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Achiral oligoamines as versatile tool for the development of aspartic protease inhibitors

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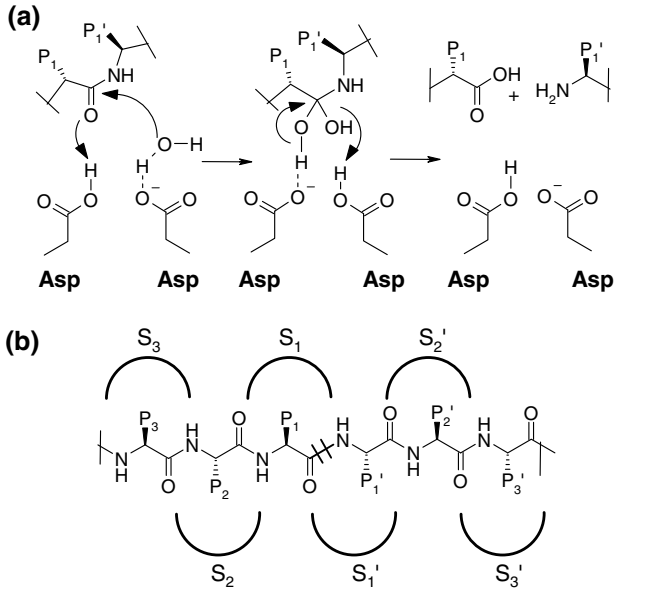
ABSTRACT

Due to the important role that aspartic proteases play in many patho-physiological processes, they have intensively been targeted by modern drug development. However, up to now, only for two family members, renin and HIV protease, approved drugs are available. Inhibitor development, mostly guided by mimicking the natural peptide substrates, resulted in very potent inhibitors for several targets, but the pharmacokinetic properties of these compounds were often not optimal. Herein we report a novel approach for lead structure discovery of non-peptidic aspartic protease inhibitors using easily accessible achiral linear oligoamines as starting point. An initial library comprising 11 inhibitors was developed and screened against six selected aspartic proteases. Several hits could be identified, among them selective as well as rather promiscuous inhibitors. The design concept was confirmed by determination of the crystal structure of two derivatives in complex with the HIV-1 protease, and represents a promising basis for the further inhibitor development.

1. Introduction

Aspartic proteases belong to the class of endopeptidases and have shown to play an important role in many physiological but also patho-physiological processes. The active site comprises two aspartic acid residues which activate a water molecule hence facilitating the nucleophilic attack at the scissile amide bond. The cleavage of the substrate follows a general catalytic acid–base mechanism in which one of the two aspartates is protonated, the other deprotonated.[1,2] The nucleophilic attack of the activated water molecule leads to a tetrahedral *gem*-diol intermediate which collapses under cleavage of the peptide bond (Scheme 1). In general, 6–10 amino acids of the natural polypeptide substrates are recognized by aspartic proteases, thus demanding an extended active site. The standard nomenclature defines the substrate residues as, for example, P3, P2, P1, P1′, P2′, and P3′ and the corresponding recognition pockets as, for example, S3, S2, S1, S1′, S2′, and S3′ as depicted in Scheme 1.[3]

Two classes of aspartic proteases have received pronounced attention as potential drug targets; the pepsin-like[5] (family A1) and the retroviral proteases (family A2).[6–8] The family of the pepsin-like proteases includes, for example, renin (cardiovascular diseases),[9,10] BACE-1 (Alzheimer's disease),[11,12] and the plasmepsins (malaria)[13,14] which all share a common folding motif: the N-terminal and the C-terminal domain, each contributing one catalytic



Scheme 1. (a) Schematic representation of the catalytic mechanism of aspartic proteases. (b) Nomenclature of the protease's subsites according to Berger and Schechter,[3] the scissile peptide bond is indicated by crossing lines.

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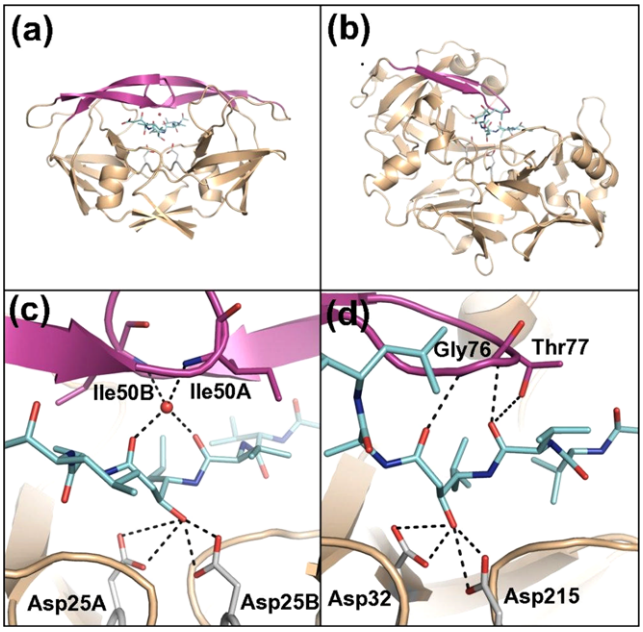


Figure 1. Crystal structures of the peptidomimetic inhibitor Pepstatin A, shown in light blue, color-coded by atom type, in complex with the HIV-1 protease (a and c) (PDB code: 5HVP) and pepsin (b and d) (PDB code: 1PSO). The proteins are represented as cartoons and flap regions establishing interactions to the inhibitor are highlighted in magenta. Hydrogen bonds are indicated by dashed lines. The catalytic aspartates are color-coded by atom type in gray and selected flap amino acid residues are color-coded by atom type in magenta. Figures 1–3 were created using PyMol 0.99.[4]

aspartic acid residue, are connected via a β-sheet domain. The active site is formed by the closure of a flexible loop region termed 'flap' which covers the active site and forms two hydrogen bonds to the substrate carbonyl groups adjacent to the cleavage site (Fig. 1b and d, PDB code: 1PSO).[15] In the S1 and S1′ pocket, preferably hydrophobic amino acids are recognized, whereas in the S2, S3, S2′, and S3′ pockets the recognition is non-uniform (Table 1). The retroviral aspartic proteases belong to the A2 family, among which the HIV-1 protease was shown to be a valuable target for treatment of HIV-infection.[16–19] The HIV-1 protease is a C_2-symmetric protein consisting of two identical subunits each contributing one catalytic aspartate. In contrast to family A1, the binding pocket is formed by the closure of two flaps. A conserved water molecule mediates the interactions of the peptide carbonyls adjacent to the cleavage site and the flap residues Ile50A and Ile50B (Fig. 1a and c, PDB code: 5HVP).[20] The HIV protease recognizes a variety of natural substrates with a strong preference for those bearing hydrophobic amino acids in P1 and P1′ and variable amino acids in farther positions (Table 1).[21]

Table 1
Cleavage sites in natural substrates of the selected aspartic proteases as annotated in the MEROPS database[7]

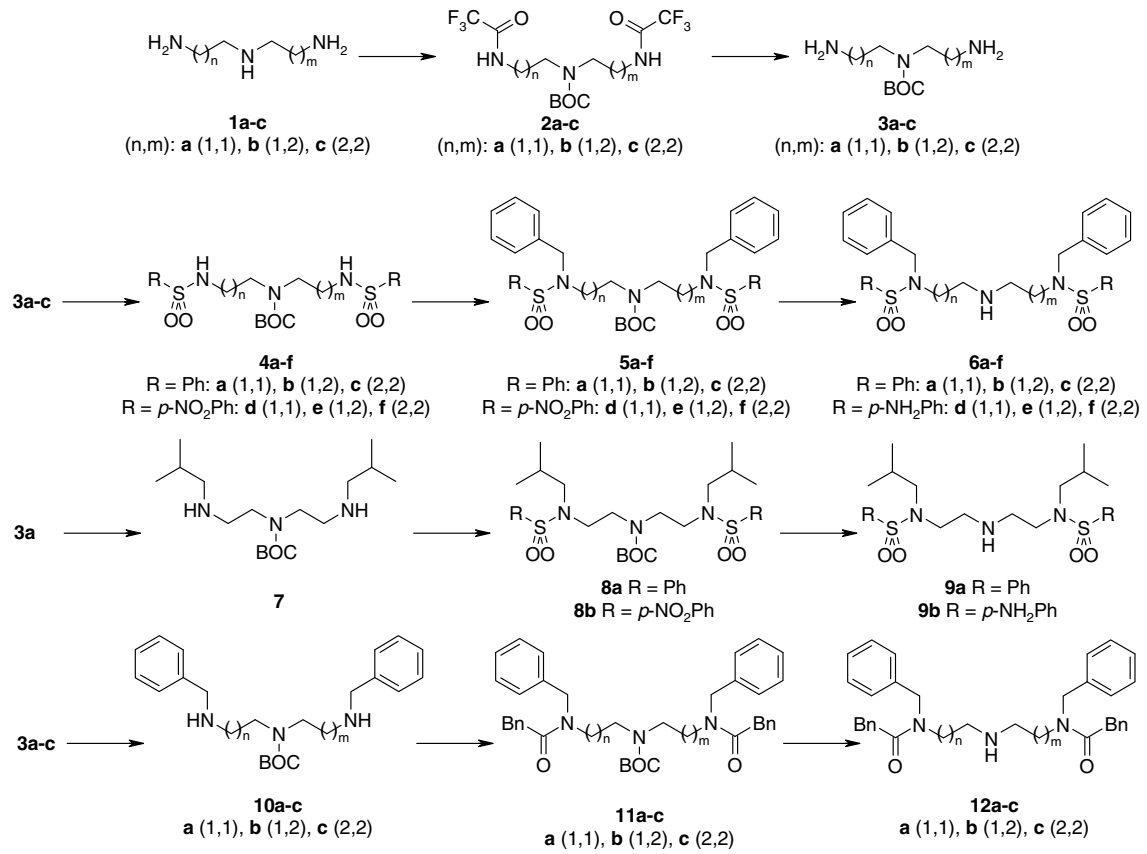
Protease	Substrate	P3	P2	P1	P1′	P2′	P3′
Pepsin A	—	—	—	F/L	F	—	—
Plm II	Hemoglobin α-chain	R	M	F	L	S	F
Plm IV	Hemoglobin α-chain	R	M	F	L	S	F
Renin	Angiotensinogen	F	H	L	L/V	V/I	H
BACE-1	Amyloid-precursor-protein	V	K	M	D	A	Q
HIV-1 protease	Gag polyprotein	Q	N	Y	P	I	V
		R	V	L	A	E	A
		T	I	M	M	Q	R
		A	N	F	L	H	K
		G	N	F	L	Q	S
	Pol polyprotein	F	N	F	P	Q	I
		L	N	F	P	I	S
		E	T	F	Y	V	D
		K	I	L	F	L	D

Despite immense efforts in the development of clinically effective drugs targeting aspartic proteases, up to now, only for HIV protease[22] and renin inhibitors have been approved for disease therapy.[23–25] The rational design of inhibitors has mostly been guided by the structure of the natural peptide substrates thus resulting in very potent inhibitors. However, the pharmacokinetic properties of these peptidic compounds bearing secondary hydroxyl groups as transition state isosters are often not optimal, thus hampering their clinical efficacy.[26] The synthesis of these complex and chiral inhibitors often turns out to be very challenging, and the optimization of the sub-pocket-addressing moieties is very resource-intensive and time-consuming. Replacement of the secondary hydroxyl group in transition state mimetics by the nearly isosteric amino functionality as well as by cyclic amidines has also been successfully pursued.[27–30] Additionally, reduced amide transition state isosters have been exploited recently as BACE-1 inhibitors.[31,32] However, for a more efficient development of novel aspartic protease inhibitors, an easily accessible and achiral core structure would be preferable. Utilizing such scaffolds would facilitate the development of promising moieties addressing the protease's sub-pockets. As suitable core structure for this approach we selected secondary amines, which have already been successfully utilized as anchoring groups for the development of aspartic protease inhibitors.

