Selective electrochemical sensing of acidic organic molecules *via* a novel guest-to-host proton transfer reaction†

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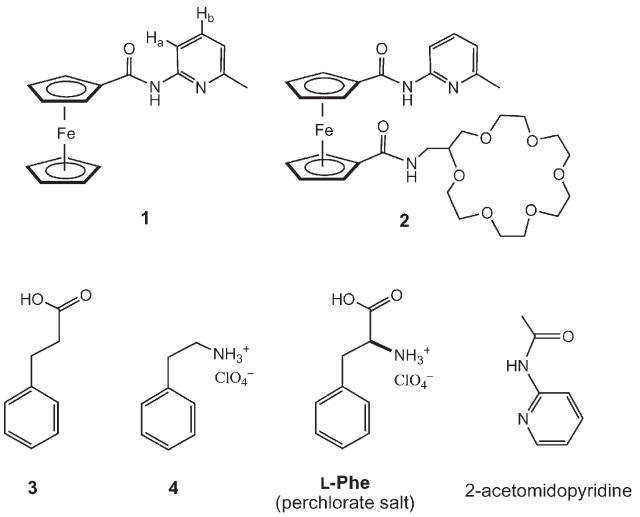
Ferrocene-containing amidopyridine receptors bind carboxylic acids and the amino acid phenylalanine in acetonitrile *via* a novel proton transfer process that enables guests to be electrochemically sensed by positive shifts in the ferrocene-centred redox potentials.

Among the numerous examples in the literature of redox-active supramolecular compounds that bind various charged and neutral species in solution,[1] receptors designed for the selective sensing of particular classes of organic molecule, for example amino acids, are very rare. This is in spite of general interest in the development of selective electrochemical sensors for bioorganic molecules[2] and recent progress in the development of related photo-active sensors for such species.[3] Herein we report two simple ferrocene receptors **1** and **2** that only bind and electrochemically respond to organic molecules containing a particularly acidic CO_2H motif, including the perchlorate salt of phenylalanine, as a result of a novel guest-to-host proton transfer reaction in acetonitrile solution.

The amidopyridine unit is well-established as a binder of the carboxylic acid motif in chlorinated organic solvents through two complementary H-bonds.[1d,4] However, in more competitive solvents such as acetonitrile, binding is much weaker, as observed here when the addition of an excess amount of hydrocinnamic acid **3** to a 10 mM solution of **1**[5] in CD_3CN brought about no change to the ^{1}H NMR spectrum of the receptor. In contrast, the addition of one molar equivalent of the perchlorate salt of **L-Phe** (*i.e.* **3** with an additional ammonium group attached, see ESI† for synthesis details) to **1** under the same conditions had a dramatic effect on its ^{1}H NMR spectrum (Fig. 1).

For example, the signal for the amide proton underwent a large downfield shift (δ = 8.34 to 9.76) to indicate a strong H-bonding interaction. Furthermore, large changes were observed to the three pyridine proton signals (*e.g.* py-H_a, δ = 8.02 to 7.57; py-H_b, 7.65 to 8.22) as well as to the two signals corresponding to the four cyclopentadienyl protons adjacent to the amidopyridine unit. The stoichiometry of the complex between the two species was established as 1 : 1 by an NMR Job plot. A titration of the downfield shift in the resonance for the H_b proton was carried out to obtain a binding constant‡ between **1** and **L-Phe** of 63 000 M^{-1} (293 K) using the EQNMR programme (see ESI† for Job plot and titration details).[1d]



