Amino Acid Binding by 2-(Guanidiniocarbonyl)pyridines in Aqueous Solvents: A Comparative Binding Study Correlating Complex Stability with Stereoelectronic Factors

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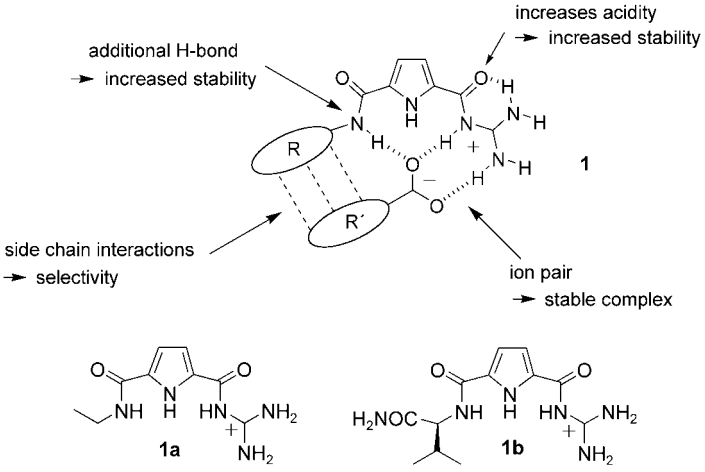
Abstract: A series of guanidiniocarbonylpyridine receptors has been synthesized, and these compounds bind amino acids (carboxylate forms) in aqueous DMSO with association constants ranging from $K = 30$ to $460\,M^{-1}$ as determined by NMR titration experiments. The differences in the complex stabilities can be correlated with steric and electrostatic effects with the aid of calculated complex structures. For example, the electrostatic repulsion between the pyridine nitrogen lone pair and the bound carboxylate makes anion binding less efficient than with the analogous pyrrole receptors previously introduced by us for carboxylate binding in water. Furthermore, steric interactions between the receptor side chain as in **2b** and the bound substrate also disfavor complexation.

Keywords: amino acids · carboxylate receptors · guanidinium cations · molecular recognition · supramolecular chemistry

Introduction

We recently introduced a de novo designed binding motif for carboxylates: the guanidiniocarbonylpyrroles **1**, which strongly bind carboxylates even in aqueous solvents through a combination of ion pairing and multiple hydrogen bonds (Scheme 1).[1] Because of the increased acidity of the acylguanidinium moiety and the additional hydrogen bonds, these complexes are much stronger than those of simple guanidinium cations, which only form stable ion pairs in organic solvents of low polarity such as chloroform or acetonitrile.[2] This recognition motif has thus already found versatile use in various fields of supramolecular[3] and bioorganic chemistry.[4]

As the guanidiniocarbonylpyrrole moiety is one of the most efficient carboxylate binding motifs known to date,[2] an interesting question is which of the multiple binding interactions present in complexes with carboxylates is mainly responsible for its unique binding properties? We have already been able to show by comparison with a neutral amidopyridine pyrrole analogue that the charge interaction within the ion pair is crucial for binding in aqueous solvents.[3a] The neutral binding motif in this analogue has exactly the same hydrogen bond pattern but is several orders of magnitude less efficient. Furthermore, a comparative thermodynamic study with a series of structurally related guanidiniocarbonylpyrroles suggested that, besides the ion-pairing, it is mainly the amide NH in the 5-position in the pyrrole ring that is important for the effective binding of the



Scheme 1. Design of guanidiniocarbonylpyrrole receptors **1** for the binding of carboxylates in aqueous solvents; the ion-pairing and the hydrogen bonds provide the binding strength, whereas additional interactions with the side chain may account for the substrate selectivity.

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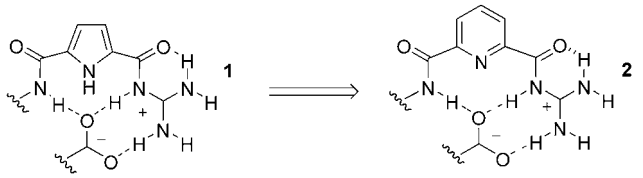
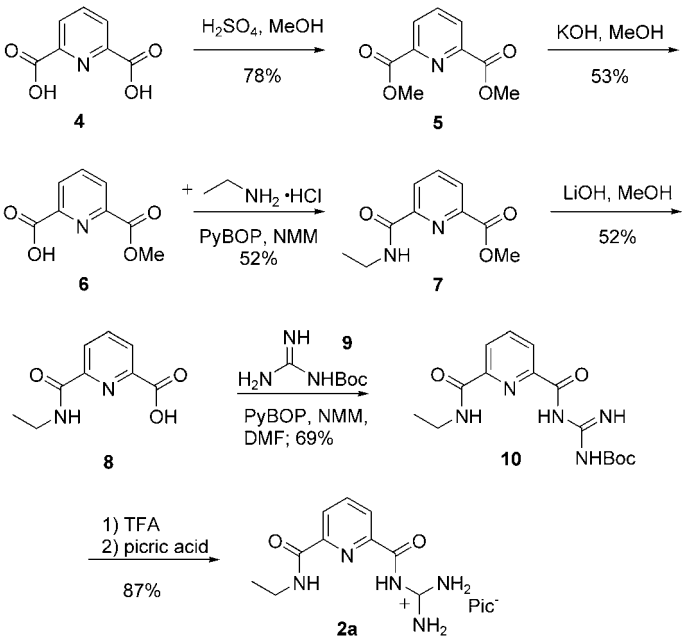
carboxylate substrate.[1] From these studies, however, it has not so far been possible to deduce the actual impact of the pyrrole ring itself. Is it only serving as a template, orientating the adjacent binding sites into the right geometry for carboxylate binding or is the pyrrole NH actively involved in the binding? If it is only a rigid template, is the ring size, and hence the angle between the two adjacent amides, crucial? To elucidate these questions we have now prepared a series of analogous guanidiniocarbonylpyridine receptors in which the pyrrole ring is replaced by a pyridine (Scheme 2).[5] Here we wish to report their syntheses and the evaluation of their binding properties.



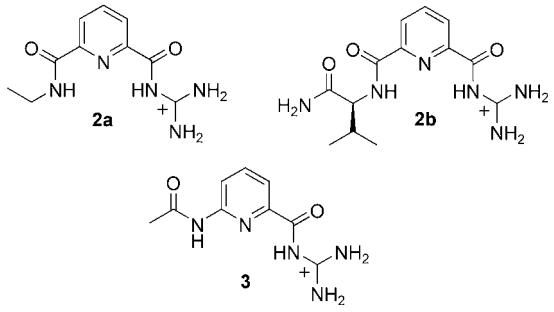
Scheme 2. Guanidiniocarbonylpyridine receptors **2** as a tool for examining the influence of the heterocycle on the binding of carboxylates.



Scheme 3. Synthesis of receptor **2a**.

Results and Discussion

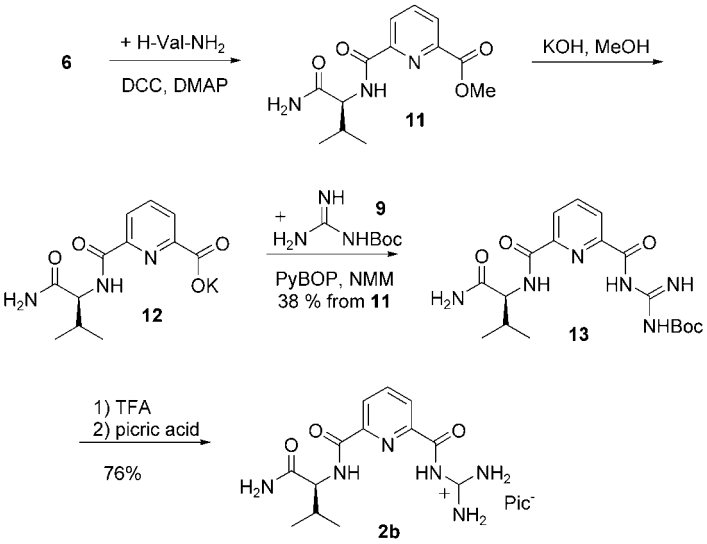
We have synthesized three prototypes of such guanidiniocarbonylpyridines. The ethyl amide- and the valine amide-substituted receptors **2a** and **2b** are the corresponding pyridine



analogues of our previously studied guanidiniocarbonylpyrroles **1a** and **1b** (Scheme 1), while in receptor **3** the direction of the amide group in position 6 of the pyridine ring is reversed relative to **2a**.

