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Potent and Highly Selective Inhibitors of the Proteasome Trypsin-like Site by Incorporation of Basic Side Chain Containing Amino Acid Derived Sulfonyl Fluorides

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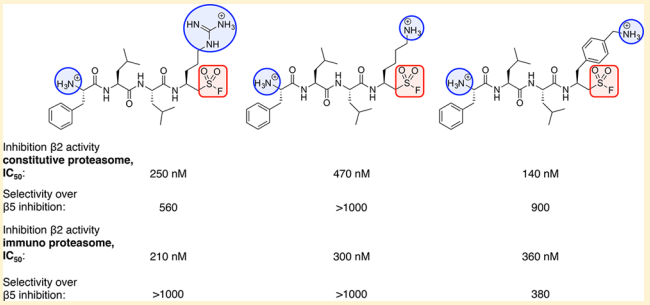
Supporting Information

ABSTRACT: A unique category of basic side chain containing amino acid derived sulfonyl fluorides (SFs) has been synthesized for incorporation into new proteasome inhibitors targeting the trypsin-like site of the 20S proteasome. Masking the former α-amino functionality of the amino acid starting derivatives as an azido functionality allowed an elegant conversion to the corresponding amino acid derived sulfonyl fluorides. The inclusion of different SFs at the P_1 site of a proteasome inhibitor resulted in 14 different peptidosulfonyl fluorides (PSFs) having a high potency and an excellent selectivity for the proteolytic activity of the β2 subunit over that of the β5 subunit. The results of this study strongly indicate that a free N-terminus of PSFs inhibitors is crucial for high selectivity toward the trypsin-like site of the 20S proteasome. Nevertheless, all compounds are slightly more selective for inhibition of the constitutive over the immunoproteasome.



INTRODUCTION

The ubiquitin−proteasome pathway (UPS) comprises the main machinery for degrading damaged, misfolded, pathogen derived, and abnormal proteins in the cell.[1] Therefore, the proteasome plays a crucial role in the regulation of many cell cycle processes especially involving antigen processing and apoptosis after protein quality control.[2] Proteolysis of the designated proteins is achieved by the 20S proteasome which consists of four stacked rings comprising 28 subunits assembled in two outer α-rings and two inner β-rings. Within the proteolytic β-rings of the 20S constitutive proteasome the β1c, β2c, and β5c subunits are found to show catalytic activity referred to as caspase-like activity (β1c), trypsin-like activity (β2c), and chymotrypsin-like activity (β5c). Upon exposure to interferon γ (IFNγ) and/or tumor necrosis factor α (TNFα) these subunits are substituted by β1i (LMP2), β2i (MECL-1), and β5i (LMP7), respectively, resulting in the so-called immunoproteasome.[3] Selective targeting of either constitutive or immunoproteasome subunits is a particular challenge and opens up further possibilities for the development of anticancer and anti-inflammatory therapeutic agents. This may even be extended to development of parasite-selective proteasome inhibitors.[4] The development of proteasome inhibitors has been an outstanding case showing that irreversible inhibitors may provide unique advantages by forming long-lived ties with their target.[5]

Since the approval of bortezomib **1** in 2003[6] and carfilzomib **2** in 2012[7] (Figure 1) for the treatment of multiple myeloma, many peptide based inhibitors containing different electrophilic traps have been reported.[8] These next generations of inhibitors like ixazomib,[9,10] oprozomib[11] (currently in clinical trials), and LU-102 (**3**)[12,13] (in preclinical testing) clearly demonstrated that other "warheads" or electrophilic traps within a peptide based inhibitor can provide attractive alternatives.

We have initiated the exploration of the sulfonyl fluoride warhead for incorporation into proteasome inhibitors and other proteases inhibitors[14] leading to peptidosulfonyl fluorides (PSFs).[15,16] Since then, this electrophilic trap has undergone considerable development as it is presently denoted as a "privileged warhead" in chemical biology, partly due to its considerable aqueous stability and chemical reactivity in the respective target enzyme.[26,17] Apart from obtaining potent proteasome inhibitors, in particular Cbz-Leu_4-SF **4** with an IC_{50} value of 7 nM for the β5c subunit of the constitutive proteasome,[15] it was found that the SF-warhead also endowed proteasome inhibitor **5** with a 25-fold higher selectivity for inhibition of the β5i subunit of the immunoproteasome over the β5c subunit although with loss of potency.[16] This showed clearly that SFs were not merely acting as a powerful

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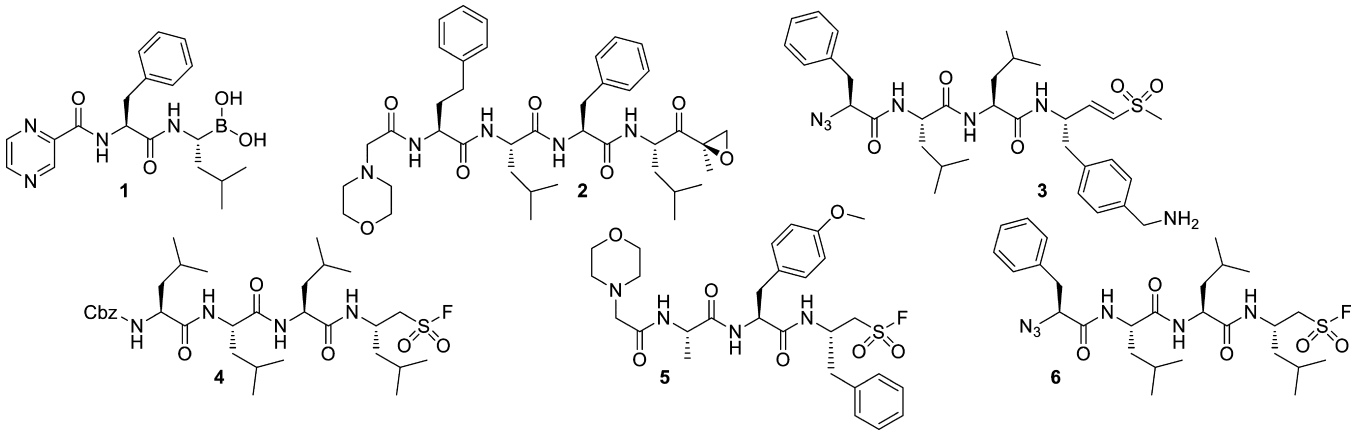

Figure 1. Structures of bortezomib (**1**), carfilzomib (**2**), LU-102 (**3**), Cbz-Leu$_4$-SF (**4**), β5i immunoproteasome selective PSF (**5**), and N$_3$-Phe-Leu$_3$-SF (**6**).

electrophilic trap but also gave rise to higher selectivity by simply changing the electrophilic trap from an epoxyketone to a sulfonyl fluoride.

Although this was an important finding, we think that selectivity for a particular proteasome subunit or in general a protease is largely determined by the character and relative position of the P$_1$ side chain with respect to the SF warhead.[27] Therefore, we focused our efforts on inhibitors with basic side chains at the P$_1$ position to evaluate whether this would be sufficient to confer selectivity of the resulting inhibitors for the proteasome trypsin-like site (β2) over the chymotrypsin-like site (β5). Moreover, development of β2 selective inhibitors will contribute to overcoming resistance against existing (β5) inhibitors.[12,13]

Recently, we found that PSF **6** was a very potent proteasome inhibitor (IC$_{50}$ = 110 nM for β5c).[15] The known inhibitor LU-102 **3** (IC$_{50}$ = 3.8 nM for β2)[19] has largely the same backbone sequence but also contains a basic non-natural amino acid residue. This latter sequence was used here for incorporation of an SF warhead leading to development of the synthesis and investigation of the selectivity and potency of basic side chain containing PSF proteasome inhibitors. The required development of amino acid derived sulfonyl fluorides containing a basic side chain is described in this paper, and their incorporation in PSF proteasome inhibitors led to both potent and highly selective inhibitors of the proteasome's trypsin-like (β2) activity.

RESULTS AND DISCUSSION

Synthesis of Basic Side Chain Containing Amino Acid Derived Sulfonyl Fluorides. *Considerations.* Synthesis of an amino acid derived sulfonyl fluoride (SF) containing a basic side chain, which upon incorporation in the remainder of the proteasome inhibitor sequence should endow the resulting molecular construct with β2-selectivity, was a significant challenge. Because of the simultaneous presence of an electrophilic site (the sulfonyl fluoride moiety) and two nucleophilic sites, that is, the former α-amino group and the basic side chain, a suitable protecting group strategy was necessary. It was known from our previous work that as long as the α-amino group is protected or protonated, it is possible to leave the SF moiety intact and ultimately incorporate it in an inhibitor construct.[15] The desired amino acid derived SFs with a basic side chain for inhibitor construction are shown in Figure 2. On the basis of the pK_a value of the side chain, it was expected that the guanidine functionality will always remain

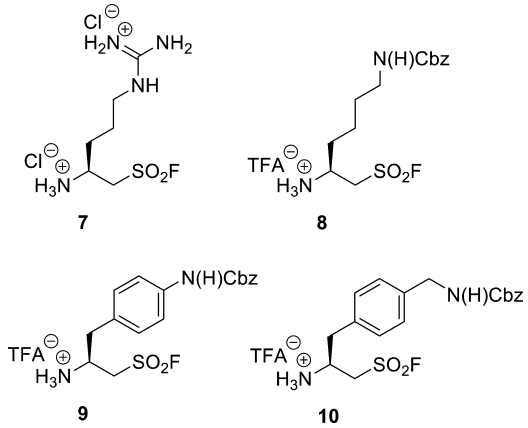

Figure 2. Basic side chain containing amino acid derived SFs **7**−**10**.

protonated. Therefore, it will not react with an SF moiety, not even after the final deprotection step in the synthesis of the arginine building block **7** (Scheme 1) and incorporation in PSFs **53**−**58** by peptide coupling reactions. The aromatic and benzylic amino groups obtained by deprotection after incorporation of **9** and **10**, respectively, will not be available for an intramolecular reaction. This is probably also the case for the lysine derived amino group obtained by deprotection after incorporation of **8**, which apart from being protonated at physiological pH can only give rise to the formation of an 8-membered ring. Nevertheless, there are possibilities for intermolecular reactions and/or (slow) hydrolysis (see stability experiments, Figure 5), and therefore amino groups of amino SF derivatives **8**−**10** remained protected also in the coupling steps leading to PSFs **59**−**67**.

Synthesis. The syntheses of amino acid derived SFs **7**−**10** was carried out following our earlier described general strategy with modifications involving the fluoronating agent and in light of the considerations above (Schemes 1−3).[14] Starting from commercially available arginine compound **11**, which has protecting groups resistant to conditions used for the introduction of the SF warhead, it was first converted to methyl ester **12**. Reduction to alcohol **13**, preparation of mesylate **14** was followed by substitution to thioacetate **15**.

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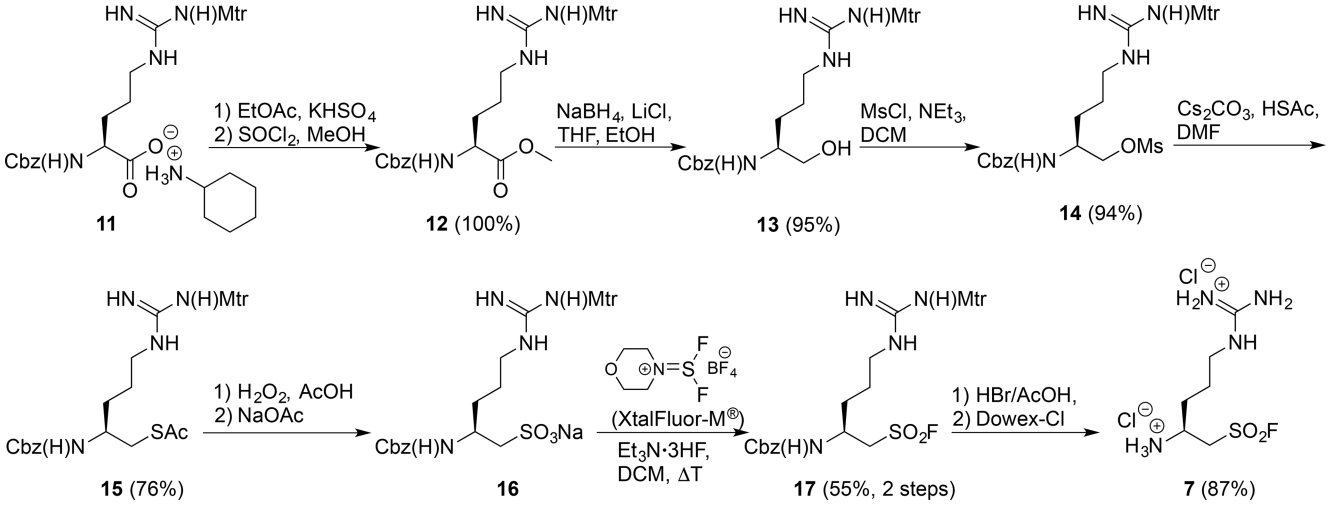
Scheme 1. Synthesis of Arginine Derived Sulfonyl Fluoride (7)

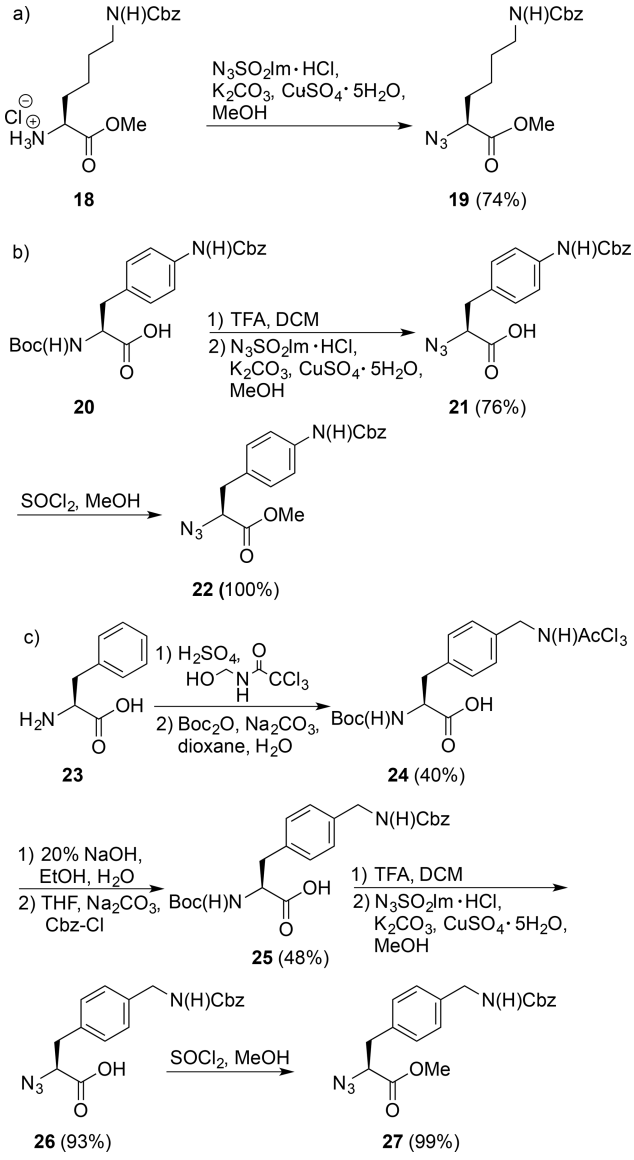


After oxidation to the sulfonic acid derivative **16** the corresponding sulfonyl fluoride **17** was obtained using XtalFluor-M.[21] Finally, simultaneous removal of the Cbz and Mtr protective groups afforded the arginine derived SF **7** ready for coupling to the remainder of the inhibitor sequence (Scheme 1).

For the synthesis of the lysine derived SF **8** and the aminophenylalanine derived SFs **9** and **10** a different synthetic strategy was necessary. To avoid manipulation of two orthogonal protecting groups present on both amino functional groups during or at the end of synthesis, it was decided to mask the α-amino group as an azide functionality until the very end, that is, after completion of the synthesis of the SF warhead (Scheme 3). This strategy was successful and allowed a relatively straightforward synthesis of the side chain protected azidoamino acid derived SFs **40**, **41**, and **42**.

Briefly, lysine derivative **18** and aminophenylalanine derivative **20** were converted to the corresponding α-azido derivatives **19** and **21** using the azido transfer reagent azidosulfonylimidazole in the presence of cupric sulfate.[22] The required aminomethylphenylalanine derivative **25** had to be prepared first in four steps from phenylalanine **23** similar to the synthesis of Geurink et al.[19] Compound **26** was then converted analogously to the required α-azido derivative **27** (Scheme 2). For these three α-azido derivatives **19**, **22**, and **27** an identical series of synthetic steps was followed to obtain the desired substituted amino SFs **8**–**10** (Scheme 3). The steps for introduction of the sulfonic acid moiety comprised reduction of the methyl ester to amino alcohol **28**–**30**, introduction of Ms-leaving group to mesylates **31**–**33**, followed by substitution to thioacetates **34**–**36** and finally oxidation to afford the sulfonates **37**–**39**, which were immediately converted to the corresponding SF-derivatives **40**–**42** using XtalFluor-M. The combined oxidation–SF conversion is still a considerable hurdle. Nevertheless, still decent yields of 33–42% over two steps (average 58–65% per step) were realized. Reduction of the azide group followed by protonation to the amino acid SF derivatives **8**–**10** was carried out using zinc powder in 15% TFA in AcOH.[23] After purification by semipreparative HPLC, SF derivatives were obtained as TFA salts in moderate yields (54–59%). The overall yields of the SF warhead containing amino acid derivatives were quite satisfactory, i.e., 11% (7 steps) for TFA·H-Lys(Cbz)ΨCH$_2$SO$_2$F (**8**), 7% (9 steps) for