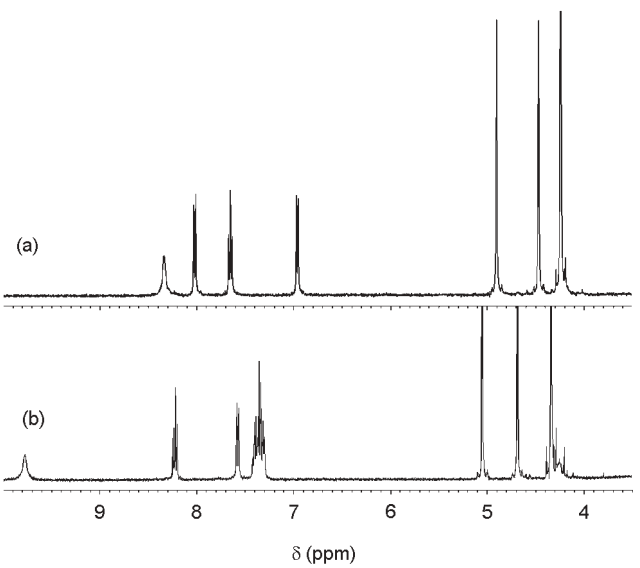


Fig. 1 ^{1}H NMR spectra in CD_3CN at 298 K of (a) compound **1** (10 mM) and (b) compound **1** plus one molar equivalent of the perchlorate salt of **L-Phe** (guest signals appear at 7.35 ppm and 4.25 ppm).

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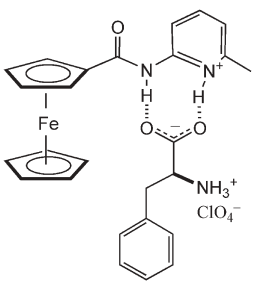
† Electronic supplementary information (ESI) available: Procedures for the synthesis of the perchlorate salt of **L-Phe** and receptor **2**, binding data (Job plot, MS, NMR), electrochemistry conditions and data. See http://dx.doi.org/10.1039/b510513g

The fact that the pyridine proton signals for **1** undergo such a marked change upon addition of L-**Phe** contrasts with the normal observation of only small changes to these signals upon binding the carboxylic acid motif in chlorinated organic solvents.[1d,4,6] This difference, and the strong binding in acetonitrile (the binding constant is at least one order of magnitude larger than those measured previously for the binding of organic molecules by related ferrocene receptors in less competitive solvents[1d]) suggest a different and much stronger H-bonding interaction with this guest, the origins of which must lie in its higher acidity (pK_a values for the CO_2H groups in L-**Phe** and **3**: 2.2[7a] and 4.7[7b] respectively§). Given that the pK_a value of the protonated amidopyridine group of the receptor is likely to lie between these values (*e.g.* protonated 2-acetomidopyridine has a pK_a value of 4.1[7c]), a thermodynamic driving force for proton transfer from the guest to the amidopyridine unit of the receptor exists in the case of the more acidic L-**Phe** but not with **3**, resulting in a strong ion-pair (salt-bridge) complex **1** : L-**Phe**, that consists of a protonated pyridine unit and a negatively charged carboxylate unit. Such an interaction would explain the large change to the amido and pyridine proton signals on the NMR spectrum. H-bond formation accompanied by proton transfer has been observed previously in the binding of acidic inorganic anions by ferrocene receptors.[8]



1 : L-**Phe** complex

In order to investigate the dependence of the guest pK_a on proton transfer in more detail, further NMR studies were carried out by adding an equimolar amount of the carboxylic acids chloroacetic acid (pK_a = 2.9), dichloroacetic acid (pK_a = 1.3) and trichloroacetic acid (pK_a = 0.7) to 10 mM solutions of **1** in CD_3CN. Significantly, chloroacetic acid gave virtually no change to the ^{1}H NMR spectrum of the receptor, whereas the addition of the two stronger acids induced large changes to the spectrum (see ESI†), supporting the notion of a strong binding interaction through guest-to-host proton transfer. However, for each of these two guests, binding was accompanied by a *downfield* shift in the signal for the H_a proton of **1**, which indicates that the phenyl group in L-**Phe** is responsible for the upfield shift in this resonance (*vide supra*), presumably through a π-stacking interaction with the H-bonding unit. This effect appears to play a role in stabilising the amino acid complex since the less acidic L-**Phe** was able to almost completely displace the more acidic dichloroacetic acid from its complex with **1**, as observed by the reappearance of an upfield resonance for the H_a proton (at *ca.* 7.7 pm) upon addition of one equivalent of L-**Phe** to an equimolar solution of **1** and dichloroacetic acid.

