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Synthesis and biological evaluation of new dipicolylamine zinc chelators as metallo-β-lactamase inhibitors

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ABSTRACT

Antibiotics are key drugs in modern healthcare, especially in hospitals, where multiresistant bacteria resides and is a potential threat to human health. In the present work, a new series of adjuvants working synergistically with the carbapenem meropenem, in which a selective zinc-chelating agent was covalently linked to the small bacterial peptide D-Ala-D-Ala, was synthesized and tested against two VIM-2 and NDM-1 metallo-β-lactamases (MBLs). The nature of the linker was modified in a structure-activity relationship study. Compound 1i, having an ethyl piperidine linker, lowered the MIC of meropenem from 32-64 mg/L to 2 and 1-2 mg/L against VIM-2- and NDM-1-producing clinical isolates, respectively. The IC_{50} value of 1i against VIM-2 was 9.8 and 2.2 µM after 5 and 20 min, respectively. Compound 1i also showed intrinsic toxicity against three eukaryotic human tumoral cell lines between 50-120 µM.

1. Introduction

Antimicrobial resistance (AMR), and in particular antibacterial resistance, is an increasingly serious threat to global public health. AMR develops when a microorganism (bacteria, fungus, virus or parasites) no longer responds to a drug to which it was originally sensitive.[1-2] Antibiotics have an enormous impact on modern medicine. They are essential in the treatment of many human diseases such as urinary tract infections, wound infections, bloodstream infections, pneumonia, tuberculosis and they are a prerequisite for chemotherapy or surgery. Without harmonized and immediate worldwide action to develop agents countering highly resistant bacteria (e.g. *Escherichia coli*, *Klebsiella pneumoniae and Staphylococcus aureus*), the world is heading towards a post-antibiotic era in which common infections could once again become life threatening.[1-2] The increase in mortality with bloodstream infections caused by methicillin-resistant *S. aureus* (MRSA) and third-generation cephalosporin-resistant *E. coli* is significant, and the prolongation of hospital stay imposes a considerable burden on health care systems.[3-4] The introduction of more potent alternatives of existing antibiotics provides only temporary

solutions, since existing resistance mechanisms rapidly adapt to accommodate these new drugs.[5-6]

β-lactam antibiotics have been the largest and most important group of antimicrobial drugs since the discovery of penicillins. The most important antibiotic resistance mechanism against β-lactam antibiotics, in terms of distribution and clinical relevance, are β-lactamases.[7] These enzymes hydrolyze β-lactam antibiotics compromising their efficacy.[8-9] β-lactamases are structurally grouped into two super families, the serine β-lactamases (SBLs) and the metallo-β-lactamases (MBLs), which hydrolyze β-lactams by two conceptually different mechanisms.[10-11] The SBLs utilize an active site serine while MBLs require divalent cations, always zinc, for the hydrolysis of β-lactams.[12-14] Inhibition of SBLs by the use of inhibitors in combination with β-lactams has been a therapeutic success and extended the therapeutic life of β-lactam antibiotics.[15] Although evolution of β-lactamases also has counteracted this approach, the recent development and introduction of new SBL inhibitors such as avibactam show the viability of this approach.[16] However, there are no clinically available MBL inhibitors and an analysis of reported MBL inhibitors[12, 17-18] makes it clear that the clinical need for a MBL inhibitor is more than ever a priority for medicinal chemists and drug developers. Since some studies revealed the importance of zinc homeostasis for the regulation of MBLs and generally bacterial pathogens,[19-20] zinc chelation has become an exciting strategy to overcome bacterial resistance.

Recent works[21-24] validated this approach by using small molecule zinc chelating agents to potentiate the activity of meropenem (MEM), a carbapenem β-lactam susceptible to inactivation by MBLs. New small molecules have also emerged very recently on the basis of 2,6-dipicolinic acid which displayed a propensity to chelate zinc.[25-26] We have earlier studied TPA as a zinc chelator,[27] however, we now wanted to study dipicolylamine (DPA) as the chelating moiety.[28-29] As metal chelators are generally toxic, we need a chelator that is selective for zinc, and by selective we mean that they bind weaker to iron, manganese, sodium, potassium, calcium and other relevant biological cations than to zinc. The DPA based zinc chelators we use fit this criterion and that is why, we think, we are able to get a decent toxicity profile. In parallel, chose to connect the bacterial dipeptide *D*-Ala-*D*-Ala to the linker-chelator construct in hope it would modulate the lipophilicity of the construct in addition to mimicking the β-lactams.[30] In order to study such bivalent hybrids, a series of linkers was selected to link the zinc chelator to the peptide (Figure 1). The aim of the study is to present simultaneously the chemical diversity of the linkers used and their impact on inhibition of MBLs and potentiation of carbapenem activity in MBL-producing bacteria.

Figure 1. General structure of a bivalent hybrid which consists of a lipophilic chelator selective for zinc linked to a vector with selective affinity for bacterial cell structure.

As shown in Figure 2, *D*-Ala-*D*-Ala was used as the peptide and DPA as the zinc chelator, respectively, and they were connected *via* ten different linkers in this study. A non-chelating negative control **14** was included together with compound **10** without linker and with the chelator directly attached to *D*-Ala-*D*-Ala.

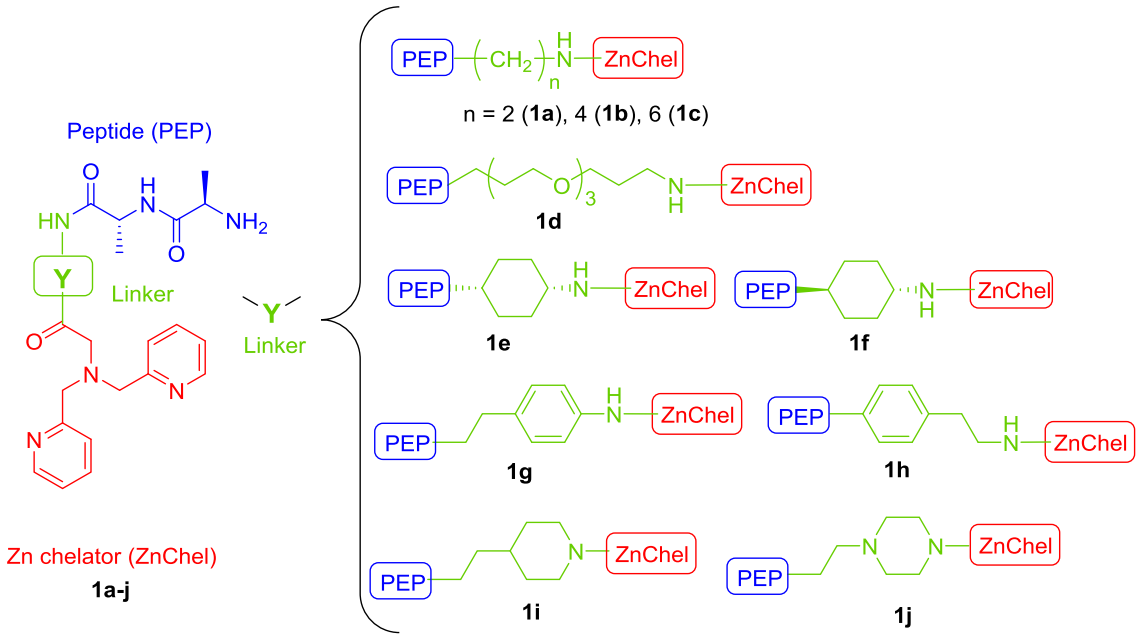


Figure 2. Structure of putative MBL inhibitors **1a-j**.

2. Results and Discussion

Synthesis of bivalent hybrids 1a-j.

All bivalent hybrids **1a-j** were obtained following a simple and straightforward chemical pathway, which mainly involved amide bond formation reactions between linker and the *D*-Ala-*D*-Ala dipeptide or linker and the zinc chelator DPA. Hence, mono Boc-protected diamines **2a-j** were used as linker precursors since they permit the coupling of two different fragments (Figure 3).

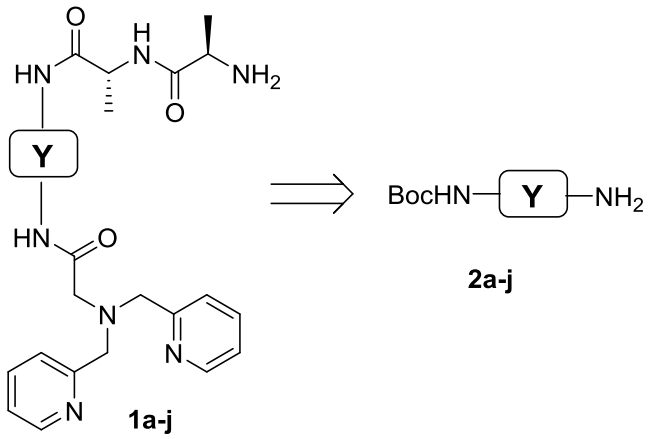
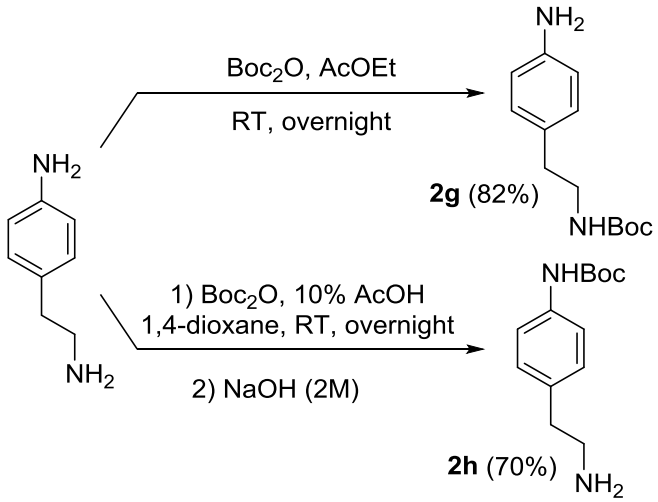


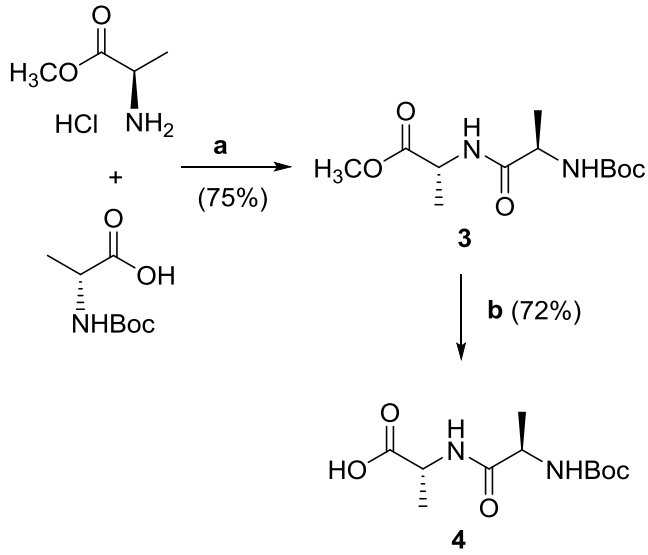
Figure 3. General structure of linker precursors.

Most of the mono Boc-protected diamines were commercially available except **2g** and **2h** which were obtained with good yields by regioselective protection from 4-(2-aminoethyl)aniline and di-*tert*-butyl dicarbonate (Scheme 1). Actually, carrying out the protection reaction under classical conditions allowed the synthesis of **2g**[31] while the use of 10% aqueous acetic acid facilitated selective introduction of the Boc group on the aromatic amine.[32]



Scheme 1. Syntheses of mono-Boc-protected diamines **2g** and **2h**.

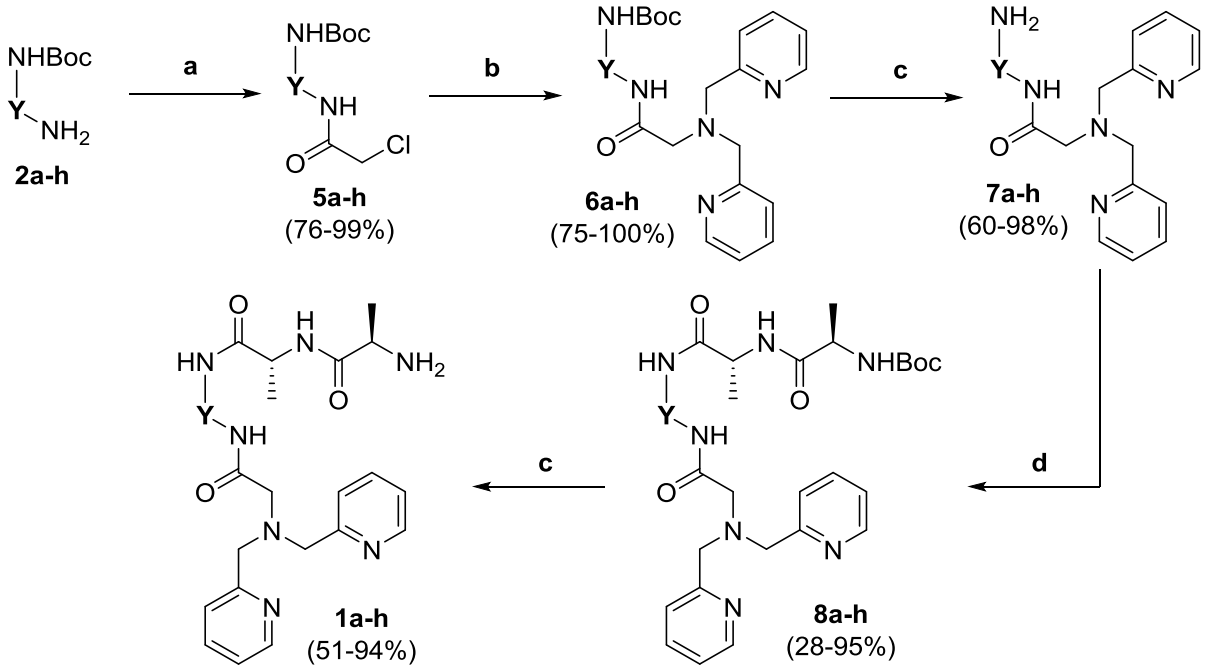
The dipeptide Boc-*D*-Ala-*D*-Ala-OH (**4**) used as the peptide precursor was prepared according to a two-step sequence described in Scheme 2. The synthesis started with the coupling reaction between the amino and carboxyl protected alanines under standard peptide synthetic procedure using HATU[33] (*O*-(7-azabenzotriazolazabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) as the coupling reagent and *N*-methylmorpholine (NMM) as a base. The fully protected dipeptide **3** was then hydrolyzed to produce **4** in a good yield.[34]



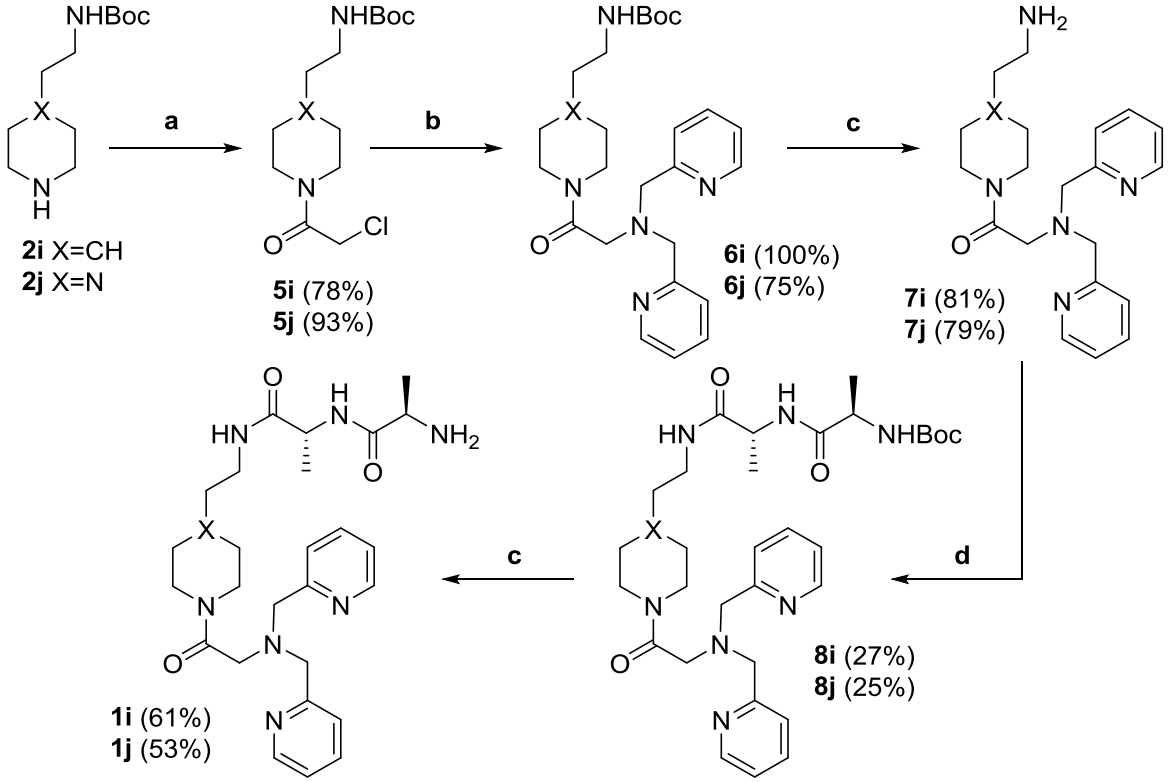
Scheme 2. Synthesis of the Boc-*D*-Ala-*D*-Ala-OH dipeptide **4** as vector precursor. Reagents and conditions: a) HATU, NMM, DMF, 0°C for 1 h then RT, overnight; b) i) NaOH 6 M, 0°C for 30 min then RT for 2 h; ii) HCl 2 M (pH 7), Et_2O.

