# Carboxylate binding in polar solvents using pyridylguanidinium salts†

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A series of thiourea and guanidinium derivatives have been prepared and their ability to bind a carboxylate group has been investigated. Guanidinium **33**, featuring two additional amides and a pyridine moiety, proved to be the most potent carboxylate binding site and was able to bind acetate in aqueous solvent systems ($K_{ass}$ = 480 $M^{-1}$ in 30% $H_2O$–DMSO). The pyridine moiety is critical to obtaining strong binding, and comparison with the binding properties of analogous compounds in which the pyridine is replaced by a benzene ring provides a striking example of enthalpy–entropy compensation.

## Introduction

The carboxylate group is a very common functional group in biological molecules and there is considerable interest in developing binding motifs for carboxylate functionality[1] which can then be incorporated into larger receptor structures for binding of complex biological molecules such as amino acids and peptides, particularly in aqueous solvents.[2,3]

Thioureas and guanidinium salts have been popular as carboxylate recognition motifs,[1] providing a pair of suitably aligned hydrogen bond donors to interact with the carboxylate oxygens, and, in the case of a guanidinium cation, providing a complementary electrostatic interaction with the carboxylate anion.[4] However, simple thioureas and guanidinium salts have limited binding properties in competitive solvents, and particularly in aqueous media. Increased binding can be achieved by incorporating further hydrogen bonds to interact with the carboxylate guest and a number of approaches have been adopted to achieve this. Thus, for example, we have studied[5] the binding properties of thioureas **1** and **2** (Fig. 1), with the amides providing hydrogen bonds to a carboxylate guest in addition to those from the thiourea. Incorporating a pyridine in **1** has the potential to help preorganise the binding cavity into the desired conformation by weak hydrogen bonds between the pyridine N-lone pair and $NH^1$ and $NH^2$, but this preorganisation is not available in the benzo-analogue **2**. A pyridine dicarboxamide motif has been used elsewhere to preorganise macrocyclic and simple acyclic receptors,[6] but the benefit of this preorganisation for the complexation of anions can be outweighed by the unfavourable electrostatic interaction between the pyridine N-lone pair and the guest anion.[7] In the case of **1** and **2** the binding constants with phenylacetate as guest were very similar in 10% DMSO-$d_6$–$CDCl_3$ indicating that the benefits of preorganisation in **1** were effectively balanced by the unfavourable electrostatic interaction in **2**.

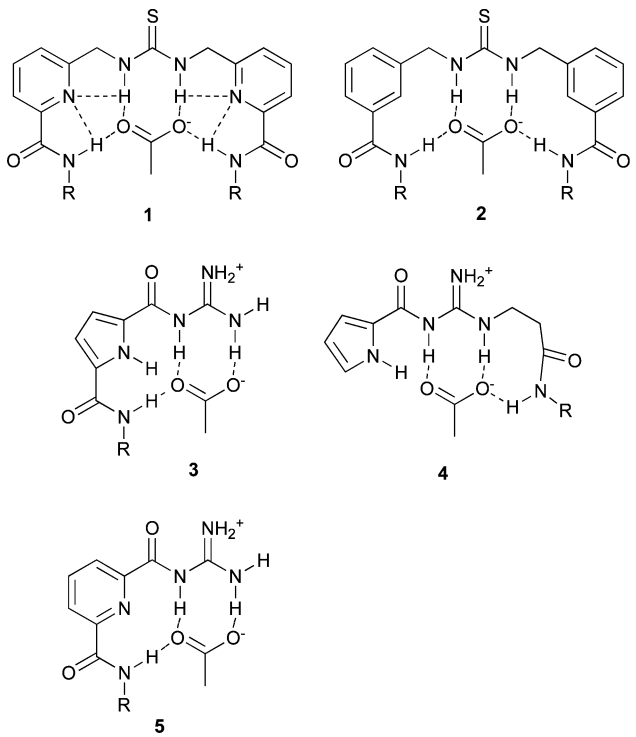


**Fig. 1** Thiourea and guanidinium derivatives as carboxylate binding sites.

Schmuck *et al.* have studied[8] a range of acylated guanidinopyrroles **3** and **4** and found that the combination of additional hydrogen bonding and the enhanced hydrogen bonding potential of the acylated guanidinium produces particularly effective carboxylate receptors (*e.g.* with the picrate salt of **3**, R = Et, using Ac–L–Ala–$O^-$ as a guest in 40% $H_2O$–DMSO (dimethylsulfoxide):[8d] $K_{ass}$ = 770 $M^{-1}$), although acylation also has the effect of lowering the p$K_a$ of the guanidinium. Schmuck and Machon have also described an analogous binding site **5**, again with an acylated guanidinium, but using a pyridine in place of the pyrrole moiety.[8b] These receptors also bound carboxylates in 40% $H_2O$–DMSO solvent mixtures, but less strongly than the pyrrole analogues.

In other work we have successfully used a combinatorial approach to prepare tweezer receptors able to bind peptides with a free carboxylate terminus in aqueous solvents, using

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† Electronic supplementary information (ESI) available: The synthesis and characterisation data of compounds **6–8**, **20**, **24**, **29** and **31**. NMR and ITC titration data for all binding studies reported in Tables 1 and 2 of this paper. See DOI: 10.1039/b700988g

a guanidinium group as the binding site for the carboxylate terminus.[3b,9] Although the combinatorial approach provides a powerful method for identifying suitable receptors it was clear that we had not necessarily optimised the receptor to maximise the binding interaction with the carboxylate, or to preorganise the side arms of the 'tweezer' to interact with the peptide backbone.

We therefore set out to prepare thiourea and guanidinium derivatives with additional hydrogen bonding functionality, designed to optimise the binding affinity with a carboxylate. At the same time we wanted to provide a functionalised scaffold which could be readily synthesised and incorporated into more sophisticated structures in the future. In particular, examination of molecular models suggested that such a scaffold could be used to produce tweezer receptors with peptidic side arms correctly orientated to form β-sheet interactions with peptide guests (Fig. 2).

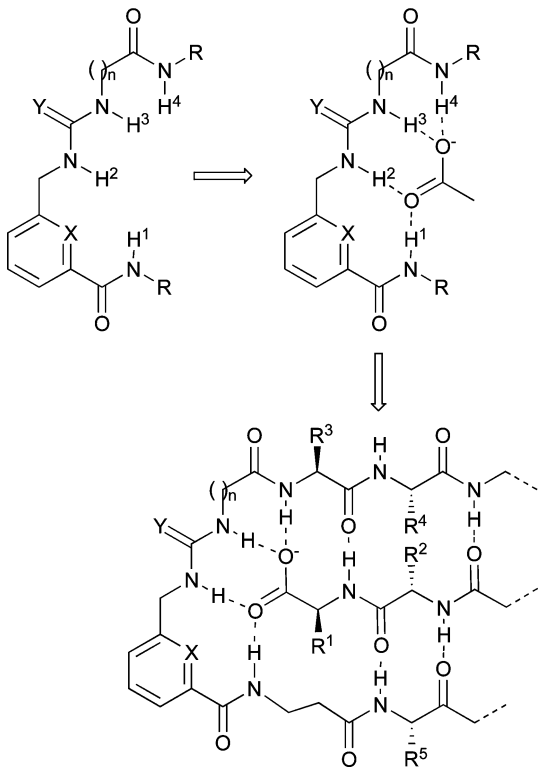


**Fig. 2** New guanidinium and thiourea derivatives as carboxylate receptors and scaffolds for peptide tweezer receptors: X = N, CH; Y = S, $NH_2^+$; $n = 1,2$.

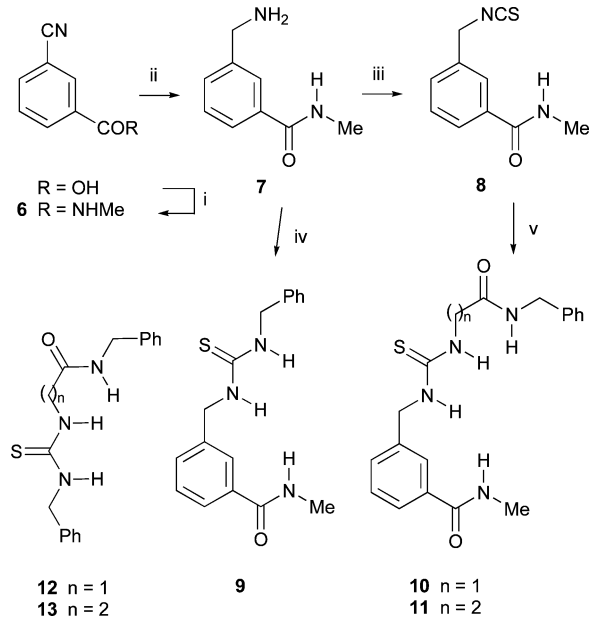
Herein are described the results of these studies which have led to the development of a potent carboxylate binding cleft and have also revealed a striking example of enthalpy–entropy compensation in the binding properties of closely related receptor structures.

