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Dimeric Self-Assembly of Pyridyl Guanidinium Carboxylates in Polar Solvents

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Abstract: A series of pyridyl guanidinium–carboxylates has been prepared and the dimeric self-assembly of these studied in H_2O/DMSO mixtures, principally using dilution isothermal calorimetry. Compounds **5** and **6**, incorporating an aromatic ring in the "tethering" region between the guanidinium and carboxylate groups, demonstrate the strongest dimerisation in neat DMSO. X-ray crystal structures of **5** and **6** reveal two different dimerisation architectures in the solid-state, but both involve carboxylate–guanidinium salt bridges as anticipated, and π–π interactions. Compounds **10–16** incorporating peptidic fragments between the guanidinium and carboxylate groups, showed reduced dimerisation strength with increased amino acid content, but also sustained dimerisation under increasingly aqueous conditions, up to 50% H_2O/DMSO in the case of **14** and **15**. The extent of our study in H_2O/DMSO mixtures was determined by substrate solubility of **10–16**, and not the limit of self-assembly.

Keywords: calorimetry · dimerization · molecular recognition · self-assembly · supramolecular chemistry

Introduction

Guanidinium salts have been extensively employed as motifs for carboxylate binding[1,2] and, in particular, are often incorporated within synthetic receptors for amino acid and peptide recognition.[3] Since ion-pair stability relies upon polar interactions, competing solvation serves to weaken binding in water and synthetic receptors for binding carboxylate in aqueous media typically employ further, cooperative binding sites for the anion, and often complex architecture (e.g., macrocyclic) for pre-organisation of binding site directionality. We recently described[2e] a structurally simple pyridylguanidinium receptor, incorporating two additional amide hydrogen bond donors, and in which the binding conformation can be preorganised as a result of two intramolecular hydrogen bonds between the pyridine lone pair and adjacent guanidinium/amide NHs.[4] Receptor preorganisation resulted in entropy-driven binding of acetate, even in polar media (Figure 1a, $K_a = 3900\,M^{-1}$ in 10% H_2O/DMSO and $480\,M^{-1}$ in 30% H_2O/DMSO) and compares favourably with other carboxylate receptors.[2h,3e]

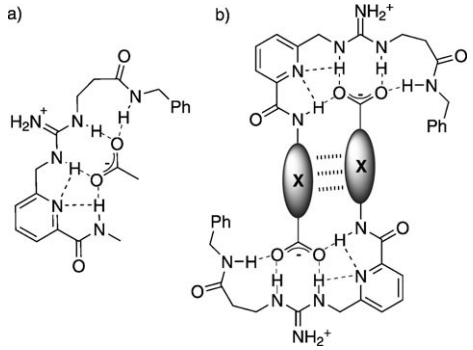

Figure 1. a) Binding of acetate (nBu_4N^+ salt) by a preorganised pyridyl guanidinium receptor (PF_6^- salt) $K_a^{1:1} = 22000\,M^{-1}$ ($\Delta G = -24.8\,kJ\,mol^{-1}$, $\Delta H = -8.0\,kJ\,mol^{-1}$, $T\Delta S = 16.8\,kJ\,mol^{-1}$) in DMSO; $3900\,M^{-1}$ ($\Delta G = -20.5\,kJ\,mol^{-1}$, $\Delta H = -7.4\,kJ\,mol^{-1}$, $T\Delta S = 13.1\,kJ\,mol^{-1}$) in 10% H_2O/DMSO; and $480\,M^{-1}$ ($\Delta G = -15.3\,kJ\,mol^{-1}$, $\Delta H = -2.0\,kJ\,mol^{-1}$, $T\Delta S = 13.3\,kJ\,mol^{-1}$) in 30% H_2O/DMSO;[2e] b) proposed (schematised) dimerisation of a pyridyl guanidinium–carboxylate for quantification of intermolecular side chain interactions (···).

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201001861: Dilution ITC data for **5**, **6**, **8**, **10**, **11** and **13–16**, ^1H NMR dilution data for **14** and ^{13}C NMR spectra for compounds **3**, **4**, and **6–16**.

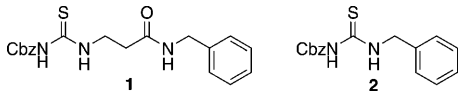
The development of well-defined, self-assembled systems is an important goal with potential for creating functional supramolecular systems, and the use of guanidinium–carboxylate binding to drive self-assembly is exemplified by Schmuck and co-workers' recent studies of polymer and vesicle formation by self-assembling (zwitterionic) guanidinium–carboxylates.[5] In order to develop functional application of the efficient host–guest association depicted in Figure 1a, we have prepared a series of pyridyl guanidinium receptors, extended in structure to incorporate a tethered carboxylate group. The success of the simple guanidinium–carboxylate motif, Figure 1a, in competitive polar solvents (H_2O/DMSO) suggests that the proposed dimerisation of pyridyl guanidinium–carboxylates, Figure 1b, could be strengthened by (designed) complementary, intermolecular, non-covalent interactions between adjacent tethering strands (**X**), and would enable us to develop a simple supramolecular assembly driven primarily by the guanidinium–carboxylate binding, in which the energetic contribution of specific intermolecular binding interactions (**X**–**X**) can be determined, and ultimately quantified in aqueous solvents.[6]

In particular, the detailed characterisation of amino acid side-chain–side-chain interactions is essential for understanding the factors controlling stability of protein secondary structural features (e.g., α-helices, β-sheets),[7] and for allowing prediction of protein folding from knowledge of the primary sequence alone, which remains a significant challenge.[8] Additionally, intermolecular β-sheet interactions are important for control of protein quaternary structure, and are, for example, also involved in the protein aggregation implicated in neurodegenerative disease states.[9] Synthetic β-sheet mimics have been used to explore inter-strand recognition and sequence selectivity, largely through NMR studies,[10] however, quantification of inter-stand interactions remains key for the development of accurate models of molecular recognition events in formation of secondary β-structure.[11] Herein we describe a proof-of-concept study of the self-assembly of pyridyl guanidinium–carboxylates, of the general structure shown in Figure 1b, using dilution microcalorimetry to establish thermodynamic parameters of dimer dissociation. The strength of dimerisation provides a measure of intermolecular interactions between the variable component of the system (**X**), antiparallel alkyl, aryl or peptide strands, relative to each other within the context of the model complex.[12]

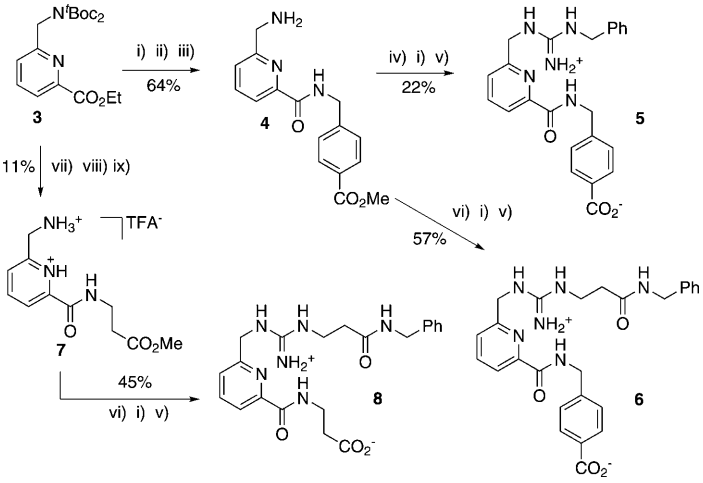
Results and Discussion

Synthesis: We chose to prepare pyridyl guanidinium–carboxylates via two-directional functionalisation of a 6-aminomethyl-2-carboxylate-substituted core pyridine. Partial reduction of diethyl pyridine-2,6-dicarboxylate, followed by mesylation and nucleophilic substitution with *tert*-butyl iminodicarboxylate, furnished this central motif, **3**.[13]

Saponification of **3** was somewhat low yielding, under various conditions, and Me_3SiOK-mediated hydrolysis proved optimal, giving the corresponding acid in reasonable yield (70%). Amide coupling with methyl 4-(aminomethyl)benzoate, followed by *N*-deprotection, then furnished **4**, carbodiimide-mediated coupling of which with carbamoyl-activiated thiourea **1** or **2** (see below), installed Cbz-protected



guanidine functionality.[14] Lastly, ester hydrolysis and Cbz-removal gave guanidinium–carboxylates **5** and **6** (Scheme 1), each incorporating aromatic tethers between the carboxylate