Scheme 3 describes the synthesis of the 2-(guanidiniocarbonyl)pyridine **2a**. The pyridinecarboxylic acid **6** was synthesized by literature methods.[6,7] Commercially available pyridine-2,6-dicarboxylic acid (**4**) was converted into the dimethyl ester **5** with methanol in the presence of a catalytic amount of H_2SO_4. The dimethyl ester was then hydrolyzed selectively at only one of its two ester functions to provide **6**. Coupling of the mono-acid with ethylamine in the presence of benzotriazolyloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) as the coupling reagent gave the corresponding amide **7**. To obtain the Boc-protected receptor **10**, the methyl ester function of **7** was hydrolyzed with LiOH in methanolic solution and after activation with PyBOP the carboxylic acid **8** was coupled with mono-Boc-protected guanidine **9**.[8] Deprotection of **10** with trifluoroacetic acid and precipitation of the picrate salt from a methanolic solution provided the ethyl amide-substituted receptor **2a**.

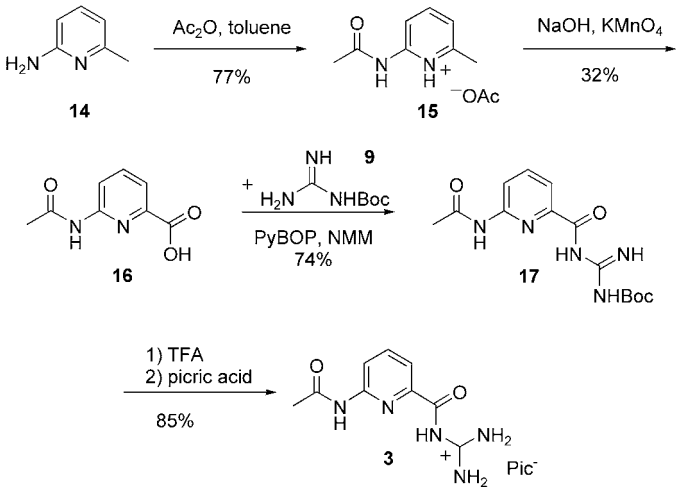
The valine-substituted receptor **2b** was synthesized accordingly (Scheme 4). Coupling of **6** with H-Val-NH_2 by use of dicyclohexylcarbodiimide (DCC) in the presence of catalytic amounts of 4-dimethylaminopyridine (DMAP) yielded the ester **11**, which was hydrolyzed by treatment with KOH in MeOH. The crude potassium salt **12** was treated directly



Scheme 4. Synthesis of receptor **2b**.

with mono-Boc-guanidine **9** without further purification. After deprotection of **13** with TFA, the picrate salt of receptor **2b** was isolated from MeOH.

The synthesis of the 2-(guanidiniocarbonyl)pyridine **3** is shown in Scheme 5. The commercially available 6-methyl-



Scheme 5. Synthesis of receptor **3**.

pyridin-2-ylamine (**14**) was acylated with acetic anhydride in toluene by a literature procedure.[9] The methyl group of the pyridinium salt **15** was oxidized with $KMnO_4$ to provide the pyridinecarboxylic acid **16**,[9,10] which after activation with PyBOP was coupled with the mono-Boc-protected guanidine **9** to yield the Boc-protected receptor **17**. The last step was the deprotection of **17** with TFA and the crystallization of the picrate salt of the receptor **3** from methanol.

The binding properties of these receptors were then studied in aqueous DMSO (40% water in DMSO) by NMR titration experiments with various amino acids in their carboxylate forms. This specific solvent mixture was chosen to allow a direct comparison of the binding data with those obtained earlier for our guanidiniocarbonylpyrroles.[1] All three receptors are indeed able to bind amino acid carboxylates even under these polar conditions, as can be seen by significant complexation-induced shift changes upon addition of the carboxylate (NMe_4^+ salt) to a solution of the receptor (1.5 mM or 1.0 mM, picrate salt).[11]

Figure 1 shows a typical data set: the chemical shift changes in the ^{1}H NMR spectrum of receptor **2b** upon addition of increasing equivalents of Ac-Gly-O^- (**18**). A downfield shift of the signal for the amide proton NH^c and upfield shifts of the signals for the protons of the amide function of the valine moiety $NH_2^{a,b}$ and the pyridine CH protons are observed. The doublet of the amide NH^c shifts from $\delta = 9.1$ ppm to $\delta = 9.4$ ppm and the broad singlets of the amide protons (a, b) shift from $\delta = 7.9$ ppm to $\delta = 7.8$ ppm and from $\delta = 7.2$ ppm to $\delta = 7.1$ ppm. The guanidinium NH protons cannot be detected under these conditions, due to fast exchange of these protons with the solvent (40% water). Equivalent shift changes are observed for the

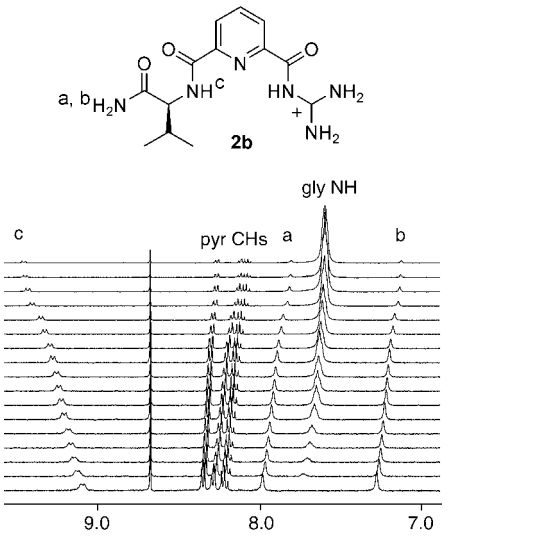


Figure 1. ^{1}H NMR spectrum of receptor **2b** (picrate salt, 1.5 mM) in the presence of increasing amounts of added Ac-Gly-O^- (**18**; NMe_4^+ salt) in 40% water in [D_6]DMSO, showing the complexation-induced shift changes (from bottom to top: 0 to 10 equivalents **18**).

protons of the substrate. The glycine amide NH shifts to higher field, from $\delta = 7.7$ ppm to $\delta = 7.6$ ppm, which means that upon complexation this proton experiences an analogous downfield shift (at the beginning of the titration the substrate is completely complexed by the excess of the receptor, whereas at the end of the titration the substrate is present in excess and hence in uncomplexed form). These shift changes not only indicate an intermolecular interaction between receptor and carboxylate but are also consistent with the general binding motif shown in Scheme 2: an ion pair between the carboxylate and the guanidinium cation, which is further enhanced by a hydrogen bond from the adjacent amide. As anion binding can be regarded as the beginning of deprotonation it would be expected that the electron density in the guanidiniocarbonylpyridine moiety should increase upon complexation, which is in good agreement with the observed upfield shifts of the pyridine CHs. Only those protons that actively participate in hydrogen bonds (the amide NH^c in this case) show a downfield shift.