## Results

### Synthesis

In order to probe the optimal structure for a carboxylate binding site scaffold a series of thioureas, **9–13** and **21–23**, and guanidinium salts, **27**, **28** and **33**, have been prepared incorporating an additional amide on one side separated by one or two methylene units, and/or with an additional amide on the other side separated by a benzyl or pyridyl moiety. Although these compounds were designed to provide a binding cleft for carboxylates (Fig. 1), it is clear that they may exist in solution in a variety of conformations stabilised by intramolecular hydrogen bonds, and some of these conformations may not be suitable for strong carboxylate binding. As outlined above, the pyridyl moiety has the potential to help preorganise the binding cavity into the desired conformation for carboxylate binding whilst potentially introducing an unfavourable electrostatic interaction between the pyridine N-lone pair and the guest anion.

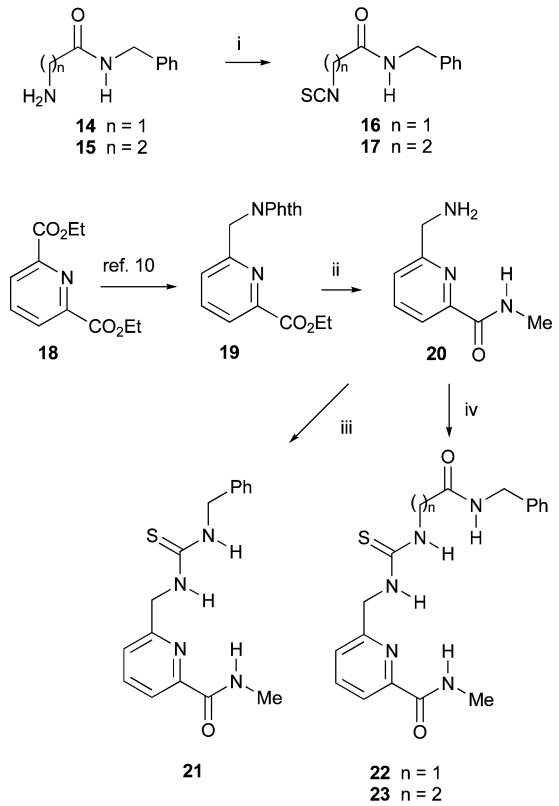
The thiourea derivatives were prepared by coupling an amine with an isothiocyanate. Thus 3-cyanobenzoic acid was converted to the corresponding benzamide **6**, the nitrile group was reduced to give amine **7** and then converted to the isothiocyanate **8** (Scheme 1). Thiourea **9** was prepared directly from amine **7** by coupling with benzylisothiocyanate, and coupling of isothiocyanate **8** with the amines **14** and **15** (Scheme 2) gave thioureas **10** and **11** respectively. Similarly, thioureas **12** and **13** were prepared by coupling amines **14** and **15** with benzylisothiocyanate.



**Scheme 1** i) a) $SOCl_2$, MeOH; b) 40% $MeNH_2$–$H_2O$, MeOH; ii) $H_2$, Pd, C; iii) $Cl_2C{=}S$, $K_2CO_3$, $H_2O$, MeOH; iv) **14** or **15**, $CH_2Cl_2$; v) amine **14** or **15**, $CH_2Cl_2$.

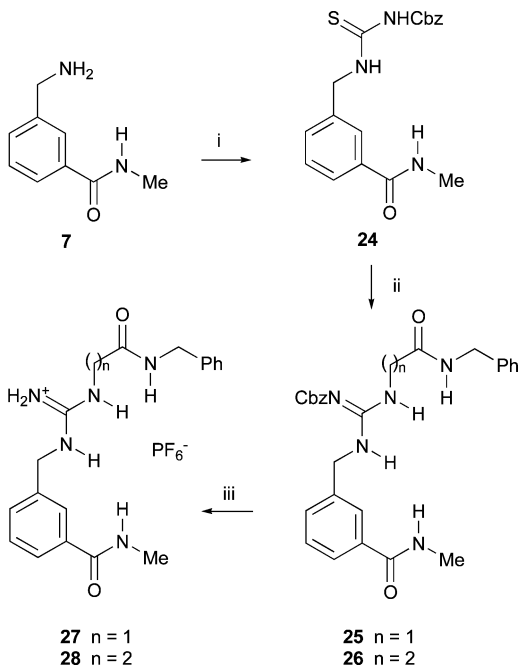
Pyrido ester **19** was prepared from pyridine dicarboxylate **18** as previously described[10] and on treatment with excess methylamine led to aminolysis of the ester, with concomitant removal of the phthalimide protecting group to give amine **20**. Amine **20** could not, however, be cleanly converted to the corresponding isothiocyanate, and instead was coupled with benzylisothiocyanate to give **21** or with isothiocyanates **16** or **17** to give thioureas **22** and **23** respectively.

Attempts to convert the various thiourea derivatives directly to guanidiniums *via* the *S*-methyl thiouroniums were largely unsuccessful. However, this difficulty was circumvented using a carbodiimide mediated coupling of an amine with a carbamoyl activated thiourea.[11] Hence amine **7** was converted to thiourea **24** and then coupled with amine **14** or **15** to give the Cbz-protected



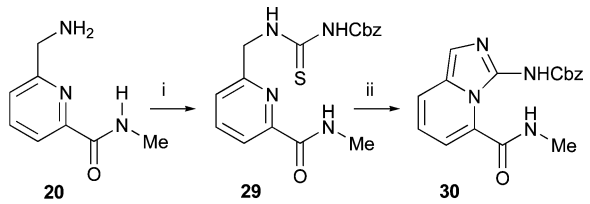

**Scheme 2** i) $Cl_2C{=}S$, $NaHCO_3$, $CH_2Cl_2$, $H_2O$; ii) 40% $MeNH_2$–$H_2O$; iii) $PhCH_2NCS$, $CH_2Cl_2$; iv) **16** or **17**, $CH_2Cl_2$.

guanidines **25** and **26** (Scheme 3). Removal of the Cbz-group and treatment with $HPF_6$ gave the guanidiniums **27** and **28** as the hexafluorophosphate salts.



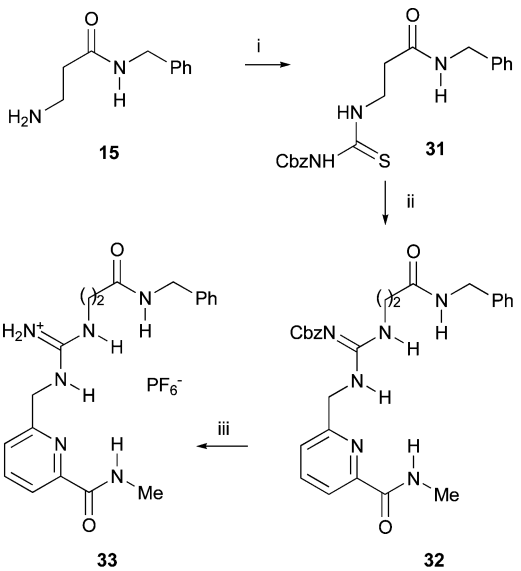

**Scheme 3** i) CbzNCS, $CH_2Cl_2$; ii) **14** or **15**, EDC, DIPEA, $CH_2Cl_2$; iii) a) $H_2$/Pd/C, MeOH; b) $HPF_6$, $H_2O$.

Amine **20** could also be converted to the corresponding carbamoyl thiourea **29** but attempted carbodiimide mediated coupling of this product with amines **14** or **15** led only to the imidazo[1,5-*a*]pyridine derivative **30** (Scheme 4).[12]




**Scheme 4** i) CbzNCS, $CH_2Cl_2$; ii) **14** or **15**, EDC, DIPEA, $CH_2Cl_2$.

However, amine **15** could be converted to Cbz activated thiourea **31** and hence coupled with amine **20** to give the corresponding Cbz-protected guanidine **32** (Scheme 5). Removal of the Cbz group and treatment with $HPF_6$ gave guanidinium **33** as the hexafluorophosphate salt. Analogous conversion of amine **14** to the corresponding guanidinium could not be successfully carried out.




