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Synthesis and Preclinical Evaluation of TPA-Based Zinc Chelators as Metallo-β-lactamase Inhibitors

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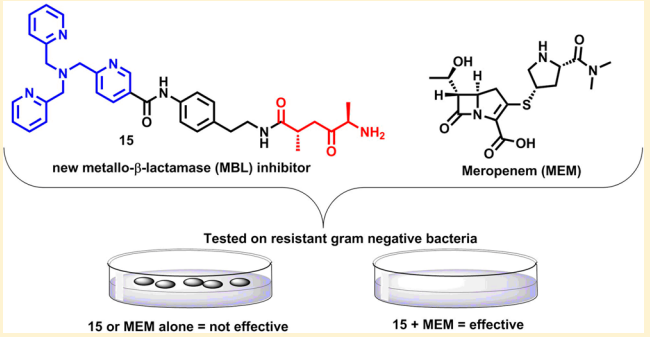
Supporting Information

ABSTRACT: The rise of antimicrobial resistance (AMR) worldwide and the increasing spread of multi-drug-resistant organisms expressing metallo-β-lactamases (MBL) require the development of efficient and clinically available MBL inhibitors. At present, no such inhibitor is available, and research is urgently needed to advance this field. We report herein the development, synthesis, and biological evaluation of chemical compounds based on the selective zinc chelator tris-picolylamine (TPA) that can restore the bactericidal activity of Meropenem (MEM) against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* expressing carbapenemases Verona integron-encoded metallo-β-lactamase (VIM-2) and New Delhi metallo-β-lactamase 1 (NDM-1), respectively. These adjuvants were prepared via standard chemical methods and evaluated in biological assays for potentiation of MEM against bacteria and toxicity (IC_{50}) against HepG2 human liver carcinoma cells. One of the best compounds, **15**, lowered the minimum inhibitory concentration (MIC) of MEM by a factor of 32−256 at 50 μM within all tested MBL-expressing clinical isolates and showed no activity toward serine carbapenemase expressing isolates. Biochemical assays with purified VIM-2 and NDM-1 and **15** resulted in inhibition kinetics with k_{inact}/K_I of 12.5 min^{-1} mM^{-1} and 0.500 min^{-1} mM^{-1}, respectively. The resistance frequency of **15** at 50 μM was in the range of 10^{-7} to 10^{-9}. **15** showed good tolerance in HepG2 cells with an IC_{50} well above 100 μM, and an in vivo study in mice showed no acute toxic effects even at a dose of 128 mg/kg.



KEYWORDS: *antimicrobial resistance, zinc chelator, metallo-β-lactamase inhibitor, enzyme inhibition kinetics, resistance frequency, toxicity*

Antibiotics are considered to be one of the cornerstones of modern medicine.[1] The rising threat of antimicrobial resistance[2,3] (AMR), however, has become a global public health challenge that has been accelerated by the overuse of antibiotics worldwide[4,5] as well as other factors.[6,7] AMR results in serious and more complicated infections, which leads to longer hospital stays and increased mortality.[8] Few genuinely new antibiotics have been introduced during the past few decades,[9] demanding

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research and development as well as international initiatives to raise awareness and fight the threat of AMR.[10]

The β-lactamases are one of the most important bacterial defense systems against β-lactam antibiotics.[11,12] These enzymes are classified according to sequence criteria (Ambler classes A–D) and can be structurally grouped into two superfamilies; the serine β-lactamases (classes A, C, and D) and metallo-β-lactamases (MBLs, class B).[13−15] MBLs require divalent zinc ions as a metal cofactor for enzyme activity[16] and are emerging as one of the most clinically important families of β-lactamases.[17] The IMP- (Imipenemase), VIM- (Verona integron-mediated metallo-β-lactamase), and NDM- (New Delhi metallo-β-lactamase) groups are now widespread in a variety of gram-negative species,[18,19] and since first being reported in 2008, NDM has spread globally.[20,21] Furthermore, MBLs are able to inactivate almost all β-lactam antibiotics, including the carbapenems, which are among the "last resort antibiotics" used in clinics.[22] Recent research afforded inhibitors of serine β-lactamases;[23] however, no clinically available inhibitor against MBLs currently exists, despite ongoing research.[24,25]

The mechanism by which β-lactam antibiotics are inactivated by MBLs is based on the hydrolysis of the β-lactam ring catalyzed by a Zn^{2+}-coordinated water molecule in the active site of the enzymes.[19] Zinc chelators could potentially be used as adjuvants[26] to inhibit MBLs by the removal of zinc[27−29] and thus restore the antibacterial activity of β-lactam antibiotics. A high degree of selectivity for zinc chelation would ensure the inhibition of MBLs without affecting host metalloenzymes.[30]

The aim of this study is to further increase the scope and chemical diversity of chelators with potential application as MBLs inhibitors. We have developed and studied compounds based on the tris-picolylamine (TPA) scaffold[31] (the chelator), something that modulates the physicochemical properties of the combination (modulator), and a linker that connects the two (Figure 1). We evaluated the biological activity in combination

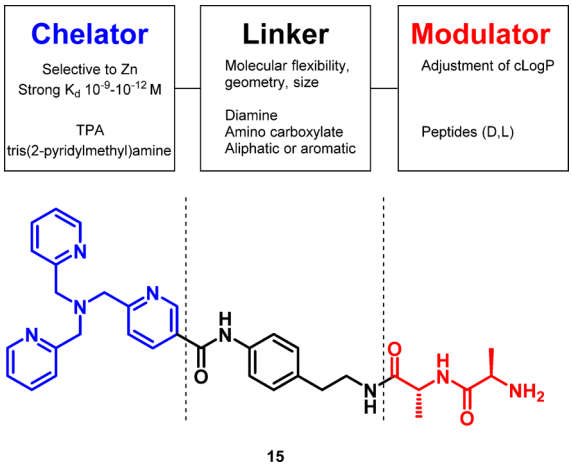

Figure 1. Design principle of the putative MBL inhibitors presented in this publication as exemplified by compound **15**.

with Meropenem (MEM) against MBL-harboring carbapenem-resistant strains as well as their toxicity toward human liver carcinoma (HepG2) cells. Initially, we chose D-ala-D-ala as the modulator on the basis that it might confer selectivity to bacteria because of its affinity for the penicillin binding proteins of bacterial cells as well as increased water solubility.[32−35] However, we also explored other small peptides as modulators in order to change the solubility of the constructs. The straightforward chemical synthesis of our putative inhibitors facilitates varying each of the three main components (Figure 1).

RESULTS AND DISCUSSION

Chemical Synthesis. To synthesize a variety of TPA analogs, we started with the preparation of building blocks based on the TPA scaffold, which were then further functionalized using standard chemical transformations. The synthesis of the key fragment, TPA methyl ester (**3**), is shown in Scheme 1 and was previously reported by starting from the methyl nicotinate **1**.[36] Methyl(6-bromomethyl)nicotinate **2** was commercially acquired from BocSci (Shirley, USA) and used as received for amination with dipicolylamine in the presence of DIPEA in THF to afford the TPA methyl ester (**3**) after precipitation from cold Et_2O as a pale-yellow solid. This reaction was convenient and scalable to afford multigram quantities of **3** as our main building block.

The TPA methyl ester (**3**) could then be further converted, giving access to the TPA acid[36] (**4**), TPA alcohol[37] (**5**), TPA amine (**6**), and TPA amide (**7**) (Scheme 2), with the first two being described in the literature. These TPA analogs were tested in biological assays to obtain the initial results (see the discussion and Table 1) and subsequently served as starting materials for the synthesis of the compounds discussed below. The TPA acid (**4**) was isolated once for characterization but otherwise used in subsequent reactions without further purification after the saponification of **3** with LiOH in H_2O/THF and neutralization with 2 M HCl. The TPA alcohol was prepared via the reduction of **3** with $NaBH_4$ in EtOH and was further reacted in a sequence of mesylation, nucleophilic substitution with NaN_3, and Staudinger reaction to TPA amine hydrochloride **6**. This sequence required no chromatography and afforded TPA amine **6** as the hydrochloride salt from the water phase cleanly in high yield after acidic extraction.

The first series of compounds containing modulators directly attached to the chelator was then prepared from TPA acid **4** or amine **6** via standard peptide coupling conditions with dipeptides or other amines (Scheme 3). The low solubility of TPA acid **4** in nonpolar and aprotic solvents limited the applicable reaction conditions. HATU and *N*-methylmorpholine (NMM) or EDCI, HOAt, and NMM in DMF were thus used, and the isolation of the coupling products was performed via C18 solid-phase extraction (SPE) or column chromatography on neutral alumina, affording mediocre to good product yields. The C18 SPE was in most cases the method of choice for the efficient chromatographic removal of byproducts from the peptide couplings, namely, tetramethylurea, DMF, and HOAt. Alternative methods would suggest the preparation of an activated ester of **4** as succinimidyl ester, acid chloride, or acid fluoride.[38,39] Neither of those variations improved the method above.

Compounds **14** and **16** were prepared via couplings facilitated by EDCI with the corresponding protected anilines, followed by deprotection of TFA or LiOH, respectively. The obtained N- and C-terminal TPA-linker fragments were then coupled via HATU with Boc-D-ala-D-ala-OH or H-D-ala-D-ala-OMe, affording after another deprotection corresponding compounds **15** and **17** (Scheme 4).

By analogy to **15** and **17**, two aliphatic linkers were implemented using similar peptide chemistry and deprotection methods (Scheme 5). Compounds **19** and **21**, derived from the

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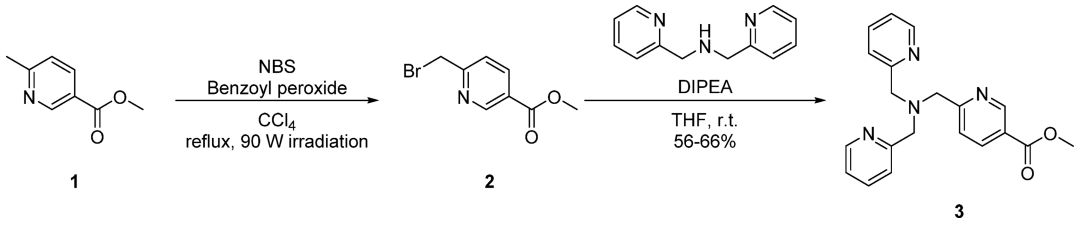
Scheme 1. Reported Synthesis of TPA Ester (3) from Methyl Nicotinate (1)[36]



Scheme 2. Synthesis of TPA Derivatives 4−7 from TPA Methyl Ester 3

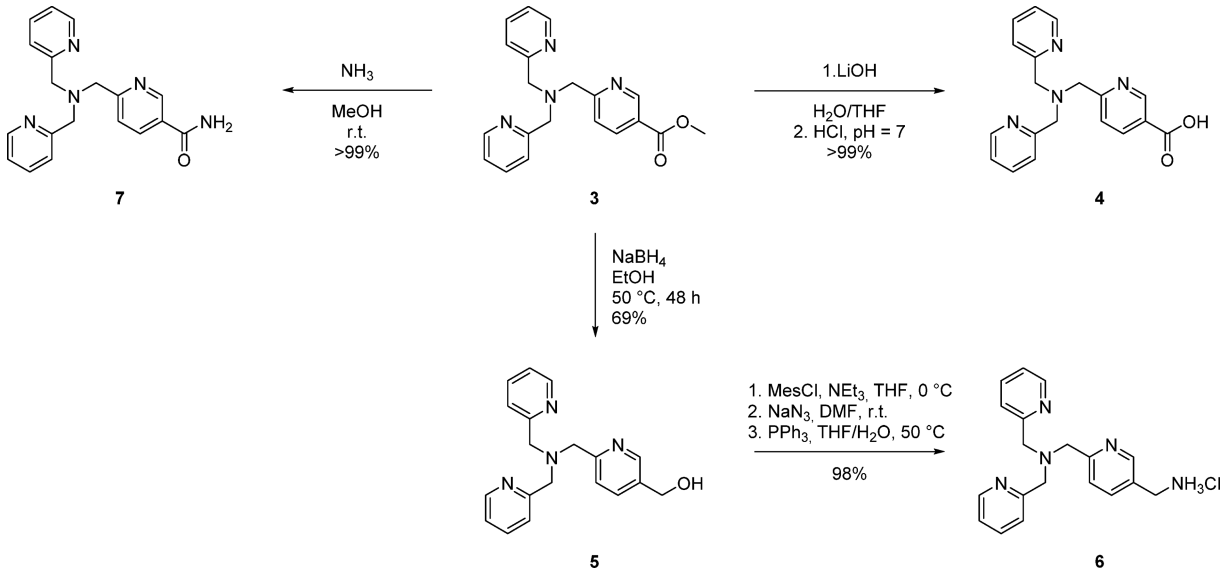


Table 1. In Vitro Testing of TPA and Derivatives as Putative MBL Inhibitors in Clinical Isolates Together with MEM and Toxicity towards HepG2 Cells[a]

		MIC MEM (mg/L)[c]		IC_{50} (μM)
compound	clog*P*[b]	*P. aeruginosa* VIM-2[d]	*K. pneumoniae* NDM-1[e]	HepG2
MEM	−1.79	32−64	32−64	ND
TPEN	3.52	1	<0.5	7.1
TPA	2.70	1	0.125	9.8
3	2.52	1	0.25	16. 5
4	2.26	1	0.25	25.9
5	2.13	2	0.125	12.4
6	1.75	2	0.125	21.4

[a]Abbreviations: MIC: minimum inhibitory concentration. ND: not determined. The MIC assay was performed as one biological replicate and two technical replicates. [b]Estimated using *ChemDraw Ultra* 15.1 from Cambridgesoft. [c]For the MIC determination, all compounds were tested at 50 μM. [d]MIC values of *P. aeruginosa* harboring MBLs VIM-2. [e]MIC values of *K. pneumoniae* harboring MBLs NDM-1.

couplings with the diamino linkers, deprotection and coupling with Boc-D-ala-D-ala-OH were obtained in 79 and 62% yields, respectively.

Compound **12**, previously prepared in Scheme 3 containing the piperazine moiety as a linker, was furthermore used and coupled to Boc-D-ala-D-ala-OH by analogy, affording **22** (Scheme 6).