From this shift change of the amide NH of the receptor, as well as the upfield shifts of the pyridine CHs, the corresponding binding constant can be calculated quantitatively by use of nonlinear least-squares fitting with a 1:1 association model.[11] This was carried out for all three receptors with the following amino acid carboxylates: Gly (**18**), Phe (**19**), Val (**20**), and Ala (**21**) in the forms of their NMe_4^+ salts. The results of these quantitative binding studies are summarized in Table 1, Table 2, and Table 3. In all cases there was an excellent fit of the measured data with the theoretical 1:1 complexation model, as exemplified by the binding isotherm shown in Figure 2. The stoichiometry was independently confirmed by Job plots from the titration data, all of which showed clean 1:1 complex formation (Figure 3).[12] Where the binding constants could be calculated by follow-

Table 1. Binding constants (K_{ass}) and free energies of complexation (ΔG_{ass}) for receptor **2a** (picrate salt, 1.0 mM) with various amino acid carboxylates **18**–**21** (NMe_4^+ salts) in water/[D_6]DMSO (40% v/v) at 25°C.

	Carboxylate	K_{ass} [M^{-1}][a]	$-\Delta G_{ass}$ [kJ mol^{-1}]
18	Ac-L-Gly-O^-	220	13.4
19	Ac-L-Phe-O^-	230	13.5
20	Ac-L-Val-O^-	330	14.4
21	Ac-L-Ala-O^-	460	15.2

[a] Error limits in K were estimated as $< \pm 10\%$.

Table 2. Binding constants (K_{ass}) and free energies of complexation (ΔG_{ass}) for receptor **2b** (picrate salt, 1.5 mM) with various amino acid carboxylates **18**–**22** (NMe_4^+ salts) in water/[D_6]DMSO (40% v/v) at 25°C.

	Carboxylate	K_{ass} [M^{-1}][a]	$-\Delta G_{ass}$ [kJ mol^{-1}]
18	Ac-L-Gly-O^-	190	13.0
19	Ac-L-Phe-O^-	240	13.6
20	Ac-L-Val-O^-	240	13.6
21	Ac-L-Ala-O^-	360	14.6
22	Ac-D-Ala-O^-	330	14.4

[a] Error limits in K were estimated as $< \pm 10\%$.

Table 3. Binding constants (K_{ass}) and free energies of complexation (ΔG_{ass}) for receptor **3** (picrate salt, 1.5 mM) with various amino acid carboxylates **18**–**21** (NMe_4^+ salts) in water/[D_6]DMSO (40% v/v) at 25°C.

	Carboxylate	K_{ass} [M^{-1}][a]	$-\Delta G_{ass}$ [kJ mol^{-1}]
18	Ac-L-Gly-O^-	90	11.1
19	Ac-L-Phe-O^-	30	10.1
20	Ac-L-Val-O^-	160	12.6
21	Ac-L-Ala-O^-	160	12.6

[a] Error limits in K were estimated as $< \pm 10\%$.

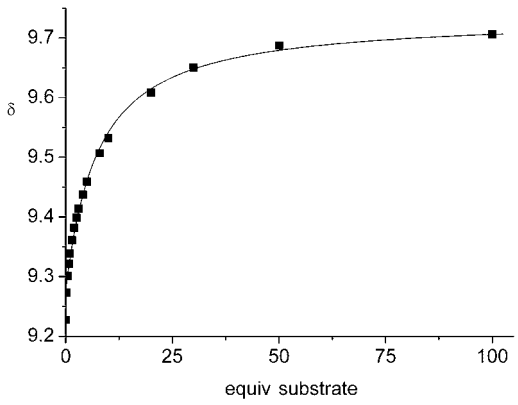


Figure 2. Binding isotherm for the amide NH of receptor **2a** for the complexation of phenylalanyl carboxylate (**19**) in 40% water in [D_6]DMSO.

ing the shift changes of more than one proton the results were consistent and within the margin of experimental error (estimated as $\pm 10\%$ in K_{ass}).

Inspection of these data showed that all the pyridine-based receptors bound carboxylates in aqueous DMSO with association constants of up to $K_{ass} \approx 5 \times 10^2\ M^{-1}$, depending both on the substrate and on the receptor. The following general trends can be deduced from these data. For all four amino acid carboxylates studied (Gly, Ala, Val, and Phe)

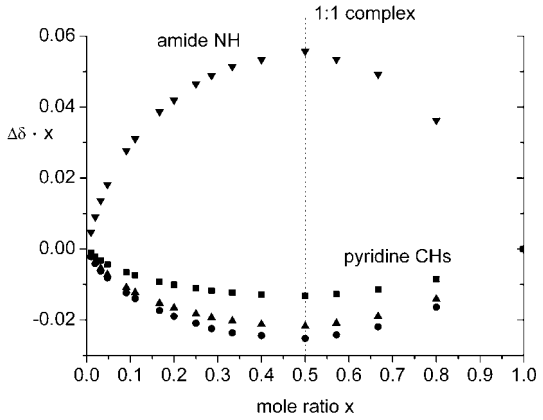


Figure 3. Job plot analysis for the complexation of receptor **2a** with Phe **19**.

the association constants increase in the order **3** < **2b** ≤ **2a** with respect to the receptor. Hence, receptor **3**, with the reversed amide group, is the least efficient, with association constants all around $10^2\ M^{-1}$ (Table 3). Binding by the valine derivative **2b** (Table 2) is about three times more efficient. For example, the binding constant for alanine increases from $K = 160\ M^{-1}$ for **3** to $K = 360\ M^{-1}$ for **2b**. For the ethyl amide receptor **2a** (Table 1) the binding constants for Ala and Val further increase significantly relative to **2b** (e.g., $K = 450\ M^{-1}$ for alanine), whereas Gly and Phe are bound essentially identically by **2a** and by **2b**. Hence, the strongest complexation for all amino acids is observed for the ethyl amide receptor **2a**.

For a given pyridine receptor, Ala is always bound better than Val, which again is always bound better than Gly. Hence, the general order of selectivity is Gly < Val < Ala. Only the binding of Phe is quite different for the three receptors. In the case of **3**, Phe is bound even more poorly than Gly ($K = 30\ M^{-1}$ versus $K = 90\ M^{-1}$, respectively). For **2b**, Phe is bound as strongly as Val ($K = 240\ M^{-1}$). Receptor **2a** binds Phe as efficiently as receptor **2b** ($K = 230\ M^{-1}$ versus $K = 240\ M^{-1}$, respectively), but as mentioned above, **2a** binds Ala and Val even better, which changes the order of selectivity to Gly < Phe < Val < Ala.

The binding with the pyridine systems is generally less efficient than that with the pyrrole receptors. For example, the valine amide-substituted guanidiniocarbonylpyrrole **1b** binds alanine (carboxylate form) with $K = 1630\ M^{-1}$, whereas the pyridine analogue **2b** binds the same substrate under the same experimental conditions only with $K = 360\ M^{-1}$. For the ethyl amide derivatives **1a** and **2a**, the binding constants for alanine carboxylate differ by a factor of roughly two ($K = 800\ M^{-1}$ and $450\ M^{-1}$, respectively). Interestingly, this results in a reversal in selectivity between **2a** and **2b** for Ala, as in the pyrrole series the analogous valine derivative showed the highest association constants, whereas in the pyridine series the ethyl amide receptor **2a** is the best. The difference between pyrrole and pyridine receptors is even more pronounced for Phe. Because of a favorable cation–π-interaction between the aromatic ring and the guanidiniocar-

bonylpyrrole moiety, Phe is bound with $K = 1200\,\text{M}^{-1}$ by the pyrrole receptor **1a**, but only with $K = 230\,\text{M}^{-1}$ by the pyridine receptor **2a**, which has a much lower electron density in the heterocycle.