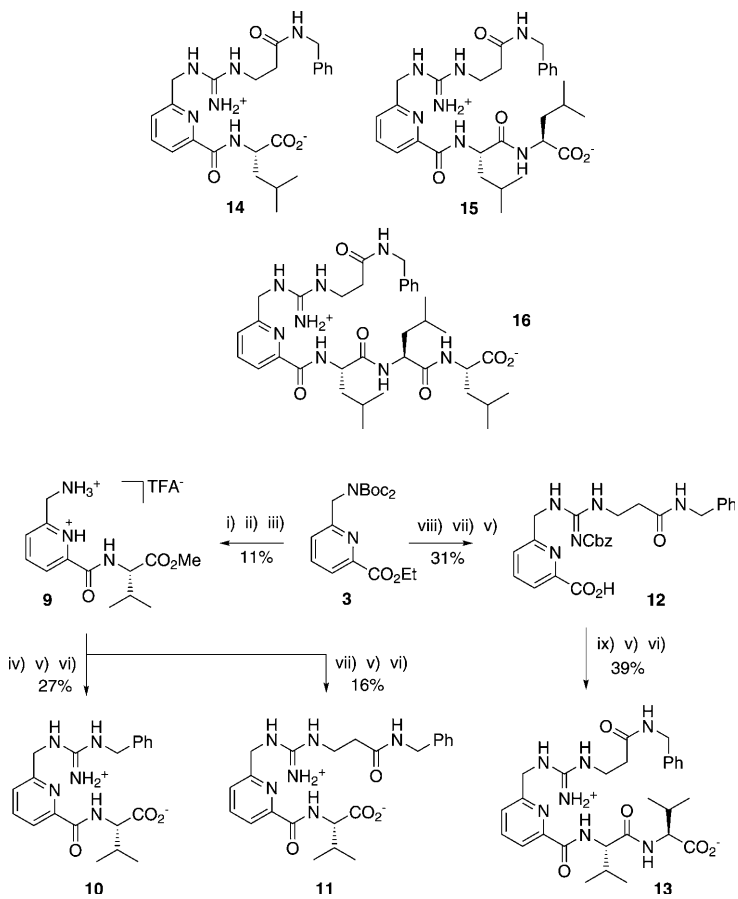


Scheme 1. Synthesis of β-alanine-, and 4-(aminomethyl)benzoate-derived pyridyl guanidinium–carboxylates. i) Me_3SiOK, THF, RT, 3–24 h; ii) methyl 4-(aminomethyl)benzoate hydrochloride, *i*Pr_2EtN, PyBOP, CH_2Cl_2, RT, 12 h; iii) 20% v/v CF_3CO_2H in CH_2Cl_2, RT, 2 h, then K_2CO_3 (aq); iv) **2**, Et_3N, EDC, CH_2Cl_2, RT, 24 h; v) H_2 (1 atm), 10% w/w Pd/C, CH_2Cl_2/MeOH, RT, 24 h; vi) **1**, Et_3N, EDC, CH_2Cl_2, RT, 48–72 h; vii) LiBr, Et_3N, 2% v/v H_2O in CH_3CN, RT, 24 h; viii) β-alanine methyl ester hydrochloride, *i*Pr_2EtN, EDC, HOBt, CH_2Cl_2/DMF 4:1, RT, 72 h; ix) 20% v/v CF_3CO_2H in CH_2Cl_2, RT, 5 h.

and guanidinium groups, potentially providing stabilisation of dimeric self-assembly via π-stacking interactions. An analogue, **8**, with simple ethylene tether, was prepared similarly; LiBr-mediated hydrolysis[15] of **3** was high yielding, although partial *N*-deprotection took place upon work-up to give mixed (separable) -*N*HBoc (32%) and -*N*Boc_2 (57%) products, the former of which was subsequently utilised for coupling of its free acid moiety with β-alanine methyl ester, giving **7** after *N*-deprotection. Subsequent coupling with thiourea **1**, ester hydrolysis and Cbz removal, were straightforward (Scheme 1).

In order to undertake a comparative study of dimerisation, and ultimately to evaluate the strength of specific amino acid side-chain–side-chain interactions, we used an

identical synthetic strategy to prepare pyridyl guanidinium carboxylates with a number of L-valine (**10**, **11** and **13**, Scheme 2) or L-leucine (**14**–**16**, see below)[16] residues incorporated in the tethering region where intermolecular interactions are anticipated upon self-assembly. Hence, 6-aminomethyl-2-carboxylate-substituted pyridine **3** was in each case 2-*C*-functionalised via hydrolysis followed by amide coupling, and 6-*N*-functionalised through Boc removal and amine coupling with thiourea **1** or **2**. Final ester hydrolysis and guanidine deprotection steps furnished the target structures.



Scheme 2. Synthesis of L-valine-derived pyridyl guanidinium–carboxylates. i) LiBr, Et_3N, 2% v/v H_2O in CH_3CN, RT, 24 h; ii) H-L-Val-OMe·HCl, iPr_2EtN, EDC, HOBt, CH_2Cl_2/DMF 50:1, 72 h; iii) 20% v/v CF_3CO_2H in CH_2Cl_2, RT, 5 h; iv) **2**, Et_3N, EDC, CH_2Cl_2, RT, 72 h; v) Me_3SiOK, THF, RT, 12–24 h; vi) H_2 (1 atm), 10% w/w Pd/C, MeOH, RT, 6–48 h; vii) **1**, Et_3N, EDC, CH_2Cl_2, RT, 48–72 h; viii) 20% v/v CF_3CO_2H in CH_2Cl_2, RT, 3 h, then K_2CO_3 (aq); ix) H-L-Val-L-Val-OMe, iPr_2EtN, PyBOP, CH_2Cl_2, RT, 12 h.

Dimerisation studies: The self-assembly of pyridyl guanidinium–carboxylates **5**, **6**, **8**, **10**, **11** and **13**–**16** was investigated using dilution isothermal titration calorimetry (ITC),[17–19] in which sequential injection of aliquots of a solution of one of these into a calorimeter cell initially containing only solvent, resulted in a series of endothermic heat signals attributable to oligomer dissociation, which decreased non-linearly as the concentration of injected molecule increased in the cell. In each case, the thermal profile which resulted from dilution was consistent with homodimer dissociation, and non-linear regression fitting to a monomer-dimer model enabled estimation of the dimerisation constant (K_{dim}) and enthalpy of dimerisation (ΔH_{dim}).[20] Thermodynamic parameters for dimeric self-assembly of pyridyl guanidinium–carboxylates, in dimethyl sulfoxide (DMSO) and DMSO/H_2O mixtures, are reported in Table 1, although caution should be exercised when interpreting the entropy–enthalpy balance in cases of weak dimerisation since empirical studies of weak affinity binding using ITC have shown that values of K_a (and therefore ΔG) are reliable, but accuracy of ΔH (and therefore $T\Delta S$) may be strongly dependent upon the error in sample concentration.[21]

Comparison of data for **5** and **6** indicates that dimerisation is only slightly enhanced by incorporation of the additional amide hydrogen bond donor in **6**, both in DMSO (entries 1 and 3) and 10% H_2O/DMSO (entries 2 and 4). Dimerisation of both **5** and **6** is significantly reduced by addition of only 10% (by volume) H_2O to the DMSO solution, but notably for **6** the reduction in dimerisation is due to a much reduced entropic contribution, in contrast to the simple guanidinium–acetate (host–guest) association shown in Figure 1a, which is entropically driven in H_2O/DMSO mixtures. X-ray crystal structures were obtained for both **5** and **6** (Figure 2)[22] and reveal two possible dimerisation architectures, both involving guanidinium–carboxylate salt bridges. The crystal structure for **6** is very close to that envisaged for the self-assembled dimer (Figure 1b) and involves a π–π stacking interaction[23] and a guanidinium–carboxylate interaction which involves just one of the carboxylate oxygens, participating in three hydrogen bonds [N5···O1 2.770 (3), N3···O1 3.180 (3) and N1···O1 3.084 (3) Å].[24] A bridging water molecule stabilises the dimer via two hydrogen-bonding interactions, with the other oxygen of the bound carboxylate [O1W···O2 2.734 (3) Å] and pendant amide [N6···O1W 2.884 (3) Å].[25] Whilst accepting that the solid-state structure is not a reliable indication of solution-phase structure, it is interesting to note that similar involvement of solvent bridging in the solution-phase dimerisation, when water is present, could account for an increase in enthalpic contribution, and significant reduction of entropic contribution, to dimerisation of **6** in 10% H_2O/DMSO. Compound **5** adopts a (solid state) dimer involving a "twisted" guanidinium–carboxylate ion-pair interaction, clearly energetically preferred to the interaction observed for **6** in the absence of the pendant amide, emphasising that more than one dimerisation architecture may be favoured, at least in the solid-state, dependent upon small structural changes.

Structure **8**, incorporating a short, flexible, β-alanine-derived tether, demonstrated only weak dimerisation in DMSO ($K_{dim} = 26\,M^{-1}$, entry 5), suggesting that an intramolecular guanidinium–carboxylate interaction is preferred if conformationally allowed, as in this example. Compounds **10**, **11** and **13**, incorporating L-valine residues in the tether-

Table 1. Thermodynamic data for the dimerisation of pyridyl guanidinium–carboxylates, determined from dilution ITC at 25 °C.[a]

Entry		Solvent	K_{dim} [M^{-1}]	ΔG_{dim} [kJ mol^{-1}]	$-\Delta H_{dim}$ [kJ mol^{-1}]	$T\Delta S_{dim}$ [kJ mol^{-1}]
1	**5**	DMSO	6622(±2105)	−21.8	8.8(±0.5)	13.0
2	**5**	10% H_2O/DMSO	148(±31)	−12.4	4.8(±0.2)	7.6
3	**6**	DMSO	11876(±1692)	−23.2	10.0(±0.4)	13.2
4	**6**	10% H_2O/DMSO	532(±23)	−15.6	11.5(±0.1)	4.1
5	**8**	DMSO	26(±4)	−8.1	6.4(±0.3)	1.7
6	**10**	DMSO	2409(±197)	−19.3	16.4(±0.2)	2.9
7	**10**	10% H_2O/DMSO	54(±3)	−9.8	5.6(±0.1)	4.2
8[b]	**10**	20% H_2O/DMSO	–	–	–	–
9	**11**	DMSO	400(±22)	−14.8	11.1(±0.1)	3.7
10[b]	**11**	10% H_2O/DMSO	–	–	–	–
11	**13**	DMSO	12(±2)	−6.1	7.9(±0.3)	−1.8
12[c]	**13**	20% H_2O/DMSO	–	–	–	–
13	**14**	DMSO	877(±100)	−16.8	16.1(±0.3)	0.7
14	**14**	10% H_2O/DMSO	68(±6)	−10.5	9.4(±0.2)	1.1
15	**14**	20% H_2O/DMSO	47(±10)	−9.6	3.5(±0.2)	6.1
16	**14**	50% H_2O/DMSO	55(±8)	−9.9	1.5(±0.1)	8.4
17	**15**	DMSO	38(±11)	−9.0	2.1(±0.2)	6.9
18	**15**	20% H_2O/DMSO	25(±3)	−7.9	4.4(±0.1)	3.5
19	**15**	50% H_2O/DMSO	50(±10)	−9.7	1.0(±0.1)	8.7
20	**16**	DMSO	71(±2)	−10.6	16.5(±0.1)	−5.9
21	**16**	20% H_2O/DMSO	42(±2)	−9.3	14.5(±0.2)	−5.2

[a] Calorimetric data were collected in duplicate except for entry 17, and are corrected for heat of mixing of blank solvent, carried out under identical conditions. K_{dim} and ΔH_{dim} are derived from non-linear regression analysis of calorimetric data in terms of a monomer-dimer equilibrium model.[20] ΔG_{dim} and $T\Delta S_{dim}$ were calculated using $\Delta G = -RT\ln K = \Delta H - T\Delta S$. All raw calorimetric data, and fits of corrected data to the dimer-dissociation model, are provided in the ESI. [b] No evidence of dimer dissociation was observed in the dilution ITC experiment. [c] A thermogram consistent with oligomer dissociation was obtained but could not be fitted to the dimer-dissociation model.