**Scheme 5** i) CbzNCS, $CH_2Cl_2$; ii) **20**, EDC, DIPEA, $CH_2Cl_2$; iii) a) $H_2$/Pd/C, MeOH; b) $HPF_6$, $H_2O$.

All of the thiourea and guanidinium derivatives were freely soluble in DMSO. Analysis of the $^1$H NMR spectra (in DMSO-$d_6$) revealed that for the pyrido derivatives the signals for the various NH were generally downfield relative to those for the analogous benzo derivatives, with the effect being most significant ($\Delta\delta \approx$ 0.25 ppm) for NH$^1$ (Fig. 2). These observations are consistent with a weak intramolecular hydrogen bond interaction of NH$^1$ and NH$^2$ with the pyridine nitrogen, and the anticipated partial preorganisation of the pyridyl series, but may also indicate stronger hydrogen bond interactions with solvent molecules.

## Binding studies

The binding properties of the various thioureas were investigated using both NMR titration experiments and isothermal calorimetry (ITC), with tetrabutyl ammonium actetate as the guest, and

**Table 1** Binding data for thiourea derivatives with tetrabutylammonium acetate in DMSO

| | $^1$H NMR titration[a] | | Isothermal calorimetry | | | |
|---|---|---|---|---|---|---|
| Thiourea | $K_{ass}$/M$^{-1}$ [b] | $\Delta G$/kJ mol$^{-1}$ | $K_{ass}$/M$^{-1}$ [a] | $\Delta G$/kJ mol$^{-1}$ | $\Delta H$/kJ mol$^{-1}$ | $T\Delta S$/kJ mol$^{-1}$ |
| **9** | 260 | −13.8 | 440 | −15.3 | −6.9 | 8.4 |
| **10** | 290 | −14.0 | 230 | −13.5 | −6.3 | 7.2 |
| **11** | 200 | −13.1 | 270 | −13.9 | −8.7 | 5.2 |
| **12** | 230 | −13.5 | 370 | −14.7 | −4.8 | 9.9 |
| **13** | 230 | −13.5 | 260 | −13.7 | −6.6 | 7.1 |
| **21** | 370 | −14.7 | 370 | −14.7 | −9.7 | 5.0 |
| **22** | 950 | −17.0 | 980 | −17.1 | −3.8 | 13.3 |
| **23** | 1740 | −18.5 | 2200 | −19.1 | −4.3 | 14.8 |

[a] Data derived by monitoring the shift of the NH protons during titration. [b] Errors in association constants were estimated as ~10%.

using DMSO as the solvent (Table 1). Dilution experiments were conducted to confirm that these receptors do not form dimers (as thioureas are prone to do in less polar solvents) at the concentrations used for the binding studies.

Data from the $^1$H NMR titrations could be fitted to a 1 : 1 (host : guest) binding isotherm using the NMRTit HG software,[13,14] and 1 : 1 binding stoichiometry was confirmed using Job plots.[15] Isothermal calorimetric binding data[16] could also be fitted using a one-site binding model, consistent with a 1 : 1 binding stoichiometry. Notably the association constants obtained with these two distinct methods were in good agreement, although the values obtained by ITC were generally slightly larger than those obtained from the $^1$H NMR titrations.

In the $^1$H NMR titration experiments, addition of tetrabutylammonium acetate to the thioureas led to significant downfield shifts ($\Delta\delta \approx$ 1–2 ppm) of both thiourea protons H$^2$ and H$^3$ (for atom labels see Fig. 2) in each case, consistent with strong hydrogen bonding interactions between the acetate and these protons. Smaller shifts of the amide protons H$^1$ and H$^4$ were observed, the most significant of these ($\Delta\delta > 0.6$ ppm) being for methyl amide proton H$^1$ in the pyridyl series **21–23**.

The binding properties of the guanidiniums **27**, **28** and **33** were initially investigated using both NMR titration experiments and ITC,[17] with tetrabutylammonium acetate as the guest, and using DMSO as the solvent. In the $^1$H NMR titration experiments, addition of acetate to the guanidiniums led to significant downfield shifts of the various NH protons ($\Delta\delta$ up to ~1 ppm) but also substantial broadening in most cases and as a consequence the data from the NMR titration experiments could not be reliably fitted to give binding constants.

Isothermal calorimetric binding data obtained using DMSO as the solvent could, however, be fitted using a two site model,[17d] and gave a large 1 : 1 binding constant and a much smaller 1 : 2 (receptor : acetate) binding constant ($K_{ass}^{1:2} < 150$ M$^{-1}$) in each case (Table 2).

Binding studies were also conducted using ITC with **27**, **28** and **33** in $H_2O$–DMSO solvent mixtures. In these more competitive solvent mixtures the data for the pyridoguanidinium **33** could be fitted to a simple 1 : 1 binding model with no evidence of appreciable 1 : 2 binding. Binding of acetate was significant even in 30% $H_2O$–DMSO (the limit of solubility of the receptor), but the presence of water effectively prevented binding by the benzo analogues **27** and **28**.

## Discussion

Comparing the binding data for the thioureas (Table 1) it is clear that the binding constants for all of the benzo derivatives **9–13** are essentially the same and indeed are little different from literature values for binding simple carboxylates with unfunctionalised thioureas in DMSO.[18] The results indicate that the incorporation of additional hydrogen bonding interactions in this series is not effective in producing more potent carboxylate binders. In the pyrido series however it is apparent that binding of acetate can be improved by incorporation of additional hydrogen bonding functionality and both **22** and **23** are stronger receptors ($\Delta\Delta G =$ 3–4 kJ mol$^{-1}$) than any of the other thioureas tested. Notably the enthalpic contribution to binding in the pyrido compounds **22** ($\Delta H = -3.8$ kJ mol$^{-1}$) and **23** ($\Delta H = -4.3$ kJ mol$^{-1}$) is in fact smaller than that for the analogous benzo compounds **10** ($\Delta H = -6.3$ kJ mol$^{-1}$) and **11** ($\Delta H = -8.7$ kJ mol$^{-1}$). However, the entropic contribution to binding is much greater for the pyrido compounds ($T\Delta S =$ 13–15 kJ mol$^{-1}$) than for the benzo compounds ($T\Delta S =$ 5–7 kJ mol$^{-1}$). The improvement in binding

**Table 2** Binding data for guanidinium salt derivatives with tetrabutylammonium acetate in various solvents

| Guanidinium salt[a] | Solvent | $K_{ass}^{1:1}$/M$^{-1}$ [b] | $\Delta G$/kJ mol$^{-1}$ | $\Delta H$/kJ mol$^{-1}$ | $T\Delta S$/kJ mol$^{-1}$ |
|---|---|---|---|---|---|
| **27** | DMSO | 6900[c] | −21.9 | −19.6 | 2.3 |
| **28** | DMSO | 5300[c] | −21.2 | −19.4 | 1.8 |
| **33** | DMSO | 22 000[c] | −24.8 | −8.0 | 16.8 |
| **33** | 10% $H_2O$–DMSO | 3900[d] | −20.5 | −7.4 | 13.1 |
| **33** | 30% $H_2O$–DMSO | 480[d] | −15.3 | −2.0 | 13.3 |
| Dibenzyl guanidinium | DMSO | 3100[c] | −20.0 | −21.8 | −1.8 |

[a] All guanidinium salts were used as the hexafluorophosphate salts. [b] Errors in association constants were estimated as ~10%. [c] ITC data were fitted using a two site model, giving the reported 1 : 1 binding constants and much smaller 1 : 2 binding constants ($K_{ass}^{1:2} < 150$ M$^{-1}$ in each case). [d] ITC data was fitted using a one site (1 : 1) binding model.

for the pyrido compounds, perhaps surprisingly, is more marked with the longer and more flexible β-alanine derived side-arm. Notably the improvement is only observed with incorporation of additional functionality on both arms of the thiourea (thiourea **21** is only a marginally stronger receptor than, for example, **9**, and neither **12** nor **13** is any stronger than simple, unfunctionalised thioureas) suggesting a significant cooperative effect for **22** and **23**.[19]

As with the thiourea series, the pyridoguanidinium **33** proved to be a much more effective receptor than the benzo compounds **27** and **28**. Indeed **27** and **28** appear to be only marginally better carboxylate receptors than unfunctionalised dibenzylguanidinium. Receptor **33**, however, proved to be sufficiently good at carboxylate binding so that a reasonably strong complex ($K_{ass}$ = 480 $M^{-1}$) could be formed with acetate even in a 30% $H_2O$–DMSO solvent mixture, which compares favourably with systems developed by Schmuck *et al*.[8] However, neither **27** nor **28** gave a measurable binding constant in 10% or 30% $H_2O$–DMSO solvent mixtures using ITC. Notably binding by the benzo derivatives **27** and **28** in neat DMSO was highly exothermic (*i.e.* almost entirely enthalpically driven) as was the case with dibenzyl guanidinium. Previous studies of guanidinium–carboxylate complexation using isothermal calorimetry have similarly found that binding is generally strongly exothermic.[17] Binding by **33**, however, was largely entropically driven in DMSO, and almost entirely so in the more competitive $H_2O$–DMSO mixtures.