Hence, the general binding features of the newly developed pyridine receptors discussed above can be summarized as follows:

- Pyridine-based receptors are all less efficient than the corresponding pyrroles.
- The stability of the complex decreases with increasing steric demand in the receptor (**2a** versus **2b**).
- The "normal" amide in **2a** and **2b** is superior to the "reversed" amide in **3**.

How can one understand these trends? Unfortunately, no exact determination of the actual complex structures in solution was possible, as no NOE (nuclear Overhauser effect) signals could be detected in the NMR. This is not surprising, however, because the complexes are still rather dynamic, as underlined by the fast proton exchange with the solvent. We therefore turned to molecular modeling calculations (Macromodel V 8.0. Amber* force field, GB/SA water solvation)[13] to investigate possible reasons for the observed differences in complex stability, and a consistent picture indeed emerged from these calculations. Of course, any interpretation based on static complex structures (whether calculated or experimentally determined) is purely enthalpic and therefore neglects entropy effects such as changes in the solvation, which are of course also quite important factors for substrate binding in polar solvents.[3b,14] However, in comparison of *relative data* within a series of structurally related systems, as is the case here, solvation effects are quite often comparable and hence not decisive for *differences* in the binding properties. An enthalpic analysis can thus still be a reasonable approximation of the real situation and so can nevertheless help us better understand the molecular recognition event in order to allow the design of even more efficient receptors in the future.

Firstly, why are the pyridine-based receptors less efficient than the pyrroles? According to our calculations the binding motif for the ethyl amide receptor **2a** is essentially the same as with the corresponding pyrrole receptor **1a** (Figure 4): the carboxylate forms an ion pair with the guanidinium moiety, and an hydrogen bond from the amide NH to the carboxylate stabilizes the complex further. The N-acetyl group is located below the aromatic ring, with an attractive interaction between the substrate amide NH and the pyridine nitrogen. This picture is completely consistent with the observed shift changes in the NMR described above (Figure 1). As there are no explicit interactions with the side chain of the amino acid, the binding constants would not be expected to differ much for the various substrates, and indeed the experimentally determined binding constants for **2a** differ only by factor of two in the order Gly < Phe < Val < Ala, probably reflecting differences in the flexibility and steric demand of the bound substrate.

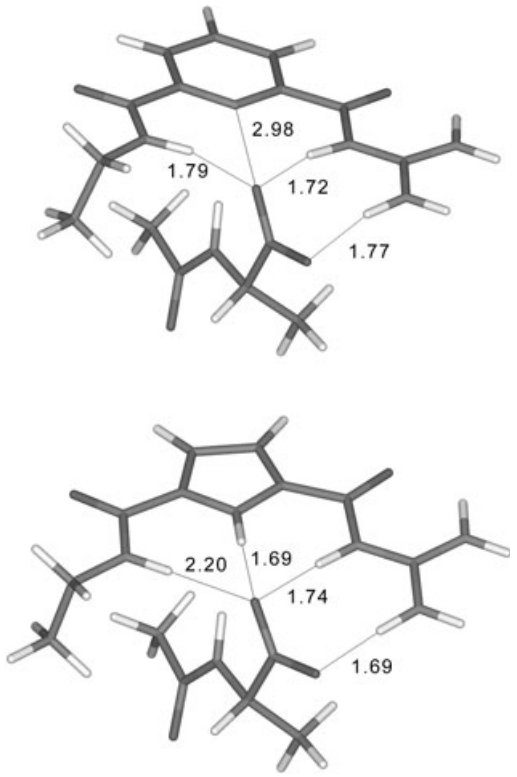
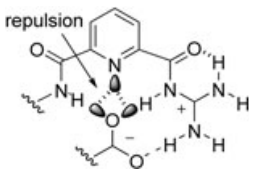


Figure 4. Calculated lowest-energy conformations of the complexes between alanine carboxylate **21** and the pyridine receptor **2a** (top) and the pyrrole receptor **1a** (bottom). The figures represent calculated O–H distances (in Å).

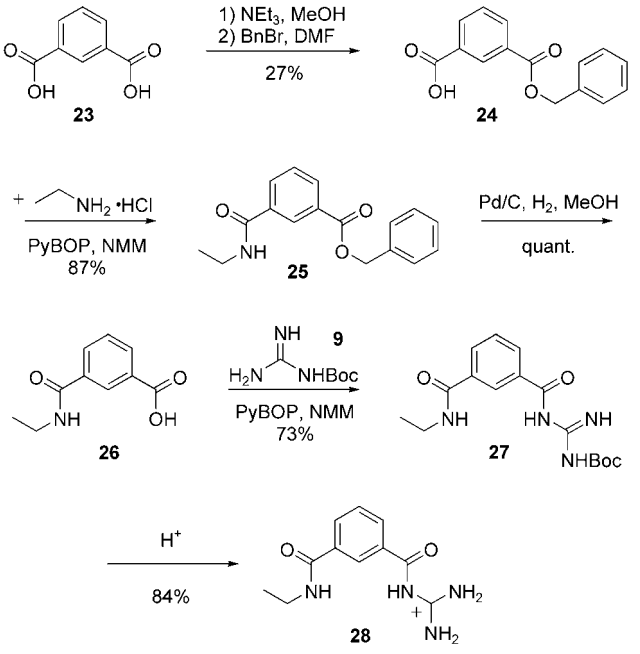
That the complexes are only half as stable as with the pyrrole receptors, despite their similar structures, is probably due to an unfavorable interaction between the pyridine nitrogen and the carboxylate.[15] Because of the difference in ring size, the calculated hydrogen-bonding distance to the ethyl amide NH is actually smaller for the pyridine receptor than for the pyrrole (1.79 versus 2.20 Å, respectively), whereas the distances to the two guanidinium amide NHs are slightly smaller for the pyrrole receptor. In the pyridine system, however, the complexation brings the carboxylate oxygen into close contact with the pyridine nitrogen (< 3 Å). The resulting dipole repulsion between the lone pairs on the two heteroatoms could be the reason for the decreased complex stability in the pyridine series (Scheme 6). On the one hand, one would expect the nitrogen lone pair to help orientate the two neighboring amide NHs inwards, thereby favoring the specific receptor conformation needed for substrate binding.[15] On the other hand, its interaction with the negatively charged substrate in the complex is repulsive. Obviously the latter effect domi-



Scheme 6. The repulsion between the lone pairs of the pyridine nitrogen and the anionic carboxylate oxygen is responsible for the less efficient binding in relation to the pyrrole systems.

nates. Calculations show that the energetic differences between the various receptor conformations are negligible in water. Hence, any conformational effect of the pyridine nitrogen on the receptor structure is energetically not advantageous for the binding event, and so the repulsive dipole interaction, which is only present in the complex, disfavors substrate binding. This finding is in good agreement with observations made by, for example, Crabtree,[16] Kilburn,[17] or Jeong[18] and their respective co-workers, who also reported similar destabilizing effects of the pyridine nitrogen on binding of both anionic and neutral substrates in organic solvents by pyridine-based receptors. This repulsive dipole interaction with the pyridine nitrogen lone pair is therefore the most likely reason why the pyridine receptors are less efficient than the guanidiniocarbonylpyrroles. However, this electrostatic effect is attenuated by the highly polar solvent, leading overall only to modest differences in complex stabilities, as electrostatic interactions strongly depend on the polarity of the surrounding medium. As an example, the destabilizing effect of a pyridine nitrogen lone pair on anion binding in methylene chloride has been reported to cause a decrease in complex stabilities of a factor of up to 40 in relation to a benzene system.[16]

This is furthermore supported by comparison of **2a** with the binding properties of an analogous benzene receptor **28**, in which the lone pair of the pyridine nitrogen atom is replaced by an aromatic CH bond. The synthesis of **28** is described in Scheme 7: the monobenzyl ester **24** was obtained

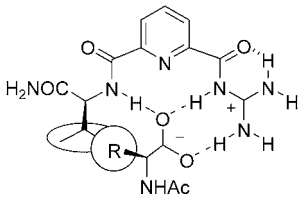


Scheme 7. Synthesis of the benzene receptor **28**.

by a known literature procedure starting from isophthalic acid (**23**).[19] Coupling of the monoacid **24** with ethylamine in the presence of PyBOP as the coupling reagent gave amide **25**. Cleavage of the benzyl ester in **25** was achieved in quantitative yields by hydrogenolysis (Pd/C) to yield **26**. The guanidiniocarbonyl moiety was again introduced by coupling with the mono-Boc-protected guanidine **9**, followed by acidic deprotection to give the free receptor **28**.