In the late 1990s, researchers at Roche discovered the piperidine moiety as a novel privileged skeleton addressing the catalytic dyad of aspartic proteases with its secondary nitrogen as revealed by X-ray crystallography.[33–35] Based on this discovery, several projects for the development of non-peptidic amine-based aspartic protease inhibitors have been pursued, which led to the development of potent inhibitors for the aspartic proteases plasmepsin (Plm) II and IV,[36–39] BACE-1,[40] and very recently for HIV-1 protease.[41] All inhibitors share in common a cyclic amino functionality addressing the enzyme's catalytic dyad directly or mediated by a water molecule. However, numerous scaffolds have been utilized. Depending on the nature of the scaffold and the target enzyme studied, the specificity pockets are addressed differently. In case of a pyrrolidine-based HIV-1 protease inhibitor, the interaction of the endocyclic, secondary amino functionality to the catalytic aspartic acid residues was studied by X-ray crystallography and Poisson–Boltzmann calculations. The latter study suggests the amine being in the protonated and the catalytic dyad in the fully deprotonated state resulting in strong electrostatic interactions.[42]

2. Results

To further exploit this promising anchoring group for the design of novel, easily accessible inhibitors, linear oligoamines were selected as novel core structure. This scaffold bears a central secondary amino function aimed to address the catalytic dyad. Via the distal amino functionalities, the introduction of appropriate acceptor groups addressing the flap regions of the respective target enzyme is easily amenable. Concomitantly hydrophobic moieties intended to address the S2 and S2′ specificity pockets are introduced. Via further alkylation of the distal nitrogen atoms, additional hydrophobic substituents can be implemented to address the S1/S1′ pockets, thereby avoiding the generation of any chiral center. This strategy allows the quick and straightforward generation of achiral inhibitors following a highly flexible and short synthetic route. To optimize the amine-acceptor distances with respect to a certain target enzyme, oligoamines with varying chain



Scheme 2. Preparation of the oligoamines: for reaction conditions and yields refer to Section 5.

length between the central and each of the distal amino functionalities can be employed.

For the design of an initial library, linkers with two and three methylene groups separating the amino groups were selected. As hydrophobic moieties intended to address the S1/S1′ sub-pockets, *iso*-butyl and benzyl groups resembling the side chains of leucine and phenylalanine of substrate peptides were introduced. For appropriate flap interactions, two types of acceptor functionalities were investigated: on the one hand carboxamides present in the natural substrate and on the other hand sulfonamides, which allow additional rotational degrees of freedom. The introduction of residues addressing the S2/S2′ pockets is easily achieved via further substitution at the lateral nitrogen atoms. In case of the sulfonamides, the hydrophobic phenyl and the more polar *p*-amino phenyl group were chosen. For carboxamides, phenyl acetic acid derivatives were utilized offering additional flexibility between the rigid amide functionality and the phenyl ring.

2.1. Chemistry

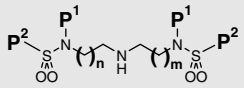
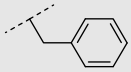
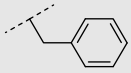
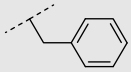
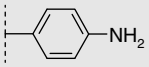
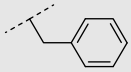
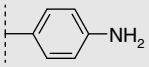
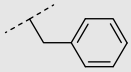
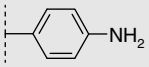
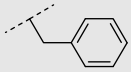
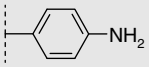
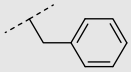
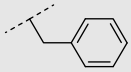
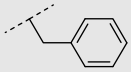
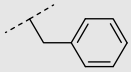
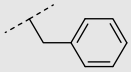
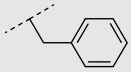
Oligoamines **3a**–**c**, Boc-protected at the pivotal, secondary amino function, are accessible from commercially available precursors **1a**–**c** by transient protection of the terminal amino groups as their trifluoroacetamides **2a**–**c** (Scheme 2).[43] Condensation with sulfonyl chlorides furnished the sulfonamides **4a**–**f** which were further alkylated with benzyl bromide giving rise to the protected inhibitors **5a**–**f**. The phenyl-substituted sulfonamides **6a**–**c** were obtained via deprotection of **5a**–**c** with HCl in Et_2O. The *p*-amino phenyl-substituted sulfonamides **6d**–**f** were synthesized by reduction of the corresponding nitro-compounds **5d**–**f** with $SnCl_2{\cdot}2H_2O$ in refluxing 32% HCl under concomitant cleavage of the Boc-protecting group. The *iso*-butyl-substituted inhibitors **9a**,**b** are accessible via reductive amination of **3a** with *iso*-butyraldehyde and $NaBH_4$ as reducing agent yielding **7**, followed by condensation with sulfonyl chlorides rendering **8a**,**b**. The final inhibitors **9a**,**b** were obtained by reduction of the nitro functionality or by acidic cleavage of the Boc-protecting group as described above. Carboxamide inhibitors **12** were synthesized following a similar synthetic sequence: the benzyl-substituted oligoamines **10a**–**c** were obtained by reductive amination of the amines **3a**–**c** and benzaldehyde utilizing $Pd/BaSO_4$ as catalyst. Subsequent condensation with phenylacetic acid chloride gave rise to the protected inhibitors **11a**–**c**, which were deprotected under acidic anhydrous conditions yielding **12a**–**c**.

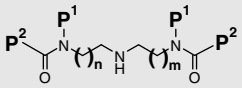
2.2. Kinetic characterization

To elucidate the potential of this new class of inhibitors, the affinity toward selected aspartic proteases was determined in fluorescence-based assays using commercially available substrates. The results are listed in Table 2. The anthranyl-HIV protease substrate was utilized in case of the HIV-1 protease (K_m = 14.6 μM), pepsin (K_m = 13.3 μM), and the plasmepsins II (K_m = 63 μM) and IV (K_m = 28 μM). For renin and BACE-1, suitably labeled oligopeptides derived from their natural substrates with comparable affinity were used (renin substrate K_m = 3.3 μM, BACE-1 substrate K_m = 7.9 μM).

Except for BACE-1, for each of the investigated target enzymes an inhibitor with an affinity in the single-digit micromolar range could be identified (Table 2). In case of the HIV-1 protease, a strong preference for inhibitors bearing ethylene linkers and a sulfonyl moiety was observed, with affinities ranging from 10.0 μM for **9a** up to 0.9 μM for **9b**. Considering the pepsin-like proteases, the tolerance of extended linkers is noteworthy. Particularly the plasmep-

Table 2
K_i-Values of the inhibitors toward selected aspartic proteases in μM

			 6a-f, 9a-b							
	n	*m*	P2	P1	HIV-1 pr.	Plm II	Plm IV	Renin	BACE-1	Pepsin
6a	1	1			3.8	7.0	36	n.i.	n.i.	5.7
6b	1	2			14	18	17	n.i.	n.i.	15
6c	2	2			n.i.	21	42	n.i.	150	n.i.
6d	1	1			9.6	4.2	7.5	n.i.	n.i.	3.9
6e	1	2			n.i.	22	7.5	n.i.	n.i.	n.i.
6f	2	2			n.i.	2.8	20	n.i.	64	4.0
9a	1	1			10	23	87	n.i.	n.i.	4.5
9b	1	1			0.9	6.8	56	n.i.	n.i.	4.8
12a	1	1			n.i.	91	98	5.7	n.i.	n.i.
12b	1	2			n.i.	63	56	n.i.	n.i.	n.i.
12c	2	2			n.i.	120	170	16	n.i.	n.i.


12a-c

n.i., IC_{50} > 250 μM.

sins II and IV reveal a remarkable tolerance toward the linker length: inhibitor **6f** exhibits the highest affinity toward Plm II (2.8 μM) and the corresponding analogues **6d** and **6e** toward Plm IV (7.5 μM). An inhibition of renin could only be observed in case of derivatives equipped with carbonyl groups as acceptor functionalities. With compound **12a** bearing the shortest linkers, the highest affinity (5.7 μM) could be achieved. An inhibition of pepsin is only accomplished with sulfonamide derivatives, **6d** being the most active compound (3.9 μM) in the series. However, no preference toward a certain chain length could be observed. In case of BACE-1, the sulfonamides equipped with the longest linkers show moderate inhibition with an affinity of 64 μM for compound **6f**.

To elucidate whether the oligoamine derivatives indeed exhibit the expected binding mode thus being in agreement with our initial design concept, the crystal structure in complex with one of the target enzymes was determined. Since the crystallization of HIV-1 protease is well established in our lab, this enzyme was selected for crystallization. The inhibitors **6a**, **6d**, and **9b** exhibiting the highest potency were selected for cocrystallization experiments. However, crystals could only be obtained in case of the better soluble compounds **6d** and **9b**. The X-ray structures of these compounds in complex with HIV-1 protease were determined with a resolution of 1.90 and 1.80 Å, respectively. The crystallographic data and refinement statistics are listed in Table 3. Both complexes adopt space group $P2_12_12$ and the inhibitors are clearly visible in the $F_o - F_c$ density at a sigma level of 3. They could be refined as one single conformer (Fig. 2).

2.3. Binding mode of 6d

The binding mode is schematically represented in Scheme 3. The pivotal secondary nitrogen of the inhibitor points toward Asp25A forming polar contacts to the terminal oxygen atoms of the side chain and to one sulfonyl oxygen atom of one of its sulfonamide functions. The *N*-benzyl moieties of the inhibitor address the S1 and S1′ pockets, whereas the *p*-amino-phenyl sulfonamide groups occupy the S2 and S2′ pockets. The *p*-amino substituents form a similar hydrogen bond network in both pockets: hydrogen bonds are observed to the main chain carbonyl oxygen atoms of Asp30A and Asp30B and to the corresponding side chain carboxyl oxygen atoms in each case mediated by a water molecule. One sulfonyl oxygen atom of each sulfonamide functionality establishes hydrogen bonds to a tetrahedrally coordinated water molecule mediating polar contacts to the main chain NHs of Ile50A and Ile50B, which are located at the tips of the flaps. One of the sulfonyl oxygen atoms remains unsatisfied showing no direct polar contacts to the protein. The ligand is deeply embedded in the binding pocket with a surface burial of 94%.

2.4. Binding mode of 9b

The crystal structure of **9b** in complex with HIV-1 protease exhibits a high similarity to the previously described structure. Comparable polar interactions of the secondary amino group to Asp25A are observed (Scheme 4). The sub-pocket occupancy

Table 3
X-ray data processing and refinement for the HIV-1 protease complexes of derivatives **6d** and **9b**

	6d	9b
Resolution (Å)	50–1.80	30–1.90
Space group	$P2_12_12$	$P2_12_12$
Cell dimensions (Å)		
a	57.4	56.0
b	85.7	85.9
c	46.5	46.5
Highest resolution shell (Å)	1.83–1.80	1.93–1.90
No. of measured reflections	49,890	65,713
No. of independent reflections	21,367	18,795
Completeness[a] (%)	97.4 [97.6]	99.0 [100]
I/σ[a]	16.0 [2.0]	15.6 [2.6]
R_{sym}[a] (%)	7.8 [44.4]	8.0 [49.0]
R_{cryst} ($F > 4\sigma F_o$; F_o)	18.4; 20.8	18.2; 20.9
R_{free} ($F > 4\sigma F_o$; F_o)	23.0; 26.0	23.9; 27.1
Mean *B*-factor (Å^2) protein (chain A; B)	25.9; 22.4	29.5; 27.1
Mean *B*-factor (Å^2) ligand	35.1	33.1
Mean *B*-factor (Å^2) water	31.0	34.0
Ramachandran plot		
Most favored geometry (%)	96.8	95.6
Additionally allowed (%)	3.2	4.4
RMSD bonds (Å)	0.006	0.005
RMSD angles (°)	2.0	2.0

[a] Values in brackets refer to the highest resolution shell.

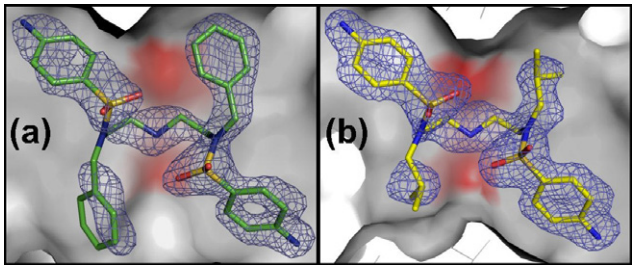
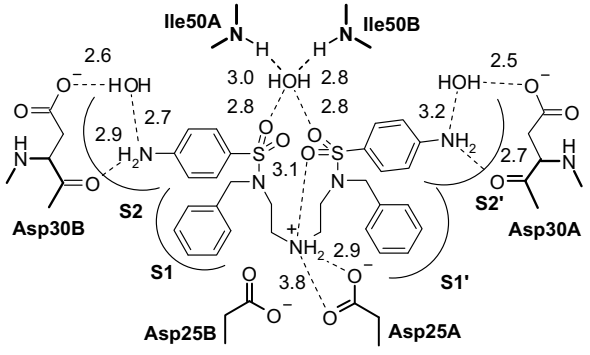
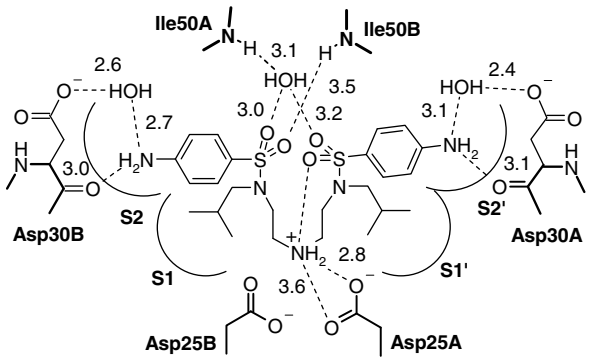


Figure 2. Ligand geometries of **6d** (a, color-coded by atom type in green) and **9b** (b, color-coded by atom type in yellow) in the cocrystal structures with the HIV-1 protease. The $F_o - F_c$ omit maps for the ligands are displayed at a σ level of 3.0 as blue mesh. The protein surface is schematically represented in gray and the catalytic aspartates Asp25A and Asp25B are highlighted in red.