In Vitro and in Vivo Biological Evaluation of the Synthesized Zinc Chelators as MBL Inhibitors. Antimicrobial resistance can be targeted in different ways depending on the mechanism of action of the active compounds.[40] Parameters such as selectivity, potency, membrane permeability, resistance development, pharmacokinetics, and toxicity are critical and need to be taken into account for the development of any drug or pharmaceutically active compound.[41,42] The MBL inhibitors could be a prodrug,[43] have additional bactericidal or bacteriostatic activity or function solely as an adjuvant being coadministered to an already existing antibiotic,[44] rendering it active again. We considered the removal of zinc from the zinc-dependent MBLs as an efficient way to inhibit these enzymes and aimed to develop a selective and strong zinc chelator. These selective zinc chelators should additionally provide the possibility of chemical modification to limit toxicity, vary lipophilicity (clog*P*), and find SAR trends leading to the development of efficient candidates with a wide therapeutic window. Synthesis simplicity, employing standard and scalable methods, was considered to be an advantage compared to the often stepwise and demanding synthesis of other putative MBL inhibitors.[45]

To be effective, compounds should have a specific zinc binding strength (K_d) of between 10^{-9} and 10^{-12} M. Earlier studies in our laboratories resulted in the initial observations that a K_d below 10^{-12} M results in toxicity, and values higher than 10^{-9} M are not effective. Two potentially active and reported Zn^{2+} chelators are *N*,*N*,*N*′,*N*′,-tetrakis(2-pyridylmethyl) ethylenediamine (TPEN)[46] and tris(2-pyridylmethyl)amine (TPA).[31] TPEN has a K_d of about 10^{-16} M[47] while TPA has a K_d of about 10^{-11} M,[31] and this seemed to be the ideal starting point for our biological testing against the MBL harboring strains. Further advantages of TPA are that it coordinates Zn^{2+}

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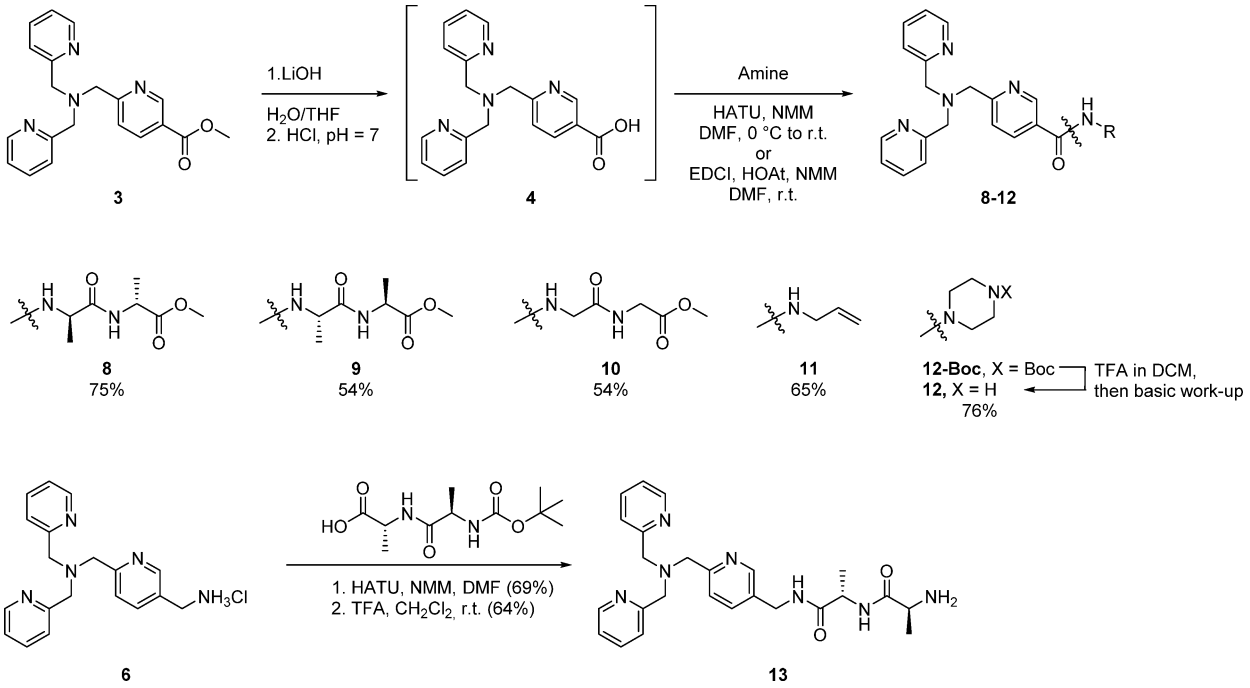
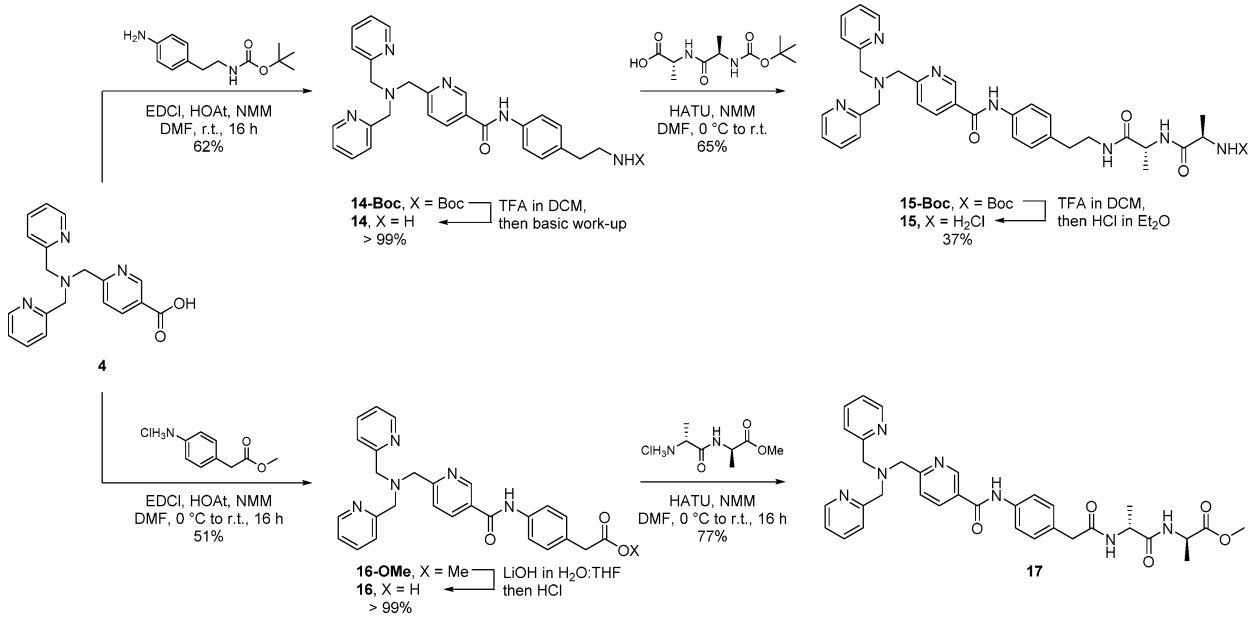
Scheme 3. Synthesis of Compounds 8−13 Containing TPA and Various Modulators



Scheme 4. Synthesis of 15 and 17



with a higher kinetic rate and that it is less toxic than TPEN.[48] TPEN was included in our biological studies for comparison.

The easily available derivatives of TPA, namely, the TPA methyl ester (**3**), TPA acid (**4**), TPA alcohol (**5**), and TPA amine (**6**) were first tested in a biological assay to obtain the initial results for activity (Table 1). *c*log*P* values for the compounds as a parameter for trends and further ongoing SAR studies and possible relations to toxicity are displayed and included in the tables.

First, the putative inhibitors were tested alone against the MBL-producing strains at concentrations of up to 1000 μM and showed no intrinsic antibacterial activity. They were then tested in vitro at 50 μM (except **13**, **19**, and **21** which were tested at 125 μM) in combination with MEM in a standard MIC assay on clinical isolates of *P. aeruginosa* and *K. pneumoniae* expressing either the VIM-2 or NDM-1 MBLs, which hydrolyze MEM.[49] The initial MIC value of MEM alone was between 32 and 64 mg/L for both strains. The addition of the zinc chelators reduced the MIC values markedly, which were in all cases equal to or less than 2 mg/L (except for **7**, which was insoluble in the medium, and **9**, Table 2), showing that the compounds behave as adjuvants.[50]

The TPA derivatives were additionally tested against HepG2 cells to determine their IC_{50} values, which corresponded to the relative toxicity toward this cell line, a critical factor in determining a therapeutic window. As expected from the

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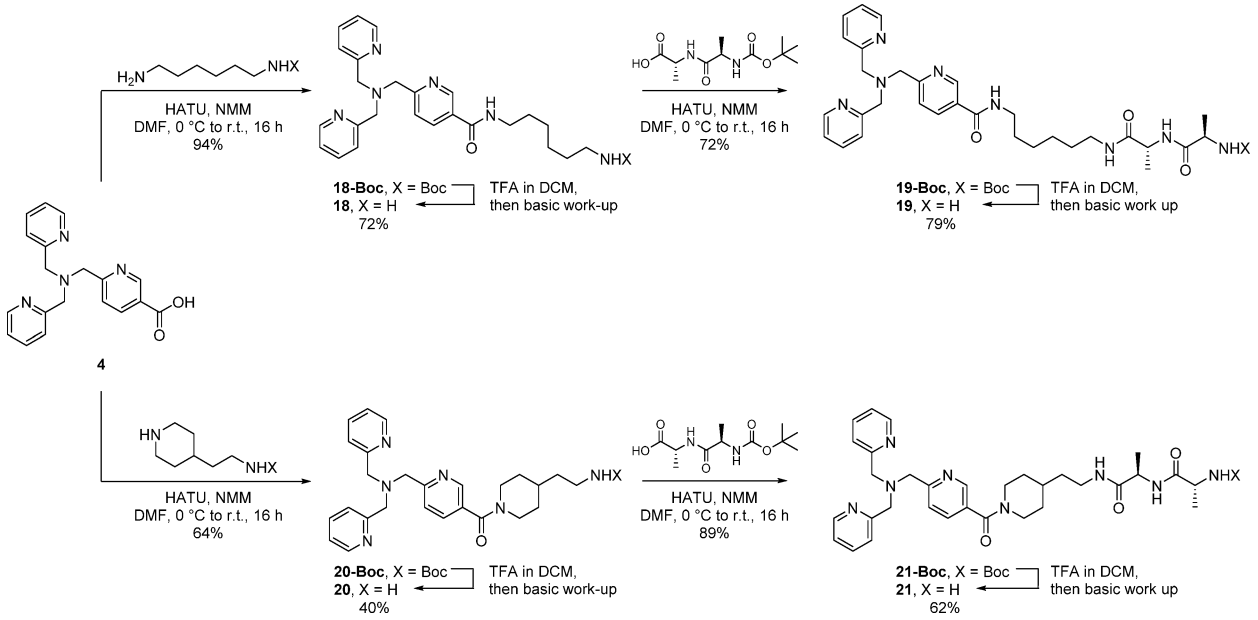
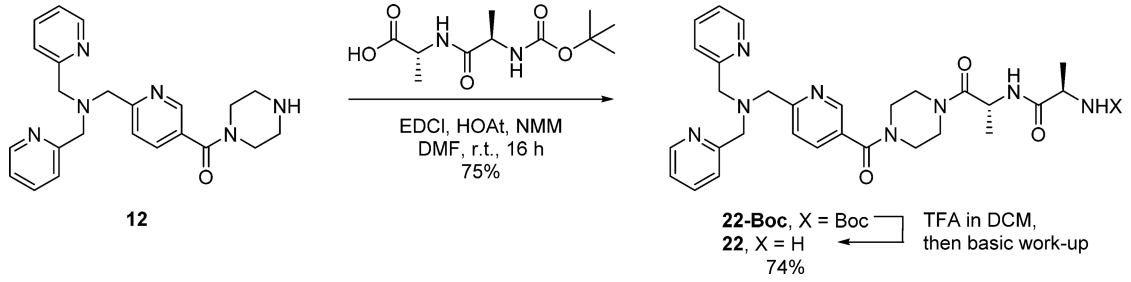
Scheme 5. Preparation of 19 and 21 Using Aliphatic Diamine Linkers



Scheme 6. Synthesis of 22 Using Piperazine as a Linker



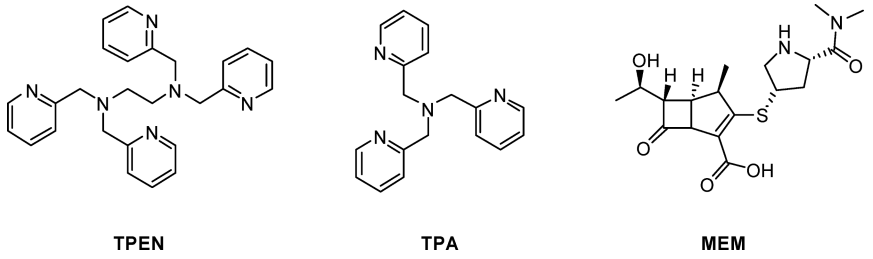


Figure 2. Structures of TPEN, TPA, and MEM.

literature,[51] the IC_{50} of TPEN (7 μM) was half that of **3** (16 μM). The low IC_{50} values of the compounds in Table 1 required chemical modification to lower the toxicity.

Peptide- or non-peptide-based side chains (modulators) were then attached to the chelators, spanning a range of *clogP* values (0.22−2.54), to obtain insight into the parameters that influenced toxicity. We started with the direct attachment of peptide side chains to the TPA moiety resulting in compounds **8**, **9**, **10**, and **13**, which contain D-ala-D-ala-OMe, L-ala-L-ala-OMe, gly-gly-OMe, and D-ala-D-ala-NH_2 as modulators (Table 2). Additionally, allylamine and piperazine were introduced to give compounds **11** and **12**. Piperazine was also used as a linker in the compounds discussed below.