The association constants for the binding of amino acid carboxylates by the benzene receptor **28** are larger than those for the pyridine system **2a** but smaller than those for the corresponding pyrrole receptor **1a**. Ala (**21**), for example, is bound with $K = 600\,\mathrm{M}^{-1}$ by **28**, in comparison with $K = 450\,\mathrm{M}^{-1}$ for **2a** and $K = 800\,\mathrm{M}^{-1}$ for **1a**. Also, glycine carboxylate (**18**) is bound with $K = 420\,\mathrm{M}^{-1}$ by **28** but only with $K = 220\,\mathrm{M}^{-1}$ by **2a**. This demonstrates that the nitrogen lone pair is indeed sterically more demanding than an aromatic CH and exerts a destabilizing electrostatic effect on anion binding, in accordance with earlier findings.[15–17] Hence, the efficiency of the guanidiniocarbonyl receptors increases in the series pyridine < benzene < pyrrole.

Secondly, why is the valine derivative **2b** less efficient than the ethyl amide receptor **2a** in binding amino acids such as Ala and Val? This probably reflects unfavorable steric interactions between the isopropyl group in the receptor and the amino acid side chains. In the pyrrole receptor **1b**, we postulated an additional hydrogen bond from the terminal carboxamide group to explain both the increased complex stability relative to the ethyl amide derivative **1a** and the stereoselectivity (L-Ala is bound three times more strongly than D-Ala).[1] However, there seems to be no such attractive binding interaction with the terminal carboxamide group in the pyridine receptor **2b**, as no stereoselectivity is observed (D- and L-Ala are bound with essentially the same binding constant: $K = 350\,\mathrm{M}^{-1}$ and $K = 330\,\mathrm{M}^{-1}$, respectively). Furthermore, no downfield shift in the NMR is observed for these NH protons (Figure 1), as would be expected were they to be participating in a hydrogen bond to the carboxylate (and was observed for **1b**). Hence, the valine residue of **2b** probably only exerts a steric effect, which is by nature repulsive (Scheme 8). Therefore, the binding constants should either be similar to those found with the ethyl amide receptor **2a** (Gly, Phe) or be even smaller in the event of steric interactions (Val, Ala), as is indeed observed.



Scheme 8. Unfavorable steric interactions between the isopropyl group of the receptor **2b** and the amino acid side chain could be the reason for the less efficient binding relative to the ethyl amide receptor **2a**.

Thirdly, why is the reversed amide in receptor **3** less efficient? Because of the geometry of the binding site, the NH of this amide is further away from the oxygens of the bound carboxylate than in **2a** or **2b**. For example, the calculated distance between the two amide NHs on either side of the

pyridine ring is 3.5 Å in **3** and 2.7 Å in **2a** and **2b** (Figure 5)! Hence, simultaneous interaction of the carboxylate both with the two guanidinium NH groups *and* with the acetyl amide NH group on the other side of the heterocycle is less efficient in **3**, which could explain the reduced complex stability. Furthermore, as there are no significant steric interactions, the binding constants for the various amino acids are again rather similar ($K = 30$–$160\,\text{M}^{-1}$).

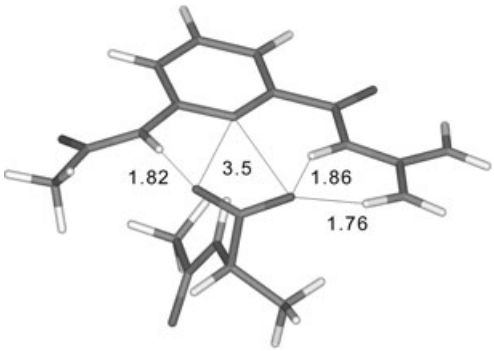


Figure 5. Calculated lowest-energy conformation of the complex between alanine carboxylate **21** and the pyridine receptor **3**.

Conclusion

In conclusion, we present here the synthesis and evaluation of the binding properties of a new class of hitherto unknown guanidiniocarbonylpyridine receptors. By NMR titration experiments it has been demonstrated that such receptors can be used to bind amino acid carboxylates effectively in aqueous solvents, with binding constants ranging from $K = 30$–$460\,\text{M}^{-1}$. Because of the electrostatic repulsion between the pyridine nitrogen lone pair and the bound carboxylate, however, these complexes are less stable than those formed with the analogous pyrrole—or even benzene—receptors. Such comparative binding studies should help us in better understanding the factors necessary to achieve strong and selective complexation of amino acid carboxylates in water.

Experimental Section

General remarks: Solvents were dried and distilled before use. The starting materials and reagents were used as obtained from Aldrich, Fluka, or Lancaster. All experiments were run in oven-dried glassware. The compounds were dried in high vacuum over phosphorus pentoxide at room temperature overnight unless otherwise stated. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer. The chemical shifts are reported relative to the deuterated solvents. The EI-mass spectra were recorded on a Finnigan MAT 90 instrument, the ESI- and HR-mass spectra were recorded on a Finnigan MAT 900 S.

6-(Methoxycarbonyl)pyridine-2-carboxylic acid (6): A solution of pyridine-2,6-dicarboxylic acid (**4**; 6.85 g, 41.0 mmol) and concentrated sulfuric acid (1 mL) in methanol (35 mL) was heated at 70 °C for two days. After cooling to room temperature the suspension was neutralized with a saturated aqueous sodium hydrogencarbonate solution. The methanol was removed under reduced pressure, and the aqueous suspension was dissolved in chloroform (30 mL). The organic layer was separated, washed with water (3×10 mL) and brine (15 mL), and dried over $MgSO_4$. The solvent was removed in vacuo to provide dimethyl pyridine-2,6-dicarboxylate (**5**; 5.89 g, 78 %) as a white solid. This crude product was used in the following step without further purification.

A solution of dimethyl pyridine-2,6-dicarboxylate (**5**; 5.85 g, 30.0 mmol) in methanol (150 mL) was cooled to 0 °C. KOH pellets (1.76 g, 31.0 mmol) were added, and the reaction mixture was stirred at 0 °C for 2 h and then at room temperature for 24 h. The solvent was removed under reduced pressure, and the residue was suspended in ethyl acetate (150 mL). The white potassium salt was collected by filtration and was then dissolved in water (100 mL). The solution was acidified to pH 3 with concentrated hydrochloric acid and extracted with chloroform (4× 40 mL). The collected organic layers were dried over $MgSO_4$, and the chloroform was removed in vacuo to provide the desired product **6** (2.90 g, 53 %) as a white solid: m.p. 146 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25 °C, TMS): $\delta = 3.91$ (s, 3H), 8.14–8.22 ppm (m, 3H); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 25 °C, TMS): $\delta = 52.6$, 127.6, 127.8, 139.0, 147.6, 148.9, 164.8, 165.6 ppm; IR (KBr): $\tilde{\nu} = 3073$, 2966, 2634, 1725, 1580, 1325 cm^{-1}.