ing region (entries 6–12) generally formed weaker dimers in comparison with compounds **5** and **6**, in identical solvent. In the case of **10**, diminution of binding upon switch of solvent from DMSO to 10% H_2O/DMSO, is of similar magnitude to that seen for **5** and **6** ($\Delta\Delta G = \sim 7$–10 kJ mol^{-1}) and no measurable self-assembly of **10** in 20% H_2O/DMSO, or of **11** in 10% H_2O/DMSO, took place (entries 8 and 10). Dilution ITC of dipeptide derivative **13** in DMSO was very weak ($K_{dim} = 12$ M^{-1}, entry 11), and in 20% H_2O/DMSO the thermal profile obtained could not be fitted to a dimer-dissociation model. Comparison of dimerisation of **11** and **13** in DMSO (entries 9 and 11) indicates that increased amino acid content has led to reduced strength of dimerisation ($\Delta\Delta G = 8.7$ kJ mol^{-1}), and the relative dimerisation strength of L-leucine analogues **14** and **15** mirrors this (entries 13 and 17, $\Delta\Delta G = 7.8$ kJ mol^{-1}). Increasing the length of the peptide component can increase the entropic penalty associated with dimer formation, and in addition may introduce additional intramolecular interactions in the monomeric species, although introduction of a third amino acid component does not have any further effect on the overall Gibbs free energy of dimerisation (**15** vs **16**). The energetic cost of breaking these upon dimerisation would lead to lower dimerisation constants.[26] Importantly, each member of the structural series **14**–**16**, which incorporates L-leucine (1–3 residues) in the tethering region, underwent dimerisation which, although weak in the presence of water (< 100 M^{-1}), was maintained with increasing water content, up to 50% H_2O/DMSO for both **14** and **15**. Unfortunately **16** proved insoluble in solvent mixtures containing > 20% H_2O. The sustained dimerisation of **14**–**16** in increasingly aqueous media presumably relies primarily upon intermolecular hydrophobic interactions, such as can be anticipated between L-leucine side-chains, although the corresponding energetic gain as the water content is in-

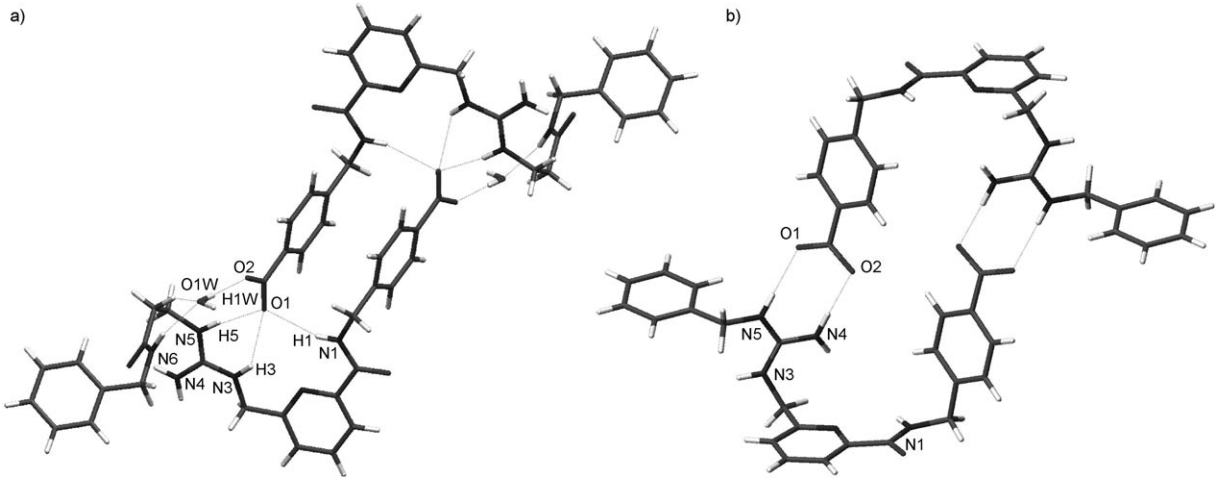


Figure 2. X-ray crystal structures of two-fold symmetric dimers of a) **6** and b) **5**. Partial numbering for clarity.[22]

creased must be balanced by the increasingly competitive nature of the solvent towards ion-pair and hydrogen bond formation.[27] As already stated, the values obtained for the entropic and enthalpic contributions to the overall dimerisation free energy must be treated with caution when the dimerisation is weak and so changes to the enthalpy-entropy balance in the series of compounds **14**–**16** cannot be interpreted with confidence, although it is notable that the results obtained for all thermodynamic parameters were closely reproducible in repeat experiments.

We have also determined the dimerisation constant for self-assembly of **14** using ^{1}H NMR dilution experiments in [D$_6$]DMSO,[28] since concentration dependence of the chemical shift of two protons was observed (Figure 3). An upfield shift of amide proton Ha, but downfield shift of a guanidinium NH proton, is observed as the concentration of **14** increases. Ha is presumably involved in intramolecular hydrogen-bonding in the monomeric species, and disruption of this intramolecular interaction upon dimerisation, and poor alignment of Ha with the carboxylate "guest" (as a consequence of a displaced ion-pair interaction similar to that described for **6**, or an alternative dimer structure) fails to "compensate" the chemical shift which consequently demonstrates an overall movement upfield. The guanidinium NH on the other hand, is probably involved in intermolecular hydrogen bonding to carboxylate in the dimer, and gives the expected downfield shift at higher concentration. The observed chemical shifts were fitted to a dimer equilibrium model,[29] and the dimerisation constant obtained, $K_{dim} = 696\,M^{-1}$ ($\pm 15\,\%$) is consistent with that determined using calorimetry (Table 1, entry 13). Thus, the dimerisation constant determined by microcalorimetry is substantiated by NMR experiments. We also investigated whether NMR could be used to provide useful NOE data and information about the structure of the dimers in solution, but in practice no useful data was obtained because of ambiguity in assigning any observed NOE to intra- or intermolecular contacts.

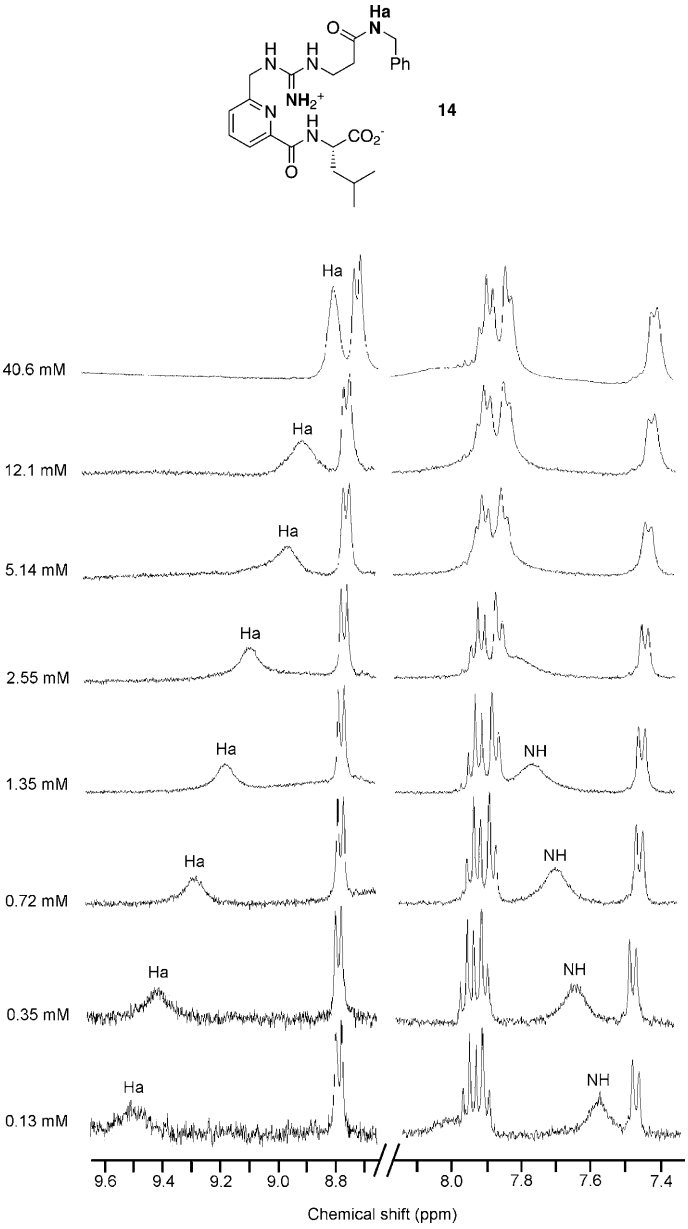


Figure 3. Concentration dependence of the chemical shifts of an amide proton (Ha) and a guanidinium proton (NH), from guanidinium–carboxylate **14**, in [D$_6$]DMSO at 400 MHz and 298 K.