All of the compounds tested in Table 2 held potential for the activity of MEM, with compounds **8**−**10** (Table 2), TPA-D-ala-D-ala-OMe, TPA-L-ala-L-ala-OMe, and TPA-gly-gly-OMe being slightly less effective at reducing the MIC for MEM compared to that for **11**−**13** (Table 2). The compounds in Table 2 were markedly less toxic than the TPA derivatives tested in Table 1 (IC_{50} > 50 μM) with the exception of compound **11** (IC_{50} = 25 μM). However, no direct correlation between the *clogP* values and toxicity could be obtained for this series of compounds.

In parallel to the synthesis of the compounds shown in Table 2, linkers of different physical properties and molecular flexibility were introduced between the chelator and modulator D-ala-D-ala to examine the dependency of activity and toxicity on the molecular structure. Four different diamine linkers were used, giving access to the N-terminal D-ala-D-ala products as well as one aminocarboxylate linker for the corresponding C-terminal derivative. Lipophilic hexyldiamine and piperidin-4-yl ethylamine as well as aromatic 4-aminophenethylamine and 2-(4-

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Table 2. In Vitro Testing of 8−13 as Putative MBL Inhibitors in Clinical Isolates Together with MEM and Toxicity towards HepG2 Cells[a]

		MIC MEM (mg/L)[c]		IC_{50} (μM)
compound	*c*log*P*[b]	*P. aeruginosa* VIM-2[d]	*K. pneumoniae* NDM-1[e]	HepG2
MEM	−1.79	32−64	32−64	ND
8	1.21	2	2	55.5
9	1.21	4	0.25	87.2[f]
10	0.22	2	0.5	127.7
11	2.54	1	0.125	24.7
12	1.46	1	0.125	89.2
13	0.43	1	0.125	[f]

[a]MIC assay performed as one biological replicate and two technical replicates. [b]Estimated using *ChemDraw Ultra* 15.1 from Cambridgesoft. [c]For the MIC determination, all compounds were tested at 50 μM except **13**, which was tested at 125 μM in coadministration with MEM. [d]MIC values of *P. aeruginosa* harboring MBLs VIM-2. [e]MIC values of *K. pneumoniae* harboring MBLs NDM-1. [f]IC_{50} could not be accurately determined from the data.

aminophenyl) carboxylate were chosen along with the piperazine from **12** (Table 3).

Table 3. In Vitro Testing of 15, 17, 19, 21, and 22 as Putative MBL Inhibitors in Clinical Isolates Together with MEM and Toxicity towards HepG2 Cells[a]

		MIC MEM (mg/L)[c]		IC_{50} (μM)
compound	*c*log*P*[b]	*P. aeruginosa* VIM-2[d]	*K. pneumoniae* NDM-1[e]	HepG2
MEM	−1.79	32−64	32−64	ND
15	2.48	1	0.125	>100[f]
17	1.96	1	0.125	>100[f]
19	1.03	1	0.125	>100[g]
21	0.67	1	0.125	>100[f]
22	0.82	1	0.25	162.6

[a]The MIC assay was performed as one biological replicate and two technical replicates. [b]Estimated using *ChemDraw Ultra* 15.1 from Cambridgesoft. [c]For the MIC determination, all compounds were tested at 50 μM except **19** and **21**, which were tested at 125 μM in coadministration with MEM. [d]MIC values of *P. aeruginosa* harboring MBLs VIM-2. [e]MIC values of *K. pneumoniae* harboring MBLs NDM-1. [f]IC_{50} could not be accurately determined from the data because cell viability did not decline to 50%. [g]Poor solubility might have affected the results.

The compounds from Table 3 were among the most potent inhibitors with MIC values for MEM of 1 mg/L against the VIM-2-producing *P. aeruginosa* strain and 0.125−0.25 mg/L for the NDM-1-producing *K. pneumoniae* strain. It was not possible to fit data obtained for the HepG2 toxicity measurements of the compounds in Table 3 to a sigmoidal pattern, and thus IC_{50}s could not be accurately determined, only estimated. A relative comparison of the IC_{50} values obtained for the compounds in Tables 1−3 allows the observation that the inhibitors in Table 3 showed lower toxicity (IC_{50} > 100 μM) as compared to the compounds in Tables 1 and 2. The estimated IC_{50} values in Table 3 corresponded to a concentration much higher than needed for activity in the MIC assay (max. 50 μM). The compounds reported herein did not show a correlation between the *c*log*P* values and toxicity in the HepG2 assays. More importantly, the activity increased, and the toxicity decreased with increasing molecule size, as compounds including linkers were the least toxic and most active.

Compound **15** was further tested at 50 μM against six clinical isolates harboring carbapenemases (Table 4). Three strains expressed MBLs (VIM-1, NDM-1, and VIM-29), while three strains carried other serine carbapenemases (GES-5, KPC-3, and OXA-48). Resensitization of all MBL-carrying strains could be achieved, and no activity against serine-carbapenemases-expressing strains could be demonstrated.

We performed enzyme kinetics based on purified protein to confirm that MBLs are the main target of our designed inhibitors. Both NDM-1 ($k_{inact}/K_I = 0.83 \times 10^{-6}$ s^{-1} M^{-1}) and VIM-2 ($k_{inact}/K_I = 20.8 \times 10^{-6}$ s^{-1} M^{-1}) were inhibited by **15** (Table 5). The corresponding graphs for the enzyme inhibition kinetics can be found in the Supporting Information.

Compound **15** was chosen for in vivo toxicity studies in mice as well as the frequency of resistance development and zinc binding ability in solution by NMR titration.

$ZnCl_2$ NMR Titration of 15. The addition of increasing amounts of $ZnCl_2$ to the test vials with dissolved **15** in DMSO-d_6 showed a linear increase in complex formation. The peaks at 10.32 ppm (**15**) and 10.67 ppm (complex) were used to visualize the ratios between complexed and uncomplexed **15**, revealing the quantitative and equimolar binding of Zn^{2+} by the TPA chelator. The amount of complex formation corresponded to the amount of $ZnCl_2$ added for each sample within experimental tolerances. No further changes in the signals were detected by the addition of 1.5 equiv of $ZnCl_2$ compared to 1.0 equiv. The detailed experimental data (NMR spectra and tables) can be found in the Supporting Information.

In Vivo Toxicity Testing of 15. Compound **15** was administered to female mice at doses doubling each week, ranging from 4 to 128 mg/kg. No acute toxicity was observed as shown by the lack of relative body mass loss, dead mice, or any other obvious health concerns (Supporting Information). In particular, the weight gain of the treated mice did not drop below 90% of the control group. This initial in vivo evaluation indicates that **15** is well tolerated in mice.

Table 4. In Vitro Testing of 15 against an Extended Panel of Clinical Isolates[a]

	MIC MEM (mg/L)[b]					
	K. pneumoniae VIM-1[c]	*E. coli* NDM-1[d]	*E. coli* VIM-29[e]	*P. aeruginosa* GES-5[f]	*K. pneumoniae* KPC-3[g]	*E. coli* OXA-48[h]
MEM	64−256	1−8	8−32	128−256	32−64	0.5−2
MEM + **15**	0.5	0.06	0.125	>64	32	2

[a]The MIC was assay performed as one biological replicate and two technical replicates. [b]For the MIC determination, compound **15** was tested at 50 μM in coadministration with MEM. [c]MIC values of *K. pneumoniae* harboring MBLs VIM-1. [d]MIC values of *E. coli* harboring MBLs NDM-1. [e]MIC values of *E. coli* harboring MBLs VIM-29. [f]MIC values of *P. aeruginosa* harboring MBLs GES-5 (Guiana extended spectrum of metallo-β-lactamase **5**). [g]MIC values of *K. pneumoniae* harboring carbapenemases KPC-3 (*Klebsiella pneumoniae* carbapenemase 3). [h]MIC values of *E. coli* harboring carbapenemases OXA-48 (oxacillinase 48).

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Table 5. Enzyme Inhibition Kinetics for 15 against VIM-2 and NDM-1[a]

	VIM-2			NDM-1		
compound	k_{inact} (s^{-1})	K_I (μM)	k_{inact}/K_I (s^{-1} M^{-1})	k_{inact} (s^{-1})	K_I (μM)	k_{inact}/K_I (s^{-1} M^{-1})
15	41.62	0.56	20.8×10^{-6}	24.41	0.81	0.83×10^{-6}
CI95	1.99	0.03	2.3×10^{-6}	1.94	0.07	0.14×10^{-6}

[a]Abbreviations: K_I, inhibitor concentration that produces the half-maximal rate of inactivation; kinact, maximum inactivation rate; CI95, confidence interval 95%.

Frequency of Resistance Development. During the development of new antimicrobials, it is common to assess the frequency of spontaneous resistant mutants within a bacterial population.[52] For NDM-1-expressing strain *K. pneumoniae* K66-45, compound **15** reduced the MIC of MEM to 0.125 mg/L (Table 3). We therefore decided to determine the frequency of resistance of *K. pneumoniae* K66-45 grown from a single colony to approximately 10^9 CFU/mL against 50 μM compound **15** in a single-step selection experiment in combination with several concentrations of MEM (1, 2, 4, and 8 mg/L). The frequency of resistance against 50 μM compound **15** and 1 mg/L MEM was 10^{-7}. Importantly, colonies were only present at the lowest concentration of MEM tested (1 mg/L), indicating that at concentrations of 2 mg/L and above the frequency of resistant mutants was less than 10^{-9}. The very low rates of resistant mutants observed at these concentrations of MEM suggest that the mechanism of MBL-inhibition by compound **15** is not easily overcome by spontaneous mutation, an important consideration for the future clinical development of any putative MBL inhibitors. Even more encouraging, from the perspective of developing a clinically useful MBL inhibitor, is that EUCAST clinical breakpoints for MEM[53] define ≤2 mg/L as susceptible. While the colonies growing on the 50 μM compound **15** and 1 mg/L combination plate were resistant when compared to the microbroth dilution MIC value of 0.125 mg/L MEM (Table 3), these mutants were still classified as susceptible on the basis of the clinical breakpoints.

CONCLUSIONS

This work presents the development, synthesis and biological evaluation of new selective zinc chelators as putative MBL inhibitors. The key structural elements include the TPA chelator modified with a linker and a modulator. The chemical synthesis of these compounds involves standard methods and allows flexible functionalization. All new compounds lowered the MIC of MEM against two gram-negative bacterial strains harboring carbapenemases VIM-2 and NDM-1. The toxicity of the compounds was evaluated as IC_{50} values on human liver cells (HepG2). The most promising compounds, those displaying the highest potentiation of MEM and lowest HepG2 toxicity, were **10**, **15**, **17**, **19**, **21** and **22**, with IC_{50} values above or equal to 100 μM. **15** was able to inhibit NDM-1 and VIM-2 and showed activity against an extended panel of gram-negative clinical isolates with MBLs, while no reduction in MIC for MEM was seen for serine-β-lactamase harboring bacteria. Initial in vivo toxicity testing (up to 128 mg/kg) of compound **15** in mice showed no acute toxic effects on the animals except a slight reduction in weight gain. The frequency of the spontaneous resistance of the NDM-1 expressing strain *K. pneumoniae* K66-45 against **15** was low (10^{-9}) for concentrations of MEM > 2 mg/L. On the basis of the high potency, low initial toxicity, and low frequency of resistance of these novel zinc chelators, we are currently undertaking further SAR studies to identify and advance compounds with suitable therapeutic windows and the potential for clinical development.

METHODS

Chemistry. Experimental procedures and spectral characterizations are described below and in the Supporting Information.

Evaluation of the Potentiation of Meropenem. The MICs of MEM alone or in combination with the synthesized compounds were determined using premade 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, U.K.). The compounds were tested at a final concentration of 50 or 125 μM. The MIC assays were performed using two MBL-producing MEM-resistant clinical strains: one NDM-1-producing *K. pneumoniae*[54] and one VIM-2-producing *P. aeruginosa*.[55] Compound **15** was also evaluated against a VIM-1-producing *K. pneumoniae*, an NDM-1-producing *E. coli*, a VIM-29-producing *E. coli*, a GES-5-producing *P. aeruginosa*, a KPC-3-producing *K. pneumoniae*, and an OXA-48-producing *E. coli* from the collection of carbapenemase-producing strains at the Norwegian National Advisory Unit on Detection of Antimicrobial Resistance. In brief, the compounds were diluted in MH broth (Becton Dickinson, Franklin Lakes, NJ, USA), and 50 μL of the suspension was 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, U.K.). The diluted bacterial suspension (50 μL) was 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 was determined in a 2-fold serial dilution series in MH broth (Becton Dickinson, Franklin Lakes, NJ, USA) ranging from 2 to 1000 μM. Each dilution step (50 μL) 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 the MIC was determined as described above.

In Vitro Toxicity in HepG2 Liver Cells. Human hepatocarcinoma cell line HepG2 (HB-8065, ATCC, Manassas, VA, USA) was cultured in DMEM-Glutamax (5.5 mM glucose) supplemented with 10% fetal bovine serum (Gibco, Life Technologies AG, Basle, Switzerland), 100 μg/mL streptomycin, and 100 units/mL penicillin (both from Gibco, Life Technologies AG, Basle, Switzerland). Cells were incubated at 37 °C under a 5% CO_2 atmosphere. For viability assays, cells were seeded in white 96-well Nunc plates at a density of 20 000 cells/well and left overnight to adhere before experiments were conducted.

Cell Viability Assay. The Zn chelators were dissolved in DMSO at concentrations ranging from 1 to 10^{-6} mM and were added to white 96-well plates (maximum DMSO concentration in wells lower than 1%) containing 20.000 HepG2 cells/well.

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Plates were incubated for 24 h at 37 °C in a 5% CO_2 atmosphere. After 24 h, AlamarBlue cell viability reagent (Thermo Fisher, Carlsbad, CA, USA) was added as a 10% solution, and plates were placed back in the incubator for 4 h. AlamarBlue is a red-ox indicator yielding a fluorescence signal proportional to the number of viable cells in each well.[56] The fluorescence signal was measured in a microplate reader (Clariostar, BMG Labtech, Ortenberg, Germany) at 550 nm/603 nm (excitation/emission). Data from eight replicates were used to calculate the half-maximal inhibitory concentration (IC_{50}) using Sigmoidal, 4PL, where X is log(concentration) analysis, and a four-parameter logistic regression from GraphPad Prism 6 (GraphPad Software Inc., USA).

clogP Calculations. The partition coefficient for all compounds was calculated using PerkinElmer Informatics ChemDraw Professional, version 15.1.0.144.