Scheme 3. Schematic representation of the binding mode of **6d**. Hydrogen bonds are indicated by dashed lines and distances are given in Å.

resembles the binding situation of **6d**: the *iso*-butyl moieties occupy the S1 and S1′ pockets, and the *p*-amino-phenyl sulfonamide groups are located in the S2 and S2′ pockets. The amino substituents form a similar hydrogen bond network as already described for **6d**: hydrogen bonds to the main chain carbonyl oxygen of Asp30A, Asp30B as well as water mediated to the corresponding side chain carboxyl oxygens are formed. The most pronounced



Scheme 4. Schematic representation of the binding mode of **9b**. Hydrogen bonds are indicated by dashed lines and distances are given in Å.

difference between both complexes is the deviating polar interaction of the sulfonyl groups of the inhibitor to the flap. Although a water molecule is involved in the mediation of polar contacts of the sulfonyl oxygen atoms in both cases, a different coordination is observed. In contrast to **6d**, the water in the enzyme–inhibitor complex of **9b** is not tetrahedrally coordinated. It only forms hydrogen bonds to two sulfonyl oxygen atoms and the main chain NH of Ile50A. One of the sulfonyl oxygen atoms establishes an intramolecular hydrogen bond to the pivotal oligoamine nitrogen atom, as observed in case of **6d**. In contrast to the previous structure, the second sulfonyl oxygen atom directly addresses Ile50 NH of the B chain, whereas in case of **6d**, this oxygen atom remains without any polar contacts. The overall burial of the inhibitor sums up to 93%.

3. Discussion

The biological data as well as the crystal structures of **6d** and **9b** in complex with HIV-1 protease clearly reveal that our initial design concept has been successful. For all of the investigated aspartic proteases inhibitors binding in the micromolar range could be identified. Noteworthy, for most of these enzymes a preference toward ethylene linkers is observed. The sulfonamide acceptor groups allowing additional rotational degrees of freedom are favored compared to the carbonyl groups for all targets except for renin. In the latter case the more rigid structure of N-alkylated carboxamides is obviously preferred for inhibition.

The binding modes of **6d** and **9b** in HIV-1 protease reveal how these compounds fulfill the initial pharmacophore hypothesis. As intended, the secondary amino group addresses the catalytic dyad, and two of the sulfonamide oxygen atoms mimic the substrate's amide carbonyl groups. This is illustrated by the C_α-superposition of the complex structures of **6d** and **9b**, respectively, with the crystal structure of a substrate analogue oligopeptide in complex with an inactive HIV-1 protease mutant (PDB code: 1KJH) (Fig. 3a and b).[21] In addition, the specificity pockets of the enzyme are occupied analogously; however, the direction in which the S1/S1′ pocket is addressed deviates from the substrate's one. The 10-fold lower binding affinity of the inhibitor **6d** compared to **9b** can most likely be attributed to this difference: the benzyl moieties of **6d** are in our case now slightly too large to occupy the S1/S1′ pockets appropriately.

The C_α-superposition of the crystal structures in complex with the HIV-1 protease of the approved peptidomimetic inhibitor Amprenavir (PDB code: 1HPV) and **9b**, which bears the same P1 and P2 substituents, is shown in Figure 3c.[44] Comparing the positions of the *N*-*iso*-butyl-*N*-*p*-amino-phenyl sulfonamide moieties of the inhibitors which address the S1 and S2 pocket, only slight differences can be observed. Although the inhibitors comprise

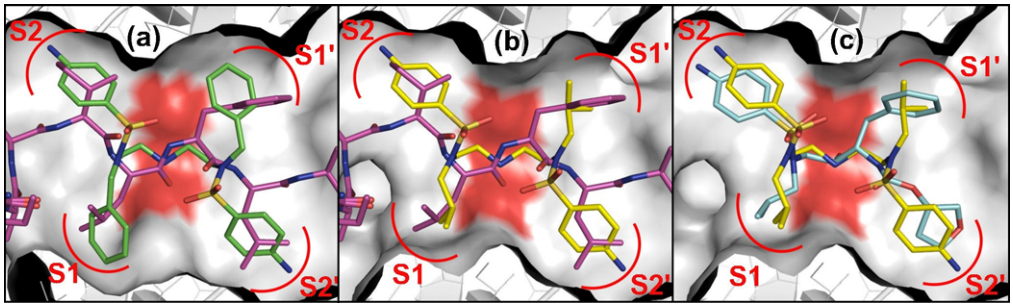


Figure 3. (a and b) C_α-superposition of the cocrystal structures of **6d** (green, color-coded by atom type) and **9b** (yellow, color-coded by atom type) with the structure of a substrate analogue polypeptide (magenta, color-coded by atom type) in complex with an inactive Asp25Asn HIV protease mutant (PDB code: 1KJH). (c) Superposition of the cocrystal structure of **9b** (yellow, color-coded by atom type) with the cocrystal structure of the approved inhibitor Amprenavir (blue, color-coded by atom type, PDB code: 1HPV). The protein surface is schematically represented in gray and the catalytic aspartates Asp25A and Asp25B are highlighted in red.

different moieties, the occupation of the S1′ and S2′ pockets appears similar. However, due to their polymethylene linkers, the oligoamine derivatives are very flexible molecules and a high degree of pre-organization prior to binding to the protein is required, resulting in an entropic penalty thus lowering the overall affinity. Additionally, the position of the flap-water molecule present in the complexes of **6d** and **9b** is different in the oligoamine structures when compared to the substrate-like oligopeptide and Amprenavir complexes. In case of **6d**, a similar tetrahedral coordination of the water molecule is observed, but it is shifted about 1 Å away from the catalytic dyad. A deviation from the usually observed coordination is detected in case of the **9b** complex leading to a direct interaction of one sulfonyl oxygen atom with the flap. These observations indicate that the flap interactions of the oligoamines **6d** and **9b** in case of HIV-1 might require some further improvement. Taking all these facts into account, the lower affinity by three orders of magnitude of the oligoamines compared to Amprenavir (K_i = 0.6 nM) seems reasonable.

The observed peptide-like binding mode in case of the ethylene-bearing inhibitors **6d** and **9b** in complex with HIV-1 protease suggests a similar binding situation in case of the other pepsin-like proteases. In this case, the acceptor functionalities of the inhibitors mimic the carbonyl groups of the substrate adjacent to the cleavage site, resulting in the same subsite occupation as observed for **6d** and **9b**. However, in contrast to HIV protease, there is no clear preference for the ethylene linkers. In pepsin-like proteases, amino acids bearing flexible polar side chains are located in the flap region: Thr77 in case of pepsin (Fig. 1d), Ser76 and Thr77 in case of renin, Thr72 and Gln73 in case of BACE-1, and Ser79 in case of Plm II and Plm IV. In addition to the peptide recognition motifs, these residues might also be addressed by the acceptor groups of the inhibitors resulting in the preference for longer linkers. The binding of inhibitors with propylene linkers results in a larger distance between the sub-pocket-addressing hydrophobic moieties and could omit occupancy of the S1 or S1′ pockets. This might explain the inhibition of BACE-1 only by the inhibitors **6c** and **6f**, because the S1′ pocket of BACE-1 is featured to recognize an aspartic acid side chain. Most likely, the studied oligoamine inhibitors are not suitable to address this site.

4. Summary and conclusion

In this study, we present a novel rational strategy for the development of aspartic protease inhibitors. This method is based on achiral linear oligoamines, which are easily accessible via a short and flexible synthetic route starting from commercially available precursors. Within the initial compound series comprising 11 inhibitors, several hits for the target enzymes could be identified. Some of them reveal remarkable selectivity, others show broad promiscuity. Noteworthy, for five out of six target enzymes, at least one single-digit micromolar inhibitor could be identified. The crystal structures of two representatives in complex with the HIV-1 protease proved the concept initially used for their design. The central amino group addresses the catalytic dyad, the acceptor groups establish polar interactions to the flap, and the hydrophobic moieties occupy the sub-pockets of the enzyme. The binding mode exhibits high similarity to the binding orientation of substrates as well as to that of peptidomimetic inhibitors. Taking this into account, the generalization of this binding situation to other aspartic proteases appears reasonable thus providing a first insight into the observed structure–activity relationships.

The straightforward synthesis allows the combinatorial introduction of numerous different substituents, thus facilitating the preparation of a plethora of putative aspartic protease inhibitors. The derived SAR data from such libraries can easily be utilized for the selection of promising moieties for selectively addressing the specificity pockets of a target enzyme in order to optimize inhibitors bearing other scaffolds. Hits from these oligoamine libraries can also be utilized as suitable starting point for further optimization. Structural variation of the oligoamine chain based on a certain inhibitor, at best guided by a crystal structure, can lead to novel derived amine-based scaffolds. Increasing the rigidity of the linker chain is the most promising approach, leading to a better preorganization of the inhibitor and a reduction of the entropic penalty upon binding hence most likely resulting in a gain of affinity.

5. Experimental

5.1. Molecular biology

5.1.1. Enzyme assays

All enzyme assays were performed at room temperature on a microplate reader (Safire^{2}™) using black 96-well microtiter plates purchased from Nunc. The assay volume was 200 µl, and inhibitors and substrates were previously dissolved in dimethyl sulfoxide (final concentration 4%). The hydrolysis of the substrates was recorded as increase in fluorescence intensity. IC_{50} values were generated by nonlinear regression analysis from plots of v_i/v_0 versus inhibitor concentration, in which v_i is the velocity in presence, and v_0 the velocity in the absence of an inhibitor. The kinetic constants were determined by the method of Lineweaver and Burk, and K_i values were consecutively calculated from the following equation: $K_i = IC_{50}/[1 + (S/K_m)]$. The overall error of the assays is estimated to be ±40%.

5.1.2. HIV-1 protease

Recombinant HIV-1 protease was expressed from *Escherichia coli* and purified as previously described.[45] The fluorogenic sub-

strate Abz-Thr-Ile-Nle-(*p*-NO_2-Phe)-Gln-Arg-NH_2 was purchased from Bachem and exhibited a K_m of 14.6 μM. The assays were performed in 100 mM MES, 300 mM KCl, 5 mM EDTA 1 mg/mL BSA, pH 5.5, and a substrate concentration of 20 μM (excitation wavelength 337 nm, emission wavelength 410 nm).[46]

5.1.3. Plasmepsin II and plasmepsin IV

Recombinant plasmepsin II and plasmepsin IV were expressed from *Escherichia coli* and purified following the protocol described by Hill et al.[47] The fluorogenic substrate Abz-Thr-Ile-Nle-(*p*-NO_2-Phe)-Gln-Arg-NH_2 was purchased from Bachem (K_m (Plm II) = 63 μM and K_m (Plm IV) = 28 μM). The assays were performed in 100 mM NaAc, pH 4.5, 1 mg/mL BSA and a substrate concentration of 18 μM (excitation wavelength 337 nm, emission wavelength 410 nm).

5.1.4. Renin

The fluorogenic substrate Arg-Glu(EDANS)-Ile-His-Pro-Phe-His-Leu-Val-Ile-His-Thr-Lys(DABCYL)-Arg and recombinant renin were purchased from Sigma–Aldrich (K_m = 3.3 μM). Assays were performed in 50 mM Tris–HCl, 100 mM NaCl, 1 mM EDTA, pH 8.0, and a substrate concentration of 5 μM (excitation wavelength 340 nm, emission wavelength 500 nm).[48]

5.1.5. β-Secretase

The fluorogenic substrate MOCAc-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Arg-(2,4-dinitrophenyl)-Lys-Arg-Arg-NH_2 and recombinant β-secretase were purchased from Sigma (K_m = 7.9 μM). Assays were performed in 100 mM NaAc, pH 4.5, and a substrate concentration of 5 μM (excitation wavelength 320 nm, emission wavelength 405 nm).

