–β7 subunits that contain the proteolytic sites.[4] In eukaryotic proteasomes, β1, β2 and β5 subunits contain proteolytically active sites, whereas the other β-type subunits are inactive. Each subunit uses the nucleophilic γ-hydroxy group of the N-terminal Thr to efficiently hydrolyze peptide bonds. They differ in substrate specificity: the β1 subunit possesses a post-glutamyl peptide hydrolyzing (PGPH) or caspase-like activity and cleaves peptides with acidic residues in the P1 position; the β2 subunit has a trypsin-like (T-L) activity and cleaves substrates with basic residues at P1; the β5 subunit possesses a chymotrypsin-like (ChT-L) activity and cleaves peptides with large hydrophobic residues at P1 (generally branched-chain amino acids).

Preferences of the various active subunits are determined solely by the substrate binding pocket S1 formed by residue 45 of the catalytic subunit and by different residues of neighboring β-subunits.[5] The S3 pocket is formed by residues of the adjacent β-subunits and is thought to influence the binding efficiency of ligands and their substrate selectivity.[6] Thus, manipulation of the corresponding P3 site of substrates opens new perspectives for design and synthesis of specific inhibitors. Crystal structure analysis also revealed that proteasome does not possess S2 pocket specificity.[7] This feature provides further advantages in the inhibitor design: bulky and space-demanding residues can be introduced in the P2 site, which enhance the specificity of the inhibitor and prevents nonspecific inhibition of other proteases that have smaller binding pockets.

Among the different enzymatic activities of proteasome, the ChT-L activity has emerged as the biological function of greatest interest and the focus of drug discovery efforts. Several compounds, both natural and synthetic, have been found to

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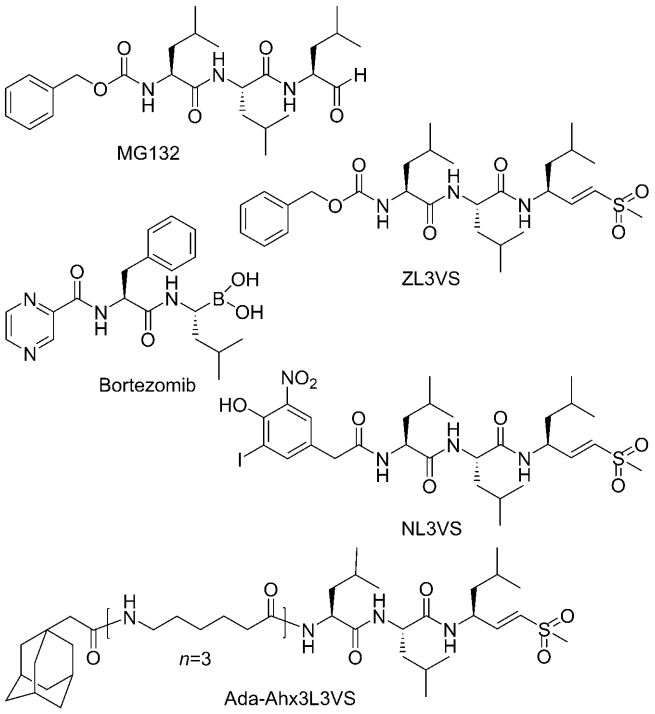
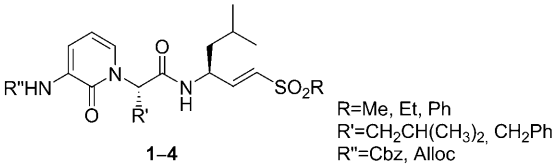
inhibit 20S proteasome and are quite selective for its ChT-L activity.[5,8] Usually they are peptide-based compounds endowed with an electrophilic warhead able to interact with the hydroxy group of the active site Thr residue. Their classification is based on the characteristics of their binding mode to the proteolytic active sites, specificity, and reversibility of binding. Natural inhibitors include lactacystin and epoxomicin, both of which bind to proteasome irreversibly. Synthetic inhibitors are represented by peptide aldehydes, such as MG132, its vinyl sulfone (VS) analogue ZL_3VS, and dipeptide boronate bortezomib (velcade), the first proteasome inhibitor approved by US Food and Drug Administration (FDA) for the treatment of hematological malignancies, such as multiple myeloma and mantle cell lymphoma.[9]



Peptide aldehydes were the first discovered inhibitors of 20S proteasome and are still under active investigation. Generally, aldehyde-containing inhibitors enter cells rapidly and reversibly inhibit the enzyme by forming reversible covalent hemiacetal bonds with the hydroxy group of the active site Thr. These agents exhibit fast dissociation rates, are inactivated by oxidation, and pumped out of the cells by the MDR carrier, affording only short-lived inhibition. In addition, these compounds also inhibit serine and cysteine proteases, and thus would not be safe for use in patients. Vinyl sulfones are less reactive than aldehydes and bind to proteasome irreversibly by acting as Michael acceptors. However, they are not particularly specific for proteasome as they also inhibit intracellular cysteine proteases, such as cathepsins, and this reason may limit their application in vivo.

In regard to vinyl sulfones, one of the most suitable recognition motif seems to be the tripeptide sequence Leu-Leu-Leu bearing a long side chain at the N-terminal residue that could confer optimal potency (e.g., the most potent vinyl sulfone, $AdaAhx_3L_3VS$) and substrate selectivity (e.g., the most selective vinyl sulfone, NL_3VS).[10] So far, no remarkable subunit selectivity has been detected for this class of compounds.[11]

Beginning with this information, the main goal of the present work was the design and synthesis of conformationally constrained peptidomimetics with reduced peptidic character compared with the lead vinyl sulfone, ZL_3VS. This strategy has been accomplished by bioisosteric replacement of the P3 Leu residue with a pyridin-2-one core. This assembly should endow the compounds with greater stability toward degradation by enzymes, improved oral bioavailability, reduced conformational freedom of peptides and thus subunit/substrate selectivity. Further modifications to the new conformationally constrained peptidomimetics entailed the P2 and P3 sites according to the features mentioned above. Specifically, the Leu residue of the P2 site was replaced by a Phe residue and the allyloxycarbonyl (Alloc) group was used in place of the carbobenzyloxy (Cbz) moiety. Finally, taking into account that a Leu residue at P1 site and the functional group downstream of the vinyl sulfone are important structural requirements to direct the selectivity towards ChT-L activity,[12] the pharmacophore portion of all designed compounds (**1**–**4**) was based on these features.

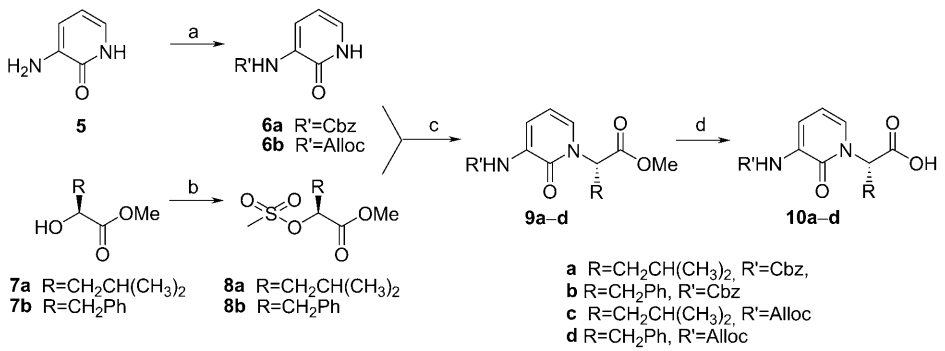


Results and Discussion

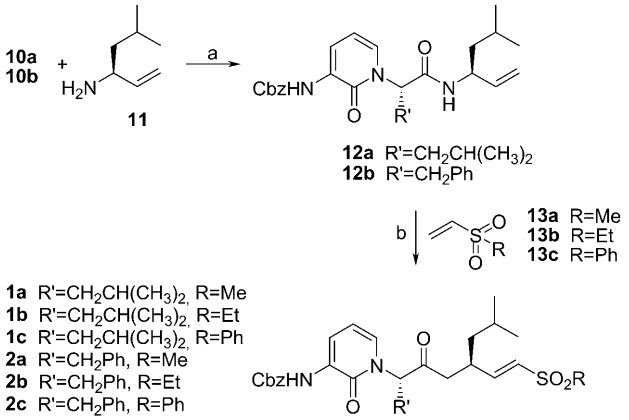
Synthesis

The synthesis of carboxylic acids **10a**–**d** with differently substituted N-terminal group, as mimics of the dipeptides Leu-Leu and Leu-Phe, respectively, is outlined in Scheme 1. The required pyridone scaffold was synthesized from commercially available 3-amino-2-pyridone **5**, by protection of the amine group as the carbamate (**6a**–**b**). The differently substituted P2 fragments were synthesized from the (*S*)-2-hydroxy-4-methylpentanoic acid methyl ester **7a**[13] or (*S*)-2-hydroxy-3-phenylpropionic acid methyl ester **7b**,[13] obtained from the corresponding commercially available acids, and activated into the more reactive methanesulfonates **8a**–**b** by reaction with mesyl chloride in the presence of triethylamine. Intermediates **8a**–**b** were then condensed with the pyridone scaffolds **6a**–**b** to give the esters **9a**–**d**, which were then hydrolized to afford the desired carboxylic acids **10a**–**d**.

Regarding vinyl sulfone series **1**–**2** bearing a Cbz group at the N terminus (Scheme 2), the synthesis of tripeptides **12a**–**b** was realized by coupling the 3-methyl-1-vinyl-butylamine **11**, made according to the work by Albeck et al.,[14] and the carboxylic acids **10a**–**b** in the presence of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU), an efficient coupling reagent for sterically hindered substrates,



Scheme 1. *Reagents and conditions:* a) benzyl or allyl chloroformate, $NaHCO_3$/dioxane (7:3), 0 °C→RT, 12 h; b) MsCl, Et_3N, CH_2Cl_2, RT, 2 h, N_2; c) NaH, DMF, 0 °C→RT, 1 h; then, **6a** or **6b**, RT, 12 h, N_2; d) LiOH, MeOH/H_2O (1:1), RT, 5 h.