Presumably in the pyrido series of compounds, the intramolecular hydrogen bonds between the pyridine nitrogen lone pair and adjacent amide and thiourea/guanidinium NH's create a preorganised binding pocket that binds tightly to solvent molecules. Hence the entropic cost of organizing the receptor into a productive conformation for binding is reduced, and there is a significant entropic gain on binding a carboxylate from the release of tightly bound solvent molecules. The enthalpic contribution to binding may be relatively small because of the unfavourable electrostatic interaction between the pyridine nitrogen lone pair and the guest anion, and because strong hydrogen bonds to the solvent have to be broken. The benzo derivatives, on the other hand, lack any preorganisation, so there is little contribution to binding from the amide moieties, and solvent is less tightly bound. Hence, on binding acetate there is less entropic gain from release of solvent, but greater enthalpic gain from the formation of strong hydrogen bonds, largely involving the thiourea/guanidinium NH's, and the carboxylate, as also observed with unfunctionalised dibenzylguanidinium.

## Conclusion

Tight binding of guest molecules is generally best achieved using highly preorganised receptors which often require complex synthesis (for example to give constrained macrocyclic systems). Conversely, ease of synthesis may come at the price that the receptor is poorly preorganised and gives only weak binding. In the case of guanidinium **33**, however, an appropriate balance between ease of synthesis and degree of preorganisation appears to have been achieved since **33** is able to bind a carboxylate in competitive solvent, while the synthesis is sufficiently straightforward to suggest that it may be readily incorporated into more sophisticated architectures such as tweezer receptors (Fig. 2). For **33** (and thioureas **22** and **23**) introduction of a pyridine moiety has a very beneficial effect in preorganising an otherwise very flexible binding pocket, which outweighs the detrimental electrostatic interaction between pyridine nitrogen lone pair and anionic guest. Isothermal calorimetry experiments show that binding a carboxylate by **33** in aqueous solvents is driven almost entirely by entropy, and comparison of the enthalpic and entropic contributions to binding of acetate by the series of benzo and pyrido derivatives provides a particularly striking example of enthalpy–entropy compensation.[19]

## Experimental

### General experimental

Reactions requiring a dry atmosphere were conducted in oven-dried glassware under nitrogen. Where degassed solvents were used, a stream of nitrogen was passed through them immediately prior to use, unless otherwise stated. Solvents were of commercial grade and were used without further purification unless otherwise stated. Dichloromethane was distilled over calcium hydride, as was petroleum ether where the fraction boiling between 40 °C and 60 °C was used. TLC analysis was carried out using foil-backed sheets coated with silica gel (0.25 mm) and containing the fluorescent indicator $UV_{254}$. Flash column chromatography was performed, on Sorbsil C60, 40–60 mesh silica.

### Instrumentation

Proton NMR spectra were obtained at 300 MHz on a Bruker AC 300 and at 400 MHz on a Bruker DPX 400 spectrometer. Carbon NMR spectra were recorded at 75 MHz on a Bruker AC 300 spectrometer and at 100 MHz on a Bruker DPX 400 spectrometer. Chemical shifts are reported in ppm on the $\delta$ scale relative to the signal of the solvent used. Coupling constants ($J$) are given in Hz. Signal multiplicities were determined using the distortionless enhancement by phase transfer (DEPT) spectral editing technique. For certain compounds quaternary carbons were not observed due to long relaxation times; these are stated in the appropriate sections.

Infra-red spectra were recorded on a BIORAD Golden Gate FTS 135. All samples were run either as neat solids or as oils. Melting points were determined in open capillary tubes using a Gallenkamp Electrothermal melting point apparatus and are uncorrected.

Mass spectra were obtained on a VG analytical 70–250 SE normal geometry double focusing mass spectrometer. All electrospray (ES) spectra were recorded on a Micromass Platform quadrupole mass analyser with an electrospray ion source using acetonitrile as the solvent. High resolution accurate mass measurements were carried out at 10 000 resolution on a Bruker Apex III FT-ICR mass spectrometer. Microanalyses were performed by MEDAC Ltd., Surrey. Calorimetry experiments were performed on an isothermal titration calorimeter from Microcal Inc., USA.

### $^1$H NMR titration experiments[14]

All $^1$H NMR titration experiments were conducted on a Brüker AM 300 spectrometer at 298 K, unless otherwise stated. A sample of host was dissolved in the deuterated solvent. A portion of this

solution was used as the host NMR sample and the remainder used to dissolve a sample of the guest, so that the concentration of the host remained constant throughout the titration. Guest stock solutions were typically prepared such that 10 μL of that solution contained 0.1 equivalents of guest with respect to host. Successive aliquots of the guest solution were added to the host NMR sample and $^1$H NMR sample recorded after each addition. The changes in chemical shifts of various signals as a function of guest concentration were analysed using the NMRTit HG software,[13] assuming a 1 : 1 or a 1 : 2 binding stoichiometry.

### Isothermal calorimetry titrations[14]

All experiments were performed using an isothermal titration calorimeter from Microcal Inc. (Northampton, MA). In a typical experiment a 1–2 mM receptor solution was added to the calorimetric cell. A solution of tetrabutylammonium acetate (48–78 mM) was introduced in 50 injections of 5 μL, to a total of 250 μL of added guest. The solution was continuously stirred to ensure rapid mixing and kept at 25 °C, through the combination of an external cooling bath and an internal heater. Dilution effects were determined by performing a blank experiment by adding the same guest solution to the pure solvent and subtracting this from the raw titration to produce the final binding curve. Binding parameters were determined by applying either one-site or two-sites models, using the Origin software provided. These methods rely on standard nonlinear least-squares regression (Levenberg–Marquard method) to fit the curves, taking into account the change in volume that occurs during the calorimetric titration.

### Synthesis

Amines **14** and **15** were prepared by hydrazinolysis of the corresponding *N*-phthalimidoyl amides.[20] The synthesis and characterisation data of compounds **6–8**, **20**, **24**, **29** and **31** are described in the electronic supplementary information.†

***N*-1-Methyl-3-([(benzylamino)carbothioyl]aminomethyl) benzamide 9.** Amine **7** (58 mg, 0.27 mmol) and benzyl isothiocyanate (0.036 cm$^3$, 0.27 mmol) were stirred in $CH_2Cl_2$ (2 cm$^3$) for 20 h. The solvent was removed *in vacuo* and purification by column chromatography (20–60% EtOAc–$CH_2Cl_2$) gave thiourea **9** (66 mg, 0.21 mmol, 78%) as a white foam. Mp 78–81 °C; (found: C, 65.30; H, 6.08; N, 13.21; $C_{17}H_{19}N_3OS$ requires C, 65.15; H, 6.11; N, 13.41%); $\nu_{max}$ (solid)/cm$^{-1}$ 3284m, 3062w, 3033w, 2935w, 1739m, 1642s, 1536s; $\delta_H$ (400 MHz, DMSO-$d_6$) 2.78 (3 H, d, *J* 4, $CH_3$), 4.69 (2 H, br, $CH_2$), 4.72 (2 H, br, $CH_2$), 7.21–7.35 (5 H, m, Ar), 7.37–7.44 (2 H, m, Ar), 7.69 (1 H, d, *J* 7, Ar), 7.77 (1 H, s, Ar), 8.00 (2 H, br, thiourea-NH), 8.40 (1 H, q, *J* 4, $CH_3NH$); $\delta_C$ (100 MHz, $CDCl_3$) 28.0 ($CH_3$), 49.4 ($CH_2$), 49.9 ($CH_2$), 126.9 (CH), 127.2 (CH), 128.9 (CH), 129.1 (CH), 130.0 (CH), 130.1 (CH), 132.1 (CH), 135.8 (C), 139.0 (C), 140.0 (C), 170.2 (C), 184.3 (C); *m*/*z* (ES) 336 ((M + Na)$^+$, 100%).