At this point, the synthesis of bivalent hybrids **1a-j** was achieved in five steps from the mono Boc-protected diamines **2a-j** used as starting materials (Scheme 3). First, compounds **2a-j** were reacted with chloroacetyl chloride, at -78°C, to give the appropriate α-chloroamide derivatives **5a-j** in excellent yield. DPA was then easily introduced by nucleophilic substitution on chlorides **5a-j** under basic conditions and in presence of catalytic amounts of potassium iodide to facilitate the reaction.[35] It is noteworthy that derivatives **6a-j** were obtained with a satisfactory purity level without the need for chromatography if one equivalent of DPA was strictly used. After deprotection of *N*-Boc amines by trifluoroacetic acid (TFA) combined with freebase generation under basic conditions, the vector, as the Boc-*D*-Ala-*D*-Ala-OH dipeptide **4**, was coupled to the free *N*-terminal amine of derivatives **7a-j** according to the same procedure described above to afford *N*-Boc-protected bivalent hybrids **8a-j** in low to excellent yields. These latter compounds were purified on C-18 reverse phase column chromatography to remove the common HATU by-

General chemical scheme for linear linkers



General chemical scheme for piperidinyl or piperazinyl linkers

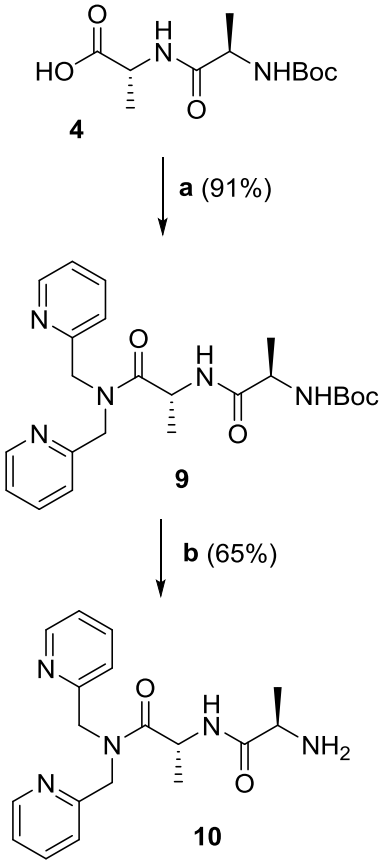


Scheme 3. General synthetic pathways to ZinChel compounds **1a-j**. Reagents and conditions: a) chloroacetyl chloride, NEt_3, CH_2Cl_2, -78 °C for 30-60 min then RT for 24 h; b) DPA (1.0 equiv.), KI (cat.), DIPEA, CH_3CN, reflux, 16 h; c) i) TFA, CH_2Cl_2, 0°C to RT, 3 h; ii) aq. K_2CO_3 (1 M); d) Boc-*D*-Ala-*D*-Ala-OH **4**, HATU, NMM, CH_2Cl_2, 0°C to RT, 4.5 h.

product *N*,*N*,*N*,*N*-tetramethylurea. Three derivatives **8a**, **8i** and **8j** were obtained with much lower yields. Coupling of the ethylene derivative **8a** led to the formation of numerous side-products and problems were encountered during the purification process of compounds **8i** and **8j**. A final Boc-deprotection step readily afforded ZnChel compounds **1a-j** with good yields. In addition, it should be noted that all the

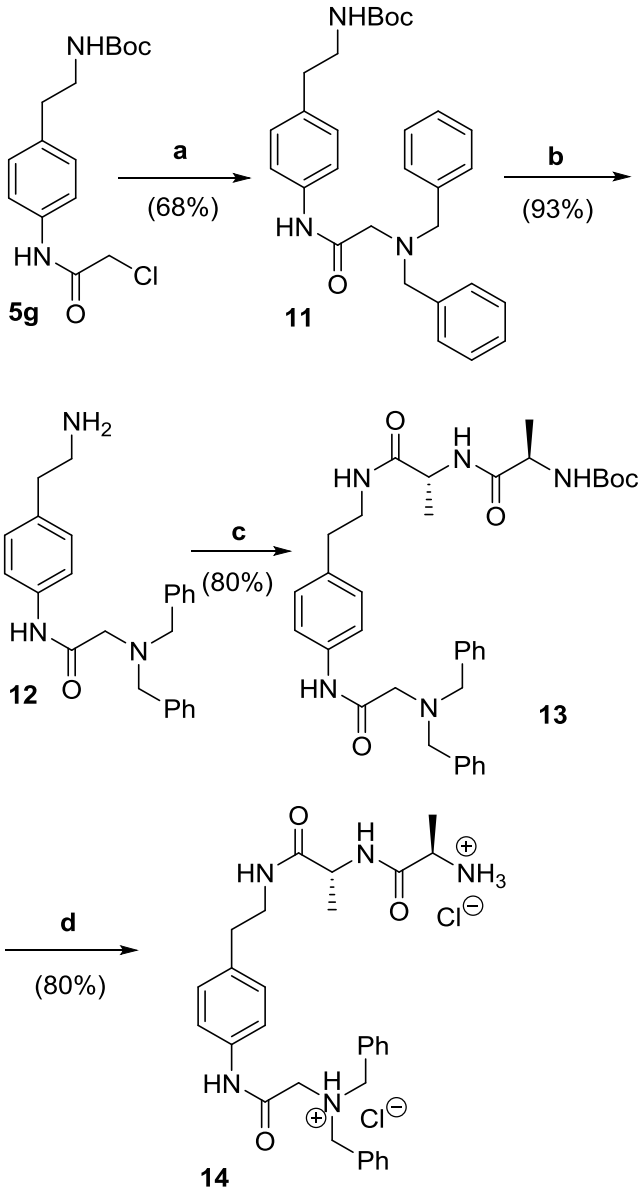
piperidinyl linker derivatives were shown to exist as a mixture of amide rotamers by NMR spectroscopy.

Two control compounds **10** and **14** were synthesized to compare their antibacterial activities to those obtained for bivalent hybrids **1a-j** and thus to examine the influence of the linker fragment and the relevance of the DPA moiety, respectively. Therefore, the construct DPA-*D*-Ala-*D*-Ala **10** was designed without any linker to conjugate the zinc chelator fragment and the vector moiety. Compound **10** was readily synthesized in two steps (59% overall yield) from dipeptide **4** by coupling reaction with DPA following by a N-Boc deprotection/neutralization sequence (Scheme 4).



Scheme 4. Synthesis of the control compound **10**. Reagents and conditions: a) DPA (1.0 equiv.), HATU, NMM, CH_2Cl_2, 0°C to RT, 4 h; b) i) TFA, CH_2Cl_2, 0 °C to RT, 3 h; ii) aq. NaOH (1 M).

To study the relevance of the zinc chelator, we designed a second construct, compound **14**, in which DPA fragment was replaced by dibenzylamine moiety. Therefore, the synthetic pathway to prepare **14** described in Scheme 5 was analogous to that performed for **1g**. A nucleophilic substitution was carried out between α-chloroacetamide **5g** and dibenzylamine in presence of DIPEA (*N*,*N*-diisopropylethylamine) and a catalytic amount of KI in refluxed acetonitrile to give **11** with 68% yield. After the same *N*-Boc deprotection/neutralization sequence as described above, the Boc-*D*-Ala-*D*-Ala-OH dipeptide **4** was coupled to primary amine **12** to afford the *N*-Boc-protected derivative **13** which was finally deprotected in acidic conditions to give the construct **14** as a hydrochloride salt.



Scheme 5. Synthesis of the reference compound **14**. Reagents and conditions: a) dibenzylamine, KI (cat.), DIPEA, CH_3CN, reflux, 17 h; b) i) TFA, CH_2Cl_2, 0°C to RT, 1 h; ii) aq. K_2CO_3 (1 M); c) Boc-*D*-Ala-*D*-Ala-OH **4**, HBTU, NMM, CH_2Cl_2, 0 °C to RT, 3 h; d) i) TFA, CH_2Cl_2, 0°C to RT, 1 h; ii) HCl (2 M), CH_2Cl_2, Et_2O.

Biological activities

Microbrothdilution MIC assay

To investigate the synergistic potential of the newly synthesized compounds in combination with MEM, they were subjected to *in vitro* testing against two clinical Gram-negative strains of *P. aeruginosa*[36] and *K. pneumoniae*[37] each harboring the metallo β-lactamases VIM-2 and NDM-1, respectively (Table 1). Both strains were resistant to MEM

alone with MIC values of 32-64 mg/L. Addition of 125 μM of the strong chelator TPEN ((*N,N,N',N'*-tetrakis(2-pyridinylmethyl)-1,2-ethanediamine) lowered the MIC values of MEM to 1 mg/L (*P. aeruginosa,* VIM-2) and ≤ 0.5 mg/L (*K. pneumoniae,* NDM-1). When evaluated alone, the compounds **1a-j** and **10** did not present any antibacterial activity up to 1000 μM (data not shown). However, synergistically all of our compounds, with the exception of **1g** and **1h**, were able to lower the MIC of MEM. Compound **1i** even demonstrated comparable MIC results to TPEN and reduced the MIC of MEM to 2 mg/L (*P. aeruginosa,* VIM-2) and 1-2 mg/L (*K. pneumoniae,* NDM-1) which is below the clinical breakpoint according to EUCAST Version 8.0, 2018. http://www.eucast.org. Compound **10**, where D-Ala-D-Ala was directly bound to the chelator via an amide bond did not lower the MIC value of meropenem despite having the same chelator structure as compound **1a-j**. This could be due to metal assisted hydrolysis of the amide in the presence of zinc or other metal ions.[38] Compound **14**, where the 2-pyridine rings were replaced with phenyl groups, showed no synergistic properties with MEM, as was expected since it lacks zinc-chelating properties.

Enzyme inhibition assay

The synthesized compounds were preincubated for 5 and 20 min with purified VIM-2[39] and residual enzymatic activity was determined (Table 1). All compounds, except **1h**, demonstrated time-dependent inhibition as the IC_{50} values dropped considerably, going for example with compound **1d** from 14.7 to 2.7 μM. Compound **1i**, which had the best synergistic effect in combination with MEM in the MIC assays, had also a low IC_{50} value (2.8 μM at 20 min). Interestingly, compounds **1b** and **1g** demonstrated enzyme inhibition comparable to compound **1i** (IC_{50} = 2.5 and 1.4 μM at 20 min, respectively). However, no synergistic activity in combination with MEM in the MIC assay (Table 1) could be shown. This could indicate permeability differences across the bacterial cell wall between the compounds.

Cell sensitivity assay

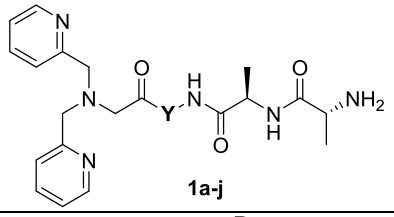
A common challenge with metal chelators is their unwanted eukaryotic toxicity. In this work, the well-described colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay[40] was used to evaluate the sensitivity of human breast cancer cells (MDA-MB-231) and pancreatic cancer cells (MIA-PaCa2 and Colo-357) towards all synthesized hybrid compounds **1a-j**. IC_{50} values were determined after 72 h exposure to compounds **1a-j** or other metal chelators as TPEN or DPA as described in the procedures. As expected, the strong chelator, TPEN, which have a dissociation constant (K_d) of 10^{-15} M - 2.6 x 10^{-16} M,[41] was more than one order of magnitude more toxic than the other compounds, with IC_{50} values between 3-5 μM, which is far below the 125 μM concentration used in the MIC assays. On the other hand, DPA had IC_{50} values ranging from 57.2 to 104.3 μM. The new compounds generally showed high IC_{50} values between 100-200 μM against the MDA-MB-231 and Mia-PaCa2 cell lines, and slightly lower for Colo-357 (50-175 μM). This is within the concentration range used in the synergistic MIC assays with MEM and points towards a possible selectivity challenge. Compound **14**, which is not a zinc chelator and did not show any synergistic activity with MEM in the MIC assays, was only slightly less toxic than TPEN. This was unexpected, and we can only speculate on why this is the case. In conclusion, the three human cancer cell lines showed a lower sensitivity to all the tripartite compounds, including the most active compound **1i**, than to TPEN or DPA. However, the intrinsic toxicity of the new compounds are too high to be used in the present form and must be optimized further.

3. Conclusions

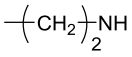
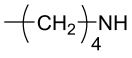
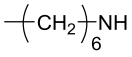
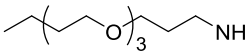
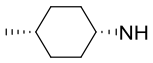
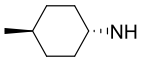
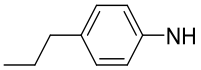
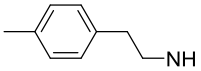
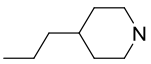
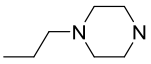
In this work, we described the straightforward synthesis of ten new DPA zinc chelators **1a-j** as MBL inhibitors. Two negative controls without linker (compound **10**) or without zinc chelator (compound **14**) were added to complete our study. As expected, the nature of the linker plays a crucial role in the ability of these bivalent hybrids **1a-j** to potentiate the activity of MEM since compound **10** did not show any activity.

Of all the new hybrids studied in this article, the compound **1i** with a 4-substituted piperidine linker chain showed the best synergistic activity in combination with MEM in the MIC assays against clinical isolates of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* harboring VIM-2 and NDM-1, respectively. **1i** demonstrated a potent inhibitory activity against purified VIM-2 enzyme (IC_{50} = 2.8 μM after 20 min). Moreover, breast cancer cell line (MDA-MB231) and pancreatic cancer cell lines (Mia-PaCa2 and Colo-357) showed a lower sensitivity to all the bivalent hybrids, including the most active compound **1i**, than to TPEN or DPA alone. However, **1i**, is still only effective at concentrations close to the eucariotic IC_{50} values and must be improved further in order to be safe and effective.

Table 1. Effect of compounds **1a-j** on the potentiation of MEM against different clinical strains of MBL-producing *P. aeruginosa* and *K. pneumoniae* (MIC values), enzymatic inhibition of the compound **1a-j** (IC_{50}) and the MDA-MB-231, Mia-PaCa2 and Colo-357 cell lines sensitivity (IC_{50}).



1a-j

Cpd	Y	MIC (mg/L)[a]		Pure enzyme inhibition VIM-2 IC_{50} (μM) Incubation time (min)		Intrinsic Toxicity IC_{50} (μM)		
		P. aeruginosa VIM-2[b]	*K. pneumoniae* NDM-1[c]	5	20	MDA-MB 231	Mia-PaCa2	Colo-357
MEM	-	32-64	32-64	ND	ND	ND	ND	ND
TPEN	-	1	≤0.5	ND	ND	4.8 ± 1.2	3.7 ± 1.2	5.0 ± 0.8
DPA	-	ND	ND	ND	ND	104.3 ± 47.8	38.7 ± 12.2	57.2 ± 22.5
1a		32	8	30.7	6.3	152.5 ± 48.1	137.6 ± 26.6	78.8 ± 6.2
1b		16	4	6.1	2.5	210.3 ± 50.7	134.2 ± 32.5	99.4 ± 17.3
1c		16	4	16.8	14.2	121.0 ± 4.2	117.5 ± 13.6	175.8 ± 86.1
1d		8	4	14.7	2.7	168.6 ± 77.5	117.9 ± 33.0	105.9 ± 20.5
1e		8	2	31.4	17.9	164.8 ± 63.0	119.5 ± 17.4	85.2 ± 11.3
1f		8	8	14.2	3.4	193.2 ± 66.1	121.6 ± 28.0	100.3 ± 17.5
1g		32	16	2.3	1.4	127.2 ± 91.6	118.4 ± 31.5	37.9 ± 19.0
1h		32	16	76.0	75.8	178.0 ± 41.2	130.1 ± 70.8	140.4 ± 50.7
1i		2	1-2	9.8	2.8	116.6 ± 53.4	56.0 ± 11.3	50.1 ± 13.1
1j		8	4	24.7	6.9	148.7 ± 18.3	142.4 ± 53.1	92.6 ± 28.6
10	-	32	32	>125	>125	ND	ND	ND
14	-	64	64	>125	>125	15.4 ± 10.0	12.7 ± 4.3	20.4 ± 5.1

ND: not determined. MIC assay performed as one biological replicate and two technical replicates. For assays on human cells, all experiments were performed in triplicate and at least three times. All values are expressed as the mean ± SD. [a] For MIC determination, all compounds were tested at 125 μM in co-administration with MEM. [b] MIC values of *P. aeruginosa* strain harboring VIM-2. [c] MIC values of *K. pneumoniae* NDM-1 strain.

However, the moderate toxicity towards three human cell lines (all IC_{50} values > 50 μM) was equal or lower than the dose used in the co-administration MIC assay (125 μM). The ability of hybrid **1i** to potentiate the activity of MEM could be explained by the fact that a zinc-atom could potentially bind to one more nitrogen piperidinyl atom in **1i** than in the other derivatives and possibly be removed from the active site in the enzyme. Finally, it is worth noting that hybrids **1b** and **1g** displayed an even higher inhibitory activity against the purified VIM-2 than compound **1i** while the former ones did not potentiate the activity of MEM. This potential difference of mechanism of action should be considered in further studies. However, more potent compounds with lower eukaryotic toxicity should be explored by changing the vector and chelator moiety in a multifactorial experimental design to optimize the MIC

performance further towards a potential drug against antimicrobial resistance.