Scheme 2. Syntheses of Azido Precursor Amino Acids 19, 22, and 27



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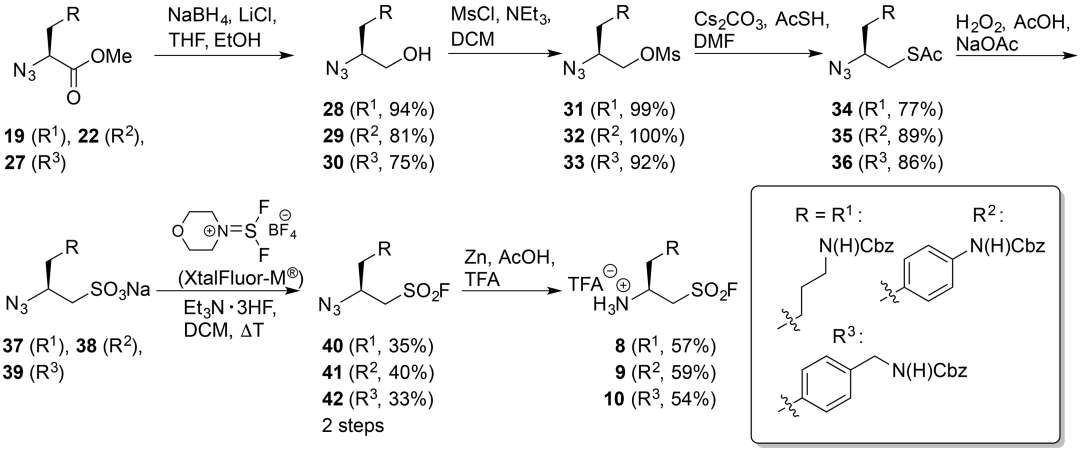
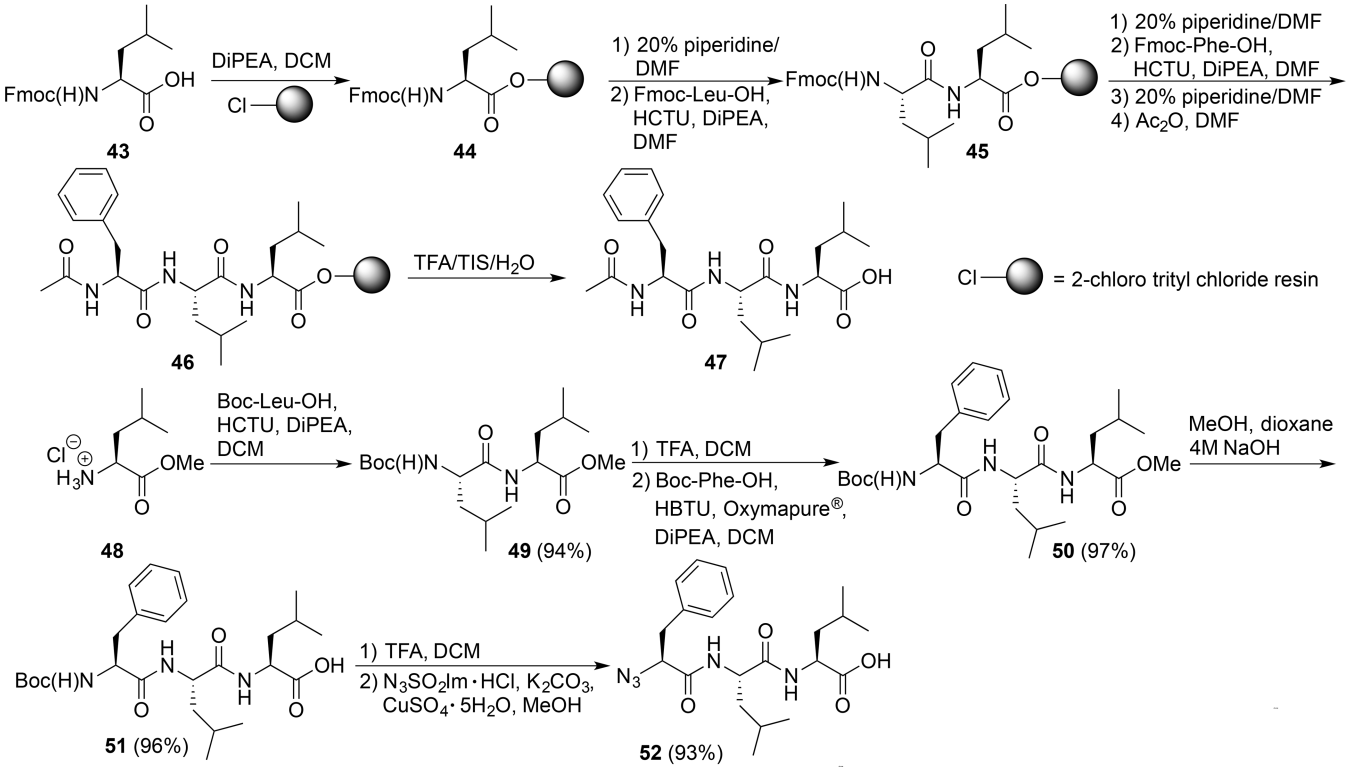
Scheme 3. Completion of the Synthesis of Basic Side Chain Containing Amino Acid Derived SFs 8−10



Scheme 4. Syntheses of the Peptides Necessary for Incorporation of the Amino Acid Derived SFs



TFA·H-Phe(4-N(H)Cbz)ΨCH$_2$SO$_2$F (**9**), and 2% (10 steps) for TFA·H-Phe(4-CH$_2$N(H)CbzΨCH$_2$SO$_2$F (**10**).

Incorporation of Amino SF Derivatives toward Syntheses of Peptidosulfonyl Fluorides (PSFs). For completion of the synthesis of the desired PSFs the amino acid derived SFs had to be incorporated into suitable peptide sequences, i.e., **47** and **52**. These sequences were based on the earlier developed powerful PSF proteasome inhibitors.[15] Their syntheses are shown in Scheme 4. Assembly of Ac-Phe-Leu-Leu-OH **47** was carried out on a 2-chlorotrityl chloride resin to afford **46** using a SPPS protocol and preparation of Boc-Phe-Leu-Leu-OH **51** was carried out in solution.[28] To prevent racemization at the C-terminus, the methyl ester of **50** was saponified first before conversion of the amino terminus to an azide functionality in **52**. This azide containing precursor peptide was prepared because the most active of our earlier described proteasome inhibitors[15] contained an azide functionality at the N-terminus of PSF **6**. As it was found that PSFs require at least a capped N-terminus for selectivity toward the immunoproteasome over the constitutive proteasome, also PSFs with an acetylated and unprotected N-terminus were synthesized to investigate if this was also crucial for β2 specificity.[16]

Introduction of SF warhead containing amino acid derivatives has always been viewed as one of the most challenging steps in the total synthesis of PSFs. First, the coupling conditions involving a nucleophilic amino group have to be selected in such a way that the SF electrophile stays as much as possible intact. Second, there is the possibility of racemization of the amino SF derivative in view of the electron-withdrawing character of the SF moiety. Therefore, several coupling reagents and conditions were attempted. These

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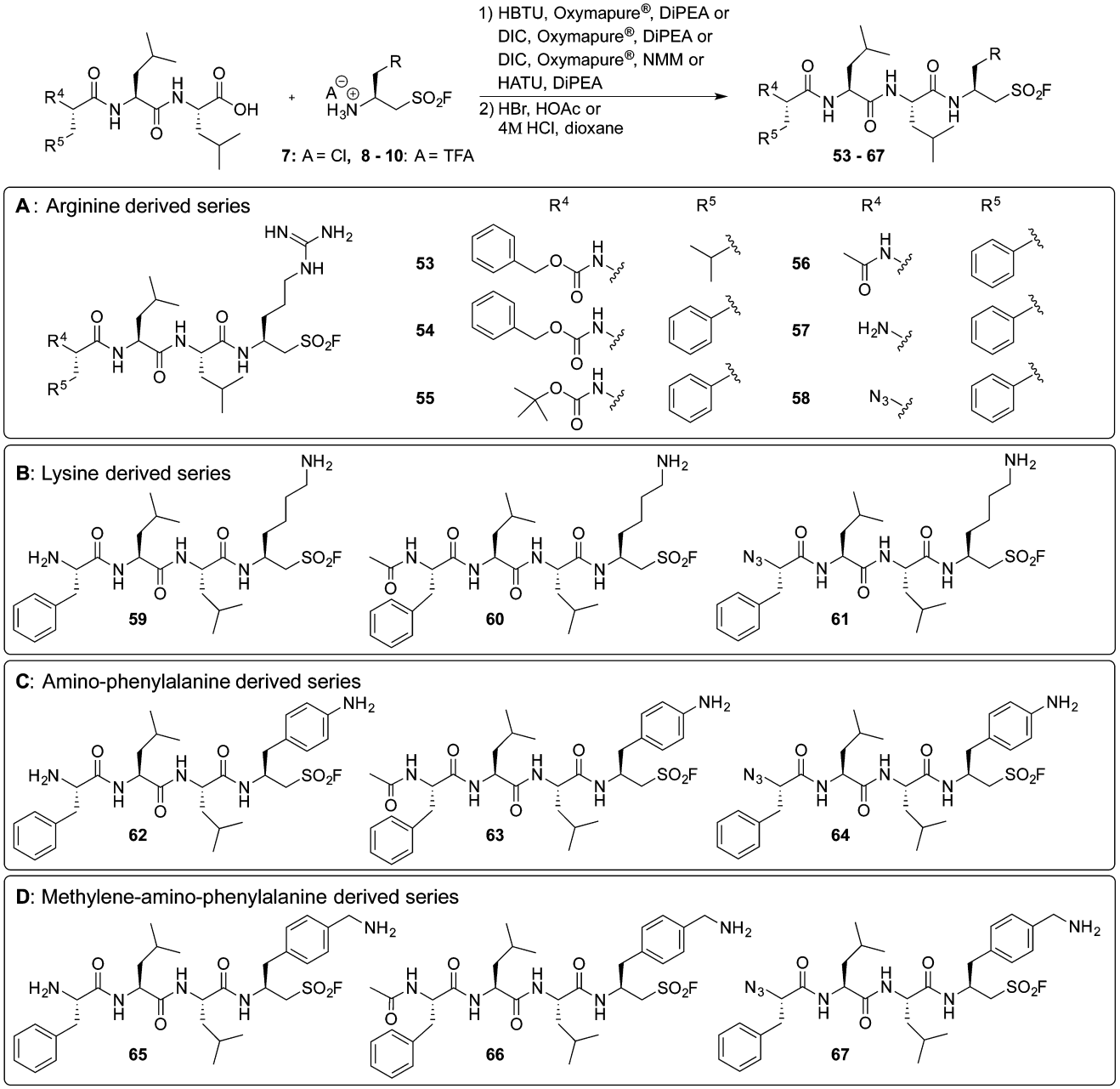
Scheme 5. Synthesis of the Peptide Sulfonyl Fluorides (PSFs) and Overview of the Structures of the Inhibitors[a]



[a]All amino and guanidine functionalities are protonated.

included HCTU, DIC/Oxymapure, HBTU/Oxymapure, BOP, and HATU. Using a different base, for example NMM instead of DiPEA, during the coupling step did not affect the yields. Nevertheless, DIC/Oxymapure and HATU in combination with DiPEA as a base gave the best results in series in terms of yields and racemization (see Supporting Information).[24,25]

For the final successful preparation of the basic side chain containing proteasome containing inhibitors **53**–**67**, the Cbz and Boc protecting groups leading to PSFs **59**, **60**, **62**, **63**, **65**, and **66** were removed by HBr in acetic acid (Scheme 5). These deprotection conditions led to substitution of the azide functionality in compounds like N_3-Phe-Leu-Leu-Phe(4-NH_2)-SF **64** by bromide. Fortuitously, a 4 M HCl solution in dioxane led to the desired deprotected proteasome inhibitor N_3-Phe-Leu-Leu-Phe(4-CH_2NH_2)-SF **67** and N_3-Phe-Leu-Leu-Lys-SF **61**. Unfortunately, this method was not successful to afford the inhibitor N_3-Phe-Leu-Leu-Phe(4-NH_2)-SF **64**. All PSF inhibitors containing an arginine at the P_1 position were coupled successfully with unprotected arginine derived SF as it was anticipated that the guanidine moiety stays protonated during the reaction and therefore would not react.

Biological Evaluation of the PSFs. The IC_{50} values of the in vitro structure–activity relationship (SAR) studies of the synthesized peptidosulfonyl fluorides were determined from the inhibitory curves in Figure 3 using constitutive human proteasome and are summarized in Table 1. The residual activity of the 20S proteasome activity was measured at time points using a fluorogenic substrate where a decrease in fluorescence corresponds to lower residual proteasome activity and therefore indicating a more potent inhibitor. In total, 14

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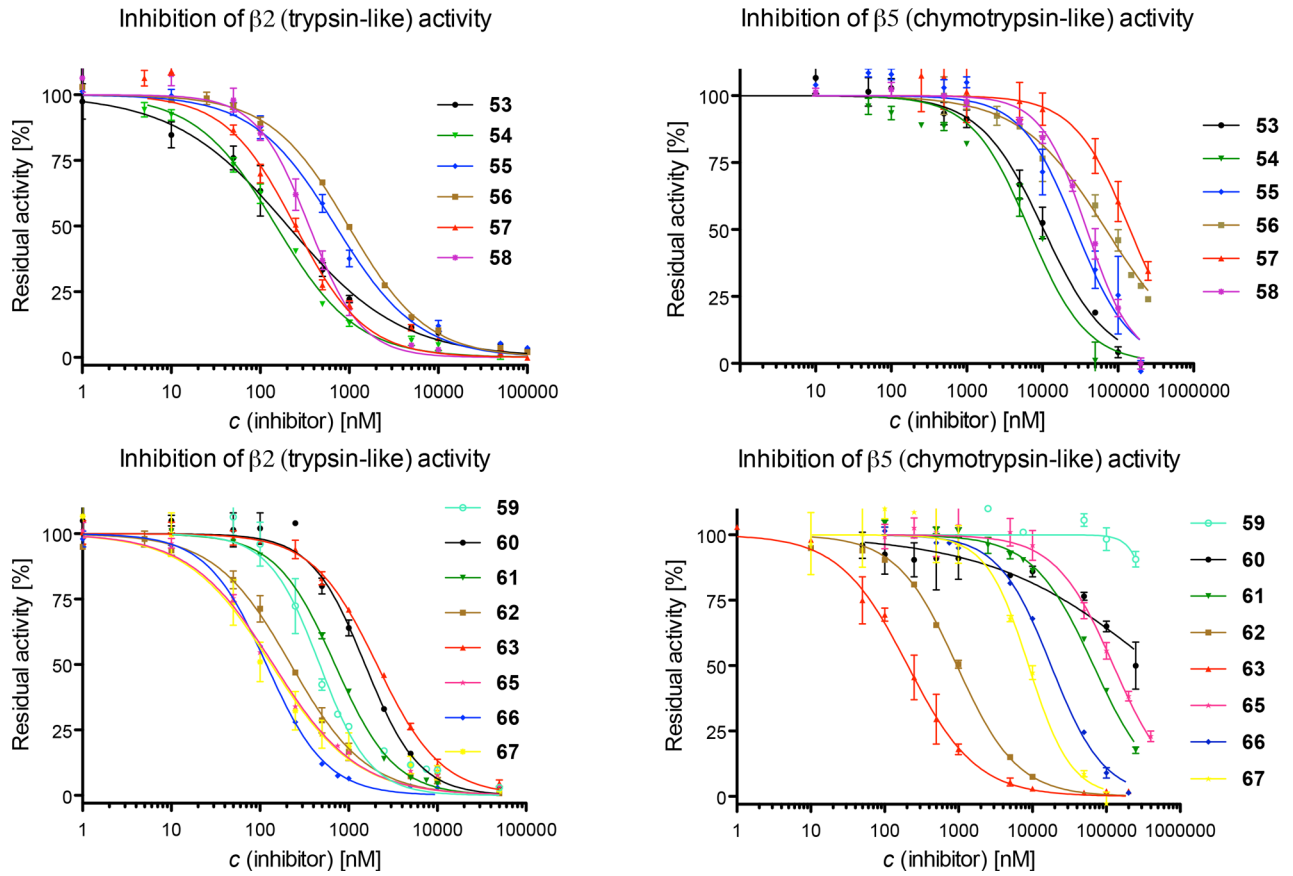

Figure 3. In vitro evaluation of PSFs using human constitutive 20S proteasome. Fluorogenic substrates were selective for the respective trypsin-like (Bz-VGR-AMC) or chymotrypsin-like (Suc-LLVY-AMC) subunit. Top: Arginine derived PSFs. Bottom: Lysine, aminophenylalanine and 4-aminomethylphenylalanine derived PSF. Left: Trypsin-like residual enzyme activity. Right: Chymotrypsin-like residual enzyme activity.

basic amino acid derived peptidosulfonyl fluorides were tested of which compounds **53**, **54**, **57**, **62**, **65**, **66**, and **67** showed IC_{50} values below 250 nM for the trypsin-like site. Particularly compounds **54**, **65**, **66**, and **67** were the most potent inhibitors of this series with IC_{50} values of 150 nM for **54**, 140 nM for **65**, 119 nM for **66**, and 130 nM for compound **67**. Although compounds **60** and **63** had IC_{50} values higher than 1 μM, all other compounds had IC_{50} values lower than 1 μM for inhibiting the trypsin-like proteasome activity. Compounds **59**, **65**, and **67** could only be obtained as a diasteromeric mixtures in which the aminosulfonyl fluoride residue had probably partially racemized; nevertheless these PSFs still had relatively low IC_{50} values. In the arginine derived PSFs the Cbz-protecting group appeared to be beneficial for the potency when compound **54** is compared to compound **55**, **56**, **57**, or **58**. For example, changing the Boc group in PSF **55** to a Cbz protecting group in PSF **54** decreased the IC_{50} value by more than 500 nM. Due to the synthetic strategy of the PSFs **59**–**67**, the synthesis of Cbz N-termini protected analogs in this series of lysine, aminophenylalanine, and 4-aminomethyl-phenylalanine derived PSFs was not feasible at this point.[29] Comparing compounds **56** with **58** and **60** with **61**, it becomes apparent that an azido functionality does enhance the potency of the inhibitor significantly (roughly by a factor of 2) compared to those of the acetyl capped counterparts. The lysine and aminophenylalanine derived PSFs **59**, **60**, **61**, **62**, and **63** were the least potent in this study, indicating that the presence of stronger basic side chains in the P_1 position was more favorable.

The key goal of this study was the development of β2 selective (trypsin-like) PSF inhibitors. It was shown that, in general, except for the aniline derived PSFs **62** and **63**, all inhibitors possessed a moderate to very high β2 selectivity. Outstanding β2-selectivity was shown by PSF inhibitors having a free terminus as in the arginine derived PSF **57** (~600-fold selectivity), the lysine derived PSF **59** (>1000-fold selectivity), and the methylene aminophenylalanine derived PSF **65** (~900-fold selectivity), emphasizing the essentiality of free amino terminus with respect to this.

Originally, azide containing PSF ligands[15] were developed to capture possibly formed ligand/proteasome covalent adducts using the copper(I) catalyzed azide–alkyne cycloaddition (click) reaction. However, later we found that formation of covalent adduct with the proteasome active site is followed by elimination of the ligand.[16] Nevertheless, PSF **6** having an azido N-terminus was uncovered as one of most active β5 proteasome inhibitors,[15] and therefore azido containing PSF inhibitors were included in this study to evaluate whether this also would be the case for β2 inhibition. Although the azido containing inhibitors (**58**, **61**, and **65**) were slightly more active or had a similar activity that N-terminally protected PSFs, as was mentioned above, a free-amino terminus generally led to both the most potent and selective β2 compounds **57**, **59**, **62**, and **65**.