***N*-1-Methyl-3-[([2-(benzylamino)-2-oxoethyl]aminocarbothioyl)-amino]methyl benzamide 10.** Isothiocyanate **8** (57 mg, 0.28 mmol), amine **14** (91 mg, 0.56 mmol) and triethylamine (TEA) (0.010 cm$^3$, 0.14 mmol) were stirred in $CH_2Cl_2$ (3 cm$^3$) for 18 h. The solvent was removed *in vacuo* and purification by column chromatography (2–10% $CH_3OH$–$CH_2Cl_2$) gave thiourea **10** (68 mg, 0.18 mmol, 64%) as a white solid. Mp 168–171 °C; (found: C, 61.40; H, 6.04; N, 14.81; $C_{19}H_{22}N_4O_2S$ requires C, 61.60; H, 5.99; N, 15.12%); $\nu_{max}$ (solid)/cm$^{-1}$ 3293m, 3064w, 2935w, 1727w, 1655s, 1638s, 1545s; $\delta_H$ (400 MHz, DMSO-$d_6$) 2.77 (3 H, d, *J* 4, $CH_3NH$), 4.17 (2 H, d, *J* 6, $CH_2C(O)$), 4.31 (2 H, d, *J* 6, $NHCH_2Ph$), 4.71 (2 H, br, $ArCH_2NHC(S)$), 7.20–7.34 (5 H, m, Ar), 7.37–7.45 (2 H, m, Ar), 7.67–7.71 (2 H, m, Ar and $NHCH_2C(O)$), 7.75 (1 H, s, Ar), 8.27 (1 H, br, $ArCH_2NHC(S)$), 8.39 (1 H, q, *J* 4, $CH_3NH$), 8.48 (1 H, br, $NHCH_2Ph$); $\delta_C$ (100 MHz, DMSO-$d_6$) 24.7 ($CH_3$), 40.6 ($CH_2$), 45.5 ($CH_2$), 47.1 ($CH_2$), 123.9 (CH), 124.6 (CH), 125.3 (CH), 125.7 (CH), 126.71 (CH), 126.73 (CH), 128.4 (CH), 133.1 (C), 137.7 (C), 165.1 (C), 167.3 (C); *m*/*z* (ES) 393 ((M + Na)$^+$, 100%); HRMS (ES) $C_{19}H_{22}N_4O_2SNa$ (M + Na)$^+$ requires calc. 393.1355, found 393.1356.

***N*-1-Methyl-3-[([3-(benzylamino)-3-oxopropyl]aminocarbothioyl)amino]methyl benzamide 11.** Isothiocyanate **8** (64 mg, 0.31 mmol) and amine **15** (61 mg, 0.34 mmol) were stirred in $CH_2Cl_2$ (4 cm$^3$) for 24 h. The solvent was removed *in vacuo* and purification by column chromatography (5% $CH_3OH$–$CH_2Cl_2$) gave thiourea **11** (86 mg, 0.22 mmol, 71%) as a white solid. Mp 147–150 °C; (found: C, 62.71; H, 6.18; N, 14.30; $C_{20}H_{24}N_4O_2S$ requires C, 62.47; H, 6.29; N, 14.57%); $\nu_{max}$ (solid)/cm$^{-1}$ 3274m, 3074w, 2921w, 1638s, 1543s; $\delta_H$ (400 MHz, DMSO-$d_6$) 2.77 (2 H, t, *J* 7, $NHCH_2CH_2$), 2.77 (3 H, d, *J* 5, $CH_3$), 3.65 (2 H, br, $NHCH_2CH_2$), 4.27 (2 H, d, *J* 6, $NHCH_2Ph$), 4.69 (2 H, br, $ArCH_2NHC(S)$), 7.19–7.33 (5 H, m, Ar), 7.35–7.43 (2 H, m, Ar), 7.56 (1 H, br, $NHCH_2CH_2$), 7.68 (1 H, d, *J* 7, Ar), 7.74 (1 H, s, Ar), 8.01 (1 H, br, $ArCH_2NHC(S)$), 8.38 (1 H, br, $CH_3NH$), 8.40 (1 H, br, $NHCH_2Ph$); $\delta_C$ (100 MHz, DMSO-$d_6$) 1 × $CH_2$ obscured by solvent, 25.7 ($CH_3$), 34.3 ($CH_2$), 40.0 ($CH_2$), 41.5 ($CH_2$), 124.8 (CH), 125.7 (CH), 126.2 (CH), 126.7 (CH), 127.6 (CH), 127.7 (CH), 129.3 (CH), 134.1 (C), 138.9 (C), 139.0 (C), 166.1 (C), 170.1 (C), 176.8 (C); *m*/*z* (ES) 407 ((M + Na)$^+$, 100%), 385 (27); HRMS (ES) for $C_{20}H_{24}N_4O_2SNa$ (M + Na)$^+$ requires 407.1512, found 407.1519.

***N*-1-Benzyl-2-[(benzylamino)carbothioyl]aminoacetamide 12.** Amine **14** (75 mg, 0.42 mmol) and benzyl isothiocyanate (0.051 cm$^3$, 0.38 mmol) were stirred in $CH_2Cl_2$ (2 cm$^3$) for 24 h. The solvent was removed *in vacuo* and purification by column chromatography (20% EtOAc–$CH_2Cl_2$ to neat EtOAc) gave thiourea **9** (85 mg, 0.26 mmol, 68%) as a white solid. Mp 146–149 °C; (found: C, 65.27; H, 5.97; N, 13.32; $C_{17}H_{19}N_3OS$ requires C, 65.15; H, 6.11; N, 13.41%); $\nu_{max}$ (solid)/cm$^{-1}$ 3286m, 3062w, 3036w, 2937w, 1738m, 1642s, 1537s; $\delta_H$ (400 MHz, DMSO-$d_6$) 4.15–4.18 (2 H, br, $CH_2C(O)$), 4.29–4.32 (2 H, m, $C(O)NHCH_2Ph$), 4.66 (2 H, br, $PhCH_2NHC(S)$), 7.22–7.33 (10 H, m, Ar), 7.64 (1 H, br, $NHCH_2C(O)$), 8.21 (1 H, br, $ArCH_2NHC(S)$), 8.45 (1 H, br, $C(O)NHCH_2Ph$); $\delta_C$ (100 MHz, 1 : 1 $CD_3OD$–$CDCl_3$) 1 × $CH_2$ obscured by solvent, 43.8 ($CH_2$), 48.1 ($CH_2$), 127.8 (CH), 128.0 (CH), 128.0 (CH), 128.1 (CH), 129.1 (2 × CH), 138.6 (C), 170.9 (C); *m*/*z* (ES) 336 ((M + Na)$^+$, 100%); HRMS (ES) $C_{17}H_{19}N_3OSNa$ (M + Na)$^+$ requires 336.1141, found 336.1147.

***N*-1-Benzyl-3-[(benzylamino)carbothioyl]aminopropanamide 13.** Amine **15** (35 mg, 0.21 mmol) and benzyl isothiocyanate (0.035 cm$^3$, 0.27 mmol) were stirred in $CH_2Cl_2$ (2 cm$^3$) for 24 h. $CH_2Cl_2$ (5 cm$^3$) was added and the mixture was washed with $H_2O$

(5 $cm^3$) and brine (5 $cm^3$), dried ($MgSO_4$) and the solvent was removed *in vacuo*. Purification by column chromatography (2–6% $CH_3OH$–$CH_2Cl_2$) and recrystallisation (EtOAc–petroleum ether) gave thiourea **13** (46 mg, 0.15 mmol, 71%) as a white solid. Mp 143–147 °C; (found: C, 66.31; H, 6.58; N, 12.61; $C_{18}H_{21}N_3OS$ requires C, 66.02; H, 6.46; N, 12.83%); $\nu_{max}$ (solid)/$cm^{-1}$ 3289m, 3062w, 3034w, 2936w, 1739m, 1643s, 1522s; $\delta_H$ (400 MHz, DMSO-$d_6$) 2.43 (2 H, t, *J* 7, NHC$H_2$C$H_2$), 3.64 (2 H, br, NHC$H_2$C$H_2$), 4.27 (2 H, d, *J* 6, C(O)NHC$H_2$Ph)), 4.64 (2 H, br, PhC$H_2$NHC(S)), 7.34–7.22 (10 H, m, Ar), 7.52 (1 H, br, N*H*C$H_2$C$H_2$)), 7.96 (1 H, br, PhC$H_2$N*H*C(S)), 8.41 (1 H, t, *J* 6, C(O)N*H*C$H_2$Ph); $\delta_C$ (100 MHz, DMSO-$d_6$) 2 × $CH_2$ obscured by solvent, 34.4 ($CH_2$), 42.0 ($CH_2$), 126.7 (CH), 126.8 (CH), 127.2 (CH), 127.3 (CH), 128.2 (2 × CH), 139.4 (2 × C), 170.6 (C), 179.1 (C); *m*/*z* (ES) 328 ((M + H)$^+$, 100%); HRMS (ES) for $C_{18}H_{21}N_3OSNa$ (M + Na)$^+$ requires 350.1297, found 350.1294.