The novel ditopic receptor **2**¶, synthesised in one step from a known ferrocene precursor[6] (see ESI† for synthesis details), was then prepared for the simultaneous binding of the carboxylic acid and ammonium groups of L-**Phe** through its amidopyridine and crown ether moieties, respectively. As observed with **1**, the addition of one equivalent of the perchlorate salt of L-**Phe** to a solution of **2** in CD_3CN (10 mM) had a dramatic effect on its ^{1}H NMR spectrum. Although solubility problems and signal broadening‖ precluded a detailed analysis of the binding process, it was clear that distinct changes in δ value were observed for a number of the proton signals, in particular those for the amidopyridine unit (*e.g.* $\Delta\delta$ for the NH protons on the pyridine and crown ether arms: *ca.* +2.6 and $\leqslant$+0.3 ppm, respectively, indicating an H-bonding interaction with only one amide unit, see ESI† for spectra). At the same time, downfield shifts were observed for the multiplet corresponding to the crown ether protons, indicating the successful formation of a ditopic complex (a 1 : 1 complex was observed by ESMS, see ESI† for details), where the guest forms a bridge between the two binding sites, as observed for related complexes between organic molecules and heteroditopic receptors.[9] No upfield shift was observed for any of the pyridine proton signals for the **2** : L-**Phe** complex, presumably because the ditopic interaction prevents any significant π-stacking interaction.

As expected, the addition of an excess amount of the ammonium salt **4** (*i.e.* L-**Phe** without the carboxylic acid group) to a CD_3CN solution of **2** only induced changes to the crown-ether proton signals of the receptor whereas the addition of an excess amount of hydrocinnamic acid **3** had no affect at all. This was in contrast to the more acidic dichloroacetic acid, which once again was found to be a strong binder when added to receptor **2**. However, as also found with **1**, the subsequent addition to this solution of an equimolar amount of the less acidic L-**Phe** resulted in a spectrum that closely resembled that of **2** and L-**Phe** alone; this indicated the preference of **2** for the amino acid over dichloroacetic acid, as would be expected for a ditopic interaction.

In order to assess how these receptors might act in a sensing capacity, the binding between L-**Phe** and receptors **1** and **2** was followed by cyclic voltammetry in CH_3CN (receptor concentration = 0.5 mM, see ESI† for other conditions). As shown in Fig. 2(a), compound **2** gave a reversible redox process centred at $E^{0'}$ = +0.820 V *vs.* Ag/AgCl, where $E^{0'} = (E_{pa} + E_{pc})/2$. Significantly, the addition of one equivalent of L-**Phe** to the solution resulted in a large positive shift of +129 mV (Fig. 2(b), Table 1) indicating the electrochemical sensing of this amino acid. No further changes were observed upon the addition of an excess amount of the guest, which was consistent with the formation of a ditopic complex of 1 : 1 stoichiometry. As expected, the addition of an excess amount of **3** to the receptor brought about no change to the appearance of the voltammogram, confirming that **2** can respond to amino acids selectively over simple carboxylic acids. Interestingly, the addition of an excess amount of **4** brought about a small positive shift in potential (+15 mV), in line with previous work on the binding of ammonium ions by ferrocenyl crown ethers.[10]

Studies were then repeated with compound **1**, the electrochemical properties of which have been described previously.[5] As expected, the addition of **3** and **4** had no effect on its voltammogram (no changes to the ^{1}H NMR spectrum of **1** were observed upon addition of excess **4**). However, the addition of L-**Phe** brought about a positive shift of +107 mV in the redox couple of the receptor. It is noteworthy that this shift is less than that obtained with **2**, which indicates that the binding of the