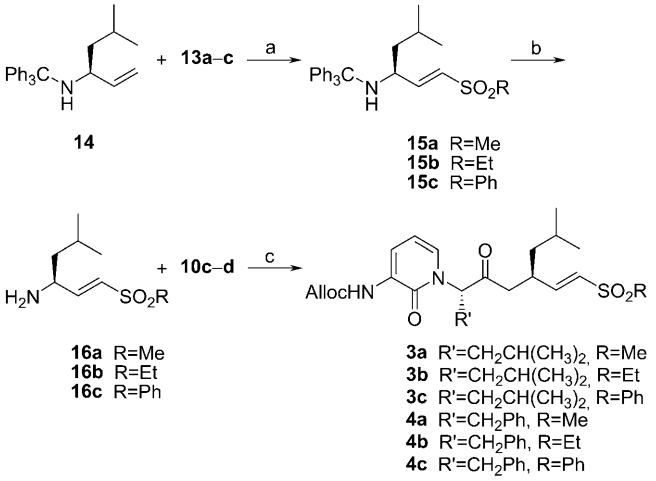
Scheme 2. *Reagents and conditions:* a) HATU, DIPEA, DMF, 100 °C, MW, 2 h; b) Hoveyda catalyst, CH_2Cl_2, 100 °C, MW, 2 h.

and employing diisopropylethylamine (DIPEA) as a base. This reaction, first attempted by stirring the mixture at room temperature overnight, was optimized performing the reaction under microwave irradiation. This strategy provided the desired coupling products **12a–b** in shorter reaction times, with higher yields, and with minimal by-product formation, when compared to standard conditions. The introduction of the required vinyl sulfone warhead on the terminal olefins **13a–b** was achieved by cross-metathesis reaction (CMR), an easy and inexpensive catalytic carbon-bond-forming reactions, using Hoveyda–Grubbs second generation catalyst,[15] a phosphine-free nitrogen-containing heterocyclic carbene ruthenium complex that is easy to handle and able to tolerate a wide range of functional groups, with minimal substrate protection necessary.

Regarding the vinyl sulfone series **3–4**, bearing an Alloc group at the N terminus, the introduction of the pharmacophore portion was realized at a different step in the synthetic route, by coupling intermediates **10c–d** with the amines **16a–c**, already functionalized by olefin metathesis, under microwaves irradiation (Scheme 3). This strategy was selected to avoid a predictable two-directional CMR at both of the terminal olefins of the final tripeptides. The synthesis of amines **16a–c** was first attempted on amine **11**, which was found to be unreactive under CMR conditions, presumably because the amine polar functionality is able to bind the catalyst, removing it from the catalytic cycle.[16] Therefore, the vinyl sulfone moiety was introduced by CMR on *N*-trityl-3-methyl-1-vinyl-butylamine (**14**) and the intermediates **15a–c** deprotected to give the desired intermediates **16a–c**.

Biological activity

All synthesized compounds **1–4** were tested for their inhibitory capacity on purified 20S proteasome isolated from human erythrocytes, using the appropriate



Scheme 3. *Reagents and conditions:* a) Hoveyda catalyst, CH_2Cl_2, 100 °C, MW, 2 h; b) 6 N HCl/acetone, Δ, 5 h; c) HATU, DIPEA, DMF, 100 °C, MW, 2 h.

fluorogenic substrate for each of the proteolytic activities (Suc-Leu-Leu-Val-Tyr-AMC for ChT-L; Boc-Leu-Arg-Arg-AMC for T-L; Z-Leu-Leu-Glu-AMC for PGPH). First, compounds underwent a preliminary screening for ChT-L activity at 20 μM using an equivalent volume of dimethyl sulfoxide (DMSO) as a negative control. The screening showed that all compounds, with the exception of **4a**, inhibited more than 40% of the enzyme activity. Thus, continuous assays (progress curve method, from 0 to 100 μM) were performed to determine the first-order rate constants of inhibition k_{inac} (min^{-1}), the dissociation constants K_I (μM), and the second-order rate constants of inhibition k_{2nd} ($M^{-1}\,min^{-1}$), as $k_{2nd} = k_{inac}/K_I$ (Table 1).

All compounds exhibit good affinity for the proteasome β5 subunit with K_I values in the sub-micromolar to micromolar range. Generally, compounds with an Alloc group as the side chain at the P3 site exhibit better affinity and inhibitory potency with respect to their Cbz group-containing derivatives, how-

Table 1. Inhibition of chymotrypsin-like proteasome activity by vinyl sulfones **1**–**4**.

Compd	K_{2nd}[a] [$M^{-1}min^{-1}$]	k_{inac}[a] [min^{-1}]	K_i[a] [μM]
1a	47000	0.044	0.94
1b	61000	0.056	0.91
1c	13000	0.058	4.5
2a	177000	0.99	5.6
2b	154000	0.051	0.33
2c	66000	0.079	1.2
3a	53000	0.049	0.93
3b	102000	0.062	0.61
3c	77000	0.073	0.95
4a	30% inhibition at 20 μM		
4b	105000	0.039	0.37
4c	47000	0.090	1.93
ZL_3VS	1740[b]		

[a] Values represent the mean of three independent determinations; variability is less than 10%. [b] Data reported in Reference [17] as $K_{obs}/[I]$ and converted into $M^{-1}min^{-1}$ for comparison purposes only.

Table 2. Inhibition of the post-glutamyl peptide hydrolyzing proteasome activity by selected vinyl sulfones.

Compd	K_{2nd}[a] [$M^{-1}min^{-1}$]	k_{inac}[a] [min^{-1}]	K_i[a] [μM]
1a	31000	0.095	3.1
1b	16000	0.083	5.2
1c	1900	0.044	23
2a	6600	[b]	[b]
2c	5000	0.032	6.9
3c	3200	0.047	15
ZL_3VS	300[c]		

[a] Values represent the mean of three independent determinations; variability is less than 10%. [b] Plot of k_{obs} versus [I] is restricted to the linear range, thus the individual constants k_{inac} and K_i could not be determined. [c] Data reported in Reference [17] as $K_{obs}/[I]$ and converted into $M^{-1}min^{-1}$ for comparison purposes only.

ever, this is only true of compounds with a Leu residue at the P2 site, for example, **3b** (K_i = 0.61 μM; k_{2nd} = 102000 $M^{-1}min^{-1}$) and **3c** (K_i = 0.95 μM; k_{2nd} = 77000 $M^{-1}min^{-1}$) versus **1b** (K_i = 0.91 μM; k_{2nd} = 61000 $M^{-1}min^{-1}$) and **1c** (K_i = 4.5 μM; k_{2nd} = 13000 $M^{-1}min^{-1}$). Conversely, compounds with a Phe residue at the P2 site show higher inhibitory potency compared to compounds with a Cbz group at the P3 site, that is, **2a** (k_{2nd} = 177000 $M^{-1}min^{-1}$), **2b** (k_{2nd} = 154000 $M^{-1}min^{-1}$) and **2c** (k_{2nd} = 66000 $M^{-1}min^{-1}$) versus **1a** (k_{2nd} = 47000 $M^{-1}min^{-1}$), **1b** (k_{2nd} = 61000 $M^{-1}min^{-1}$) and **1c** (k_{2nd} = 13000 $M^{-1}min^{-1}$). Modifications to the pharmacophore fragment follow a common trend with higher affinity and inhibitory potency for ethyl-vinyl sulfones with respect to the methyl and phenyl analogues, for example, **3b** (K_i = 0.61 μM, k_{2nd} = 102000 $M^{-1}min^{-1}$) versus **3a** (K_i = 0.93 μM, k_{2nd} = 53000 $M^{-1}min^{-1}$). Ethyl derivative **2b** represents an exception to this trend as it is less potent than its methyl analogue **2a** but possesses a higher affinity, that is, **2b** (k_{2nd} = 154000 $M^{-1}min^{-1}$, K_i = 0.33 μM) versus **2a** (k_{2nd} = 177000 $M^{-1}min^{-1}$, K_i = 5.6).

Noteworthy, the most potent compound of this series (**2a**, k_{2nd} = 177000 $M^{-1}min^{-1}$) also has the weakest affinity towards the enzyme (K_i = 5.6 μM), but the highest first-order rate constant of inhibition (k_i = 0.99 min^{-1}). Interestingly, all the synthesized vinyl sulfones **1**–**4**, with the exception of compound **4a**, turned out to be more potent than the elected lead ZL_3VS,[17] validating our rational drug design strategy.

The main issue of these newly synthesized vinyl sulfones is their subunit selectivity. As shown in Table 2, six out of twelve compounds did not pass the preliminary screening for proteasome PGPH inhibitory activity, using the same selection criteria as for the ChT-L activity. Compounds considered for continuous assays (**1a–c**, **2a**, **2c**, **3c**) displayed moderate inhibition of proteasome β1 subunit, with K_i values in the micromolar range and second-order rate constants one or two order of magnitude less than those detected for the β5 subunit, that is, **2c** and **3c** (k_{2nd} = 5000 and 3200 $M^{-1}min^{-1}$, respectively) or **2a** (k_{2nd} = 6,600 $M^{-1}min^{-1}$). None of these peptide-like vinyl sulfones exhibited any inhibition in the T-L activity assay at 20 μM.

The selectivity of these compounds towards the target enzyme was verified by testing them against bovine pancreatic α-chymotrypsin and human cathepsins B and L at 20 μM. The preliminary screening against α-chymotrypsin showed no significant inhibition with values ranging from 4.2% (**3a**) to 35% (**3c**). Regarding the cathepsins, since the vinyl sulfone warhead is expected to be the most reactive toward cysteine thiolates, the degree of inhibition is slightly higher, ranging from 27% (**2a**) to 38% (**1b**) and from 21% (**1c**) to 37% (**1b**) for cathepsins B and L, respectively.