Although the β5-inhibitory activity, that is, inhibition of chymotrypsin activity, of all compounds was understandably poor, perhaps with the exception of **62** and **63** (both IC_{50} values of <1 μM), this activity improves slightly by having a

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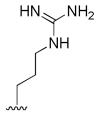
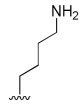
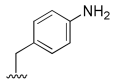
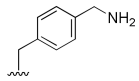
Table 1. Overview of the IC_{50} Values of Inhibition of the Constitutive Proteasome by Synthesized PSFs[a]

Compound				IC_{50} [nM]		IC_{50} (β5)/ IC_{50} (β2)
	R^4	R^5	R	β2 (Trypsin-like)	β5 (Chymotrypsin-like)	
53	Cbz(H)N			200 ± 100	10,000 ± 3,400	50
54	Cbz(H)N			150 ± 30	6,700 ± 1,600	45
55	Boc(H)N			700 ± 200	27,800 ± 15,700	40
56	Ac(H)N			980 ± 10	69,000 ± 29,000	70
57	H_2N			250 ± 45	140,000 ± 12,100	560
58	N_3			350 ± 65	39,000 ± 8,000	110
59	H_2N			470 ± 110	>> 100 μM	> 1,000
60	Ac(H)N			1,500 ± 35	>> 100 μM	300
61	N_3			700 ± 45	67,000 ± 3,200	96
62	H_2N			220 ± 90	950 ± 65	4.3
63	Ac(H)N			2,100 ± 35	210 ± 80	0.1
64	N_3			n.d.	n.d.	n.d.
65	H_2N			140 ± 7	125,000 ± 26,000	900
66	Ac(H)N			119 ± 7	18,000 ± 380	150
67	N_3			130 ± 117	8,900 ± 360	66

[a]Compounds **59**, **65**, and **67** could only be obtained as a diasteromeric mixture in which the aminosulfonyl fluoride residue had probably partially racemized as was apparent from ^{1}H NMR. n.d.: not determined as compound **64** could not be synthesized. A higher $IC_{50}(\beta5)/IC_{50}(\beta2)$ ratio indicates a higher β2 selectivity. In this assay synthesized LU-102 showed IC_{50} values of 10.8 ± 2.4 nM (β2) and 2600 ± 500 nM (β5) with an $IC_{50}(\beta5)/IC_{50}(\beta)$ of 241, which is agreement with the literature.[19]

protected or masked (azide) N-terminus. This finding is in accordance with earlier findings that β5-selectivity (toward the chymotrypsin-like site) requires at least a capped N-terminus of the PFSs.[15] Interestingly, compound **63** is the only one in this series that gave rise to stronger inhibition of β5-activity as compared to inhibition of β2 activity. This might indicate that the hydrophobic character of "aniline" side chain plays a dominant role in determination of the selectivity toward β5 inhibition.

Recently[16] we found that PSFs showed selective inhibition of the immunoproteasome. Therefore, a subset of the most powerful inhibitors were taken and evaluated in an immunoproteasome assay as in shown in Table 2 and Figure 4. The data showed that the selectivity of β2 inhibition PSFs is maintained. However, in contrast to earlier developed PSFs[19] selectivity for inhibition of the immunoproteasome was virtually absent. This was also the case for LU-102, which although a highly potent inhibitor, also showed selectivity for constitutive proteasome inhibition.

PSFs containing the SF-warhead comprise relatively new inhibitor ligands of which stability and reactivity properties have not been investigated as yet. In order to start determination of the former properties, we have evaluated the degree of hydrolysis of PSFs in buffers of different pHs. Therefore, stability tests of several PSFs were carried out to study the rate of hydrolysis of the SF moiety at a physiological pH (7.4) as well as at pH 6.5 and pH 8.0. Acetyl capped PSFs were chosen to prevent a possible intermolecular reaction involving the N-terminus and the SF moiety. Of course intermolecular reactions involving the amino groups in **60**, **63**, and **66** cannot be ruled out but are highly unlikely because they are largely protonated at the studied pH's or poorly nucleophilic (**63**). An intermolecular reaction involving the guanidinium functionality in **56** is even less likely because it is virtually completely protonated at the studied pH range. So it was assumed that a possible decrease in stability of the SF moiety is due to an increased inclination toward hydrolysis. In order to study only the hydrolytic stability and to exclude nucleophilic reactions of buffer components (for example, tris(hydroxylmethyl)-

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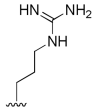
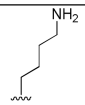
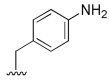
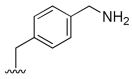
Table 2. Overview of the IC_{50} Values of Inhibition of the Immuno Proteasome by Selected PSFs[a]

Compound				IC_{50} [nM]		IC_{50} (β5)/ IC_{50} (β2)
	R^4	R^5	R	β2 (Trypsin-like)	β5 (Chymotrypsin-like)	
53	Cbz(H)N			1,110 ± 260	14,000 ± 7,300	13
54	Cbz(H)N			400 ± 130	6,700 ± 12,800	17
57	H_2N			210 ± 60	>> 100 μM	>1000
59	H_2N			300 ± 60	>> 100 μM	>1000
62	H_2N			200 ± 60	450 ± 100	2.3
65	H_2N			360 ± 130	136,500 ± 68,600	380
66	Ac(H)N			395 ± 40	17,200 ± 6,700	44
67	N_3			130 ± 10	3,100 ± 1,500	24

[a]Compounds **59**, **65**, and **67** could only be obtained as a diasteromeric mixture in which the aminosulfonyl fluoride residue had probably partially racemized as was apparent from ^{1}H NMR. A higher $IC_{50}(\beta5)/IC_{50}(\beta2)$ ratio indicates a higher β2 selectivity. In this assay synthesized LU-102 showed IC_{50} values of 27.5 ± 6.9 nM (β2) and 1800 ± 2,400 nM (β5) with an $IC_{50}(\beta5)/IC_{50}(\beta)$ of 241, which is agreement with the literature.[19]

aminomethane in "Tris" buffer), phosphate buffered saline (PBS) was chosen as a buffer system.

As anticipated, hydrolysis of PSFs was slower at slightly acidic pH (6.5) compared to physiological pH (7.4) and slightly basic pH (8.0). Even after 12 h about 75% of the initial used PSF remained unaffected at pH 6.5. After 1 h at pH 7.4 90% of the PSF was still intact. Thus, during an incubation time of 1 h for the biological evaluation of a PSF, a large majority is still available for interaction with the proteasome. After 6 h at pH 7.4, ≥50% of the PSFs were still intact. However, rapid hydrolysis was observed at pH 8.0 at which 50% of the PSFs were hydrolyzed after 2 h (Figure 5).

CONCLUSIONS

We have shown that our general route for the preparation of amino acid derived SFs could be extended to SF derivatives containing nucleophilic groups in their side chains in the presence of the SF electrophilic trap. The route to the SF derived from arginine **7** was largely based on our earlier developed synthesis. The preparation of SF derivatives containing an amino group in their side chains was achieved using a judicious strategy masking the α-amino group as an azide functionality, thereby circumventing complicated (de)-protection strategies. This resulted in the successful and convenient synthesis of lysine derived SF **8**, aminophenylalanine **9** and methylene aminophenylalanine derived SF **10** ready for incorporation in proteasome inhibitor sequences.

Although incorporation of the SF warhead into a peptide inhibitor sequence remains a challenging step in the total synthesis of PSFs, we have successfully synthesized several potent and highly selective PSFs capable of inhibiting the β2 (trypsin-like) activity over the β5 (chymotrypsin-like) activity for the first time.

In addition, we have established the crucial role of the N-terminus. PSFs with a free amino terminus gave rise to highly selective β2 proteasome inhibitors. Having an azide functionality as N-terminus did not lead to a tremendous enhanced potency as was found earlier with the β5-proteasome inhibitors. Although the described PSFs were less potent than, for example, LU-102, their selectivity for β2 was similar. Both PSFs and LU-102 did not show selective inhibition of the immunoproteasome. Since PSFs gave rise to permanent inhibition of the proteasome by ligand-induced cross-linking of the active site, it would be interesting to evaluate the significance of proteasome inhibition by irreversible inhibitors versus reversible inhibitors.

In view of the promising electrophilic trap properties leading to highly active and selective compounds, we embarked on initial stability studies in buffer of the SF-warhead. It was found that the stability at physiological pH was quite satisfactory. Clearly, this is just the start toward gaining insights in the behavior of these relatively novel aliphatic amino acid derived SFs and PSFs. Thus, there will be significant chemical challenges ahead with respect to modulating both reactivity and stability. This research will be guided by future studies directed toward investigations of cellular permeability and stability of the most promising PSFs described above.

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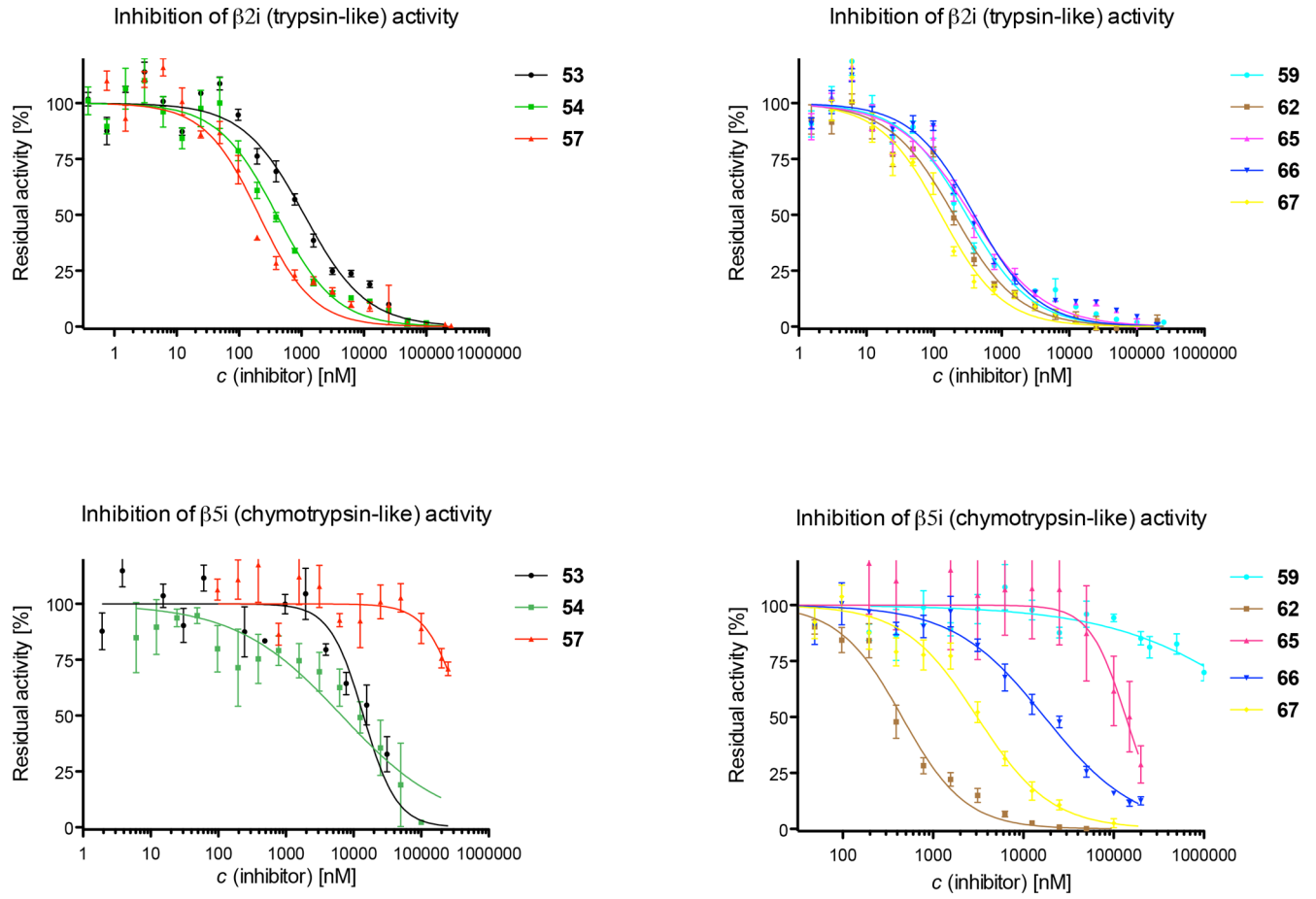

Figure 4. In vitro evaluation of PSFs using human immuno 20S proteasome. Fluorogenic substrates were selective for the respective trypsin-like (Bz-VGR-AMC) or chymotrypsin-like (Suc-LLVY-AMC) subunit. Top: Arginine derived PSFs. Bottom: Lysine, aminophenylalanine and 4-aminomethylphenylalanine derived PSF. Left: Trypsin-like residual enzyme activity. Right: Chymotrypsin-like residual enzyme activity.

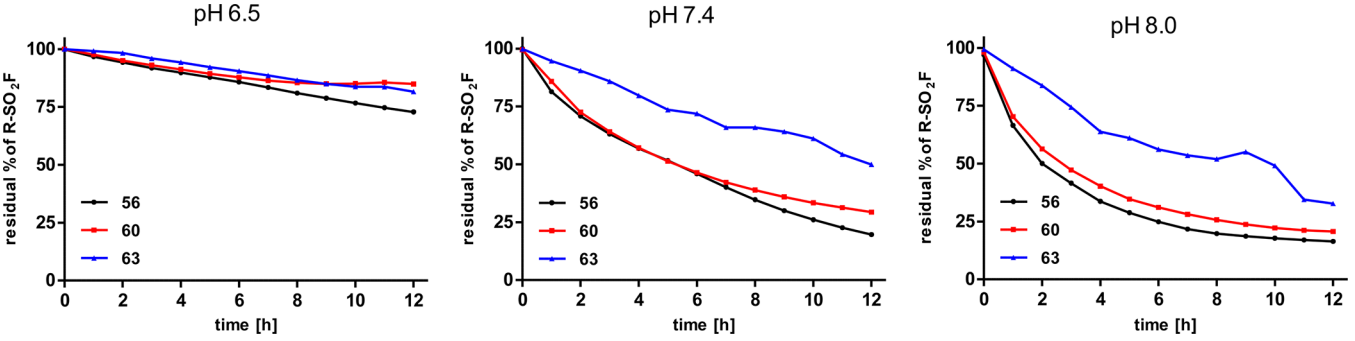

Figure 5. PBS buffer stability test at pH 6.5, 7.4, and 8.0. Hydrolysis of selected PSFs was measured via analytical HPLC over 12 h.

EXPERIMENTAL PROCEDURES

General Procedures. All starting materials, reagents, and solvents were obtained from commercial sources and used as received. Dry solvents were obtained from a PureSolv 500 MD solvent purification system. Reactions requiring dry conditions were performed in heat-gun dried glassware. All reactions were performed at ambient temperature unless stated otherwise. Reactions in solution were monitored by TLC analysis on Merck precoated silica gel 60 F_{254} (0.25 mm) glass backed plates. Spots were visualized by UV light (254 and 366 nm) and by heating plates after dipping in a ninhydrin or cerium/molybdenum solution. Column chromatography was performed on Siliaflash P60 (40−63 μm) from Silicycle (Canada). Petroleum ether (40−60 °C fraction) and *n*-hexane were used for flash column chromatography. ^{1}H NMR, ^{13}C NMR, and ^{19}F NMR spectra were recorded on a Bruker DPX 400 spectrometer or Bruker 500 spectrometer with chemical shift values reported in parts per million (ppm) relative to TMS (δ_H = 0.00 and δ_C = 0.0) or residual $CHCl_3$ (δ_H = 7.28 and δ_C = 77.16) or residual DMSO-d_6 (δ_H = 2.50 and δ_C = 39.52) as standard. Assignments of ^{1}H and ^{13}C NMR signals are based on two-dimensional COSY, HSQC, HMBC, DEPT, and DEPTQ experiments, respectively. High-resolution electrospray ionization (ESI) mass spectra were measured on a Bruker micrOTOF-Q II in positive or negative mode and calibrated with an ESI tuning mix from Agilent Technologies. Infrared spectra were recorded using a Shimadzu FTIR 8400S apparatus. Optical rotations were determined as solutions irradiating with the sodium D line (λ = 589 nm) using an Auto pol V polarimeter. $[\alpha]_D$ values are given in units 10^{-1} deg cm^2 g^{-1}. Semipreparative high performance liquid chromatography (HPLC) was performed on an Agilent Technologies 1260 Infinity

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system (12.5 mL min^{-1}). Analytical HPLC chromatograms were recorded on a Shimadzu Prominence system (1 mL min^{-1}). Buffers used for HPLC: buffer A (0.1% TFA in MeCN/H_2O v/v 5:95) and buffer B (0.1% TFA in MeCN/H_2O v/v 95:5). Semipreparative runs started with an isocratic flow of buffer A (100% for 5 min), followed by a linear gradient of buffer B (in 60 min to *X*%). Subsequently, an isocratic flow of buffer B (100% for 5 min) was performed followed by a linear gradient to buffer A (in 5 min to 100%). Runs ended with an isocratic flow of buffer A (100% for 5 min). Used columns are mentioned in the Supporting Information. In addition, all HPLC chromatograms and retention times of all purified compounds are supplied in the Supporting Information. All compounds have purities of ≥95%.

Proteasome Inhibitory Assays. Inhibition of the constitutive and immuno proteasome enzymatic activity was determined using the VIVAdetect 20S assay kit PLUS (Viva bioscience, U.K.) utilizing a Clariostar microplate reader (BMG LABTECH, Germany). All working solutions were freshly prepared for each measurement. Kinetic enzyme assays were performed using 96-well Corning half-area plates using 50 μL of total amount of liquid. Incubation of all measured inhibitors was at ambient temperature on a shaker for 60 min. Fluorescence measurements were carried out at λ_{ex} = 360 nm and λ_{ex} = 460 nm at 25 °C for 2 h. All assays were carried out in duplicate with three repetitions. Each well contained 35 μL of VIVAdetect buffer. The final enzyme concentration in a well was 2.5 nM (5 μL of a 25 nM enzyme working solution in VIVAdetect buffer). Final substrate concentration was 100 μM (5 μL of a 1 mM substrate working solution in VIVAdetect buffer; Suc-LLVY-AMC for the chymotrypsin-like activity; Bz-VGR-AMC for the trypsin-like activity). In order to use a final minimal concentration of DMSO, stock solutions of each inhibitor were prepared: Arginine derived PSFs were dissolved in 10% DMSO/H_2O, lysine derived PSFs were dissolved in 30% DMSO/H_2O, 4-aminophenylalanine and 4-methyleneaminophenylalanine derived PSFs were dissolved in 50% DMSO/H_2O. Dilution series of inhibitors were prepared using the appropriate stock solution. For the positive controls 5 μL of the stock DMSO-percentage solution was added instead of the respective inhibitor solution. MG132 (Cbz-Leu-Leu-leucinal) was used as a negative control with a final concentration of 5 μM (5 μL of a 50 μM working solution, supplied in the VIVAdetect 20S assay kit PLUS). Final inhibitor concentrations were 400 μM, 200 μM, 100 μM, 50 μM, 10 μM, 5 μM, 2.5 μM, 1 μM, 750 nM, 500 nM, 250 nM, 100 nM, 50 nM, 10 nM, 1 nM, 0.1 nM. The inhibitory activities of the compounds were expressed as IC_{50} values. IC_{50} values were obtained by plotting the residual percentage of enzymatic activity against the logarithm of the inhibitor concentrations. Experimental data were fitted to the equation % residual activity = $100/(1 + 10^{((\log IC_{50} \log c(\text{inhibitor}))\text{Hill slope})})$ using GraphPad Prism software version 5.