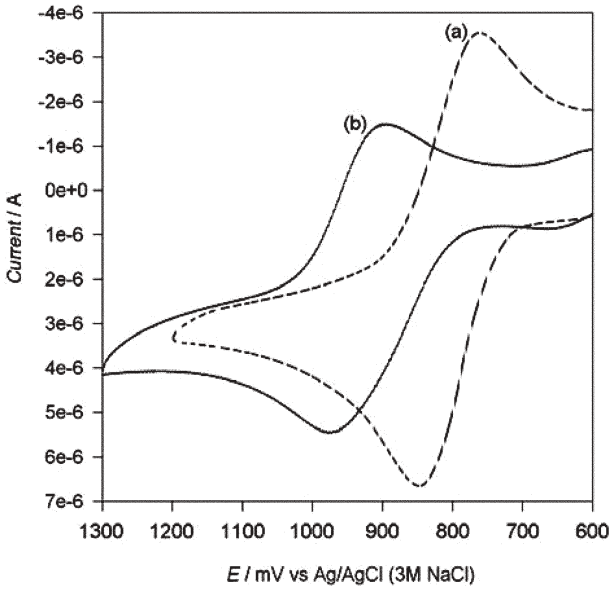


Fig. 2 Cyclic voltammograms in CH_3CN of **2** (0.5 mM) in (a) the absence and (b) the presence of five molar equivalents of the perchlorate salt of **L-Phe**.

Table 1 Electrochemical data detailing the shift in the formal electrode potential, $\Delta E^{0'}$, (± 5 mV) at 293 K of compounds **1** and **2** in CH_3CN (0.5 mM) upon the addition of an excess amount of guest species

Receptor	**L-Phe**	**3**	**4**
1	+107	$< +5$	$< +5$
2	+129	$< +5$	+15

ammonium moiety of **L-Phe** in the crown-ether cavity of the ditopic receptor makes a small contribution to the overall $\Delta E^{0'}$ value, giving further support for a ditopic interaction with this guest. Finally, the addition of an excess amount of chloroacetic acid to **1** had no observable effect on in its cyclic voltammogram, whereas the addition of excess dichloroacetic acid brought about a shift of +105 mV (see ESI†), indicating the selective sensing of stronger carboxylic acids over their weaker counterparts.

The observation of *positive* shifts in the redox couples of **1** and **2** contrasts with previous examples of the binding of carboxylic acids and carboxylates by ferrocene receptors,[1d] where guests have been sensed through *negative* shifts in potential. The fact that oxidation is more difficult upon complexation in this case is consistent with a guest-to-host proton transfer reaction,[11] since a positive charge is introduced onto the receptor, in close proximity to the ferrocene centre. In fact, previous studies by us[5] have shown that protonation of the pyridine nitrogen of **1** through the addition of the acid HBF_4 produces spectroscopic changes and shifts in potential that are similar to those reported here** for the addition of **L-Phe**.

In conclusion, we have shown how strongly acidic carboxylic acids and their derivatives can be selectively sensed as a result of their binding to redox-active amidopyridine receptors *via* a novel proton transfer process. Work on the binding and sensing of similar guests by related ferrocene receptors will be reported in due course.

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Notes and references

‡ The error in this value is large (>10%) since the very strong binding interaction precludes the determination of an accurate binding constant at NMR concentrations.

§ The pK_a values quoted are for water as a solvent; it is reasonable to expect the same trend in acetonitrile.

¶ Selected characterisation data for **2**: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.76 (s, NH, 1H), 8.04 (d, py-H, 1H), 7.67 (tr, py-H, 1H), 6.98 (m, py-H and NH, 2H), 4.85 (s, Cp-H, 2H), 4.72 (s, Cp-H, 2H), 4.51 (s, Cp-H, 2H), 4.43 (s, Cp-H, 2H), 3.78 (m, OCH, 1H), 3.45–3.75 (m, OCH_2, 22H), 3.41 (m, NCH_2, 2H), 2.46 (s, CH_3, 3H); HRMS (ES+): calc. for $C_{31}H_{42}N_3O_8Fe$ *m/z* 640.2321; found 640.2321.

‖ Compound **2** is chiral, resulting in the presence of two diastereomeric complexes that may affect the appearance of the NMR spectrum. Work is in progress comparing binding studies with related achiral and chiral ferrocene receptors.

** In the light of these studies, it is possible that protonation of **1** by HBF_4, as reported in ref. 5, leads to a similar H-bonded ion-pair complex involving the tetrafluoroborate anion.

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