4. Experimental Section

General

All reagents and solvents were of analytical grade and were used as received, without further purification. Compounds **2g**[31] and **2h**[32] were synthesized according to literature procedures. ^{1}H spectra were recorded with Bruker DRX400 or DRX300 Fourier transform spectrometers, using an internal deuterium lock, operating at 400 MHz or 300 MHz. ^{13}C NMR spectra were recorded with a Bruker DRX400 or DRX300 Fourier transform spectrometers, using an internal deuterium lock, operating at 100 MHz or 75 MHz. All spectra were recorded at 25°C. Chemical shifts are reported in parts per million (ppm) relative to residual protons or carbons thirteen of deuterated solvent (δ = 2.50 ppm for ^{1}H NMR and δ = 39.52 ppm for ^{13}C NMR for DMSO-d6, δ = 7.26 ppm for ^{1}H NMR and δ = 77.16 ppm for ^{13}C NMR for $CDCl_3$, δ = 3.31 ppm for ^{1}H NMR and δ = 49.00 ppm for ^{13}C NMR for CD_3OD, δ = 1.94 ppm for ^{1}H NMR and δ = 1.32 and 118.26 ppm for ^{13}C NMR for CD_3CN and δ = 4.79 ppm for ^{1}H NMR for D_2O). Carbon multiplicity was determined by DEPT experiments. Electron-Spray low-resolution mass spectra were recorded on a Thermo ALCQ Advantage spectrometer or Agilent 6120 spectrometer. High-resolution mass spectra were recorded on a Bruker MicroTOF Q or ThermoQuest FINNIGAN MAT 95 XL apparatus operating at 70eV (for compounds 5a-j, chlorine-35 isotope was the only isotope to be analyzed in mass spectrometric measurements). TLC analyses were carried out using Meck Aluminum Oxide 60 F_{256} pates visualized by UV light.

Chemistry

*Synthesis of methyl Boc-D-alanyl-D-alaninate (**3**)*

D-Ala-OMe HCl (3.688 g, 26.4 mmol, 1 equiv.), Boc-*D*-Ala-OH (5.00 g, 26.4 mmol, 1 equiv.), and HATU (10.048 g, 26.4 mmol, 1 equiv.) were dissolved in DMF (25 mL) and cooled to 0 °C in an ice bath. *N*-methylmorpholine (28.14 mL, 52.53 mmol, 2 equiv.) was slowly added and the mixture was left to stir at 0 °C for 1 h before warming up to room temperature. The reaction was left stirring overnight before it was diluted with 350 mL of water and extracted with small portions of EtOAc (3-10 x 20-40 mL). Combined organic extracts were washed with 0.1 M HCl (2 x 50 mL), 0.5 M $NaHCO_3$ (2 x 50 mL), and with brine (50 mL). Resulting organic phase was dried over $MgSO_4$, filtered and evaporated to give the title compound **3** as a white powder (5.432 g, 75% yield): ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.9 (bs, 1H, NH), 5.06 (s, 1H, NH), 4.56 (pentet, *J* = 7.3 Hz, 1H), 4.17 (s, 1H), 3.73 (s, 3H, $COOCH_3$), 1.43 (s, 9H, $C(CH_3)_3$), 1.39 (d, *J* = 7.2 Hz, 3H, CH_3), 1.35 (d, *J* = 7.1 Hz, 3H, CH_3).

*Synthesis of Boc-D-alanyl-D-alanine-OH (**4**)*

To a 6 M NaOH solution (30 mL, 180 mmol, 10 equiv.) cooled to 0°C was added Boc-*D*-alanyl-*D*-alanine methyl ester (4.94 g, 18 mmol, 1 equiv.). The mixture was left to stir at 0°C for 30 min and for 2 more h at room temperature. HCl was added to adjust the pH to 7. The compound was extracted using EtOAc (3 x 100 mL) and dried over $MgSO_4$. Filtration and solvent removal under reduced pressure gave title compound **4** as a white solid (3.375 g, 72% yield): ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.96 (s, 1H, COOH), 7.04 (d, *J* = 7.5 Hz, 1H, NH), 5.35 (s, 1H, NH), 4.56 (pentet, *J* = 7.2 Hz, 1H, CHα), 4.26 (m, 1H, CHα), 1.46 – 1.41 (m, 12H, $C(CH_3)_3$, CH_3), 1.35 (d, *J* = 7.0 Hz, 3H, CH_3).

*General procedure for synthesis of 2-chloroacetamide derivatives (**5a-j**) from mono-Boc protected diamines (**2a-j**)*

A solution of the corresponding mono-Boc-protected diamine **2a-j** (1.0 equiv.) in CH_2Cl_2 (0.5 M) was cooled to -78ºC (acetone/dry ice). NEt_3 (1.5 equiv.) was rapidly added before a solution of chloroacetyl chloride (1.2 equiv.) in CH_2Cl_2 (0.5 M) was added dropwise for 30-60 min. The resulting white slurry mixture was left to warm up to room temperature to become dark brown, and then was stirred at room temperature for 24 h. The resulting mixture was washed with 0.5 M citric acid (3 times) and then with water (twice). The organic layer was extracted, dried over K_2CO_3 and the solvent removed under reduced pressure to give the title compound as a dark brown oil.

Tert-butyl (2-(2-chloroacetamido)ethyl)carbamate (**5a**)[42]

Yield (6.298 g, 85%): ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.18 (bs, 1H, NH), 4.89 (bs, 1H, NH), 4.03 (s, 2H, CH_2Cl), 3.40 (m, 2H), 3.31 (m, 2H), 1.44 (s, 9H, $C(CH_3)_3$).

Tert-butyl (4-(2-chloroacetamido)butyl)carbamate (**5b**)[42]

Yield (1.166 g, 83%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.68 (bs, 1H, NH), 4.60 (bs, 1H, NH), 4.04 (s, 2H, CH_2Cl), 3.32 (quartet, *J* = 6.5 Hz, 2H, $C\underline{H}_2NH$), 3.14 (quartet, *J* = 6.5 Hz, 2H, $C\underline{H}_2NH$), 1.63 – 1.47 (m, 4H), 1.43 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ=166.0 (CO), 156.1 (NCOOtBu), 79.4 ($\underline{C}(CH_3)_3$), 42.8 (CH_2), 40.2 (CH_2), 39.6 (CH_2), 28.5 ($C(\underline{C}H_3)_3$), 27.6 (CH_2), 26.7 (CH_2); HRMS-ESI *m/z* $[M+Na]^+$ calculated for $C_{11}H_{21}ClNaN_2O_3$: 287.1133, found: 287.1133.

Tert-butyl (6-(2-chloroacetamido)hexyl)carbamate (**5c**)

Yield (730 mg, 93 %): ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.63 (bs, 1H, NH), 4.52 (bs, 1H, NH), 4.04 (s, 2H, CH_2Cl), 3.29 (td, *J* = 7.2, 6.0 Hz, 2H, $C\underline{H}_2NH$), 3.10 (t, *J* = 7.0 Hz, 2H, $C\underline{H}_2NH$), 1.54 (m, 2H), 1.47 (t, *J* = 6.8 Hz, 2H), 1.43 (s, 9H, $C(CH_3)_3$), 1.37 – 1.31 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=165.9 (CO), 156.2 (NCOOtBu), 79.4 ($\underline{C}(CH_3)_3$), 42.8 (CH_2), 39.8 (CH_2), 30.1 (CH_2), 29.4 (CH_2),

28.6 (C(CH$_3$)$_3$), 26.5 (CH$_2$), 26.4 (CH$_2$); HRMS-ESI *m/z* [*M*+Na]$^+$ calculated for $C_{13}H_{25}ClNaN_2O_3$: 315.1445, found: 315.1451.

Tert-butyl (1-chloro-2-oxo-7,10,13-trioxa-3-azahexadecan-16-yl)carbamate (**5d**)

Yield (801 mg, 72%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=4.95 (bs, 1H, NH), 4.01 (bs, 2H, CH_2Cl), 3.66 – 3.55 (m, 10H), 3.52 (t, *J* = 6.0 Hz, 2H), 3.42 (quartet, *J* = 5.9 Hz, 2H, CH_2NH), 3.21 (quartet, *J* = 6.4 Hz, 2H, CH_2NH), 1.92 (s, 1H), 1.81 (pentet, *J* = 5.9 Hz, 2H, $CH_2CH_2CH_2$), 1.74 (pentet, *J* = 6.3 Hz, 2H, $CH_2CH_2CH_2$), 1.42 (s, 9H, C(CH$_3$)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ=166.0 (CO), 156.1 (NCOOtBu), 79.0 (C(CH$_3$)$_3$), 70.69 (CH$_2$), 70.67 (CH$_2$), 70.60 (CH$_2$), 70.57 (CH$_2$), 70.3 (CH$_2$), 69.7 (CH$_2$), 42.8 (CH$_2$), 38.9 (CH$_2$), 38.7 (CH$_2$), 29.8 (CH$_2$), 28.7 (CH$_2$), 28.6 (C(CH$_3$)$_3$); HRMS-ESI *m/z* [*M*+Na]$^+$ calculated for $C_{17}H_{33}ClNaN_2O_6$: 419.1921, found: 419.1924.

Tert-butyl (cis-4-(2-chloroacetamido)cyclohexyl)carbamate (**5e**)

Yield (875 mg, 80%): ^{1}H NMR (300 MHz, $CDCl_3$): δ=6.61 (d, *J* = 7.9 Hz, 1H, NH), 4.77 (s, 1H, NH), 3.94 (s, 2H, CH_2Cl), 3.83 (tt, *J* = 7.7, 4.1 Hz, 1H), 3.53 (m, 1H), 1.73 – 1.59 (m, 4H), 1.58 – 1.43 (m, 4H), 1.35 (s, 9H, C(CH$_3$)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ=165.0 (CO), 155.1 (NCOOtBu), 79.1 (C(CH$_3$)$_3$), 46.5 (CH), 46.2 (CH), 42.7 (CH_2Cl), 28.6 (CH$_2$), 28.4 (C(CH$_3$)$_3$), 27.8 (CH$_2$); HRMS-ESI *m/z* [*M*+Na]$^+$ calculated for $C_{13}H_{23}ClNaN_2O_3$: 313.1292, found: 313.1294.

Tert-butyl (trans-4-(2-chloroacetamido)cyclohexyl)carbamate (**5f**)

Reaction solvent THF, yield (1.032 g, 76%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.39 (m, 1H, NH), 4.44 (s, 1H, NH), 4.01 (s, 2H, CH_2Cl), 3.74 (m, 1H), 3.42 (m, 1H), 2.08-1.96 (m, 4H), 1.43 (s, 9H, C(CH$_3$)$_3$), 1.35 – 1.13 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=165.3 (CO), 155.3 (NCOOtBu), 79.5 (C(CH$_3$)$_3$), 48.9 (CH), 48.3 (CH), 42.8 (CH_2Cl), 32.0 (CH$_2$), 31.5 (CH$_2$), 28.5 (C(CH$_3$)$_3$); HRMS-ESI *m/z* [*M*+Na]$^+$ calculated for $C_{13}H_{23}ClNaN_2O_3$: 313.1292, found: 313.1289.

Tert-butyl (4-(3-chloro-2-oxopropyl)phenethyl)carbamate (**5g**)

Yield (7.46 g, 75%): ^{1}H NMR (400 MHz, DMSO-d6): δ=10.21 (s, 1H, NH), 7.49 (d, *J*=8.4 Hz, 2H), 7.14 (d, *J*=8.4 Hz, 2H), 6.84 (t, *J*=5.6 Hz, 1H, NH), 4.23 (s, 2H, CH_2Cl), 3.08 (m, 2H), 2.65 (m, 2H), 1.36 (s, 9H, C(CH$_3$)$_3$); ^{13}C NMR (100 MHz, DMSO-d6): δ=164.4 (CO), 155.5 (NCOOtBu), 136.5 (Cquat arom), 134.9 (Cquat arom), 128.9 (2×CH arom), 119.4 (2×CHarom), 77.4 (C(CH$_3$)$_3$), 43.5 (CH$_2$), 41.5 (CH$_2$), 34.9 (CH$_2$), 28.2 (C(CH$_3$)$_3$).

Tert-butyl (4-(2-(2-chloroacetamido)ethyl)phenyl)carbamate (**5h**)

Yield (1.44 g, 99%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.31 (d, *J* = 8.5 Hz, 2H), 7.11 (d, *J* = 8.5 Hz, 2H), 6.59 (s, 1H, NH), 6.52 (s, 1H, NH), 4.01 (s, 2H, CH_2Cl), 3.52 (td, *J* = 6.9, 5.8 Hz, 2H, CH_2NH), 2.79 (t, *J* = 7.0 Hz, 2H, ArCH_2), 1.51 (s, 9H, C(CH$_3$)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ=165.9 (CO), 152.9 (NCOOtBu), 137.1 (Cquat arom), 133.0 (Cquat arom), 129.4 (2×CHarom), 119.0 (2×CHarom), 80.7 (C(CH$_3$)$_3$), 42.8 (CH$_2$), 41.1 (CH$_2$), 34.9 (CH$_2$), 28.5 (C(CH$_3$)$_3$); HRMS-ESI *m/z* [*M*+Na]$^+$ calculated for $C_{15}H_{21}ClNaN_2O_3$: 335.1128, found: 335.1133.

Tert-butyl (2-(1-(2-chloroacetyl)piperidin-4-yl)ethyl)carbamate (**5i**)

The title compound was isolated as a mixture of amide rotamers (519 mg, 78% yield): ^{1}H NMR (400 MHz, $CDCl_3$): δ=4.62 – 4.43 (m, 2H), 4.07 (d, *J* = 12 Hz, AB system, 1H), 4.02 (d, *J* = 12 Hz, AB system, 1H), 3.88 – 3.73 (m, 1H), 3.21 – 2.89 (m, 3H), 2.59 and 2.50 (td, *J* = 12.9, 2.9 Hz, 1H), 1.88 – 1.65 (m, 2H), 1.63 – 1.48 (m, 1H), 1.49 – 1.36 (s, 11H), 1.30 – 1.05 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=168.9 and 165.0 (CO), 156.1 (NCOOtBu), 79.4 (C(CH$_3$)$_3$), 53.5, 46.7, 42.7, 41.8, 41.3, 38.1, 36.74 and 36.68 (5×CH$_2$), 33.7 and 33.5 (CH), 32.6, 32.5, 31.8 and 31.7 (2×CH$_2$), 28.5 (C(CH$_3$)$_3$); HRMS-ESI *m/z* [*M*+Na]$^+$ calculated for $C_{14}H_{25}ClNaN_2O_3$: 327.1456, found: 327.1446.

Tert-butyl (2-(piperazin-1-yl)ethyl)carbamate (**5j**)

pH of the mixture was adjusted to 10-11 with 0.5 M K_2CO_3 before extraction.

Yield (617 mg, 93%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=4.91 (bs, 1H, NH), 4.05 (s, 2H, CH_2Cl), 3.62 (t, *J* = 5.1 Hz, 2H), 3.51 (t, *J* = 5.0 Hz, 2H), 3.23 (quartet, *J* = 6.0 Hz, 2H), 2.54 – 2.37 (m, 6H), 1.44 (s, 9H, C(CH$_3$)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ=165.1 (CO), 156.0 (NCOOtBu), 79.4 (C(CH$_3$)$_3$), 57.2 (CH$_2$), 52.9 (CH$_2$), 52.5 (CH$_2$), 46.4 (CH$_2$), 42.2 (CH$_2$), 41.0 (CH$_2$), 37.1 (CH$_2$), 28.5 (C(CH$_3$)$_3$); HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{13}H_{25}ClN_3O_3$: 306.1580, found: 306.1579.

General procedure for nucleophilic substitution by dipicolylamine [14]

The corresponding 2-chloroacetamide **5a-j** (1.0 equiv.) and KI (0.6 equiv.) were dissolved in MeCN (0.05 M) and DPA (1.0 equiv.) was added to the stirring mixture. DIPEA (9.7 equiv.) was then added and the mixture was heated to reflux for 16 h. After cooling to room temperature, the mixture was concentrated under reduced pressure, dissolved in CH_2Cl_2, and washed with a 1 M NaOH solution, to give title compounds **6a-j** as orange sticky oils generally employed without further purification in the following step. In the case of trace amounts of DPA, the compound was purified by neutral alumina column chromatography (1-2% MeOH in CH_2Cl_2).

Tert-butyl (2-(di-2-picolylaminoacetamido)ethyl)carbamate (**6a**)

Yield (3.37 g, quantitative yield): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.82 (t, *J* = 6.3 Hz, 1H, NH), 8.62 (d, *J* = 4.9 Hz, 2H), 7.67 (t, *J* = 7.7, 1.8 Hz, 2H), 7.30 (d, *J* = 7.7 Hz, 2H), 7.21 (m, 2H), 5.97 (t, *J* = 5.7 Hz, 1H), 3.85 (s, 4H, 2×CH_2Pyr), 3.43 (m, 2H, CH_2NH), 3.35 – 3.23 (m, 4H, CH_2NH, CH_2CO), 1.42 (s, 9H C(CH$_3$)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ=171.1 (CO), 157.3 (2×Cquat Pyr), 156.1 (NCOOtBu), 149.2 (2×CH Pyr), 137.3 (2×CH Pyr), 123.9 (2×CH Pyr), 122.8 (2×CH Pyr), 79.1 (C(CH$_3$)$_3$), 59.9 (2×CH_2Pyr), 58.2

(N<u>C</u>H$_2$CO), 40.9 (CH$_2$), 39.3 (CH$_2$), 28.5 (C(<u>C</u>H$_3$)$_3$); HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{21}H_{30}N_5O_3$: 400.2343, found: 400.2348.

Tert-butyl (4-(di-2-picolylaminoacetamido)butyl)carbamate (**6b**)

Yield (1.611 g, quantitative): ^{1}H NMR (400 MHz, CDCl$_3$): δ=8.77 (t, *J* = 5.6 Hz, 1H, NH), 8.54 (ddd, *J* = 4.9, 1.8, 0.9 Hz, 2H), 7.60 (m, 2H), 7.24 (dt, *J* = 7.7, 1.0 Hz, 2H), 7.15 (ddd, *J* = 7.5, 4.8, 1.2 Hz, 2H), 4.65 (s, 1H, NH), 3.82 (s, 4H, 2×C<u>H</u>$_2$Pyr), 3.32 – 3.23 (m, 4H), 3.11 (quartet, *J* = 6.5 Hz, 2H, C<u>H</u>$_2$NH), 1.62 – 1.45 (m, 4H), 1.41 (s, 9H, C(CH$_3$)$_3$); ^{13}C NMR (100 MHz, CDCl$_3$): δ=171.3 (CO), 158.3 (2×Cquat Pyr), 156.1 (NCOOtBu), 149.5 (2×CH Pyr), 136.6 (2×CH Pyr), 123.3 (2×CH Pyr), 122.5 (2×CH Pyr), 79.1 (<u>C</u>(CH$_3$)$_3$), 60.6 (2×<u>C</u>H$_2$Pyr), 58.1 (N<u>C</u>H$_2$CO), 40.4 (CH$_2$), 38.8 (CH$_2$), 28.5 (C(<u>C</u>H$_3$)$_3$), 27.6 (CH$_2$), 26.9 (CH$_2$); HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{23}H_{34}N_5O_3$: 428.2639, found: 428.2656.