Conclusion

We have carried out a preliminary study of the self-assembly of pyridyl guanidinium–carboxylates and demonstrated that dimerisation of these structures occurs in competitive media (up to 50 % H_2O in DMSO). These zwitterionic structures are simple to prepare and the tethering region is readily varied. The potential for incorporation, in this region, of functional groups which assist dimerisation via π-stacking or hydrophobic interactions has been demonstrated, the latter leading to sustained dimerisation strength under increasingly aqueous conditions such that the extent of our study in H_2O/DMSO mixtures was determined by substrate solubility and not the limit of self-assembly. Although a detailed discussion of the entropy–enthalpy balance cannot be reliably substantiated by thermodynamic parameters determined from dilution ITC when dimerisation is weak, the technique permits accurate calculation of the dimerisation constant under these conditions and is therefore a valuable tool for characterisation of individual intermolecular binding interactions within the context of an overall weakly bound supramolecular assembly. Successful application of ^{1}H NMR dilution spectroscopy for study of self-assembly in one case, corroborates the use of dilution ITC for characterisation of the dimerisation phenomenon and, importantly, also highlights the value of the latter technique for application to monomer–dimer equilibria which exist outside the concentration range of ^{1}H NMR spectroscopy. The dimerisation constants of pyridyl guanidinium–carboxylates described herein are lower than the previously reported association constants for (host–guest) carboxylate recognition by pyridyl guanidinium

receptors, and for related zwitterionic guanidinium–carboxylate systems, suggesting that we have not optimised the cooperativity of possible intermolecular interactions in our structures. Obtaining detailed structural information about the dimers in solution has not proved possible for the structures we have investigated so far, but the X-ray crystal structure of **6** indicates that the anticipated dimerisation architecture (Figure 1b) is plausible. We are currently addressing design of the carboxylate binding pocket in order to generate improved dimeric, self-assemblies as plausible models of β-sheet structure, in which the pyridyl guanidinium–carboxylate region acts as a non-covalent "hairpin" linking antiparallel peptide strands and potentially enabling future, context-specific characterisation of intermolecular amino acid side-chain - side-chain interactions.

Experimental Section

General techniques: Reagents and solvents were obtained from commercial suppliers and if necessary dried and distilled before use. THF was freshly distilled from sodium/benzophenone under argon. Toluene was distilled from sodium under argon. Dichloromethane, acetonitrile and triethylamine were freshly distilled from CaH_2. *N*,*N*-Dimethylformamide was distilled from CaH_2 and stored over 4 Å molecular sieves. Reactions requiring a dry atmosphere were conducted in oven dried glassware under nitrogen. Petrol refers to the fraction boiling between 40 and 60 °C.

^{1}H and ^{13}C NMR spectra were recorded on Bruker AV300, AM300 or DPX400 spectrometers. ^{1}H chemical shifts are reported as values in ppm referenced to residual solvent. The following abbreviations are used to denote multiplicity and may be compounded: s = singlet, d = doublet, t = triplet, q = quartet. ^{13}C spectra were proton decoupled and referenced to solvent. Signals are reported as s, d, t, q, depending on the number of directly attached protons (0, 1, 2, 3, respectively), this being determined by DEPT experiments. Infrared spectra were recorded either as neat solids or as oils on a Bio-Rad Golden Gate ATR FT-IR spectrometer fitted with an ATR accessory. Absorptions are given in wavenumbers (cm^{-1}) and the following abbreviations used to denote peak intensities: s = strong, m = medium, w = weak and/or br (broad). Low-resolution mass spectra were recorded on a Micromass platform single quadrupole mass spectrometer in methanol or acetonitrile. Accurate mass spectra were recorded on a double focusing mass spectrometer. Melting points were determined in open capillary tubes using a Gallenkamp Electrothermal melting point apparatus and are uncorrected.

Isothermal calorimetry dilution experiments: All data from ITC dilution experiments are provided in the ESI. All experiments were performed in duplicate using a VP-ITC isothermal titration calorimeter from MicroCal at 25 °C. In a typical experiment sequential injections of concentrated substrate solution (29 × 10 μL of a 3–40 mM solution) were made, at intervals of 262 s, into the stirred calorimeter cell (1.6 mL volume) initially containing solvent alone. Resulting data were corrected for heat of mixing of solvent, carried out separately under identical conditions, and analysed by non-linear regression using a corrected version of MicroCal Origin 7.0 software.[20]

^{1}H NMR dilution experiments: All data from ^{1}H NMR dilution experiments are provided in the Supporting Information. All experiments were conducted on a Bruker DPX400 spectrometer at 298 K. [D_6]DMSO of commercial grade was used. ^{1}H NMR spectra were recorded for a series of fifteen samples of increasing concentration of compound **14**, each of approx. 5% incremental increase in dimer, from 0.13 up to 40.6 mM. Each of these samples was prepared directly in a single dilution, from 40.6 mM stock solution. Changes in chemical shift of proton signals, as a function of sample concentration, were analysed using NMRDil_Dimer software[29] and the error calculated on the basis of the average for each dimerisation constant measured.

Synthesis: The synthesis and full characterisation of thiourea **1** has been reported by us previously.[2e] Benzyl (benzylcarbamothioyl)carbamate (**2**) was prepared using the published procedure.[14]

Methyl-4-[({[6-(aminomethyl)pyridin-2-yl]carbonyl}amino)methyl] benzoate (4): To a stirred solution of ethyl 6-[[bis(*tert*-butoxycarbonyl)amino]methyl]pyridine-2-carboxylate (**3**) (780 mg, 2.05 mmol) in THF (10 mL) at room temperature, was added Me_3SiOK (316 mg, 2.64 mmol) in a single portion and the resulting mixture stirred for 12 h before cooling to 0 °C and addition of H_2O (50 mL) and CH_2Cl_2 (50 mL). The mixture was adjusted to pH ~6 by dropwise addition of citric acid (0.1 M aqueous solution) at 0 °C and the biphasic mixture stirred vigorously at 0 °C for 1 h before separation and extraction of the aqueous phase with EtOAc (3 × 50 mL). The combined organic phase was dried over $MgSO_4$ and concentrated in vacuo to yield the carboxylic acid, **17**, as a white solid (540 mg, 70%), which was not purified further.

To a stirred suspension of **17** (500 mg, 1.4 mmol) and methyl 4-(aminomethyl)benzoate hydrochloride (340 mg, 1.18 mmol) in CH_2Cl_2 (15 mL), was added iPr$_2$NEt (1.22 mL, 7 mmol) followed by PyBop (875 mg, 1.68 mmol). The resulting mixture was stirred at room temperature for 12 h, before addition of $KHSO_4$ (25 mL of a 1 M aqueous solution), separation of the organic phase, and washing with K_2CO_3 (25 mL of a saturated aqueous solution) and brine (25 mL). The organic phase was dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (SiO_2 eluted with petrol/EtOAc 85:15) gave the amide coupled product, **18**, as a white solid (685 mg, 98%). This amide (240 mg, 0.48 mmol) was treated with trifluoroacetic acid (10 mL of a 20% v/v solution in CH_2Cl_2) at room temperature and the mixture stirred for 2 h. CH_2Cl_2 was removed in vacuo and the residue washed with toluene (3 × 10 mL), removal of the azeptrope with TFA was made in vacuo each time. The crude oil was taken into CH_2Cl_2 (25 mL) and washed with K_2CO_3 (25 mL of a saturated aqueous solution) before drying over $MgSO_4$ and concentration in vacuo, to give the title compound **4** as a colourless oil without further purification (134 mg, 94%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.61 (br s, 1H), 8.05 (d, J = 8.0 Hz, 1H), 7.93 (d, J = 8.4 Hz, 2H), 7.76 (t, J = 7.7 Hz, 1H), 7.37 (m, 4H), 4.66 (d, J = 5.9 Hz, 2H), 3.99–3.85 (m, 2H), 3.85 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 166.81 (s), 164.51 (s), 160.12 (s), 149.07 (s), 143.69 (s), 138.01 (d), 129.91 (d), 129.18 (s), 127.51 (d), 124.11 (d), 120.73 (d), 52.07 (q), 47.03 (t), 43.00 ppm (t); IR (oil): $\tilde{\nu}$ = 3350 (w, br), 1716 (s), 1666 (s), 1522 (s), 1108 cm^{-1} (m); MS (ES$^+$): m/z (%): 300 (100) $[M+H]^+$.

Guanidinium–carboxylate 5: To a stirred mixture of amine **4** (244 mg, 0.82 mmol) and benzyl (benzylcarbamothioyl)carbamate (**2**) (295 mg, 0.98 mmol) in CH_2Cl_2 (15 mL), was added Et_3N (0.34 mL, 2.46 mmol) followed by EDC·HCl (472 mg, 2.46 mmol). The mixture was stirred at room temperature for 24 h, before addition of $KHSO_4$ (25 mL of a 1 M aqueous solution), separation of the organic phase, and washing with K_2CO_3 (25 mL of a saturated aqueous solution) and brine (25 mL). The organic phase was dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (SiO_2 eluted with 1% MeOH in CH_2Cl_2) gave the Cbz-guanidine product, **19**, as a white solid (372 mg, 80%). To a stirred solution of **19** (165 mg, 0.29 mmol) in THF (10 mL) at room temperature, was added Me_3SiOK (56 mg, 0.44 mmol) in a single portion and the resulting mixture stirred for 12 h before cooling to 0 °C and addition of H_2O (20 mL) and CH_2Cl_2 (20 mL). The mixture was adjusted to pH ~7 by dropwise addition of citric acid (0.1 M aqueous solution) at 0 °C before separation and extraction of the aqueous phase with CH_2Cl_2 (3 × 50 mL). The combined organic phase was dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (SiO_2 eluted with 3% MeOH in CH_2Cl_2) gave carboxylic acid product **20** as a white solid (87 mg, 54%). A stirred solution of **20** (100 mg, 0.18 mmol) in MeOH (5 mL) was treated with 10% Pd/C (19 mg, 0.18 mmol) and stirred under H_2 (1 atm) at room temperature for 24 h before filtration through Celite, washing of the filter-cake with MeOH (10 mL), and concentration of the filtrate in vacuo. Recrystallisation from CH_2Cl_2/MeOH gave the title compound **5** as a white solid (37 mg, 50%). Mp. 302–304 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 11.0 (br s, 1H), 9.61 (br s,

1H), 8.0–7.92 (m, 2H), 7.51 (d, J=7.5 Hz, 1H), 7.43 (d, J=7.2 Hz, 2H), 7.30–7.19 (m, 5H), 6.73 (d, J=5.5 Hz, 2H), 4.65 (d, J=5.1 Hz, 2H), 4.42 (d, J=5.5 Hz, 2H), 4.34 ppm (d, J=6.1 Hz, 2H); ^{13}C NMR (100.5 MHz, [D$_6$]DMSO): δ=140.59 (d), 130.89 (d), 130.54 (d), 129.34 (d), 129.06 (d), 127.30 (d), 126.73 (d), 122.76 (d), 47.49 (t), 45.96 (t), 44.52 ppm (t); IR (solid): $\tilde{\nu}$ = 3252 (br, w), 1667 (s), 1545 (m), 1375 cm^{-1} (s); MS (ES$^+$): m/z (%): 418 (100) [M+H]$^+$; HRMS (ES$^+$): m/z: calcd for $C_{23}H_{24}N_5O_3$: 418.1879; found: 418.1875 [M+H]$^+$.