Stability Evaluation in Buffer. Compounds **56**, **60**, and **61** were dissolved in 90 μL of DMSO and added to an aqueous 1× PBS buffer solution (prepared from 10× PBS buffer solution (Gibco) adjusted with aq 2 M HCl or 2 M NaOH solution, respectively, pH 6.5 or 7.4 or 8.0, 910 μL) resulting in a final concentration of ∼1 mM. The hydrolysis was monitored via analytical HPLC (C_{A3}) over 12 h. 0 h was measured directly after the addition of the PBS buffer solution and was taken as the reference peak.

Synthesis. The synthetic procedures and characterization data of crucial synthetic intermediates as well as those of the final PSFs are described below. All other synthetic procedures and characterization data are included in the Supporting Information, which also contains all NMR spectra, MS data, and HLPC traces of the final PSFs.

2HCl·H-Arg-ψ[CH_2SO_2]-F (7). Cbz-Arg(Mtr)-ψ[CH_2SO_2]-F (**17**) (310 mg, 541 μmol, 1.00 equiv) was dissolved in CH_2Cl_2 (8 mL), and 33% HBr/AcOH solution (8 mL) was added. The reaction mixture was stirred for 1 h and the solvents removed in vacuo. H_2O (20 mL) was added and the aqueous layer extracted with EtOAc (20 mL) and treated with Dowex 1X8 chloride form (777 mg) for 10 min. The mixture was filtered and freeze-dried. The crude product was obtained as a yellowish solid (166 mg, 555 μmol, 100%). ^{1}H NMR (500 MHz, DMSO-d_6, 298 K): δ_H (ppm) = 8.63 (s, 3H, $NH_3\alpha$C), 7.79 (t, *J* = 5.9 Hz, 1H, CN*H*CH_2), 4.48 (dt, *J* = 15.5, 6.0 Hz, 1H, CH_aSO_2F), 4.40 (dt, $^3J_{H,H,F}$ = 15.5, 5.5 Hz, 1H, CH_bSO_2F), 3.86−3.72 (m, 1H, $H_3N\alpha CH$), 3.22−3.05 (m, 2H, αCNHCH_2), 1.87−1.72 (m, 2H, αCHCH_2), 1.72−1.55 (m, 2H, αCHCH_2CH_2). ^{13}C NMR (126 MHz, DMSO-d_6, 298 K): δ_C (ppm) = 157.4 ($NHCNH_2$), 52.3 (d, $J_{C,S,F}$ = 14.8 Hz, CH_2SO_2F), 46.3 (αCH), 40.6 (αCNHCH_2), 29.3 ($NHCH_2CH_2$), 24.3 (αCNHCH$_2$CH_2). ^{19}F NMR (471 Hz, DMSO-d_6, 298 K): δ_F (ppm) = 60.3 (s, 1F, SO_2F). HRMS (ESI positive) calcd for $C_6H_{16}N_4O_2SF$ $[M + H]^+$ 227.0973, found 227.0971.

TFA·H-Lys(Cbz)-ψ[CH_2SO_2]-F (8). N_3-Lys(Cbz)-ψ[CH_2SO_2]-F (**40**) (300 mg, 837 μmol, 1.00 equiv) was dissolved in AcOH (12.5 mL). Next, zinc powder (547 mg, 8.37 mmol, 10.0 equiv) and TFA (1.9 mL) were added and the reaction mixture was stirred overnight at rt. The solvents were removed in vacuo, and AcOH (10 mL) was added. The crude product was purified via semipreparative HPLC (0 to 100% B, C_{P1}), and fractions containing the pure product were pooled and lyophilized. The pure product was obtained as a white solid (212 mg, 474 μmol, 57%). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ_H (ppm) = 8.39 (s, 3H, NH_3^+), 7.41−7.28 (m, 5H, Ar-H (Cbz)), 7.24 (t, *J* = 5.6 Hz, 1H, N*H*CH_2), 5.01 (s, 2H, CH_2 (Cbz)), 4.41 (dt, *J* = 15.6, 5.6 Hz, 1H, CH_aSO_2F), 4.25 (dt, *J* = 15.6, 5.7 Hz, 1H, CH_bSO_2F), 3.79−3.65 (m, 1H, NH_3CH), 3.04−2.92 (m, 2H, NHCH_2), 1.79−1.68 (m, 2H, αCHCH_2), 1.48−1.32 (m, 4H, NHCH_2CH_2CH_2). ^{13}C NMR (126 MHz, $CDCl_3$, 298 K): δ_c (ppm) = 156.6 (C═O), 137.7 (C-Ar), 128.8, 128.2, 128.2 (CH-Ar), 65.6 (CH_2 (Cbz)), 52.3 (d, $J_{C,F}$ = 15.0 Hz, CH_2SO_2F), 46.6 (NH_3CH), 40.3 ($NHCH_2$), 31.8 (NH_3CHCH_2), 29.3 (CH_2), 21.5 (CH_2). ^{19}F NMR (471 MHz, $CDCl_3$, 298 K): δ_F (ppm) = 62.7 (SO_2F). HRMS (ESI positive) calcd for $C_{14}H_{23}N_2O_4SF$ $[M + H]^+$ 333.1279, found 333.1260.

TFA·H-Phe(4-NHCbz)-ψ[CH_2SO_2]-F (9). N_3-Phe(4-NHCbz)-ψ-[CH_2SO_2]-F (**41**) (314 mg, 799 μmol, 1.00 equiv) was dissolved in AcOH (12 mL). Next, zinc powder (522 mg, 7.99 mmol, 10.0 equiv) and TFA (1.73 mL) were added and the reaction mixture was stirred overnight at rt. The solvents were removed in vacuo, and AcOH (10 mL) was added. The crude product was purified via semipreparative HPLC (0 to 100% B, C_{P1}), and fractions containing the pure product were pooled and lyophilized. The pure product was obtained as a white solid (209 mg, 468 μmol, 59%). ^{1}H NMR (400 MHz, DMSO-d_6, 298 K): δ_H (ppm) = 9.84 (s, 1H, NH), 8.20 (br s, 3H, NH_3^+), 7.46 (d, *J* = 8.4 Hz, 2H, CH (Phe)), 7.44−7.32 (m, 5H, Ar-H (Cbz)), 7.23 (d, *J* = 8.4 Hz, 2H, CH (Phe)), 5.15 (s, 2H, CH_2 (Cbz)), 4.24 (app dt, *J* = 15.5, 5.3 Hz, 1H, CH_aSO_2F), 4.16 (app dt, *J* = 15.5, 6.0 Hz, 1H, CH_bSO_2F), 4.02−3.93 (m, 1H, $NH_3^+\alpha CH$), 3.03−2.92 (m, 2H, $N_3\alpha CHCH_2$). ^{13}C NMR (101 MHz, DMSO-d_6, 298 K): δ_c (ppm) = 153.8 (C═O), 138.9 (C-Ar), 137.1 (C-Ar), 130.5 (CH-Ar (Phe)), 128.9 (CH-Ar (Cbz)), 128.6 (CH-Ar (Cbz)), 128.5 (CH-Ar (Cbz)), 128.0 (C-Ar), 119.0 (CH-Ar (Phe)), 66.2 (CH_2 (Cbz)), 52.2 (d, *J* = 15.7 Hz, CSO_2F), 47.8 (αCH), 37.3 (αCH CH_2). ^{19}F NMR (377 MHz, $CDCl_3$, 298 K): δ_F (ppm) = 60.6 (t, *J* = 5.8 Hz, 1F, SO_2F). HRMS (ESI positive) calcd for $C_{17}H_{19}N_2O_4SFNa$ $[M + Na]^+$ 389.0942, found 389.0926.

TFA·H-Phe(4-CH_2NHCbz)-ψ[CH_2SO_2]-F (10). N_3-Phe(4-CH_2NHCbz)-ψ[CH_2SO_2]-F (**42**) (62 mg, 153 μmol, 1.00 equiv) was dissolved in AcOH (2.3 mL). Next, zinc powder (99.8 mg, 1.53 mmol, 10.0 equiv) and TFA (342 μL) were added and the reaction mixture was stirred overnight at rt. Then, the solvents were removed in vacuo and AcOH (5 mL) was added. The crude product was purified via semipreparative HPLC (0 to 100% B, C_{P1}), and fractions containing the pure product were pooled and lyophilized. The pure product was obtained as a white solid (40.9 mg, 82.7 μmol, 54%). ^{1}H NMR (400 MHz, DMSO-d_6, 298 K): δ_H (ppm) = 8.53 (br s, 3H, NH_3^+), 7.86 (t, *J* = 6.3 Hz, 1H, N*H*CH_2), 7.41−7.20 (m, 9H, Ar-H (Cbz), CH (Phe)), 5.05 (s, 2H, CH_2 (Cbz)), 4.31−4.16 (m, 4H, NHCH_2, CH_2SO_2F), 4.12−3.97 (m, 1H, NH_3CH), 3.17−2.97 (m, 2H, αCHCH_2Phe). ^{13}C NMR (101 MHz, DMSO-d_6, 298 K): δ_c (ppm) = 156.9 (C═O), 139.5 (C-Ar), 137.6 (C-Ar), 133.3 (C-Ar), 130.0 (CH-Ar (Phe[4-CH_2NHCbz])), 128.8 (CH-Ar (Cbz)), 128.3 (CH-Ar (Phe[4-CH_2NHCbz])), 128.2 (CH-Ar (Cbz)), 127.9 (CH-Ar (Cbz)), 65.9 (CH_2 (Cbz)), 52.1 (d, *J* = 15.4 Hz, CH_2SO_2F), 47.7

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(αCHCH$_2$Phe), 43.9 (CH$_2$NHCbz), 37.5 (αCHCH$_2$Phe). ^{19}F NMR (471 MHz, DMSO-d_6, 298 K): δ_F (ppm) = 60.6 (s, J = 5.6 Hz, SO$_2$F). HRMS (ESI positive) calcd for $C_{18}H_{21}N_2O_4SFNa$ [M + Na]$^+$ 403.1098, found 403.1091.

Cbz-Arg(Mtr)-ψ[CH$_2$O]-H (13). Sodium borohydride (1.81 g, 47.9 mmol, 2.50 equiv) was added to a mixture of N_α-Z-N_ω-(4-methoxy-2,3,6-trimethylbenzenesulfonyl)-L-arginine methyl ester **12** (10.2 g, 19.2 mmol, 1.00 equiv) and LiCl (2.03 g, 47.9 mmol, 2.50 equiv) in dry THF (40 mL) at rt and was stirred for 15 min. EtOH (55 mL) was added carefully and the resulting cloudy mixture stirred for 5 h (TLC monitored). The reaction mixture was cooled to 0 °C and quenched with saturated NH$_4$Cl (45 mL) and H$_2$O (13 mL). The mixture was extracted with EtOAc (3 × 100 mL), and the combined organic phases were dried over MgSO$_4$. The solvent was removed in vacuo and the crude product purified via column chromatography (EtOAc). The pure product was obtained as a white solid (9.19 g, 18.1 mmol, 95%). ^{1}H NMR (400 MHz, CDCl$_3$, 298 K): δ_H (ppm) = 7.29−7.14 (m, 5H, Ar-H (Cbz)), 6.42 (s, 1H, Ar-H (Mtr)), 6.26−6.03 (m, 3H, 3 × NH (guanidine)), 5.49 (d, J = 8.5 Hz, 1H, HNαCH), 4.96 (s, 2H, OCH$_2$Ph), 3.73 (s, 3H, OCH$_3$ (Mtr)), 3.61−3.41 (m, 3H, HNαCH, CH$_2$OH), 3.21 (br s, 1H, CH$_2$OH), 3.15−3.04 (m, 2H, NHCH$_2$), 2.57 (s, 3H, Ar-CH$_3$ (Mtr)), 2.50 (s, 3H, Ar-CH$_3$ (Mtr)), 2.03 (s, 3H, Ar-CH$_3$ (Mtr)), 1.52−1.32 (m, 4H, αCHCH$_2$CH$_2$). ^{13}C NMR (101 MHz, CDCl$_3$, 298 K): δ_C(ppm) = 158.5 (C═O), 157.0 (C-guanidine), 156.4 (C-Ar), 138.5 (C-Ar), 136.4 (C-Ar (Cbz)), 133.3 (C-Ar), 128.5, 128.1, 128.0 (CH-Ar (Cbz)), 124.9 (C-Ar), 111.8 (CH-Ar (Mtr)), 66.8 (CH$_2$ (Cbz)), 64.7 (CH$_2$OH), 55.4 (OCH$_3$), 41.0 (NHCH$_2$), 28.5 (αCHCH$_2$CH$_2$), 25.6 (αCHCH$_2$CH$_2$), 24.1 CH$_3$ (Mtr)), 18.3 CH$_3$ (Mtr)), 11.9 (CH$_3$ (Mtr)). HRMS (ESI positive) calcd for $C_{24}H_{34}N_4O_3S$ [M + Na]$^+$ 529.2091, found 529.2073.

Cbz-Arg(Mtr)-ψ[CH$_2$O]-Ms (14). Methanesulfonyl chloride (1.72 mL, 22.2 mmol, 1.30 equiv) was added dropwise to a solution of Cbz-Arg(Mtr)-ψ[CH$_2$O]-H (**13**) (8.64 g, 17.1 mmol, 1.00 equiv) in dry CH$_2$Cl$_2$ (200 mL) at 0 °C. NEt$_3$ (3.1 mL, 22.2 mmol, 1.30 equiv) was added dropwise, and the reaction mixture was stirred for 2 h at rt (TLC monitored). After the reaction was finished, the organic phase was washed with an aqueous 1 M KHSO$_4$ solution (250 mL), H$_2$O (250 mL), and brine (250 mL). The organic layer was dried over MgSO$_4$, the solvent was removed in vacuo and the crude product obtained as a white solid (9.36 g, 16.0 mmol, 94%). ^{1}H NMR (400 MHz, CDCl$_3$, 298 K): δ_H (ppm) = 7.31−7.20 (m, 5H, Ar-H (Cbz)), 6.44 (s, 1H, Ar-H (Mtr)), 6.11 (br s, 2H, 2 × NH (guanidine)), 5.99 (s, 1H, NH (guanidine)), 5.46 (d, J = 7.7 Hz, 1H, HNαCH), 5.01 (d, J = 12.2 Hz, 1H, OCH$_a$Ph), 4.95 (d, J = 12.2 Hz, 1H, OCH$_b$Ph), 4.11 (dd, J = 10.3, 4.3 Hz, 1H, CH$_a$OSO$_2$CH$_3$), 4.05 (dd, J = 9.9, 4.3 Hz, 1H, CH$_b$OSO$_2$CH$_3$), 3.85−3.76 (m, 1H, HNαCH), 3.74 (s, 3H, OCH$_3$ (Mtr)), 3.17−3.05 (m, 2H, HNCH$_2$), 2.86 (s, 3H, SO$_2$CH$_3$), 2.57 (s, 3H, Ar-CH$_3$(Mtr)), 2.50 (s, 3H, Ar-CH$_3$(Mtr)), 2.04 (s, 3H, Ar-CH$_3$ (Mtr)), 1.57−1.37 (m, 4H, αCHCH$_2$CH$_2$). ^{13}C NMR (101 MHz, CDCl$_3$, 298 K): δ_C (ppm) = 158.6 (C═O), 156.4 (C-guanidino), 156.3 (C-Ar), 138.5 (C-Ar), 136.6 (C-Ar), 136.3 (C-Ar), 128.5, 128.2, 128.0 (CH-Ar (Cbz)), 124.9 (C-Ar), 111.8 (CH-Ar (Mtr)), 71.1 (CH$_2$OSO$_2$CH$_3$), 66.9 (OCH$_2$(Cbz)), 55.5 (OCH$_3$), 50.1 (αCH), 40.7 (NHCH$_2$), 37.2 (SO$_2$CH$_3$), 28.1 (αCHCH$_2$CH$_2$), 25.5 (αCHCH$_2$CH$_2$), 24.1 (CH$_3$ (Mtr)), 18.3 (CH$_3$ (Mtr)), 12.0 (CH$_3$ (Mtr)). HRMS (ESI positive) calcd for $C_{25}H_{36}N_4O_8S_2Na$ [M + Na]$^+$ 607.1867, found 607.1853

Cbz-Arg(Mtr)-ψ[CH$_2$S]-Ac (15). Thioacetic acid (2.27 mL, 31.7 mmol, 2.00 equiv) was added to a suspension of Cs$_2$CO$_3$ (5.17 g, 15.9 mmol, 1.00 equiv) in DMF (10 mL) under argon atmosphere. Most of the Cs$_2$CO$_3$ was dissolved when added to a solution of Cbz-Arg(Mtr)-ψ[CH$_2$O]-Ms (**14**) (9.28 g, 15.9 mmol, 1.00 equiv) in DMF (38 mL) under argon atmosphere. The reaction mixture was stirred overnight at rt with the flask covered in aluminum foil. EtOAc (160 mL) and H$_2$O (160 mL) were added to the reaction mixture and the organic layer was washed with aq solution of 1 M KHSO$_4$ (160 mL), aq solution of 1 M NaHCO$_3$ (160 mL), brine (160 mL) and dried over MgSO$_4$. The solvent was removed in vacuo and the crude product purified via column chromatography (EtOAc/petroleum ether v/v 8:2 → EtOAc). The pure product was obtained as a yellowish solid (6.85 g, 12.1 mmol, 76%). ^{1}H NMR (400 MHz, CDCl$_3$, 298 K): δ_H (ppm) = 7.30−7.15 (m, 5H, Ar-H (Cbz)), 6.43 (s, 1H, Ar-H (Mtr)), 6.27−5.97 (m, 3H, 3 × NH (guanidine)), 5.15 (d, J = 9.2 Hz, 1H, HNαCH), 5.00 (d, J = 12.4 Hz, 1H, OCH$_a$Ph), 4.92 (d, J = 12.4 Hz, 1H, OCH$_b$Ph), 3.72 (s, 3H, OCH$_3$ (Mtr)), 3.68−3.57 (m, 1H, HNαCH), 3.13−2.99 (m, 2H, HNCH$_2$), 2.89 (dd, J = 14.0, 4.7 Hz, 1H, CH$_a$SC(O)CH$_3$), 2.79 (dd, J = 14.0, 7.9 Hz, 1H, CH$_b$SC(O)CH$_3$), 2.58 (s, 3H, Ar-CH$_3$ (Mtr)), 2.51 (s, 3H, Ar-CH$_3$ (Mtr)), 2.19 (s, 3H, SC(O)CH$_3$), 2.03 (s, 3H, Ar-CH$_3$ (Mtr)), 1.51−1.28 (m, 4H, αCHCH$_2$CH$_2$). ^{13}C NMR (101 MHz, CDCl$_3$, 298 K): δ_C (ppm) = 196.1 (SC(O)CH$_3$), 158.4 (C═O), 156.6 (C-guanidino), 156.3 (C-Ar), 138.5 (C-Ar), 136.5 (C-Ar), 136.4 (C-Ar), 133.6 (C-Ar), 128.5, 128.1, 127.8 (CH-Ar (Cbz)), 124.8 (C-Ar), 111.7 (CH-Ar (Mtr)), 66.7 (OCH$_2$ (Cbz)), 55.4 (OCH$_3$), 51.1 (CH$_2$S), 40.8 (NHCH$_2$), 33.8 (NHαCH), 31.8 (αCHCH$_2$CH$_2$), 30.5 (SC(O)CH$_3$), 25.7 (αCHCH$_2$CH$_2$), 24.1 (CH$_3$ (Mtr)), 18.3 (CH$_3$ (Mtr)), 12.0 (CH$_3$ (Mtr)). HRMS (ESI negative) calcd for $C_{26}H_{36}N_4O_6S_2$ [M − H]$^-$ 563.2003, found 563.1980.