Methyl 6-(ethylcarbamoyl)pyridine-2-carboxylate (7): A solution of PyBOP (2.87 g, 5.52 mmol) and *N*-methyl-morpholine (NMM; 410 mg, 4.05 mmol) in DMF (10 mL) was added to a solution of 6-(methoxycarbonyl)pyridine-2-carboxylic acid (**6**; 1.00 g, 5.52 mmol), ethylamine hydrochloride (450 mg, 5.52 mmol), and NMM (410 mg, 4.05 mmol) in DMF (10 mL). The reaction mixture was stirred overnight at room temperature, and water (40 mL) and ethyl acetate (50 mL) were added. After separation of the organic layer, the aqueous layer was extracted with ethyl acetate (5×20 mL). The solvent was removed from the organic layers, and the residue was purified by flash column chromatography on silica gel (ethyl acetate/cyclohexane 1:1) to give **7** (598 mg, 52 %) as a white solid: m.p. 68 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25 °C, TMS): $\delta = 1.14$ (t, 3H), 3.35–3.40 (m, 2H), 3.93 (s, 3H), 8.15–8.25 (m, 3H), 8.56 ppm (brs, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 25 °C, TMS): $\delta = 14.8$, 33.8, 52.7, 125.1, 127.0, 139.3, 146.5, 150.5, 162.9, 164.7 ppm; IR (KBr): $\tilde{\nu} = 3541$, 3288, 2979, 1725, 1666, 1543, 1441, 1302, 1250 cm^{-1}; EI-MS: m/z: 208.1 $[M]^+$.

6-(Ethylcarbamoyl)pyridine-2-carboxylic acid (8): LiOH (115 mg, 4.80 mmol) was added to a solution of methyl 6-(ethylcarbamoyl)pyridine-2-carboxylate (**7**; 500 mg, 2.40 mmol) in methanol (10 mL). The reaction mixture was stirred at room temperature for 30 min, the methanol was removed under reduced pressure, and the residue was dissolved in water (25 mL). The solution was acidified to pH 5 with hydrochloric acid and extracted with ethyl acetate (5×20 mL). The collected organic layers were dried over Na_2SO_4 and evaporated to dryness to give the desired product **8** (242 mg, 52 %) as a white solid: m.p. 102 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25 °C, TMS): $\delta = 1.17$ (t, 3H), 3.35–3.42 (m, 2H), 8.18–8.27 (m, 3H), 8.56 ppm (brs, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 25 °C, TMS): $\delta = 14.8$, 33.7, 125.2, 126.4, 139.8, 146.0, 149.5, 162.6, 164.8 ppm; IR (KBr): $\tilde{\nu} = 3407$, 3260, 3080, 2979, 1729, 1657, 1558, 1460, 1362 cm^{-1}; EI-MS: m/z : 294.1 $[M]^+$.

***tert*-Butoxycarbonyl-[6-(ethylcarbamoyl)pyridine-2-carbonyl]guanidine (10)**: A solution of PyBOP (780 mg, 1.50 mmol) and NMM (205 mg, 2.03 mmol) in DMF (5 mL) was added to a solution of 6-(ethylcarbamoyl)pyridine-2-carboxylic acid (**8**; 291 mg, 1.50 mmol), Boc-protected guanidine (**9**; 239 mg, 1.50 mmol), and NMM (205 mg, 2.03 mmol) in DMF (5 mL). The reaction mixture was stirred at room temperature for one day. After addition of water (15 mL), a white solid crystallized. This solid was collected by filtration and washed several times with water and dried in vacuo to provide the desired product **10** (347 mg, 69 %) as a white solid: m.p. 208 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25 °C, TMS): $\delta = 1.20$ (t, 3H), 1.45 (s, 9H), 3.43 (m, 2H), 8.24–8.37 (m, 4H), 8.78 (brs, 1H), 9.28 ppm (brs, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 25 °C, TMS): $\delta = 15.1$, 27.9, 33.7, 77.9, 125.4, 125.8, 140.1, 149.2, 157.5, 162.5 ppm; IR (KBr): $\tilde{\nu} = 3395$, 2974, 1752, 1685, 1573, 1531, 1420, 1313 cm^{-1}; HR-MS (ESI) calcd for $[M+Na]^+$: 358.1491; found 358.149.

6-(Ethylcarbamoyl)pyridine-2-carbonylguanidinium picrate (2a): Trifluoroacetic acid (6 mL) was added to the protected receptor **10** (150 mg,

0.447 mmol). The solution was stirred at room temperature for 15 min. The excess trifluoroacetic acid was removed in vacuo, and the obtained residue was dissolved in methanol (25 mL). A saturated solution of picric acid in water (20 mL) was then added, and the mixture was stirred for one day at room temperature. The picrate salt **2a** crystallized and was filtered, washed several times with methanol, and dried to provide a yellow solid (180 mg, 87%): m.p. 260°C; ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25°C, TMS): δ = 1.22 (t, 3H), 3.45 (m, 2H), 8.30–8.39 (m, 3H), 8.50 (brs, 3H), 8.58 (s, 2H), 8.97 (brs, 1H), 11.39 ppm (brs, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 25°C, TMS): δ = 14.8, 34.0, 124.2, 125.2, 125.6, 126.5, 140.5, 141.8, 145.6, 149.2, 154.3, 160.8, 162.3, 164.1 ppm; IR (KBr): $\tilde{\nu}$ = 3421, 3330, 3089, 1731, 1656, 1530, 1326 cm^{-1}; ESI-MS: m/z: 236.35 $[M]^+$.

Methyl 6-[((*S*)-1-carbamoyl-2-methylpropyl)carbamoyl]pyridine-2-carboxylate (11): A solution of 6-(methoxycarbonyl)pyridine-2-carboxylic acid (**6**; 322 mg, 1.78 mmol), *N*,*N*-dimethylpyridin-4-ylamine (435 mg, 3.56 mmol), H-Val-NH$_2$ hydrochloride (272 mg, 1.78 mmol), and DCC (367 mg, 1.78 mmol) in dichloromethane (20 mL) was stirred at room temperature for three days. The solvent was then removed in vacuo and the crude product was purified by flash column chromatography on silica gel (ethyl acetate/cyclohexane/ methanol 4:4:1) to give **11** (383 mg, 77%) as a white solid: m.p. 150°C; ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25°C, TMS): δ = 0.91 (dd, 6H), 2.10 (m, 1H), 3.94 (s, 3H), 4.43 (dd, 1H), 7.24 (brs, 1H), 7.70 (brs, 1H), 8.18–8.26 (m, 3H), 8.39 ppm (d, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 25°C, TMS): δ = 17.7, 19.3, 31.4, 52.8, 57.1, 125.2, 127.4, 139.6, 146.4, 149.5, 162.4, 164.4, 172.2 ppm; IR (KBr): $\tilde{\nu}$ = 3384, 2956, 1731, 1665, 1530, 1328 cm^{-1}; EI-MS: m/z : 279.1 $[M]^+$.

***tert*-Butoxycarbonyl-{6-[((*S*)-1-carbamoyl-2-methylpropyl)carbamoyl]pyridine-2-carbonyl}guanidine (13)**: A solution of methyl 6-[((*S*)-1-carbamoyl-2-methylpropyl)carbamoyl]pyridine-2-carboxylate (**11**; 900 mg, 3.22 mmol) and KOH (181 mg, 3.22 mmol) in methanol (30 mL) was heated at 45°C for 5 h. The solvent was removed under reduced pressure. The obtained white solid, Boc-protected guanidine (**9**; 513 mg, 3.22 mmol), and NMM (410 mg, 4.05 mmol) were dissolved in DMF (10 mL). To this solution was added a solution of PyBOP (1.67 g, 3.22 mmol) and NMM (410 mg, 4.05 mmol) in DMF (10 mL). The reaction mixture was stirred at room temperature for one day. After addition of water (100 mL), the solution was extracted with diethyl ether (3× 50 mL). The combined organic phases were dried over MgSO$_4$, and the solvent was removed in vacuo. The yellow crude product was purified by flash column chromatography on silica gel (ethyl acetate/cyclohexane/ methanol 8:8:3) to give **13** (500 mg, 38%) as a white solid: m.p. 165°C; ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25°C, TMS): δ = 0.96 (dd, 6H), 1.43 (s, 9H), 2.22 (m, 1H), 4.37 (dd, 1H), 7.15 (brs, 1H), 7.62 (brs, 1H), 8.23–8.35 (m, 3H), 8.72 (brs, 1H) 8.87 ppm (brs, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 25°C, TMS): δ = 18.9, 19.4, 27.8, 30.5, 58.2, 78.5, 126.1, 139.8, 149.1, 157.6, 162.7, 172.3 ppm; IR (KBr): $\tilde{\nu}$ = 3385, 2972, 1670, 1573, 1414, 1308, 1142 cm^{-1}; HR-MS (ESI) calcd for $[2\times M+\mathrm{Na}]^+$: 835.3826; found 835.383.