Guanidinium–carboxylate 6: To a stirred mixture of amine **4** (127 mg, 0.42 mmol) and thiourea **1** (187 mg, 0.5 mmol) in CH_2Cl_2 (4 mL), was added Et_3N (0.18 mL, 1.26 mmol) followed by EDC·HCl (242 mg, 1.26 mmol). The mixture was stirred at room temperature for 48 h, before addition of $KHSO_4$ (20 mL of a 1 M aqueous solution), separation of the organic phase, and washing with Na_2CO_3 (20 mL of a saturated aqueous solution) and brine (20 mL). The organic phase was dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (SiO_2 eluted with 1% MeOH in CH_2Cl_2 → 5% MeOH in CH_2Cl_2) gave the Cbz-guanidine product, **21**, as a white solid (235 mg, 88%). To a stirred solution of **21** (168 mg, 0.26 mmol) in THF (15 mL) at room temperature, was added Me_3SiOK (51 mg, 0.4 mmol) in a single portion and the resulting mixture stirred for 24 h before cooling to 0 °C and addition of H_2O (20 mL) and CH_2Cl_2 (20 mL). The mixture was adjusted to pH ~7 by dropwise addition of citric acid (0.1 M aqueous solution) at 0 °C before separation and extraction of the aqueous phase with CH_2Cl_2 (3× 20 mL). The combined organic phase was dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (SiO_2 eluted with 5% MeOH in CH_2Cl_2) gave the carboxylic acid product, **22**, as a white solid (121 mg, 75%). A stirred solution of **22** (200 mg, 0.32 mmol) in MeOH/CH_2Cl_2 (5 mL) was treated with 10% Pd/C (34 mg) and stirred under H_2 (1 atm) at room temperature for 24 h before filtration through Celite, washing of the filter-cake with MeOH (15 mL), and concentration of the filtrate in vacuo. Precipitation from Et_2O/MeOH gave the title compound **6** as a white solid (134 mg, 86%). M.p. 168–170 °C; ^{1}H NMR (300 MHz, [D$_4$]MeOD): δ=8.04 (d, J=7.7 Hz, 1H), 7.93 (t, J=7.7 Hz, 1H), 7.82 (d, J=8.0 Hz, 2H), 7.49 (d, J=7.7 Hz, 1H), 7.25–7.12 (m, 7H), 4.56 (s, 2H), 4.54 (s, 2H), 4.25 (s, 2H), 3.44 (t, J=5.9 Hz, 2H), 2.41 ppm (m, 2H); ^{13}C NMR (100.5 MHz, [D$_6$]DMSO): δ=171.91 (s), 170.29 (s), 164.23 (s), 156.86 (s), 155.15 (s), 149.32 (s), 140.16 (s), 139.23 (s), 138.42 (d), 136.77 (s), 128.67 (d), 128.25 (d), 127.20 (d), 126.72 (d), 125.26 (d), 124.42 (d), 120.57 (d), 45.31 (t), 42.29 (t), 42.19 (t), 37.74 (t), 34.86 ppm (t); IR (solid): $\tilde{\nu}$ = 3206 (m), 1667 (s), 1515 (s), 1297 cm^{-1} (s); MS (ES$^+$): m/z (%): 489 (100) [M+H]$^+$; HRMS (ES$^+$): m/z: calcd for $C_{26}H_{29}N_6O_4$: 489.2250; found: 489.2237 [M+H]$^+$.

Methyl 3-(6-(aminomethyl)picolinamido)propanoate·TFA salt (7). To a stirred solution of ethyl ester **3** (1.8 g, 5 mmol) in CH_3CN (20 mL containing 2% (v/v) of H_2O), was added Et_3N (2.2 mL, 15.8 mmol) followed by LiBr (4.6 g, 52.9 mmol). The resulting mixture was stirred vigorously at room temperature for 24 h before addition of H_2O (100 mL) followed by EtOAc (100 mL). The mixture was adjusted to pH 2–3 by dropwise addition of HCl (2 M aqueous) and the organic phase then separated and washed with H_2O (50 mL), before drying over $MgSO_4$ and concentration in vacuo. Purification by column chromatography (SiO_2 eluted with 5% MeOH in CH_2Cl_2) gave separable -*N*HBoc (**23a**, 404 mg, 32%) and -*N*Boc$_2$ (**23b**, 1.0 g, 57%) products, each as a white solid. To a stirred solution of **23a** (3.4 g, 9.7 mmol) in CH_2Cl_2 (50 mL) was added iPr$_2$NEt (5.95 mL, 34.2 mmol) followed by EDC·HCl (4.4 g, 24.3 mmol) and HOBt (3.9 g, 29.1 mmol). To the resulting mixture was then added a solution of β-alanine methyl ester hydrochloride (2.6 g, 19.4 mmol) in CH_2Cl_2/DMF (25 mL of a 4:1 mixture), before stirring at room temperature for 72 h. Solvents were then removed in vacuo. Purification by column chromatography (SiO_2 eluted with CH_2Cl_2/MeOH 20:1) gave the amide product, **24**, as a white gummy solid (2.3 g, 48%). A solution of **24** (500 mg, 1.14 mmol) in CH_2Cl_2 (50 mL) was treated with trifluoroacetic acid (5 mL of a 20% v/v solution in CH_2Cl_2) at room temperature and the mixture stirred for 5 h. CH_2Cl_2 was removed in vacuo and the residue washed with toluene (3×2 mL), removal of the azeptrope with TFA was made in vacuo each time to give the title salt **7** as a white gummy solid (0.5 g, ~72%). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=9.19 (t, J=6.2 Hz, 1H), 8.40 (brs, 3H), 8.04 (t, J=7.7 Hz, 1H), 7.98 (dd, J=7.7, 1.1 Hz, 1H), 7.63 (dd, J=7.7, 1.1 Hz, 1H), 4.31 (q, J=5.7 Hz, 2H), 3.60 (s, 3H), 3.56 (apparent q, J=6.4 Hz, 2H), 2.63 ppm (t, J=6.8 Hz, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=171.67 (s), 163.50 (s), 152.27 (s), 148.79 (s), 138.94 (d), 124.86 (d), 121.06 (d), 51.47 (q), 42.14 (t), 35.05 (t), 33.72 ppm (t); MS (ES$^+$): m/z (%): 238 (100) [M]$^+$; HRMS (ES$^+$): m/z: calcd for $C_{11}H_{16}N_3O_3$: 238.1186; found: 238.1187 [M]$^+$.

Guanidinium–carboxylate 8: To a stirred suspension of **7**·TFA salt (230 mg, ~1.04 mmol) in CH_2Cl_2 (40 mL) was added Et_3N (500 μL, 3.12 mmol) followed by EDC·HCl (480 mg, 2.6 mmol) and thiourea **1** (456 mg, 1.23 mmol), each in a single portion. The resulting mixture was stirred for 72 h at room temperature before addition of $KHSO_4$ (50 mL of a 1 M aqueous solution), separation of the organic phase, and washing with brine (50 mL). The organic phase was dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (SiO_2 eluted with 1% MeOH in CH_2Cl_2 → 5% MeOH in CH_2Cl_2) gave the Cbz-guanidine product, **25**, as a white solid (500 mg, 77%). A stirred solution of **25** (450 mg, 0.8 mmol) in THF (30 mL) was added to a suspension of Me_3SiOK (100 mg, 0.8 mmol) in THF (10 mL) and the resulting mixture stirred at room temperature for 16 h before cooling to 0 °C and addition of H_2O (50 mL) and CH_2Cl_2 (50 mL). The mixture was adjusted to pH ~6 by dropwise addition of citric acid (0.1 M aqueous solution) at 0 °C and the resulting biphasic mixture stirred vigorously at 0 °C for 1 h before separation of the organic phase, drying over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (SiO_2 eluted with 2% MeOH in CH_2Cl_2 → 5% MeOH in CH_2Cl_2) gave the carboxylic acid, **26**, as a white solid (300 mg, 75%). A stirred solution of **26** (330 mg, 0.6 mmol) in MeOH (50 mL) was treated with 10% Pd/C (60 mg) and stirred under H_2 (1 atm) at room temperature for 48 h before filtration through Celite. Concentration of the filtrate in vacuo, followed by precipitation of the residue from Et_2O/MeOH, gave the title compound **8** as a white solid (200 mg, 78%). M. p. 218–220 °C; ^{1}H NMR (300 MHz, [D$_4$]MeOD): δ=8.00–7.85 (m, 2H), 7.47 (d, J=8.0 Hz, 1H), 7.24–6.86 (m, 5H), 4.50 (brs, 2H), 4.29 (brs, 2H), 3.57–3.47 (m, 4H), 2.68 (t, J= 5.8 Hz, 2H), 2.42 ppm (m, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ= 178.45 (s), 170.32 (s), 162.43 (s), 156.08 (s), 152.12 (s), 148.98 (s), 139.35 (s), 138.75 (d), 128.00 (d), 127.03 (d), 126.46 (d), 123.85 (d), 119.92 (d), 44.64 (t), 42.12 (t), 38.42 (t), 36.91 (t), 36.12 (t), 34.59 ppm (t); IR (solid): $\tilde{\nu}$ = 3286 (w), 1644 (s), 1567 (s), 1537 (s), 697 (m), 607 cm^{-1} (m); MS (ES$^+$): m/z (%): 427 (100) [M+H]$^+$; HRMS (ES$^+$): m/z: calcd for $C_{21}H_{27}N_6O_4$: 427.2088; found: 427.2077 [M+H]$^+$.