Time-Dependent Inactivation Kinetics. Stock solutions of NDM-1 and VIM-2 were prepared in 50 mM HEPES buffer at pH 7.5. The inhibition of MBLs by zinc chelators has been shown to be time-dependent.[57] Therefore, the enzyme inhibition for compound **15** was measured at different concentrations of inhibitor after preincubation times of 2, 8, 15, 25, and 32 min in 50 mM HEPES buffer at pH 7.5 supplemented with 1 μM $ZnSO_4$ and BSA (final concentration 2 μg/mL) at 25 °C. Concentrations of 1 nM VIM-2 and 30 nM NDM-1 were used, and the reaction was initiated by the addition of 30 μM nitrocefin (VIM-2) or 100 μM imipenem (NDM-1). The reaction was measured at 482 nm (VIM-2) or 300 nm (NDM-1) in either standard (VIM-2) or UV-transparent (NDM-1) 96-well plates (Thermo Fisher Scientific, Roskilde, Denmark and Corning, Kennebunk, ME, USA) at 25 °C in a SpectraMax Plus plate reader (Molecular Devices). All enzyme and substrate concentrations indicated are final concentrations in the assay. The % enzyme activity was calculated on the basis of the initial velocity and compared to the control without inhibitor. All tests were performed at least in duplicate.

The observed rate constant (k_{obs}) per inhibitor concentration was calculated from the slope of the semilog plot of % enzyme activity versus preincubation time. The individual values of k_{obs} were plotted against the inhibitor concentration, and saturation kinetics were fitted into eq 1 by using Graph Pad Prism 4 based on the following model (eq 2)

$$k_{obs} = \frac{k_{inact}[I]}{\frac{[S]}{K_I} + [I]} \quad (1)$$

$$E{:}Zn + I \overset{K_I}{\leftrightarrow} E{:}Zn{:}I \xrightarrow{k_{inact}} E + Zn{:}I \text{ or } E^*{:}Zn{:}I \quad (2)$$

where K_I represents the inhibitor concentration that leads to a half-maximum inactivation of the enzyme, k_{inact} is the first-order rate constant, E:Zn is the holoenzyme, I is the inhibitor, E:Zn:I is the enzyme:Zn:inhibtior ternary complex, E is the inactive Zn-depleted enzyme, Zn:I is the zinc-inhibitor complex, and E*:Zn:I is the inactive enzyme:Zn:inhibitor ternary complex.[58]

By fitting these values (eq 1), the irreversible kinetic parameters' maximum inactivation rate (k_{inact}) and the inhibitor concentration that produces the half-maximal rate of inactivation (K_I) were obtained. Finally, the inhibitors were characterized by calculating k_{inact}/K_I. Where no saturation curve could be observed, K_I and k_{inact} were determined from the linear part of the plot of $1/k_{obs}$ versus $1/[I]$.

In Vivo Toxicity of Compound 15 in Mice. In vivo toxicity experiments were performed by Antineo (www.antineo.fr). Female Balb/c mice (4 weeks old, approximately 20 g, Charles River, L'Arbresle, France) were acclimatized for 4 days in the animal facility before the initiation of experiments. A group of six mice were treated with 200 μL of **15** intraperitoneally once a week, with increasing doses each time from 4−128 mg/kg. Another group, also containing six mice, was left untreated and used as control group. Individual weights were followed 4 days a week. Relative weight was calculated as the ratio between the weight of the day and the weight of the first day. The protocol for experiments in mice was approved by the University of Lyon Animal Ethics Committee (Comité d'Ethique en Expérimentation Animale de l'Université Claude Bernard Lyon 1, authorization number DR2015-09).

Frequency of Resistance Determination for Compound 15. To determine the frequency of resistant mutants, a modified single-step selection experiment was carried out as previously described.[59] Briefly, *K. pneumoniae* K66-45 was grown from a single colony to approximately 10^9 CFU/mL and plated on Muellar-Hinton (Becton Dickinson, Franklin Lakes, NJ, USA) agar containing 50 μM compound **15** and 1, 2, 4, or 8 mg/L MEM. Colonies were counted after overnight incubation at 37 °C. The concentrations of MEM were chosen on the basis of the EUCAST clinical breakpoints for Enterobacteriaceae that define ≤2 mg/L as susceptible and >8 mg/L as resistant to MEM.[53]

Synthesis Procedures and Characterization. All reagents and solvents were of analytical grade and were used as received, without further purification. ^{1}H spectra were recorded with a Bruker DRX400, DRX300, or AVI 600 Fourier transform spectrometer using an internal deuterium lock, operating at 400, 300, or 600 MHz. ^{13}C NMR spectra were recorded with a Bruker DRX400, DRX300, or AVI 600 Fourier transform spectrometer using an internal deuterium lock, operating at 100, 75, or 150 MHz. All spectra were recorded at 25 °C. Chemical shifts are reported in parts per million (ppm) relative to residual protons or carbons of deuterated solvents (δ = 2.50 ppm for ^{1}H NMR and δ = 39.52 ppm for ^{13}C NMR for DMSO-*d*6, 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). The carbon multiplicity was determined by DEPT experiments. Mass spectra were recorded at 70 eV on a Waters Prospec Q or Micromass QTOF 2W spectrometer using ESI or APCI as the method of ionization. High-resolution mass spectra were recorded at 70 eV on a Waters Prospec Q or a Micromass QTOF 2W spectrometer using ESI or APCI as the method of ionization. TLC analyses were carried out using Merck Aluminum Oxide 60 F_{256} or Merck Silica gel 60 RP-18 plates visualized by UV light. Agilent Bondesil C18-OH or Versaflash C18 column material supplied by Sigma-Aldrich was used as the stationary phase for reverse-phase dry vacuum chromatography. The yields reported are of isolated material and are uncorrected for purity.

*Methyl 6-((Bis(pyridin-2-ylmethyl)amino)methyl)-nicotinate (**3**).* Methyl 5-(bromomethyl)picolinate (12.979 g, 56.4 mmol, 1.0 equiv) was suspended in 400 mL of THF at room temperature. Dipicolylamine (13.49 g, 12.15 mL, 67.7 mmol, 1.2 equiv) and DIPEA (16.7 mL, 95.89 mmol, 1.7 equiv) were added, and the reaction mixture was stirred at room temperature for 16 h and then concentrated under reduced pressure to approximately 200 mL. The suspension was filtered through a paper filter, the solid was washed with THF (2 × 50 mL), and the obtained solution was concentrated under reduced pressure. The residual dark-brown oil was dissolved in 100 mL of diethyl

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ether, filtered through a plug of Celite, and stored in the freezer to obtain 6-((bis(pyridin-2-ylmethyl)amino)methyl)nicotinate **3** (11.14 g, 31.9 mmol, 56%) as a pale-yellow solid. The obtained ^{1}H NMR and ^{13}C NMR data were in agreement with reported data.[36] ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.11 (dd, J = 2.1, 0.7 Hz, 1H), 8.53 (ddd, J = 4.8, 1.6, 0.8 Hz, 2H), 8.24 (dd, J = 8.2, 2.2 Hz, 1H), 7.73−7.59 (m, 1H), 7.53 (d, J = 7.8 Hz, 2H), 7.14 (ddd, J = 7.3, 4.9, 1.1 Hz, 2H), 3.95 (s, 2H), 3.92 (s, 3H), 3.89 (s, 4H).

*6-((Bis(pyridin-2-ylmethyl)amino)methyl)nicotinic Acid (**4**).* Methyl 6-((bis(pyridin-2-ylmethyl)amino)methyl)-nicotinate (**3**, 1.734 g, 4.97 mmol, 1.0 equiv) was dissolved in 20 mL of THF and cooled in an ice bath. A solution of LiOH hydrate (626 mg, 14.92 mmol, 3.0 equiv) in 20 mL of distilled H_2O was added, and the solution was stirred at 0 °C until TLC (alumina, 5% MeOH/CH_2Cl_2) indicated full conversion. The THF was removed under reduced pressure, and the residual aqueous solution was adjusted to pH 6 using 4 N HCl. The solvent was removed under reduced pressure, affording product **4** in quantitative yield, which was used in the next step without further purification. NMR data were in agreement with the reported data.[60]

*(6-((Bis(pyridin-2-ylmethyl)amino)methyl)pyridin-3-yl) Methanol (**5**).* Methyl 6-((bis(pyridin-2-ylmethyl)amino)-methyl)nicotinate (**3**, 330 mg, 0.95 mmol, 1.0 equiv) was dissolved in absolute ethanol (10 mL) and placed under argon. $NaBH_4$ pellets (250 mg, 6.95 mmol, 7.0 equiv) were added to the stirring mixture, and the slurry was held at 50 °C for 48 h. The mixture was then quenched by the addition of NH_4Cl solution and concentrated under reduced pressure to give a sticky white solid. The crude material was suspended in 1 M K_2CO_3 (25 mL) and extracted with DCM (3 × 25 mL). The combined organic phases were dried over K_2CO_3, filtered, and concentrated under reduced pressure to give 221 mg (69%) of the title product as a pale-yellow oil. NMR data were in agreement with the published data.[36]

*(6-((Bis(pyridin-2-ylmethyl)amino)methyl)pyridin-3-yl)-methylmethanesulfonate (**5-Mes**).* (6-((Bis(pyridin-2-ylmethyl)amino)methyl)pyridin-3-yl)methanol (**5**, 2.822 g, 8.81 mmol, 1.0 equiv) was dissolved in 150 mL of dry THF under Ar and cooled to 0 °C in an ice bath. To this solution was added NEt_3 (2.45 mL, 17.62 mmol, 2.0 equiv), followed by a solution of mesyl chloride (1.363 mL, 17.62 mmol, 2.0 equiv) in 30 mL of dry THF dropwise. A precipitate formed, and the suspension was stirred at 0 °C for 30 min until TLC (alumina, 3% MeOH in CH_2Cl_2) indicated full conversion. The mixture was filtered into a new flask and concentrated under reduced pressure to a volume of ca. 100 mL at 40 °C, and DMF (80 mL) was added. The remaining THF was removed under reduced pressure, and the obtained solution of (6-((bis(pyridin-2-ylmethyl)amino)methyl)pyridin-3-yl)methylmethanesulfonate (5-Mes) in DMF was used in the next reaction without further treatment under the assumption of quantitative conversion. ^{1}H NMR (300 MHz, chloroform-d) δ 10.59 (s, 1H), 8.70 (ddd, J = 5.5, 1.6, 0.8 Hz, 2H), 8.50 (dd, J = 2.2, 0.8 Hz, 1H), 8.05 (td, J = 7.8, 1.7 Hz, 2H), 7.80 (d, J = 7.9 Hz, 1H), 7.69 (dd, J = 8.1, 2.3 Hz, 1H), 7.61−7.37 (m, 2H), 4.47 (s, 2H), 4.42 (s, 4H), 4.16 (s, 2H), 3.06 (s, 3H).

1-(5-(Azidomethyl)pyridin-2-yl)-N,N-bis(pyridin-2-ylmethyl)methanamine (TPA-N_3). To the solution of (6-((bis(pyridin-2-ylmethyl)amino)methyl)pyridin-3-yl)methyl-methanesulfonate prepared in the previous reaction (**5-Mes**, 3.51 g, 8.81 mmol, 1.0 equiv) in 80 mL of DMF was added NaN_3 (2.864 g, 44.04 mmol, 5.0 equiv) at room temperature. The mixture was stirred at room temperature for 20 h and then filtered into a new flask and concentrated under reduced pressure to a volume of approximately 30 mL. The mixture was diluted with 100 mL of H_2O, transferred to a separation funnel, and extracted with EtOAc (2 × 100 mL). The combined organics were washed with saturated aqueous K_2CO_3 solution (50 mL) and brine (50 mL), dried over K_2CO_3, filtered, and concentrated under reduced pressure. The obtained compound was used in the next reaction without further treatment. ^{1}H NMR (300 MHz, methanol-d_4) δ 8.45−8.40 (m, 3H), 7.82−7.74 (m, 3H), 7.66 (dd, J = 8.0, 2.4 Hz, 3H), 7.26 (ddd, J = 7.4, 5.0, 1.2 Hz, 2H), 4.42 (s, 2H), 3.86 (s, 6H).

*1-(5-(Aminomethyl)pyridin-2-yl)-N,N-bis(pyridin-2-ylmethyl)methanamine Hydrochloride (**6**).* The 1-(5-(Azidomethyl)pyridin-2-yl)-*N*,*N*-bis(pyridin-2-ylmethyl)methanamine prepared in the previous reaction (TPA-N_3, 2.783 g, 8.06 mmol, 1.0 equiv) was dissolved in 50 mL of THF. To this solution was added 5 mL of distilled H_2O followed by PPh_3 (4.228 g, 16.12 mmol, 2.0 equiv) in one portion. The mixture was heated to 50 °C and stirred for 3 h until TLC (alumina, 3% MeOH in CH_2Cl_2) indicated full conversion. The mixture was concentrated under reduced pressure, the residue was treated with CH_2Cl_2 and H_2O (100 mL each) and transferred to a separation funnel, and the phases were separated. The pH of the aqueous phase was adjusted to 1 with conc HCl under rapid stirring and was transferred to a separation funnel. The aqueous phase was washed with CH_2Cl_2 (50 mL) and concentrated under reduced pressure to afford 2.825 g (7.93 mmol, 98%) of 1-(5-(aminomethyl)pyridin-2-yl)-*N*,*N*-bis(pyridin-2-ylmethyl)-methanamine **6** as the hydrochloride salt. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.00 (s (b), 3H), 8.91 (d, J = 1.6 Hz, 1H), 8.81 (dd, J = 5.7, 0.9 Hz, 2H), 8.47 (dtd, J = 9.3, 8.1, 1.4 Hz, 3H), 8.16 (d, J = 7.9 Hz, 2H), 8.09 (d, J = 8.2 Hz, 1H), 7.93−7.84 (m, 2H), 4.39 (s, 4H), 4.30 (s, 2H), 4.17 (q, J = 5.3 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 153.2, 152.5, 145.4, 142.6, 131.7, 127.0, 125.9, 125.8, 56.2, 55.6, 38.7. APCI-HRMS m/z calcd for $C_{19}H_{22}N_5^+$, 320.1875; found, 320.1869.