Conclusions

We designed and synthesized novel constrained peptidomimetics bearing a vinyl sulfone (VS) warhead (compounds **1**–**4**), which proved to be potent irreversible inhibitors of proteasome chymotrypsin-like activity. Peptidomimetic derivatives **2b**, **3b** and **4b**, which possess high inhibitory potencies coupled with a good target selectivities, could be considered as lead compounds for further development in the design of novel antitumor agents.

Experimental Section

Chemistry

All reagents and solvents were obtained from commercial suppliers and were used without further purification. HPLC grade acetonitrile was obtained from Merck. Elemental analyses were carried out on a C. Erba 1106 elemental analyzer for C, H and N, and the results were within ±0.4% of the theoretical values. Reactions under microwave (MW) irradiation were performed on a CEM Discover apparatus. Merck silica gel 60 F_{254} plates were used for analytical TLC; flash column chromatography was performed on Merck silica gel (200–400 mesh) or conducted using prepacked cartridges on a MP-LC Buchi system. Semipreparative HPLC was performed on a Waters 1525 binary HPLC pump system equipped with a Rheodyne model 3725i injector (2 mL sample loop). Reverse-phase chroma-

tography was carried out on a Merck Chromolith SemiPrep RP-18e column (100×10 mm i.d.), at a temperature of 20 °C, with a mobile phase of water/acetonitrile (90:10) and a flow rate of 5 mL min^{-1}. A Waters 2489 UV/Vis dual-wavelength absorbance detector was used, and chromatograms were processed using Waters HPLC Breeze 2 software. The detector wavelength was set at 266 and 310 nm. All solutions employed in the analysis were filtered through 25 mm×0.45 μm polypropylene (PP) membrane membrane. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 spectrometer at 300 K and 300 and 75 MHz, respectively. ^{1}H chemical shifts (δ) are reported in parts per million (ppm) relative to the internal tetramethylsilane (TMS) and coupling constants (J) are reported in hertz (Hz). ^{13}C NMR chemical shifts (δ) are reported relative to the residual solvent peak ($CHCl_3$, central peak, δ = 77.0 ppm).

(2-Oxo-1,2-dihydropyridin-3-yl)-carbamic acid benzyl ester (6a): A solution of **5** (900 mg, 8.17 mmol) in saturated aq $NaHCO_3$/dioxane (7:3, 50 mL) was treated dropwise with benzyl chloroformate (1.84 mL, 12.26 mmol) at 0 °C. The reaction mixture was then stirred at RT for 12 h, diluted with EtOAc, and washed with HCl (3 N). The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo. The crude was by flash column chromatography (light petroleum/EtOAc, 2:8) to afford **6a** as a white solid (1.65 g, 90%): R_f = 0.39 (light petroleum/EtOAc, 2:8); mp: 175–178 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.21 (s, 2H, CH_2Bz), 6.32 (t, J = 7.0 Hz, 1H, ArH), 6.98 (dd, J = 7.0, 1.6 Hz, 1H, ArH), 7.26–7.40 (m, 5H, ArH), 7.79 (s, 1H, NH), 8.11 ppm (d, J = 7.0 Hz, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 67.5, 108.1, 122.7, 126.3, 128.6, 129.0, 129.7, 136.3, 153.7, 159.2 ppm; Anal. calcd (%) for $C_{13}H_{12}N_2O_3$: C 63.93, H 4.95, N 11.47, found: C 63.85, H 4.83, N 11.64.

(2-Oxo-1,2-dihydropyridin-3-yl)-carbamic acid allyl ester (6b): Compound **5** (600 mg, 5.45 mmol) was reacted with allyl chloroformate (0.90 mL, 8.17 mmol) according to the same procedure described for **6a**. The title compound was obtained after purification by flash column chromatography (light petroleum/EtOAc, 2:8) as pale pink solid (845 mg, 80%): R_f = 0.35 (light petroleum/EtOAc, 2:8); mp: 140–143 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.84 (d, J = 5.5 Hz, 2H, CH_2O), 5.43 (d, J = 10.2 Hz, 2H, CH_2=), 5.54 (d, J = 10.2 Hz, 2H, CH_2=), 6.12 (m, 1H, CH), 6.51 (t, J = 7.0 Hz, 1H, ArH), 7.18 (d, J = 7.0 Hz, 1H, ArH), 7.92 (s, 1H, NH), 8.28 ppm (d, J = 7.0 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 66.3, 108.1, 118.6, 122.9, 126.4, 129.6, 132.6, 153.6, 159.2 ppm; Anal. calcd (%) for $C_9H_{10}N_2O_3$: C 55.67, H 5.19, N 14.43, found: C 55.81, H 5.03, N 14.31.

(*S*)-2-Methanesulfonyloxy-4-methyl-pentanoic acid methyl ester (8a): A solution of **7a** (360 mg, 2.46 mmol) in dry CH_2Cl_2 (40 mL) was treated with Et_3N (622 mg, 6.15 mmol) and methanesulfonyl chloride (561 mg, 4.9 mmol). The resulting solution was stirred, under N_2, at RT for 2 h. The reaction mixture was washed with water, dried (Na_2SO_4), filtered and concentrated in vacuo to give **8a** as a pale yellow oil (546 mg, 99%): R_f = 0.68 (light petroleum/EtOAc, 4:6, detected by $KMnO_4$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.99–1.00 (m, 6H, $CH(CH_3)_2$), 1.64–1.80 (m, 2H, CH_2), 1.85 (m, 1H, $CH(CH_3)_2$), 3.15 (s, 3H, CH_3SO_2), 3.80 (s, 3H, OCH_3), 5.07 ppm (m, 1H, $CHOSO_2$); Anal. calcd (%) for $C_8H_{16}O_5S$: C 42.84, H 7.19, found: C 42.99, H 7.06.

(*S*)-2-Methanesulfonyloxy-3-phenyl-propionic acid methyl ester (8b): Starting from **7b** (350 mg, 1.9 mmol) and following the same procedure described for **8a** gave the title compound **8b** as a pale yellow oil (486 mg, 99%): R_f = 0.73 (light petroleum/EtOAc, 4:6); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.87 (s, 3H, CH_3SO_2), 3.26 (dd, J = 8.8, 14.3 Hz, 1H, CH_2) 3.43 (dd, J = 4.1, 14.3 Hz, 1H, CH_2), 3.90 (s, 3H, OCH_3), 5.31 (m, 1H, $CHOSO_2$), 7.38–7.49 ppm (m, 5H, ArH); Anal. calcd (%) for $C_{11}H_{14}O_5S$: C 51.15, H 5.46, found: C 51.33, H 5.28.

(*S*)-2-(3-Benzyloxycarbonylamino-2-oxo-2*H*-pyridin-1-yl)-4-methyl-pentanoic acid methyl ester (9a): A suspension of NaH (118 mg, 2.95 mmol) in DMF at 0 °C under N_2 was treated with **6a** (690 mg, 2.83 mmol) and stirred for 1 h. Methanesulfonate **8a** was then added (552 mg, 2.46 mmol) and the reaction mixture was stirred for a further 12 h at RT. The reaction was quenched with saturated aq $NaHCO_3$ and extracted with EtOAc. The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo. The crude was purified by flash column chromatography (light petroleum/EtOAc, 6:4) to give the title compound **9a** as a pale yellow oil (675 mg, 64%): R_f = 0.80 (light petroleum/EtOAc, 6:4); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.92 (d, J = 6.6 Hz, 6H, $CH(CH_3)_2$), 0.98 (d, J = 6.6 Hz, 6H, $CH(CH_3)_2$), 1.77–2.04 (m, 3H, $CH_2CH(CH_3)_2$), 3.74 (s, 3H, OCH_3), 5.19 (s, 2H, CH_2O), 5.64 (m, 1H, NCHCO), 6.30 (t, J = 7.3 Hz, ArH), 6.99 (dd, J = 1.6, 7.3 Hz, 1H, ArH), 7.34–7.39 (m, 5H, ArH), 7.85 (s, 1H, NH), 8.02 ppm (d, J = 7.3 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.7, 23.2, 24.9, 39.7, 52.9, 67.2, 76.7, 106.89, 120.2, 127.3, 127.4, 128.5, 128.6, 129.5, 136.5, 153.6; 157.5, 171.0 ppm; Anal. calcd (%) for $C_{20}H_{24}N_2O_5$: C 64.50, H 6.50, N 7.52, found: C 64.38, H 6.41, N 7.76.

(*S*)-2-(3-Benzyloxycarbonylamino-2-oxo-2*H*-pyridin-1-yl)-3-phenyl-propionic acid methyl ester (9b): Compound **6a** (730 mg, 3.0 mmol) was reacted with methanesulfonate **8b** (646 mg, 2.5 mmol) according to the same procedure described for **9a** to give the title compound **9b** as a pale yellow oil (670 mg, 55%,): R_f = 0.70 (light petroleum/EtOAc, 6:4); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.25–3.53 (m, 2H, CH_2Ph), 3.93 (s, 3H, OCH_3), 5.36 (s, 2H, CH_2O), 5.51 (m, 1H, NCHCO), 6.25 (t, J = 7.0 Hz, ArH), 6.87 (d, J = 7.0 Hz, ArH), 7.22–7.52 (m, 10H, ArH), 8.01 (s, 1H, NH), 8.12 ppm (d, J = 7.0 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 39.7, 52.8, 78.9, 67.2, 76.8, 106.8, 120.2, 127.3, 127.4, 128.5, 128.6, 129.3, 129.6, 129.9, 130.5, 136.4, 153.6, 157.4, 171.0 ppm; Anal. calcd (%) for $C_{23}H_{22}N_2O_5$: C 67.97, H 5.46, N 6.89, found: C 68.29, H 5.31, N 6.75.