5.1.6. Pepsin

Pepsin from porcine gastric mucosa was purchased from Sigma–Aldrich, and the HIV protease substrate Abz-Thr-Ile-Nle-(*p*-NO_2-Phe)-Gln-Arg-NH_2 was used for kinetic measurements (K_m = 13.3 μM). Assays were performed in 10 mM NaHCOO, pH 3.5, and a substrate concentration of 20 μM (excitation wavelength 337 nm, emission wavelength 410 nm).

5.1.7. Crystallization of the HIV-1 protease inhibitor complexes

The HIV-1 protease inhibitor complexes were crystallized at 18 °C in 0.1 M Bis–Tris, pH 6.5, 2.5–3.0 M NaCl and a protein concentration of 7 mg/mL in the space group $P2_12_12$ (crystal data, Table 3). The crystals were obtained by cocrystallization of the enzyme with inhibitor concentrations ranging from 20- to 100-fold the K_i value. Crystals were further optimized using streak-seeding techniques. For cryoprotection, the crystals were briefly soaked in mother liquor containing 25% glycerol.

5.1.8. Data collection, phasing, and refinement

The data sets were collected on a Rigaku R-AXIS IV image plate detector using CuK_α radiation from an in-house RU-H3R Rigaku rotating anode. Data were processed and scaled with Denzo and Scalepack as implemented in HKL2000.[49] The structures were determined by the molecular replacement method using Phaser,[50] one monomer of the 1.50 Å HIV-1 protease in complex with a pyrrolidine-based inhibitor (PDB code: 2PQZ) was used as the search model. The structure refinement was continued with SHELXL-97,[51] for each refinement step, at least 10 cycles of conjugate gradient minimization were performed, with restraints on bond distances, angles, and *B*-values. Intermittent cycles of model building were done with the program COOT.[52] The coordinates have been deposited in the PDB (http://www.rcsb.org/pdb/) with access codes: 3BGC and 3BGB.

5.2. Chemistry

5.2.1. General

Reported yields refer to the analytically pure product obtained by column chromatography or recrystallization. All proton and carbon nuclear magnetic resonance spectra were recorded on a Jeol Eclipse+ Spectrometer (^1H NMR: 500 MHz, ^{13}C NMR: 125 MHz) using TMS as internal standard (0.00 ppm). ^{13}C-spectra were referenced to $CDCl_3$ (77.0 ppm), DMSO-d_6 (39.7 ppm), or CD_3OD (49.0 ppm). The values of chemical shifts (δ) are given in ppm and coupling constants (J) are given in Hz. Abbreviations: br = broad, s = singulet, d = doublet, t = triplet, q = quartet, sm = symmetric multiplet, m = multiplet. Mass spectra were obtained from a double-focusing sectorfield Micromass VG-Autospec spectrometer. Combustion analyses were determined on a Vario Micro Cube by Elementar Analysen GmbH. Melting Points were determined using a Leitz HM-Lux apparatus and are uncorrected. Flash chromatography was performed with a Büchi Sepacore Flash MPLC system using silica gel 60 (0.04–0.063 mm) purchased from Macherey-Nagel and solvents as indicated. TLC was carried out using 0.2 mm aluminium plates coated with silica gel 60 F_{254} by Merck and visualized by UV-detection or ninhydrin staining. Solvents and reagents that are commercially available were used without further purification unless otherwise noted. All moisture-sensitive reactions were carried out using oven-dried glassware under a positive stream of argon.

5.2.2. General procedure A for the preparation of *N*-Boc-protected bis-trifluoromethylacetamides of type 2

In accordance to a procedure of Hodačová et al.,[43] ethyl trifluoromethylacetate (15.6 g, 12.5 mL, 110 mmol) in CH_2Cl_2 (50 mL) was added dropwise to a stirred solution of 50.0 mmol of the corresponding oligoamine **1** in CH_2Cl_2 (100 mL) at 0 °C, and the reaction mixture was stirred for 30 min at 0 °C. After addition of CH_2Cl_2 (20 mL) to dissolve the resulting precipitate, the mixture was allowed to warm to rt and stirred for additional 60 min. A mixture of triethylamine (11.1 g, 15.3 mL, 110.0 mmol) and Boc_2O (24.0 g, 110.0 mmol) in CH_2Cl_2 (50 mL) was then added dropwise to the solution and stirring was continued for 5 h. The reaction mixture was washed with a satd $NaHCO_3$-solution (2× 80 mL) and water (2× 80 mL), the organic layer separated, dried over K_2CO_3, and filtered. After concentration of the organic layer to about one third of its original volume, an equal volume of hexanes was added. The mixture was kept in the freezer (−12 °C) rendering colorless crystals, which were separated by filtration, washed with water followed by hexanes, and finally dried under vacuum giving rise to the respective *N*-Boc-protected bis-trifluoroacetamides **2a**–**c**.

5.2.3. General procedure B for the preparation of *N*-Boc-protected oligoamines of type 3

According to a procedure of Hodačová et al.,[43] an aqueous solution of NaOH (5.00 g, 125.0 mmol, in 42 mL water) was added to a solution of 10.0 mmol of the respective *N*-Boc-bis-trifluoroacetamide **2** in MeOH (50 mL), and the reaction mixture stirred for 6 h at rt. The major amount of methanol was removed under reduced pressure and the remaining solution extracted with CH_2Cl_2 (3× 40 mL). The combined organic layers were dried over K_2CO_3, filtered, evaporated, and finally dried in vacuum furnishing the respective *N*-Boc-protected oligoamines **3a**–**c** as colorless oils.

5.2.4. General procedure C for the preparation of *N*-Boc-protected bis-arylsulfonamides of type 4

To a stirred solution of the corresponding *N*-Boc-protected oligoamine **3** (5.0 mmol) in CH_2Cl_2 (17 mL), triethylamine (1.42 g, 1.95 mL, 14.0 mmol) was added, followed by slow addition of the respective arylsulfonyl chloride (11.0 mmol) in CH_2Cl_2 (10 mL) at

0 °C. The reaction mixture was allowed to reach rt, stirred for additional 12 h, and quenched with H_2O (42 mL). After addition of MTBE (42 mL), the aqueous phase was separated and extracted with MTBE (3× 17 mL). The combined organic layers were washed with a satd $NaHCO_3$-solution (1× 50 mL) and brine (1× 50 mL), dried over $MgSO_4$, filtered, and evaporated. The residual oil was further purified by FC yielding the analytically pure *N*-Boc-protected bis-arylsulfonamides **4a**–**f**.

5.2.5. General procedure D for benzylation of *N*-Boc-protected bis-arylsulfonamides of type 4

To a suspension of the respective *N*-Boc-protected bis-arylsulfonamide **4** (1.50 mmol) and anhydrous K_2CO_3 (4.50 mmol) in dry CH_3CN (12 mL), a catalytic amount of KI and benzyl bromide (3.30 mmol) were added, and the reaction mixture was stirred for 3 h under reflux. After cooling to rt, insoluble material was filtered off, the remaining residue washed with MTBE, and the combined filtrates concentrated in vacuum. The remaining oily residue was purified by FC giving rise to the analytically pure bis-benzylated *N*-Boc-protected bis-arylsulfonamides **5a**–**f**.

5.2.6. General procedure E for Boc-deprotection of *N*-Boc-protected bis-arylsulfonamides of type 5

A solution of the respective *N*-Boc-protected bis-arylsulfonamide **5** (~0.5 mmol) in 2 M HCl in Et_2O (8 mL) was stirred at rt until TLC indicated completion of the reaction (12–36 h). The solution was decanted from the resulting precipitate, which was thoroughly washed with dry ether and finally dried in vacuum rendering the *N*-Boc-deprotected bis-arylsulfonamides **6a**–**c** as their hydrochlorides.

5.2.7. General procedure F for the reduction of nitro groups and concomitant Boc-deprotection of *N*-Boc-protected bis-nitro-arylsulfonamides 5d–f and 8b

The corresponding Boc-protected nitroarylsulfonamide (0.13–0.46 mmol) was suspended in 32% HCl (5–15 mL). After addition of $SnCl_2{\cdot}2H_2O$ (8 equiv), the suspension was refluxed for 3.5 h and subsequently allowed to reach rt. A 10 M NaOH-solution was carefully added until pH 12 was obtained. The resulting mixture was extracted with CH_2Cl_2 (3× 10 mL), and the combined organic layers were washed with brine (2× 10 mL), dried over $MgSO_4$, filtered, and evaporated. The residual oil was purified by FC (CH_2Cl_2/MeOH 95:5) and the product-containing fractions were combined and concentrated. After treatment with 2 M HCl in Et_2O (8–20 mL), a precipitate was formed which was triturated with Et_2O and dried in vacuum yielding compounds **6d**–**f** and **9b** as their hydrochlorides.

5.2.8. General procedure G for the preparation of *N*-Boc-protected bis-benzylamines of type 10 via reductive amination

To a solution of the corresponding *N*-Boc-protected oligoamine of type **3** (6.1–9.8 mmol) in MeOH (100 mL), benzaldehyde (2 equiv) and 10% of Pd/$BaSO_4$ were added. A permanent H_2-atmosphere was applied to the reaction flask and the mixture stirred for 16 h. The catalyst was removed by filtration through a pad of Celite, and the filtrate was concentrated in vacuum yielding the corresponding *N*-Boc-protected bis-benzylamine derivatives **10a**–**c**.

5.2.9. General procedure H for the preparation of *N*-Boc-protected bis-benzylated carboxamides of type 11 utilizing phenylacetic acid chloride

To a stirred solution of the corresponding *N*-Boc-protected bis-benzylated oligoamine of type **10** (0.68–1.5 mmol) in CH_2Cl_2 (5–10 mL), phenylacetic acid chloride (2.2 equiv), triethylamine (2.5 equiv), and a catalytic amount of DMAP were added at 0 °C. After allowing the reaction mixture to reach rt, stirring was continued overnight. The reaction was quenched by addition of a satd $NaHCO_3$-solution (10–20 mL) followed by CH_2Cl_2 (10–20 mL). The aqueous layer was extracted with CH_2Cl_2 (2× 25–50 mL). The combined organic phases were washed with brine (2× 30–60 mL), dried over $MgSO_4$, filtered, and evaporated. The resid was further purified by MPLC rendering the respective derivatives **11a**–**c**.

5.2.10. Bis-[2-(2,2,2-trifluoro-acetylamino)-ethyl]-carbamic acid *tert*-butyl ester (2a)

Following the general procedure A, employment of oligoamine **1a** (5.15 g, 50.0 mmol) yielded 14.12 g (71%) of **2a**. The compound exhibited identical physical and spectroscopic data to those described earlier.[43] Colorless crystals; mp: 125 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ = 1.36 (s, 9H), 3.30 (s, 8H), 9.41 (br s, 2H).

5.2.11. [2-(2,2,2-Trifluoro-acetylamino)-ethyl]-[3-(2,2,2-trifluoro-acetylamino)-propyl]-carbamic acid *tert*-butyl ester (2b)

According to general procedure A, utilization of oligoamine **1b** (5.86 g, 50.0 mmol) gave rise to 17.66 g (86%) of **2b**. Colorless crystals; mp: 113 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ = 1.38 (s, 9H), 1.69 (sm, 2H, J = 6.9), 3.16 (sm, 4H, J = 7.0), 3.26–3.30 (m, 4H), 9.39 (br s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers) δ = 27.1, 27.7, 28.0, 37.2, 38.0, 44.3, 44.9, 45.1, 45.3, 78.9, 116.0 (q, J = 287.9), 116.1 (q, J = 287.9), 154.8, 155.0, 156.4 (q, J = 36.5), 156.5 (q, J = 36.5); MS (ESI) m/z = 432 (100, $[M+Na]^+$), 841 (25, $[2M+Na]^+$); HRMS (ESI) $[M+NH_4]^+$ Calcd 427.1780, found 427.1806; Anal. Calcd for $C_{14}H_{21}F_6N_3O_4$: C, 41.08; H, 5.17; N, 10.27. Found: C, 41.20; H, 4.96; N, 10.20.