Cbz-Arg(Mtr)-ψ[CH$_2$SO$_2$]-F (17). Cbz-Arg(Mtr)-ψ[CH$_2$S]-Ac (**15**) (6.63 g, 11.7 mmol, 1.00 equiv) was dissolved in AcOH (40 mL), and 30% H$_2$O$_2$ aq solution (13.5 mL) was added. The reaction mixture was stirred for 48 h, and additional H$_2$O$_2$ aq solution (3.5 mL) was added. NaOAc (963 mg, 11.7 mmol, 1.00 equiv) was added and the mixture stirred for 1 h. DMF (20 mL) was added, and the solution was concentrated in vacuo until a quarter of the volume. This procedure was repeated 3 more times and finally DMF was removed completely. After coevaporation with H$_2$O (2 × 100 mL) the crude product was lyophilized and **16** obtained as a white solid (6.82 g). The crude sodium salt **16** (600 mg, 1.01 mmol, 1.00 equiv) was dissolved in dry CH$_2$Cl$_2$ (25 mL) under argon atmosphere, and XtalFluor-M (442 mg, 1.82 mmol, 1.80 equiv) and 3HF·NEt$_3$ (7.1 μL, 43.6 μmol, 0.04 equiv) were added. The reaction mixture was heated to 40 °C and stirred overnight. Silica was added to quench the reaction and the solvent removed in vacuo. Column chromatography (EtOAc) yielded the pure product as a white solid (320 mg, 559 μmol, 55% over two steps). ^{1}H NMR (400 MHz, CDCl$_3$, 298 K): δ_H (ppm) = 7.37−7.24 (m, 5H, Ar-H (Cbz)), 6.51 (s, 1H, Ar-H (Mtr)), 6.36−6.04 (m, 3H, 3 × NH (guanidine)), 5.89 (br s, 1H, HNαCH), 5.13−4.99 (m, 2H, CH$_2$ (Cbz)), 4.20−4.00 (m, 1H, HNαCH), 3.80 (s, 3H, OCH$_3$ (Mtr)), 3.65 (dd, J = 15.2, 6.9 Hz, 1H, CH$_a$SO$_2$F), 3.52−3.39 (m, 1H, CH$_b$SO$_2$F), 3.15 (br s, 2H, HNCH$_2$), 2.63 (s, 3H, Ar-CH$_3$ (Mtr)), 2.56 (s, 3H, Ar-CH$_3$ (Mtr)), 2.10 (s, 3H, Ar-CH$_3$ (Mtr)), 1.79−1.38 (m, 4H, αCHCH$_2$CH$_2$). ^{13}C NMR (126 MHz, CDCl$_3$, 298 K): δ_C (ppm) = 158.7 (C═O), 156.3 (C-guanidino), 156.0 (C-Ar), 138.4 (C-Ar), 136.6 (C-Ar), 136.1 (C-Ar), 132.9 (C-Ar), 128.5 (CH-Ar (Cbz)), 128.2 (CH-Ar (Cbz)), 127.9 (CH-Ar (Cbz)), 125.0 (C-Ar), 111.8 (CH (Mtr)), 67.1 (CH$_2$ (Cbz)), 55.4 (OCH$_3$), 54.5 (d, $J_{C,F}$ = 13.2 Hz, CH$_2$SO$_2$F), 47.2 (αCH), 40.4 (αCHCH$_2$CH$_2$CH$_2$), 30.5 (αCHCH$_2$), 25.6 (αCHCH$_2$CH$_2$), 24.0 (CH$_3$ (Mtr)), 18.3 (CH$_3$ (Mtr)), 11.9 (CH$_3$ (Mtr)). ^{19}F NMR (377 MHz, CDCl$_3$, 298 K): δ_F (ppm) = 61.6 (s, 1F, SO$_2$F). HRMS (ESI positive) calcd for $C_{24}H_{33}FN_4O_7S_2$ [M + Na]$^+$ 595.1667, found 595.1670. $[\alpha]_D^{23}$ +1.29 (*c* 1.14, CDCl$_3$). IR (neat, cm^{-1}) = 1705, 1622, 1550, 1456 (SO$_2$), 1408, 1307, 1256, 1120, 731.

Boc-Phe(4-NHCbz)-OH (20). 4-Amino-(*N*-*tert*-butoxycarbonyl)-L-phenylalanine (9.95 g, 35.5 mmol, 1.00 equiv) was dissolved in a 1:1 mixture of H$_2$O/dioxane (200 mL). Benzyloxycarbonyl chloride (6.25 mL, 43.8 mmol, 1.23 equiv) was added and the pH adjusted to 8 by addition of sodium bicarbonate. The reaction mixture was stirred overnight. Dioxane was removed in vacuo, and the aqueous layer was washed with EtOAc (2 × 200 mL). The aqueous layer was acidified with aq solution of 2 M HCl and the pH adjusted to 1. The precipitate was extracted with EtOAc (3 × 200 mL), dried over MgSO$_4$, and filtered. The solvent was removed in vacuo, and the crude product purified by flash column chromatography (EtOAc/Hex v/v 1:1 + 1% AcOH). The pure product was obtained as a white solid (11.1 g, 26.8 mmol, 73%). ^{1}H NMR (400 MHz, CDCl$_3$, 298 K): δ_H (ppm) = 7.39−7.11 (m, 7H, Ar-H (Cbz), Ar-H (Phe)), 7.00 (d, J = 8.2 Hz, 2H, Ar-H (Phe)), 5.20−5.01 (m, 2H, CH$_2$ (Cbz)), 4.93 (d, J = 8.1 Hz, 1H, HNαCH), 4.61−4.45 (m, 1H, HNαCH), 3.10−2.96 (m, 2H,

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αCHCH$_2$), 1.33 (s, 9H, CH$_3$ (Boc)). ^{13}C NMR (126 MHz, CDCl$_3$, 298 K): δ_c (ppm) = 176.3 (CO$_2$H), 155.3 (C=O (Cbz)), 153.7 (C=O (Boc)), 136.9 (C-Ar), 136 (C-Ar), 130.8 (C-Ar), 130.1 (CH-Ar (Phe)), 128.6, 128.3, 128.3 (CH-Ar (Cbz)), 118.9 (CH-Ar (Phe)), 80.3 (C(CH$_3$)$_3$), 67.1 (CH$_2$ (Cbz)), 54.2 (αCH), 37.0 (αCHCH$_2$), 28.3 (C(CH$_3$)$_3$). HRMS (ESI positive) calcd for $C_{22}H_{26}N_2O_6Na$ [M + Na]$^+$ 437.1683, found 437.1666.

N$_3$-Phe(4-NHCbz)-OH (21). Boc-Phe(4-NHCbz)-OH (**20**) (11.1 g, 26.8 mmol, 1.00 equiv) was dissolved in CH_2Cl_2 (250 mL), and TFA (250 mL) was added and the reaction mixture stirred for 1 h at rt. The solvents were removed under reduced pressure, and the residue coevaporated with toluene. The crude salt was obtained as a white solid. The crude TFA salt (11.5 g, 26.8 mmol, 1.00 equiv), N_3SO_2Im·HCl (6.74 g, 32.2 mmol, 1.20 equiv), $CuSO_4$·5H$_2$O (335 mg, 1.34 mmol, 0.05 equiv), and K_2CO_3 (9.26 g, 67.0 mmol, 2.50 equiv) were dissolved in MeOH (88 mL) and stirred at rt. After 18 h more N_3SO_2Im·HCl (5.62 g, 26.8 mmol, 1.00 equiv) was added, and the reaction mixture was stirred for 2 days. The solvent was removed in vacuo and dissolved in H$_2$O (350 mL), and the aqueous mixture was acidified with aq solution of 2 M HCl. The precipitate was extracted with EtOAc (3 × 200 mL), and the combined organic layers were dried over $MgSO_4$ and filtered. The solvent was removed in vacuo and the crude product purified by flash column chromatography (EtOAc/Hex v/v 7:3 + 1% AcOH) The pure product was obtained as a clear colorless oil (6.96 g, 20.5 mmol, 76%). ^{1}H NMR (400 MHz, CDCl$_3$, 298 K): δ_H (ppm) = 9.93 (s, 1H, CO$_2$H), 7.33−7.21 (m, 7H, CH (Phe), Ar-H (Cbz)), 7.13−7.05 (m, 2H, CH (Phe)), 6.86 (s, 1H, NH) 5.11 (s, 2H, CH$_2$ (Cbz)), 4.04 (dd, J = 8.0, 5.4 Hz, 1H, N$_3$αCH), 3.07 (dd, J = 14.1, 5.4 Hz, 1H, αCHCH$_a$), 2.93 (dd, J = 14.1, 8.0 Hz, 1H, αCHCH$_b$). ^{13}C NMR (101 MHz, CDCl$_3$, 298 K): δ_c (ppm) = 174.9 (CO$_2$H), 137.0 (C-Ar$_t$), 135.8 (C-Ar), 130.0 (C-Ar), 128.7 (CH (Phe)), 128.4 (CH (Cbz)), 128.3 (CH (Cbz)), 128.2 (CH (Cbz)), 119.0 (CH (Phe)), 67.3 (CH$_2$ (Cbz)), 63.0 (αCH), 36.9 (αCHCH$_2$). HRMS (ESI negative) calcd for $C_{17}H_{15}N_4O_4$ [M − H]$^-$ 339.1099, found 339.1092.

N$_3$-Phe(4-NHCbz)-OMe (22). N$_3$-Phe(4-NHCbz)-OH (**21**) (6.96 g, 20.5 mmol, 1.00 equiv) was dissolved in MeOH (60 mL) under N$_2$ atmosphere and cooled to −20 °C. Thionyl chloride (1.6 mL, 22.1 mmol, 1.05 equiv) was added, and the mixture was allowed to warm up to rt and stirred overnight. The solvent was removed under reduced pressure and the residue coevaporated with $CHCl_3$ (3 × 90 mL). The product was obtained as a yellow oil (7.26 g, 20.5 mmol, 100%). ^{1}H NMR (400 MHz, CDCl$_3$, 298 K): δ_H (ppm) = 7.44−7.30 (m, 7H, Ar-H (Cbz), Ar-H (Phe)), 7.16 (d, J = 8.5 Hz, 2H, Ar-H (Phe)), 6.74 (s, 1H, NH), 5.19 (s, 2H, CH$_2$ (Cbz)), 4.04 (dd, J = 8.6, 5.5 Hz, 1H, N$_3$αCH), 3.76 (s, 3H, CH$_3$), 3.12 (dd, J = 14.0, 5.5 Hz, 1H, αCHCH$_a$), 2.96 (dd, J = 14.0, 8.6 Hz, 1H, αCHCH$_b$). ^{13}C NMR (101 MHz, CDCl$_3$, 298 K): δ_c (ppm) = 170.4 (CO$_2$CH$_3$), 153.3 (C=O (Cbz)), 137.0 (C-Ar), 136.0 (C-Ar), 130.9 (C-Ar), 129.9 (CH-Ar (Phe)), 128.6 (CH-Ar (Cbz)), 128.4 (CH-Ar (Cbz)), 128.3 (CH-Ar (Cbz)), 118.9 (CH-Ar (Phe)), 67.1 (CH$_2$ (Cbz)), 63.3 (αCH), 52.7 (CH$_3$), 37.0 (αCHCH$_2$). HRMS (ESI positive) calcd for $C_{18}H_{18}N_4O_4Na$ [M + Na]$^+$ 377.1220, found 377.1206.

N$_3$-Phe(4-CH$_2$NHCbz)-OH (26). Boc-Phe(4-CH$_2$NHCbz)-OH (**25**) (4.93 g, 11.5 mmol, 1.00 equiv) was dissolved in CH_2Cl_2 (60 mL), and TFA (22 mL) was added and the reaction mixture stirred for 45 min at rt. The solvents were removed in vacuo, and the residue was coevaporated with toluene (3 × 100 mL) and $CHCl_3$ (3 × 100 mL). The crude was dissolved in MeOH (38 mL), and $CuSO_4$·5H$_2$O (144 mg, 0.58 mmol, 0.05 equiv), K_2CO_3 (3.98 g, 28.8 mmol, 2.50 equiv), and N_3SO_2Im·HCl (2.89 g, 13.8 mmol, 1.20 equiv) were added. The reaction mixture was stirred overnight at rt and the solvent removed in vacuo. H$_2$O (200 mL) was added and the pH adjusted to 1 with aq solution of 1 M HCl. The aqueous layer was extracted with EtOAc (3 × 200 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and the solvent was removed in vacuo. The crude was purified via column chromatography (EA/petroleum ether v/v 1:1 + 1% AcOH) and the pure product was obtained as a yellowish oil (3.78 g, 10.7 mmol, 93%). ^{1}H NMR (500 MHz, CDCl$_3$, 298 K): δ_H (ppm) = 7.33−7.19 (m, 5H, Ar-H (Cbz)), 7.18−7.07 (m, 4H, Phe), 5.09 (br s, 1H, CH$_2$NHCbz), 5.06 (s, 1H, CH$_2$ (Cbz)), 4.28 (d, J = 5.9 Hz, 2H, CH$_2$NHCbz), 4.06 (dd, J = 8.5, 5.2 Hz, 1H, N$_3$αCH), 3.12 (dd, J = 14.1, 5.2 Hz, 1H, N$_3$αCHCH$_a$), 2.94 (dd, J = 14.1, 8.5 Hz, 1H, N$_3$αCHCH$_b$). ^{13}C NMR (126 MHz, CDCl$_3$, 298 K): δ_c (ppm) = 173.8 (C(O)OH), 156.7 (C=O), 137.4 (CH-Ar), 136.3 (CH-Ar), 135.1 (CH-Ar), 129.6 (CH-Ar (Phe)), 128.6 (CH-Ar (Cbz)), 128.3 (CH-Ar (Cbz)), 128.2 (CH-Ar (Cbz)), 127.9 (CH-Ar (Phe)), 67.1 (OCH$_2$Ph), 63.0 (αCH), 44.8 (CH$_2$NHCbz), 37.1 (N$_3$αCHCH$_2$). HRMS (ESI positive) calcd for $C_{18}H_{18}N_4O_4Na$ [M + Na]$^+$ 377.1220, found 377.1212

N$_3$-Phe(4-CH$_2$NHCbz)-OMe (27). N$_3$-Phe(4-CH$_2$NHCbz)-OH (**26**) (3.78 g, 10.7 mmol, 1.00 equiv) was dissolved in MeOH (50 mL) and cooled to −20 °C under nitrogen atmosphere. Thionyl chloride (813 μL, 11.2 mmol, 1.05 equiv) was added dropwise, and the reaction mixture was allowed to warm up to rt and stirred overnight. The solvent was removed in vacuo and coevaporated with $CHCl_3$ (3 × 100 mL). The crude product was obtained as a white oil (3.92 g, 10.6 mmol, 99%). ^{1}H NMR (400 MHz, CDCl$_3$, 298 K): δ_H (ppm) = 7.41−7.28 (m, 5H, Ar-H (Cbz)), 7.25 (d, J = 8.3 Hz, 2H, 2 × CH (Phe)), 7.19 (d, J = 8.0 Hz, 2H, 2 × CH (Phe)), 5.14 (s, 2H, CH$_2$ (Cbz)), 5.08 (br s, 1H, CH$_2$NHCbz), 4.37 (d, J = 6.0 Hz, 2H, CH$_2$NHCbz), 4.06 (dd, J = 8.8, 5.3 Hz, 1H, N$_3$αCH), 3.77 (s, 1H, OCH$_3$), 3.15 (dd, J = 14.0, 5.3 Hz, 1H, N$_3$αCHCH$_a$), 2.98 (dd, J = 14.0, 8.8 Hz, 1H N$_3$αCHCH$_b$). ^{13}C NMR (101 MHz, CDCl$_3$, 298 K): δ_c (ppm) = 170.3 (C(O)OMe), 156.4 (C=O), 137.5 (C-Ar), 136.5 (C-Ar), 135.2 (C-Ar), 129.5 (CH-Ar (Phe)), 128.5 (CH-Ar (Cbz)), 128.2 (CH-Ar (Cbz)), 128.2 (CH-Ar (Cbz)), 127.9 (CH-Ar (Phe)), 66.9 (OCH$_2$Ph), 63.2 (αCH), 52.7 (OCH$_3$), 44.8 (CH$_2$NHCbz), 37.2 (N$_3$αCHCH$_2$). HRMS (ESI positive) calcd for $C_{19}H_{20}N_4O_4Na$ [M + Na]$^+$ 391.1377, found 391.1368. IR (neat, cm^{-1}) = 2110 (N$_3$), 1742, 1720, 1516, 1244, 1043.