(*S*)-Methyl 2-(6-(aminomethyl)picolinamido)-3-methylbutanoate·TFA salt (9). To a stirred solution of carboxylic acid **23a** (100 mg, 0.36 mmol) in CH_2Cl_2 (25 mL) was added iPr$_2$NEt (0.2 mL, 1.26 mmol) followed by EDC·HCl (165 mg, 0.9 mmol) and HOBt (146 mg, 1.08 mmol). To the resulting mixture was then added a solution of L-valine methyl ester hydrochloride (120 mg, 0.72 mmol) in CH_2Cl_2/DMF (26 mL of a 25:1 mixture), before stirring at room temperature for 72 h. CH_2Cl_2 (40 mL) and $KHSO_4$ (40 mL of a 1 M aqueous solution) were then added before separation of the organic layer, washing with Na_2CO_3 (30 mL of a saturated aqueous solution) and brine (30 mL), drying over MgSO4 and concentration in vacuo. Purification by column chromatography (SiO_2 eluted with CH_2Cl_2/petrol 1:1 → 2% MeOH in CH_2Cl_2) gave the amide coupled product, **27**, as a pale yellow oil (80 mg, 50%). A solution of **27** (56 mg, 0.15 mmol) in CH_2Cl_2 (10 mL) was treated with trifluoroacetic acid (2 mL of a 20% v/v solution in CH_2Cl_2) at room temperature and the mixture stirred for 5 h. CH_2Cl_2 was removed in vacuo and the residue washed with toluene (3×2 mL), removal of the azeptrope with TFA was made in vacuo each time to give the title salt **9** as a yellow oil (30 mg, ~70%). ^{13}C NMR (75 MHz, $CDCl_3$): δ=174.34 (s), 164.34 (s), 151.04 (s), 149.26 (s), 139.09 (d), 125.31 (d), 122.95 (d), 58.47 (d), 52.62 (q), 43.38 (t), 31.16 (d), 19.22 (q), 18.57 ppm (q); IR (oil): $\tilde{\nu}$ = 2961 (w), 1668 (m), 1594 cm^{-1} (m); MS (ES$^+$): m/z (%): 266 (100) [M]$^+$; HRMS (ES$^+$): m/z: calcd for $C_{13}H_{20}N_3O_3$: 266.1499; found: 266.1495 [M]$^+$.

Guanidinium–carboxylate 10: To a stirred suspension of **9**·TFA salt (40 mg, ~0.15 mmol) in CH_2Cl_2 (15 mL) was added Et_3N (0.1 mL, 0.7 mmol) followed by EDC·HCl (55 mg, 0.2 mmol) and benzyl (benzylcarbamothioyl)carbamate (**2**) (67 mg, 0.2 mmol), each in a single portion. The resulting mixture was stirred for 72 h at room temperature before re-

moval of solvents in vacuo. Purification by column chromatography (SiO_2 eluted with $CH_2Cl_2 \rightarrow 3\%$ MeOH in CH_2Cl_2) gave the Cbz-guanidine product, **28**, as a white solid (1.35 g, 75%). A stirred solution of **28** (350 mg, 0.66 mmol) in THF (25 mL) was added to a suspension of Me_3SiOK (85 mg, 0.66 mmol) in THF (25 mL) and the resulting mixture stirred at room temperature for 16 h before cooling to 0°C and addition of H_2O (50 mL) and CH_2Cl_2 (50 mL). The mixture was adjusted to pH ~6 by dropwise addition of citric acid (0.1 M aqueous solution) at 0°C and the resulting biphasic mixture stirred vigorously at 0°C for 1 h before separation of the organic phase, drying over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (SiO_2 eluted with 2% MeOH in $CH_2Cl_2 \rightarrow 5\%$ MeOH in CH_2Cl_2) gave the carboxylic acid, **29**, as a white solid (310 mg, 71%). A stirred solution of **29** (300 mg, 0.6 mmol) in MeOH (30 mL) was treated with 10% Pd/C (64 mg) and stirred under H_2 (1 atm) at room temperature for 48 h before filtration through Celite. Concentration of the filtrate in vacuo, followed by precipitation of the residue from Et_2O/MeOH, gave the title compound **10** as a white solid (110 mg, 50%). M.p. 260–262°C; ^{1}H NMR (300 MHz, [D$_4$]MeOD): $\delta = 7.96$ (d, $J = 8.0$ Hz, 1H), 7.89 (tt, $J = 7.5$, 2.0 Hz, 1H), 7.43 (d, $J = 7.5$ Hz, 1H), 7.26–7.18 (m, 5H), 4.60 (s, 2H), 4.42 (s, 2H), 4.35 (br d, $J = 4.5$ Hz, 1H), 2.20 (m, 1H), 0.93 (d, $J = 7.0$ Hz, 3H), 0.90 ppm (d, $J = 7.0$ Hz, 3H); ^{13}C NMR (75 MHz, [D$_4$]MeOD): $\delta = 177.94$ (s), 165.15 (s), 158.42 (s), 156.27 (s), 150.87 (s), 139.92 (d), 137.64 (s), 129.86 (d), 128.94 (d), 128.30 (d), 125.58 (d), 122.15 (d), 61.50 (d), 46.64 (t), 46.16 (t), 33.50 (d), 20.23 (q), 1858 ppm (q); IR (solid): $\tilde{\nu} = 3188$ (br, w), 1651 (s), 1393 cm^{-1} (m); MS (ES$^+$): m/z (%): 384 (100) $[M+H]^+$; HRMS (ES$^+$): m/z: calcd for $C_{20}H_{26}N_5O_3$: 384.2030; found: 384.2036 $[M+H]^+$.

Guanidinium–carboxylate 11: To a stirred suspension of **9**·TFA salt (460 mg, ~1.75 mmol) in CH_2Cl_2 (30 mL) was added Et_3N (0.74 mL, 5.25 mmol) followed by EDC·HCl (840 mg, 4.4 mmol) thiourea **1** (650 mg, 1.75 mmol), each in a single portion. The resulting mixture was stirred for 72 h at room temperature before addition of $KHSO_4$ (50 mL of a 1 M aqueous solution), separation of the organic layer, washing with brine (50 mL), drying over $MgSO_4$ and concentration in vacuo. Purification by column chromatography (SiO_2 eluted with 1% MeOH in $CH_2Cl_2 \rightarrow 3\%$ MeOH in CH_2Cl_2) gave the Cbz-guanidine product, **30**, as a white solid (500 mg, 50%). A stirred solution of **30** (470 mg, 0.8 mmol) in THF (30 mL) was added to a suspension of Me_3SiOK (133 mg, 1.04 mmol) in THF (10 mL) and the resulting mixture stirred at room temperature for 16 h before cooling to 0°C and addition of H_2O (50 mL) and CH_2Cl_2 (50 mL). The mixture was adjusted to pH ~6 by dropwise addition of citric acid (0.1 M aqueous solution) at 0°C and the resulting biphasic mixture stirred vigorously at 0°C for 1 h before separation of the organic phase, drying over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (SiO_2 eluted with 3% MeOH in $CH_2Cl_2 \rightarrow$ 10% MeOH in CH_2Cl_2) gave the carboxylic acid, **31**, as a white solid (250 mg, 60%). A stirred solution of **31** (240 mg, 0.41 mmol) in MeOH (30 mL) was treated with 10% Pd/C (43 mg) and stirred under H_2 (1 atm) at room temperature for 48 h before filtration through Celite. Concentration of the filtrate in vacuo, followed by precipitation of the residue from Et_2O/MeOH, gave the title compound **11** as a white solid (120 mg, 55%). M.p. 242–244°C; ^{1}H NMR (400 MHz, [D$_4$]MeOD): $\delta =$ 8.00 (dd, $J = 6.4$, 1.1 Hz, 1H), 7.95 (t, $J = 7.5$ Hz, 1H), 7.50 (dd, $J = 7.5$, 1.1 Hz, 1H), 7.29–7.17 (m, 5H), 4.59 (s, 2H), 4.40 (d, $J = 4.9$ Hz, 1H), 4.31 (s, 2H), 3.55 (m, 2H), 2.48 (t, $J = 6.0$ Hz, 2H), 2.17 (m, 1H), 0.88 (d, $J = 7.1$ Hz, 3H), 0.85 ppm (t, $J = 7.1$ Hz, 3H);^{13}C NMR (100.5 MHz, [D$_4$]MeOD): $\delta = 173.02$ (s), 165.18 (s), 158.41 (s), 156.13 (s), 150.83 (s), 139.93 (d), 139.83 (s), 129.53 (d), 128.59 (d), 128.24 (d), 125.67 (d), 122.20 (d), 61.53 (d), 46.56 (t), 44.19 (t), 39.19 (t), 36.00 (t), 33.50 (d), 20.24 (q), 18.59 ppm (q); IR (solid): $\tilde{\nu} = 3208$ (w, br), 2957 (s), 2927 (s), 2858 (w), 2361 (s), 2342 (m), 1728 cm^{-1} (s); MS (ES$^+$): m/z (%): 455 (100) $[M+H]^+$; HRMS (ES$^+$): m/z: calcd for $C_{23}H_{31}N_6O_4$: 455.2401; found: 455.2389 $[M+H]^+$.