***N*-2-Methyl-6-{[(benzylamino)carbothioyl]aminomethyl)-2-pyridine carboxamide 21.** Amine **20** (76 mg, 0.46 mmol) and benzyl isothiocyanate (0.055 $cm^3$, 0.46 mmol) were stirred in $CH_2Cl_2$ (2 $cm^3$) for 18 h. The solvent was removed *in vacuo* and purification by column chromatography (EtOAc) gave thiourea **21** (96 mg, 0.30 mmol, 73%) as a white foam. (Found: C, 61.30; H, 6.03; N, 17.81; $C_{16}H_{18}N_4OS$ requires C, 61.12; H, 5.77; N, 17.82%); $\nu_{max}$ (solid)/$cm^{-1}$ 3288m, 3061w, 3031w, 2939w, 1739m, 1643s, 1537s; $\delta_H$ (400 MHz, DMSO-$d_6$) 2.85 (3 H, d, *J* 5, $CH_3$), 4.72 (2 H, br, $CH_2$), 4.86 (2 H, d, *J* 5, $CH_2$), 7.23–7.36 (5 H, m, Ar), 7.46 (1 H, br, pyr), 7.88–7.97 (2 H, m, Ar), 8.13 (1 H, t, *J* 5, thiourea-NH), 8.21 (1 H, br, thiourea-NH), 8.65 (1 H, q, *J* 5, $CH_3$N*H*); $\delta_C$ (100 MHz, $CDCl_3$) 26.10 ($CH_3$), 48.7 ($CH_2$), 49.4 ($CH_2$), 120.8 (CH), 124.9 (CH), 127.7 (2 × CH), 128.8 (CH), 137.6 (C), 138.3 (CH), 148.7 (C), 155.8 (C), 165.2 (C), 183.0 (C); *m*/*z* (ES) 337 ((M + Na)$^+$, 100%), 315 (61); HRMS (ES) $C_{16}H_{18}N_4OSNa$ (M + Na)$^+$ requires 337.1093, found 337.1097.

***N*-2-Methyl-6-[([2-(benzylamino)-2-oxoethyl]aminocarbothioyl)-amino]methyl-2-pyridine carboxamide 22.** Amine **14**[20] (100 mg, 0.61 mmol) was dissolved in $CH_2Cl_2$ (5 $cm^3$) at 4 °C and sat. $NaHCO_3$ (2.5 $cm^3$) was added. Thiophosgene (0.093 $cm^3$, 1.21 mmol) was added to the organic phase and the reaction was stirred at 4 °C for 10 min. $CH_2Cl_2$ (5 $cm^3$) was added, the phases were separated, the organic phase was washed with 2 M HCl (5 $cm^3$) and brine (5 $cm^3$), and dried ($MgSO_4$) and the solvent was removed *in vacuo* to give isothiocyanate **16** which was used without further purification. Crude isothiocyanate **16** (37 mg, 0.18 mmol) was added to amine **20** (30 mg, 0.18 mmol) in $CH_2Cl_2$ (3 $cm^3$) and the mixture was stirred for 18 h. The solvent was removed *in vacuo* and purification by column chromatography (1–6% $CH_3OH$–$CH_2Cl_2$) gave thiourea **22** (37 mg, 0.10 mmol, 55%) as a brown waxy solid. (Found: C, 58.46; H, 5.76; N, 18.97; $C_{18}H_{21}N_5OS$ requires C, 58.20; H, 5.70; N, 18.85%); $\nu_{max}$ (solid)/$cm^{-1}$: 3294br, 3086w, 2924w, 1656s, 1592w, 1543s; $\delta_H$ (400 MHz, DMSO-$d_6$) 2.86 (3 H, d, *J* 4, $CH_3$), 4.20 (2 H, br, $CH_2$C(O)), 4.33 (2 H, d, *J* 6, NHC$H_2$Ph), 4.84 (2 H, br, pyrC$H_2$), 7.20–7.33 (5 H, m, Ph), 7.51 (1 H, d, *J* 8, pyr), 7.88–7.97 (3 H, m, pyr and N*H*C$H_2$C(O)), 8.43 (1 H, br, pyrC$H_2$N*H*), 8.58 (1 H, t, *J* 6, N*H*C$H_2$Ph), 8.76 (1 H, q, *J* 4, $CH_3$N*H*); $\delta_C$ (100 MHz, DMSO-$d_6$) 26.0 ($CH_3$), 42.1 ($CH_2$), 47.1 ($CH_2$), 48.7 ($CH_2$), 120.0 (CH), 123.9 (CH), 126.8 (CH), 127.2 (CH), 128.3 (CH), 138.3 (C), 139.3 (CH), 149.2 (C), 156.8 (C), 158.7 (C), 164.2 (C), 168.8 (C); *m*/*z* (ES) 372 ((M + H)$^+$, 100%); HRMS (ES) $C_{18}H_{21}N_5O_2SNa$ (M + H)$^+$ requires 394.1308, found 394.1308.

***N*-2-Methyl-6-[([3-(benzylamino)-3-oxopropyl]aminocarbothioyl)amino]methyl-2-pyridine carboxamide 23.** Amine **15**[20] (89 mg, 0.50 mmol) was stirred in $CH_2Cl_2$ (5 $cm^3$) at 0 °C and sat. $NaHCO_3$ (2.5 $cm^3$) was added. Thiophosgene (0.076 $cm^3$, 1.00 mmol) was added to the organic phase and the reaction was stirred vigorously for 20 min. $CH_2Cl_2$ (5 $cm^3$) was added, the organic phase was washed with 2 M HCl (5 $cm^3$) and brine (5 $cm^3$), and dried ($MgSO_4$) and the solvent was removed *in vacuo* to give isothiocyanate **17** which was used without further purification. Isothiocyanate **17** was added to amine **20** (42 mg, 0.25 mmol) in $CH_2Cl_2$ (3 $cm^3$) and the mixture was stirred for 48 h. The solvent was removed *in vacuo* and purification by column chromatography (1–5% $CH_3OH$–$CH_2Cl_2$) gave thiourea **23** (30 mg, 0.08 mmol, 31% overall) as a brown waxy solid. (Found: C, 59.34; H, 5.84; N, 18.37; $C_{19}H_{23}N_5O_2S$ requires C, 59.20; H, 6.01; N, 18.17%); $\nu_{max}$ (solid)/$cm^{-1}$ 3298br, 3087w, 2941w, 1648s, 1593w, 1536s; $\delta_H$ (400 MHz, DMSO-$d_6$) 2.47 (2 H, t, *J* 6, NHC$H_2$C$H_2$), 2.96 (3 H, d, *J* 5, $CH_3$), 3.70 (2 H, br, NHC$H_2$C$H_2$), 4.29 (2 H, d, *J* 6, C$H_2$Ph), 4.81 (2 H, br, pyrC$H_2$), 7.18–7.32 (5 H, m, Ph), 7.47 (1 H, d, *J* 7, pyr), 7.83 (1 H, br, N*H*C$H_2$C$H_2$), 7.87–7.96 (2 H, m, pyr), 8.16 (1 H, br, pyrC$H_2$N*H*), 8.43 (1 H, br, N*H*C$H_2$Ph), 8.73 (1 H, q, *J* 5, $CH_3$N*H*); $\delta_C$ (100 MHz, $CD_3OD$) 26.4 ($CH_3$), 36.4 ($CH_2$), 41.6 ($CH_2$), 44.1 ($CH_2$), 54.8 ($CH_2$), 121.5 (CH), 125.6 (CH), 128.1 (CH), 128.4 (CH), 129.49 (CH), 129.51 (CH), 139.4 (C), 139.9 (C), 150.3 (C), 158.0 (C), 167.1 (C), 173.8 (C); *m*/*z* (ES) 386 ((M + H)$^+$, 100%); HRMS (ES) $C_{19}H_{23}N_5O_2SNa$ (M + Na)$^+$ requires 408.1464, found 408.1467.

**Benzyl *N*-{[2-(benzylamino)-2-oxoethyl]amino(3-[(methylamino)-carbonyl]benzylamino)methylene}carbamate 25.** Thiourea **24** (70 mg, 0.20 mmol), amine **14** (64 mg, 0.39 mmol) and diisopropylethylamine (DIPEA, 0.070 $cm^3$, 0.39 mmol) were stirred in dry $CH_2Cl_2$ (4 $cm^3$) and dimethylformamide (DMF, 0.5 $cm^3$) under $N_2$ at 4 °C. 1-Ethyl-3-(3′-dimethylaminopropyl)carbodiimide (EDC, 74 mg, 0.39 mmol) was added and the reaction was stirred at 4 °C for 1 h and at room temperature for 48 h. The solvent was removed *in vacuo* and purification by column chromatography (1–4% $CH_3OH$–$CH_2Cl_2$) gave guanidine **25** (71 mg, 0.15 mmol, 75%) as a white solid. Mp 173–176 °C; $\nu_{max}$ (solid)/$cm^{-1}$ 3309w, 1635s, 1605s, 1556s; $\delta_H$ (400 MHz, DMSO-$d_6$) 9.25 (1 H, br, NH), 8.45 (1 H, br, NH), 8.40 (2 H, m, 2 × NH), 7.80 (1 H, s, Ar), 7.71 (1 H, d, *J* 8, Ar), 7.38–7.48 (2 H, m, Ar), 7.18–7.38 (10 H, m, Ar), 4.96 (2 H, s, OC$H_2$Ph), 4.50 (2 H, d, *J* 6, $CH_2$), 4.31 (2 H, d, *J* 6, $CH_2$), 3.92 (2 H, br, $CH_2$), 2.76 (3 H, d, *J* 5, $CH_3$); $\delta_C$ (100 MHz, DMSO-$d_6$): 1 × $CH_2$ obscured by solvent, 26.2 ($CH_3$), 42.1 ($CH_2$), 43.7 ($CH_2$), 65.6 ($CH_2$), 125.9 (CH), 126.7 (CH), 127.1 (CH), 127.5 (CH), 127.8 (CH), 128.2 (CH), 129.7 (CH), 134.5 (C), 137.8 (C), 142.1 (C), 159.9 (C × 2), 166.5 (C), 170.9 (C); *m*/*z* (ES) 510 ((M + Na)$^+$, 100%); HRMS (ES) $C_{27}H_{30}N_5O_4$ (M + H)$^+$ requires 488.2293, found 488.2295.