(*S*)-2-(3-Allyloxycarbonylamino-2-oxo-2*H*-pyridin-1-yl)-4-methyl-pentanoic acid methyl ester (9c): Compound **6b** (600 mg, 3.0 mmol) was reacted with methanesulfonate **8a** (560 mg, 2.5 mmol) according to the same synthetic procedure described for **9a** to give the title compound **9c** as a pale yellow oil (550 mg, 60%): R_f = 0.76 (light petroleum/EtOAc, 6:4); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.92 (d, J = 6.6 Hz, $CH(CH_3)_2$), 0.98 (d, J = 6.6 Hz, $CH(CH_3)_2$), 1.88–2.04 (m, 3H, CH_2CH), 3.74 (s, 3H, OCH_3), 4.66 (d, J = 5.5 Hz, 2H, CH_2O), 5.25 (d, J = 10.4 Hz, 1H, CH_2=), 5.36 (d, J = 17.3 Hz, 1H, CH_2=), 5.69 (m, 1H, NCHCO), 5.95 (m, 1H, =CH), 6.30 (t, J = 7.1 Hz, ArH), 6.99 (d, J = 7.1 Hz, 1H, ArH), 7.85 (bs, 1H, NH), 8.02 ppm (d, J = 7.1 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.8, 23.3, 25.0, 40.0, 53.4, 56.8, 66.2, 107.1, 118.4, 120.2, 126.9, 129.6, 133.5, 153.4, 157.3, 171.1 ppm; Anal. calcd (%) for $C_{16}H_{22}N_2O_5$: C 59.62, H 6.88, N 8.69, found: C 59.39, H 6.97, N 8.81.

(*S*)-2-(3-Allyloxycarbonylamino-2-oxo-2*H*-pyridin-1-yl)-3-phenyl-propionic acid methyl ester (9d): Compound **6b** (442 mg, 2.28 mmol) was reacted with methanesulfonate **8b** (491 mg, 1.9 mmol) according to the same synthetic procedure described for **9a** to give the title compound **9d** as a pale yellow oil (552 mg, 68%): R_f = 0.66 (light petroleum/EtOAc, 6:4); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.19 (dd, J = 6.9, 13.7 Hz, 1H, CH_2Ar), 3.38 (dd, J = 4.4, 13.7 Hz, 1H, CH_2Ar), 3.88 (s, 3H, OCH_3), 4.80 (d, J = 5.5 Hz, 2H, CH_2O), 5.40 (d, J = 10.2 Hz, 1H, CH_2=), 5.51 (d, J = 17.0 Hz, 1H, CH_2=), 6.12 (m, 1H, =CH), 6.35 (t, J = 7.4 Hz, 1H, ArH), 6.91 (d, J =

7.4 Hz, 1H, ArH), 7.22–7.41 (m, 5H, ArH), 7.95 (s, 1H, NH), 8.18 ppm (d, J=7.4 Hz, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=36.3, 53.2, 62.6, 66.2, 106.7, 118.4, 120.4, 127.5, 127.9, 128.5, 129.3, 129.6, 129.9, 132.7, 135.3, 136.3, 153.6, 157.2, 169.8 ppm; Anal. calcd (%) for $C_{19}H_{20}N_2O_5$: C 64.04, H 5.66, N 7.86, found: C 64.25, H 5.71, N 7.62.

(*S*)-2-(3-Benzyloxycarbonylamino-2-oxo-2*H*-pyridin-1-yl)-4-methyl-pentanoic acid (10a): A solution of ester **9a** (675 mg, 1.8 mmol) in MeOH/H_2O (1:1, 50 mL) at 0 °C was treated with LiOH (86 mg, 3.6 mmol) and stirred at 20 °C for 5 h. The reaction was concentrated in vacuo, and the residue was treated with 10% citric acid (until pH 4) and extracted with EtOAc. The organic phase was dried (Na_2SO_4), filtered and concentrated to give acid **10a** as a brown oil (612 mg, 95%): ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.91 (d, J=6.6 Hz, CH(CH_3)$_2$), 0.98 (d, J=6.6 Hz, CH(CH_3)$_2$), 1.87–2.03 (m, 3H, CH_2CH), 5.20 (s, 2H, CH_2O), 5.63 (m, 1H, NCHCO), 6.29 (t, J=7.2 Hz, ArH), 6.98 (d, J=7.2 Hz, 1H, ArH), 7.35–7.39 (m, 5H, ArH), 7.84 (s, 1H, NH), 8.01 (d, J=7.2 Hz, ArH), 9.25 ppm (bs, 1H, COOH); Anal. calcd (%) for $C_{19}H_{22}N_2O_5$: C 63.68, H 6.19, N 7.82, found: C 63.79, H 6.31, N 7.62.

(*S*)-2-(3-Benzyloxycarbonylamino-2-oxo-2*H*-pyridin-1-yl)-3-phenyl-propionic acid (10b): Ester **9b** (500 mg, 1.23 mmol) was reacted with LiOH (59 mg, 2.46 mmol) according to the same synthetic procedure described for compound **10a** to give the title compound **10b** as a brown oil (470 mg, 97%): ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.23–3.50 (m, 2H, CH_2Ph), 5.34 (s, 2H, CH_2O), 5.50 (m, 1H, NCHCO), 6.23 (t, J=7.3 Hz, ArH), 6.85 (d, J=7.3 Hz, ArH), 7.20–7.51 (m, 10H), 8.00 (s, 1H, NH), 8.11 (d, J=7.3 Hz, ArH), 9.29 ppm (bs, 1H, COOH); Anal. calcd (%) for $C_{22}H_{20}N_2O_5$: C 67.34, H 5.14, N 7.14, found: C 67.53, H 5.05, N 7.01.

(*S*)-2-(3-Allyloxycarbonylamino-2-oxo-2*H*-pyridin-1-yl)-4-methyl-pentanoic acid (10c): Ester **9c** (550 mg, 1.55 mmol) was reacted with LiOH (74 mg, 3.10 mmol) according to the same synthetic procedure described for compound **10a** to give the title compound **10c** as a brown oil (468 mg, 90%): ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.84 (d, J=4.9 Hz, CH(CH_3)$_2$), 0.86 (d, J=4.9 Hz, CH(CH_3)$_2$), 1.84–2.07 (m, 3H, CH_2CH), 4.59 (d, J=5.2 Hz, 2H, CH_2O), 5.16 (d, J=10.4 Hz, 1H, CH_2=), 5.28 (d, J=17.0 Hz, 1H, CH_2=), 5.56 (m, 1H, NCHCO), 5.86 (m, 1H, =CH), 6.28 (t, J=7.3 Hz, ArH), 6.94 (dd, J=1.6, 7.3 Hz, 1H, ArH), 7.84 (bs, 1H, NH), 8.00 (d, J=7.3 Hz, ArH) 9.29 ppm (bs, 1H, COOH); Anal. calcd (%) for $C_{15}H_{20}N_2O_5$: C 58.43, H 6.54, N 9.09, found: C 58.31, H 6.66, N 9.16.

(*S*)-2-(3-Allyloxycarbonylamino-2-oxo-2*H*-pyridin-1-yl)-3-phenyl-propionic acid (10d): Ester **9d** (552 mg, 1.87 mmol) was reacted with LiOH (90 mg, 3.74 mmol) according to the same synthetic procedure described for compound **10a** to give the title compound **10d** as a brown oil (499 mg, 94%): ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.20 (dd, J=6.9, 13.7 Hz, 1H, CH_2Ph), 3.35 (dd, J=4.4, 13.7 Hz, 1H, CH_2Ph), 4.81 (d, J=5.5 Hz, 2H, CH_2O), 5.38 (d, J=10.2 Hz, 1H, CH_2=), 5.53 (d, J=17.0 Hz, 1H, CH_2=), 6.09 (m, 1H, =CH), 6.30 (t, J=7.4 Hz, 1H, ArH), 6.92 (d, J=7.4 Hz, 1H, ArH), 7.20–7.38 (m, 5H, ArH), 7.93 (s, 1H, NH), 8.15 (d, J=7.4 Hz, 1H, ArH), 9.23 ppm (bs, 1H, COOH); Anal. calcd (%) for $C_{18}H_{18}N_2O_5$: C 63.15, H 5.30, N 8.18, found: C 63.31, H 5.15, N 8.07.

Benzyl-1-((*S*)-4-methyl-1-((*S*)-5-methylhex-1-en-3-ylamino)-1-oxo-pentan-2-yl)-2-oxo-1,2-dihydropyridin-3-ylcarbamate (12a): A solution of acid **10a** (201 mg, 0.56 mmol) in CH_2Cl_2 was treated with HATU (300 mg, 0.84 mmol), amine **11** (126 mg, 0.84 mmol) and DIPEA (0.10 mL, 0.56 mmol) and stirred at 100 °C for 1 h under microwave irradiation. After that time, additional HATU (100 mg, 0.28 mmol) and DIPEA (0.048 mL, 0.28 mmol) were added, and the mixture was stirred under the same conditions for an additional 1 h. The solution was diluted with CH_2Cl_2 and washed with H_2O. The organic phase was dried (Na_2SO_4), filtered and evaporated in vacuo. The crude was purified by flash column chromatography (light petroleum/EtOAc, 8:2) to give compound **12a** as a pale yellow oil (150 mg, 60%): R_f=0.78 (light petroleum/EtOAc, 8:2); ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.91–0.97 (m, 12H, CH(CH_3)$_2$), 1.40–1.64 (m, 4H, CH_2CH), 1.79–2.12 (m, 2H, CH_2CH), 4.24 (m, 1H, NHCH), 4.81–4.99 (m, 2H, CH=CH_2), 5.21 (s, 2H, CH_2O), 5.65 (m, 1H, NCHCO), 5.83 (m, 1H, CH=CH_2), 6.30 (t, 1H, J=7.1 Hz, ArH), 6.99 (d, 1H, J=7.1 Hz, ArH), 7.34–7.39 (m, 5H), 7.85 (s, 1H, NH), 8.02 ppm (d, 1H, J=7.1 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=22.5, 23.3, 24.8, 25.5, 40.9, 45.2, 52.4, 64.2, 67.0, 109.5, 111.7, 117.3, 127.1, 127.7, 129.0, 131.8, 134.2, 136.2, 137.7, 153.3, 158.4, 171.0 ppm; Anal. calcd (%) for $C_{26}H_{35}N_3O_4$: C 68.85, H 7.78, N 9.26, found: C 68.99, H 7.91, N 9.02.