5.2.12. Bis-[3-(2,2,2-trifluoro-acetylamino)-propyl]-carbamic acid *tert*-butyl ester (2c)

Following general procedure A, employment of oligoamine **1c** (6.56 g, 50.0 mmol) furnished 15.28 g (72%) of **2c**. Colorless crystals; mp: 68 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ = 1.38 (s, 9H), 1.68 (sm, 4H, J = 7.1), 3.11–3.18 (m, 8H); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers) δ = 27.1, 27.9, 28.2, 36.1, 37.4, 43.0, 44.4, 81.0, 115.9 (q, J = 286.9), 156.8, 157.3 (q, J = 37.0); MS (ESI) m/z = 324 (100, $[M-CO_2-C_4H_8+H]$), 441 (100, $[M+NH_4]^+$), 864 (9, $[2M+NH_4]^+$); HRMS (ESI) Calcd 441.1937, found 441.1930; Anal. Calcd for $C_{15}H_{23}F_6N_3O_4{\cdot}H_2O$: C, 40.82; H, 5.71; N, 9.52. Found: C, 40.73; H, 5.56; N, 9.34.

5.2.13. Bis-(2-amino-ethyl)-carbamic acid *tert*-butyl ester (3a)

According to general procedure B, usage of bis-trifluoroacetamide **2a** (3.95 g, 10.0 mmol) yielded 1.89 g (93%) of **3a**. The compound exhibited identical physical and spectroscopic data to those described earlier.[43] Colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 1.23 (s, 4H), 1.43 (s, 9H), 2.81 (t, 4H, J = 6.9), 3.25 (br s, 4H).

5.2.14. (2-Amino-ethyl)-(3-amino-propyl)-carbamic acid *tert*-butyl ester (3b)

According to general procedure B, utilization of bis-trifluoroacetamide **2b** (4.09 g, 10.0 mmol) gave rise to 1.71 g (79%) of **3b**. Colorless oil; ^{1}H NMR (500 MHz, DMSO-d_6) δ = 1.44 (s, 9H), 1.57 (br s, 4H), 1.65 (sm, 2H, J = 6.9), 2.69 (t, 2H, J = 6.6), 2.80 (t, 2H, J = 6.6), 3.21 (br s, 4H); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers) δ = 28.2, 31.4, 32.2, 38.8, 40.3, 40.5, 44.3, 44.8, 49.8, 79.3, 155.6; MS (EI) m/z = 217 (1, $[M]^+$), 188 (100, $[M-C_2H_5]^+$), 175 (89, $[M-C_3H_6]^+$), 132 (78), 131 (76), 127 (64), 119 (17); HRMS (ESI) Calcd 218.1869, found 218.1865.

5.2.15. Bis-(3-amino-propyl)-carbamic acid *tert*-butyl ester (3c)

Following general procedure A, employment of bis-trifluoroacetamide **2c** (4.23 g, 10.0 mmol) gave rise to 2.22 g (96%) of **3c**. Colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 1.30 (s, 4H), 1.42 (s, 9H),

1.62 (sm, 4H, J = 6.9), 2.66 (t, 4H, J = 6.6), 3.23 (br s, 4H); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers) δ = 28.0, 31.5, 32.1, 38.8, 39.0, 43.4, 43.9, 78.8, 155.3; MS (ESI) m/z = 232 (100, $[M+H]^+$), 463 (80, $[2M+H]^+$); HRMS (ESI) Calcd 232.2025, found 232.2049; Anal. Calcd for $C_{11}H_{25}N_3O_2{\cdot}0.5H_2O$: C, 54.97; H, 10.90; N, 17.48. Found: C, 54.59; H, 10.90; N, 17.08.

5.2.16. Bis-(2-benzenesulfonylamino-ethyl)-carbamic acid *tert*-butyl ester (4a)

According to general procedure C, utilization of **3a** (1.02 g, 5.0 mmol) and benzenesulfonyl chloride (1.42 mL, 11.0 mmol) furnished, after FC (hexanes/EtOAc 1:1), 1.78 g (74%) of bis-sulfonamide **4a**. Colorless crystals; mp: 58–62 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ = 1.32 (s, 9H), 2.80 (sm, 4H, J = 6.5), 3.12 (br s, 4H), 7.51–7.75 (m, 8H), 7.76–7.80 (m, 4H); ^{13}C NMR (125 MHz, DMSO-d_6, rotamers) δ = 28.2, 41.9, 42.2, 47.8, 48.5, 80.9, 126.6, 129.1, 132.6, 139.7, 156.1; MS (ESI) m/z = 506 (57, $[M+Na]^+$), 989 (100, $[2M+Na]^+$), 1472 (29, $[3M+Na]^+$); HRMS (ESI) Calcd 484.1576, found 484.1553; Anal. Calcd for $C_{21}H_{29}N_3O_6S_2$: C, 52.16; H, 6.04; N, 8.69. Found: C, 52.39; H, 5.89; N, 8.71.

5.2.17. (2-Benzenesulfonylamino-ethyl)-(3-benzenesulfonyl-amino-propyl)-carbamic acid *tert*-butyl ester (4b)

Following general procedure C, employment of **4b** (1.09 g, 5.0 mmol) and benzenesulfonyl chloride (1.42 mL, 11.0 mmol) yielded, after purification via FC (hexanes/EtOAc 1:1), 1.60 g (66%) of bis-sulfonamide **4b**. Colorless crystals; mp: 55–60 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ = 1.29 (s, 9H), 1.46–1.54 (m, 2H), 2.65 (sm, 2H, J = 7.3), 2.79 (sm, 4H, J = 6.9), 3.01–3.10 (m, 4H), 7.40–7.61 (m, 8H), 7.77 (t, 4H, J = 6.8); ^{13}C NMR (125 MHz, DMSO-d_6) δ = 27.9, 28.2, 28.7, 39.8, 40.5, 41.7, 42.3, 44.1, 45.2, 46.9, 80.8, 126.6, 126.9, 129.0, 129.1, 132.4, 132.7, 139.7, 139.8, 156.1; MS (ESI) m/z = 515 (56, $[M+H_2O]^+$), 1012 (100, $[2M+H_2O]^+$); HRMS (ESI) Calcd 498.1733, found 498.1748; Anal. Calcd for $C_{22}H_{31}N_3O_6S_2$: C, 53.10; H, 6.28; N, 8.44; S, 12.89. Found: C, 52.75; H, 6.08; N, 8.26; S, 12.29.

5.2.18. Bis-(3-benzenesulfonylamino-propyl)-carbamic acid *tert*-butyl ester (4c)

According to general procedure C, utilization of **3c** (1.12 g, 5.0 mmol) and benzenesulfonyl chloride (1.42 mL, 11.0 mmol) gave rise to 2.13 g (83%) of bis-sulfonamide **4c** after FC (hexanes/EtOAc 1:1). Colorless crystals; mp: 55–60 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 1.35 (s, 9H), 1.61 (br s, 4H), 2.88 (br s, 4H), 3.03–3.21 (m, 4H), 4.91 (s, 1H), 6.03 (s, 1H), 7.40–7.63 (m, 6H), 7.84 (d, 4H, J = 7.1); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers) δ = 27.8, 28.2, 28.4, 39.8, 40.6, 43.1, 44.0, 80.3, 126.9, 129.0, 132.3, 132.6, 139.8, 140.2, 156.2; MS (ESI) m/z = 534 (85, $[M+Na]^+$), 1045 (100, $[2M+Na]^+$), 1556 (20, $[3M+Na]^+$); HRMS (ESI) Calcd 534.1708, found 534.1674; Anal. Calcd for $C_{23}H_{33}N_3O_6S_2$: C, 53.99; H, 6.50; N, 8.21; S, 12.53. Found: C, 53.98; H, 6.46; N, 8.23; S, 12.45.

5.2.19. Bis-[2-(4-nitro-benzenesulfonylamino)-ethyl]-carbamic acid *tert*-butyl ester (4d)

According to general procedure C, utilization of **3a** (1.02 g, 5.0 mmol) and 4-nitrobenzenesulfonyl chloride (2.44 g, 11.0 mmol) furnished 1.71 g (60%) of bis-sulfonamide **4d** after FC (hexanes/EtOAc 3:2, then CH_2Cl_2/MeOH 4:1). Colorless crystals; mp: 158 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ = 1.33 (s, 9H), 2.89 (t, 4H, J = 6.6), 3.14 (t, 4H, J = 6.4), 8.02 (d, 4H, J = 8.7), 8.40 (d, 4H, J = 8.5); ^{13}C NMR (125 MHz, DMSO-d_6) δ = 28.1, 40.8, 41.2, 47.5, 47.9, 79.2, 124.7, 128.1, 146.3, 149.7, 154.5; MS (ESI) m/z = 596 (100, $[M+Na]^+$), 612 (27, $[M+K]^+$); HRMS (ESI) Calcd 596.1097, found 596.1118; Anal. Calcd for $C_{21}H_{27}N_5O_{10}S_2$: C, 43.97; H, 4.74; N, 12.21; S, 11.18. Found: C, 44.32; H, 4.67; N, 12.13; S, 10.82.

5.2.20. [2-(4-Nitro-benzenesulfonylamino)-ethyl]-[3-(4-nitro-benzenesulfonylamino)-propyl]-carbamic acid *tert*-butyl ester (4e)

Following the general procedure C, employment of **3b** (0.71 g, 3.3 mmol) and 4-nitrobenzenesulfonyl chloride (1.61 g, 7.3 mmol) yielded, after purification via FC (hexanes/EtOAc 3:2, then CH_2Cl_2/MeOH 4:1), 1.41 g (73%) of bis-sulfonamide **4e**. Colorless crystals; mp: 81 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ = 1.29 (s, 9H), 1.53 (br s, 2H), 2.72 (t, 2H, J = 7.3), 2.87 (t, 2H, J = 6.6), 3.05 (t, 2H, J = 7.1), 3.09 (t, 2H, J = 6.4), 8.01 (d, 4H, J = 8.9), 8.40 (d, 4H, J = 8.7); ^{13}C NMR (125 MHz, DMSO-d_6, rotamers) δ = 28.0, 28.6, 40.5, 40.9, 41.2, 44.7, 45.2, 46.6, 46.8, 79.0, 124.7, 128.1, 128.2, 146.1, 146.3, 149.7, 154.6; MS (ESI) m/z = 610 (100, $[M+Na]^+$), 1197 (39, $[2M+Na]^+$); HRMS (ESI) Calcd 610.1254, found 610.1286; Anal. Calcd for $C_{22}H_{29}N_5O_{10}S_2$: C, 44.97; H, 4.97; N, 11.92; S, 10.91. Found: C, 44.95; H, 4.85; N, 11.85; S, 10.56.

5.2.21. Bis-[3-(4-nitro-benzenesulfonylamino)-propyl]-carbamic acid *tert*-butyl ester (4f)

According to general procedure C, utilization of **3c** (1.00 g, 4.3 mmol) and 4-nitrobenzenesulfonyl chloride (2.11 g, 9.5 mmol) rendered **4f**, which partly crystallized during work-up. The remaining oily portion was purified by FC (hexanes/EtOAc 3:2, then CH_2Cl_2/MeOH 3:1), overall 1.92 g (74%) of bis-sulfonamide **4f** was obtained. Yellow crystals; mp: 152 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ = 1.27 (s, 9H), 1.53 (sm, 4H, J = 6.7), 2.72 (t, 4H, J = 7.1), 3.01 (t, 4H, J = 7.1), 3.30 (br s, 2H), 8.02 (d, 4H, J = 8.7), 8.40 (d, 4H, J = 8.7); ^{13}C NMR (125 MHz, DMSO-d_6) δ = 28.0, 28.2, 40.2, 44.2, 78.7, 124.7, 128.2, 146.1, 149.7, 154.6; MS (ESI) m/z = 602 (42, $[M+H]^+$), 619 (100, $[M+NH_4]^+$), 624 (52, $[M+Na]^+$); HRMS (ESI) Calcd 624.1410, found 624.1403; Anal. Calcd for $C_{23}H_{31}N_5O_{10}S_2$: C, 45.91; H, 5.19; N, 11.64; S, 10.66. Found: C, 46.23; H, 5.37; N, 11.30; S, 10.50.