Tert-butyl (6-(di-2-picolylaminoacetamido)hexyl)carbamate (**6c**)

Yield (675 mg, 87%): ^{1}H NMR (400 MHz, CDCl$_3$): δ=8.70 (t, *J* = 5.7 Hz, 1H, NH), 8.55 (m, 2H), 7.63 (td, *J* = 7.7, 1.7 Hz, 2H), 7.30 (d, *J* = 7.8 Hz, 2H), 7.18 (dd, *J* = 7.5, 5.0 Hz, 2H), 4.55 (m, 1H, NH), 3.88 (s, 4H, 2×C<u>H</u>$_2$Pyr), 3.34 (s, 2H, NCH$_2$CO), 3.26 (quartet, *J* = 6.7 Hz, 2H, C<u>H</u>$_2$NH), 3.06 (quartet, *J* = 6.8 Hz, 2H, C<u>H</u>$_2$NH), 1.61 – 1.47 (m, 2H), 1.48 – 1.38 (m, 11H, CH$_2$, C(CH$_3$)$_3$), 1.36 – 1.26 (m, 4H); ^{13}C NMR (100 MHz, CDCl$_3$): δ=170.7 (CO), 157.8 (2×Cquat Pyr), 156.0 (NCOOtBu), 149.0 (2×CH Pyr), 136.9 (2×CH Pyr), 123.4 (2×CH Pyr), 122.6 (2×CH Pyr), 79.0 (<u>C</u>(CH$_3$)$_3$), 60.1 (2×<u>C</u>H$_2$Pyr), 58.1 (N<u>C</u>H$_2$CO), 40.5 (CH$_2$), 39.0 (CH$_2$), 30.0 (CH$_2$), 29.4 (CH$_2$), 28.4 (C(<u>C</u>H$_3$)$_3$), 26.6 (CH$_2$), 26.5 (CH$_2$); HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{25}H_{38}N_5O_3$: 456.2982, found: 456.2974.

Tert-butyl (4-oxo-1-(pyridin-2-yl)-2-(pyridin-2-ylmethyl)-9,12,15-trioxa-2,5-diazaoctadecan-18-yl)carbamate (**6d**)

Yield (705 mg, quantitative): ^{1}H NMR (300 MHz, CDCl$_3$): δ=8.70 (t, *J* = 5.7 Hz, 1H, NH), 8.54 (m, 2H), 7.59 (td, *J* = 7.7, 1.8 Hz, 2H), 7.26 (m, 2H), 7.15 (ddd, *J* = 7.6, 4.9, 1.3 Hz, 2H), 5.01 (s, 1H, NH), 3.83 (s, 4H, 2×C<u>H</u>$_2$Pyr), 3.65 – 3.46 (m, 12H), 3.35 (quartet, *J* = 6.6 Hz, 2H, C<u>H</u>$_2$NH), 3.29 (s, 2H, NCH$_2$CO), 3.20 (quartet, *J* = 6.3 Hz, 2H, C<u>H</u>$_2$NH), 1.84 (pentet, *J* = 6.7 Hz, 2H, CH$_2$C<u>H</u>$_2$CH$_2$), 1.74 (pentet, *J* = 6.2 Hz, 2H, CH$_2$C<u>H</u>$_2$CH$_2$), 1.41 (s, 9H, C(CH$_3$)$_3$); ^{13}C NMR (75 MHz, CDCl$_3$): δ=171.3 (CO), 158.4 (2×Cquat Pyr), 156.1 (NCOOtBu), 149.5 (2×CH Pyr), 136.6 (2×CH Pyr), 123.3 (2×CH Pyr), 122.5 (2×CH Pyr), 79.0 (<u>C</u>(CH$_3$)$_3$), 70.7 (2×CH$_2$), 70.4 (CH$_2$), 70.3 (CH$_2$), 69.7 (CH$_2$), 69.2 (CH$_2$), 60.6 (2×<u>C</u>H$_2$Pyr), 58.2 (CH$_2$), 54.9 (CH$_2$), 38.7 (CH$_2$), 36.5 (CH$_2$), 29.8 (CH$_2$), 28.6 (C(<u>C</u>H$_3$)$_3$); HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{29}H_{46}N_5O_6$: 560.3455, found: 560.3448.

Cis tert-butyl (4-(di-2-picolylaminoacetamido)cyclohexyl)carbamate (**6e**)

Yield (1.272 g, 98%): ^{1}H NMR (300 MHz, CDCl$_3$): δ=8.65 (d, *J* = 7.8 Hz, 1H, NH), 8.57 (ddd, *J* = 4.9, 1.8, 0.9 Hz, 2H), 7.62 (td, *J* = 7.6, 1.9 Hz, 2H), 7.28 (m, 2H), 7.18 (ddd, *J* = 7.6, 4.9, 1.2 Hz, 2H), 4.50 (s, 1H, NH), 3.92 (m, 1H, C<u>H</u>NH), 3.84 (s, 4H, 2×NC<u>H</u>$_2$Py), 3.60 (m, 1H, C<u>H</u>NH), 3.29 (s, 2H, NCH$_2$CO), 1.80 – 1.59 (m, 8H), 1.47 (s, 9H, C(CH$_3$)$_3$); ^{13}C NMR (75 MHz, CDCl$_3$): δ=170.7 (CO), 158.7 (2×Cquat Pyr), 155.5 (NCOOtBu), 149.8 (2×CH Pyr), 136.9 (2×CH Pyr), 123.7 (2×CH Pyr), 122.8 (2×CH Pyr), 78.9 (<u>C</u>(CH$_3$)$_3$), 60.7 (2×<u>C</u>H$_2$Pyr), 58.4 (N<u>C</u>H$_2$CO), 45.4 (2×CH), 28.9 (4×CH$_2$), 28.7 (C(<u>C</u>H$_3$)$_3$); HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{25}H_{36}N_5O_3$: 454.2827, found: 454.2818.

Trans tert-butyl (4-(di-2-picolylaminoacetamido)cyclohexyl)carbamate (**6f**)

Yield (1.048 g, 75%): ^{1}H NMR (400 MHz, CDCl$_3$): δ=8.88 (d, *J* = 8.4 Hz, 1H, NH), 8.54 (tdd, *J* = 4.9, 1.9, 0.9 Hz, 2H), 7.58 (td, *J* = 7.7, 1.8 Hz, 2H), 7.22 (dt, *J* = 7.8, 1.1 Hz, 2H), 7.15 (m, 2H), 4.46 (d, *J* = 8.3 Hz, 1H, NH), 3.82 (s, 4H, 2×NC<u>H</u>$_2$Py), 3.72 (m, 1H, C<u>H</u>NH), 3.45 (m, 1H, C<u>H</u>NH), 3.29 (s, 2H, NCH$_2$CO), 2.06 – 1.89 (m, 4H), 1.43 (s, 9H, C(CH$_3$)$_3$), 1.36 (td, *J* = 12.4, 3.3 Hz, 2H), 1.26 (m, 2H); ^{13}C NMR (100 MHz, CDCl$_3$): δ=170.7 (CO), 158.4 (2×Cquat Pyr), 155.4 (NCOOtBu), 149.4 (2×CH Pyr), 136.6 (2×CH Pyr), 123.2 (2×CH Pyr), 122.5 (2×CH Pyr), 79.3 (<u>C</u>(CH$_3$)$_3$), 60.5 (2×<u>C</u>H$_2$Pyr), 58.2 (N<u>C</u>H$_2$CO), 49.2 (CH), 47.3 (CH), 32.2 (2×CH$_2$), 31.7 (2×CH$_2$), 28.5 (C(<u>C</u>H$_3$)$_3$); HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{25}H_{36}N_5O_3$: 454.2798, found: 454.2813.

Tert-butyl (4-(di-2-picolylaminoacetamido)phenethyl)carbamate (**6g**)

Yield (11.3 g, quantitative yield): ^{1}H NMR (400 MHz, CDCl$_3$): δ=10.86 (s, 1H, NH), 8.61 (d, *J* = 4.1 Hz, 2H), 7.70 (d, *J* = 4.1 Hz, 2H), 7.61 (td, *J* = 7.7, 1.7 Hz, 2H), 7.27 (m, 2H), 7.22–7.11 (m, 4H), 4.54 (s, 1H, NH), 3.93 (s, 4H, 2×NC<u>H</u>$_2$Py), 3.42-3.25 (m, 2H), 2.76 (t, *J* = 6.8 Hz, 2H), 1.42 (s, 9H, C(CH$_3$)$_3$); ^{13}C NMR (100 MHz, CDCl$_3$): δ=169.8 (CO), 158.2 (2×Cquat Pyr), 155.9 (NCOOtBu), 149.5 (2×CH Pyr), 137.1 (Cquat Phenyl), 136.7 (2×CH Pyr), 134.4 (Cquat Phenyl), 129.2 (2×CH Phenyl), 123.3 (2×CH Pyr), 122.6 (2×CH Pyr), 112.0 (2×CH Phenyl), 79.2 (<u>C</u>(CH$_3$)$_3$), 60.4 (2×<u>C</u>H$_2$Pyr), 58.8 (N<u>C</u>H$_2$CO), 41.9 (CH$_2$), 35.7 (CH$_2$), 28.5 (C(<u>C</u>H$_3$)$_3$).

Tert-butyl (4-(2-(di-2-picolylaminoacetamido)ethyl)phenyl)carbamate (**6h**)

Yield (2,149 g, quantitative yield): ^{1}H NMR (400 MHz, CDCl$_3$): δ=8.66 (t, *J* = 5.8 Hz, 1H, NH), 8.49 (ddd, *J* = 4.7, 1.8, 1.1 Hz, 2H), 7.56 (td, *J* = 7.7, 1.8 Hz, 2H), 7.22 (d, *J* = 8.0 Hz, 2H), 7.17 – 7.07 (m, 6H), 6.47 (bs, 1H, NH), 3.76 (s, 4H, 2×NC<u>H</u>$_2$Py), 3.54 (td, *J* = 7.0, 5.7 Hz, 2H, C<u>H</u>$_2$NH), 3.27 (s, 2H, NCH$_2$CO), 2.81 (t, *J* = 7.0 Hz, 2H, PhC<u>H</u>$_2$), 1.51 (s, 9H, C(CH$_3$)$_3$); ^{13}C NMR (100 MHz, CDCl$_3$): δ=171.3 (CO), 158.3 (2×Cquat Pyr), 152.8 (NCOOtBu), 149.4 (2×CH Pyr), 136.7 (Cquat Phenyl), 136.6 (2×CH Pyr), 134.0 (Cquat Phenyl), 129.3 (2×CH Pyr), 123.2 (2×CH Phenyl), 122.4 (2×CH Pyr), 118.6 (2×CH Phenyl), 80.5 (<u>C</u>(CH$_3$)$_3$), 60.6 (2×<u>C</u>H$_2$Pyr), 58.1 (N<u>C</u>H$_2$CO), 40.4 (CH$_2$), 35.0 (CH$_2$), 28.5

(C(CH$_3$)$_3$); HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{27}H_{34}N_5O_3$: 476.2673, found: 476.2656.

Tert-butyl (2-(1-(bis(pyridin-2-ylmethyl)glycyl)piperidin-4-yl)ethyl)carbamate (**6i**)

The title compound was isolated as a mixture of amide rotamers (758 mg, quantitative yield): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.57 – 8.46 (m, 2H), 7.68 – 7.59 (m, 2H), 7.53 and 7.34 (d, *J* = 7.8 Hz, 2H), 7.14 (ddd, *J* = 7.5, 4.9, 1.2 Hz, 2H), 4.63 – 4.44 (m, 3H), 3.91 (d, *J* = 14 Hz, AB system, 2H), 3.86 (d, *J* = 14 Hz, AB system, 2H), 3.75 (m, 1H), 3.42 (d, *J* = 14.7 Hz, AB system, 1H), 3.37 (d, *J* = 14.7 Hz, AB system, 1H), 3.21 – 3.05 (m, 3H), 2.80 (td, *J* = 12.8, 2.7 Hz, 1H), 2.46 (m, 2H), 1.79 – 1.55 (m, 2H), 1.55 – 1.32 (m, 11H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=168.9 and 168.6 (CO), 159.9 and 159.1 (2×Cquat Pyr), 156.0 (NCOOtBu), 149.4 and 149.1 (2×CH Pyr), 136.6 and 136.5 (2×CH Pyr), 123.8 and 122.4 (2×CH Pyr), 122.2 and 122.0 (2×CH Pyr), 79.3 (C(CH$_3$)$_3$), 60.5 (2×CH$_2$Pyr), 56.1, 54.9, 46.7, 45.5, 42.2, 41.8, 38.1 and 36.8 (5×CH$_2$), 33.6 (CH), 32.6, 32.0 and 31.8 (2×CH$_2$), 28.5 (C(CH$_3$)$_3$); HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{26}H_{38}N_5O_3$: 468.2973, found: 468.2969.

Tert-butyl (2-(4-(bis(pyridin-2-ylmethyl)glycyl)piperazin-1-yl)ethyl)carbamate (**6j**)

Yield (1.05 g, 75%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.53 (ddd, *J* = 4.9, 1.8, 0.9 Hz, 2H), 7.65 (m, 2H), 7.52 (dt, *J* = 7.8, 1.1 Hz, 2H), 7.16 (dtd, *J* = 7.5, 4.3, 3.8, 1.2 Hz, 2H), 4.92 (bs, 1H, NH), 3.89 (s, 4H, 2×NCH$_2$Py), 3.57 (t, *J* = 4.9 Hz, 2H), 3.42 (s, 2H, NCH$_2$CO), 3.38 (t, *J* = 5.0 Hz, 2H), 3.22 (m, 2H), 2.43 (t, *J* = 6.0 Hz, 2H), 2.38 (t, *J* = 5.1 Hz, 2H), 2.33 (t, *J* = 5.1 Hz, 2H), 1.45 (s, 9H, C(CH$_3$)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ=168.9 (CO), 159.0 (2×Cquat Pyr), 156.0 (NCOOtBu), 149.2 (2×CH Pyr), 136.6 (2×CH Pyr), 123.8 (2×CH Pyr), 122.3 (2×CH Pyr), 79.4 (C(CH$_3$)$_3$), 60.5 (2×CH$_2$Pyr), 56.2 (NCH$_2$CO), 55.0 (CH$_2$), 53.1 (CH$_2$), 52.8 (CH$_2$), 45.2 (CH$_2$), 41.7 (CH$_2$), 37.2 (CH$_2$), 28.6 (C(CH$_3$)$_3$); HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{25}H_{37}N_6O_3$: 469.2913, found: 469.2922.

*General procedure for N-Boc deprotection of compounds **6a-j***

The corresponding *N*-Boc-protected amine **6a-j** (1.0 equiv.) was dissolved in CH_2Cl_2 (0.25 M) and cooled to 0°C in an ice-water bath. Trifluoroacetic acid (60.0 equiv.) in CH_2Cl_2 (13 M) was then slowly added to the stirring mixture. The reaction was left at 0°C for 20 min before warming up to room temperature. The mixture was stirred for an additional 3 h at room temperature, until TLC (2% MeOH in CH_2Cl_2, alumina plates) indicated consumption of the carbamate. After solvent removal under reduced pressure, excess 1 M aqueous K_2CO_3 (50 mL) was added to the mixture, and the compound was extracted with CH_2Cl_2 (3-20 x 20 mL). The combined organic layers were washed with fresh 0.5 M K_2CO_3 (3 x 50 mL), dried on K_2CO_3, filtered, and the solvent removed under reduced pressure, to give title compound **7a-j** as a brown oil.

N-(2-Aminoethyl)-2-(di-2-picolylamino)acetamide (**7a**)

Yield (1.52 g, 60%): ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.81 (t, *J* = 5.8 Hz, 1H, NH), 8.50 (m, 2H), 7.55 (td, *J* = 7.6, 1.8 Hz, 2H), 7.20 (dt, *J* = 7.7, 1.1 Hz, 2H), 7.10 (m, 2H), 3.78 (s, 4H, 2×NCH$_2$Py), 3.33 (quartet, *J* = 5.8 Hz, 2H), 3.28 (s, 2H, NCH$_2$CO), 2.83 (dd, *J* = 6.5, 5.2 Hz, 2H), 2.59 (s, 2H, NH$_2$); ^{13}C NMR (75 MHz, $CDCl_3$): δ=171.6 (CO), 158.1 (2×Cquat Pyr), 149.4 (2×CH Pyr), 136.6 (2×CH Pyr), 123.4 (2×CH Pyr), 122.5 (2×CH Pyr), 60.4 (2×CH$_2$Pyr), 58.2 (NCH$_2$CO), 42.1 (CH$_2$), 41.6 (CH$_2$); HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{16}H_{22}N_5O$: 300.1831, found: 300.1824.

N-(4-Aminobutyl)-2-(di-2-picolylamino)acetamide (**7b**)

Yield (910 mg, 74%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.74 (t, *J* = 6.1 Hz, 1H, NH), 8.53 (ddd, *J* = 4.9, 1.8, 0.9 Hz, 2H), 7.58 (td, *J* = 7.6, 1.8 Hz, 2H), 7.24 (dt, *J* = 7.8, 1.1 Hz, 2H), 7.14 (ddd, *J* = 7.6, 4.9, 1.2 Hz, 2H), 3.81 (s, 4H, 2×NCH$_2$Py), 3.32 – 3.23 (m, 4H), 2.67 (t, *J* = 6.9 Hz, 2H), 1.62 – 1.53 (m, 2H), 1.51 – 1.41 (m, 2H), 1.38 (br s, 2H, NH$_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ=171.2 (CO), 158.4 (2×Cquat Pyr), 149.4 (2×CH Pyr), 136.6 (2×CH Pyr), 123.3 (2×CH Pyr), 122.5 (2×CH Pyr), 60.5 (2×CH$_2$Pyr), 58.1 (NCH$_2$CO), 42.0 (CH$_2$), 39.0 (CH$_2$), 31.3 (CH$_2$), 26.9 (CH$_2$); HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{18}H_{26}N_5O$: 328.2116, found: 328.2132.