TFA.Cbz-Leu$_3$-Arg-ψ[CH$_2$SO$_2$]-F (53). Cbz-Leu$_3$-OH[20] (78.3 mg, 159 μmol, 1.10 equiv) was dissolved in DMF (1.5 mL), and HBTU (60.4 mg, 159 μmol, 1.10 equiv), Oxymapure (22.6 mg, 159 μmol, 1.10 equiv), and DiPEA (25.2 μL, 145 μmol, 1.00 equiv) were added, and the reaction mixture was stirred for 5 min. Next, a solution of 2HCl·H-Arg-[CH$_2$SO$_2$]-F (**7**) (65.8 mg, 145 μmol, 1.00 equiv) in DMF (2 mL) was added to the reaction mixture followed by DiPEA (37.9 μL, 217 mmol, 1.50 equiv) and stirring was continued for 4 h. The solvent was removed in vacuo and the crude product purified by semipreparative HPLC (0 to 100% B, C$_{P1}$). Fractions containing the product were pooled, lyophilized, and the pure product was obtained as a white solid (44.5 mg, 54.7 μmol, 37%). ^{1}H NMR (500 MHz, DMSO-d_6, 298 K): δ_H (ppm) = 8.14 (d, J = 8.6 Hz, 1H, NHαCH (Arg)), 7.98 (d, J = 8.1 Hz, 1H, NHαCH (Leu)), 7.82 (d, J = 8.0 Hz, 1H, NHαCH (Leu)), 7.57 (br s, 1H, NHCH$_2$ (Arg)), 7.44 (d, J = 8.1 Hz, 1H, NHαCH, (Leu)), 7.41−7.25 (m, 5H, Ar-H (Cbz)), 5.02 (s, 2H, CH$_2$ (Cbz)), 4.36−4.18 (m, 3H, 2 × NHαCH (Leu), NHαCH (Arg)), 4.15 (ddd, J = 15.0, 7.1, 3.4 Hz, 1H, CH$_a$SO$_2$F), 4.09−4.01 (m, 1H, NHαCH (Leu)), 3.93 (dd, J = 15.0, 9.2 Hz, 1H, CH$_b$SO$_2$F), 3.15−2.99 (m, 2H, NHCH$_2$ (Arg)), 1.70−1.54 (m, 3H, CH(CH$_3$)$_2$), 1.54−1.30 (m, 10H, 3 × CH$_2$ (Leu), NHCH$_2$CH$_2$CH$_2$ (Arg)), 0.95−0.73 (m, 18H, 6 × CH$_3$ (Leu)). ^{13}C NMR (126 MHz, DMSO-d_6, 298 K): δ_c (ppm) = 171.7 (C=O), 171.1 (C=O), 171.0 (C=O), 156.1 (C (guanidine)), 155.3 (C=O (Cbz)), 136.4 (C$_{quart}$ (Phe)), 127.7 (CH (Phe)), 127.2 (CH (Phe)), 127.0 (CH (Phe)), 64.8 (CH2 (Cbz)), 53.2 (d, J = 11.3 Hz, CH$_2$SO$_2$F), 52.5(CbzNHαCH), 50.5 (NHαCH), 50.3 (NHαCH), 43.6 (αCH (Arg)), 40.0 (CH$_2$ (Leu)), 39.9 (CH$_2$ (Leu)), 39.8 (CH$_2$ (Leu)), 39.7 (NHCH$_2$ (Arg)), 30.1 (αCHCH$_2$ (Arg)), 24.0 (αCHCH$_2$CH$_2$ (Arg)), 23.6 (CH (Leu)), 23.5 (CH (Leu)), 23.4 (CH (Leu)), 22.44 (CH$_3$), 22.42 (CH$_3$), 22.38 (CH$_3$), 21.0 (CH$_3$), 20.94 (CH$_3$), 20.88 (CH$_3$). ^{19}F NMR (471 MHz, DMSO-d_6, 298 K): δ_F (ppm) = 59.8 (s, 1F, SO$_2$F). HRMS (ESI positive) calcd for $C_{32}H_{55}N_7O_7SF$ [M + H]$^+$ 700.3862, found 700.3841. t_R (0 to 100% B, 30 min, C$_{A1}$) = 23.0 min.

TFA·Cbz-Phe-Leu$_2$-Arg-ψ[CH$_2$SO$_2$]-F (54). Cbz-Phe-Leu$_2$-OH (see Supporting Information) (112 mg, 213 μmol, 1.10 equiv) was dissolved in DMF (1.5 mL), and HBTU (80.7 mg, 213 μmol, 1.10 equiv), Oxyma (30.3 mg, 213 μmol, 1.10 equiv), and DiPEA (33.7 μL, 194 μmol, 1.00 equiv) were added and stirred for 5 min. Next, a

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solution of 2HCl·H-Arg-ψ[CH_2SO_2]-F (**7**) (112 mg, 194 μmol, 1.00 equiv) in DMF (2.5 mL) was added to reaction mixture followed by DiPEA (50.6 μL, 290 mmol, 1.50 equiv), and stirring was continued for 6 h. The solvent was removed in vacuo, and the crude product was purified by semipreparative HPLC (0 to 100% B, C_{P1}). Fractions containing the product were pooled, lyophilized, and the pure product was obtained as a white solid (46.6 mg, 54.9 μmol, 28%). ^{1}H NMR (500 MHz, DMSO-d_6, 298 K): δ_H (ppm) = 8.16 (d, J = 8.6 Hz, 1H, NHαCH), 8.10 (d, J = 8.1 Hz, 1H, NHαCH), 7.94 (d, J = 8.1 Hz, 1H, NHαCH), 7.62−7.56 (m, 1H, NHCH$_2$ (Arg)), 7.49 (d, J = 8.5 Hz, 1H, NHαCH (Phe)), 7.37−7.15 (m, 10H, Ar-H (Phe, Cbz)), 4.94 (s, 2H, CH$_2$ (Cbz)), 4.37−4.20 (m, 4H, 2 × NHαCH (Leu), NHαCH (Arg), NHαCH (Phe)), 4.15 (ddd, J = 15.0, 6.9, 3.3 Hz, 1H, CH$_a$SO$_2$F), 3.93 (dd, J = 15.0, 9.1 Hz, 1H, CH$_b$SO$_2$F), 3.14−3.02 (m, 2H, NHCH$_2$ (Arg)), 2.98 (dd, J = 13.9, 3.8 Hz, 1H, CH$_a$ (Phe)), 2.73 (dd, J = 13.9, 10.7 Hz, 1H, CH$_b$ (Phe)), 1.66−1.56 (m, 2H, 2 × CH(CH$_3$)$_2$), 1.56−1.37 (m, 8H, 2 × CH$_2$ (Leu), NHCHCH$_2$CH$_2$ (Arg)), 0.92−0.80 (m, 12H, 4 × CH$_3$ (Leu)). ^{13}C NMR (126 MHz, DMSO-d_6, 298 K): δ_c (ppm) = 172.19 (C═O), 172.17 (C═O), 172.0 (C═O), 157.2 (C (guanidine)), 156.3 (C═O (Cbz)), 138.5 (C-Ar), 137.4 (C-Ar), 129.6 (CH-Ar), 128.7 (CH-Ar), 128.5 (CH-Ar), 128.1 (CH-Ar), 127.9 (CH-Ar), 126.7 (CH-Ar), 65.7 (CH$_2$ (Cbz)), 56.5 (CbzNHαCH), 54.3 (d, J = 11.2 Hz, CH$_2$SO$_2$F), 51.6 (αCH (Leu)), 51.57 (αCH (Leu)), 44.6 (αCH (Arg)), 41.1 (CH$_2$ (Leu)), 40.9 (CH$_2$ (Leu)), 40.8 (CH$_2$NH (Arg)), 37.7 (CH$_2$ (Phe)), 31.2 (αCHCH$_2$ (Arg)), 25.1 (αCHCH$_2$CH$_2$ (Arg)), 24.6 (CH (Leu)), 24.5 (CH (Leu)), 23.50 (CH$_3$ (Leu)), 23.48 (CH$_3$ (Leu)), 22.2 (CH$_3$ (Leu)), 22.0 (CH$_3$ (Leu)). ^{19}F NMR (471 MHz, DMSO-d_6, 298 K): δ_F (ppm) = 59.8 (s, 1F, SO$_2$F). HRMS (ESI positive) calcd for $C_{35}H_{53}N_7O_7SF$ [M + H]$^+$ 734.3706, found 734.3673. t_R (0 to 100% B, 30 min, C_{A1}) = 23.2 min

TFA·Boc-Phe-Leu$_2$-Arg-ψ[CH$_2$SO$_2$]-F (55). Boc-Phe-Leu$_2$-OH (99.8 mg, 203 μmol, 1.10 equiv) was dissolved in DMF (2 mL) under nitrogen atmosphere, and HBTU (77.0 mg, 203 μmol, 1.10 equiv), Oxyma (29 mg, 203 μmol, 1.10 equiv), and DiPEA (32.0 μL, 184 μmol, 1.00 equiv) were added and stirred for 5 min. Next, a solution of 2HCl·H-Arg-[CH_2SO_2]-F (**7**) (55.0 mg, 184 μmol, 1.00 equiv) in DMF (2 mL) was added to reaction mixture followed by DiPEA (48.0 μL, 276 mmol, 1.50 equiv) and stirring was continued for 2 h. The solvent was removed in vacuo, and the crude product was purified by semipreparative HPLC (0 to 100% B, C_{P1}). Fractions containing the product were pooled, lyophilized, and the pure product was obtained as a white solid (58.5 mg, 71.9 μmol, 39%). ^{1}H NMR (400 MHz, DMSO-d_6, 298 K): δ_H (ppm) = 8.17 (d, J = 8.6 Hz, 1H, NH (Leu)), 7.99−7.92 (m, 2H, NH (Leu), BocNH), 7.54 (t, J = 5.8 Hz, 1H, NHCH$_2$ (Arg)), 7.34−7.16 (m, 5H, Ar-H (Phe)), 6.96 (d, J = 8.5 Hz, 1H, NHαCH (Arg)), 4.39−4.10 (m, 5H, BocNHαCH, 2 × NHαCH (Leu), NHαCH (Arg), CH$_a$SO$_2$F), 3.93 (ddd, J = 14.9, 9.2, 1.9 Hz, 1H, CH$_b$SO$_2$F), 3.14−3.00 (m, 2H, NHCH$_2$ (Arg)), 2.94 (dd, J = 13.8, 4.1 Hz, 1H, CH$_a$ (Phe)), 2.72 (dd, J = 13.8, 10.4 Hz, 1H, CH$_b$ (Phe)), 1.68−1.55 (m, 2H, 2 × CH(CH$_3$)$_2$), 1.54−1.34 (m, 8H, 2 × CH$_2$ (Leu), NHCH$_2$CH$_2$CH$_2$ (Arg)), 1.30 (s, 9H, C(CH$_3$)$_3$), 0.90−0.81 (m, 12H, 4 × CH$_3$ (Leu)). ^{13}C NMR (101 MHz, CDCl$_3$, 298 K): δ_c (ppm) = 172.19 (C═O), 172.16 (C═O), 172.02 (C═O), 157.1 (C_guanidino), 155.7 (C═O (Boc)), 138.6 (C-Ar), 129.6 (CH-Ar), 128.5 (CH-Ar), 126.6 (CH-Ar), 78.6 (C(CH$_3$)$_3$), 56.1 (BocNHC), 54.3 (d, J = 10.8 Hz, CSO$_2$F), 51.6 (αCH (Leu)), 51.4 (αCH (Leu)), 44.6 (αCH (Arg)), 41.3 (CH$_2$ (Leu)), 40.9 (CH$_2$ (Leu)), 40.8 (NHCH$_2$), 37.5 (CH$_2$ (Phe)), 31.2 (αCHCH$_2$ (Arg)), 28.6 (C(CH$_3$)$_3$), 25.1 (αCHCH$_2$CH$_2$ (Arg)), 24.5 (CH (Leu)), 24.45 (CH (Leu)), 23.6 (CH$_3$ (Leu)), 23.5 (CH$_3$ (Leu)), 22.1 (CH$_3$ (Leu)), 22.0 (CH$_3$ (Leu)). ^{19}F NMR (377 MHz, DMSO-d_6, 298 K): δ_F (ppm) = 59.8 (d, J = 7.2 Hz, 1F, SO$_2$F). HRMS (ESI positive) calcd for $C_{32}H_{55}N_7O_7SF$ [M + H]$^+$ 642.3444, found 642.3414. t_R (0 to 100% B, 30 min, C_{A1}) = 23.1 min.

TFA·Ac-Phe-Leu$_2$-Arg-ψ[CH$_2$SO$_2$]-F (56). Ac-Phe-Leu$_2$-OH (**47**) (88.0 mg, 203 μmol, 1.10 equiv) was dissolved in DMF (2 mL) under nitrogen atmosphere, and HBTU (77.0 mg, 203 μmol, 1.10 equiv), Oxymapure (29 mg, 203 μmol, 1.10 equiv), and DiPEA (32.0 μL, 184 μmol, 1.00 equiv) were added, and the mixture was stirred for 5 min. A solution of 2HCl·H$_2$N-Arg-ψ[CH_2SO_2]-F (**7**) (55.0 mg, 184 μmol, 1.00 equiv) in DMF (2 mL) was added to reaction mixture followed by DiPEA (48.0 μL, 276 mmol, 1.50 equiv), and stirring was continued for 2 h. The solvent was removed in vacuo and the crude product was purified by semipreparative HPLC (0 to 100% B, C_{P1}). Fractions containing the product were pooled, lyophilized, and the pure product was obtained as a white solid (44.6 mg, 59.0 μmol, 32%). ^{1}H NMR (500 MHz, DMSO-d_6, 298 K): δ_H (ppm) = 8.13 (d, J = 8.6 Hz, 1H, NHαCH (Arg)), 8.09 (d, J = 8.1 Hz, 1H, AcNH), 8.04 (d, J = 8.1 Hz, 1H, NH (Leu)), 7.88 (d, J = 8.0 Hz, 1H, NH (Leu)), 7.52 (t, J = 5.7 Hz, 1H, NHCH$_2$ (guanidine)), 7.29−7.15 (m, 5H, Ar-H (Phe)), 4.50 (ddd, J = 10.0, 8.1, 4.2 Hz, 1H, AcNHαCH), 4.33−4.19 (m, 3H, CHCH$_2$SO$_2$F, NHαCH (Leu^1), NHαCH (Leu^2)), 4.14 (ddd, J = 14.9, 6.8, 3.4 Hz, 1H, CH$_b$SO$_2$F), 3.93 (dd, J = 14.9, 9.2 Hz, 1H, CH$_b$SO$_2$F), 3.14−3.00 (m, 2H, NHCH$_2$ (guanidine)), 2.97 (dd, J = 14.0, 4.2 Hz, 1H, CH$_b$ (Phe)), 2.72 (dd, J = 14.0, 10.0 Hz, 1H, CH$_b$ (Phe)), 1.75 (s, 3H, CH$_3$C(O)), 1.66−1.37 (m, 10H, 2 × CH(CH$_3$)$_2$, 2 × CH$_2$ (Leu), NHCH$_2$CH$_2$CH$_2$ (Arg)), 0.89 (d, J = 2.6 Hz, 3H, CH$_3$), 0.87 (d, J = 2.7 Hz, 2H, CH$_3$), 0.84 (s, 3H, CH$_3$), 0.83 (s, 3H, CH$_3$). ^{13}C NMR (126 MHz, DMSO-d_6, 298 K): δ_c (ppm) = 172.2 (C═O), 172.1 (C═O), 171.8 (C═O), 169.8 (C═O), 157.1 (C-guanidine), 138.4 (C-Ar), 129.6, 128.5, 126.7 (CH-Ar), 54.4 (AcNHC), 54.3 (d, J = 10.2 H, CH$_2$SO$_2$F), 51.6 (αCH (Leu)), 51.5 (αCH (Leu)), 44.6 (αCH (Arg)), 41.0 (CH$_2$ (Leu)), 40.9 (CH$_2$ (Leu)), 40.8 (CH$_2$ (Arg)), 37.7 (CH$_2$-Phe), 31.2 (αCHCH$_2$ (Arg)), 25.1 (αCHCH$_2$CH$_2$ (Arg)), 24.6 (CH (Leu)), 24.5 (CH (Leu)), 23.5 (CH$_3$ (Ac)), 22.9 (2 × CH$_3$ (Leu)), 22.1 (CH$_3$ (Leu)), 22.0 (CH$_3$ (Leu)). ^{19}F NMR (471 MHz, DMSO-d_6, 298 K): δ_F (ppm) = 59.8 (s, 1 F, SO$_2$F). HRMS (ESI positive) calcd for $C_{29}H_{49}N_7O_6SF$ [M + H]$^+$ 642.3444, found 642.3414. t_R (0 to 100% B, 50 min, C_{A1}) = 27.0 min.

2TFA·H-Phe-Leu$_2$-Arg-ψ[CH$_2$SO$_2$]-F (57). Boc-Phe-Leu$_2$-Arg-ψ-[CH_2SO_2]-F (**55**) (49.7 mg, 61.1 μmol, 1.00 equiv) was dissolved in CH_2Cl_2 (5 mL), and TFA (5 mL) was added. The reaction mixture was stirred for 30 min at rt. The solvent was removed in vacuo and the crude purified by semipreparative HPLC (0 to 100% B, C_{P1}). Fractions containing the product were pooled, lyophilized, and the pure product was obtained as a white solid (31.2 mg, 37.7 μmol, 62%). ^{1}H NMR (500 MHz, DMSO-d_6, 298 K): δ_H (ppm) = 8.63 (d, J = 8.4 Hz, 1H, NH (Leu)), 8.27−8.22 (m, 2H, NH (Leu), NHαCH (Arg)), 8.13 (s, 3H, NH$_3^+$), 7.83−7.72 (m, 1H, NHCH$_2$ (Arg)), 7.33−7.23 (m, 5H, Ar-H (Phe)), 4.44−4.38 (m, 1H, NHαCH (Leu)), 4.32−4.23 (m, 2H, NHαCH (Leu), NHαCH (Arg)), 4.15 (ddd, J = 14.9, 6.9, 3.3 Hz, 1H, CH$_a$SO$_2$F), 4.12−4.04 (m, 1H, NH$_3^+$CH), 3.94 (dd, J = 14.9, 9.3 Hz, 1H, CH$_b$SO$_2$F), 3.15−3.00 (m, 3H, NHCH$_2$ (Arg), CH$_a$ (Phe)), 2.92 (dd, J = 14.2, 7.6 Hz, 1H, CH$_a$ (Phe)), 1.67−1.57 (m, 2H, 2 × CH(CH$_3$)$_2$), 1.57−1.39 (m, 8H, 2 × CH$_2$ (Leu), NHCH$_2$CH$_2$CH$_2$ (Arg)), 0.92−0.88 (m, 6H, 2 × CH$_3$ (Leu)), 0.87 (d, J = 4.3 Hz, 3H, CH$_3$ (Leu)), 0.86 (d, J = 4.3 Hz, 3H, CH$_3$ (Leu)). ^{13}C NMR (126 MHz, DMSO-d_6, 298 K): δ_c (ppm) = 171.1 (C═O), 170.6 (C═O), 167.0 (C═O), 156.2 (C-guanidino), 134.1 (C-Ar), 129.0 (CH-Ar), 127.8 (CH-Ar), 126.5 (CH-Ar), 53.2 (d, J = 10.8 Hz, CSO$_2$F), 52.5 (NH$_3^+$C), 50.6 (αCH (Leu)), 50.4 (αCH (Leu)), 43.6 (αCH (Arg)), 40.4 (CH$_2$ (Leu)), 39.9 (CH$_2$ (Leu)), 39.7 (CH$_2$NH (Arg)), 36.3 (CH$_2$ (Phe)), 30.2 (αCHCH$_2$ (Arg)), 24.0 (αCHCH$_2$CH$_2$ (Arg)), 23.5 (CH (Leu)), 23.4 (CH (Leu)), 22.5 (CH$_3$ (Leu)), 22.4 (CH$_3$ (Leu)), 21.1 (CH$_3$ (Leu)), 21.0 (CH$_3$ (Leu)). ^{19}F NMR (471 MHz, DMSO-d_6, 298 K): δ_F (ppm) = 59.8 (s, 1 F, SO$_2$F). HRMS (ESI positive) calcd for $C_{27}H_{47}N_7O_5SF$ [M + H]$^+$ 600.3338, found 600.3321. t_R (0 to 100% B, 30 min, C_{A1}) = 17.1 min.