6-[((*S*)-1-Carbamoyl-2-methylpropyl)carbamoyl]pyridine-2-carbonylguanidinium picrate (2b): Trifluoroacetic acid (6 mL) was added to the protected receptor **13** (150 mg, 0.369 mmol). The reaction mixture was stirred at room temperature for 90 min, the excess trifluoroacetic acid was removed in vacuo, and the obtained residue was dissolved in methanol (2 mL). A saturated solution of picric acid in water (5 mL) was then added and the mixture was stirred for one day. The salt **2b** crystallized, and was filtered, washed with mixture of water and methanol (1:1), and dried to provide a yellow solid (150 mg, 76%): m.p. 162°C; ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25°C, TMS): δ = 0.97 (d, 6H), 2.18 (m, 1H), 4.31 (dd, 1H), 7.16 (brs, 1H), 7.67 (brs, 1H), 8.30–8.39 (m, 3H), 8.44 (brs, 4H), 8.58 (s, 2H), 8.65 (d, 1H), 11.73 ppm (brs, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 25°C, TMS): δ = 19.0, 19.4, 30.0, 58.8, 124.2, 125.2, 126.0, 127.0, 140.3, 141.9, 146.3, 149.1, 154.5, 154.6, 160.8, 162.8, 164.6, 168.8, 172.6 ppm; IR (KBr): $\tilde{\nu}$ = 3381, 3200, 2993, 1728, 1643, 1566, 1313 cm^{-1}; ESI-MS: m/z: 307.30 $[M]^+$.

6-Acetylamino-2-methylpyridinium acetate (15): A mixture of 6-methylpyridin-2-ylamine (**14**; 21.6 g, 200 mmol) and acetic anhydride (43.3 g, 424 mmol) in toluene (150 mL) was heated at 95°C. The solvent and the excess acetic anhydride was removed in vacuo. The product had slowly crystallized after one day and was filtered and washed several times with hexane. The product was dried to provide **15** (32.4 g, 77%) as a white solid: m.p. 62°C; ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25°C, TMS): δ = 1.80 (s, 3H), 2.06 (s, 3H), 2.38 (s, 3H), 6.92 (d, 1H), 7.62 (t, 1H), 7.86 (d, 1H), 10.36 (s, 1H), 11.90 ppm (brs, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 25°C, TMS): δ = 21.2, 23.7, 24.1, 110.4, 118.6, 138.6, 151.8, 156.6, 169.4, 172.3 ppm; IR (KBr): $\tilde{\nu}$ = 3242, 3067, 1903, 1705, 1580, 1455, 1309 cm^{-1}.

6-Acetylaminopyridine-2-carboxylic acid (16): A solution of NaOH (1.90 g, 47.6 mmol) in water (15 mL) was added to a solution of 6-acetylamino-2-methylpyridinium acetate (**15**; 10.0 g, 47.6 mmol) in water (70 mL). The obtained suspension was heated to 70°C, and potassium permanganate (45.1 g, 94.9 mmol) was added in small increments over a period of 30 min. The reaction mixture was heated at reflux for 30 min and filtered, the residue was washed several times with boiling water (20 mL), the washings and the filtrate were combined, and the volume was decreased to 40 mL in vacuo. The yellow solution was acidified to pH ≈4 with concentrated hydrochloric acid. The white solid was filtered and dried to provide the desired product **16** (2.74 g, 32%) as a white solid: m.p. 217°C; ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25°C, TMS): δ = 2.09 (s, 3H), 7.71 (d, 1H), 7.92 (t, 1H), 8.26 (d, 1H), 10.79 ppm (s, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 25°C, TMS): δ = 23.9, 116.9, 120.1, 139.4, 146.9, 152.1, 165.9, 169.8 ppm; IR (KBr): $\tilde{\nu}$ = 3413, 3251, 2925, 2607, 1726, 1644, 1538, 1452, 1306 cm^{-1}.

(6-Acetylaminopyridine-2-carbonyl)-*tert*-butoxycarbonylguanidine (17): A solution of PyBOP (2.89 g, 5.55 mmol) and NMM (615 mg, 6.08 mmol) in DMF (15 mL) was added to a solution of 6-acetylaminopyridine-2-carboxylic acid (**16**; 1.00 g, 5.55 mmol), Boc-protected guanidine (**9**, 884 mg, 5.55 mmol), and NMM (615 mg, 6.08 mmol) in DMF (15 mL). The reaction mixture was stirred overnight at room temperature. After addition of water (100 mL) and diethyl ether (50 mL) a white solid crystallized and the suspension was stirred for 10 min at room temperature. The solid was filtered, washed with diethyl ether, and dried to provide the desired product **17** (1.32 g, 74%) as a white solid: m.p. 154°C; ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25°C, TMS): δ = 1.42 (s, 9H), 2.13 (s, 3H), 7.84 (d, 1H), 8.04 (t, 1H), 8.35 (d, 1H), 8.85 (brs, 1H), 10.36 (brs, 1H), 10.91 ppm (brs, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 25°C, TMS): δ = 23.9, 27.9, 78.1, 117.5, 117.9, 140.4, 151.2, 157.5, 169.7, 169.7 ppm; IR (KBr): $\tilde{\nu}$ = 3467, 3386, 3130, 2983, 1703, 1672, 1555, 1407, 1313, 1149 cm^{-1}; HR-MS (ESI) calcd for $[M+\mathrm{Na}]^+$: 344.1335; found 344.134.

(6-Acetylaminopyridine-2-carbonyl)guanidinium picrate (3): Trifluoroacetic acid (10 mL) was added to the protected receptor **17** (150 mg, 0.467 mmol), and the reaction mixture was stirred at room temperature for 15 min. The excess trifluoroacetic acid was removed in vacuo, and the obtained residue was dissolved in methanol (40 mL). A saturated solution of picric acid in water (50 mL) was then added and the mixture was stirred for 1 h. The salt **3** crystallized and was filtered and dried to provide a yellow solid (179 mg, 85%): m.p. 245°C; ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25°C, TMS): δ = 2.16 (s, 3H), 7.86 (dd, 1H), 8.09 (t, 1H), 8.29 (d, 1H), 8.40 (brs, 2H), 8.58 (s, 2H), 8.61 (brs, 2H), 10.38 (s, 1H), 11.21 ppm (brs, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 25°C, TMS): δ = 23.9, 119.0, 119.3, 124.2, 125.2, 140.4, 141.8, 145.6, 151.1, 154.6, 160.8, 163.2, 165.0, 169.6 ppm; IR (KBr): $\tilde{\nu}$ = 3361, 3200, 1739, 1692, 1643, 1569, 1298 cm^{-1}; ESI-MS: m/z: 222.26 $[M]^+$.