N-(6-Aminohexyl)-2-(di-2-picolylamino)acetamide (**7c**)

Yield (47 mg, 60%): ^{1}H NMR (400 MHz, CD_3OD): δ=8.78 (ddd, *J* = 5.6, 1.6, 0.8 Hz, 2H), 8.29 (td, *J* = 7.8, 1.6 Hz, 2H), 7.83 (dt, *J* = 8.0, 1.0 Hz, 2H), 7.77 (ddd, *J* = 7.7, 5.6, 1.2 Hz, 2H), 4.39 (s, 4H, 2×NCH$_2$Py), 3.63 (s, 2H, NCH$_2$CO), 3.17 (t, *J* = 7.2 Hz, 2H), 2.91 (t, *J* = 7.7 Hz, 2H), 1.70 – 1.28 (m, 10H, 4×CH$_2$, NH$_2$); ^{13}C NMR (100 MHz, CD_3OD): δ=172.1 (CO), 155.7 (2×Cquat Pyr), 145.3 (2×CH Pyr), 144.9 (2×CH Pyr), 127.1 (2×CH Pyr), 126.4 (2×CH Pyr), 58.4 (2×CH$_2$Pyr), 58.0 (NCH$_2$CO), 40.6 (CH$_2$), 40.3 (CH$_2$), 30.1 (CH$_2$), 28.5 (CH$_2$), 27.5 (CH$_2$), 27.1 (CH$_2$); HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{20}H_{30}N_5O$: 356.2443, found: 356.2450.

N-(3-(2-(2-(3-Aminopropoxy)ethoxy)ethoxy)propyl)-2-(di-2-picolylamino)acetamide (**7d**)

Yield (362 mg, 89%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.71 (t, *J* = 5.8 Hz, 1H, NH), 8.54 (ddd, *J* = 5.2, 2.0, 0.9 Hz, 2H), 7.60 (m, 2H), 7.26 (m, 2H), 7.14 (m, 2H), 3.82 (s, 4H, 2×NCH$_2$Py), 3.70 – 3.45 (m, 12H), 3.35 (quartet, *J* = 6.6 Hz, 2H), 3.29 (s, 2H, NCH$_2$CO), 2.76 (t, *J* = 6.7 Hz, 2H), 1.84 (pentet, *J* = 6.7 Hz, 2H), 1.70 (pentet, *J* = 6.5 Hz, 2H), 1.54 (bs, 2H, NH$_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ=171.3 (CO), 158.4 (2×Cquat Pyr), 149.5 (2×CH Pyr), 136.6 (2×CH Pyr), 123.3 (2×CH Pyr), 122.5 (2×CH Pyr), 70.72 (CH$_2$), 70.69 (CH$_2$), 70.4 (CH$_2$), 70.3 (CH$_2$), 69.5 (CH$_2$), 69.2 (CH$_2$), 60.5 (2×CH$_2$Pyr), 58.1 (NCH$_2$CO), 39.7 (CH$_2$), 36.5 (CH$_2$), 33.5 (CH$_2$), 29.7 (CH$_2$); HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{24}H_{38}N_5O_4$: 460.2927, found: 460.2923.

Cis N-(4-aminocyclohexyl)-2-(di-2-picolylamino)acetamide (**7e**)

Yield (608 mg, 78%): ^{1}H NMR (300 MHz, DMSO-d6): δ=8.55 (m, 2H), 8.34 (d, *J* = 7.8 Hz, 1H, NH), 7.75 (m, 2H), 7.42 (m, 2H), 7.27

(m, 2H), 3.80 (s, 4H, 2×NC$\underline{H}_2$Py), 3.70 (m, 1H, CH), 3.16 (s, 2H, NCH_2CO), 2.73 (m, 1H, CH), 1.70 – 1.31 (m, 10H, 4×CH_2, NH_2); ^{13}C NMR (75 MHz, DMSO-d6): δ=169.0 (CO), 158.4 (2×Cquat Pyr), 149.1 (2×CH Pyr), 136.6 (2×CH Pyr), 123.0 (2×CH Pyr), 122.3 (2×CH Pyr), 59.6 (2×C$\underline{H}_2$Pyr), 57.4 (NC$\underline{H}_2$CO), 47.6 (CH), 44.6 (CH), 31.1 (2×CH_2), 27.8 (2×CH_2); HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{20}H_{28}N_5O$: 354.2300, found: 354.2293.

Trans N-(4-aminocyclohexyl)-2-(di-2-picolylamino)acetamide (**7f**)

Yield (631 mg, 83%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.67 (d, *J* = 8.3 Hz, 1H, NH), 8.53 (ddd, *J* = 4.9, 1.8, 0.9 Hz, 2H), 7.59 (td, *J* = 7.6, 1.8 Hz, 2H), 7.23 (dt, *J* = 7.8, 1.1 Hz, 2H), 7.14 (m, 2H), 3.81 (s, 4H, 2×NC$\underline{H}_2$Py), 3.70 (tdt, *J* = 11.8, 8.3, 4.0 Hz, 1H, C$\underline{H}$NH), 3.27 (s, 2H, NCH_2CO), 2.67 (tt, *J* = 10.6, 3.9 Hz, 1H, C$\underline{H}$NH_2), 1.96 – 1.82 (m, 4H), 1.43 – 1.12 (m, 6H, 2×CH_2, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ=170.5 (CO), 158.4 (2×Cquat Pyr), 149.4 (2×CH Pyr), 136.6 (2×CH Pyr), 123.3 (2×CH Pyr), 122.5 (2×CH Pyr), 60.5 (2×C$\underline{H}_2$Pyr), 58.2 (NC$\underline{H}_2$CO), 50.2 (CH), 47.6 (CH), 35.5 (2×CH_2), 31.8 (2×CH_2); HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{20}H_{28}N_5O$: 354.2276, found: 354.2288.

N-(4-(2-Aminoethyl)phenyl)-2-(di-2-picolylamino)acetamide (**7g**)

Yield (155 mg, 98%): ^{1}H NMR (400 MHz, DMSO-d6): δ=10.53 (s, 1H, NH), 8.52 (m, 2H), 7.75 (td, *J* = 7.6, 1.8 Hz, 2H), 7.58 (d, *J* = 8.4 Hz, 2H), 7.45 (d, *J* = 7.8 Hz, 2H), 7.27 (ddd, *J* = 7.5, 4.9, 1.0 Hz, 2H), 7.15 (d, *J* = 8.5 Hz, 2H), 3.90 (m, 4H, 2×NC$\underline{H}_2$Py), 3.41 (m, 2H, NCH_2CO), 2.73 (t, *J* = 7.1 Hz, 2H), 2.58 (t, *J* = 7.1 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=169.0 (CO), 158.4 (2×Cquat Pyr), 149.0 (2×CH Pyr), 136.7 (Cquat Phenyl), 136.6 (2×CH Pyr), 135.3 (Cquat Phenyl), 128.9 (2×CH Phenyl), 123.0 (2×CH Pyr), 122.4 (2×CH Pyr), 119.0 (2×CH Phenyl), 59.4 (2×C$\underline{H}_2$Pyr), 57.8 (NC$\underline{H}_2$CO), 43.7 (CH_2); HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{22}H_{26}N_5O$: 376.2059, found: 376.2133.

N-(4-Aminophenethyl)-2-(di-2-picolylamino)acetamide (**7h**)

Yield (1.42 g, 90%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.54 (m, 1H, NH), 8.50 (ddd, *J* = 4.8, 1.8, 1.0 Hz, 2H), 7.58 (td, *J* = 7.6, 1.8 Hz, 2H), 7.18 – 7.10 (m, 4H), 6.99 (m, 2H), 6.58 (m, 2H), 3.77 (s, 4H, 2×NC$\underline{H}_2$Py), 3.50 (td, *J* = 7.1, 5.7 Hz, 2H, C$\underline{H}_2$NH), 3.26 (s, 2H, NCH_2CO), 2.75 (t, *J* = 7.1 Hz, 2H, PhCH_2), 1.04 (bs, 2H, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ=171.1 (CO), 158.3 (2×Cquat Pyr), 149.4 (2×CH Pyr), 144.8 (Cquat Phenyl), 136.6 (2×CH Pyr), 129.6 (2×CH Phenyl), 129.1 (Cquat Phenyl), 123.2 (2×CH Pyr), 122.4 (2×CH Pyr), 115.3 (2×CH Phenyl), 60.6 (2×C$\underline{H}_2$Pyr), 58.1 (NC$\underline{H}_2$CO), 40.5 (CH_2), 34.8 (CH_2). HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{22}H_{26}N_5O$: 376.2133, found: 376.2132.

1-(4-(2-Aminoethyl)piperidin-1-yl)-2-(di-2-picolylamino)ethan-1-one (**7i**)

The title compound was isolated as a mixture of amide rotamers (465 mg, 81% yield): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.57 – 8.47 (m, 2H), 7.69 – 7.59 (m, 2H), 7.53 and 7.34 (dt, *J* = 7.9, 1.1 Hz, 2H), 7.18 – 7.09 (m, 2H), 4.52 (ddt, *J* = 13.3, 4.2, 2.4 Hz, 1H), 3.91 (d, *J* = 14.1 Hz, AB system, 2H), 3.86 (d, *J* = 14.1 Hz, AB system, 2H), 3.74 (m, 1H), 3.42 (d, *J* = 14.7 Hz, AB system, 1H), 3.38 (d, *J* = 14.7 Hz, AB system, 1H), 2.82 (m, 1H), 2.71 (m, 2H), 2.48 (td, *J* = 13.0, 3.0 Hz, 1H), 1.74 – 1.46 (m, 5H), 1.37 (q, *J* = 6.9 Hz, 2H), 1.05 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=168.6 (CO), 159.8 and 159.1 (2×Cquat Pyr), 149.3 and 149.1 (2×CH Pyr), 136.5 (2×CH Pyr), 123.8 and 122.4 (2×CH Pyr), 122.2 and 122.0 (2×CH Pyr), 60.5 (2×C$\underline{H}_2$Pyr), 56.0 and 54.9 (NC$\underline{H}_2$CO), 45.5 (CH_2), 42.2 (CH_2), 40.2 (CH_2), 39.4 (CH_2), 33.7 (CH), 32.8 (CH_2), 32.1 (CH_2); HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{21}H_{30}N_5O$: 368.2449, found: 368.2445.

1-(4-(2-Aminoethyl)piperazin-1-yl)-2-(di-2-picolylamino)ethan-1-one (**7j**)

Yield (500 mg, 79%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.52 (ddd, *J* = 4.9, 1.8, 0.9 Hz, 2H), 7.64 (td, *J* = 7.6, 1.8 Hz, 2H), 7.51 (dt, *J* = 7.8, 1.1 Hz, 2H), 7.15 (ddd, *J* = 7.5, 4.9, 1.3 Hz, 2H), 3.89 (s, 4H, 2×NC$\underline{H}_2$Py), 3.56 (t, *J* = 5.2 Hz, 2H), 3.41 (s, 2H, NCH_2CO), 3.37 (m, 2H), 2.76 (t, *J* = 6.1 Hz, 2H), 2.49 – 2.28 (m, 6H), 1.78 (bs, 2H, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ=168.8 (CO), 158.9 (2×Cquat Pyr), 149.1 (2×CH Pyr), 136.6 (2×CH Pyr), 123.8 (2×CH Pyr), 122.3 (2×CH Pyr), 61.1 (CH_2), 60.5 (2×C$\underline{H}_2$Pyr), 56.1 (NC$\underline{H}_2$CO), 53.5 (CH_2), 53.0 (CH_2), 45.3 (CH_2), 41.8 (CH_2), 38.7 (CH_2); HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{20}H_{29}N_6O$: 369.2387, found: 369.2397.

General procedure for peptide coupling reaction

The corresponding primary amine **7a-j** (1.0 equiv.) was dissolved in CH_2Cl_2 (0.25 M) and cooled to 0°C in an ice-water bath. *N*-Boc-*D*-Ala-*D*-Ala-OH **4** (1.0 equiv.) and HATU (1.0 equiv.) were added, before *N*- methylmorpholine (2 equiv.) was added to the stirring mixture. The mixture was stirred in the ice-water bath for 15 min before slowly warming up to room temperature. After 4.5 h at room temperature, the mixture was washed with 0.5 M K_2CO_3 (3 times), dried over K_2CO_3, filtered and concentrated under reduced pressure. The oily residue was dissolved in CH_2Cl_2 and purified by C-18 reverse phase column chromatography (20-75% MeOH in water) to afford title compounds **8a-j** as brown oils.

Tert-butyl ((10R,13R)-10-methyl-4,9,12-trioxo-1-(pyridin-2-yl)-2-(pyridin-2-ylmethyl)-2,5,8,11-tetraazatetradecan-13-yl)carbamate (**8a**)

Yield (250 mg, 28%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.90 (t, *J* = 5.2 Hz, 1H, NH), 8.48 (ddd, *J* = 4.9, 1.8, 0.9 Hz, 2H), 7.59 – 7.48 (m, 3H, 2*CH Pyr, NH), 7.20 (dt, *J* = 7.7, 1.2 Hz, 2H), 7.1 – 7.06 (m, 3H, 2*CH Pyr, NH), 5.49 (d, *J* = 7.2 Hz, 1H, NH), 4.32 (pentet, *J* = 7.1 Hz, 1H, CHα), 4.09 (m, 1H, CHα), 3.76 (s, 4H, 2×NC$\underline{H}_2$Py), 3.42 – 3.27 (m, 4H), 3.24 (s, 2H, NCH_2CO), 1.34 (s, 9H, C(CH_3)$_3$), 1.25 (d, *J* = 7.1 Hz, 3H, CH_3), 1.21 (d, *J* = 7.0 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ=172.5 (2×CO), 172.2

(CO), 157.9 (2×Cquat Pyr), 155.6 (NCOOtBu), 149.3 (2×CH Pyr), 136.7 (2×CH Pyr), 123.4 (2×CH Pyr), 122.5 (2×CH Pyr), 79.9 (C(CH$_3$)$_3$), 60.3 (2×CH$_2$Pyr), 58.0 (NCH$_2$CO), 50.2 (CHα), 48.8 (CHα), 39.9 (CH$_2$), 38.6 (CH$_2$), 28.3 (C(CH$_3$)$_3$), 18.6 (CH$_3$), 18.3 (CH$_3$); HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{27}H_{40}N_7O_5$: 542.3061, found: 542.3085.

Tert-butyl ((12R,15R)-12-methyl-4,11,14-trioxo-1-(pyridin-2-yl)-2-(pyridin-2-ylmethyl)-2,5,10,13-tetraazahexadecan-15-yl)carbamate (**8b**)

Yield (800 mg, 92%): ^{1}H NMR (400 MHz, CDCl$_3$): δ=8.82 (t, *J* = 5.8 Hz, 1H, NH), 8.54 (ddd, *J* = 4.9, 1.8, 0.9 Hz, 2H), 7.60 (td, *J* = 7.7, 1.8 Hz, 2H), 7.24 (dt, *J* = 7.9, 1.1 Hz, 2H), 7.16 (ddd, *J* = 7.6, 4.9, 1.2 Hz, 2H), 6.96 (t, *J* = 5.6 Hz, 1H, NH), 6.87 (d, *J* = 7.7 Hz, 1H, NH), 5.22 (d, *J* = 6.1 Hz, 1H, NH), 4.43 (pentet, *J* = 7.1 Hz, 1H, CHα), 4.11 (m, 1H, CHα), 3.82 (s, 4H, 2×NCH$_2$Py), 3.34 – 3.17 (m, 6H), 1.56 (m, 4H), 1.41 (s, 9H, C(CH$_3$)$_3$), 1.34 (d, *J* = 7.1 Hz, 3H, CH$_3$), 1.32 (d, *J* = 6.1 Hz, 3H, CH$_3$); ^{13}C NMR (100 MHz, CDCl$_3$): δ=172.6 (CO), 172.1 (CO), 171.5 (CO), 158.2 (2×Cquat Pyr), 155.8 (NCOOtBu), 149.5 (2×CH Pyr), 136.7 (2×CH Pyr), 123.4 (2×CH Pyr), 122.6 (2×CH Pyr), 80.4 (C(CH$_3$)$_3$), 60.6 (2×CH$_2$Pyr), 58.1 (NCH$_2$CO), 50.7 (CHα), 49.0 (CHα), 39.4 (CH$_2$), 38.7 (CH$_2$), 28.4 (C(CH$_3$)$_3$), 27.1 (CH$_2$), 26.4 (CH$_2$), 18.4 (CH$_3$), 18.3 (CH$_3$); HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{29}H_{44}N_7O_5$: 570.3388, found: 570.3398.