*6-((Bis(pyridine-2-ylmethyl)amino)methyl)-nicotinate (**7**).* NH_3 (7 M) in MeOH (10.29 mL, 72 mmol, 50 equiv) was slowly added to solid methyl 6-((bis(pyridin-2-ylmethyl)-amino)methyl)nicotinate (**3**, 502 mg, 1.44 mmol, 1 equiv) under rapid stirring. The reaction mixture stirred for 24 h at room temperature before another 50 equiv of NH_3 was added, and the reaction mixture was stirred for another 24 h at room temperature. The mixture was concentrated under reduced pressure to give 475 mg (1.43 mmol, >99%) of the title compound as a clear yellow liquid/oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.94 (d, J = 2.0 Hz, 1H), 8.53−8.46 (m, 2H), 8.18 (dd, J = 8.1, 2.2 Hz, 1H), 8.09 (s (b), 1H), 7.77 (td, J = 7.7, 1.8 Hz, 2H), 7.68 (d, J = 8.1 Hz, 1H), 7.58 (d, J = 7.8 Hz, 2H), 7.53 (s (b), 1H), 7.29−7.20 (m, 2H), 3.84 (s, 2H), 3.79 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.4, 161.9, 158.8, 148.9, 148.1, 136.6, 135.7, 128.1, 122.6, 122.2, 121.9, 59.4, 59.2, 48.6. MS: m/z calcd for $C_{19}H_{20}N_5O$ $[M + H]^+$, 334.17; found, 334.16.

*(R)-Methyl 2-((R)-2-(6-((Bis(pyridin-2-ylmethyl)amino)-methyl)nicotinamido)propanamido)propanoate (**8**).* 6-((Bis-(pyridin-2-ylmethyl)amino)methyl)nicotinic acid (**4**, 179 mg, 0.535 mmol, 1.0 equiv) was dissolved in 2 mL of dry DMF at room temperature. D-Ala-D-Ala-OMe hydrochloride (93 mg, 0.535 mmol, 1.0 equiv) was added, and the mixture was cooled to 0 °C in an ice bath. HATU (203 mg, 0.535 mmol, 1.0 equiv) and NMM (247 μL, 2.247 mmol, 4.2 equiv) were added, and the

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mixture was stirred at 0 °C for 1 h and then at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure, and product purification was achieved by way of dry column vacuum chromatography on C18 material using a stepwise elution from 10 to 90% methanol in water affording 195 mg (0.398 mmol, 75%) of product **8**. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.91 (d, J = 2.0 Hz, 1H), 8.44 (d, J = 4.9 Hz, 2H), 8.19 (dd, J = 8.2, 2.2 Hz, 1H), 7.79 (td, J = 7.8, 1.4 Hz, 2H), 7.74 (d, J = 8.2 Hz, 1H), 7.66 (d, J = 7.8 Hz, 2H), 7.32−7.23 (m, 2H), 4.58 (q, J = 7.2 Hz, 1H), 4.44 (q, J = 7.3 Hz, 1H), 3.92 (s, 2H), 3.88 (s, 4H), 3.71 (s, 3H), 1.48 (d, J = 7.2 Hz, 3H), 1.41 (d, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, methanol-d_4) δ 174.9, 174.6, 167.7, 163.5, 159.9, 149.6, 149.0, 138.7, 137.4, 130.0, 125.0, 124.2, 123.9, 61.2, 61.0, 52.7, 50.8, 49.5, 18.0, 17.3. APCI-HRMS m/z calcd for $C_{26}H_{31}N_6O_4$ $[M + H]^+$, 491.2401; found, 491.2399.

*(S)-Methyl 2-((S)-2-(6-((bis(pyridin-2-ylmethyl)amino)-methyl)nicotinamido)propanamido)propanoate (**9**).* 6-((Bis-(pyridin-2-ylmethyl)amino)methyl)nicotinic acid (**4**, 119 mg, 0.35 mmol, 1.0 equiv) was dissolved in 3 mL of dry DMF at room temperature. Ala-Ala-OMe hydrochloride (74 mg, 0.35 mmol, 1.0 equiv) was added, and the mixture was cooled to 0 °C in an ice bath. HATU (133 mg, 0.35 mmol, 1.0 equiv) and NMM (85 μL, 0.77 mmol, 2.2 equiv) were added, and the mixture was stirred at 0 °C for 1 h and then at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure, and the purification of the product was achieved by way of dry column vacuum chromatography on C18 material using stepwise elution from 10 to 90% methanol in water affording 93 mg (0.189 mmol, 54%) of product **9**. ^{1}H NMR (300 MHz, MeOH) δ 8.91 (d, J = 1.6 Hz, 1H), 8.48−8.38 (m, 2H), 8.20 (dd, J = 8.2, 2.3 Hz, 1H), 7.78 (ddd, J = 11.9, 8.9, 5.1 Hz, 3H), 7.66 (d, J = 7.8 Hz, 2H), 7.27 (ddd, J = 7.4, 5.0, 1.1 Hz, 2H), 4.58 (q, J = 7.2 Hz, 1H), 4.44 (q, J = 7.3 Hz, 1H), 3.90 (d, J = 5.4 Hz, 2H), 3.87 (s, 4H), 3.71 (s, 3H), 1.48 (d, J = 7.2 Hz, 3H), 1.41 (d, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, methanol-d_4) δ 174.9, 174.6, 167.7, 163.5, 159.9, 149.6, 149.0, 138.7, 137.4, 130.0, 124.9, 124.2, 123.9, 61.2, 60.9, 52.7, 50.8, 49.5, 18.0, 17.3. APCI-HRMS m/z calcd for $C_{26}H_{31}N_6O_4$ $[M + H]^+$, 491.2401; found, 491.2399.

*Methyl 2-(2-(6-((Bis(pyridin-2-ylmethyl)amino)methyl)-nicotinamido)acetamido)acetate (**10**).* 6-((Bis(pyridin-2-ylmethyl)amino)methyl)nicotinic acid (**4**, 194 mg, 0.581 mmol, 1.0 equiv) was dissolved in 5 mL of dry DMF at room temperature. Gly-Gly-OMe hydrochloride (111 mg, 0.61 mmol, 1.05 equiv) was added, and the mixture was cooled to 0 °C in an ice bath. HATU (232 mg, 0.61 mmol, 1.05 equiv) and NMM (141 μL, 1.28 mmol, 2.2 equiv) were added, and the mixture was stirred at 0 °C for 1 h and then at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure, and the purification of the product was achieved by way of dry column vacuum chromatography on C18 material using a stepwise elution from 10 to 90% methanol in water affording 145 mg (0.312 mmol, 54%) of product **10**. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.93 (s, 1H), 8.44 (d, J = 3.3 Hz, 2H), 8.21 (d, J = 8.1 Hz, 1H), 7.77 (dd, J = 18.2, 10.2 Hz, 3H), 7.65 (d, J = 7.8 Hz, 2H), 7.27 (t, J = 5.7 Hz, 2H), 4.11 (s, 2H), 3.98 (s, 2H), 3.91 (d, J = 7.4 Hz, 2H), 3.88 (s, 4H), 3.71 (s, 3H). ^{13}C NMR (101 MHz, methanol-d_4) δ 172.1, 171.7, 168.1, 163.6, 159.9, 149.6, 148.9, 138.7, 137.4, 129.8, 124.9, 124.3, 123.9, 61.2, 60.9, 52.6, 43.8, 41.8. APCI-HRMS m/z calcd for $C_{24}H_{27}N_6O_4$ $[M + H]^+$, 466.2088; found, 463.2087.

*N-Allyl-6-((bis(pyridin-2-ylmethyl)amino)methyl)-nicotinamide (**11**).* 6-((Bis(pyridin-2-ylmethyl)amino)-methyl)nicotinic acid (**4**, 1.0 g, 2.99 mmol, 1.0 equiv) was dissolved in 20 mL of dry DMF at room temperature. HATU (1.140 g, 2.99 mmol, 1.0 equiv), the amine (751 μL, 9.99 mmol, 3.3 equiv), and NMM (751 μL, 4.00 mmol, 1.3 equiv) were added. The mixture was stirred at room temperature overnight before it was concentrated under reduced pressure. The crude material was suspended in 250 mL of 1 M K_2CO_3 and extracted with 5 × 25 mL EtOAc. The combined organic fractions were dried over K_2CO_3, filtered, and concentrated under reduced pressure to give a pale-brown oil. The material was purified on neutral Al_2O_3 using 0−5% MeOH in CH_2Cl_2 as the eluent. A total of 726 mg (65%) of clean product was obtained. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.93 (dd, J = 2.3, 0.8 Hz, 1H), 8.77 (t, J = 5.7 Hz, 1H), 8.49 (ddd, J = 4.9, 1.8, 0.9 Hz, 2H), 8.17 (dd, J = 8.1, 2.3 Hz, 1H), 7.77 (td, J = 7.6, 1.9 Hz, 2H), 7.69 (dd, J = 8.2, 0.8 Hz, 1H), 7.58 (dt, J = 7.8, 1.1 Hz, 2H), 7.25 (ddd, J = 7.4, 4.8, 1.2 Hz, 2H), 5.89 (ddt, J = 17.2, 10.4, 5.3 Hz, 1H), 5.27−5.01 (m, 2H), 3.91 (tt, J = 5.5, 1.7 Hz, 2H), 3.85 (s, 2H), 3.80 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.5, 161.8, 158.8, 148.8, 147.7, 136.5, 135.4, 135.1, 128.3, 122.6, 122.1, 122.0, 115.3, 59.4, 59.2, 41.4. MS. APCI-HRMS m/z calcd for $C_{22}H_{24}N_5O$ $[M + H]^+$, 374.1975; found, 374.1975.

*tert-Butyl 4-(6-((Bis(pyridin-2-ylmethyl)amino)methyl)-nicotinoyl)piperazine-1-carboxylate (**12-Boc**).* 6-((Bis-(pyridin-2-ylmethyl)amino)methyl)nicotinic acid (**4**, 180 mg, 0.537 mmol, 1.0 equiv) was dissolved in 3 mL of dry DMF at room temperature. *tert*-Butyl piperazine-1-carboxylate (150 mg, 0.806 mmol, 1.5 equiv), EDCl (134 mg, 0.806 mmol, 1.5 equiv), HOAt (109.6 mg, 0.806 mmol, 1.5 equiv), and NMM (89 μL, 0.806 mmol, 1.5 equiv) were added, and the mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure, and the purification of the product was achieved by way of dry column vacuum chromatography on C18 material using stepwise elution from 10 to 90% methanol in water, affording 244 mg (0.486 mmol, 91%) of product 12-Boc. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.52 (d, J = 1.6 Hz, 1H), 8.44 (d, J = 4.3 Hz, 2H), 7.86−7.75 (m, 3H), 7.73 (d, J = 8.0 Hz, 1H), 7.67 (d, J = 7.9 Hz, 2H), 7.32−7.22 (m, 2H), 3.91 (s, 2H), 3.89 (s, 4H), 3.72 (s (b), 2H), 3.45 (s (b), 6H), 1.46 (s, 9H). ^{13}C NMR (101 MHz, methanol-d_4) δ 169.8, 162.2, 160.0, 156.2, 149.6, 148.2, 138.6, 137.2, 131.4, 125.0, 124.4, 123.9, 81.7, 61.3, 61.0, 28.6. APCI-HRMS m/z calcd for $C_{28}H_{35}N_6O_3$ $[M + H]^+$, 503.2765; found, 503.2765.

*(6-((Bis(pyridin-2-ylmethyl)amino)methyl)pyridin-3-yl)-(piperazin-1-yl)methanone (**12**).* *tert*-Butyl 4-(6-((bis(pyridin-2-ylmethyl)amino)methyl)nicotinoyl)piperazine-1-carboxylate prepared in the previous reaction (**12-Boc**, 234 mg, 0.465 mmol, 1.0 equiv) was dissolved in 10 mL of CH_2Cl_2 at room temperature. To this solution was added TFA (2.84 mL, 80 equiv), and the mixture was stirred at room temperature until NMR indicated full conversion. The mixture was concentrated under reduced pressure, and the residue was dissolved in distilled H_2O, neutralized with saturated aqueous K_2CO_3 solution, and concentrated under reduced pressure. Purification of the product was achieved by way of dry column vacuum chromatography on C18 material using stepwise elution from 10 to 90% methanol in water, affording 80 mg (0.35 mmol, 76%) of product **12**. ^{1}H NMR (300 MHz, MeOH) δ 8.50 (dd, J = 2.1, 0.7 Hz, 1H), 8.44 (ddd, J = 5.0, 1.7, 0.9 Hz, 2H), 7.79 (ddd, J = 9.6, 6.2, 2.3 Hz, 3H), 7.73 (dd, J = 8.1, 0.4 Hz, 1H), 7.67 (d, J = 7.8 Hz, 2H), 7.27 (ddd, J = 7.4, 5.0, 1.2 Hz, 2H), 3.90 (s, 2H), 3.89

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(s, 4H), 3.72 (s, J = 15.5 Hz, 2H), 3.40 (s, J = 18.3 Hz, 2H), 2.84 (d, J = 17.0 Hz, 4H). ^{13}C NMR (101 MHz, methanol-d_4) δ 169.6, 162.0, 160.0, 149.6, 148.0, 138.6, 137.1, 131.6, 125.0, 124.4, 123.9, 61.7, 61.1, 46.6, 45.7, 44.0. APCI-HRMS m/z calcd for $C_{23}H_{27}N_6O$ $[M + H]^+$, 403.2241; found, 403.2240.