5.2.22. Bis-[2-(benzenesulfonyl-benzyl-amino)-ethyl]-carbamic acid *tert*-butyl ester (5a)

According to general procedure D, utilization of bis-sulfonamide **4a** (0.73 g, 1.5 mmol) furnished, after FC (hexanes/EtOAc 3:1), 0.47 g (47%) of bis-benzylated bis-sulfonamide **5a**. Colorless crystals; mp: 44 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 1.35 (s, 9H), 2.95–3.03 (m, 4H), 3.05–3.14 (m, 4H), 4.33 (d, 4H, J = 14.0), 7.10–7.29 (m, 10H), 7.49–7.53 (m, 6H), 7.80 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers) δ = 28.3, 45.7, 45.9, 46.0, 46.8, 52.1, 52.7, 80.0, 127.1, 127.8, 128.0, 128.4, 128.6, 128.7, 129.1, 129.2, 132.5, 132.6, 135.9, 139.6, 140.0, 154.8; MS (ESI) m/z = 686 (100, $[M+Na]^+$), 1349 (49, $[2M+Na]^+$); HRMS (ESI) Calcd 664.2515, found 664.2508; Anal. Calcd for $C_{35}H_{41}N_3O_6S_2$: C, 63.32; H, 6.23; N, 6.33; S, 9.66. Found: C, 63.50; H, 6.11; N, 6.22; S, 13.52.

5.2.23. [2-(Benzenesulfonyl-benzyl-amino)-ethyl]-[3-(benzenesulfonyl-benzyl-amino)-propyl]-carbamic acid *tert*-butyl ester (5b)

Following the general procedure D, employment of bis-sulfonamide **4b** (1.39 g, 2.8 mmol) yielded, after purification via FC (hexanes/EtOAc 3:1), 1.25 g (66%) of bis-benzylated bis-sulfonamide **5b**. Colorless crystals; mp: 46 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 1.30 (s, 9H), 1.36–1.39 (m, 2H), 2.60–3.22 (m, 8H), 4.21–4.33 (m, 4H), 7.21–7.35 (m, 10H), 7.45–7.64 (m, 6H), 7.81–7.88 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers) δ = 27.3, 28.3, 44.6, 45.8, 46.1, 46.2, 52.1, 52.2, 53.2, 79.7, 127.06, 127.09, 127.8, 128.1, 128.3, 128.4, 128.6, 129.1, 132.5, 132.7, 136.0, 136.2, 139.3, 139.7, 155.0; MS (ESI) m/z = 700 (84, $[M+Na]^+$), 1377 (100, $[2M+Na]^+$); HRMS (ESI) Calcd 700.2491, found 700.2522; Anal. Calcd for $C_{36}H_{43}N_3O_6S_2$: C, 63.79; H, 6.39; N, 6.20; S, 9.46. Found: C, 63.70; H, 6.29; N, 6.12; S, 9.07.

5.2.24. Bis-[3-(benzenesulfonyl-benzyl-amino)-propyl]-carbamic acid *tert*-butyl ester (5c)

According to general procedure D, utilization of bis-sulfonamide **4c** (0.73 g, 1.4 mmol) gave rise to 0.79 g (80%) of bis-benzylated bis-sulfonamide **5c** after FC (hexanes/EtOAc 3:1). Colorless crystals; mp: 46 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ = 1.24 (s, 9H), 1.15–1.32 (m, 4H), 2.65 (br s, 4H), 2.95 (t, 4H, J = 7.3), 4.30 (s, 4H), 7.21–7.45 (m, 10H), 7.64 (t, 4H, J = 7.3), 7.70 (t, 2H, J = 7.6), 7.84 (d, 4H, J = 7.3); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers) δ = 27.2, 28.3, 43.9, 44.2, 46.2, 46.3, 52.4, 79.3, 126.8, 127.0, 127.9, 128.3, 128.6, 129.1, 132.5, 136.4, 139.6, 155.1; MS (ESI) m/z = 714 (71, $[M+Na]^+$), 1405 (100, $[2M+Na]^+$); HRMS (ESI) Calcd 714.2648, found 714.2680; Anal. Calcd for $C_{37}H_{45}N_3O_6S_2$: C, 64.23; H, 6.56; N, 6.07; S, 9.27. Found: C, 64.15; H, 6.51; N, 6.02; S, 8.89.

5.2.25. Bis-{2-[benzyl-(4-nitro-benzenesulfonyl)-amino]-ethyl}-carbamic acid *tert*-butyl ester (5d)

According to general procedure D, utilization of bis-nitrosulfonamide **4d** (1.15 g, 2.0 mmol) furnished, after FC (hexanes/EtOAc 3:2), 0.99 g (80%) of bis-benzylated bis-nitrosulfonamide **5d**. Colorless crystals; mp: 167 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 1.34 (s, 9H), 3.03–3.11 (m, 4H), 3.17–3.21 (m, 2H), 3.22–3.27 (m, 2H), 4.35 (s, 2H), 4.40 (s, 2H), 7.16–7.32 (m, 10H), 7.90 (d, 2H, J = 8.5), 7.94 (d, 2H, J = 8.5), 8.28 (d, 2H, J = 8.5), 8.33 (d, 2H, J = 8.5); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers) δ = 28.3, 45.9, 46.2, 46.5, 47.1, 52.3, 52.8, 80.5, 124.3, 124.4, 128.3, 128.4, 128.6, 128.8, 128.9, 135.1, 145.4, 145.7, 149.9, 150.0, 154.8; MS (ESI) m/z = 776 (100, $[M+Na]^+$), 1529 (37, $[2M+Na]^+$); HRMS (ESI) Calcd 776.2036, found 776.2079; Anal. Calcd for $C_{35}H_{39}N_5O_{10}S_2$: C, 55.76; H, 5.21; N, 9.29; S, 8.51. Found: C, 55.56; H, 5.31; N, 8.96; S, 8.80.

5.2.26. {2-[Benzyl-(4-nitro-benzenesulfonyl)-amino]-ethyl}-{3-[benzyl-(4-nitro-benzenesulfonyl)-amino]-propyl}-carbamic acid *tert*-butyl ester (5e)

Following the general procedure D, employment of bis-nitrosulfonamide **4e** (0.93 g, 1.6 mmol) yielded, after purification via FC (hexanes/EtOAc 3:2, then CH_2Cl_2), 0.89 g (73%) of bis-benzylated bis-nitrosulfonamide **5e**. Colorless crystals; mp: 68 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ = 1.27 (s, 9H), 2.72 (br s, 2H), 2.81–2.90 (m, 2H), 2.97 (br s, 2H), 3.13 (br s, 2H), 4.35 (s, 2H), 4.37 (s, 2H), 7.16–7.33 (m, 10H), 8.07–8.12 (m, 4H), 8.42 (d, 4H, J = 8.7); ^{13}C NMR (125 MHz, DMSO-d_6) δ = 26.6, 27.1, 28.0, 43.9, 44.6, 44.8, 45.4, 45.7, 45.9, 46.3, 51.5, 51.7, 52.2, 79.1, 124.9, 127.9, 128.0, 128.2, 128.6, 128.68, 128.72, 136.2, 136.4, 144.8, 144.9, 149.9, 154.3; MS (ESI) m/z = 790 (100, $[M+Na]^+$), 1557 (42, $[2M+Na]^+$); HRMS (ESI) Calcd 790.2193, found 790.2199; Anal. Calcd for $C_{36}H_{41}N_5O_{10}S_2$: C, 56.31; H, 5.38; N, 9.12; S, 8.35. Found: C, 56.13; H, 5.36; N, 8.98; S, 8.01.

5.2.27. Bis-{3-[benzyl-(4-nitro-benzenesulfonyl)-amino]-propyl}-carbamic acid *tert*-butyl ester (5f)

According to general procedure D, utilization of bis-sulfonamide **4f** (1.40 g, 2.3 mmol) gave rise to 0.99 g (55%) of bis-benzylated bis-sulfonamide **5f** after FC (hexanes/EtOAc 4:1, then CH_2Cl_2). Colorless crystals; mp: 113 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ = 1.34 (s, 9H), 1.42–1.51 (m, 4H), 2.80 (t, 4H, J = 6.4), 3.10 (t, 4H, J = 7.3), 4.34 (s, 4H), 7.19–7.32 (m, 10H), 7.97 (d, 4H, J = 8.5), 8.35 (d, 4H, J = 8.3); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 27.4, 28.3, 44.3, 46.4, 52.4, 79.7, 124.4, 128.2, 128.3, 128.8, 135.5, 145.5, 149.9, 155.2; MS (ESI) m/z = 782 (13, $[M+H]^+$), 804 (100, $[M+Na]^+$), 820 (15, $[M+K]^+$); HRMS (ESI) Calcd 804.2349, found 804.2368; Anal. Calcd for $C_{37}H_{43}N_5O_{10}S_2$: C, 56.84; H, 5.54; N, 8.96; S, 8.20. Found: C, 56.84; H, 5.65; N, 8.81; S, 8.27.

5.2.28. Bis-(2-(phenylsulfonyl-*N*-benzyl-amino)-ethyl)-amine hydrochloride (6a)

According to general procedure E, utilization of Boc-protected bis-sulfonamide **5a** (302 mg, 0.46 mmol) furnished 261 mg (96%) of hydrochloride **6a**. Colorless crystals; mp: 205 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ = 2.68 (br s, 4H), 3.29–3.33 (m, 4H), 4.34 (s, 4H), 7.25–7.32 (m, 10H), 7.70 (t, 4H, J = 7.6), 7.74 (t, 2H, J = 7.5), 7.86 (d, 4H, J = 7.3), 8.93 (br s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ = 43.6, 44.9, 52.0, 126.9, 127.7, 128.1, 128.4, 129.5, 133.1, 136.1, 138.4; MS (ESI) m/z = 564 (100, $[M-HCl+H]^+$), 586 (19, $[M-HCl+Na]^+$), 1127 (26, $[2M-2HCl+H]^+$), 1149 (19, $[2M-2HCl+Na]^+$); HRMS (ESI) Calcd 564.1991, found 564.1963; Anal. Calcd for $C_{30}H_{34}N_3O_4S_2Cl\cdot H_2O$: C, 58.28; H, 5.87; N, 6.80; S, 10.37. Found: C, 58.28; H, 5.56; N, 6.84; S, 10.43.

5.2.29. (2-(Phenylsulfonyl-*N*-benzylamino)-ethyl)-(3-(phenylsulfonyl-*N*-benzylamino)-propyl)-amine hydrochloride (6b)

Following the general procedure E, employment of Boc-protected bis-sulfonamide **5b** (358 mg, 0.53 mmol) yielded 279 mg (86%) of hydrochloride **6b**. Colorless crystals; mp: 185 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ = 1.56 (sm, 2H, J = 7.6), 2.54 (s, 2H), 2.62 (s, 2H), 3.12 (t, 2H, J = 7.2), 3.32 (t, 2H, J = 7.3), 4.30 (s, 2H), 4.34 (s, 2H), 7.22–7.38 (m, 10H), 7.61–7.75 (m, 6H), 7.87 (t, 4H, J = 7.1), 8.89 (br s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ = 24.6, 43.8, 44.2, 45.0, 45.6, 51.6, 52.3, 127.0, 127.1, 127.7, 127.9, 128.2, 128.4, 128.6, 128.7, 129.6, 129.7, 133.1, 133.3, 136.4, 136.8, 138.5, 139.1; MS (ESI) m/z = 578 (100, $[M-HCl+H]^+$), 1155 (19, $[2M-2HCl+H]^+$); HRMS (ESI) Calcd 578.2147, found 578.2148; Anal. Calcd for $C_{31}H_{36}N_3O_4S_2Cl\cdot 0.5H_2O$: C, 59.74; H, 5.98; N, 6.74; S, 10.29. Found: C, 60.14; H, 5.74; N, 6.79; S, 9.89.

5.2.30. Bis-(3-(phenylsulfonyl-*N*-benzylamino)-propyl)-amine hydrochloride (6c)

According to general procedure E, utilization of Boc-protected bis-sulfonamide **5c** (321 mg, 0.46 mmol) gave rise to 260 mg (89%) of hydrochloride **6c**. Colorless crystals; mp: 175 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ = 1.59 (sm, 4H, J = 7.3), 2.50 (s, 4H), 3.14 (t, 4H, J = 7.3), 4.32 (s, 4H), 7.20–7.33 (m, 10H), 7.63 (t, 4H, J = 7.3), 7.71 (t, 2H, J = 7.3), 7.88 (d, 4H, J = 7.3), 8.74 (br s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ = 24.5, 44.1, 45.7, 51.6, 127.0, 127.7, 128.2, 128.5, 129.5, 133.0, 136.8, 139.1; MS (ESI) m/z = 592 (100, $[M-HCl+H]^+$); HRMS (ESI) Calcd 592.2304, found 592.2311; Anal. Calcd for $C_{32}H_{38}N_3O_4S_2Cl$: C, 61.18; H, 6.10; N, 6.69; S, 10.21. Found: C, 61.10; H, 5.99; N, 6.55; S, 9.67.