Tert-butyl ((14R,17R)-14-methyl-4,13,16-trioxo-1-(pyridin-2-yl)-2-(pyridin-2-ylmethyl)-2,5,12,15-tetraazaoctadecan-17-yl)carbamate (**8c**)

Yield (40 mg, 58%): ^{1}H NMR (300 MHz, CDCl$_3$): δ=8.73 (bs, 1H, NH), 8.53 (m, 2H), 7.59 (td, *J* = 7.6, 1.9 Hz, 2H), 7.25 (d, *J* = 9.0 Hz, 2H), 7.16 (dt, *J* = 7.4, 4.6 Hz, 2H), 6.89 (d, *J* = 7.6 Hz, 1H, NH), 6.69 (d, *J* = 5.8 Hz, 1H, NH), 5.22 (bs, 1H, NH), 4.44 (quartet, *J* = 7.0 Hz, 1H, CHα), 4.11 (m, 1H, CHα), 3.83 (s, 4H, 2×NCH$_2$Py), 3.35 – 2.92 (m, 6H), 1.53 (m, 2H), 1.47 – 1.39 (m, 11H), 1.38 – 1.22 (m, 10H); ^{13}C NMR (75 MHz, CDCl$_3$): δ=172.6 (CO), 172.0 (CO), 171.3 (CO), 158.4 (2×Cquat Pyr), 155.8 (NCOOtBu), 149.5 (2×CH Pyr), 136.6 (2×CH Pyr), 123.3 (2×CH Pyr), 122.5 (2×CH Pyr), 80.4 (C(CH$_3$)$_3$), 60.5 (2×CH$_2$Pyr), 58.2 (NCH$_2$CO), 50.7 (CHα), 49.0 (CHα), 39.3 (CH$_2$), 38.8 (CH$_2$), 29.4 (CH$_2$), 29.3 (CH$_2$), 28.4 (C(CH$_3$)$_3$), 26.3 (CH$_2$), 26.2 (CH$_2$), 18.4 (CH$_3$), 18.3 (CH$_3$); HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{31}H_{48}N_7O_5$: 598.3726, found: 598.3716.

Tert-butyl ((21R,24R)-21-methyl-4,20,23-trioxo-1-(pyridin-2-yl)-2-(pyridin-2-ylmethyl)-9,12,15-trioxa-2,5,19,22-tetraazapentacosan-24-yl)carbamate (**8d**)

Yield (264 mg, 87%): ^{1}H NMR (400 MHz, CDCl$_3$): δ=8.77 (m, 1H, NH), 8.55 (ddd, *J* = 5.0, 1.9, 0.9 Hz, 2H), 7.60 (td, *J* = 7.6, 1.8 Hz, 2H), 7.26 (dt, *J* = 7.8, 1.1 Hz, 2H), 7.15 (ddd, *J* = 7.5, 4.8, 1.2 Hz, 2H), 6.90 (t, *J* = 5.6 Hz, 1H, NH), 6.85 (d, *J* = 7.5 Hz, 1H, NH), 5.14 (m, 1H, NH), 4.38 (pentet, *J* = 7.1 Hz, 1H, CHα), 4.12 (t, *J* = 7.5 Hz, 1H, CHα), 3.83 (s, 4H, 2×NCH$_2$Py), 3.65 – 3.47 (m, 12H), 3.37 (m, 2H), 3.30 (s, 2H, NCH$_2$CO), 2.76 (m, 2H), 1.84 (pentet, *J* = 6.7 Hz, 2H), 1.76 (pentet, *J* = 6.2 Hz, 2H), 1.42 (s, 9H, C(CH$_3$)$_3$), 1.35 (s, 3H, CH$_3$), 1.33 (s, 3H, CH$_3$); ^{13}C NMR (100 MHz, CDCl$_3$): δ=172.4 (CO), 172.0 (CO), 171.4 (CO), 158.3 (2×Cquat Pyr), 155.6 (NCOOtBu), 149.5 (2×CH Pyr), 136.7 (2×CH Pyr), 123.4 (2×CH Pyr), 122.5 (2×CH Pyr), 80.2 (C(CH$_3$)$_3$), 70.7 (CH$_2$), 70.6 (CH$_2$), 70.3 (CH$_2$), 70.2 (CH$_2$), 69.7 (CH$_2$), 69.1 (CH$_2$), 60.5 (2×CH$_2$Pyr), 58.1 (NCH$_2$CO), 50.5 (CHα), 49.0 (CHα), 37.8 (CH$_2$), 36.5 (CH$_2$), 29.7 (CH$_2$), 29.0 (CH$_2$), 28.4 (C(CH$_3$)$_3$), 18.7 (CH$_3$), 18.6 (CH$_3$); HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{35}H_{56}N_7O_8$: 702.4211, found: 702.4190.

Tert-butyl ((R)-1-(((R)-1-((cis-4-(2-(bis(pyridin-2-ylmethyl)amino)acetamido)cyclohexyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (**8e**)

Yield (660 mg, 78%): ^{1}H NMR (300 MHz, CDCl$_3$): δ=8.66 (d, *J* = 7.6 Hz, 1H, NH), 8.53 (ddd, *J* = 4.9, 1.9, 0.9 Hz, 2H), 7.58 (td, *J* = 7.7, 1.8 Hz, 2H), 7.23 (dt, *J* = 7.8, 1.2 Hz, 2H), 7.17 – 7.04 (m, 3H, 2H Pyr, NH), 6.64 (d, *J* = 7.4 Hz, 1H, NH), 5.35 (d, *J* = 7.1 Hz, 1H, NH), 4.41 (pentet, *J* = 7.0 Hz, 1H, CHα), 4.10 (m, 1H, CHα), 3.84 (m, 2H), 3.78 (s, 4H, 2×NCH$_2$Py), 3.23 (s, 2H, NCH$_2$CO), 1.64 (m, 8H), 1.35 (s, 9H, C(CH$_3$)$_3$), 1.32 (d, *J* = 7.0 Hz, 3H, CH$_3$), 1.27 (d, *J* = 7.1 Hz, 3H, CH$_3$); ^{13}C NMR (75 MHz, CDCl$_3$): δ=173.0 (CO), 171.2 (CO), 170.4 (CO), 158.2 (2×Cquat Pyr), 155.5 (NCOOtBu), 149.4 (2×CH Pyr), 136.5 (2×CH Pyr), 123.3 (2×CH Pyr), 122.5 (2×CH Pyr), 80.0 (C(CH$_3$)$_3$), 60.3 (2×CH$_2$Pyr), 58.0 (NCH$_2$CO), 50.3 (CHα), 48.9 (CHα), 46.3 (CH$_2$), 45.3 (CH$_2$), 38.6 (CH$_2$), 28.3 (C(CH$_3$)$_3$), 28.0 (CH$_2$), 18.5 (CH$_3$), 17.8 (CH$_3$). HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{31}H_{46}N_7O_5$: 596.3572, found: 596.3560.

Tert-butyl ((R)-1-(((R)-1-((trans-4-(2-(bis(pyridin-2-ylmethyl)amino)acetamido)cyclohexyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (**8f**)

Yield (800 mg, 95%): ^{1}H NMR (400 MHz, CDCl$_3$): δ=8.90 (d, *J* = 8.3 Hz, 1H, NH), 8.54 (ddd, *J* = 4.9, 1.8, 0.9 Hz, 2H), 7.58 (td, *J* = 7.7, 1.8 Hz, 2H), 7.22 (dt, *J* = 7.8, 1.1 Hz, 2H), 7.15 (ddd, *J* = 7.6, 4.8, 1.2 Hz, 2H), 6.72 (d, *J* = 7.5 Hz, 1H, NH), 6.60 (d, *J* = 8.2 Hz, 1H, NH), 5.08 (m, 1H, NH), 4.40 (pentet, *J* = 7.1 Hz, 1H, CHα), 4.10 (pentet, *J* = 6.7 Hz, 1H, CHα), 3.81 (s, 4H, 2×NCH$_2$Py), 3.73 (m, 2H), 3.29 (s, 2H, NCH$_2$CO), 1.95 (bs, 4H), 1.43 (s, 9H, C(CH$_3$)$_3$), 1.40 – 1.23 (m, 10H); ^{13}C NMR (100 MHz, CDCl$_3$): δ=172.5 (CO), 171.5 (CO), 170.7 (CO), 158.4 (2×Cquat Pyr), 155.8 (NCOOtBu), 149.4 (2×CH Pyr), 136.6 (2×CH Pyr), 123.2 (2×CH Pyr), 122.5 (2×CH Pyr), 80.6 (C(CH$_3$)$_3$), 60.4 (2×CH$_2$Pyr), 58.2 (NCH$_2$CO), 50.8 (CHα), 49.2 (CHα), 47.9 (CH), 47.3 (CH), 31.62 (CH$_2$), 31.60 (CH$_2$), 31.5 (CH$_2$), 31.4 (CH$_2$), 28.4 (C(CH$_3$)$_3$), 18.4 (CH$_3$), 18.3 (CH$_3$); HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{31}H_{46}N_7O_5$: 596.3542, found: 596.3555.

Tert-butyl ((R)-1-(((R)-1-((4-(2-(bis(pyridin-2-ylmethyl)amino)acetamido)phenethyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (**8g**)

Yield (3.65 g, 77%): ^{1}H NMR (400 MHz, DMSO-d6): δ=10.53 (s, 1H, NH), 8.57 (ddd, *J* = 4.9, 1.8, 0.9 Hz, 2H), 7.90 (d, *J* = 5.6 Hz, 1H, NH), 7.82-7.67 (m, 3H, 2H arom, NH), 7.58 (m, 2H), 7.43 (m, 2H), 7.28 (m, 2H), 7.14 (m, 2H), 6.98 (d, *J* = 7.3 Hz, 1H, NH), 4.20 (m, 1H, CHα), 3.90 (m, 5H), 3.41 (s, 2H, NCH_2CO), 3.21 (m, 2H), 2.63 (m, 2H), 1.37 (s, 9H, $C(CH_3)_3$), 1.15 (d, *J* = 7.0 Hz, 6H, 2×CH_3); ^{13}C NMR (100 MHz, DMSO-d6): δ=171.8 (CO), 169.0 (CO), 158.4 (2×Cquat Pyr), 156.1 (NCOOtBu), 149.0 (2×CH Pyr), 136.9 (Cquat Phenyl), 136.7 (2×CH Pyr), 134.2 (Cquat Phenyl), 128.9 (2×CH Phenyl), 123.0 (2×CH Pyr), 122.4 (2×CH Pyr), 119.0 (2×CH Phenyl), 78.1 ($\underline{C}(CH_3)_3$), 59.4 (2×$\underline{C}H_2$Pyr), 57.8 (N$\underline{C}H_2$CO), 49.8 (CHα), 48.0 (CHα), 40.2 (CH_2), 34.4 (CH_2), 28.1 (C($\underline{C}H_3)_3$), 17.9 (2×CH_3).

Tert-butyl ((R)-1-(((R)-1-((4-(2-(2-(bis(pyridin-2-ylmethyl)amino)acetamido)ethyl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (**8h**)

Yield (2.15 g, 94%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.80 (s, 1H, NH), 8.73 (t, *J* = 5.9 Hz, 1H, NH), 8.47 (m, 2H), 7.56 (td, *J* = 7.7, 1.8 Hz, 2H), 7.47 (d, *J* = 8.0 Hz, 2H), 7.15 – 7.07 (m, 6H), 6.95 (d, *J* = 7.5 Hz, 1H, NH), 5.21 (d, *J* = 5.8 Hz, 1H, NH), 4.58 (quartet, *J* = 7.1 Hz, 1H, CHα), 4.16 (bs, 1H, CHα), 3.74 (s, 4H, 2×N$\underline{C}H_2$Py), 3.53 (quartet, *J* = 6.7 Hz, 2H, C$\underline{H}_2$NH), 3.26 (s, 2H, NCH_2CO), 2.80 (t, *J* = 6.9 Hz, 2H, PhC$\underline{H}_2$), 1.43 (d, *J* = 7.1 Hz, 3H, CH_3), 1.40 (s, 9H, $C(CH_3)_3$), 1.37 (d, *J* = 7.1 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ=173.0 (CO), 171.4 (CO), 170.3 (CO), 158.2 (2×Cquat Pyr), 156.1 (NCOOtBu), 149.4 (2×CH Pyr), 136.7 (2×CH Phenyl), 136.5 (Cquat Phenyl), 135.1 (Cquat Phenyl), 129.1 (2×CH Pyr), 123.2 (2×CH Pyr), 122.5 (2×CH Pyr), 120.0 (2×CH Phenyl), 80.8 ($\underline{C}(CH_3)_3$), 60.5 (2×$\underline{C}H_2$Pyr), 58.1 (N$\underline{C}H_2$CO), 51.0 (CHα), 49.7 (CHα), 40.4 (CH_2), 35.1 (CH_2), 28.4 (C($\underline{C}H_3)_3$), 18.1 (CH_3), 18.0 (CH_3); HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{33}H_{44}N_7O_5$: 618.3385, found: 618.3398.

Tert-butyl ((R)-1-(((R)-1-((2-(1-(bis(pyridin-2-ylmethyl)glycyl)piperidin-4-yl)ethyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (**8i**)

The title compound was isolated as a mixture of amide rotamers (200 mg, 27% yield): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.52 (ddd, *J* = 4.9, 2.0, 1.1 Hz, 2H), 7.65 (td, *J* = 7.7, 1.8 Hz, 2H), 7.53 (d, *J* = 7.8 Hz, 2H), 7.15 (ddd, *J* = 7.5, 4.9, 1.2 Hz, 2H), 6.68 – 6.53 (m, 2H, 2*NH), 5.00 (s, 1H, NH), 4.51 (d, *J* = 13.3 Hz, 1H, NH), 4.41 (pentet, *J* = 7.2 Hz, 1H, CHα), 4.07 (m, 1H, CHα), 3.91 (d, *J* = 14.1 Hz, AB system, 2H), 3.86 (d, *J* = 14.1 Hz, AB system, 2H), 3.75 (m, 1H), 3.40 (s, 2H, NCH_2CO), 3.29 (m, 1H), 3.16 (m, 1H), 2.80 (m, 1H), 2.47 (m, 1H), 2.04 (bs, 1H), 1.76 – 1.56 (m, 2H), 1.55 – 1.39 (m, 11H, $C(CH_3)_3$, CH_2), 1.37 (d, *J* = 3.0 Hz, 3H, CH_3), 1.36 (d, *J* = 3.1 Hz, 3H, CH_3), 1.11 – 0.94 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=172.6 (CO), 171.9 (CO), 168.7 (CO), 159.1 (2×Cquat Pyr), 156.1 (NCOOtBu), 149.1 (2×CH Pyr), 136.6 (2×CH Pyr), 123.8 (2×CH Pyr), 122.3 (2×CH Pyr), 81.0 ($\underline{C}(CH_3)_3$), 60.5 (2×$\underline{C}H_2$Pyr), 56.1 (N$\underline{C}H_2$CO), 51.2 (CHα), 49.1 (CHα), 45.49 and 45.44 (CH_2), 42.2 (CH_2), 37.1 and 37.0 (CH_2), 36.4, 36.0 and 35.9 (CH_2), 33.7 and 33.5 (CH), 32.6 (CH_2), 31.94 and 31.87 (CH_2), 28.4 (C($\underline{C}H_3)_3$), 18.0 (2×CH_3); HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{32}H_{48}N_7O_5$: 610.3700, found: 610.3711.

Tert-butyl ((R)-1-(((R)-1-((2-(4-(bis(pyridin-2-ylmethyl)glycyl)piperazin-1-yl)ethyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (**8j**)

Yield (200 mg, 25%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.52 (ddd, *J* = 5.0, 1.8, 0.9 Hz, 2H), 7.65 (td, *J* = 7.6, 1.8 Hz, 2H), 7.51 (dt, *J* = 7.8, 1.2 Hz, 2H), 7.16 (ddd, *J* = 7.5, 4.9, 1.3 Hz, 2H), 6.73 (d, *J* = 7.6 Hz, 1H, NH), 6.65 (bs, 1H, NH), 5.04 (d, *J* = 6.4 Hz, 1H, NH), 4.42 (pentet, *J* = 7.2 Hz, 1H, CHα), 4.12 (m, 1H, CHα), 3.88 (s, 4H, 2×N$\underline{C}H_2$Py), 3.57 (m, 2H), 3.45 – 3.20 (m, 6H), 2.46 (t, *J* = 6.6 Hz, 2H), 2.43 – 2.29 (m, 4H), 1.43 (s, 9H, $C(CH_3)_3$), 1.36 (d, *J* = 6.3 Hz, 3H, CH_3), 1.34 (d, *J* = 6.2 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ=172.5 (CO), 172.0 (CO), 168.8 (CO), 158.9 (2×Cquat Pyr), 155.8 (NCOOtBu), 149.2 (2×CH Pyr), 136.6 (2×CH Pyr), 123.8 (2×CH Pyr), 122.3 (2×CH Pyr), 80.6 ($\underline{C}(CH_3)_3$), 60.4 (2×$\underline{C}H_2$Pyr), 56.6 (CH_2), 56.2 (N$\underline{C}H_2$CO), 53.1 (CH_2), 52.8 (CH_2), 50.6 (CHα), 49.0 (CHα), 45.1 (CH_2), 41.7 (CH_2), 36.2 (CH_2), 28.4 (C($\underline{C}H_3)_3$), 18.4 (CH_3), 18.2 (CH_3); HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{31}H_{47}N_8O_5$: 611.3659, found: 611.3664.

*General procedure for N-Boc deprotection of compounds **8a-j***

The compounds **1a-j** were synthesized and purified according the previously described method (see general procedure for N-Boc deprotection of compounds **6a-j**). All the compounds, except **1g**, were obtained as oils.

(R)-2-Amino-N-((R)-1-((2-(2-(di-2-picolylamino)acetamido)ethyl)amino)-1-oxopropan-2-yl)propanamide (**1a**)

Yield (80 mg, 54%): ^{1}H NMR (400 MHz, CD_3CN): δ=8.53 (m, 2H), 8.28 (bs, 1H, NH), 8.23 (bs, 1H, NH), 7.69 (m, 2H), 7.51 (m, 1H, NH), 7.32 (dt, *J* = 7.8, 1.1 Hz, 2H), 7.22 (ddd, *J* = 7.7, 4.8, 1.2 Hz, 2H), 4.17 (pentet, *J* = 7.2 Hz, 1H, CHα), 4.00 (t, *J* = 7.2 Hz, 1H, CHα), 3.74 (s, 4H, 2×N$\underline{C}H_2$Py), 3.34 – 3.14 (m, 4H), 1.47 (d, *J* = 7.0 Hz, 3H, CH_3), 1.34 – 1.25 (m, 4H), 1.22 (d, *J* = 7.2 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, CD_3CN): δ=173.3 (CO), 171.4 (CO), 161.5 (CO), 159.0 (2×Cquat Pyr), 150.3 (2×CH Pyr), 137.9 (2×CH Pyr), 124.8 (2×CH Pyr), 123.5 (2×CH Pyr), 60.9 (2×$\underline{C}H_2$Pyr), 58.7 (N$\underline{C}H_2$CO), 54.6 (CHα), 50.7 and 50.6 (CHα), 40.6 (CH_2), 39.4 (CH_2), 17.73 (CH_3), 17.66 (CH_3); HRMS-ESI *m/z* [*M*+Na]$^+$ calculated for $C_{22}H_{31}NaN_7O_3$: 464.2372, found: 464.2381.