**Benzyl *N*-{[3-(benzylamino)-3-oxopropyl]amino(3-[(methyl amino)carbonyl]benzylamino)methylene}carbamate 26.** Thiourea **24** (115 mg, 0.31 mmol), amine **15** (100 mg, 0.61 mmol) and dry TEA (0.083 $cm^3$, 0.61 mmol) were stirred in dry $CH_2Cl_2$ (5 $cm^3$). EDC (117 mg, 0.61 mmol) was added and the reaction

was stirred for 8 h at room temperature. The solvent was removed *in vacuo* and purification by column chromatography (EtOAc to 10% $CH_3OH$–$CH_2Cl_2$) gave guanidine **26** (115 mg, 0.21 mmol, 69%) as a white foam. Mp 162–166 °C; (found: C, 66.98; H, 6.22; N, 13.71; $C_{28}H_{31}N_5O_4$ requires C, 67.05; H, 6.23; N, 13.96%); $\nu_{max}$ (solid)/cm$^{-1}$ 3285m, 3064w, 2930w, 1738w, 1640s, 1537s; $\delta_H$ (400 MHz, DMSO-$d_6$) 2.45 (2 H, br, NHCH$_2$C$H_2$), 2.77 (3 H, d, *J* 4, $CH_3$), 3.47 (2 H, dt, *J* 6 and 6, NHC$H_2$CH$_2$), 4.27 (2 H, d, *J* 5, NHC$H_2$Ph), 4.46 (2 H, d, *J* 6, ArC$H_2$NHC(S)), 4.96 (2 H, s, OC$H_2$Ph), 7.19–7.35 (11 H, m, Ar and NH), 7.36–7.41 (2 H, m, Ar), 7.69 (1 H, d, *J* 8, Ar), 7.76 (1 H, s, Ar), 8.40 (1 H, q, *J* 4, CH$_3$N*H*), 8.47 (1 H, t, *J* 5, N*H*CH$_2$Ph), 9.01 (1 H, br, NH); $\delta_C$ (100 MHz, DMSO-$d_6$) 1 × C not observed, 1 × $CH_2$ obscured by solvent, 26.2 ($CH_3$), 35.1 ($CH_2$), 37.3 ($CH_2$), 42.1 ($CH_2$), 65.5 ($CH_2$), 118.0 (C), 126.0 (CH), 126.7 (CH), 127.2 (2 × CH), 127.4 (CH), 127.7 (CH), 128.2 (3 × CH), 129.6 (CH), 137.8 (C), 139.3 (2 × C), 166.6 (2 × C), 170.3 (C); *m*/*z* (ES) 524 ((M + Na)$^+$, 100%); HRMS (ES) $C_{28}H_{32}N_5O_4$ (M + H)$^+$ requires 502.2449, found 502.2451.

***N*-1-Methyl-3-[(ammonio[2-(benzylamino)-2-oxoethyl]amino-methyl)amino]methylbenzamide hexafluorophosphate 27.** Guanidine **25** (71 mg, 0.15 mmol) and 10% Pd/C (cat.) in $CH_3OH$ (3 cm$^3$) and $CH_2Cl_2$ (1 cm$^3$) were stirred under a hydrogen atmosphere (1 atm) for 2 h. The palladium residues were removed by filtration and washed with $CH_3OH$ (10 cm$^3$). The filtrate was concentrated *in vacuo* and the residue was dissolved in $H_2O$ (4 cm$^3$), 60% $HPF_6$–$H_2O$ (10 drops) was added and the aqueous solution was extracted with EtOAc (3 × 5 cm$^3$). The combined organic phases were washed with brine (3 cm$^3$), and dried ($MgSO_4$), and the solvent was removed *in vacuo* to give guanidinium salt **27** (51 mg, 0.10 mmol, 70%) as a white foam. (Found: C, 39.64; H, 4.22; N, 12.46; $C_{19}H_{24}N_5O_2PF_6$ requires C, 39.70; H, 4.21; N, 12.18%); $\nu_{max}$ (solid)/cm$^{-1}$ 3357w, 1737w, 1631s, 1556s; $\delta_H$ (400 MHz, $CD_3CN$) 3.00 (3 H, s, $CH_3$), 4.01 (2 H, br, $CH_2$), 4.43 (1 H, d, *J* 6, $CH_2$), 4.51 (2 H, d, *J* 5, $CH_2$), 6.51 (2 H, br, $NH_2$), 6.69 (1 H, br, NH), 7.22 (1 H, br, NH), 7.28–7.48 (5 H, m, Ar), 7.52–7.61 (3 H, m, Ar and NH), 7.71 (1 H, d, *J* 8, Ar), 7.76 (1 H, s, Ar), 8.39 (1 H, br, NH); $\delta_C$ (100 MHz, $CD_3OD$) 27.0 ($CH_3$), 44.3 ($CH_2$), 44.9 ($CH_2$), 45.7 ($CH_2$), 126.8 (CH), 127.6 (CH), 128.4 (CH), 128.6 (CH), 129.6 (CH), 130.2 (CH), 131.4 (CH), 136.0 (C), 138.2 (C), 139.4 (C), 158.5 (C), 169.7 (C), 170.3 (C); *m*/*z* (ES) 354 ((M − $PF_6$)$^+$, 100%); HRMS (ES) $C_{19}H_{24}N_5O_2$ (M − $PF_6$)$^+$ requires 354.1934, found 354.1929.

***N*-1-Methyl-3-[(ammonio[3-(benzylamino)-3-oxopropyl]amino-methyl)amino]methylbenzamide hexafluorophosphate 28.** Guanidine **26** (52 mg, 0.10 mmol) and 10% Pd/C (cat.) in $CH_3OH$ (3 cm$^3$) was stirred under a hydrogen atmosphere (1 atm) for 2 h. The palladium residues were removed by filtration and washed with $CH_3OH$ (10 cm$^3$). The filtrate was concentrated *in vacuo* and the residue was dissolved in $H_2O$ (4 cm$^3$), 60% $HPF_6$–$H_2O$ (10 drops) was added and the aqueous solution was extracted with EtOAc (3 × 5 cm$^3$). The combined organic phases were washed with brine (3 cm$^3$), and dried ($MgSO_4$), and the solvent was removed *in vacuo* to give guanidinium salt **28** (44 mg, 0.09 mmol, 83%) as a white foam. (Found: C, 40.98; H, 4.33; N, 11.76; $C_{20}H_{26}N_5O_2PF_6$ requires C, 40.80; H, 4.45; N, 11.89%); $\nu_{max}$ (solid)/cm$^{-1}$ 3275w, 2969w, 1738s, 1630s, 1543s; $\delta_H$ (400 MHz, DMSO-$d_6$) 1 × $CH_2$ obscured by solvent, 2.78 (3 H, d, *J* 4, C$H_3$NH), 3.42 (2 H, q, *J* 6, NHC$H_2$CH$_2$), 4.29 (2 H, d, *J* 6, NHC$H_2$Ph), 4.43 (2 H, d, *J* 6, ArC$H_2$NHC(N)), 7.21–7.34 (5 H, m, Ar), 7.41–7.46 (2 H, m, Ar), 7.51 (3 H, br, $NH_2$ and N*H*CH$_2$CH$_2$), 7.75 (1 H, d, *J* 7, Ar), 7.79 (1 H, s, Ar), 7.94 (1 H, br, ArCH$_2$N*H*C(N)), 8.45 (1 H, q, *J* 4, CH$_3$N*H*), 8.52 (1 H, t, *J* 6, N*H*CH$_2$Ph); $\delta_C$ (100 MHz, $CD_3OD$) 27.0 ($CH_3$), 36.0 ($CH_2$), 39.0 ($CH_2$), 44.2 ($CH_2$), 45.6 ($CH_2$), 127.1 (CH), 127.5 (CH), 128.2 (CH), 128.5 (CH), 129.5 (CH), 130.1 (CH), 131.4 (CH), 136.0 (C), 138.3 (C), 139.7 (C), 157.7 (C), 170.3 (C), 173.2 (C); *m*/*z* (ES) 368 ((M − $PF_6$)$^+$, 100%); HRMS (ES) $C_{20}H_{26}N_5O_2$ (M − $PF_6$)$^+$ requires 368.2081, found 368.2079.