*1-(5-(Aminomethyl)pyridin-2-yl)-N,N-bis(pyridin-2-ylmethyl)methanamine (**13-Boc**).* Boc-D-Ala-D-Ala-OH (60 mg, 0.23 mmol, 1.0 equiv) was dissolved in 1 mL of dry DMF and cooled to 0 °C in an ice-water bath. 1-(5-(Aminomethyl)-pyridin-2-yl)-*N*,*N*-bis(pyridin-2-ylmethyl)methanamine (73 mg, 0.23 mmol, 1.0 equiv) and HATU (88 mg, 0.23 mmol, 1.0 equiv) were added, before NMM (50.5 μL, 0.46 mmol, 2.0 equiv) was added to the stirring mixture. The mixture was stirred in an ice-water bath for 15 min before slowly warming to room temperature and was left to stir overnight. The mixture was diluted with 0.5 M K_2CO_3 (30 mL) and extracted with EtOAc (3 × 20 mL). The combined extracts were washed with 0.5 M K_2CO_3 (3 × 20 mL), dried over K_2CO_3, filtered, and concentrated under reduced pressure. The orange oily residue was dissolved in CH_2Cl_2 and purified by column chromatography on neutral alumina (1−2% MeOH in CH_2Cl_2) to afford the title compound. Yield (89 mg, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (dd, J = 5.0, 1.8 Hz, 2H), 8.40 (d, J = 2.3 Hz, 1H), 7.67−7.49 (m, 7H), 7.12 (ddd, J = 7.4, 4.9, 1.3 Hz, 2H), 6.76 (d, J = 7.6 Hz, 1H), 5.12 (broad s, 1H), 4.48 (pentet, J = 7.5 Hz, 1H), 4.38 (d, J = 5.9 Hz, 2H), 4.05 (m, 1H), 3.84 (s, 4H), 3.82 (s, 2H), 1.47−1.21 (m, 15H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8, 172.3, 162.6, 159.3, 158.5, 149.2, 148.4, 136.6, 136.1, 132.4, 123.1, 122.9, 122.2, 80.7, 60.2, 59.9, 51.0, 49.1, 40.8, 28.3, 19.01, 18.0. HRMS (ESI) calcd for $C_{30}H_{40}N_7O_4$ $[M + H]^+$, 562.3136; found, 562.3136.

*(R)-2-Amino-N-((R)-1-(((6-((bis(pyridin-2-ylmethyl)-amino)methyl)pyridin-3-yl)methyl)amino)-1-oxopropan-2-yl)propanamide (**13**).* The N-Boc-protected amine (**13-Boc**, 84 mg, 0.15 mmol, 1.0 equiv) was dissolved in 1 mL of CH_2Cl_2 and cooled to 0 °C in an ice-water bath. Trifluoroacetic acid (0.69 mL, 9.0 mmol, 60 equiv) in 1 mL of CH_2Cl_2 was then slowly added to the stirring mixture. The reaction was left at 0 °C for 20 min before warming 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 the 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 mL). The combined organic layers were washed with fresh 0.5 M K_2CO_3 (3 × 50 mL), dried on K_2CO_3, and filtered, and the solvent was removed under reduced pressure to give the title compound. Yield (44 mg, 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (dd, J = 4.9, 1.8 Hz, 2H), 8.39 (d, J = 2.2 Hz, 1H), 7.83 (d, J = 7.9 Hz, 1H), 7.63 (td, J = 7.6, 1.8 Hz, 2H), 7.59−7.47 (m, 4H), 7.33 (t, J = 6.1 Hz, 1H), 7.12 (ddd, J = 7.5, 4.9, 1.3 Hz, 2H), 4.44 (pentet, J = 7.1 Hz, 1H), 4.37 (d, J = 5.9 Hz, 2H), 3.83 (s, 4H), 3.82 (s, 2H), 3.41 (q, J = 6.9 Hz, 1H), 1.87 (broad s, 2H), 1.37 (d, J = 6.9 Hz, 3H), 1.25 (d, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.1, 172.4, 159.2, 158.4, 149.1, 148.3, 136.5, 136.0, 132.2, 123.0, 122.8, 122.1, 60.1, 59.8, 50.4, 48.5, 40.7, 21.4, 17.6. HRMS (ESI) calcd for $C_{25}H_{32}N_7O_2$ $[M + H]^+$, 462.2612; found, 462.2612.

*tert-Butyl 4-(6-((Bis(pyridin-2-ylmethyl)amino)methyl) nicotinamido)phenethylcarbamate (**14-Boc**).* 6-((Bis-(pyridin-2-ylmethyl)amino)methyl)nicotinic acid (**4**, 1.660 g, 4.96 mmol, 1.0 equiv) was dissolved in 20 mL of dry DMF at room temperature and filtered into a 50 mL round-bottomed flask prior to reaction to remove the insoluble salts. To this solution was added *tert*-butyl 4-aminophenethylcarbamate (1.76 g, 7.45 mmol, 1.5 equiv), followed by EDCI (1.428 g, 7.45 mmol, 1.5 equiv), HOAt (1.014 g, 7.45 mmol, 1.5 equiv), and NMM (0.821 mL, 7.45 mmol, 1.5 equiv). The reaction mixture was stirred at room temperature for 16 h and then concentrated under reduced pressure. The residual crude mixture was dissolved in 100 mL of $CHCl_3$, transferred to a separation funnel, and washed with 100 mL of saturated aqueous K_2CO_3 solution and 100 mL of brine. The organic phase was separated, dried oved Na_2SO_4, filtered, and concentrated under reduced pressure. Purification of the product was performed by column chromatography on neutral Al_2O_3 using 1% MeOH in DCM to give the product with minor impurities, followed by C18-SPE using gradient elution (10% MeOH to 90% MeOH in H_2O), affording 1.697 g (3.07 mmol, 62%) of the product as a yellow oil. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.96 (d, J = 1.9 Hz, 1H), 8.42 (d, J = 4.4 Hz, 2H), 8.23 (dd, J = 8.2, 2.2 Hz, 1H), 7.75 (t, J = 7.7 Hz, 3H), 7.64 (d, J = 7.8 Hz, 2H), 7.60 (d, J = 8.2 Hz, 2H), 7.28−7.20 (m, 2H), 7.16 (d, J = 8.3 Hz, 2H), 3.88 (s, 2H), 3.84 (s, 4H), 3.23 (t, J = 7.4 Hz, 2H), 2.72 (t, J = 7.3 Hz, 2H), 1.40 (s, 9H). ^{13}C NMR (101 MHz, methanol-d_4) δ 166.1, 163.4, 159.9, 158.3, 149.6, 148.9, 138.6, 137.8, 137.4, 137.2, 130.9, 130.2, 124.8, 124.1, 123.8, 122.2, 79.9, 61.1, 60.8, 43.0, 36.6, 28.8. APCI-HRMS m/z calcd for $C_{32}H_{37}N_6O_3$ $[M + H]^+$, 553.2922; found, 553.2920.

*N-(4-(2-Aminoethyl)phenyl)-6-((bis(pyridin-2-ylmethyl) amino)methyl)nicotinamide (**14**).* The *tert*-butyl 4-(6-((bis-(pyridin-2-ylmethyl)amino)methyl)-nicotinamido) phenethyl-carbamate (**14-Boc**, 1.697 g, 3.07 mmol, 1 equiv) prepared in the previous reaction was dissolved in 10 mL of DCM at room temperature. To this solution was added TFA (5 mL), and the mixture was stirred at room temperature until TLC (Al_2O_3, 5% MeOH in DCM) or NMR indicated full conversion. The mixture was then concentrated under reduced pressure, and the residue was dissolved in a mixture of $CHCl_3$/dest. H_2O/sat. aq. K_2CO_3 (100 mL/10 mL/100 mL) and transferred to a separation funnel. The organic phase was separated, the aqueous phase was extracted twice with 50 mL of $CHCl_3$, and the combined organics were washed with 100 mL of brine, dried over K_2CO_3/Na_2SO_4, filtered, and concentrated under reduced pressure to afford *N*-(4-(2-aminoethyl)phenyl)-6-((bis(pyridin-2-ylmethyl)amino)methyl)nicotinamide (**14**) in quantitative yield. ^{1}H NMR (300 MHz, MeOH) δ 8.97 (d, J = 1.7 Hz, 1H), 8.45 (ddd, J = 5.0, 1.7, 0.9 Hz, 2H), 8.26 (dd, J = 8.2, 2.3 Hz, 1H), 7.80 (td, J = 7.6, 1.6 Hz, 3H), 7.68 (d, J = 7.8 Hz, 2H), 7.62 (d, J = 8.5 Hz, 2H), 7.28 (ddd, J = 7.4, 5.0, 1.2 Hz, 2H), 7.23 (d, J = 8.5 Hz, 2H), 3.94 (s, 2H), 3.90 (s, 4H), 2.89 (dd, J = 10.6, 4.1 Hz, 2H), 2.76 (t, J = 6.9 Hz, 2H). ^{13}C NMR (101 MHz, methanol-d_4) δ 163.6, 160.0, 149.6, 148.9, 138.7, 137.5, 131.0, 130.2, 125.0, 124.3, 123.9, 122.5, 61.2, 61.0, 44.0, 39.2. APCI-HRMS m/z calcd for $C_{27}H_{29}N_6O$ $[M + H]^+$, 453.2397; found, 453.2396.

*tert-Butyl (R)-1-((R)-1-(4-(6-((Bis(pyridin-2-ylmethyl)-amino)methyl)nicotinamido)phenethylamino)-1-oxo-propan-2-ylamino)-1-oxopropan-2-ylcarbamate (**15-Boc**).* *N*-(4-(2-aminoethyl)phenyl)-6-((bis(pyridin-2-ylmethyl)-amino)methyl)nicotinamide prepared in the previous reaction (**14**, 218 mg, 0.48 mmol, 1.0 equiv) was dissolved in 3 mL of dry DMF and cooled to 0 °C in an ice bath. To this solution was added Boc-D-Ala-D-Ala-OH (132 mg, 0.506 mmol, 1.05 equiv), HATU (192 mg, 0.506 mmol, 1.05 equiv), and NMM (116 μL, 1.056 mmol, 2.2 equiv), and the solution was stirred at 0 °C for

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30 min and then at room temperature for 16 h. The mixture was concentrated under reduced pressure, and the product was purified by dry column vacuum chromatography on C18 material using stepwise elution from 10 to 90% methanol in water, affording 217.7 mg (0.313 mmol, 65%) of the product as a pale-yellow oil. ^{1}H NMR (600 MHz, methanol-d_4) δ 8.96 (d, J = 1.7 Hz, 1H), 8.44 (d, J = 4.3 Hz, 2H), 8.25 (dd, J = 8.2, 2.2 Hz, 1H), 7.78 (ddd, J = 10.3, 6.1, 2.2 Hz, 3H), 7.67 (d, J = 7.9 Hz, 2H), 7.61 (d, J = 8.4 Hz, 2H), 7.27 (ddd, J = 7.3, 5.1, 0.8 Hz, 2H), 7.20 (d, J = 8.5 Hz, 2H), 4.30 (q, J = 7.0 Hz, 1H), 4.03 (q, J = 7.0 Hz, 1H), 3.92 (s, 2H), 3.88 (s, 4H), 3.48−3.41 (m, 1H), 3.35 (dd, J = 20.2, 7.1 Hz, 1H), 2.78 (t, J = 7.0 Hz, 2H), 1.44 (s, 9H), 1.30 (t, J = 6.6 Hz, 6H). ^{13}C NMR (151 MHz, methanol-d_4) δ 175.6, 174.7, 166.2, 163.4, 159.9, 158.0, 149.6, 148.9, 138.7, 137.9, 137.5, 136.9, 131.0, 130.2, 124.9, 124.2, 123.9, 122.3, 80.7, 61.2, 60.9, 52.0, 50.4, 42.0, 35.9, 28.7, 18.2, 18.0. APCI-HRMS m/z calcd for $C_{38}H_{47}N_8O_5$ $[M + H]^+$, 695.3664; found, 695.3659.

*N-(4-(2-((R)-2-((R)-2-Aminopropanamido)propanamido)-ethyl)phenyl)-6-((bis(pyridin-2-ylmethyl)amino)methyl)nicotinamide Hydrochloride (**15**).* (*R*)-1-((*R*)-1-(4-(6-((Bis-(pyridin-2-ylmethyl)amino)methyl)nicotinamido)phenethylamino)-1-oxopropan-2-ylamino)-1-oxopropan-2-ylcarbamate prepared in the previous reaction (15-Boc, 176 mg, 0.253 mmol, 1.0 equiv) was dissolved in 5 mL of CH_2Cl_2 and cooled to 0 °C in an ice bath. To this solution was added TFA (0.97 mL, 12.65 mmol, 50 equiv), and the mixture was stirred at room temperature until full conversion as monitored by ^{1}H NMR. The mixture was concentrated under reduced pressure, and the residue was dissolved in a mixture of EtOAc/dist. H_2O/sat. aq. K_2CO_3 (30 mL/10 mL/30 mL) and transferred to a separation funnel. The organic phase was separated and the aq. phase was extracted with EtOAc (2 × 30 mL), and the combined organics were dried over K_2CO_3, filtered, and concentrated under reduced pressure to afford a white, foamy solid. This was dissolved in 2 mL of CH_2Cl_2, and 2 M HCl in diethyl ether was added in excess, resulting in a white precipitate. The mixture was stored in the refrigerator for 4 h and filtered with suction, and the solid was washed with diethyl ether, turning into a yellowish oil. This oil was dissolved in warm H_2O, collected in a flask, and concentrated under reduced pressure to afford 59 mg (0.094 mmol, 37%) of product as a yellowish oil. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.96 (d, J = 1.8 Hz, 1H), 8.44 (dd, J = 4.9, 0.7 Hz, 2H), 8.25 (dd, J = 8.2, 2.3 Hz, 1H), 7.83−7.75 (m, 3H), 7.67 (d, J = 7.8 Hz, 2H), 7.61 (d, J = 8.5 Hz, 2H), 7.30−7.25 (m, 2H), 7.22 (d, J = 8.4 Hz, 2H), 4.30 (q, J = 7.1 Hz, 1H), 3.93 (s, 2H), 3.88 (s, 4H), 3.53−3.36 (m, 3H), 2.78 (t, J = 7.1 Hz, 2H), 1.30 (d, J = 7.1 Hz, 3H), 1.27 (d, J = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, methanol-d_4) δ 177.7, 174.9, 166.3, 163.5, 159.9, 149.6, 148.9, 138.7, 137.9, 137.5, 137.0, 131.0, 130.3, 124.9, 124.3, 123.9, 122.3, 61.2, 60.9, 51.4, 50.3, 41.9, 35.9, 21.2, 18.5. APCI-HRMS m/z calcd for $C_{33}H_{39}N_8O_3$ ($[M + H]^+$ free amine), 595.3140; found, 595.3135.