Benzyl-1-((*S*)-1-((*S*)-5-methylhex-1-en-3-ylamino)-1-oxo-3-phenyl-propan-2-yl)-2-oxo-1,2-dihydropyridin-3-ylcarbamate (12b): Acid **10b** (278 mg, 0.71 mmol) was reacted with amine **11** (120 mg, 1.06 mmol) according to the same synthetic procedure described for compound **12a** to give the title compound **12b** as a pale yellow oil (225 mg, 65%): R_f=0.73 (light petroleum/EtOAc, 8:2); ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.93 (d, 6H, J=6.5 Hz, CH(CH_3)$_2$), 1.22–1.50 (m, 3H, CH_2CH), 3.24 (m, 1H, CH_2Ph), 3.67 (m, 1H, CH_2Ph), 4.51 (m, 1H, NHCH), 4.99–5.17 (m, 3H, CH=CH_2 and NCHCO), 5.35 (s, 2H, CH_2O), 5.73 (m, 1H, CH=CH_2), 6.35 (s, 1H, NH), 6.48 (t, J=7.1 Hz, ArH), 7.30–7.68 (m, 11H, ArH), 7.92 (bs, 1H, NH), 8.21 ppm (d, 1H, J=7.1 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=23.3, 24.8, 35.3, 45.2, 52.4, 66.9, 69.1, 109.4, 111.7, 117.3, 125.9, 127.1, 127.6, 127.7, 128.6, 129.0, 131.8, 134.3, 134.1, 136.2, 137.7, 153.3, 158.3, 171.1 ppm; Anal. calcd (%) for $C_{29}H_{33}N_3O_4$: C 71.44, H 6.82, N 8.62, found: C 71.71, H 6.67, N 8.56.

Benzyl-1-((*S*)-4-methyl-1-((*S,E*)-5-methyl-1-(methylsulfonyl)hex-1-en-3-ylamino)-1-oxopentan-2-yl)-2-oxo-1,2-dihydropyridin-3-yl-carbamate (1a): A solution of **12a** (60 mg, 0.132 mmol) in dry CH_2Cl_2 (5 mL) was treated with methyl vinyl sulfone **13a** (140 mg, 1.32 mmol) and Hoveyda–Grubbs second generation catalyst [(1,3-bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene)-dichloro-(*o*-isopropoxyphenylmethylene)ruthenium] (16 mg, 0.026 mmol). The resulting mixture was heated under microwave irradiation at 100 °C for 2 h, the concentrated in vacuo. Purification first by flash column chromatography (light petroleum/EtOAc, 7:3) and subsequently by semipreparative RP-HPLC gave the title compound **1a** as a white solid (14 mg, 20%): R_f=0.65 (light petroleum/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.86–0.90 (m, 12H, CH(CH_3)$_2$), 1.68–2.08 (m, 6H, CH_2CH), 3.01 (s, 3H, CH_3), 4.32 (m, 1H, NCH), 4.63 (m, 1H, NCHCO), 5.21 (s, 2H, CH_2O), 6.30 (t, 1H, J=7.3 Hz, ArH), 6.94 (d, 1H, J=15.2 Hz, =CHSO_2), 6.96 (d, 1H, J=7.3 Hz, ArH), 7.12 (dd, 1H, J=4.2, 15.2 Hz, CH=), 7.34–7.39 (m, 5H), 7.83 (s, 1H, NH), 8.04 ppm (d, 1H, J=7.3 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=22.4, 22.5, 23.2, 23.4, 39.1, 43.2, 45.3, 47.1, 55.8, 66.1, 109.5, 111.8, 127.3, 129.0, 129.4, 131.8, 134.5, 141.1, 141.2, 148.5, 158.4, 171.2 ppm; Anal. calcd (%) for $C_{27}H_{37}N_3O_6S$: C 61.00, H 7.01, N 7.90, found: C 61.17, H 7.12, N 7.79.

Benzyl-1-((*S*)-1-((*S,E*)-1-(ethylsulfonyl)-5-methylhex-1-en-3-yl-amino)-4-methyl-1-oxopentan-2-yl)-2-oxo-1,2-dihydropyridin-3-ylcarbamate (1b): Starting from olefin **12a** (100 mg, 0.22 mmol) and ethyl vinyl sulfone **13b** (264 mg, 2.2 mmol) and following the same procedure described for **1a** gave the title compound **1b** as a white solid (26 mg, 22%): R_f=0.70 (light petroleum/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.89–0.94 (m, 12H, CH(CH_3)$_2$), 1.41 (t, 3H, $SO_2CH_2CH_3$), 1.61–2.01 (m, 6H, CH_2CH), 3.07–3.11 (m, 2H,

$SO_2CH_2CH_3$), 4.22 (m, 1H, NCH), 4.67 (m, 1H, NCHCO), 5.20 (s, 2H, CH_2O), 6.29 (t, 1H, $J=7.0$ Hz, ArH), 6.95 (d, 1H, $J=15.2$ Hz, $=CHSO_2$), 6.99 (d, 1H, $J=7.0$ Hz, ArH), 7.05 (dd, 1H, $J=4.2$, 15.2 Hz, CH=), 7.36–7.41 (m, 5H), 7.74 (s, 1H, NH), 7.99 ppm (d, 1H, $J=7.0$ Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=5.1$, 22.4, 22.5, 23.2, 23.4, 39.1, 45.4, 45.3, 47.1, 55.7, 66.1, 109.5, 111.8, 127.3, 129.0, 129.4, 131.8, 134.5, 141.0, 141.3, 148.4, 158.4, 171.2 ppm; Anal. calcd (%) for $C_{28}H_{39}N_3O_6S$: C 61.63, H 7.20, N 7.70, found: C 61.47, H 7.52, N 7.59.

Benzyl-1-((*S*)-4-methyl-1-((*S,E*)-5-methyl-1-(phenylsulfonyl)hex-1-en-3-ylamino)-1-oxopentan-2-yl)-2-oxo-1,2-dihydropyridin-3-ylcarbamate (1c): Starting from olefin **15a** (100 mg, 0.22 mmol) and phenyl vinyl sulfone **13c** (370 mg, 2.2 mmol) and following the same procedure described for **1a** gave the title compound **1c** as a white solid (26 mg 20%): $R_f=0.79$ (light petroleum/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=0.88$–0.93 (m, 12H, $CH(CH_3)_2$), 1.78–2.06 (m, 6H, CH_2CH), 4.23 (m, 1H, NCH), 4.69 (m, 1H, NCHCO), 5.20 (s, 2H, CH_2O), 6.29 (t, 1H, $J=7.1$ Hz, ArH), 6.91 (d, 1H, $J=15.2$ Hz, $=CHSO_2$), 6.93 (d, 1H, $J=7.1$ Hz, ArH), 7.06 (dd, 1H, $J=4.2$, 15.2 Hz, CH=), 7.33–7.55 (m, 10H), 7.86 (s, 1H, NH), 8.02 ppm (d, 1H, $J=7.1$ Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=22.4$, 22.5, 23.2, 23.4, 39.1, 45.2, 47.1, 55.7, 66.1, 109.5, 111.8, 127.2, 128.3, 129.0, 129.4, 129.8, 131.8, 133.8, 134.5, 139.4, 141.0, 141.2, 148.4, 158.4, 171.2 ppm; Anal. calcd (%) for $C_{32}H_{39}N_3O_6S$: C 64.73, H 6.62, N 7.08, found: C 64.57, H 6.74, N 7.17.

Benzyl-1-((*S*)-1-((*S,E*)-5-methyl-1-(methylsulfonyl)hex-1-en-3-ylamino)-1-oxo-3-phenylpropan-2-yl)-2-oxo-1,2-dihydropyridin-3-ylcarbamate (2a): Starting from olefin **12b** (150 mg, 0.30 mmol) and methyl vinyl sulfone **13a** (318 mg, 3.0 mmol) and following the same procedure described for **1a** gave the title compound **2a** as a white solid (46 mg, 27%): $R_f=0.46$ (light petroleum/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=0.72$ (m, 1H, CH_2CH), 0.92 (d, 6H, $J=6.2$ Hz, $CH(CH_3)_2$), 1.16 (t, 2H, $J=7.4$ Hz, CH_2CH), 1.58 (t, 2H, $J=7.4$ Hz, CH_2CH), 2.86 (s, 3H, SO_2CH_3), 3.02 (dd, 1H, $J=4.4$ Hz, 12.4 Hz, CH_2Ph), 3.26 (dd, 1H, $J=7.0$ Hz, 12.4 Hz, CH_2Ph), 4.16 (m, 1H, NHCH), 5.25 (s, 2H, CH_2O), 5.64 (t, 1H, $J=6.4$ Hz, NCHCO), 5.89 (d, 1H, $J=7.3$ Hz, ArH), 6.55 (d, 1H, $J=15.2$ Hz, $=CHSO_2$), 6.72 (dd, 1H, $J=6.1$ Hz, 15.2 Hz, CH=), 6.79 (d, 1H, $J=7.3$ Hz, ArH), 7.15–7.29 (m, 10H), 7.79 (bs, 1H, NH), ppm 8.12 (d, 1H, $J=7.3$ Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=23.2$, 23.4, 35.5, 43.2, 45.3, 47.1, 60.7, 66.1, 109.5, 111.8, 126.0, 127.3, 127.8, 128.7, 129.0, 129.4, 131.8, 134.5, 139.5, 141.1, 141.2, 148.5, 158.4, 171.2 ppm; Anal. calcd (%) for $C_{30}H_{35}N_3O_6S$: C 63.70, H 6.24, N 7.43, found: C 63.53, H 6.35, N 7.59.