(R)-2-Amino-N-((R)-1-((4-(2-(bis(pyridin-2-ylmethyl)amino)acetamido)butyl)amino)-1-oxopropan-2-yl)propanamide (**1b**)

Yield (94 mg, 57%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.78 (t, *J* = 5.9 Hz, 1H, NH), 8.54 (ddd, *J* = 4.9, 1.8, 0.9 Hz, 2H), 7.76 (d, *J* = 8.0 Hz, 1H, NH), 7.60 (td, *J* = 7.7, 1.8 Hz, 2H), 7.23 (dt, *J* = 7.8, 1.1 Hz, 2H), 7.16 (ddd, *J* = 7.6, 4.9, 1.2 Hz, 2H), 7.03 (bt, *J* = 5.5 Hz, 1H, NH), 4.47 – 4.33 (pentet, *J* = 7.2 Hz, 1H, CHα), 3.81 (s, 4H, 2×NC$\underline{H}_2$Py), 3.45 (quartet, *J* = 7.0 Hz, 1H, CHα), 3.29 – 3.18 (m, 6H), 1.63 – 1.38 (m, 6H), 1.34 (d, *J* = 7.0 Hz, 3H, CH_3), 1.29 (d, *J* = 7.0 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ=176.0 (CO), 172.4 (CO), 171.5 (CO), 158.1 (2×Cquat Pyr), 149.5 (2×CH Pyr), 136.8 (2×CH Pyr), 123.5 (2×CH Pyr), 122.6 (2×CH Pyr), 60.6 (2×$\underline{C}H_2$Pyr), 58.1 (N$\underline{C}H_2$CO), 50.7 (CHα), 48.5 (CHα), 39.2 (CH_2), 38.7 (CH_2), 27.1 (CH_2), 26.4 (CH_2), 21.6 (CH_3), 18.2 (CH_3); HRMS-ESI *m/z* $[M+H]^+$ calculated for $C_{24}H_{36}N_7O_3$: 470.2871, found: 470.2874.

(R)-2-Amino-N-((R)-1-((6-(2-(di-2-picolylamino)acetamido)hexyl)amino)-1-oxopropan-2-yl)propanamide (**1c**)

Yield (10 mg, 51%): ^{1}H NMR (600 MHz, $CDCl_3$): δ=8.69 (m, 1H, NH), 8.55 (ddd, *J* = 5.2, 1.7, 0.9 Hz, 2H), 7.71 (dd, *J* = 17.4, 7.7 Hz, 1H, NH), 7.61 (td, *J* = 7.6, 1.9 Hz, 2H), 7.26 (m, 2H), 7.16 (m, 2H), 6.51 (t, *J* = 5.5 Hz, 1H, NH), 4.39 (m, 1H, CHα), 3.84 (s, 4H, 2×NC$\underline{H}_2$Py), 3.48 (m, 1H, CHα), 3.30 (s, 2H, NCH_2CO), 3.26 (m, 2H), 3.19 (m, 2H), 2.08 (m, 2H), 1.53 (m, 2H), 1.43 (m, 3H), 1.36 (d, *J* = 7.0 Hz, 3H, CH_3), 1.34 – 1.29 (m, 6H); ^{13}C NMR (151 MHz, $CDCl_3$): δ=176.1 (CO), 172.2 (CO), 171.3 (CO), 158.4 (2×Cquat Pyr), 149.5 (2×CH Pyr), 136.7 (2×CH Pyr), 136.6 (2×CH Pyr), 123.3 (2×CH Pyr), 122.5 (2×CH Pyr), 60.6 (2×$\underline{C}H_2$Pyr), 58.2 (N$\underline{C}H_2$CO), 50.7 (CHα), 48.6 (CHα), 39.3 (CH_2), 38.8 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 26.4 (CH_2), 26.3 (CH_2), 21.7 (CH_3), 17.8 (CH_3); HRMS-ESI *m/z* $[M+H]^+$ calculated for $C_{26}H_{40}N_7O_3$: 498.3198, found: 498.3192.

(R)-2-Amino-N-((R)-4,20-dioxo-1-(pyridin-2-yl)-2-(pyridin-2-ylmethyl)-9,12,15-trioxa-2,5,19-triazadocosan-21-yl)propanamide (**1d**)

Yield (34 mg, 94%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.74 (t, *J* = 6.0 Hz, 1H, NH), 8.53 (ddd, *J* = 4.9, 1.7, 0.9 Hz, 2H), 7.74 (d, *J* = 7.8 Hz, 1H, NH), 7.59 (td, *J* = 7.7, 1.8 Hz, 2H), 7.25 (m, 2H), 7.14 (ddd, *J* = 7.3, 4.9, 1.2 Hz, 2H), 7.02 (t, *J* = 5.3 Hz, 1H, NH), 4.36 (pentet, *J* = 7.1 Hz, 1H, CHα), 3.80 (s, 4H, 2×NC$\underline{H}_2$Py), 3.65 – 3.41 (m, 13H), 3.39 – 3.28 (m, 4H), 3.26 (s, 2H, NCH_2CO), 2.83 (bs, 2H), 1.81 (pentet, *J* = 6.7 Hz, 2H), 1.74 (pentet, *J* = 6.1 Hz, 2H), 1.32 (d, *J* = 7.0, 3H, CH_3), 1.28 (d, *J* = 6.9 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ=175.8 (CO), 172.3 (CO), 171.3 (CO), 158.2 (2×Cquat Pyr), 149.4 (2×CH Pyr), 136.7 (2×CH Pyr), 123.4 (2×CH Pyr), 122.5 (2×CH Pyr), 70.6 (CH_2), 70.5 (CH_2), 70.3 (CH_2), 70.2 (CH_2), 69.6 (CH_2), 69.0 (CH_2), 60.5 (2×$\underline{C}H_2$Pyr), 58.1 (N$\underline{C}H_2$CO), 50.7 (CHα), 48.5 (CHα), 37.7 (CH_2), 36.5 (CH_2), 29.6 (CH_2), 28.9 (CH_2), 21.6 (CH_3), 18.5 (CH_3); HRMS-ESI *m/z* $[M+H]^+$ calculated for $C_{30}H_{48}N_7O_6$: 602.3658, found: 602.3661.

(R)-2-amino-N-((R)-1-((4-(2-(di-2-picolylamino)acetamido)cyclohexyl)amino)-1-oxopropan-2-yl)propanamide (**1e**)

Yield (115 mg, 87%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.60 (d, *J* = 7.9 Hz, 1H, NH), 8.57 (m, 2H), 7.82 (d, *J* = 7.8 Hz, 1H, NH), 7.62 (td, *J* = 7.7, 1.9 Hz, 2H), 7.27 (m, 2H), 7.17 (ddd, *J* = 7.5, 4.8, 1.2 Hz, 2H), 6.66 (d, *J* = 7.6 Hz, 1H, NH), 4.41 (pentet, *J* = 7.2 Hz, 1H, CHα), 3.87 (m, 2H), 3.82 (s, 4H, 2×NC$\underline{H}_2$Py), 3.52 (quartet, *J* = 6.9 Hz, 1H, CHα), 3.26 (s, 2H, NCH_2CO), 2.51 (bs, 2H), 1.75 – 1.60 (m, 8H), 1.38 (d, *J* = 6.9 Hz, 3H, CH_3), 1.33 (d, *J* = 6.9 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ=175.8 (CO), 171.5 (CO), 170.4 (CO), 158.3 (2×Cquat Pyr), 149.5 (2×CH Pyr), 136.7 (2×CH Pyr), 123.5 (2×CH Pyr), 122.6 (2×CH Pyr), 60.6 (2×$\underline{C}H_2$Pyr), 58.1 (N$\underline{C}H_2$CO), 50.6 (CHα), 48.8 (CHα), 46.2 (CH), 45.3 (CH), 28.4 (2*CH_2), 28.20 (CH_2), 28.17 (CH_2), 21.5 (CH_3), 17.8 (CH_3); HRMS-ESI *m/z* $[M+H]^+$ calculated for $C_{26}H_{38}N_7O_3$: 496.3026, found: 496.3031.

Trans (R)-2-amino-N-((R)-1-((4-(2-(di-2-picolylamino)acetamido)cyclohexyl)amino)-1-oxopropan-2-yl)propanamide (**1f**)

Yield (45 mg, 55%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.88 (d, *J* = 8.3 Hz, 1H, NH), 8.52 (ddd, *J* = 4.8, 1.8, 0.9 Hz, 2H), 7.78 (d, *J* = 7.9 Hz, 1H, NH), 7.57 (td, *J* = 7.7, 1.8 Hz, 2H), 7.21 (dt, *J* = 7.7, 1.1 Hz, 2H), 7.14 (ddd, *J* = 7.6, 4.9, 1.2 Hz, 2H), 6.80 (d, *J* = 8.1 Hz, 1H, NH), 4.40 (pentet, *J* = 7.1 Hz, 1H, CHα), 3.80 (s, 4H, 2×NC$\underline{H}_2$Py), 3.71 (m, 2H), 3.45 (quartet, *J* = 6.9 Hz, 1H, CHα), 3.27 (s, 2H, NCH_2CO), 2.02 – 1.86 (m, 4H), 1.46 – 1.19 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=175.8 (CO), 171.8 (CO), 170.7 (CO), 158.3 (2×Cquat Pyr), 149.4 (2×CH Pyr), 136.6 (2×CH Pyr), 123.2 (2×CH Pyr), 122.5 (2×CH Pyr), 60.4 (2×$\underline{C}H_2$Pyr), 58.2 (N$\underline{C}H_2$CO), 50.7 (CHα), 48.6 (CHα), 47.8 (CH), 47.3 (CH), 31.49 (2×CH_2), 31.46 (2×CH_2), 21.7 (CH_3), 18.5 (CH_3); HRMS-ESI *m/z* $[M+H]^+$ calculated for $C_{26}H_{38}N_7O_3$: 496.3023, found: 496.3031.

(R)-1-(((R)-1-((4-(2-(Di-2-picolylamino)acetamido)phenethyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-aminium chloride (**1g**)

Yield (2.60 g, 83%): ^{1}H NMR (400 MHz, D_2O): δ=8.77 (d, *J* = 5.4 Hz, 2H), 8.53 (td, *J* = 8.0, 1.4 Hz, 2H), 8.07 (d, *J* = 8.0 Hz, 2H), 7.96 (t, *J* = 6.8 Hz, 2H), 7.27 (m, 4H), 4.51 (s, 4H, 2×NC$\underline{H}_2$Py), 4.29-4.02 (m, 2H), 3.78 (s, 2H, NCH_2CO), 3.65–3.32 (m, 2H), 2.84 (q, *J* = 6.7 Hz, 2H), 1.51 (d, *J*=7.1 Hz, 3H, CH_3), 1.27 (d, *J*=7.2 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, D_2O): δ=174.8 (CO), 170.9 (CO), 170.8 (CO), 152.7 (2×Cquat Pyr), 147.3 (2×CH Pyr), 142.0 (Cquat Phenyl), 137.1 (Cquat Phenyl), 134.7 (2×CH Pyr), 129.9 (2×CH Phenyl), 127.5 (2×CH Phenyl), 126.7 (2×CH Pyr), 122.1 (2×CH Pyr), 58.3 (2×$\underline{C}H_2$Pyr), 57.1 (N$\underline{C}H_2$CO), 50.3 (CHα), 49.2 (CHα),

40.7 (CH_2), 34.4 (CH_2), 17.1 (CH_3), 16.9 (CH_3); HRMS-ESI *m/z* [*M*+H+Na]$^+$ calculated for $C_{26}H_{37}NaN_7O_3$: 518.2880, found: 518.2879.

(R)-2-Amino-N-((R)-1-((4-(2-(2-(di-2-picolylamino)acetamido)ethyl)phenyl)amino)-1-oxopropan-2-yl)propanamide (**1h**)

Yield (1.111 g, 88%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=9.34 (s, 1H, NH), 8.70 (t, J = 5.7 Hz, 1H, NH), 8.43 (ddd, J = 4.8, 1.9, 1.0 Hz, 2H), 7.99 (d, J = 7.8 Hz, 1H, NH), 7.51 (td, J = 7.7, 1.8 Hz, 2H), 7.41 (m, 2H), 7.12 – 7.00 (m, 6H), 4.63 (pentet, J = 7.1 Hz, 1H, CHα), 3.70 (s, 4H, 2×NC$\underline{H_2}$Py), 3.47 (m, 3H, CHα, C$\underline{H_2}$NH), 3.22 (s, 2H, NCH_2CO), 1.46 (bs, 2H, NH_2), 1.40 (d, J = 6.9 Hz, 3H, CH_3), 1.29 (d, J = 6.9 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ=176.3 (CO), 171.2 (CO), 170.5 (CO), 158.1 (2×Cquat Pyr), 149.2 (2×CH Pyr), 136.6 (Cquat Phenyl), 136.5 (2×CH Pyr), 134.8 (Cquat Phenyl), 129.0 (2×CH Phenyl), 123.1 (2×CH Pyr), 122.3 (2×CH Pyr), 119.8 (2×CH Phenyl), 60.4 (2×C$\underline{H_2}$Pyr), 58.0 (NC$\underline{H_2}$CO), 50.6 (CHα), 49.2 (CHα), 40.2 (CH_2), 34.9 (CH_2), 21.5 (CH_3), 18.1(CH_3); HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{28}H_{36}N_7O_3$: 518.2864, found: 518.2874.

(R)-2-Amino-N-((R)-1-((2-(1-(bis(pyridin-2-ylmethyl)glycyl)piperidin-4-yl)ethyl)amino)-1-oxopropan-2-yl)propanamide (**1i**)

The title compound was isolated as a mixture of amide rotamers (92 mg, 61% yield): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.52 (ddd, *J* = 4.9, 1.8, 0.9 Hz, 2H), 7.76 (d, *J* = 7.8 Hz, 1H, NH), 7.65 (td, *J* = 7.6, 1.8 Hz, 2H), 7.53 (dt, *J* = 7.9, 1.1 Hz, 2H), 7.15 (ddd, *J* = 7.4, 4.9, 1.3 Hz, 2H), 6.52 (t, *J* = 5.8 Hz, 1H, NH), 4.52 (d, *J* = 13.2 Hz, 1H), 4.36 (pentet, *J* = 7.2 Hz, 1H, CHα), 3.91 (d, *J* = 14.1 Hz, AB system, 2H), 3.86 (d, *J* = 14.1 Hz, AB system, 2H), 3.76 (m, 1H), 3.49 (quartet, *J* = 7.0 Hz, 1H, CHα), 3.40 (s, 2H, NCH_2CO), 3.24 (m, 2H), 2.79 (m, 1H), 2.47 (tt, *J* = 12.8, 3.3 Hz, 1H), 1.83 (bs, 2H, NH_2), 1.72 – 1.55 (m, 3H), 1.33 (m, 2H), 1.36 (d, *J* = 7.0 Hz, 3H, CH_3), 1.33 (d, *J* = 7.0 Hz, 3H, CH_3), 1.04 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=176.1, 172.2, 170.2 and 168.7 (3×CO), 159.1 (2×Cquat Pyr), 149.1 (2×CH Pyr), 136.6 (2×CH Pyr), 123.8 (2×CH Pyr), 122.3 (2×CH Pyr), 60.5 (2×C$\underline{H_2}$Pyr), 56.1 (NC$\underline{H_2}$CO), 50.6 (CHα), 48.6 (CHα), 45.4 (CH_2), 42.1 (CH_2), 37.2 and 37.1 (CH_2), 36.11 and 36.05 (CH_2), 33.9 and 33.8 (CH), 32.6 and 32.5 (CH_2), 32.0 and 31.9 (CH_2), 21.7 (CH_3), 17.5 (CH_3); HRMS-ESI *m/z* [*M*+Na]$^+$ calculated for $C_{27}H_{39}NaN_7O_3$: 532.2990, found: 532.3007.

(R)-2-Amino-N-((R)-1-((2-(4-(bis(pyridin-2-ylmethyl)glycyl)piperazin-1-yl)ethyl)amino)-1-oxopropan-2-yl)propanamide (**1j**)

Yield (80 mg, 53%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.52 (ddd, *J* = 4.9, 1.8, 0.9 Hz, 2H), 7.72 (d, *J* = 7.9 Hz, 1H, NH), 7.64 (td, *J* = 7.6, 1.8 Hz, 2H), 7.50 (dt, *J* = 7.8, 1.1 Hz, 2H), 7.15 (ddd, *J* = 7.5, 4.9, 1.3 Hz, 2H), 6.62 (t, *J* = 5.2 Hz, 1H, NH), 4.38 (pentet, *J* = 7.1 Hz, 1H, CHα), 3.87 (s, 4H, 2×NC$\underline{H_2}$Py), 3.54 (m, 2H), 3.46 (quartet, *J* = 7.0 Hz, 1H, CHα), 3.43 – 3.21 (m, 6H), 2.44 (t, *J* = 6.1 Hz, 2H), 2.34 (m, 4H), 1.66 (s, 2H), 1.36 (d, *J* = 7.0 Hz, 3H, CH_3), 1.30 (d, *J* = 7.0 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ=175.8 (CO), 172.3 (CO), 168.8 (CO), 158.9 (2×Cquat Pyr), 149.1 (2×CH Pyr), 136.6 (2×CH Pyr), 123.8 (2×CH Pyr), 122.3 (2×CH Pyr), 60.4 (2×C$\underline{H_2}$Pyr), 56.7 (CH_2), 56.5 (CH_2), 56.1 (NC$\underline{H_2}$CO), 53.0 (CH_2), 52.7 (CH_2), 50.7 (CHα), 48.5 (CHα), 45.2 (CH_2), 41.7 (CH_2), 36.1 (CH_2), 21.7 (CH_3), 18.1 (CH_3); HRMS-ESI *m/z* [*M*+Na]$^+$ calculated for $C_{26}H_{38}NaN_8O_3$: 533.2940, found: 533.2959.