**Benzyl *N*-5-[(methylamino)carbonyl]imidazo[1,5-*a*]pyridin-3-yl-carbamate 30.** Thiourea **29** (74 mg, 0.21 mmol) and *N*-1-benzyl-2-aminoacetamide **14** (68 mg, 0.41 mmol) were stirred in dry $CH_2Cl_2$ (3 cm$^3$) and DMF (1 cm$^3$) at 4 °C. EDC (78 mg, 0.41 mmol) was added and the reaction was stirred at room temperature for 8 h. The solvents were removed *in vacuo*, the residue was dissolved in $CHCl_3$ (10 cm$^3$) and the solution was extracted with 1% HCl (3 × 5 cm$^3$). The aqueous phases were combined, basified (2 M NaOH, pH ≈ 12) and extracted with EtOAc (3 × 10 cm$^3$). The combined EtOAc phases were dried ($MgSO_4$), the solvent was removed *in vacuo* and the product was purified by column chromatography ($CH_2Cl_2$ to 4% $CH_3OH$–$CH_2Cl_2$) to give imidazole **30** (27 mg, 0.08 mmol, 40%) as a yellow foam. $\nu_{max}$ (solid)/cm$^{-1}$ 3251w, 3048w, 2933w, 1732m, 1717m, 1652m, 1625m, 1542s; $\delta_H$ (400 MHz, DMSO-$d_6$) 2.78 (3 H, d, *J* 3, C$H_3$NH), 5.12 (2 H, s, OC$H_2$Ph), 6.79–6.88 (2 H, m, Ar), 7.36–7.46 (5 H, m, Ph), 7.48 (1 H, s, NCH), 7.68 (1 H, dd, *J* 9 and 1, Ar), 8.77 (1 H, q, *J* 3, CH$_3$N*H*), 9.42 (1 H, br, CbzN*H*); $\delta_C$ (100 MHz, DMSO-$d_6$) 26.5 ($CH_3$), 66.7 ($CH_2$), 114.2 (C), 118.5 (CH), 118.9 (C), 120.2 (CH), 128.3 (CH), 128.5 (CH), 128.9 (CH), 130.4 (CH), 130.5 (C), 130.9 (CH), 137.0 (C), 155.1 (C), 163.6 (C); *m*/*z* (ES) 325 ((M + H)$^+$, 100%); HRMS (ES) $C_{17}H_{17}N_4O_3$ (M + H)$^+$ requires 325.1295, found 325.1295.

**Benzyl *N*-[3-(benzylamino)-3-oxopropyl]amino[(6-[(methylamino)-carbonyl]-2-pyridylmethyl)amino]methylenecarbamate 32.** EDC (111 mg, 0.58 mmol) was added to a mixture of thiourea **31** (106 mg, 0.29 mmol), *N*-2-methyl-6-(aminomethyl)-2-pyridinecarboxamide **20** (81 mg, 0.49 mmol) and dry TEA (0.082 cm$^3$, 0.58 mmol) in dry $CH_2Cl_2$ (5 cm$^3$) and the reaction was stirred for 5 h. The solvent was removed *in vacuo* and purification by column chromatography (5% $CH_3OH$–$CH_2Cl_2$) gave guanidine **32** (111 mg, 0.22 mmol, 76%) as a white solid. Mp 118–120 °C; (found: C, 64.58; H, 6.05; N, 16.45; $C_{27}H_{30}N_6O_4$ requires C, 64.53; H, 6.02; N, 16.71%); $\nu_{max}$ (solid)/cm$^{-1}$ 3349w, 2945w, 1738m, 1667m, 1620s, 1597s, 1542s; $\delta_H$ (400 MHz, DMSO-$d_6$) 1 × $CH_2$ obscured by solvent, 2.83 (3 H, d, *J* 3, C$H_3$NH), 3.52 (2 H, d, *J* 6, NHC$H_2$CH$_2$), 4.28 (2 H, d, *J* 4, NHC$H_2$Ph), 4.57 (2 H, d, *J* 4, pyr-C$H_2$), 4.97 (2 H, br, OC$H_2$Ph), 7.16–7.36 (10 H, m, Ar), 7.48 (1 H, m, pyr), 7.84–8.00 (2 H, m, pyr), 8.50 (1 H, br, N*H*CH$_2$Ph), 8.72 (1 H, br, CH$_3$N*H*), 9.03 (1 H, br, guanidine-NH), 10.14 (1 H, br, guanidine-NH); $\delta_C$ (100 MHz, $CD_3OD$) 1 × C not observed, 26.5 ($CH_3$), 37.3 ($CH_2$), 38.8 ($CH_2$), 44.2 ($CH_2$), 46.4 ($CH_2$), 67.8 ($CH_2$), 121.6 (CH), 125.6 (CH), 128.1 (CH), 128.5 (CH), 128.7 (CH), 128.8 (CH), 129.4 (CH), 129.5 (CH), 139.0 (2 × C), 139.7 (C), 139.9 (C), 143.3 (CH), 165.0 (C), 173.8 (2 × C); *m*/*z* (ES) 503 ((M + H)$^+$, 100%); HRMS (ES) $C_{27}H_{31}N_6O_4$ (M + H)$^+$ requires 503.2411, found 503.2402.

***N*-2-Methyl-6-[(ammonio[3-(benzylamino)-3-oxopropyl]amino methyl)amino]methyl-2-pyridinecarboxamide hexafluorophosphate 33.** Guanidine **32** (44 mg, 0.17 mmol) and 10% Pd/C (cat.) in $CH_3OH$ (3 $cm^3$) was stirred under a hydrogen atmosphere (1 atm) for 2 h. The palladium residues were removed by filtration and washed with $CH_3OH$ (10 $cm^3$). The filtrate was concentrated *in vacuo* and the residue was dissolved in $H_2O$ (4 $cm^3$), 60% $HPF_6$–$H_2O$ (10 drops) was added and the aqueous solution was extracted with EtOAc (3 × 5 $cm^3$). The combined organic phases were washed with brine (3 $cm^3$), and dried ($MgSO_4$), and the solvent was removed *in vacuo* to give guanidinium salt **33** (42 mg, 0.11 mmol, 91%) as a white foam. Mp 173 °C (dec.); (found: C, 38.74; H, 4.39; N, 14.41; $C_{19}H_{25}N_6O_2PF_6$ requires C, 38.69; H, 4.27; N, 14.25%); $\nu_{max}$ (solid)/$cm^{-1}$ 3468w, 3395w, 3336w, 3205w, 2970w, 1738w, 1641s, 1540m; $\delta_H$ (400 MHz, DMSO-$d_6$) 1 × $CH_2$ obscured by solvent, 2.81 (3 H, d, *J* 5, $CH_3$), 3.40 (2 H, dt, *J* 6 and 6, NHC$H_2$$CH_2$), 4.25 (2 H, d, *J* 6, C$H_2$Ph), 4.51 (2 H, d, *J* 6, pyrC$H_2$), 7.15–7.28 (5 H, m, Ph), 7.45 (1 H, d, *J* 8, pyr), 7.55 (3 H, br, N*H*$CH_2CH_2$ and $NH_2$), 7.84 (1 H, br, pyr$CH_2$N*H*), 7.90–7.99 (2 H, m, pyr), 8.49 (1 H, t, *J* 6, N*H*$CH_2$Ph), 8.61 (1 H, q, *J* 5, $CH_3$N*H*); $\delta_C$ (100 MHz, $CD_3OD$) 26.4 ($CH_3$), 36.0 ($CH_2$), 39.2 ($CH_2$), 44.3 ($CH_2$), 46.9 ($CH_2$), 122.3 (CH), 125.7 (CH), 128.3 (CH), 128.5 (CH), 129.5 (CH), 139.8 (C), 140.0 (CH), 150.7 (C), 155.6 (C), 158.1 (C), 166.9 (C), 173.3 (C); *m*/*z* (ES) 369 ((M − $PF_6$)$^+$, 100%); HRMS (ES) $C_{19}H_{25}N_6O_2$ (M − $PF_6$)$^+$ requires 369.2033, found 369.2031.

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