Benzyl-1-((*S*)-1-((*S,E*)-1-(ethylsulfonyl)-5-methylhex-1-en-3-ylamino)-1-oxo-3-phenylpropan-2-yl)-2-oxo-1,2-dihydropyridin-3-ylcarbamate (2b): Starting from olefin **12b** (150 mg, 0.30 mmol) and ethyl vinyl sulfone **13b** (360 mg, 3.0 mmol) and following the same procedure described for **1a** gave the title compound **2b** as a white solid (52 mg, 30%): $R_f=0.51$ (light petroleum/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=0.73$ (m, 1H, CH_2CH), 0.94 (d, 6H, $J=6.2$ Hz, $CH(CH_3)_2$), 1.19 (t, 1H, $J=7.4$ Hz, CH_2CH), 1.37 (t, 3H, $J=6.8$ Hz, $SO_2CH_2CH_3$), 1.60 (t, 1H, $J=7.4$ Hz, CH_2CH), 3.04 (dd, 1H, $J=4.4$ Hz, 12.4 Hz, CH_2Ph), 3.13 (q, 2H, $J=6.8$ Hz, $SO_2CH_2CH_3$), 3.28 (dd, 1H, $J=7.0$ Hz, 12.4 Hz, CH_2Ar), 4.18 (m, 1H, NHCH), 5.27 (s, 2H, CH_2O), 5.66 (t, 1H, $J=6.4$ Hz, NCHCO), 5.90 (d, 1H, $J=7.3$ Hz, ArH), 6.57 (d, 1H, $J=15.6$ Hz, $=CHSO_2$), 6.74 (dd, 1H, $J=6.1$ Hz, 15.6 Hz, CH=), 6.81 (d, 1H, $J=7.3$ Hz, ArH), 7.19–7.32 (m, 10H), 7.80 (bs, 1H, NH), 8.15 ppm (d, 1H, $J=7.3$ Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=5.2$, 23.2, 23.3, 35.5, 45.4, 45.3, 47.1, 60.6, 66.1, 109.5, 111.8, 126.0, 127.3, 127.8, 128.7, 129.0, 129.4, 131.8, 134.5, 139.5, 141.0, 141.2, 148.5, 158.4, 171.2 ppm; Anal. calcd (%) for $C_{31}H_{37}N_3O_6S$: C 64.23, H 6.43, N 7.25, found: C 64.51, H 6.29, N 7.11.

Benzyl-1-((*S*)-1-((*S,E*)-5-methyl-1-(phenylsulfonyl)hex-1-en-3-ylamino)-1-oxo-3-phenylpropan-2-yl)-2-oxo-1,2-dihydropyridin-3-ylcarbamate (2c): Starting from olefin **12b** (150 mg, 0.30 mmol) and phenyl vinyl sulfone **13c** (504 mg, 3.0 mmol) and following the same procedure described for **1a** gave the title compound **2c** as a white solid (60 mg, 32%): $R_f=0.65$ (light petroleum/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=0.73$ (m, 1H, CH_2CH), 0.94 (d, 6H, $J=6.2$ Hz, $CH(CH_3)_2$), 1.17 (t, 2H, $J=7.4$ Hz, CH_2CH), 1.59 (t, 2H, $J=7.4$ Hz, CH_2CH), 3.04 (dd, 1H, $J=4.4$ Hz, 12.4 Hz, CH_2Ph), 3.28 (dd, 1H, $J=7.0$ Hz, 12.4 Hz, CH_2Ph), 4.17 (m, 1H, NHCH), 5.27 (s, 2H, CH_2O), 5.66 (t, 1H, $J=6.4$ Hz, NCHCO), 5.90 (d, 1H, $J=7.3$ Hz, ArH), 6.56 (d, 1H, $J=15.2$ Hz, $=CHSO_2$), 6.75 (dd, 1H, $J=6.1$ Hz, 15.2 Hz, CH=), 6.81 (d, 1H, $J=7.3$ Hz, ArH), 7.17–7.38 (m, 15H), 7.79 (bs, 1H, NH), 8.11 ppm (d, 1H, $J=7.3$ Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=23.2$, 23.3, 35.5, 45.2, 47.1, 60.6, 66.1, 109.5, 111.7, 126.0, 127.3, 127.8, 128.3, 128.7, 129.0, 129.4, 129.8, 131.8, 133.8, 134.6, 139.5, 139.5, 141.0, 141.2, 148.4, 158.4, 171.2 ppm; Anal. calcd (%) for $C_{35}H_{37}N_3O_6S$: C 66.97, H 5.94, N 6.69, found: C 66.61, H 6.19, N 6.81.

(*S*)-[1-(2-Methanesulfonyl-vinyl)-3-methyl-butyl]-tritylamine (15a): Starting from olefin **14** (125 mg, 0.35 mmol) and methyl vinyl sulfone **13a** (371 mg, 3.5 mmol) and using the same procedure described for **1a** gave the title compound **15a**, following purification by flash column chromatography (light petroleum/EtOAc, 9:1), as a brown oil (68 mg, 45%): $R_f=0.30$ (light petroleum/EtOAc, 98:2); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=0.69$ (d, 6H, $J=6.4$ Hz, $CH(CH_3)_2$), 0.74 (d, 6H, $J=6.4$ Hz, $CH(CH_3)_2$), 1.38–1.54 (m, 3H, CH_2CH), 2.92 (s, 3H, SO_2CH_3), 3.10 (m, 1H, NHCH), 6.59 (d, $J=15.2$ Hz, 1H, $=CHSO_2$), 6.81 (dd, $J=15.2$ Hz, 4.2 Hz, 1H, CH=), 7.12–7.78 ppm (m, 15H, ArH); Anal. calcd (%) for $C_{27}H_{31}NO_2S$: C 74.79, H 7.21, N 3.23, found: C 74.58, H 7.39, N 3.31.

(*S*)-[1-(2-Ethanesulfonyl-vinyl)-3-methyl-butyl]-tritylamine (15b): Starting from olefin **14** (125 mg, 0.35 mmol) and ethyl vinyl sulfone **13b** (420 mg, 3.5 mmol) and using the same procedure described for **1a** gave the title compound **15b**, following flash column chromatography (light petroleum/EtOAc, 9:1), as a brown oil (78 mg, 50%): $R_f=0.34$ (light petroleum/EtOAc, 98:2); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=0.70$ (d, 6H, $J=6.4$ Hz, $CH(CH_3)_2$), 0.78 (d, 6H, $J=6.4$ Hz, $CH(CH_3)_2$), 1.30 (t, $J=7.5$ Hz, $SO_2CH_2CH_3$) 1.40–1.56 (m, 3H, CH_2CH), 2.97 (q, $J=7.5$ Hz, $SO_2CH_2CH_3$), 3.11 (m, 1H, NHCH), 6.58 (d, $J=15.2$ Hz, 1H, $=CHSO_2$), 6.80 (dd, $J=15.2$ Hz, 4.2 Hz, 1H, CH=), 7.12–7.78 ppm (m, 15H, ArH); Anal. calcd (%) for $C_{28}H_{33}NO_2S$: C 75.13, H 7.43, N 3.13, found: C 75.28, H 7.22, N 3.24.

(*S*)-[1-(2-Benzenesulfonyl-vinyl)-3-methyl-butyl]-tritylamine (15c): Starting from olefin **14** (125 mg, 0.35 mmol) and phenyl vinyl sulfone **13c** (589 mg, 3.5 mmol) and using the same procedure described for **1a** gave the title compound **15c**, following flash column chromatography (light petroleum/EtOAc, 9:1), as a brown oil (69 mg, 40%): $R_f=0.57$ (light petroleum/EtOAc, 98:2); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=0.70$ (d, 6H, $J=6.4$ Hz, $CH(CH_3)_2$), 0.78 (d, 6H, $J=6.4$ Hz, $CH(CH_3)_2$), 1.40–1.56 (m, 3H, CH_2CH), 3.11 (m, 1H, NHCH), 6.58 (d, $J=15.2$ Hz, 1H, $=CHSO_2$), 6.80 (dd, $J=15.2$ Hz, 4.2 Hz, 1H, CH=), 7.12–7.80 ppm (m, 20H, ArH); Anal. calcd (%) for $C_{32}H_{33}NO_2S$: C 77.54, H 6.71, N 2.83, found: C 77.32, H 6.85, N 2.98.

(*S*)-1-(2-Methanesulfonyl-vinyl)-3-methyl-butylamine (16a): A solution of olefin **15a** (68 mg, 0.15 mmol) in acetone was treated dropwise over 5 min with 6 N HCl (40 μL, 0.22 mmol) and then refluxed for 5 h. The solvent was evaporated in vacuo, the residue

was taken up in CH_2Cl_2, and the solution was extracted with 1 N HCl. The aqueous phase was brought to pH 10 by addition of $Na_2CO_{3(s)}$ and extracted with CH_2Cl_2. The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo to give compound **16a** as a brown oil (12 mg, 41%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.68 (d, 6H, J = 6.4 Hz, CH(CH_3)$_2$), 0.73 (d, 6H, J = 6.4 Hz, CH(CH_3)$_2$), 1.36–1.53 (m, 3H, CH_2CH), 2.90 (s, 3H, SO_2CH_3), 3.09 (m, 1H, NHC*H*), 6.57 (d, J = 15.2 Hz, 1H, =CHSO$_2$), 6.80 ppm (dd, J = 15.2 Hz, 4.2 Hz, 1H, CH=); Anal. calcd (%) for $C_8H_{17}NO_2S$: C 50.23, H 8.96, N 7.32, found: C 50.01, H, 8.82, N 7.59.

(*S*)-1-(2-Ethanesulfonyl-vinyl)-3-methyl-butylamine (16b): Starting from olefin **15b** (68 mg, 0.15 mmol) and following the same procedure described for **16a** gave the title compound **16b** as a brown oil (14 mg, 46%): ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.69 (d, 6H, J = 6.4 Hz, CH(CH_3)$_2$), 0.75 (d, 6H, J = 6.4 Hz, CH(CH_3)$_2$), 1.29 (t, J = 7.5 Hz, $SO_2CH_2CH_3$) 1.38–1.54 (m, 3H, CH_2CH), 2.95 (q, J = 7.5 Hz, $SO_2CH_2CH_3$), 3.09 (m, 1H, NHC*H*), 6.56 (d, J = 15.2 Hz, 1H, =CHSO$_2$), 6.81 ppm (dd, J = 15.2 Hz, 4.2 Hz, 1H, CH=); Anal. calcd (%) for $C_9H_{19}NO_2S$: C 52.65, H 9.33, N 6.82, found: C 52.92, H 9.18, N 6.67.