Isophthalic acid monobenzyl ester (24): A solution of triethylamine (2.45 g, 24.2 mmol) in methanol (25 mL) was added to a suspension of isophthalic acid (**23**; 4.00 g, 24.1 mmol) in methanol (50 mL) and water (5 mL). The reaction mixture was stirred at room temperature overnight. The methanol was removed under reduced pressure and the residue was dried in vacuo. The oily residue was dissolved in DMF (60 mL), benzyl bromide (4.53 g, 26.5 mmol) was added dropwise, and the solution was heated at 100°C for 2 h. After cooling to room temperature, the mixture was poured into aqueous sodium hydrogen carbonate (5%, 120 mL) and extracted with ethyl acetate (3×70 mL) to remove diester impurities. The aqueous layer was acidified to pH 3–4 with concentrated hydrochloric acid and was then extracted with ethyl acetate (3×70 mL). The collected organic layers were dried over MgSO$_4$ and evaporated to dryness. The white solid was purified by flash column chromatography on silica gel

(diethyl ether/hexane 1:1) to give the desired product **24** (1.67 g, 27%) as a white solid: m.p. 108°C; ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25°C, TMS): δ = 5.38 (s, 2H), 7.34–7.50 (m, 5H), 7.67 (t, 1H), 8.19–8.23 (m, 2H), 8.50 (dd, 1H), 13.32 ppm (brs, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 25°C, TMS): δ = 66.6, 128.1, 128.2, 128.6, 129.5, 129.8, 130.0, 131.4, 133.3, 133.9, 135.9, 164.9, 166.4 ppm; IR (KBr): $\tilde{\nu}$ = 3028–2551, 1719, 1705, 1690, 1609, 1269, 728 cm^{-1}.

Benzyl 3-(ethylcarbamoyl)benzoate (25): A solution of ethylamine hydrochloride (382 mg, 4.68 mmol) and NMM (437 mg, 4.68 mmol) in DMF (5 mL) was added to a solution of isophthalic acid monobenzyl ester (**24**; 1.20 g, 4.68 mmol), PyBOP (2.44 g, 4.68 mmol), and NMM (947 mg, 9.36 mmol) in DMF (5 mL). The reaction mixture was stirred overnight at room temperature, and water (40 mL) was then added. The solution was extracted with diethyl ether (3×50 mL), the collected organic layers were washed with brine (2×20 mL) and dried over $MgSO_4$, the solvent was removed under reduced pressure, and the residue was purified by flash column chromatography on silica gel (diethyl ether/hexane 1:1) to give **25** (1.16 g, 87%) as a white solid: m.p. 92°C; ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25°C, TMS): δ = 1.13 (t, 3H), 3.30 (s, 2H), 5.39 (s, 2H), 7.34–7.50 (m, 5H), 7.62 (t, 1H), 8.10–8.13 (m, 2H), 8.45 (t, 1H), 8.69 ppm (t, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 25°C, TMS): δ = 14.7, 34.2, 66.4, 127.9, 128.0, 128.2, 128.5, 128.9, 129.7, 131.6, 131.9, 135.2, 136.0, 165.0, 165.2 ppm; IR (KBr): $\tilde{\nu}$ = 3366, 2971, 1715, 1637, 1545, 1258 cm^{-1}; EI-MS: m/z: 283.2 $[M]^+$.

3-(Ethylcarbamoyl)benzoic acid (26): A mixture of **25** (1.00 g, 3.53 mmol) and Pd/C (100 mg) in methanol (30 mL) was hydrogenated at room temperature for 30 min. The mixture was filtered over Celite to remove Pd/C, and the solvent was evaporated to give **26** (680 mg, 100%) as a white solid: m.p. 227°C; ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25°C, TMS): δ = 1.13 (t, 3H), 3.30 (m, 2H), 7.58 (t, 1H), 8.05–8.07 (dd, 2H), 8.42 (t, 1H), 8.65 ppm (t, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 25°C, TMS): δ = 14.7, 34.1, 128.0, 128.6, 131.1, 131.3, 131.6, 135.0, 165.1, 167.0 ppm; IR (KBr): $\tilde{\nu}$ = 3307, 3075–2551, 1686, 1635, 1542, 1322 cm^{-1}; EI-MS: m/z: 193.1 $[M]^+$.

***tert*-Butoxycarbonyl-(3-ethylcarbamoylbenzoyl)guanidine (27)**: A solution of Boc-protected guanidine (**9**; 412 mg, 2.59 mmol) and NMM (262 mg, 2.59 mmol) in DMF (5 mL) was added to a solution of the acid **26** (500 mg, 2.59 mmol), PyBOP (1.35 g, 2.59 mmol), and NMM (524 mg, 5.18 mmol) in DMF (5 mL). The reaction mixture was stirred overnight at room temperature, and water (40 mL) was then added. The solution was extracted with ethyl acetate (3×50 mL), the collected organic layers were washed with brine (2×25 mL) and dried over Na_2SO_4, the solvent was removed under reduced pressure, and the residue was purified by flash column chromatography on silica gel (diethyl ether/hexane/triethylamine 6:2:1) to give **27** (633 mg, 73%) as a white solid: m.p. 70°C; ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25°C, TMS): δ = 1.13 (t, 3H), 3.29 (m, 2H), 7.51 (t, 1H), 7.94 (d, 1H), 8.21 (d, 1H), 8.51–8.53 (m, 2H), 8.61 (brs, 1H), 9.63 (brs, 1H), 11.00 ppm (brs, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 25°C, TMS): δ = 14.7, 27.7, 34.1, 127.7, 127.9, 130.0, 131.1, 134.8, 137.4, 158.9, 165.8 ppm; IR (KBr): $\tilde{\nu}$ = 3379, 2965, 2925, 1730, 1638, 1541, 1243, 1151 cm^{-1}; HR-MS (ESI) calcd for $[M+Na]^+$: 357.1539; found 357.154.

(3-Ethylcarbamoylbenzoyl)guanidinium picrate (28): Trifluoroacetic acid (6 mL) was added to the Boc-protected receptor (**27**; 150 mg, 0.449 mmol). The solution was stirred at room temperature for 2 h, the excess trifluoroacetic acid was removed in vacuo, and the obtained residue was dissolved in methanol (5 mL). A saturated solution of picric acid in water (10 mL) was then added, and the mixture was stirred for 30 min at room temperature. The picrate salt **28** crystallized, and was filtered, washed several times with cold water, and dried to provide a yellow solid (175 mg, 84%): m.p. 230°C; ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25°C, TMS): δ = 1.15 (t, 3H), 3.32 (m, 2H), 7.72 (t, 1H), 8.05–8.07 (m, 1H), 8.15–8.17 (m, 1H), 8.29 (brs, 4H), 8.41 (t, 1H), 8.58 (s, 2H), 8.70 (t, 1H), 11.28 ppm (brs, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 25°C, TMS): δ = 14.7, 34.2, 124.1, 125.2, 127.1, 129.1, 130.6, 131.8, 131.9, 135.3, 141.8, 155.0, 160.8, 164.9, 167.1 ppm; IR (KBr): $\tilde{\nu}$ = 3410–3090, 1714, 1627, 1605, 1269 cm^{-1}; ESI-MS: m/z: 235.11 $[M]^+$.

NMR titrations: All NMR titrations were carried out by addition of aliquots of a 150 mM solution of the carboxylate (NMe_4^+ salt) to a 1 mM or 1.5 mM solution of the receptor (picrate salt) and recording of the chemical shifts after each addition. Presaturation of the water signal was used. Dilution was taken into account in analysis of the data. Each titration was performed with 15–20 measurements. Where possible, different NMR signals of the carboxylate were used to calculate the binding constants.

Molecular modeling: All calculations described in this paper were performed with the aid of the Macromodel 8.0 software package. Conformational searches were carried out with at least 10000 steps until the resulting minimum structure was found several times. The Amber* force field and the GB/SA water solvation model implemented in Macromodel were used in all studies.

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