TFA·N$_3$-Phe-Leu$_2$-Arg-ψ[CH$_2$SO$_2$]-F (58). N$_3$-Phe-Leu$_2$-OH (**52**) (81.5 mg, 195 μmol, 1.05 equiv) was dissolved in DMF (2 mL) under nitrogen atmosphere and cooled to 0 °C. HATU (74.2 mg, 195 μmol, 1.05 equiv) was added, and stirring was continued for 20 min. 2HCl·H-Arg-ψ[CH_2SO_2]-F (**7**) (55.6 mg, 186 μmol, 1.00 equiv) in DMF (2 mL) was added to the reaction mixture. After 30 min DiPEA (93.7 μL, 539 μmol, 2.90 equiv) was added and stirring was continued overnight at rt. The solvent was removed in vacuo, and the crude product was purified by semipreparative HPLC (0 to 70% B, C_{P3}). The obtained product (80 mg) required a second purification step by semi-preparative HPLC (0 to 80% B, C_{P3}). Fractions containing the product

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were pooled, lyophilized, and the pure product was obtained as a white solid (16.9 mg, 22.8 μmol, 12%). ^{1}H NMR (500 MHz, DMSO-d_6, 298 K): δ_H (ppm) = 8.35 (d, J = 8.2 Hz, 1H, NH (Leu)), 8.18 (d, J = 8.6 Hz, 1H, N$H\alpha$CH (Arg)), 8.10 (d, J = 8.1 Hz, 1H, NH (Leu)), 7.60 (t, J = 5.9 Hz, 1H, NHCH$_2$ (guanidine)), 7.33−7.22 (m, 5H, Ar-H (Phe)), 7.01 (br s, 3H), 4.38 (app q, J = 7.7 Hz, 1H, NHαCH (Leu)), 4.31−4.20 (m, 2H, CHCH$_2$SO$_2$F, NHαCH (Leu)), 4.15 (ddd, J = 14.0, 6.9, 3.4 Hz, 1H, CH$_a$SO$_2$F), 4.10 (dd, J = 9.1, 5.1 Hz, 1H, N$_3$CH), 3.93 (dd, J = 14.0, 9.5 Hz, 1H, CH$_b$SO$_2$F), 3.13−3.01 (m, 3H, NHCH_2 (guanidine), CH$_a$ (Phe)), 2.89 (dd, J = 14.1, 9.1 Hz, 1H, CH$_b$ (Phe)), 1.70−1.36 (m, 10H, 2 × CH(CH$_3$)$_2$, 2 × CH$_2$ (Leu), NHCH$_2$CH_2CH$_2$ (Arg)), 0.89 (d, J = 2.2 Hz, 3H, CH$_3$), 0.88 (d, J = 2.2 Hz, 3H, CH$_3$), 0.86 (d, J = 4.0 Hz, 3H, CH$_3$), 0.84 (d, J = 3.9 Hz, 3H, CH$_3$). ^{13}C NMR (126 MHz, DMSO-d_6, 298 K): δ_c (ppm) = 171.1 (C═O), 170.7 (C═O), 168.2 (C═O), 156.1 (C-(guanidine), 136.3 (C-Ar), 128.4, 127.8, 126.1 (CH-Ar), 61.7 (N$_3$C), 53.2 (d, J = 11.1 Hz, CH$_2$SO$_2$F), 50.6 (αCH (Leu)), 50.5 (αCH (Leu)), 43.6 (αCH (Arg)), 40.2 (CH$_2$ (Leu)), 39.8 (CH$_2$ (Leu)), 39.7 (NHCH$_2$ (Arg)), 36.2 (CH$_2$-Phe), 30.1 (αCH$\underline{C}$H$_2$ (Arg)), 24.0 (NHCH$_2\underline{C}$H$_2$ (Arg)), 23.5 (CH (Leu)), 23.5 (CH (Leu)), 22.4 (CH$_3$ (Leu)), 22.4 (CH$_3$ (Leu)), 21.0 (CH$_3$ (Leu)), 20.9 (CH$_3$ (Leu)). ^{19}F NMR (471 MHz, DMSO-d_6, 298 K): δ_F (ppm) = 59.8 (s, 1F, SO$_2$F). HRMS (ESI positive) calcd for $C_{27}H_{45}N_9O_5SF$ [M + H]$^+$ 626.3243, found 626.3216. t_R (0 to 100% B, 50 min, C$_{A3}$) = 33.6 min.

2TFA·H-Phe-Leu$_2$-Lys-ψ[CH$_2$SO$_2$]-F (59). BocHN-Phe-Leu$_2$-Lys-(Cbz)-ψ[CH$_2$SO$_2$]-F (31.8 mg, 34.6 μmol, 1.00 equiv) was dissolved in CH$_2$Cl$_2$ (5 mL), and a 33% HBr/AcOH solution (5 mL) was added and the reaction mixture stirred for 45 min at rt. The solvent was removed in vacuo and the crude product purified by semipreparative HPLC (0 to 50% B, C$_{P3}$). Fractions containing the product were pooled, lyophilized, and the pure product was obtained as a white solid (21.5 mg, 26.9 μmol, 78%). ^{1}H NMR (500 MHz, DMSO-d_6, 298 K): δ_H (ppm) = 8.66−8.60 (m, 1H, NH), 8.28 (d, J = 7.9 Hz, 1H, NH), 8.22−8.06 (m, 4H, NH, NH$_3^+$ (Phe)), 7.79 (br s, 3H, NH$_3^+$ (Lys)), 7.37−7.19 (m, 5H, CH-Ar), 4.42 (td, J = 8.7, 4.2 Hz, 1H, αCH (Leu)), 4.32−4.22 (m, 2H, αCH (Lys), αCH (Leu)), 4.14 (ddd, J = 15.0, 6.8, 3.3 Hz, 1H, CH$_a$SO$_2$F), 4.11−4.03 (m, 1H, αCH (Phe)), 3.90 (dd, J = 15.0, 9.1 Hz, 1H, CH$_b$SO$_2$F), 3.09 (dd, J = 14.2, 5.1 Hz, 1H, CH$_a$Phe), 2.92 (dd, J = 14.2, 7.7 Hz, 1H, CH$_b$Phe), 2.78−2.66 (m, 2H, CH_2NH$_3^+$), 1.69−1.38 (m, 10H, 2 × CH (Leu), CH$_2$ (Leu), CH_2 CH$_2$CH_2CH$_2$NH$_3^+$ (Lys)), 1.38−1.21 (m, 2H, CH_2CH$_2$CH$_2$NH$_3^+$ (Lys)), 0.96−0.79 (m, 12H, 4 × CH$_3$ (Leu)). ^{13}C NMR (126 MHz, DMSO-d_6, 298 K): δ_C (ppm) = 172.0, 171.7, 168.0 (C═O), 135.2 (C-Ar), 130.0, 128.9, 127.6 (CH-Ar), 54.4 (d, J = 10.9 Hz, CSO$_2$F), 53.5 (αCH (Phe)), 51.6, 51.4 (αCH (Leu)), 44.5 (αCH (Lys)), 40.9, 40.8 (CH$_2$ (Leu)), 39.0 (CH$_2$NH$_3^+$), 37.4 (CH$_2$Phe), 33.4 ($\underline{C}$H$_2$CH$_2$CH$_2$CH$_2$NH$_3^+$ (Lys)), 26.8 ($\underline{C}$H$_2$CH$_2$NH$_3^+$ (Lys)), 24.5, 24.4 (CH (Leu)), 23.6, 23.5 (CH$_3$ (Leu)), 22.1 ($\underline{C}$H$_2$CH$_2$CH$_2$NH$_3^+$ (Lys)), 22.1, 22.0 (CH$_3$ (Leu)). ^{19}F NMR (471 MHz, DMSO-d_6, 298 K): δ_F (ppm) = 59.6 (s, 1F, SO$_2$F). HRMS (ESI positive) calcd for $C_{27}H_{47}N_5O_5SF$ [M + H]$^+$ 572.3276, found 572.3263. t_R (0 to 100% B, 50 min, C$_{A3}$) = 24.6 min.

TFA·Ac-Phe-Leu$_2$-Lys-ψ[CH$_2$SO$_2$]-F (60). Ac-Phe-Leu$_2$-Lys-(Cbz)-ψ[CH$_2$SO$_2$]-F (20.0 mg, 23.2 μmol, 1.00 equiv) was dissolved in CH$_2$Cl$_2$ (5 mL), and 33% HBr/AcOH solution (5 mL) was added, and the reaction mixture was stirred for 1 h at rt. The solvents were removed in vacuo, and the crude was purified by semipreparative HPLC (0 to 90% B, C$_{P3}$). Fractions containing the product were pooled, lyophilized, and the pure product was obtained as a white solid (11.1 mg, 15.2 μmol, 66%). ^{1}H NMR (400 MHz, DMSO-d_6, 298 K): δ_H (ppm) = 8.16−8.01 (m, 3H, 2 × N$H\alpha$CH, N$H\alpha$CH (Lys)), 7.93 (d, J = 8.1 Hz, 1H, N$H\alpha$CH), 7.72 (br s, 3H, NH$_3^+$), 7.30−7.15 (m, 5H, Ar-H (Phe)), 4.50 (ddd, J = 10.0, 8.1, 4.2 Hz, 1H, AcNHαCH), 4.36−4.17 (m, 3H, 2 × NHαCH, NHαCH (Lys)), 4.12 (ddd, J = 14.8, 6.8, 3.4 Hz, 1H, CH$_a$SO$_2$F), 3.89 (dd, J = 14.8, 9.1 Hz, 1H, CH$_b$SO$_2$F), 2.97 (dd, J = 13.9, 4.2 Hz, 1H, CH$_a$ (Phe)), 2.79−2.66 (m, 3H, CH$_b$ (Phe), NH$_3^+$CH$_2$), 1.75 (s, 3H, CH$_3$ (AcNH)), 1.67−1.38 (m, 10H, 2 × CH(CH$_3$)$_2$ (Leu), 2 × CH$_2$ (Leu), NH$_3^+$CH$_2$CH_2, αCHCH_2 (Lys)), 1.36−1.22 (m, 2H, NH$_3^+$CH$_2$CH$_2$CH_2 (Lys)), 0.89 (d, J = 6.6 Hz, 3H, CH$_3$ (Leu)), 0.88 (d, J = 6.6 Hz, 3H, CH$_3$ (Leu)), 0.84 (d, J = 6.5 Hz, 3H, CH$_3$ (Leu)), 0.84 (d, J = 6.5 Hz, 3H, CH$_3$ (Leu)). ^{13}C NMR (101 MHz, DMSO-d_6, 298 K): δ_c (ppm) = 171.6 (C═O), 171.5 (C═O), 171.2 (C═O), 169.2 (C═O), 137.9 (C-Ar (Phe)), 129.0 (CH-Ar), 127.9 (CH-Ar), 126.1 (CH-Ar), 124.1 (CH-Ar), 53.8 (AcNHC), 53.7 (CSO$_2$F), 51.1 (αCH (Leu)), 50.9 (αCH (Leu)), 43.9 (αCH (Lys)), 40.5 (2 × CH$_2$ (Leu)), 37.2 (CH$_2$ (Phe)), 32.8 (αCH$\underline{C}$H$_2$ (Lys)), 26.2 (NHCH$_2\underline{C}$H$_2$ (Lys)), 24.0 ($\underline{C}$H(CH$_3$)$_2$), 23.9 ($\underline{C}$H(CH$_3$)$_2$), 23.0 (CH$_3$ (Leu)), 22.9 (CH$_3$ (Leu)), 22.3 (CH$_3$ (Ac)), 21.6 (αCHCH$_2\underline{C}$H$_2$ (Lys)), 21.54 (CH$_3$ (Leu)), 21.47 (CH$_3$ (Leu)) ^{19}F NMR (377 MHz, DMSO-d_6, 298 K): δ_F (ppm) = 59.6 (d, $J_{C,F}$ = 6.6 Hz, 1F, SO$_2$F). HRMS (ESI positive) calcd for $C_{29}H_{48}N_5O_6SF$ [M + H]$^+$ 614.3382, found 614.3371. t_R (0 to 100% B, 50 min, C$_{A3}$) = 29.0 min.

TFA·N$_3$-Phe-Leu$_2$-Lys-ψ[CH$_2$SO$_2$]-F (61). N$_3$-Phe-Leu$_2$-Lys-(Cbz)-ψ[CH$_2$SO$_2$]-F (20.0 mg, 23.6 μmol, 1.00 equiv) was set under nitrogen atmosphere, and 4 m HCl solution in dioxane (10 mL) was added and the reaction mixture stirred overnight at rt. The solvent was removed in vacuo, and the crude was purified by semipreparative HPLC (0 to 100% B, C$_{P3}$). Fractions containing the product were pooled, lyophilized, and the pure product was obtained as a white solid (6.5 mg, 9.13 μmol, 39%). ^{1}H NMR (400 MHz, DMSO-d_6, 298 K): δ_H (ppm) = 8.33 (d, J = 8.2 Hz, 1H, N$H\alpha$CH (Leu)), 8.13−8.06 (m, 2H, N$H\alpha$CH (Leu), N$H\alpha$CH (Lys)), 7.63 (br s, 3H, NH$_3^+$), 7.35−7.21 (m, 5H, Ar-H (Phe)), 4.38 (app q, J = 7.7 Hz, 1H, NHαCH (Leu)), 4.33−4.18 (m, 2H, NHαCH (Leu), NHαCH (Lys)), 4.17−4.07 (m, 2H, N$_3\alpha$CH, CH$_a$SO$_2$F), 3.89 (dd, J = 15.0, 9.1 Hz, 1H, CH$_b$SO$_2$F), 3.08 (dd, J = 14.1, 5.2 Hz, 1H, CH$_a$ (Phe)), 2.88 (dd, J = 14.1, 9.1 Hz, 1H, CH$_b$ (Phe)), 2.78−2.69 (m, 2H, NHCH$_2$ (Lys)), 1.67−1.39 (m, 4H, 2 × CH(CH$_3$)$_2$ (Leu), αCHCH_2 (Lys)), 1.33−1.19 (m, 8H, 2 × CH$_2$, NHCH$_2$CH_2CH$_2$ (Lys)), 0.95−0.77 (m, 12H, 4 × CH$_3$ (Leu)). ^{19}F NMR (471 MHz, DMSO-d_6, 298 K): δ_F (ppm) = 59.7 (d, J = 6.8 Hz, 1F, SO$_2$F). HRMS (ESI positive) calcd for $C_{27}H_{45}N_7O_5SF$ [M + H]$^+$ 598.3181, found 598.3157. t_R (0 to 100% B, 50 min, C$_{A3}$) = 30.3 min.

2TFA·H-Phe-Leu$_2$-Phe(4-NH$_2$)-ψ[CH$_2$SO$_2$]-F (62). TFA·Boc-Phe-Leu$_2$-Phe(4-NHCbz)-ψ[CH$_2$SO$_2$]-F (29.7 mg, 31.1 μmol, 1.00 equiv) was dissolved in CH$_2$Cl$_2$ (10 mL), and a 33% HBr/AcOH solution (3 mL) was added and the reaction mixture stirred for 1.5 h at rt. The solvent was removed in vacuo, and the crude was purified via semipreparative HPLC (0 to 100% B, C$_{P3}$). The obtained product required a second purification step by semipreparative HPLC (0 to 50% B, C$_{P3}$). Fractions containing the product were pooled, lyophilized, and the pure product was obtained as a white solid (20.6 mg, 24.7 μmol, 79%). ^{1}H NMR (500 MHz, DMSO-d_6, 298 K): δ_H (ppm) = 8.65 (d, J = 8.3 Hz, 1H, N$H\alpha$CH (Leu)), 8.31 (d, J = 8.3 Hz, 1H, N$H\alpha$CH (4-H$_3$N$^+$-Phe)), 8.20 (d, J = 8.2 Hz, 1H, N$H\alpha$CH (Leu)), 7.34−7.23 (m, 5H, Ar-H (Phe)), 7.14 (d, J = 8.0 Hz, 2H, 2 × CH (4-H$_3$N$^+$-Phe)), 6.95 (d, J = 8.0 Hz, 2H, 2 × CH (4-H$_3$N$^+$-Phe)), 4.46−4.35 (m, 2H, NHαCH (4-H$_3$N$^+$-Phe), NHαCH (Leu)), 4.31−4.19 (m, 1H, NHαCH (Leu)), 4.11−4.02 (m, 2H, NHαCH (H$_3$N$^+\alpha$CH), CH$_a$SO$_2$F), 3.91 (dd, J = 15.4, 9.2 Hz, 1H, CH$_b$SO$_2$F), 3.10 (dd, J = 14.2, 5.0 Hz, 1H, CH$_a$ (Phe)), 2.92 (dd, J = 14.2, 7.7 Hz, 1H, CH$_b$ (Phe)), 2.85−2.76 (m, 2H, αCHCH_2 (4-H$_3$N$^+$-Phe)), 1.68−1.60 (m, 1H, CH(CH$_3$)$_2$ (Leu)), 1.59−1.51 (m, 1H, CH(CH$_3$)$_2$ (Leu), 1.50−1.30 (m, 4H, 2 × CH$_2$ (Leu)), 0.91 (d, J = 6.6 Hz, 3H, CH$_3$ (Leu)), 0.89−0.85 (m, 6H, 2 × CH$_3$ (Leu)), 0.83 (d, J = 6.5 Hz, 3H, CH$_3$ (Leu)). ^{13}C NMR (126 MHz, DMSO-d_6, 298 K): δ_c (ppm) = 171.4 (C═O), 171.0 (C═O), 167.5 (C═O), 134.6 (C-Ar), 130.2 (CH-Ar (4-H$_3$N$^+$-Phe)), 129.5 (CH-Ar), 128.3 (CH-Ar), 127.0 (CH-Ar), 119.2 (CH-Ar (4-H$_3$N$^+$-Phe)), 53.0 (d, J = 12.3 Hz, CSO$_2$F), 52.96 (H$_3$N$^+\alpha$CH), 50.99 (αCH (Leu)), 50.95 (αCH (Leu)), 46.1 ($\alpha\underline{C}$HCH$_2$SO$_2$F), 41.0 (CH$_2$ (Leu)), 40.6 (CH$_2$ (Leu)), 38.3 (αCH$\underline{C}$H$_2$ (4-H$_3$N$^+$-Phe)), 36.8 (CH$_2$ (Phe)), 24.0 ($\underline{C}$H(CH$_3$)$_2$), 23.9 ($\underline{C}$H(CH$_3$)$_2$), 23.0 (CH$_3$ (Leu)), 22.9 (CH$_3$ (Leu)), 21.6 (CH$_3$ (Leu)), 21.5 (CH$_3$ (Leu)). ^{19}F NMR (471 MHz, CDCl$_3$, 298 K): δ_F (ppm) = 59.7 (d, J = 6.6 Hz, 1F, SO$_2$F). HRMS (ESI positive) calcd for $C_{30}H_{44}N_5O_5SFNa$ [M + Na]$^+$ 628.2939, found 628.2907. t_R (0 to 100% B, 30 min, C$_{A3}$) = 17.1 min.