(*S*)-1-(2-Benzenesulfonyl-vinyl)-3-methyl-butylamine (16c): Starting from olefin **15c** (69 mg, 0.14 mmol) and following the same procedure described for **16a** gave the title compound **16c** as a brown oil (16 mg, 45%): ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.69 (d, 6H, J = 6.4 Hz, CH(CH_3)$_2$), 0.75 (d, 6H, J = 6.4 Hz, CH(CH_3)$_2$), 1.40–1.55 (m, 3H, CH_2CH), 3.13 (m, 1H, NHC*H*), 6.50 (d, J = 15.2 Hz, 1H, =CHSO$_2$), 6.75 (dd, J = 15.2 Hz, 4.2 Hz, 1H, CH=), 7.36–7.74 ppm (m, 5H, Ar); Anal. calcd (%) for $C_{13}H_{19}NO_2S$: C 61.63, H 7.56, N 5.53, found: C 61.82, H 7.25, N 5.78.

Allyl-1-((*S*)-4-methyl-1-((*S,E*)-5-methyl-1-(methylsulfonyl)hex-1-en-3-ylamino)-1-oxopentan-2-yl)-2-oxo-1,2-dihydropyridin-3-yl-carbamate (3a): Starting from acid **10c** (60 mg, 0.20 mmol) and amine **16a** (44 mg, 0.22 mmol) and using the same procedure described for **12a** gave the title compound **3a**, following preliminary purification by flash column chromatography (light petroleum/EtOAc, 7:3) and subsequent semipreparative RP-HPLC, a white solid (32 mg, 34%): R_f = 0.50 (light petroleum/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.72 (m, 1H, CH_2CH), 0.91 (d, 12H, J = 6.2 Hz, CH(CH_3)$_2$), 1.04 (d, 12H, J = 6.2 Hz, CH(CH_3)$_2$), 1.19 (t, 2H, J = 7.2 Hz, CH_2CH), 1.51 (t, 2H, J = 7.2 Hz, CH_2CH), 1.56 (m, 1H, CH_2CH), 1.64 (t, 2H, J = 7.2 Hz, CH_2CH), 1.79 (t, 2H, J = 7.2 Hz, CH_2CH), 2.85 (s, 3H, SO_2CH_3), 4.18 (m, 1H, NHC*H*), 4.80 (d, 2H, J = 6.2 Hz, CH_2O), 5.21 (d, 2H, J = 10.2, CH_2=), 5.67 (t, 1H, J = 7.5 Hz, NCHCO), 5.85 (m, 1H, CH_2=C*H*), 5.91 (d, 1H, J = 7.1 Hz, ArH), 6.11 (d, 1H, J = 7.1 Hz, ArH), 6.65 (dd, 1H, J = 6.1 Hz, 15.2 Hz, CH=), 6.78 (d, 1H, J = 15.2 Hz, = $CHSO_2$) 7.88 (bs, 1H, NH), 7.96 ppm (d, 1H, J = 7.1 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 22.4, 22.5, 23.2, 23.3, 39.2, 43.2, 45.3, 47.1, 55.7, 65.1, 109.5, 111.8, 116.4, 129.4, 131.9, 133.6, 134.5, 141.1, 148.4, 158.5, 171.1 ppm; Anal. calcd (%) for $C_{23}H_{35}N_3O_6S$: C 57.36, H 7.33, N 8.72, found: C 57.12, H 7.54, N 8.61.

Allyl-1-((*S*)-1-((*S,E*)-1-(ethylsulfonyl)-5-methylhex-1-en-3-ylamino)-4-methyl-1-oxopentan-2-yl)-2-oxo-1,2-dihydropyridin-3-yl-carbamate (3b): Starting from acid **10c** (74 mg, 0.24 mmol) and amine **16b** (52 mg, 0.26 mmol) and using the same procedure described for **12a** gave the title compound **3b**, following preliminary purification by flash column chromatography (light petroleum/EtOAc, 7:3) and subsequent semipreparative RP-HPLC, a white solid (42 mg, 35%): R_f = 0.55 (light petroleum/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.97–0.99 (m, 12H, CH(CH_3)$_2$), 1.22 (m, 1H, CH_2CH), 1.32 (t, 1H, J = 7.2 Hz, CH_2CH), 1.38 (t, 3H, J = 6.8 Hz, $SO_2CH_2CH_3$), 1.40 (m, 1H, CH_2CH), 1.47 (t, 3H, J = 7.2 Hz, CH_2CH), 1.54 (t, 3H, J = 7.2 Hz, CH_2CH), 2.01 (t, 3H, J = 7.2 Hz, CH_2CH), 3.43 (q, 2H, J = 6.8 Hz, $SO_2CH_2CH_3$), 4.18 (m, 1H, NHC*H*), 4.78 (d, 2H, J = 6.2 Hz, CH_2O), 5.10 (d, 2H, J = 10.2, CH_2=), 5.15 (t, 1H, J = 7.5 Hz, NCHCO), 5.84 (m, 1H, CH_2=C*H*), 5.92 (d, 1H, J = 7.1 Hz, ArH), 6.70 (d, 1H, J = 7.1 Hz, ArH), 6.74 (dd, 1H, J = 6.0 Hz, 15.1 Hz, CH=), 6.85 (d, 1H, J = 15.1 Hz, =CHSO$_2$), 7.85 (bs, 1H, NH), 7.99 ppm (d, 1H, J = 7.1 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 5.2, 22.4, 22.5, 23.2, 23.3, 39.2, 45.2, 45.4, 47.2, 55.8, 65.2, 109.5, 111.8, 116.4, 129.4, 131.8, 133.6, 134.5, 141.0, 148.4, 158.5, 171.1 ppm; Anal. calcd (%) for $C_{24}H_{37}N_3O_6S$: C 58.16, H 7.52, N 8.48, found: C 58.33, H 7.74, N 8.19.

Allyl-1-((*S*)-4-methyl-1-((*S,E*)-5-methyl-1-(phenylsulfonyl)hex-1-en-3-ylamino)-1-oxopentan-2-yl)-2-oxo-1,2-dihydropyridin-3-yl-carbamate (3c): Starting from acid **10c** (56 mg, 0.18 mmol) and amine **18c** (50 mg, 0.20 mmol) and using the same procedure described for **12a** gave the title compound **3c**, following preliminary purification by flash column chromatography (light petroleum/EtOAc, 7:3) and subsequent semipreparative RP-HPLC, a white solid (35 mg, 36%): R_f = 0.73 (light petroleum/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.73 (m, 1H, CH_2CH), 0.93 (d, 12H, J = 6.2 Hz, CH(CH_3)$_2$), 1.05 (d, 12H, J = 6.2 Hz, CH(CH_3)$_2$), 1.21 (t, 2H, J = 7.2 Hz, CH_2CH), 1.50 (t, 2H, J = 7.2 Hz, CH_2CH), 1.55 (m, 1H, CH_2CH), 1.64 (t, 2H, J = 7.2 Hz, CH_2CH), 1.78 (t, 2H, J = 7.2 Hz, CH_2CH), 4.19 (m, 1H, NHC*H*), 4.81 (d, 2H, J = 6.2 Hz, CH_2O), 5.20 (d, 2H, J = 10.2, CH_2=), 5.69 (t, 1H, J = 7.5 Hz, NCHCO), 5.86 (m, 1H, CH_2=C*H*), 5.89 (d, 1H, J = 7.3 Hz, ArH), 6.13 (d, 1H, J = 7.3 Hz, ArH), 6.67 (dd, 1H, J = 6.1 Hz, 15.2 Hz, CH=), 6.79 (d, 1H, J = 15.2 Hz, =CHSO$_2$), 7.36–7.74 (m, 5H, Ar), 7.84 (bs, 1H, NH), 8.01 ppm (d, 1H, J = 7.3 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 22.4, 22.5, 23.2, 23.3, 39.2, 45.3, 47.2, 55.7, 65.2, 109.5, 111.7, 116.4, 128.3, 129.4, 129.8, 131.9, 133.7, 133.8, 134.6, 139.4, 141.1, 148.4, 158.5, 171.1 ppm; Anal. calcd (%) for $C_{28}H_{37}N_3O_6S$: C 61.86, H 6.86, N 7.73, found: C 61.67, H 6.71, N 7.99.

Allyl-1-((*S*)-1-((*S,E*)-5-methyl-1-(methylsulfonyl)hex-1-en-3-ylamino)-1-oxo-3-phenylpropan-2-yl)-2-oxo-1,2-dihydropyridin-3-yl-carbamate (4a): Starting from acid **10d** (62 mg, 0.18 mmol) and amine **16a** (40 mg, 0.20 mmol) and using the same procedure described for **12a** gave the title compound **4a**, following preliminary purification by flash column chromatography (light petroleum/EtOAc, 7:3) and subsequent semipreparative RP-HPLC, a white solid (35 mg, 38%): R_f = 0.31 (light petroleum/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.84 (d, 6H, J = 6.4 Hz, CH(CH_3)$_2$), 0.90 (d, 6H, J = 6.4 Hz, CH(CH_3)$_2$), 1.52–1.94 (m, 6H, CH_2CH), 3.00 (s, 3H, SO_2CH_3), 3.15 (dd, 1H, J = 6.9 Hz, 13.7 Hz, CH_2Ph), 3.38 (dd, J = 4.4 Hz, 13.7 Hz, 1H, CH_2Ph), 4.75 (d, J = 5.5 Hz, 2H, CH_2O), 4.15–4.19 (m, 1H, NHC*H*), 4.40 (m, 1H, NCHCO), 5.33 (d, 1H, J = 10.2 Hz, CH_2=), 5.45 (d, 1H, J = 17.0 Hz, CH_2=), 6.00 (m, 1H, CH_2=C*H*), 6.30 (t, 1H, J = 7.1 Hz, ArH), 6.81 (d, 1H, J = 15.2 Hz, =CHSO$_2$), 6.94 (d, 1H, J = 7.1 Hz, ArH), 6.99 (dd, 1H, J = 15.2 Hz, 4.2 Hz, CH=), 7.21–7.33 (m, 5H), 7.79 (s, 1H, NH), 8.01 ppm (d, 1H, J = 7.1 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.2, 23.4, 35.5, 43.2, 45.3, 47.1, 60.6, 65.1, 109.5, 111.8, 116.5, 126.1, 127.8, 128.7, 129.5, 131.8, 133.6, 134.5, 139.5, 141.1, 148.4, 158.5, 171.1 ppm; Anal. calcd (%) for $C_{26}H_{33}N_3O_6S$: C 60.56, H 6.45, N 8.15, found: C 60.78, H 6.23, N 8.02.