*Methyl 2-(4-(6-((Bis(pyridin-2-ylmethyl)amino)methyl) nicotinamido)phenyl)acetate (**16-OMe**).* 6-((Bis(pyridin-2-ylmethyl)amino)methyl)nicotinic acid (**4**, 976 mg, 2.92 mmol, 1.0 equiv) was dissolved in 20 mL of dry DMF and cooled to 0 °C in an ice bath. Methyl 2-(4-aminophenyl)acetate hydrochloride (883 mg, 4.38 mmol, 1.5 equiv), EDCI (839 mg, 4.38 mmol, 1.5 equiv), and HOAt (596 mg, 4.38 mmol, 1.5 equiv) were added, followed by NMM (740 μL, 6.71 mmol, 2.3 equiv) dropwise over a period of 30 min at 0 °C. The mixture was allowed to warm to room temperature, stirred for 16 h, and concentrated under reduced pressure. The residue was dissolved in 100 mL of $CHCl_3$ and transferred to a separation funnel. The organic phase was washed with a mixture of saturated aqueous K_2CO_3 solution and H_2O (50 mL each) followed by brine (50 mL). The organic phase was separated, dried over Na_2SO_4, filtered, and concentrated under reduced pressure, and the product was purified by dry column vacuum chromatography on C18 material using stepwise elution from 10 to 90% methanol in water, affording 724 mg (1.5 mmol, 51%) of product as a yellow oil. ^{1}H NMR (400 MHz, methanol-d_4) δ 9.01 (s, 1H), 8.56 (d, J = 4.6 Hz, 2H), 8.24 (d, J = 8.1 Hz, 1H), 7.89 (t, J = 7.7 Hz, 2H), 7.62 (t, J = 8.6 Hz, 5H), 7.42−7.35 (m, 2H), 7.26 (d, J = 8.0 Hz, 2H), 4.14 (s, 6H), 3.67 (s, 3H), 3.63 (s, 2H). ^{13}C NMR (101 MHz, methanol-d_4) δ 173.9, 166.1, 161.5, 157.7, 157.7, 149.2, 148.6, 140.2, 138.5, 137.7, 132.1, 131.3, 130.8, 125.5, 124.7, 124.5, 122.2, 60.5, 60.2, 60.1, 52.5, 41.1. APCI-HRMS m/z calcd for $C_{28}H_{28}N_3O_3$ $[M + H]^+$, 482.2187; found, 482.2184.

*2-(4-(6-((Bis(pyridin-2-ylmethyl)amino)methyl)-nicotinamido) phenyl)acetic Acid (**16**).* Methyl 2-(4-(6-((bis(pyridin-2-ylmethyl)amino)methyl)nicotinamido)-phenyl)acetate (**16-OMe**, 288 mg, 0.59 mmol, 1.0 equiv) was dissolved in 10 mL of THF and cooled to 0 °C in an ice bath. A solution of LiOH hydrate (49.5 mg, 1.18 mmol, 2.0 equiv) in 10 mL of distilled H_2O was added, and the solution was stirred at 0 °C until TLC (alumina, 5% MeOH in CH_2Cl_2) indicated full conversion. The THF was removed under reduced pressure, and the residual aqueous solution was adjusted to pH 6 using 4 N HCl. The solvent was removed under reduced pressure, affording the product in quantitative yield, which was used in the next step without further purification.

*(R)-Methyl-2-((R)-2-(2-(4-(6-((bis(pyridin-2-ylmethyl)-amino)methyl)nicotinamido)phenyl)acetamido)-propanamido)propanoate (**17**).* 2-(4-(6-((Bis(pyridin-2-ylmethyl)amino)methyl)nicotinamido)phenyl)acetic acid prepared in the previous reaction (**16**, 94 mg, 0.202 mmol, 1.0 equiv) was dissolved in 3 mL of dry DMF at room temperature. D-Ala-D-Ala-OMe hydrochloride (44.6 mg, 0.212 mmol, 1 equiv) was then added, and the mixture was cooled to 0 °C in an ice bath. HATU (80.6 mg, 0.212 mmol, 1.05 equiv) and NMM (91 μL, 0.828 mmol, 4.2 equiv) were added, and the mixture was stirred at 0 °C for 30 min and then at room temperature for 16 h. The mixture was concentrated under reduced pressure, and the product was purified by dry column vacuum chromatography on C18 material using stepwise elution from 10 to 90% methanol in water, affording 97 mg (0.155 mmol, 77%) of product. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.44 (d, J = 4.8 Hz, 2H), 8.25 (dd, J = 8.2, 2.2 Hz, 1H), 7.79 (ddd, J = 8.4, 5.9, 2.0 Hz, 3H), 7.67 (d, J = 7.9 Hz, 2H), 7.64 (d, J = 8.5 Hz, 2H), 7.33−7.24 (m, 4H), 4.38 (qd, J = 7.2, 2.7 Hz, 2H), 3.94 (s, 2H), 3.89 (s, 4H), 3.70 (s, 3H), 3.54 (s, 2H), 1.36 (s, 3H), 1.35 (d, J = 0.8 Hz, 3H. ^{13}C NMR (101 MHz, methanol-d_4) δ 174.8, 174.5, 173.7, 166.3, 163.5, 159.9, 149.6, 149.0, 138.7, 138.4, 137.5, 133.3, 131.0, 130.6, 125.0, 124.3, 123.9, 122.3, 61.2, 61.0, 52.7, 50.2, 49.1 (from DEPT), 42.9, 18.1, 17.3. APCI-HRMS m/z calcd for $C_{34}H_{38}N_7O_5$ $[M + H]^+$, 624.2929; found, 624.2927.

*tert-Butyl (6-(6-((Bis(pyridin-2-ylmethyl)amino)methyl)-nicotinamido)hexyl)carbamate (**18-Boc**).* 6-((Bis(pyridin-2-ylmethyl)amino)methyl)nicotinic acid (**4**, 772 mg, 1.45 mmol, 1.0 equiv) was dissolved in 6.0 mL of dry DMF and cooled to 0 °C in an ice-water bath. *tert*-Butyl-6-aminohexylcarbamate (313.7 mg, 1.45 mmol, 1.0 equiv) and HATU (551.5 mg, 1.45 mmol, 1.0 equiv) were added before NMM (318 μL, 2.9 mmol, 2 equiv) was added to the stirring mixture. The mixture was

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stirred in an ice-water bath for 15 min before slowly warming to room temperature and was left to stir overnight. The mixture was diluted with 0.5 M K_2CO_3 (30 mL) and extracted with EtOAc (3 × 20 mL). The combined extracts were washed with 0.5 M K_2CO_3 (3 × 20 mL), dried over K_2CO_3, filtered, and concentrated under reduced pressure. The orange oily residue was dissolved in CH_2Cl_2 and purified by column chromatography on neutral alumina (1−2% MeOH in CH_2Cl_2) to afford 724 mg (94%) of the title compound as an orange oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (d, J = 2.7 Hz, 1H), 8.51 (dd, J = 4.9, 1.8 Hz, 2H), 8.10 (dd, J = 8.1, 2.4 Hz, 1H), 7.64 (m, 3H), 7.53 (d, J = 8.0 Hz, 2H), 7.13 (dd, J = 7.5, 4.9 Hz, 2H), 6.72 (broad s, 1H), 4.61 (broad s, 1H), 3.90 (s, 2H), 3.85 (s, 4H), 3.42 (td, J = 6.9, 5.1 Hz, 2H), 3.11 (m, 2H), 1.60 (pentet, J = 6.1 Hz, 2H), 1.51−1.21 (m, 15H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7, 165.7, 159.2, 156.3, 149.2, 147.5, 136.6, 135.7, 128.94, 128.91, 123.1, 122.7, 122.2, 79.2, 60.3, 60.0, 40.0, 39.9, 39.5, 39.4, 30.2, 29.4, 28.5, 26.8, 26.7, 26.0, 25.8. HRMS (ESI) calcd for $C_{30}H_{41}N_6O_3$ $[M + H]^+$, 533.3235; found, 533.3235.

*N-(6-Aminohexyl)-6-((bis(pyridin-2-ylmethyl)amino)-methyl)nicotinamide (**18**).* The N-Boc-protected amine (**18-Boc**, 693 mg, 1.3 mmol, 1.0 equiv) was dissolved in 5.0 mL of CH_2Cl_2 and cooled to 0 °C in an ice-water bath. Trifluoroacetic acid (5.9 mL, 78 mmol, 60.0 equiv) in 6.0 mL of CH_2Cl_2 was then slowly added to the stirring mixture. The reaction was left at 0 °C for 20 min before warming 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 the 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 mL). The combined organic layers were washed with fresh 0.5 M K_2CO_3 (3 × 50 mL), dried on K_2CO_3 and filtered, and the solvent was removed under reduced pressure to give 410 mg (72%) of the title compound as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.93 (d, J = 2.3 Hz, 1H), 8.51 (m, 2H), 8.10 (dd, J = 8.1, 2.3 Hz, 1H), 7.67−7.58 (m, 3H), 7.50 (dt, J = 7.9, 1.1 Hz, 2H), 7.14 (ddd, J = 7.5, 4.9, 1.2 Hz, 2H), 6.72 (t, J = 5.6 Hz, 1H), 3.89 (s, 2H), 3.85 (s, 4H), 3.44 (td, J = 7.0, 5.7 Hz, 2H), 2.73 (t, J = 6.9 Hz, 2H), 2.46 (broad s, 2H), 1.61 (m, 2H), 1.48 (m, 2H), 1.42−1.35 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165. 8, 162.4, 159.0, 149.3, 147.4, 136.7, 135.9, 129.1, 123.3, 122.9, 122.3, 60.3, 60.0, 41.8, 40.0, 32.9, 29.5, 26.7, 26. HRMS (ESI) calcd for $C_{25}H_{33}N_6O$ $[M + H]^+$, 433.2710; found, 433.2710.

*tert-Butyl ((R)-1-(((R)-1-((6-(6-((Bis(pyridin-2-ylmethyl)-amino)methyl)nicotinamido)hexyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (**19-Boc**).* Free amine **18** (389 mg, 0.9 mmol, 1.0 equiv) was dissolved in 4 mL of dry DMF and cooled to 0 °C in an ice-water bath. Boc-D-Ala-D-Ala-OH (234 mg, 0.9 mmol, 1.0 equiv) and HATU (342.4 mg, 0.9 mmol, 1.0 equiv) were added before NMM (198 μL, 1.8 mmol, 2.0 equiv) was added to the stirring mixture. The mixture was stirred in the ice-water bath for 15 min before slowly warming to room temperature and was left to stir overnight. The mixture was diluted with 0.5 M K_2CO_3 (30 mL) and extracted with EtOAc (3 × 20 mL). The combined extracts were washed with 0.5 M K_2CO_3 (3 × 20 mL), dried over K_2CO_3, filtered, and concentrated under reduced pressure. The orange oily residues can undergo further purification by either column chromatography on neutral alumina (1−2% MeOH in CH_2Cl_2) or C18 reverse-phase chromatography (20−75% MeOH in water). Yield (436 mg, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.95 (d, J = 2.4 Hz, 1H), 8.51 (dd, J = 4.9, 1.8 Hz, 2H), 8.14 (dd, J = 8.2, 2.3 Hz, 1H), 7.68−7.61 (m, 3H), 7.53 (dt, J = 7.8, 1.1 Hz, 2H), 7.14 (ddd, J = 7.5, 4.9, 1.2 Hz, 2H), 7.02 (broad s, 1H), 6.82−6.67 (m, 2H), 5.23 (broad s, 1H), 4.41 (pentet, J = 7.2 Hz, 1H), 4.08 (m, 1H), 3.91 (s, 2H), 3.86 (s, 4H), 3.51−3.36 (m, 2H), 3.30 (m, 1H), 3.15 (m, 1H), 2.13 (broad s, 2H), 1.66−1.55 (m, 2H), 1.55−1.46 (m, 2H), 1.41 (s, 9H), 1.38−1.29 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8, 172.2, 165.9, 162.4, 159.2, 156.1, 149.3, 147.6, 136.6, 135.9, 129.1, 123.1, 122.8, 122.3, 80.8, 60.3, 60.0, 51.1, 49.2, 39.4, 39.0, 29.2, 28.4, 25.8, 25.6, 18.1. HRMS (ESI) calcd for $C_{36}H_{51}N_8O_5$ $[M + H]^+$, 675.3977; found, 675.3977.

*N-(6-((R)-2-((R)-2-Aminopropanamido)propanamido)-hexyl)-6-((bis(pyridin-2-ylmethyl)amino)methyl)-nicotinamide (**19**).* N-Boc-protected amine **19-Boc** (412 mg, 0.61 mmol, 1.0 equiv) was dissolved in 3.0 mL of CH_2Cl_2 and cooled to 0 °C in an ice-water bath. Trifluoroacetic acid (2.8 mL, 36.6 mmol, 60.0 equiv) in 3.0 mL of CH_2Cl_2 was then slowly added to the stirring mixture. The reaction was left at 0 °C for 20 min before warming 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 the 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 mL). The combined organic layers were washed with fresh 0.5 M K_2CO_3 (3 × 50 mL), dried on K_2CO_3, and filtered, and the solvent was removed under reduced pressure to give 280 mg (79%) of the title compound as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.95 (d, J = 2.3 Hz, 1H), 8.51 (dd, J = 4.9, 1.8 Hz, 2H), 8.14 (dd, J = 8.1, 2.3 Hz, 1H), 7.78 (d, J = 7.7 Hz, 1H), 7.68−7.59 (m, 3H), 7.52 (dt, J = 7.9, 1.1 Hz, 2H), 7.13 (ddd, J = 7.5, 4.9, 1.2 Hz, 2H), 7.05 (t, J = 5.9 Hz, 1H), 6.72 (t, J = 6.0 Hz, 1H), 4.38 (pentet, J = 7.1 Hz, 1H), 3.90 (s, 2H), 3.85 (s, 4H), 3.50−3.32 (m, 3H), 3.22 (m, 2H), 2.27 (broad s, 2H), 1.64−1.42 (m, 4H), 1.42−1.22 (m, 4H), 1.35 (d, J = 7.0 Hz, 3H), 1.29 (d, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.2, 172.5, 165.8, 162.5, 159.1, 149.3, 147.6, 136.6, 135.9, 129.0, 123.1, 122.7, 122.2, 60.3, 60.0, 50.7, 48.7, 39.4, 38.8, 29.4, 29.7, 25.8, 25.6, 21.7, 17.9. HRMS (ESI) calcd for $C_{31}H_{43}N_8O_3$ $[M + H]^+$, 575.3453; found, 575.3453.