6-((3-(3-(Benzylamino)-3-oxopropyl)-2-((benzyloxy)carbonyl) guanidino)methyl)picolinic acid (12): Ethyl ester **3** (2.5 g, 3.81 mmol) was treated with trifluoroacetic acid (30 mL of a 20% v/v solution in CH_2Cl_2) at room temperature and the mixture stirred for 3 h. CH_2Cl_2 was removed in vacuo and the residue washed with toluene (3×20 mL), removal of the azeptrope with TFA was made in vacuo each time. The crude oil was taken into CH_2Cl_2 (50 mL) and washed with K_2CO_3 (50 mL of a saturated aqueous solution) before drying over $MgSO_4$ and concentration in vacuo, to give the amine product **32** as a colourless oil without further purification (886 mg, 75%). To a stirred mixture of amine **32** (1.2 g, 6.63 mmol) and thiourea **1** (2.95 g, 7.96 mmol) in CH_2Cl_2 (50 mL), was added Et_3N (2.7 mL, 19.9 mmol) followed by EDC·HCl (3.82 g, 19.9 mmol). The mixture was stirred at room temperature for 48 h, before addition of $KHSO_4$ (50 mL of a 1 M aqueous solution), separation of the organic phase, and washing with K_2CO_3 (50 mL of a saturated aqueous solution) and brine (50 mL). The organic phase was dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (SiO_2 eluted with 1% MeOH in CH_2Cl_2) gave the Cbz-guanidine product, **33**, as a white solid (1.57 g, 46%). To a stirred solution of **33** (1.54 g, 3.06 mmol) in THF (20 mL) at room temperature, was added Me_3SiOK (590 mg, 4.6 mmol) in a single portion and the resulting mixture stirred for 12 h before cooling to 0°C and addition of H_2O (25 mL) and CH_2Cl_2 (25 mL). The mixture was adjusted to pH ~6 by dropwise addition of citric acid (0.1 M aqueous solution) at 0°C and the biphasic mixture stirred vigorously at 0°C for 1 h before separation and extraction of the aqueous phase with CH_2Cl_2 (3×100 mL). The combined organic phase was dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (SiO_2 eluted with 7% MeOH in CH_2Cl_2) gave the title compound, **12**, as an off-white solid (1.35 g, 90%). M.p. 79–81°C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 9.0$ (br s, 1H), 8.48 (br s, 1H), 7.91 (m, 2H), 7.47 (t, $J = 4.5$ Hz, 1H), 7.33–7.17 (m, 9H), 4.91 (m, 2H), 4.56 (m, 2H), 4.27 (d, $J = 4.5$ Hz, 2H), 3.49 ppm (m, 2H); ^{13}C NMR (100.5 MHz, [D$_6$]DMSO): $\delta = 180.45$ (s), 170.29 (s), 166.06 (s), 162.81 (s), 159.86 (s), 139.31 (s), 138.11 (d), 137.88 (s), 128.22 (d), 128.21 (d), 127.66 (d), 127.42 (d), 127.17 (d), 126.70 (d), 122.91 (d), 65.49 (t), 42.06 (t), 37.31 (t), 35.12 ppm (t); IR (solid): $\tilde{\nu} = 3269$ (br, m), 1589 (m), 1381 cm^{-1} (m); MS (ES$^+$): m/z (%): 490 (100) $[M+H]^+$; HRMS (ES$^+$): m/z: calcd for $C_{26}H_{28}N_5O_5$: 490.2085; found: 490.2074 $[M+H]^+$.

Guanidinium–carboxylate 13: Boc-L-Val-L-Val-OMe (141 mg, 0.43 mmol) was treated with trifluoroacetic acid (5 mL of a 20% v/v solution in CH_2Cl_2) at room temperature and the mixture stirred for 3 h. CH_2Cl_2 was removed in vacuo and the residue washed with toluene (3×10 mL), removal of the azeptrope with TFA was made in vacuo each time. The crude oil was taken into CH_2Cl_2 (10 mL) and washed with K_2CO_3 (10 mL of a saturated aqueous solution) before drying over $MgSO_4$ and concentration in vacuo, to give the amine dipeptide product as a colourless oil which was used directly (98 mg, ~98%). To a mixture of this amine product (98 mg, 0.43 mmol) and carboxylic acid **12** (208 mg, 0.43 mmol) in CH_2Cl_2 (20 mL) was added iPr$_2$NEt (0.37 mL, 2.14 mmol) and PyBop (266 mg, 0.51 mmol), and the resulting mixture stirred at room temperature for 12 h before addition of $KHSO_4$ (25 mL of a 1 M aqueous solution), separation of the organic phase, and washing with K_2CO_3 (25 mL of a saturated aqueous solution) and brine (25 mL). The organic phase was dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (SiO_2 eluted with EtOAc/MeCN 85:15) gave the amide product, **34**, as a white solid (288 mg, 96%). To a stirred solution of **34** (183 mg, 0.26 mmol) in THF (10 mL) at room temperature, was added Me_3SiOK (50 mg, 0.39 mmol) in a single portion and the resulting mixture stirred for 12 h before cooling to 0°C and addition of H_2O (20 mL) and CH_2Cl_2 (20 mL). The mixture was adjusted to pH ~6 by dropwise addition of citric acid (0.1 M aqueous solution) at 0°C and the biphasic mixture stirred vigorously at 0°C for 1 h before separation and extraction of the aqueous phase with CH_2Cl_2 (3×50 mL). The combined organic phase was dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (SiO_2 eluted with 10% MeOH in CH_2Cl_2) gave the carboxylic acid product, **35**, as a white solid (96 mg, 54%). A stirred solution of **35** (163 mg, 0.24 mmol) in MeOH (10 mL) was treated with 10% Pd/C (25 mg) and stirred under H_2 (1 atm) at room temperature for 6 h before filtration through Celite. Concentration of the filtrate in vacuo, washing of the filter cake with MeOH (20 mL) and precipitation of the residue from Et_2O/MeOH, gave the title compound **13** as a white solid (100 mg, 76%). m.p. 228–230°C. IR (solid): 3387 (br, w), 1647 (m), 1016 (m) cm^{-1}. ^{1}H NMR (400 MHz, [D$_4$]MeOD): $\delta = 8.04$ (d, $J = 7.5$ Hz, 1H), 7.98 (t, $J =$ 7.5 Hz, 1H), 7.53 (d, $J = 7.5$ Hz, 1H), 7.24–7.17 (m, 5H), 4.60 (s, 2H),

4.53 (d, $J=7.0$ Hz, 1H), 4.35 (s, 2H), 4.16 (d, $J=5.5$ Hz, 1H), 3.55–3.51 (m, 2H), 2.62 (t, $J=6.5$ Hz, 2H), 2.22 (m, 1H), 2.12 (m, 1H), 1.02 (d, $J=6.5$ Hz, 3H), 0.97 (d, $J=7.0$ Hz, 3H), 0.93–0.91 ppm (m, 6H); ^{13}C NMR (100.5 MHz, [D$_4$]MeOD): $\delta=182.22$ (s), 178.21 (s), 173.18 (s), 172.64 (s), 165.78 (s), 158.35 (s), 156.25 (s), 140.01 (d), 139.85 (s), 129.52 (d), 128.57 (d), 128.22 (d), 125.92 (d), 122.41 (d), 61.95 (d), 60.35 (d), 46.56 (t), 44.19 (t), 39.25 (t), 35.97 (t), 33.02 (d), 32.48 (d), 20.21 (q), 20.01 (q), 18.73 (q), 18.67 ppm (q); MS (ES$^+$): m/z (%): 554 (82) $[M+H]^+$; HRMS (ES$^+$): m/z: calcd for $C_{28}H_{40}N_7O_5$: 554.3085; found: 554.3073 $[M+H]^+$.

Guanidinium–carboxylate 14: To a mixture of H-L-Leu-OMe·HCl (167 mg, 0.92 mmol) and carboxylic acid **12** (300 mg, 0.61 mmol) in CH_2Cl_2 (20 mL) was added iPr$_2$NEt (0.54 mL, 3.1 mmol) and PyBop (385 mg, 0.74 mmol), and the resulting mixture stirred at room temperature for 12 h before addition of $KHSO_4$ (25 mL of a 1 M aqueous solution), separation of the organic phase, and washing with K_2CO_3 (25 mL of a saturated aqueous solution) and brine (25 mL). The organic phase was dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (SiO_2 eluted with EtOAc/MeCN 3:1) gave the amide product, **36**, as a white solid (340 mg, 90%). To a stirred solution of **36** (273 mg, 0.44 mmol) in THF (10 mL) at room temperature, was added Me_3SiOK (85 mg, 0.66 mmol) in a single portion and the resulting mixture stirred for 12 h before cooling to 0°C and addition of H_2O (20 mL) and CH_2Cl_2 (20 mL). The mixture was adjusted to pH ~6 by dropwise addition of citric acid (0.1 M aqueous solution) at 0°C and the biphasic mixture stirred vigorously at 0°C for 1 h before separation and extraction of the aqueous phase with CH_2Cl_2 (3×50 mL). The combined organic phase was dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (SiO_2 eluted with 5% MeOH in CH_2Cl_2) gave the carboxylic acid product, **37**, as a white solid (240 mg, 90%). A stirred solution of **37** (150 mg, 0.25 mmol) in MeOH (10 mL) was treated with 10% Pd/C (27 mg) and stirred under H_2 (1 atm) at room temperature for 6 h before filtration through Celite. Concentration of the filtrate in vacuo, washing of the filter cake with MeOH (20 mL) and purification by column chromatography (SiO_2 eluted with 5 → 12% MeOH (saturated with NH_3) in CH_2Cl_2) followed by precipitation from Et$_2$O/MeOH, gave the title compound **14** as a white solid (94 mg, 80%). M.p. 181–183°C; $[\alpha]_D^{25} = -2.9°$ ($c = 0.005$, MeOH); ^{1}H NMR (400 MHz, [D$_4$]MeOD): $\delta=$ 7.99–7.91 (m, 2H), 7.49 (m, 1H), 7.26–7.17 (m, 5H), 4.58–4.56 (m, 3H), 4.35 (s, 2H), 3.56 (m, 2H), 2.60 (t, $J=6.5$ Hz, 2H), 1.80–1.66 (m, 3H), 0.98 (d, $J=6.6$ Hz, 3H), 0.96 ppm (d, $J=6.0$ Hz, 3H); ^{13}C NMR (100.5 MHz, [D$_4$]MeOD): $\delta=182.26$ (s), 179.42 (s), 173.06 (s), 164.99 (s), 158.34 (s), 155.90 (s), 150.80 (s), 139.84 (d), 129.52 (d), 128.58 (d), 128.22 (d), 125.54 (d), 122.09 (d), 55.18 (d), 46.57 (t), 44.21 (t), 44.08 (t), 39.21 (t), 36.09 (t), 26.39 (d), 23.75 (q), 22.69 ppm (q); IR (solid): $\tilde{\nu}=3232$ (br, w), 1651 (s), 1538 (s), 1392 cm^{-1} (s); MS (ES$^+$): m/z (%): 469 (100) $[M+H]^+$; HRMS (ES$^+$): m/z: calcd for $C_{24}H_{33}N_6O_4$: 469.2558; found: 469.2562 $[M+H]^+$.