Allyl-1-((*S*)-1-((*S,E*)-1-(ethylsulfonyl)-5-methylhex-1-en-3-ylamino)-1-oxo-3-phenylpropan-2-yl)-2-oxo-1,2-dihydropyridin-3-yl-carbamate (4b): Starting from acid **10d** (62 mg, 0.18 mmol) and amine **16b** (50 mg, 0.20 mmol) and using the same procedure described for **12a** gave the title compound **4b**, following preliminary purification by flash column chromatography (light petroleum/EtOAc, 7:3) and subsequent semipreparative RP-HPLC, a white solid (38 mg, 40%): R_f = 0.34 (light petroleum/EtOAc, 7/3); ^{1}H NMR

(300 MHz, $CDCl_3$): $\delta = 0.84$ (d, 6H, $J = 6.4$ Hz, $CH(CH_3)_2$), 0.90 (d, 6H, $J = 6.4$ Hz, $CH(CH_3)_2$), 1.41 (t, 3H, $SO_2CH_2CH_3$), 1.52–1.94 (m, 6H, CH_2CH), 3.07 (q, 2H, SO_2CH_2), 3.15 (dd, 1H, $J = 6.9$ Hz, 13.7 Hz, CH_2Ph), 3.38 (dd, 1H, $J = 4.40$ Hz, 13.7 Hz, CH_2Ph), 4.76 (d, 2H, $J = 5.5$ Hz, CH_2O), 4.19 (m, 1H, NHCH), 4.38 (m, 1H, NCHCO), 5.35 (d, 1H, $J = 10.2$ Hz, $CH_2=$), 5.46 (d, 1H, $J = 17.0$ Hz, $CH_2=$), 5.99 (m, 1H, $CH_2=CH$), 6.33 (t, 1H, $J = 7.1$ Hz, ArH), 6.82 (d, 1H, $J = 15.2$ Hz, $=CHSO_2$), 6.96 (d, 1H, $J = 7.1$ Hz, ArH), 6.97 (dd, 1H, $J = 15.2$ Hz, 4.2 Hz, CH=), 7.19–7.28 (m, 5H), 7.81 (s, 1H, NH), 8.00 ppm (d, 1H, $J = 7.1$ Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 5.1$, 23.2, 23.3, 35.5, 45.3, 45.4, 47.1, 60.7, 65.1, 109.6, 111.8, 116.5, 126.1, 127.8, 128.7, 129.5, 131.8, 133.6, 134.5, 139.5, 141.1, 148.3, 158.4, 171.1 ppm; Anal. calcd (%) for $C_{27}H_{35}N_3O_6S$: C 61.23, H 6.66, N 7.93, found: C 61.58, H 6.38, N 7.72.

Allyl-1-((*S*)-1-((*S,E*)-5-methyl-1-(phenylsulfonyl)hex-1-en-3-ylamino)-1-oxo-3-phenylpropan-2-yl)-2-oxo-1,2-dihydropyridin-3-ylcarbamate (4c): Starting from acid **10d** (48 mg, 0.14 mmol) and amine **16c** (48 mg, 0.16 mmol) and using the same procedure described for **12a** gave the title compound **4c**, following preliminary purification by flash column chromatography (light petroleum/EtOAc, 7:3) and subsequent semipreparative RP-HPLC, a white solid (27 mg, 34%); $R_f = 0.59$ (light petroleum/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.83$ (d, 6H, $J = 6.4$ Hz, $CH(CH_3)_2$), 0.91 (d, 6H, $J = 6.4$ Hz, $CH(CH_3)_2$), 1.51–1.93 (m, 6H, CH_2CH), 3.12 (dd, 1H, $J = 6.9$ Hz, 13.7 Hz, CH_2Ph), 3.36 (dd, 1H, $J = 4.40$ Hz, 13.7 Hz, CH_2Ph), 4.74 (d, 2H, $J = 5.5$ Hz, CH_2O), 4.18 (m, 1H, NHCH), 4.39 (m, 1H, NCHCO), 5.34 (d, 1H, $J = 10.2$ Hz, $CH_2=$), 5.45 (d, 1H, $J = 17.0$ Hz, $CH_2=$), 5.99 (m, 1H, $CH_2=CH$), 6.29 (t, 1H, $J = 7.0$ Hz, ArH), 6.79 (d, 1H, $J = 15.2$ Hz, $=CHSO_2$), 6.96 (d, 1H, $J = 7.0$ Hz, ArH), 7.01 (dd, 1H, $J = 15.2$ Hz, 4.2 Hz, CH=), 7.26–7.53 (m, 10H), 7.77 (s, 1H, NH), 8.03 ppm (d, 1H, $J = 7.0$ Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta =$ 23.2, 23.3, 35.5, 45.3, 47.1, 60.7, 65.1, 109.6, 111.8, 116.4, 126.1, 127.8, 128.3, 128.7, 129.5, 129.8, 131.8, 133.6, 133.8, 134.6, 139.4, 139.6, 141.1, 148.5, 158.5, 171.2 ppm; Anal. calcd (%) for $C_{31}H_{35}N_3O_6S$: C 64.45, H 6.11, N 7.27, found: C 64.69, H 6.28, N 7.11.

Biology

In vitro 20S proteasome inhibition assays: The three distinct proteolytic activities of human 20S proteaseome (Biomol GmbH, Hamburg, Germany) were measured by monitoring the hydrolysis of peptidyl 7-amino-4-methyl coumarin substrates (all obtained from Bachem), Suc-Leu-Leu-Val-Tyr-AMC, Boc-Leu-Arg-Arg-AMC, and Cbz-Leu-Leu-Glu-AMC, for ChT-L, T-L or PGPH enzyme activity, respectively. Hydrolysis of the given substrates was measured using a Varian Cary Eclipse fluorescence spectrophotometer (Varian, Darmstadt, Germany) at 30 °C with a 370 nm excitation filter and a 465 nm emission filter. Preliminary screening for inhibition of the three proteolytic activities of 20S proteaseome was performed at 20 μM inhibitor concentrations using an equivalent amount of DMSO as a negative control. Compounds showing at least 40% inhibition at 20 μM were subjected to detailed assays. The inhibition constants were obtained from progress curves (30 min) at various concentrations of inhibitor by fitting the progress curves to Equation (1), yielding the pseudo-first order rate constants of inhibition k_{obs}, which were then fitted to the inhibitor concentrations using Equation (2), yielding the first-order rate constant k_{inac} and the apparent dissociation constant K_I^{app}.

$$F = A(1-\exp(-k_{obs}t)) + B \quad (1)$$

$$k_{obs} = k_{inac}[I]/(K_I^{app} + [I]) \quad (2)$$

Dissociation constants K_I were obtained by correction to zero substrate concentration using Equation (3), the Cheng–Prusoff equation. Second-order rate constants were calculated using Equation (4). In cases where the plots of k_{obs} versus inhibitor concentration [I] were restricted to the linear range only, k_{2nd} values were calculated using Equation (5). The K_m values were determined in separate experiments: ChT-L activity with Suc-Leu-Leu-Val-Tyr-AMC (13 μM), PGPH activity with Cbz-Leu-Leu-Glu-AMC (53 μM).

$$K_I = K_I^{app}/(1 + [S]/K_m) \quad (3)$$

$$k_{2nd} = k_{inac}/K_I \quad (4)$$

$$k_{2nd} \approx k_{obs}[I]^{-1}(1 + [S]K_m^{-1}) \quad (5)$$

Assaying the chymotryptic activity of 20S proteasome: Human 20S proteasome was incubated at 30 °C at a final concentration of 0.004 mg mL−1 with test compound present at varying concentrations. The reaction buffer consisted of 50 mm Tris pH 7.5, 10 mm NaCl, 25 mm KCl, 1 mm MgCl2, 0.03% sodium dodecyl sulfate (SDS), and 5% DMSO. Product release from substrate hydrolysis (75 μm) was monitored continuously over a period of 30 min.

Assaying the tryptic activity of the 20S proteasome: Human 20S proteasome was incubated at 30 °C at a final concentration of 0.0025 mg mL^{-1} with test compound at varying concentrations. The reaction buffer consisted of 50 mM Tris buffer pH 7.4, 50 mM NaCl, 0.5 mM EDTA, 0.03% SDS, and 7.5% DMSO. Product release from substrate hydrolysis (85 μM) was monitored continuously over a period of 30 min.

Assaying the post-glutamyl-peptide hydrolyzing activity of the 20S proteasome: Human 20S proteasome was incubated at 30 °C at a final concentration of 0.004 mg mL^{-1} with the test compound at varying concentrations. The reaction buffer consisted of 50 mM Tris buffer pH 7.5 containing 25 mM KCl, 10 mM NaCl, 1 mM $MgCl_2$, 0.03% SDS, and 5% DMSO. Product release from substrate hydrolysis (80 μM) was monitored continuously over a period of 30 min.

Assays for bovine pancreatic α-chymotrypsin inhibition: The enzyme (250 μg mL^{-1}) was incubated at 25 °C with test compound. The reaction buffer consisted of 50 mM Tris buffer pH 8.0 containing 100 mM NaCl, 5 mM EDTA and 7.5% DMSO. Product release from substrate hydrolysis (75 μM final concentration, Suc-Leu-Tyr-AMC) was determined over a period of 10 min.

Assays for cathepsin B and L inhibition: Inhibition of cathepsin B and L was determined using the methods previously described.[18] Cbz-Phe-Arg-AMC was used as the substrate (80 μM for cathepsin B, 5 μM for cathepsin L).

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