5.2.31. Bis-(2-(4-amino-phenylsulfonyl-*N*-benzylamino)-ethyl)-amine hydrochloride (6d)

According to general procedure F, utilization of Boc-protected bis-nitrosulfonamide **5d** (100 mg, 0.13 mmol) furnished 60 mg (64%) of bis-aminosulfonamide **6d** as hydrochloride. Colorless crystals; mp: 140 °C; ^{1}H NMR (500 MHz, MeOH-d_4) δ = 2.67 (br s, 4H), 3.31 (br s, 4H), 4.30 (s, 4H), 7.05 (d, 4H, J = 8.0), 7.32 (s, 10H), 7.72 (d, 4H, J = 8.0); ^{13}C NMR (125 MHz, DMSO-d_6) δ = 43.9, 45.4, 52.4, 115.3, 125.6, 127.9, 128.3, 128.7, 129.2, 136.7, 150.3; MS (ESI) m/z = 594 (100, $[M-HCl+H]^+$), 1187 (4, $[2M-2HCl+H]^+$); HRMS (ESI) Calcd 594.2209, found 594.2237.

5.2.32. (2-(4-Aminophenylsulfonyl-*N*-benzylamino)-ethyl)-(3-(4-aminophenylsulfonyl-*N*-benzylamino)-propyl)-amine hydrochloride (6e)

Following the general procedure F, employment of Boc-protected bis-nitrosulfonamide **5e** (200 mg, 0.26 mmol) yielded 65 mg (35%) of bis-aminosulfonamide **6e** as hydrochloride. Colorless crystals; mp: 115 °C; ^{1}H NMR (500 MHz, MeOH-d_4) δ = 1.23–

1.31 (m, 2H), 2.01 (t, 2H, J = 7.1), 2.26 (t, 2H, J = 6.6), 2.95 (t, 2H, J = 6.9), 3.06 (t, 2H, J = 6.6), 4.20 (s, 4H), 6.72 (d, 4H, J = 8.5), 7.21–7.38 (m, 10H), 7.51–7.55 (m, 4H); ^{13}C NMR (125 MHz, MeOH-d_4) δ = 26.6, 46.4, 46.9, 47.4, 48.3, 54.7, 55.5, 119.4, 120.5, 129.5, 129.8, 130.2, 130.4, 130.5, 130.8, 131.0, 133.6, 137.5, 138.1, 145.7, 148.0; MS (ESI) m/z = 608 (100, [M−HCl+H]$^+$); HRMS (ESI) Calcd 608.2365, found 608.2366; Anal. Calcd for $C_{31}H_{38}N_5O_4S_2Cl{\cdot}3H_2O$: C, 53.32; H, 6.35; N, 10.03. Found: C, 53.09; H, 6.03; N, 10.06.

5.2.33. Bis-(3-(4-Aminophenylsulfonyl-*N*-benzylamino)-propyl)-amine hydrochloride (6f)

According to general procedure F, utilization of Boc-protected bis-nitrosulfonamide **5f** (156 mg, 0.2 mmol) gave rise to 46 mg (31%) of bis-aminosulfonamide **6f** as hydrochloride. Yellow crystals; mp: 103 °C; ^{1}H NMR (500 MHz, MeOH-d_4) δ = 1.54–1.62 (m, 4H), 2.67 (t, 4H, J = 7.2), 3.20 (t, 4H, J = 6.4), 4.28 (s, 4H), 6.86 (d, 4H, J = 8.7), 7.33–7.40 (m, 10H), 7.65 (d, 4H, J = 8.7); ^{13}C NMR (125 MHz, DMSO-d_6) δ = 24.6, 44.2, 45.7, 51.8, 113.0, 123.5, 127.5, 128.2, 128.5, 129.1, 137.4, 153.1; MS (ESI) m/z = 622 (100, [M−HCl+H]$^+$); HRMS (ESI) Calcd 622.2522, found 622.2556; Anal. Calcd for $C_{32}H_{40}N_5O_4S_2Cl{\cdot}3H_2O$: C, 53.96; H, 6.51; N, 9.83. Found: C, 53.89; H, 6.19; N, 9.56.

5.2.34. Bis-(2-isobutylamino-ethyl)-carbamic acid *tert*-butyl ester (7)

To a solution of **3a** (0.85 g, 4.2 mmol) in dry MeOH (15 mL), *iso*-butyric aldehyde (0.84 mL, 9.2 mmol) and triethyl orthoformate (2.77 mL, 16.8 mmol) were added at rt. The reaction mixture was stirred overnight, subsequently $NaBH_4$ (0.95 g, 25.2 mmol) was added, and stirring was continued for 2 h. The reaction mixture was quenched by careful addition of concd HCl. The resulting precipitate was filtered off, washed with MTBE, and discarded. The filtrate was made alkaline by addition of a concd aqueous NaOH-solution and extracted with MTBE (2× 50 mL). The combined organic phases were washed with brine (2× 40 mL), dried over $MgSO_4$, filtered, and evaporated. The residual oil was purified by FC (CH_2Cl_2/MeOH 7:3 + 1% NEt_3) yielding 346 mg (24%) of mono-Boc-protected oligoamine **7**. Colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 0.88 (d, 12H, J = 6.6), 1.44 (s, 9H), 1.64–1.75 (m, 4H), 2.40 (d, 4H, J = 6.9), 2.74 (t, 4H, J = 6.6), 3.31 (br s, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 20.5, 28.26, 28.30, 47.6, 48.2, 57.7, 79.3, 155.7; MS (ESI) m/z = 216 (100, [M−CO_2−C_4H_8+H]$^+$), 316 (49, [M+H]$^+$); HRMS (ESI) Calcd 316.2964, found 316.2989; Anal. Calcd for $C_{17}H_{37}N_3O_2{\cdot}0.5H_2O$: C, 62.92; H, 11.80; N, 12.95. Found: C, 63.28; H, 11.59; N, 13.21.

5.2.35. Bis-[2-(benzenesulfonyl-isobutyl-amino)-ethyl]-carbamic acid *tert*-butyl ester (8a)

According to general procedure C, utilization of mono-Boc-protected oligoamine **7** (423 mg, 1.3 mmol) and benzenesulfonyl chloride (0.40 mL, 3.1 mmol) furnished, after FC (hexanes/EtOAc 4:1), 670 mg (83%) of **8a**. Colorless crystals; mp: 111 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 0.89 (d, 6H, J = 6.6), 0.94 (d, 6H, J = 6.6), 1.45 (s, 9H), 1.83–1.94 (m, 2H), 2.88 (d, 2H, J = 7.3), 2.90 (d, 2H, J = 7.3), 3.15 (t, 4H, J = 8.0), 3.35–3.43 (m, 4H), 7.49–7.60 (m, 6H), 7.85 (t, 4H, J = 8.6); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers) δ = 19.9, 20.0, 27.0, 27.1, 28.4, 46.9, 47.1, 47.6, 57.2, 57.5, 80.2, 127.16, 127.21, 129.1, 132.4, 139.1, 139.3, 155.0; MS (ESI) m/z = 618 (100, [M+Na]$^+$), 1213 (23, [2M+Na]$^+$); HRMS (ESI) Calcd 618.2648, found 618.2616; Anal. Calcd for $C_{29}H_{45}N_3O_6S_2$: C, 58.46; H, 7.61; N, 7.05; S, 10.76. Found: C, 58.14; H, 7.59; N, 7.09; S, 10.90.

5.2.36. Bis-{2-[isobutyl-(4-nitro-benzenesulfonyl)-amino]-ethyl}-carbamic acid *tert*-butyl ester (8b)

According to general procedure C, utilization of mono-Boc-protected oligoamine **7** (627 mg, 2.1 mmol) and 4-nitro benzenesulfonyl chloride (1.03 g, 4.6 mmol) gave rise to 1.15 g (80%) of **8b** after FC (hexanes/EtOAc 4:1). Colorless crystals; mp: 125 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 0.88 (d, 6H, J = 6.4), 0.92 (d, 6H, J = 6.4), 1.45 (s, 9H), 1.84–1.94 (m, 2H), 2.94 (d, 2H, J = 7.3), 2.97 (d, 2H, J = 7.6), 3.19–3.27 (m, 4H), 3.38–3.45 (m, 4H), 8.04 (d, 4H, J = 7.8), 8.38 (d, 4H, J = 8.5); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 19.7, 19.8, 26.9, 27.0, 28.3, 46.7, 47.0, 47.5, 57.1, 57.2, 80.5, 124.1, 124.4, 128.3, 128.4, 144.9, 145.1, 149.9, 154.8; MS (ESI) m/z = 708 (100, [M+Na]$^+$), 1393 (19, [2M+Na]$^+$); HRMS (ESI) Calcd 708.2349, found 708.2371; Anal. Calcd for $C_{29}H_{43}N_5O_{10}S_2$: C, 50.79; H, 6.32; N, 10.21; S, 9.35. Found: C, 50.81; H, 6.45; N, 10.17; S, 9.24.

5.2.37. Bis-(2-(phenylsulfonyl-*N*-isobutyl-amino)-ethyl)-amine hydrochloride (9a)

According to general procedure E, utilization of Boc-protected bis-sulfonamide **8a** (280 mg, 0.46 mmol) furnished, after purification via FC (CH_2Cl_2/MeOH 98:2), 150 mg (62%) of hydrochloride **9a**. Colorless crystals; mp: 45 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 0.89 (d, 12H, J = 6.6), 1.86 (sm, 2H, J = 6.9), 2.89 (d, 4H, J = 7.6), 3.15 (t, 4H, J = 6.5), 3.38 (t, 4H, J = 6.6), 7.53 (t, 4H, J = 7.8), 7.59 (t, 2H, J = 7.3), 7.86 (d, 4H, J = 8.7); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 19.9, 27.1, 46.5, 47.7, 58.0, 127.4, 129.2, 132.8, 138.0; MS (ESI) m/z = 496 (100, [M−HCl+H]$^+$), 518 (8, [M−HCl+Na]$^+$); HRMS (ESI) Calcd 496.2304, found 496.2323; Anal. Calcd for $C_{24}H_{38}N_3O_4S_2Cl$: C, 54.17; H, 7.20; N, 7.90. Found: C, 54.63; H, 7.27; N, 7.97.

5.2.38. Bis-(2-(4-amino-phenylsulfonyl-*N*-isobutylamino)-ethyl)-amine hydrochloride (9b)

According to general procedure F, utilization of bis-nitrosulfonamide **8b** (300 mg, 0.44 mmol) furnished 77 mg (28%) of **9b** as hydrochloride. Colorless crystals; mp: 123 °C; ^{1}H NMR (500 MHz, MeOH-d_4) δ = 0.94 (d, 12H, J = 6.4), 1.91 (sm, 2H, J = 6.6), 2.94 (d, 4H, J = 7.3), 3.33 (br s, 4H), 3.41 (t, 4H, J = 6.2), 7.12 (d, 4H, J = 8.5), 7.78 (d, 4H, J = 8.7); ^{13}C NMR (125 MHz, MeOH-d_4) δ = 20.7, 28.6, 47.0, 49.4, 59.8, 119.5, 131.0, 131.3, 147.4; MS (ESI) m/z = 526 (100, [M−HCl+H]$^+$), 1051 (4, [2M−2HCl+H]$^+$); HRMS (ESI) Calcd 526.2522, found 526.2515; Anal. Calcd for $C_{24}H_{40}N_5O_4S_2Cl{\cdot}2.5H_2O$: C, 47.47; H, 7.47; N, 11.53. Found: C, 47.45; H, 7.02; N, 11.27.

5.2.39. Bis-(2-benzylamino-ethyl)-carbamic acid *tert*-butyl ester (10a)

According to general procedure G, utilization of mono-Boc-protected oligoamine **3a** (1.34 g, 6.1 mmol) furnished 0.89 g (49%) of mono-Boc-protected bis-benzylamine **10a** after FC (CH_2Cl_2/MeOH, 95:5 then 9:1). Yellow oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 1.38 (s, 9H), 2.76 (br s, 4H), 3.33 (br s, 4H), 3.76 (s, 4H) 7.22–7.32 (m, 10H); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 28.3, 47.5, 47.7, 53.5, 79.5, 126.9, 127.9, 128.3, 140.0, 155.8; MS (ESI) m/z = 384 (100, [M+H]$^+$), 767 (34, [2M+H]$^+$); HRMS (ESI) Calcd 384.2651, found 384.2624; Anal. Calcd for $C_{23}H_{33}N_3O_2{\cdot}H_2O$: C, 68.80; H, 8.79; N, 10.46. Found: C, 68.80; H, 8.44; N, 10.22.