Guanidinium–carboxylate 15. Boc-L-Leu-L-Leu-OMe (760 mg, 2.12 mmol) was treated with trifluoroacetic acid (15 mL of a 20% v/v solution in CH_2Cl_2) at room temperature and the mixture stirred for 2 h. CH_2Cl_2 was removed in vacuo and the residue washed with toluene (3× 10 mL), removal of the azeptrope with TFA was made in vacuo each time. The crude oil was taken into CH_2Cl_2 (15 mL) and washed with K_2CO_3 (20 mL of a saturated aqueous solution) before drying over $MgSO_4$ and concentration in vacuo, to give the amine dipeptide product as a colourless oil which was used directly (529 mg, ~97%). To a mixture of this dipeptide (527 mg, 2.1 mmol) and carboxylic acid **17** (534 mg, 2.12 mmol) in CH_2Cl_2 (50 mL) was added iPr$_2$NEt (1.84 mL, 10.6 mmol) and PyBop (1.32 g, 2.54 mmol), and the resulting mixture stirred at room temperature for 12 h before addition of $KHSO_4$ (25 mL of a 1 M aqueous solution), separation of the organic phase, and washing with K_2CO_3 (25 mL of a saturated aqueous solution) and brine (25 mL). The organic phase was dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (SiO_2 eluted with EtOAc/MeCN 85:15) gave the amide product, **38**, as a white solid (876 mg, 84%). This product, **38**, (980 mg, 1.99 mmol) was treated with trifluoroacetic acid (20 mL of a 20% v/v solution in CH_2Cl_2) at room temperature and the mixture stirred for 2 h. CH_2Cl_2 was removed in vacuo and the residue washed with toluene (3×10 mL), removal of the azeptrope with TFA was made in vacuo each time. The crude oil was taken into CH_2Cl_2 (15 mL) and washed with K_2CO_3 (20 mL of a saturated aqueous solution) before drying over $MgSO_4$ and concentration in vacuo, to give the amine product, **39**, as a colourless oil which was used directly (744 mg, 95%). To a stirred solution of **39** (744 mg, 1.9 mmol) in CH_2Cl_2 (70 mL) was added Et_3N (0.8 mL, 5.7 mmol) followed by EDC·HCl (1.1 g, 5.7 mmol) and thiourea **1** (846 mg, 2.28 mmol), each in a single portion. The resulting mixture was stirred for 12 h at room temperature before addition of $KHSO_4$ (50 mL of a 1 M aqueous solution), separation of the organic phase, and washing with K_2CO_3 (50 mL of a saturated aqueous solution) and brine (50 mL). The organic phase was dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (SiO_2 eluted with 2% MeOH in CH_2Cl_2) gave the Cbz-guanidine product, **40**, as a white solid (838 mg, 60%). A stirred solution of **40** (635 mg, 0.87 mmol) in THF (30 mL) was added to a suspension of Me_3SiOK (168 mg, 1.31 mmol) in THF (25 mL) and the resulting mixture stirred at room temperature for 12 h before cooling to 0°C and addition of H_2O (25 mL) and CH_2Cl_2 (25 mL). The mixture was adjusted to pH ~6 by dropwise addition of citric acid (0.1 M aqueous solution) at 0°C and the resulting biphasic mixture stirred vigorously at 0°C for 1 h before separation of the organic phase, drying over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (SiO_2 eluted with 7% MeOH in CH_2Cl_2) gave the carboxylic acid, **41**, as a white solid (493 mg, 80%). A stirred solution of **41** (277 mg, 0.39 mmol) in MeOH (10 mL) was treated with 10% Pd/C (41 mg) and stirred under H_2 (1 atm) at room temperature for 6 h before filtration through Celite. Concentration of the filtrate in vacuo, and purification by column chromatography (SiO_2 eluted with 5% → 12% MeOH (saturated with NH_3) in CH_2Cl_2) followed by precipitation from Et$_2$O/MeOH, gave the title compound **15** as a white solid (180 mg, 79%). M.p. 170–172°C; $[\alpha]_D^{25} = -26.8°$ ($c = 0.01$, MeOH); ^{1}H NMR (400 MHz, [D$_4$]MeOD): $\delta=8.02$ (dd, $J=7.5$, 1.0 Hz, 1H), 7.98 (t, $J=7.5$ Hz, 1H), 7.53 (dd, $J=7.5$, 1.0 Hz, 1H), 7.25–7.17 (m, 5H), 4.57 (m, 3H), 4.35 (s, 2H), 4.29 (m, 1H), 3.65–3.52 (m, 2H), 2.61 (t, $J=6.6$ Hz, 2H), 1.78–1.57 (m, 6H), 0.97 (d, $J=6.5$ Hz, 3H), 0.96 (d, $J=6.5$ Hz, 3H), 0.91 (d, $J=6.5$ Hz, 3H), 0.897 ppm (d, $J=6.5$ Hz, 3H); ^{13}C NMR (100.5 MHz, [D$_4$]MeOD): $\delta=179.70$ (s), 173.55 (s), 173.26 (s), 165.87 (s), 158.22 (s), 155.98 (s), 150.54 (s), 139.99 (d), 139.81 (s), 129.51 (d), 128.57 (d), 128.21 (d), 125.94 (d), 122.45 (d), 55.23 (d), 53.69 (d), 46.66 (t), 44.25 (t), 43.09 (t), 42.71 (t), 39.31 (t), 36.14 (t), 26.25 (d), 26.18 (d), 23.69 (q), 23.54 (q), 22.41 (q), 22.22 ppm (q); IR (solid): 3274 (br, w), 1634 (s), 1519 (s), 1384 cm^{-1} (m); MS (ES$^+$): m/z (%): 582 (100) $[M+H]^+$; HRMS (ES$^+$): m/z: calcd for $C_{30}H_{44}N_7O_5$: 582.3398; found: 582.3392 $[M+H]^+$.

Guanidinium–carboxylate 16. Boc-L-Leu-L-Leu-L-Leu-OMe (350 mg, 0.74 mmol) was treated with TFA (15 mL of a 20% v/v solution in CH_2Cl_2) at room temperature and the mixture stirred for 3 h. CH_2Cl_2 was removed in vacuo and the residue washed with toluene (3×10 mL), removal of the azeptrope with TFA was made in vacuo each time. The crude oil was taken into CH_2Cl_2 (15 mL) and washed with K_2CO_3 (20 mL of a saturated aqueous solution) before drying over $MgSO_4$ and concentration in vacuo, to give the amine dipeptide product as a colourless oil which was used directly (190 mg, ~68%). To a mixture of this tripeptide (185 mg, 0.5 mmol) and carboxylic acid **12** (244 mg, 0.5 mmol) in CH_2Cl_2 (20 mL) was added iPr$_2$NEt (0.43 mL, 2.5 mmol) and PyBop (312 mg, 0.6 mmol), and the resulting mixture stirred at room temperature for 12 h before addition of $KHSO_4$ (20 mL of a 1 M aqueous solution), separation of the organic phase, and washing with K_2CO_3 (20 mL of a saturated aqueous solution). The organic phase was dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (SiO_2 eluted with EtOAc/MeCN 85:15) gave the amide product, **42**, as a white solid (322 mg, 76%). To a stirred solution of **42** (308 mg, 0.36 mmol) in THF (15 mL) at room temperature, was added Me_3SiOK (71 mg, 0.55 mmol) in a single portion and the resulting mixture stirred for 12 h before cooling to 0°C and addition of H_2O (25 mL) and CH_2Cl_2 (25 mL). The mixture was adjusted to pH ~6 by dropwise addition of citric acid (0.1 M aqueous solution) at 0°C and the biphasic mixture stirred vigorously at 0°C for 1 h before separation and extraction of the aqueous phase with CH_2Cl_2 (3×100 mL). The combined organic phase was dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (SiO_2 eluted with 7% MeOH in CH_2Cl_2) gave the carboxylic acid product, **43**,

as a white solid (294 mg, 97%). A stirred solution of **43** (208 mg, 0.25 mmol) in MeOH (10 mL) was treated with 10% Pd/C (27 mg) and stirred under H_2 (1 atm) at room temperature for 6 h before filtration through Celite and washing of the filter cake with MeOH (20 mL). Concentration of the filtrate in vacuo and purification by column chromatography (SiO_2 eluted with 5 → 12% MeOH (saturated with NH_3) in CH_2Cl_2) followed by precipitation from Et_2O/MeOH, gave the title compound **16** as a white solid (125 mg, 72%). M.p. 188–190 °C; $[\alpha]_D^{25} = -45.1°$ (c = 0.01, MeOH); ^{1}H NMR (400 MHz, [D$_4$]MeOD): δ = 8.04 (d, J = 7.0 Hz, 1H), 7.97 (t, J = 7.7 Hz, 1H), 7.55 (d, J = 7.0 Hz, 1H), 7.26–7.20 (m, 5H), 4.58 (s, 2H), 4.40 (m, 1H), 4.35 (s, 2H), 4.30 (m, 1H), 3.60 (m, 2H), 3.35 (s, 1H), 2.62 (t, J = 6.0 Hz, 2H), 1.72–1.61 (m, 7H), 1.55 (m, 1H), 1.00 (d, J = 6.5 Hz, 3H), 0.98 (d, J = 6.5 Hz, 3H), 0.92–0.91 (m, 9H), 0.86 ppm (d, J = 6.0 Hz, 3H); ^{13}C NMR (100.5 MHz, [D$_4$]MeOD): δ = 179.53 (s), 174.36 (s), 173.33 (s), 165.77 (s), 158.32 (s), 156.16 (s), 150.35 (s), 140.00 (d), 139.84 (s), 129.52 (d), 128.57 (d), 128.21 (d), 125.99 (d), 122.44 (d), 54.86 (d), 53.80 (d), 52.90 (d), 46.65 (t), 44.24 (t), 43.78 (t), 42.90 (t), 41.30 (t), 39.28 (t), 36.20 (t), 26.19 (d), 26.13 (d), 25.92 (d), 23.81 (q), 23.62 (q), 23.44 (q), 22.66 (q), 22.10 (q), 22.04 ppm (q); IR (solid): $\tilde{\nu}$ = 3272 (br, w), 1642 (s), 1520 (s), 1385 cm^{-1} (m); MS (ES$^+$): m/z (%): 695 (100) $[M+H]^+$; HRMS (ES$^+$): m/z: calcd for $C_{36}H_{55}N_8O_6$: 695.4239; found: 695.4235 $[M+H]^+$.

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