*Synthesis of tert-butyl ((R)-1-(((R)-1-(di-2-picolylamino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (**9**)*

N-Boc-*D*-alanyl-*D*-alanioic acid **4** (100 mg, 0.384 mmol, 1.0 equiv.), DPA (0.07 mL, 0.384 mmol, 1.0 equiv.) and HATU (144.5 mg, 0.384 mmol, 1.0 equiv.) dissolved in CH_2Cl_2 (4 mL) and cooled to 0°C in an ice-water bath. *N*-methylmorpholine (0.085 mL, 0.768 mmol, 2 equiv.) was added and the stirring mixture was left at 0°C for 15 min before slowly warming up to room temperature. After 4 h at room temperature, the mixture was washed with 1 M NaOH (4 x 20 mL), dried over K_2CO_3, filtered and concentrated under reduced pressure. The yellow oily residue was dissolved in CH_2Cl_2 and purified by column chromatography on neutral alumina (0-5% MeOH in CH_2Cl_2) to afford the title compound **9** as a mixture of amide rotamers which is a clear yellow oil. Yield (150 mg, 91%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.50 (m, 2H), 7.62 (m, 2H), 7.23 – 7.11 (m, 4H), 7.03 (t, *J* = 8.5 Hz, 1H, NH), 5.08 (bs, 1H, NH), 4.99 (m, 1H, CHα), 4.89 – 4.63 (m, 4H), 4.16 (bs, 1H, CHα), 1.43 and 1.42 (s, 9H, C(CH_3)$_3$), 1.38 (d, *J* = 6.7 Hz, 3H, CH_3), 1.32 (d, *J* = 7.0 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ=173.5 (CO), 171.9 (CO), 156.9 (Cquat Pyr), 155.8 (Cquat Pyr), 155.3 (NCOOtBu), 150.0 (CH Pyr), 149.4 (CH Pyr), 137.0 (CH Pyr), 136.9 (CH Pyr), 122.8 (CH Pyr), 122.5 (CH Pyr), 122.2 (CH Pyr), 121.6 (CH Pyr), 121.5 (CH Pyr), 80.1 (C(CH_3)$_3$), 51.4 (NCH_2Py), 50.2 (CHα), 45.9 and 45.8 (CHα), 28.4 (C(C$\underline{H_3}$)$_3$), 19.1, 19.0 and 18.8 (2*CH_3); HRMS-ESI *m/z* [*M*+H]$^+$ calculated for $C_{23}H_{32}N_5O_4$: 442.2442, found: 442.2449.

*Synthesis of (R)-2-((R)-2-aminopropanamido)-N,N-bis(pyridine-2-ylmethyl)propanamide (**10**)*

The *N*-Boc-protected amine **9** (60 mg, 0.136 mmol, 1.0 equiv.) was dissolved in CH_2Cl_2 (2 mL) and cooled to 0°C in an ice-water bath. Trifluoroacetic acid (0.84 mL, 6.8 mmol, 60.0 equiv.) in CH_2Cl_2 (2 mL) was then slowly added to the stirring mixture. The reaction was left at 0°C for 20 min before warming up to room temperature for an additional 3 h. After solvent removal under reduced pressure, 1 M NaOH (20 mL) was added to the mixture, and the compound was extracted with CH_2Cl_2 (6 x 5 mL). The combined organic layers were washed with fresh 1 M NaOH (2 x

20 mL), dried on K_2CO_3, filtered, and the solvent removed under reduced pressure, to give the title compound **10** as a mixture of rotamers which is a clear yellow oil. Yield (30 mg, 65%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.50 (dd, *J* = 4.9, 1.9 Hz, 2H), 7.87 (d, *J* = 7.9 Hz, 1H, NH), 7.62 (td, *J* = 7.7, 1.8 Hz, 2H), 7.23 (dt, *J* = 8.1, 1.1 Hz, 2H), 7.14 (m, 2H), 4.99 (dq, *J* = 8.2, 6.8 Hz, 1H, CHα), 4.94 – 4.59 (m, 4H, 2×NCH_2Py), 3.43 (m, 1H, CHα), 1.68 (s, 2H, NH_2), 1.38 (d, *J* = 6.7 Hz, 3H, CH_3), 1.30 (d, *J* = 7.0 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ=175.3 and 175.2 (CO), 173.84 and 173.77 (CO), 157.0 (Cquat Pyr), 156.04 and 156.01 (Cquat Pyr), 149.93 and 149.91 (CH Pyr), 149.4 and 149.3 (CH Pyr), 136.94 (CHPyr), 136.89 and 136.86 (CH Pyr), 122.7 (CH Pyr), 122.5 and 122.4 (CH Pyr), 122.19 and 122.15 (CH Pyr), 121.54 and 121.52 (CH Pyr), 52.8 and 52.6 (CH_2), 51.44 and 51.39 (CH_2), 50.82 and 50.80 (CHα), 45.4 and 45.3 (CHα), 21.7 (CH_3), 19.0 (CH_3); HRMS-ESI *m/z* $[M+H]^+$ calculated for $C_{18}H_{24}N_5O_2$: 342.1930, found: 342.1925.

*Synthesis of tert-butyl (4-(2-dibenzylamino)acetamido)phenethyl)carbamate (**11**)*

Chloroacetamide **5g** (1.002g, 3.20 mmol) and KI (0.597g, 3.60 mmol) were dissolved in 350 mL MeCN and dibenzylamine (0.74 mL, 3.85 mmol) was added to the stirring mixture. DIPEA (5.5 mL, 31.6 mmol) was then added and the mixture was heated to reflux and left for 17 h. After cooling to room temperature, the mixture was concentrated under reduced pressure and purified using colum chromatography on silica. The product was eluted using 20-50% ethyl acetate in heptane, yielding 1.03 g (68%) of the title compound **11** as a pale light oil: ^{1}H NMR (400 MHz, DMSO-d6): δ=9.54 (s, 1H, NH), 7.48 (d, *J* = 8.1 Hz, 2H), 7.42 (d, *J*=7.0 Hz, 4H), 7.34 (t, *J*=7.2 Hz, 4H), 7.30-7.19 (m, 2H), 7.11 (d, *J* = 8.2 Hz, 2H), 6.83 (t, *J* = 5.7 Hz, 1H, NH), 3.76 (s, 4H, 2×NCH_2Ph), 3.21 (s, 2H, NCH_2CO), 3.09 (t, *J*=8.0 Hz, 2H), 2.63 (t, *J*=8.4 Hz, 2H), 1.37 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (100 MHz, DMSO-d6): δ=168.7 (CO), 155.45 (NCOOtBu), 138.5 (2×Cquat Phenyl), 136.55 (Cquat Phenyl), 134.35 (Cquat Phenyl), 128.7 (4×CH Phenyl), 128.3 (4×CH Phenyl), 128.04 (CH Phenyl), 127.85 (2×CH Phenyl), 127.1 (CH Phenyl), 119.3 (2×CH Phenyl), 77.4 ($\underline{C}(CH_3)_3$), 57.5 (2×NCH_2Ph), 56.1 (N$\underline{C}H_2CO$), 40.2 (CH_2), 34.9 (CH_2), 28.2 (C($\underline{C}H_3)_3$); HRMS-TOF MS ES *m/z* $[M+H]^+$ calculated for calculated for $C_{29}H_{25}N_3O_3$: 474.2756, found: 474.2763.

*Synthesis of N-(4-(2-aminoethyl)phenyl)-2-(dibenzylamino)acetamide (**12**)*

The *N*-Boc-protected amine **11** (550 mg, 1.16 mmol) was dissolved in 25 mL CH_2Cl_2 and cooled to 0°C in an ice-water bath. Trifluoroacetic acid (6 mL, 78.35 mmol) was then added dropwise to the stirring solution over the course of 5 min. The reaction was left for 1 h at room temperature, before it was concentrated under reduced pressure to a light brown oil. The crude material was dissolved in 25 mL ethyl acetate and washed with 50 mL 0.5 M aqueous K_2CO_3. The water phase was extracted with 3 × 20 mL ethyl acetate. The combined organic phases were pooled and washed with 25 mL 0.5 M aqueous K_2CO_3 and then dried over K_2CO_3, filtered and concentrated under reduced pressure to give the title compound **12** (0.404 g, 93%) as a pale light oil: ^{1}H NMR (400 MHz, DMSO-d6): δ=9.52 (s, 1H, NH), 7.46 (m, 2H), 7.41 (d, *J* = 7.0 Hz, 4H), 7.34 (t, *J* = 7.5 Hz, 4H), 7.29-7.21 (m, 2H), 7.11 (m, 2H), 3.75 (s, 4H, 2×NCH_2Ph), 3.20 (s, 2H, NCH_2CO), 2.72 (t, *J* = 7.2 Hz, 2H), 2.57 (t, *J* = 7.2 Hz, 2H), 1.34 (bs, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d6): δ=168.65 (CO), 138.5 (2×Cquat Phenyl), 136.3 (Cquat Phenyl), 135.4 (Cquat Phenyl), 128.75 (4×CH Phenyl), 128.3 (4×CH Phenyl), 128.05 (CH Phenyl), 127.85 (CH Phenyl), 127.1 (2×CH Phenyl), 119.3 (2×CH Phenyl), 57.5 (2×NCH_2Ph), 56.1 (N$\underline{C}H_2CO$), 52.1 (CH_2), 43.7 (CH_2); HRMS-TOF MS ES *m/z* $[M+H]^+$ calculated for calculated for $C_{24}H_{27}N_3O$: 374.2232, found: 374.2234.

*Synthesis of tert-butyl ((R)-1-((R)-1-((4-(2-(dibenzylamino)acetamido)phenethyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (**13**)*

Amine **12** (0.404g, 1.017 mmol) was dissolved in 4 mL DMF. Boc-*D*-alanyl-*D*-alanine-OH hydrochloride **4** (0.285g, 1.095 mmol) and HBTU (0.413g, 1.090 mmol) were added to the stirring solution and cooled to 0°C in an ice-water bath. NMM (0.115 mL, 1,044 mmol) was added and the solution was left 30 min at 0°C and then 3 h in room temperature. The mixture was diluted with 200 mL water and extracted with 3*50 mL ethyl acetate. The combined organic phases were pooled and washed with 25 mL 0.5 M aqueous $NaHCO_3$ and then dried over K_2CO_3, filtered and concentrated under reduced pressure to give the title compound **13** (0.498g, 80%) as a pale light oil: ^{1}H NMR (400 MHz, DMSO-d6): δ=9.57 (s, 1H, NH), 7.91 (t, *J* = 5.7 Hz, 1H, NH), 7.78 (d, *J* = 7.5 Hz, 1H, NH), 7.51-7.30 (m, 10H), 7.30-7.21 (m, 2H), 7.15-7.08 (m, 2H), 7.01 (d, *J* = 7.4 Hz, 1H, NH), 4.19 (pentet, *J* = 7.1 Hz, 1H, CHα), 3.93 (pentet, *J* = 7.3 Hz, 1H, CHα), 3.75 (s, 4H, 2×NCH_2Ph), 3.24 (m, 2H, NCH_2CO), 2.64 (t, *J*=7.1 Hz, 2H), 1.38 (s, 9H, $C(CH_3)_3$), 1.15 (d, *J*=7.2 Hz, 6H, 2×CH_3); ^{13}C NMR (100 MHz, DMSO-d6): δ=172.15 (CO), 171.8 (CO), 168.7 (CO), 155.1 (NCOOtBu), 138.5 (2×Cquat Phenyl), 136.6 (Cquat Phenyl), 134.2 (Cquat Phenyl), 128.79 (CH Phenyl), 128.76 (4×CH Phenyl), 128.3 (4×CH Phenyl), 127.1 (2×CH Phenyl), 119.2 (2×CH Phenyl), 78.1 ($\underline{C}(CH_3)_3$), 57.5 (2×NCH_2Ph), 56.0 (N$\underline{C}H_2CO$), 49.7 (CHα), 47.95 (CHα), 40.1 (CH_2), 34.4 (CH_2), 28.1 (C($\underline{C}H_3)_3$), 18.5 (CH_3), 17.9 (2×CH_3); HRMS-TOF MS ES *m/z* $[M+H]^+$ calculated for calculated for $C_{35}H_{45}N_5O_5$: 616.3498, found: 616.3511.

*Synthesis of (R)-2-amino-N-((R)-1-((4-(2-(dibenylamino)acetamido)phenethyl)amino)-1-oxopropan-2-yl)propanamide hydrochloride (**14**)*

The *N*-Boc-protected amine **13** (0.254g, 0.413 mmol) was dissolved in 25 mL CH_2Cl_2 and cooled to 0°C in an ice-water bath. Trifluoroacetic acid (2 mL, 26.12 mmol) was dissolved in 25 mL CH_2Cl_2 and added dropwise to the stirring solution over the course of 5 min. The reaction was left at room temperature for 1 h. The solution was concentrated under reduced pressure. The residue was diluted in 5 mL dry CH_2Cl_2 and 1 mL 2 M HCl in diethyl ether was added to get a precipitate which was filtered, washed with diethyl ether and dried under reduced pressure to obtain the compound **14** as a powder: ^{1}H NMR (400 MHz, D_2O): δ=7.46 (dd, *J*=6.8, 2.9 Hz, 4H), 7.42-7.29 (m, 6H), 7.07 (m, 2H), 6.92 (m, 2H), 4.50 (s, 3H, $^+NH_3$), 4.06 (q, *J* = 7.2 Hz, 1H, CHα), 3.93 (q, *J*=7.1 Hz, 1H, CHα), 3.87 (s, 4H), 3.49-3.37 (m, 5H), 3.22 (m, 1H), 2.74-2.58 (m, 2H), 1.36 (d, *J*=7.2, 3H, CH_3), 1.11 (d, *J*=7.2, 3H, CH_3); ^{13}C NMR (100 MHz, D_2O): δ=175.04 (CO), 171.09 (CO), 164.46 (CO), 137.52, 134.34, 132.09, 131.21, 130.16, 130.13, 130.01, 129.05, 122.49, 60.54 (2×NCH_2Ph), 53.02 (NCH_2CO), 50.56 (CHα), 49.47 (CHα), 40.89 (CH_2), 34.64 (CH_2), 17.33 (CH_3), 17.14 (CH_3); HRMS-TOF MS ES *m/z* $[M]^+$ calculated for calculated for $C_{30}H_{38}N_5O_3$: 516.2974, found: 516.2985.

Biological studies

Microdilution MIC assay

The MIC values of MEM alone or in combination with the compounds were determined using pre-made broth microdilution plates containing MEM in 2-fold dilution steps ranging from 0.03 to 64 mg/L (TREK Diagnostic Systems/Thermo Fisher Scientific, East Grinstead, UK). The compounds were tested at a final concentration of 125 µM. The MIC assays were performed using two MBL-producing MEM resistant strains, one NDM-1-producing *K. pneumoniae*[43-44] and one VIM-2-producing *P. aeruginosa*[45]. In brief, the compounds were diluted in MH-broth (Becton Dickinson, Franklin Lakes, NJ USA) and 50 µL of the suspension were added to each well of the MEM plate. Bacterial colonies were suspended in 0.9% saline buffer to 0.5 McFarland and further diluted 1:100 in MH broth (Thermo Fisher Scientific, East Grinstead, UK). 50 µL of the diluted bacterial suspension were added to the MEM plates to a final volume of 100 µL. The plates were incubated for 20 h at 37°C. Each MIC was determined in duplicate. To investigate any intrinsic antibacterial activity of the compounds the MIC of each compound were determined in a 2-fold serial dilution series in MH broth (Becton Dickinson, Franklin Lakes, NJ USA) ranging from 2 – 1000 µM. 50 µL of each dilution step was then mixed with 50 µL of a bacterial inoculum, prepared as described above, in 96 well microtiter plates (Thermo Fisher Scientific, Roskilde, Denmark). The plates were incubated, and MIC determined as described above.

Enzyme inhibition assay

For the determination of the inhibitor concentration 50% (IC_{50}), we preincubated purified VIM-2 (1 nM)[39] at inhibitor concentrations ranging from 0 to 250 µM. The incubation was carried out in 50 mM HEPES buffer pH 7.5 containing BSA (2 µg/mL) and $ZnSO_4$ (1 µM) at 25°C for 5 and 20 min. The initial reaction velocity was measured by adding nitrocefin (50 µM) as a reporter substrate. The kinetics were recorded at 482 nm under stable temperature conditions (25°C) using a SpectraMax Plus plate reader. Data evaluation as well as the IC_{50} calculation of at least two independent measurements was done by GraphPad Prism$^{(R)}$ 4.0. All reported concentrations are final values for 100 µL assay volume.

Cell sensitivity assay

Human breast cancer cells (MDA-MB-231) and pancreatic cancer cells (MIA-PaCa2 and Colo-357) were cultured at 37°C with 5% CO_2 in DMEM media containing 10% fetal bovine serum and antibiotics. For cell survival assays, cells were seeded in 96 well plates (3000 cells per well, 100 µL medium), adhered for 24 h and exposed to different concentrations (from 300 µM to 0.3 µM) of indicated compounds for an additional 72 h. Then, metabolically active cells were quantified by the MTT assay[40] by adding 100 ng MTT per well, incubating cells for 2 h, replacing media with 100 µL isopropanol/HCl/$H_2$0 (90/1/9, v/v/v) and determining optical density at 540 nm. IC_{50} values were determined using the CompuSyn software. Results were expressed as mean values of IC_{50} ± SD (µM) of three independent experiments performed in triplicate.

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