5.2.40. (2-Benzylamino-ethyl)-(3-benzylamino-propyl)-carbamic acid *tert*-butyl ester (10b)

Following the general procedure G, employment of mono-Boc-protected oligoamine **3b** (2.10 g, 9.6 mmol) yielded 1.87 g (98%) of mono-Boc-protected bis-benzylamine **10b**. Brownish oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 1.30–1.40 (m, 9H), 1.71 (sm, 2H, J = 6.9), 2.60 (t, 2H, J = 6.9), 2.74–2.81 (m, 2H), 3.29 (br s, 4H), 3.76 (s, 2H), 3.79 (s, 2H), 7.20–7.37 (m, 10H); ^{13}C NMR

(125 MHz, $CDCl_3$, rotamers) δ = 28.3, 28.9, 45.0, 45.4, 46.3, 46.8, 47.4, 53.5, 53.8, 79.1, 126.58, 126.64, 127.76, 127.83, 128.07, 128.09, 140.2, 155.5; MS (ESI) *m/z* = 298 (100, $[M-CO_2-C_4H_8+H]^+$), 398 (91, $[M+H]^+$), 795 (12, $[2M+H]^+$); HRMS (ESI) Calcd 398.2808, found 398.2779; Anal. Calcd for $C_{24}H_{35}N_3O_2$: C, 72.51; H, 8.87; N, 10.57. Found: C, 71.96; H, 8.90; N, 10.80.

5.2.41. Bis-(3-benzylamino-propyl)-carbamic acid *tert*-butyl ester (10c)

According to general procedure G, utilization of mono-Boc-protected oligoamine **3c** (2.26 g, 9.8 mmol) gave rise to 1.76 g (96%) of mono-Boc-protected bis-benzylamine **10c**. Brownish oil; ^1H NMR (500 MHz, $CDCl_3$) δ = 1.42 (s, 9H), 1.71 (sm, 4H, *J* = 6.9), 2.60 (t, 4H, *J* = 6.9), 3.20 (br s, 2H), 3.26 (br s, 2H), 3.77 (s, 4H), 7.20–7.35 (m, 10H); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 28.2, 29.0, 44.3, 44.8, 46.3, 46.5, 53.9, 79.0, 126.6, 127.9, 128.1, 140.3, 155.5; MS (ESI) *m/z* = 312 (100, $[M-CO_2-C_4H_8+H]^+$), 412 (95, $[M+H]^+$), 823 (23, $[2M+H]^+$); HRMS (ESI) Calcd 412.2964, found 412.2967; Anal. Calcd for $C_{25}H_{37}N_3O_2$: C, 72.95; H, 9.06; N, 10.21. Found: C, 72.27; H, 8.98; N, 9.90.

5.2.42. Bis-[2-(benzyl-phenylacetyl-amino)-ethyl]-carbamic acid *tert*-butyl ester (11a)

According to general procedure H, utilization of bis-benzylamine **10a** (260 mg, 0.68 mmol) furnished 312 mg (74%) of bis-carboxamide **11a** after FC (hexanes/EtOAc 7:3). Colorless resin; ^1H NMR (500 MHz, $CDCl_3$) δ = 1.39–1.47 (m, 9H), 3.05–3.52 (m, 8H), 3.64–3.81 (m, 8H), 4.50–4.61 (m, 4H), 7.05–7.13 (m, 2H), 7.15–7.40 (m, 18H); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers) δ = 28.30, 28.33, 29.6, 40.2, 40.4, 40.6, 40.8, 44.8, 45.1, 45.3, 45.4, 45.7, 45.9, 48.1, 48.4, 51.3, 51.7, 52.3, 79.7, 80.2, 80.4, 81.1, 126.3, 126.6, 126.7, 126.8, 126.9, 127.0, 127.2, 127.3, 127.5, 127.6, 127.8, 127.9, 128.1, 128.5, 128.6, 128.7, 128.8, 128.9, 134.6, 134.8, 134.96, 135.04, 135.3, 136.2, 136.5, 137.2, 137.4, 137.5, 154.8, 155.0, 155.1, 155.4, 171.0, 171.4, 171.5, 171.7; MS (ESI) *m/z* = 642 (100, $[M+Na]^+$), 1261 (26, $[2M+Na]^+$); HRMS (ESI) Calcd 642.3308, found 642.3322; Anal. Calcd for $C_{39}H_{45}N_3O_4{\cdot}0.5H_2O$: C, 74.49; H, 7.37; N, 6.68. Found: C, 74.76; H, 7.19; N, 6.28.

5.2.43. [2-(Benzyl-phenylacetyl-amino)-ethyl]-[3-(benzyl-phenylacetyl-amino)-propyl]-carbamic acid *tert*-butyl ester (11b)

Following the general procedure H, employment of bis-benzylamine **10b** (0.50 g, 1.3 mmol) yielded, after purification via FC (hexanes/EtOAc 7:3), 0.68 g (77%) of bis-carboxamide **11b**. Colorless resin; ^1H NMR (500 MHz, $CDCl_3$) δ = 1.35–1.40 (m, 9H), 1.61–1.75 (m, 2H), 2.89–3.54 (m, 8H), 3.61–3.84 (m, 4H), 4.41–4.59 (m, 4H), 7.08–7.14 (m, 2H), 7.18–7.38 (m, 18H); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers) δ = 26.1, 26.5, 26.8, 27.2, 27.6, 28.2, 40.4, 40.6, 40.7, 40.9, 43.7, 43.9, 44.1, 44.3, 44.9, 45.0, 45.6, 45.8, 46.4, 48.1, 48.5, 48.8, 51.3, 51.7, 52.0, 79.4, 79.7, 79.9, 126.20, 126.24, 126.6, 126.8, 127.2, 127.3, 127.4, 127.9, 128.2, 128.56, 128.64, 128.8, 129.2, 134.7, 134.9, 135.0, 136.3, 136.6, 137.5, 154.9, 155.1, 170.8, 171.16, 171.24, 171.5; MS (ESI) *m/z* = 656 (100, $[M+Na]^+$); HRMS (ESI) Calcd 656.3464, found 656.3469; Anal. Calcd for $C_{40}H_{47}N_3O_4{\cdot}0.5H_2O$: C, 74.74; H, 7.53; N, 6.54. Found: C, 74.44; H, 7.48; N, 6.28

5.2.44. Bis-[3-(benzyl-phenylacetyl-amino)-propyl]-carbamic acid *tert*-butyl ester (11c)

According to general procedure H, utilization of bis-benzyl amine **10c** (615 mg, 1.5 mmol) gave rise to 693 mg (72%) of bis-carboxamide **11c** after FC (hexanes/EtOAc 3:2, then 1:1). Colorless resin; ^1H NMR (500 MHz, $CDCl_3$) δ = 1.32–1.36 (m, 9H), 1.46–1.78 (m, 4H), 2.65–3.35 (m, 8H), 3.69 (s, 2H), 3.75 (s, 2H), 4.49 (s, 2H), 4.59 (s, 2H), 7.03–7.13 (m, 2H), 7.15–7.40 (m, 18H); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers) δ = 26.0, 26.4, 27.3, 28.2, 40.8, 43.9, 44.2, 45.0, 48.1, 48.2, 51.25, 51.33, 79.1, 79.4, 79.8, 126.2, 126.6, 126.7, 127.1, 127.2, 127.4, 127.5, 127.9, 128.4, 128.5, 128.72, 128.77, 134.86, 134.94, 136.4, 136.5, 137.4, 154.9, 155.1, 155.2, 170.7, 171.2; MS (ESI) *m/z* = 670 (100, $[M+Na]^+$), 1316 (66, $[2M+Na]^+$); HRMS (ESI) Calcd 670.3621, found 670.3620; Anal. Calcd for $C_{41}H_{49}N_3O_4{\cdot}0.5H_2O$: C, 74.97; H, 7.67; N, 6.40. Found: C, 74.94; H, 7.59; N, 6.56.

5.2.45. Bis-(2-(*N*-benzyl-phenylacetamido)-ethyl)-amine hydrochloride (12a)

According to general procedure E, utilization of Boc-protected bis-carboxamide **11a** (244 mg, 0.39 mmol) furnished after FC (CH_2Cl_2/MeOH 98:2) 127 mg (58%) of hydrochloride **12a**. Colorless crystals; mp: 38 °C; ^1H NMR (500 MHz, $CDCl_3$) δ = 2.49–3.09 (m, 4H), 3.49–3.69 (m, 4H), 3.78–3.84 (m, 4H), 4.57–4.68 (m, 4H), 7.00–7.38 (m, 20H); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 40.4, 40.5, 40.7, 43.0, 43.4, 44.8, 45.7, 46.0, 46.2, 48.5, 48.7, 51.5, 51.8, 126.3, 126.5, 126.7, 127.3, 127.7, 127.9, 128.1, 128.3, 128.4, 128.6, 128.9, 129.1, 129.3, 129.6, 134.9, 135.0, 136.0, 136.1, 137.4, 171.5, 173.4, 174.0; MS (ESI) *m/z* = 520 (100, $[M-HCl+H]^+$), 1039 (11, $[2M-2HCl+H]^+$); HRMS (ESI) Calcd 520.2964, found 520.2995; Anal. Calcd for $C_{34}H_{38}N_3O_2Cl{\cdot}H_2O$: C, 71.12; H, 7.02; N, 7.32. Found: C, 71.51; H, 7.01; N, 7.51.

5.2.46. (2-(*N*-benzyl-phenylacetamido)-ethyl)-(3-(*N*-benzyl-phenylacetamido)-propyl)-amine hydrochloride (12b)

Following general procedure E, employment of Boc-protected bis-carboxamide **11b** (359 mg, 0.39 mmol) yielded after column chromatography (CH_2Cl_2/MeOH 95:5) 150 mg (67%) of hydrochloride **12b**. Colorless crystals; mp: 51 °C; ^1H NMR (500 MHz, $CDCl_3$) δ = 1.82–2.01 (m, 2H), 2.45–3.25 (m, 4H), 3.43–3.88 (m, 8H), 4.55–4.73 (m, 4H), 7.02–7.43 (m, 20H); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 24.3, 25.0, 25.4, 40.3, 40.4, 40.7, 42.6, 43.3, 45.1, 46.0, 47.6, 48.6, 51.3, 51.5, 51.7, 52.1, 126.3, 126.4, 126.54, 126.65, 126.72, 127.0, 127.2, 127.3, 127.7, 127.9, 128.0, 128.1, 128.3, 128.38, 128.42, 128.5, 128.7, 128.9, 129.0, 129.4, 129.5, 134.6, 134.8, 135.2, 135.5, 135.7, 136.0, 137.3, 171.0, 171.1, 171.4, 173.7, 173.8; MS (ESI) *m/z* = 534 (100, $[M-HCl+H]^+$), 1067 (7, $[2M-2HCl+H]^+$); HRMS (ESI) Calcd 534.3121, found 534.3099; Anal. Calcd for $C_{35}H_{40}N_3O_2Cl{\cdot}H_2O$: C, 71.47; H, 7.20; N, 7.14. Found: C, 71.12; H, 7.12; N, 6.87.

5.2.47. Bis-(3-(*N*-benzyl-phenylacetamido)-propyl)-amine hydrochloride (12c)

According to general procedure E, utilization of Boc-protected bis-carboxamide **11c** (320 mg, 0.49 mmol) furnished after FC (CH_2Cl_2/MeOH 98:2, then 4:1) 181 mg (62%) of hydrochloride **12c**. Colorless resin; ^1H NMR (500 MHz, $CDCl_3$) δ = 1.95–2.15 (m, 4H), 2.49–2.81 (m, 4H), 3.29–3.51 (m, 4H), 3.70–3.80 (m, 4H), 4.56–4.61 (m, 4H), 7.09–7.43 (m, 20H), 9.55 (br s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 24.3, 24.4, 24.7, 40.3, 40.5, 40.9, 42.8, 43.1, 44.2, 45.0, 45.1, 45.4, 47.6, 51.3, 51.6, 126.3, 126.8, 127.0, 127.1, 127.3, 127.8, 128.0, 128.1, 128.5, 128.6, 128.7, 128.8, 128.97,129.04, 134.4, 134.6, 135.0, 135.4, 135.7, 137.2, 171.0, 173.1, 173.6; MS (ESI) *m/z* = 548 (100, $[M-HCl+H]^+$); HRMS (ESI) Calcd 548.3277, found 548.3263; Anal. Calcd for $C_{36}H_{42}N_3O_2Cl{\cdot}2H_2O$: C, 69.71; H, 7.48; N, 6.77. Found: C, 69.54; H, 7.15; N, 6.71.

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