TFA·Ac-Phe-Leu$_2$-Phe(4-NH$_2$)-ψ[CH$_2$SO$_2$]-F (63). TFA·Ac-Phe-Leu$_2$-Phe(4-NHCbz)-ψ[CH$_2$SO$_2$]-F (20 mg, 22.3 μmol, 1.00 equiv)

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was dissolved in CH_2Cl_2 (5 mL), and a 33% HBr/AcOH solution (5 mL) was added, and the reaction mixture was stirred for 1 h at rt. The solvents were removed in vacuo and the crude product was purified by semipreparative HPLC (0 to 100% B, C_{P3}). Fractions containing the product were pooled, lyophilized, and the pure product was obtained as a white solid (8.7 mg, 11.4 μmol, 51%). ^{1}H NMR (400 MHz, DMSO-d_6, 298 K): δ_H (ppm) = 8.21 (d, J = 8.3 Hz, 1H, N*H*αCH (4-H_3N^+-Phe)), 8.13−8.07 (m, 2H, N*H*αCH (Leu), AcN*H*αCH)), 7.81 (d, J = 8.3 Hz, 1H, N*H*αCH (Leu)), 7.28−7.22 (m, 4H, Ar-H (Phe)), 7.22−7.14 (m, 3H, Ar-H (Phe), Ar-H, 2 × CH (4-H_3N^+-Phe)), 7.01 (d, J = 7.8 Hz, 2H,, 2 × CH (4-H_3N^+-Phe)) 4.50 (ddd, J = 10.1, 8.2, 4.0 Hz, 1H, AcNHαC*H*), 4.45−4.33 (m, 1H, NHαC*H* (4-H_3N^+-Phe)), 4.29 (app q, J = 7.7 Hz, 1H, NHαC*H* (Leu)), 4.25−4.17 (m, 1H, NHαC*H* (Leu)), 4.07 (ddd, J = 14.9, 7.4, 3.1 Hz, 1H, CH_aSO_2F), 3.91 (dd, J = 14.9, 10.2 Hz, 1H, CH_bSO_2F), 2.97 (dd, J = 13.9, 4.0 Hz, 1H, CH_a (Phe)), 2.81 (d, J = 6.7 Hz, 2H, αCHC*H*$_2$ (4-H_3N^+-Phe)), 2.70 (dd, J = 13.9, 10.1 Hz, 1H, CH_b (Phe)), 1.75 (s, 3H, CH_3 (Ac)), 1.65−1.50 (m, 2H, 2 × C*H*$(CH_3)_2$ (Leu)), 1.49−1.27 (m, 4H, 2 × CH_2 (Leu)), 0.89 (d, J = 6.5 Hz, 3H, CH_3 (Leu)), 0.84 (d, J = 6.5 Hz, 6H, 2 × CH_3 (Leu)), 0.81 (d, J = 6.5 Hz, 3H, CH_3 (Leu)). ^{13}C NMR (101 MHz, DMSO-d_6, 298 K): δ_c (ppm) = 171.38 (C═O), 171.35 (C═O), 171.30 (C═O), 169.2 (C═O), 137.9 (C-Ar), 130.3 (C-Ar), 130.3 (C-Ar), 129.0 (CH-Ar), 127.9 (CH-Ar), 126.1 (CH-Ar), 119.8 (CH-Ar (4-H_3N^+-Phe)), 53.8 (AcNHC), 53.0 (d, $J_{C,F}$ = 11.5 Hz, CSO_2F), 51.0 (αCH (Leu)), 50.9 (αCH (Leu)), 46.1 (αCH (4-H_3N^+-Phe)), 40.6 (C*H*$(CH_3)_2$ (Leu)), 40.4 (C*H*$(CH_3)_2$ (Leu)), 38.4 (αCHC*H*$_2$ (4-H_3N^+-Phe)), 37.2 (CH_2 (Phe)), 24.0 (C*H*$(CH_3)_2$ (Leu)), 23.9 (C*H*$(CH_3)_2$ (Leu)), 23.0 (CH_3 (Leu)), 22.9 (CH_3 (Leu)), 22.3 (CH_3 (Ac)), 21.6 (CH_3 (Leu)), 21.5 (CH_3 (Leu)). ^{19}F NMR (377 MHz, $CDCl_3$, 298 K): δ_F (ppm) = 59.7 (d, J = 6.8 Hz, 1F, SO_2F). HRMS (ESI positive) calcd for $C_{32}H_{46}N_5O_6SFNa$ $[M + Na]^+$ 670.3045, found 670.3016. t_R (0 to 100% B, 50 min, C_{A3}) = 29.7 min.

TFA·N_3-Phe-Leu$_2$-Phe(4-NH_2)-ψ[CH_2SO_2]-F (64). TFA·N_3-Phe-Leu$_2$-Phe(4-NHCbz)-ψ[CH_2SO_2]-F (14.1 mg, 16.0 μmol, 1.00 equiv) was set under nitrogen atmosphere, and 4 M HCl in dioxane (10 mL) was added and the reaction mixture stirred for 48 h at 60 °C. The solvent was removed in vacuo and the crude product purified by semipreparative HPLC (0 to 100% B, C_{P3}). The starting material was recovered (13.0 mg), and no product could be isolated.

2TFA·H-Phe-Leu$_2$-Phe(4-CH_2NH_2)-ψ[CH_2SO_2]-F (65). TFA·Boc-Phe-Leu$_2$-Phe(4-CH_2NH_2)-ψ[CH_2SO_2]-F (12.7 mg, 13.1 μmol, 1.00 equiv) was dissolved in CH_2Cl_2 (5 mL), and 33% HBr/AcOH solution (5 mL) was added, and the reaction mixture was stirred for 45 min at rt. The solvent was removed in vacuo, and the crude product was purified by semipreparative HPLC (0 to 60% B, C_{P2}). Fractions containing the product were pooled, lyophilized, and the pure product was obtained as a white solid (7.5 mg, 8.84 μmol, 68%). ^{1}H NMR (500 MHz, DMSO-d_6, 298 K): δ_H (ppm) = 8.68 (d, J = 8.0 Hz, 0.5H, NH rotamer), 8.62 (d, J = 8.3 Hz, 0.5H, NH rotamer), 8.39−8.30 (m, 2H, 2 × NH), 8.22−8.14 (m, 3H, NH_3^+), 8.09 (br s, 3H, NH_3^+), 7.40−7.21 (m, 9H, Ar-H), 4.51−4.38 (m, 2H, αCH (Leu), αCH (Phe$CH_2NH_3^+$)), 4.31−4.12 (m, 2H αCH (Leu), CH_aSO_2F), 4.11−4.02 (m, 1H, αCH (Phe)), 4.02−3.90 (m, 3H, CH_bSO_2F, $CH_2NH_3^+$), 3.09 (dd, J = 14.3, 4.8 Hz, 1H, CH_aPhe), 2.98−2.78 (m, 3H, CH_bPhe, (C*H*$_2$Phe$CH_2NH_3^+$)), 1.68−1.51 (m, 1H, C*H*$(CH_3)_2$), 1.50−1.39 (m, 3H, C*H*$(CH_3)_2$, CH_2 (Leu)), 1.38−1.28 (m, 1H, CH_2−a (Leu)), 1.29−1.14 (m, 1H, CH_2−b (Leu)), 0.93−0.74 (m, 12H, CH_3 (Leu)). ^{13}C NMR (126 MHz, DMSO-d_6, 298 K): δ_C (ppm) = 171.9, 171.6, 168.2 (C═O), 137.9, 135.2, 132.8 (C-Ar), 130.0, 129.4, 129.1, 129.0, 127.6 (CH-Ar), 53.6 (d, J = 12.0 Hz, CSO_2F), 53.4 (NH_3^+αCH), 51.5, 51.4 (2 × αCH), 46.7 (αCH (Phe$CH_2NH_3^+$)), 42.5 ($CH_3NH_3^+$), 41.5, 41.2 (2 × CH_2 (Leu)), 39.2 (CH_2Phe$CH_2NH_3^+$), 37.5 (CH_2Phe), 24.6, 24.5 (2 × CH (Leu)), 23.5, 23.4, 22.1, 22.0 (4 × CH_3 (Leu)). ^{19}F NMR (471 MHz, DMSO-d_6, 298 K): δ_F (ppm) = 59.8 (d, J = 5.9 Hz, SO_2F). HRMS (ESI positive) calcd for $C_{31}H_{47}N_5O_5SF$ $[M + H]^+$ 620.3276, found 620.3275. t_R (0 to 100% B, 50 min, C_{A1}) = 40.5 min.

TFA·Ac-Phe-Leu$_2$-Phe(4-CH_2NH_2)-ψ[CH_2SO_2]-F (66). TFA·Ac-Phe-Leu$_2$-Phe(4-CH_2NHCbz)-ψ[CH_2SO_2]-F (24.7 mg, 27.2 μmol, 1.00 equiv) was dissolved in CH_2Cl_2 (5 mL), and a 33% HBr/AcOH solution (5 mL) was added, and the reaction mixture was stirred for 1 h at rt. The solvent was removed in vacuo and the crude product purified via semipreparative HPLC (0 to 100% B, C_{P3}). The obtained product (15.5 mg) required a second purification step by semi-HPLC (0 to 50% B, C_{P3}). Fractions containing the product were pooled, lyophilized, and the pure product was obtained as a white solid (6.2 mg, 8.0 μmol, 29%). ^{1}H NMR (500 MHz, DMSO-d_6, 298 K): δ_H (ppm) = 8.27 (d, J = 8.4 Hz, 1H, N*H*αCH (4-$H_3N^+CH_2$-Phe)), 8.16 (br s, 3H, NH_3^+), 8.12−8.06 (m, 2H, AcN*H*αCH, N*H*αCH (Leu)), 7.78 (d, J = 8.3 Hz, 1H, N*H*αCH (Leu)), 7.37 (d, J = 7.9 Hz, 2H, Ar-H (4-$H_3N^+CH_2$-Phe)), 7.31−7.14 (m, 7H, 5 × Ar-H (Phe), 2 × Ar-H (4-$H_3N^+CH_2$-Phe)), 4.49 (ddd, J = 10.1, 8.1, 4.0 Hz, 1H, AcNHαC*H*), 4.47−4.39 (m, 1H, αC*H*CH_2SO_2F), 4.32−4.25 (m, 1H, NHαC*H* (Leu)), 4.22 (ddd, J = 10.2, 8.3, 5.0 Hz, 1H, NHαC*H* (Leu)), 4.08−3.98 (m, 3H, CH_aSO_2F, C*H*$_2NH_3^+$), 3.95 (dd, J = 14.1, 9.1 Hz, 1H, CH_bSO_2F), 2.97 (dd, J = 14.0, 4.0 Hz, 1H, CH_a (Phe)), 2.87 (d, J = 6.9 Hz, 2H, C*H*$_2$Phe(4-$CH_2NH_3^+$)), 2.70 (dd, J = 14.0, 10.1 Hz, 1H, CH_b (Phe)), 1.75 (s, 3H, CH_3 (Ac)), 1.63−1.51 (m, 2H, 2 × C*H*$(CH_3)_2$ (Leu)), 1.49−1.38 (m, 3H, CH_2 (Leu), CH_a (Leu)), 1.33 (ddd, J = 13.7, 9.0, 5.0 Hz, 1H, CH_b (Leu)), 0.89 (d, J = 6.6 Hz, 3H, CH_3 (Leu)), 0.85 (d, J = 6.5 Hz, 3H, CH_3 (Leu)), 0.84 (d, J = 6.5 Hz, 3H, CH_3 (Leu)), 0.81 (d, J = 6.5 Hz, 3H, CH_3 (Leu)). ^{13}C NMR (101 MHz, DMSO-d_6, 298 K): δ_C (ppm) = 171.98 (C═O), 171.95 (C═O), 171.88 (C═O), 169.8 (C═O), 138.5 (C-Ar), 137.9 (C-Ar), 132.7 (C-Ar), 130.0 (CH-Ar), 129.6 (CH-Ar), 129.3 (CH-Ar), 128.5 (CH-Ar), 126.7 (CH-Ar)), 54.4 (AcNHαC), 53.8 (d, $J_{C,F}$ = 11.7 Hz, CSO_2F), 51.6 (αCH (Leu)), 51.4 (αCH (Leu)), 46.6 (αCHCH_2SO_2F), 42.5 ($CH_2NH_3^+$), 41.2 (CH_2 (Leu)), 41.0 (CH_2 (Leu)), 39.3 (CH_2Phe), 37.7 (CH_2Phe[4-$CH_2NH_3^+$]), 24.6 (C*H*$(CH_3)_2$), 24.4 (C*H*$(CH_3)_2$), 23.6 (CH_3 (Leu)), 23.5 (CH_3 (Leu)), 22.9 (CH_3 (Ac)), 22.1 (CH_3 (Leu)), 22.0 (CH_3 (Leu)). ^{19}F NMR (471 MHz, DMSO-d_6, 298 K): δ_F (ppm) = 59.8 (d, J = 6.0 Hz, SO_2F). HRMS (ESI positive) calcd for $C_{33}H_{49}N_5O_6SF$ $[M + H]^+$ 662.3382, found 662.3370. t_R (0 to 100% B, 50 min, C_{A3}) = 29.6 min.

TFA·N_3-Phe-Leu$_2$-Phe(4-CH_2NH_2)-ψ[CH_2SO_2]-F (67). TFA·N_3-Phe-Leu$_2$-Phe(4-CH_2NHCbz)-ψ[CH_2SO_2]-F (21.0 mg, 23.5 μmol, 1.00 equiv) was placed under nitrogen atmosphere, and 4 M HCl solution in dioxane (10 mL) was added, and the reaction mixture was stirred for 30 h at 50 °C. The solvent was removed in vacuo, and the crude product was purified via semipreparative HPLC (0 to 50% B, C_{P3}). Fractions containing the product were pooled, lyophilized, and the pure product was obtained as a white solid (9.8 mg, 12.9 μmol, 55%). ^{1}H NMR (500 MHz, DMSO-d_6, 298 K): δ_H (ppm) = 8.37 (d, J = 8.3 Hz, 1H, N*H*αCH (Leu)), 8.30 (d, J = 8.4 Hz, 1H, N*H*αCH (Phe[4-$CH_2NH_3^+$])), 8.16 (br s, 3H, $CH_2NH_3^+$), 8.00 (d, J = 8.3 Hz, 1H, N*H*αCH (Leu)), 7.37 (d, J = 7.9 Hz, 2H, Ar-H (Phe[4-$CH_2NH_3^+$])), 7.33−7.21 (m, 7H, Ar-H (Phe), 2 × Ar-H (Phe[4-$CH_2NH_3^+$])), 4.49−4.41 (m, 1H, NHαC*H* (Phe[4-$CH_2NH_3^+$])), 4.40−4.33 (m, 1H, NHαC*H* (Leu)), 4 4.26−4.19 (m, 1H, NHαC*H* (Leu)), 4.10 (dd, J = 9.3, 5.0 Hz, 1H, N_3αC*H*), 4.08−3.91 (m, 4H, C*H*$_2NH_3^+$, CH_2SO_2F), 3.08 (dd, J = 14.1, 5.0 Hz, 1H, N_3αCHC*H*$_a$), 2.91−2.84 (m, 2H, N_3αCHC*H*$_b$, C*H*$_2$Phe(4-$CH_2NH_3^+$)), 1.62−1.50 (m, 2H, 2 × C*H*$(CH_3)_2$ (Leu)), 1.50−1.27 (m, 4H, 2 × CH_2 (Leu)), 0.89 (d, J = 6.7 Hz, 3H, CH_3 (Leu)), 0.87−0.84 (m, 6H, 2 × CH_3 (Leu)), 0.82 (d, J = 6.5 Hz, 3H, CH_3 (Leu)). ^{13}C NMR (126 MHz, DMSO-d_6, 298 K): δ_C (ppm) = 171.4 (C═O), 171.0 (C═O), 168. Seven (C═O), 137.3 (C-Ar), 136.8 (C-Ar), 132.1 (C-Ar), 129.4 (CH-Ar), 128.9 (CH-Ar), 128.8 (CH-Ar), 128.3 (CH-Ar), 126.6 (CH-Ar), 62.2 (N_3αC), 53.2 (d, $J_{C,F}$ = 12.4 Hz, CSO_2F), 51.0 (αCH (Leu)), 50.9 (αCH (Leu)), 46.1 (αCHCH_2SO_2F), 41.9 (C$H_2NH_3^+$), 40.64 (CH_2 (Leu)), 40.58 (CH_2 (Leu)), 38.7 (CH_2Phe[4-$CH_2NH_3^+$]), 36.7 (N_3αCHCH_2), 24.0 (C*H*$(CH_3)_2$), 23.9 (C*H*$(CH_3)_2$), 23.0 (CH_3 (Leu), 22.9 (CH_3 (Leu), 21.5 (2 × CH_3 (Leu)). ^{19}F NMR (471 MHz, DMSO-d_6, 298 K): δ_F (ppm) = 59.8 (d, J = 5.9 Hz, SO_2F). HRMS (ESI positive) calcd for $C_{31}H_{45}N_7O_5SF$ $[M + H]^+$ 646.3181, found 646.3161. t_R (0 to 100% B, 50 min, C_{A1}) = 34.3 min, 34.6 min.

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■ ASSOCIATED CONTENT

Supporting Information

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Analytical and preparative HPLC-columns; synthesis of peptides required for incorporation of aminosulfonyl fluoride derivatives; synthesis of precursors of aminosulfonyl fluorides (**28**−**30**, **32**−**36**); synthesis of aminosulfonyl fluorides (**40**−**42**); synthesis of protected peptide sulfonyl fluorides; proton, carbon, and fluoride NMR spectra; HPLC traces of peptide sulfonyl fluorides (PDF)

Molecular formula strings and some data (CSV)

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

Notes

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■ ABBREVIATIONS USED

BOP, (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate;; HCTU, 2-(6-chloro-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HATU, 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]-pyridinium 3-oxide hexafluorophosphate; HBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; THF, tetrahydrofuran; DMF, *N*,*N*-dimethylformamide; DiPEA, *N*,*N*-diisopropylethylamine; Boc, *tert*-butyloxycarbonyl; Cbz, carboxybenzyl; EtOAc, ethyl acetate; Hex, *n*-hexane

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(26) Several transformations (e.g., oxidation, reduction, hydrolysis, etc.) can be carried out in the presence of an aromatic SF moiety, which is indicative of the difference in stability of aliphatic and aromatic SFs.[18]
(27) Recently it was found in peptidovinyl sulfonyl fluorides (PVSFs) that although a PVSF may be more reactive than a peptidosulfonyl fluoride (PSF), the sulfonyl fluoride warhead part may occupy a less favorable P_1' position because it is further positioned from the P_1 side chain, leading to a reduced inhibition.[20]
(28) There was no specific reason for synthesis of one tripeptide on the solid phase and the other tripeptide in solution. Either method can be used for both tripeptides.
(29) The final deprotection step was removal of the side-chain Cbz-protecting group of the amino protected sulfonyl fluoride, which also would remove any terminal Cbz-protecting group.

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