*tert-Butyl (2-(1-(6-((Bis(pyridin-2-ylmethyl)amino)-methyl)nicotinoyl)piperidin-4-yl)ethyl)carbamate (**20-Boc**).* 6-((Bis(pyridin-2-ylmethyl)amino)methyl)nicotinic acid (**4**, 485 mg, 1.45 mmol, 1.0 equiv) was dissolved in 6.0 mL of dry DMF and cooled to 0 °C in an ice-water bath. *tert*-Butyl 2-(piperidin-4-yl)ethylcarbamate (331.1 mg, 1.45 mmol, 1.0 equiv) and HATU (551.5 mg, 1.45 mmol, 1.0 equiv) were added before NMM (318 μL, 2.9 mmol, 2.0 equiv) was added to the stirring mixture. The mixture was stirred in the ice-water bath for 15 min before slowly warming to room temperature and was left to stir overnight. The mixture was diluted with 0.5 M K_2CO_3 (30 mL) and extracted with EtOAc (3 × 20 mL). The combined extracts were washed with 0.5 M K_2CO_3 (3 × 20 mL), dried over K_2CO_3, filtered, and concentrated under reduced pressure. The orange oily residue was dissolved in CH_2Cl_2 and purified by column chromatography on neutral alumina (1−2% MeOH in CH_2Cl_2) to afford 505 mg (64%) of the title compound as an orange oil. The title compound was isolated as a mixture of rotamers (conformational isomers). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59−8.55 (m, 3H), 7.73−7.66 (m, 3H), 7.59 (d, J = 8.0 Hz, 1H), 7.51 (d, J = 8.0 Hz, 2H), 7.20 (ddd, J = 7.7, 5.1, 1.3 Hz, 2H), 4.78−4.44 (m, 2H), 4.07 (m, 6H), 3.77 (m,

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1H), 3.15 (q, J = 6.8 Hz, 2H), 3.00 (ddd, J = 13.4, 12.4, 2.8 Hz, 1H), 2.51 (td, J = 12.9, 2.9 Hz, 1H), 1.82−1.64 (m, 2H), 1.64−1.38 (m, 12H), 1.19−1.00 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 168.9, 167.6, 162.6, 156.1, 149.2, 147.4, 137.2, 135.8, 123.3, 122.9, 122.8, 79.4, 60.5, 59.6, 46.7, 41.8, 38.1, 36.8, 33.7, 32.7, 31.9, 28.5. HRMS (ESI) calcd for $C_{31}H_{41}N_6O_3$ $[M + H]^+$, 545.3235; found, 545.3235.

*(4-(2-Aminoethyl)piperidin-1-yl)(6-((bis(pyridin-2-ylmethyl)amino)methyl)pyridin-3-yl)methanone (**20**).* N-Boc-protected amine **20-Boc** (463 mg, 0.85 mmol, 1.0 equiv) was dissolved in 4.0 mL of CH_2Cl_2 and cooled to 0 °C in an ice-water bath. Trifluoroacetic acid (3.9 mL, 51 mmol, 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 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 the 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 mL). The combined organic layers were washed with fresh 0.5 M K_2CO_3 (3 × 50 mL), dried on K_2CO_3, and filtered, and the solvent was removed under reduced pressure to give 151 mg (40%) of the title compound as a brown oil. The title compound was isolated as a mixture of rotamers (conformational isomers). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56−8.50 (m, 3H), 7.73−7.61 (m, 4H), 7.54 (dt, J = 7.9, 1.2 Hz, 2H), 7.14 (ddd, J = 7.5, 4.9, 1.3 Hz, 2H), 4.68 (m, 1H), 3.90 (s, 2H), 3.88 (s, 4H), 3.69 (m, 1H), 3.01 (m, 1H), 2.74 (m, 2H), 1.80 (m, 1H), 1.73−1.59 (m, 2H), 1.48−1.05 (m, 7H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.9, 160.9, 159.2, 149.3, 147.2, 136.6, 135.6, 130.5, 123.1, 122.7, 122.2, 60.4, 60.0, 48.3, 42.7, 40.3, 39.4, 33.7, 33.1, 32.0. HRMS (ESI) calcd for $C_{26}H_{33}N_6O$ $[M + H]^+$, 445.2710; found, 445.2710.

*tert-Butyl ((R)-1-(((R)-1-((2-(1-(6-((bis(pyridin-2-ylmethyl)amino)methyl)nicotinoyl)piperidin-4-yl)ethyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (**21-Boc**).* Free amine **20** (133 mg, 0.3 mmol, 1.0 equiv) was dissolved in DMF (0.25M) and cooled to 0 °C in an ice-water bath. Boc-D-Ala-D-Ala-OH (78 mg, 0.3 mmol, 1.0 equiv) and HATU (114 mg, 0.3 mmol, 1.0 equiv) were added before NMM (132 μL, 0.6 mmol, 2.0 equiv) was added to the stirring mixture. The mixture was stirred in the ice-water bath for 15 min before slowly warming to room temperature and was left to stir overnight. The mixture was diluted with 0.5 M K_2CO_3 (30 mL) and extracted with EtOAc (3 × 20 mL). The combined extracts were washed with 0.5 M K_2CO_3 (3 × 20 mL), dried over K_2CO_3, filtered, and concentrated under reduced pressure. The orange oily residues can undergo further purification by either column chromatography on neutral alumina (1−2% MeOH in CH_2Cl_2) or C18 reverse-phase chromatography (20−75% MeOH in water). Yield (183 mg, 89%). The title compound was isolated as a mixture of rotamers (conformational isomers). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56−8.48 (m, 3H), 7.71−7.59 (m, 4H), 7.54 (d, J = 7.8 Hz, 2H), 7.17−7.10 (m, 2H), 6.66 (s, 1H), 6.58 (d, J = 7.4 Hz, 1H), 5.02 (s, 1H), 4.66 (m, 1H), 4.40 (pentet, J = 7.2 Hz, 1H), 3.91 (s, 2H), 3.89 (s, 4H), 3.67 (m, 1H), 3.32 (m, 1H), 3.18 (m, 1H), 3.00 (m, 1H), 2.76 (m, 1H), 1.89−1.45 (m, 6H), 1.43 (s, 9H), 1.37 (d, J = 2.7 Hz, 3H), 1.35 (d, J = 2.8 Hz, 3H), 1.17−1.03 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 172.0, 171.3, 167.8, 160.9, 159.1, 149.2, 147.3, 136.6, 135.6, 130.5, 123.1, 122.7, 122.2, 80.9, 60.4, 59.9, 51.2, 49.01, 48.2, 42.7, 37.0, 35.9, 33.6, 32.9, 31.7, 28.4, 21.2, 17.9. HRMS (ESI) calcd for $C_{37}H_{51}N_8O_5$ $[M + H]^+$, 687.3977; found, 687.3977.

*(R)-2-Amino-N-((R)-1-((2-(1-(6-((bis(pyridin-2-ylmethyl)amino)methyl)nicotinoyl)piperidin-4-yl)ethyl)amino)-1-oxopropan-2-yl)propanamide (**21**).* N-Boc-protected amine **21-Boc** (186 mg, 0.27 mmol, 1.0 equiv) was dissolved in 1.0 mL of CH_2Cl_2 and cooled to 0 °C in an ice-water bath. Trifluoroacetic acid (1.24 mL, 16.2 mmol, 60.0 equiv) in 1 mL of CH_2Cl_2 was then slowly added to the stirring mixture. The reaction was left at 0 °C for 20 min before warming 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 the 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 mL). The combined organic layers were washed with fresh 0.5 M K_2CO_3 (3 × 50 mL), dried on K_2CO_3, and filtered, and the solvent was removed under reduced pressure to give 97 mg (62%) of the title compound as a brown oil. The title compound was isolated as a mixture of rotamers (conformational isomers). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57−8.47 (m, 3H), 7.73 (d, J = 7.8 Hz, 1H), 7.71−7.60 (m, 4H), 7.53 (d, J = 7.9 Hz, 2H), 7.16−7.09 (m, 2H), 6.62 (m, 1H), 4.65 (m, 1H), 4.36 (pentet, J = 7.1 Hz, 1H), 3.89 (s, 2H), 3.87 (s, 4H), 3.69 (m, 1H), 3.46 (q, J = 7.0 Hz, 1H), 3.33−3.16 (m, 2H), 2.99 (m, 1H), 2.74 (m, 1H), 2.27−2.06 (m, 2H), 1.89−1.38 (m, 5H), 1.35 (d, J = 7.0, 0.9 Hz, 3H), 1.32 (d, J = 7.0, 1.0 Hz, 3H), 1.25−1.06 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.2, 172.2, 167.9, 161.0, 159.2, 149.3, 147.2, 136.6, 135.6, 130.4, 123.1, 122.7, 122.2, 60.4, 60.0, 50.6, 48.6, 48.2, 42.6, 37.0, 36.0, 33.9, 32.9, 31.8, 21.7, 17.6. HRMS (ESI) calcd for $C_{32}H_{43}N_8O_3$ $[M + H]^+$, 587.3453; found, 587.3453.

*tert-Butyl (R)-1-((R)-1-(4-(6-((Bis(pyridin-2-ylmethyl)amino)methyl)nicotinoyl)piperazin-1-yl)-1-oxopropan-2-ylamino)-1-oxopropan-2-ylcarbamate (**22-Boc**).* (6-((Bis(pyridin-2-ylmethyl)amino)methyl)pyridin-3-yl)(piperazin-1-yl)methanone (**12**, 80 mg, 0.197 mmol, 1.0 equiv) was dissolved in 2 mL of dry DMF at room temperature. Boc-D-Ala-D-Ala-OH (57 mg, 0.2175 mmol, 1.1 equiv), EDCl (42 mg, 0.2175 mmol, 1.1 equiv), HOAt (30 mg, 0.2175 mmol, 1.1 equiv), and NMM (24 μL, 0.2175 mmol, 1.1 equiv) were added, and the mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure, and the purification of the product was achieved by way of dry column vacuum chromatography on C18 material using a stepwise elution from 10 to 90% methanol in water, affording 95 mg (0.147 mmol, 75%) of product. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.53 (d, J = 1.5 Hz, 1H), 8.44 (dd, J = 4.9, 0.8 Hz, 2H), 7.87−7.76 (m, 3H), 7.73 (d, J = 8.0 Hz, 1H), 7.68 (d, J = 7.8 Hz, 2H), 7.33−7.23 (m, 2H), 4.06 (d, J = 6.4 Hz, 1H), 3.91 (s, 2H), 3.90 (s, 4H), 3.86−3.37 (m, J = 45.0 Hz, 8H), 1.43 (s, 9H), 1.35−1.25 (m, 6H). ^{13}C NMR (101 MHz, methanol-d_4) δ 175.2, 172.9, 169.8, 162.3, 160.0, 157.6, 149.6, 148.2, 138.7, 137.2, 131.3, 125.0, 124.4, 123.9, 80.6, 61.4, 61.1, 51.5, 46.4, 28.7, 18.2, 17.9. APCI-HRMS m/z calcd for $C_{34}H_{45}N_8O_5$ $[M + H]^+$, 645.3507; found, 645.3506.

*(R)-2-Amino-N-((R)-1-(4-(6-((bis(pyridin-2-ylmethyl)amino)methyl)nicotinoyl)piperazin-1-yl)-1-oxopropan-2-yl)propanamide (**22**).* The *tert*-butyl (*R*)-1-((*R*)-1-(4-(6-((bis(pyridin-2-ylmethyl)amino)methyl)nicotinoyl)piperazin-1-yl)-1-oxopropan-2-ylamino)-1-oxopropan-2-ylcarbamate prepared in the previous reaction (**22-Boc**, 78 mg, 0.12 mmol, 1.0 equiv) was dissolved in 5 mL of CH_2Cl_2 and cooled in an ice bath to 0 °C. To this solution was added TFA (0.739 mL, 80 equiv) in 2 mL of CH_2Cl_2 dropwise, and the mixture was stirred at room

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temperature for 16 h. The mixture was then concentrated under reduced pressure, the residue was dissolved in distilled H_2O, the pH was adjusted to 8 with saturated aqueous K_2CO_3 solution, and the mixture was concentrated under reduced pressure. Purification of the product was achieved by way of dry column vacuum chromatography on C18 material using stepwise elution from 10 to 90% methanol in water, affording 48 mg (0.088 mmol, 74%) of product. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.54 (s, 1H), 8.44 (d, J = 4.7 Hz, 2H), 7.85 (d, J = 8.0 Hz, 1H), 7.79 (t, J = 7.7 Hz, 2H), 7.74 (d, J = 8.0 Hz, 1H), 7.67 (d, J = 7.8 Hz, 2H), 7.28 (t, J = 6.0 Hz, 2H), 3.91 (s, 2H), 3.90 (s, 4H), 3.86−3.39 (m, 9H), 1.33 (d, J = 6.3 Hz, 3H), 1.28 (d, J = 6.7 Hz, 3H). ^{13}C NMR (101 MHz, methanol-d_4) δ 177.6, 173.2, 169.8, 162.3, 160.0, 149.6, 148.2, 138.7, 137.2, 131.3, 125.0, 124.4, 123.9, 61.4, 61.1, 51.3, 46.4, 21.3, 17.9. APCI-HRMS m/z calcd for $C_{29}H_{37}N_8O_3$ $[M + H]^+$, 545.2983; found, 545.2981.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsinfecdis.8b00137.

^{1}H and ^{13}C spectra, NMR titration experimental details for compound **15**, HepG2 toxicity curves, and in vivo toxicity data (PDF)

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Notes

The authors declare the following competing financial interest(s): OAHA, ØS, PR, AB, HKSL, GKA and CS have filed a patent on these results.

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