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Determination of Binding Constants of Hydrogen-Bonded Complexes by ITC, NMR CIS, and NMR Diffusion Experiments[‡]

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The host–guest complex formation between barbital and various acylaminopyridyl isophthalamides (Hamilton receptors) has been determined quantitatively. The syntheses of nine isophthalamides are described. Their structures differ in the substitution patterns on the central isophthalic unit, and the natures of the acyl residues. Ethylhexanoyl derivatives proved to be more soluble than pentanoyl amides. The association constants were determined by ^{1}H NMR titrations monitoring chemically induced shifts (CIS values), by ^{1}H NMR diffusion experiments, and by isothermal titration calorimetry (ITC), giving K_{ass} values in chloroform at 298 K between 33×10^3 and 100×10^3 M^{-1}.

Introduction

Noncovalent interactions are the fundamental forces in supramolecular chemistry and molecular recognition.[1] In order to design supramolecular structures based on multiple hydrogen bonds, it is necessary to use complementary acceptor and donor patterns.[2,3] Various linear hydrogen-bond acceptor/donor sequences have been synthesized and their host–guest chemistry has been investigated during recent years.

When four hydrogen bonds are situated next to one another, the pattern determines whether homodimers, as described by Meijer,[4,5] or heterodimers, such as the quadruple hydrogen bond system first synthesized in our group,[6] are formed. For controlled self-assembly of large supramolecules through hydrogen bonds it is not enough to use just one complementary pair: various orthogonal recognition domains are necessary. For four neighboring hydrogen bonds, several orthogonal pairs have already been synthesized.[7–12] Another type of orthogonality can be achieved if the recognition domain shows nonlinear geometry.

In 1988, Chang and Hamilton synthesized a macrocyclic angulate receptor.[13] Containing six converging hydrogen bonds, this phthalamide host was able to bind barbituric acid derivatives (Figure 1, R = R′ = H, with the two amide groups in the 6-positions of the pyridine rings being connected to give a macrocycle).[13] ^{1}H NMR titrations of several related receptors with different guests gave association constants in the 10^5 M^{-1} range (in chloroform) for such bent arrays of six hydrogen bonds.[14] The original macrocyclic Hamilton receptors[13] were synthesized from isophthalic acid as starting material. Molecular recognition between open structures such as **1** and barbiturates, however, is also strong and orthogonal to other hydrogen bond patterns.[7] Consequently, nonmacrocyclic "Hamilton receptors" have been used preferentially, and today quite a number of supramolecular systems use the "Hamilton receptor" and barbituric acid derivatives; examples include self-assembled dendrimers,[15,16] multifunctional block copolymers[17] or double dynamers.[18]

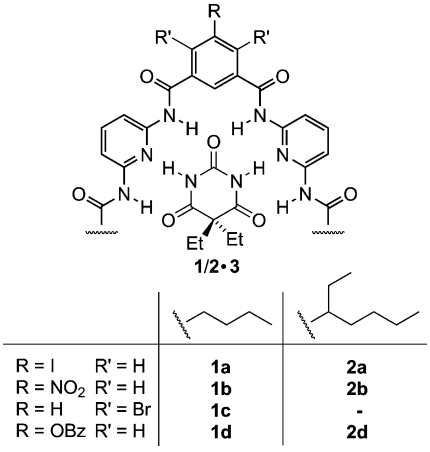

Figure 1. Host–guest systems **1**·**3** with *n*-butyl residues and **2**·**3** with ethylpentyl residues at the amido groups at the 6-positions of the pyridine rings.

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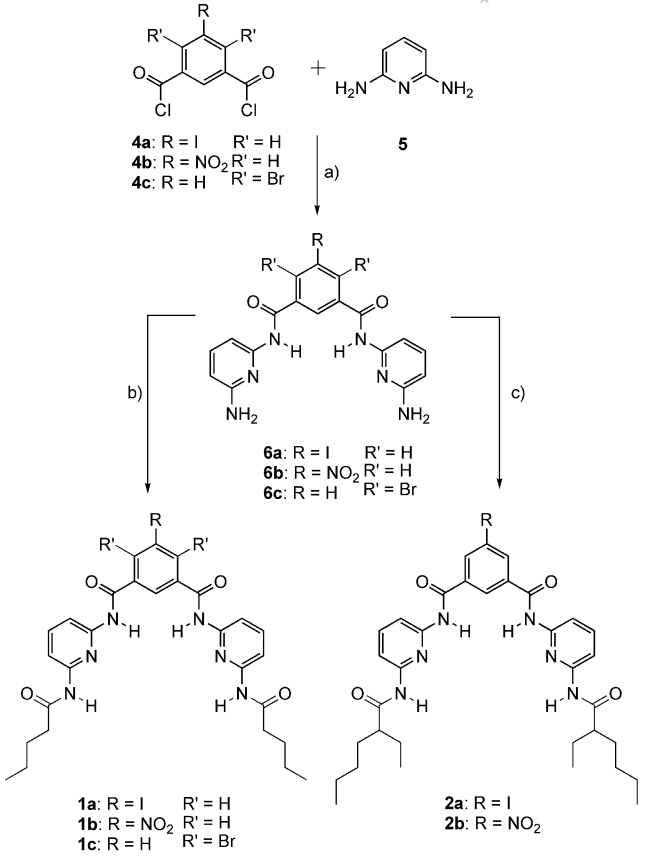
A prerequisite for introduction of a Hamilton receptor into a larger structure is a covalent connection to another moiety, so syntheses of receptors functionalized in the isophthalic component have been carried out. 5-Iodo,[7] -amino,[15] or -hydroxy[16] substitution have been reported. For practical applications, however, not every combination of Hamilton hosts and corresponding guests can be used, due to solubility problems. Firstly, the hosts and guests are flat, so they can stack, and secondly, the heterocycles contain many hetero elements that favor intermolecular interactions through dipole–dipole interactions, including hydrogen bonds. All these forces have to be overcome if applications are to be carried out in solution. To enhance solubility, we therefore synthesized a set of Hamilton receptors **2** (Figure 1), each bearing a branched alkyl residue rather than the *n*-alkyl substituent of receptors **1**.

Here we report on the syntheses of different Hamilton receptors of both families **1** and **2** and on the determination of their association constants when forming a complex with barbital (**3**, Figure 1). Besides the two different residues at the amido group in the 6-positions in the pyridine rings, the receptors differ in the character and position of the functional group(s) in the isophthalic acid moiety. The influence of these changes on binding constants was studied. Three techniques for the determination of the association constants – ^{1}H NMR titrations monitoring chemically induced shifts (CIS values), ^{1}H NMR diffusion experiments, and isothermal titration calorimetry (ITC) – were selected and the results were compared.



Scheme 1. Synthesis of the Hamilton receptors **1a**–**c** and **2a** and **2b**. a) Et_3N, THF, room temp., 3 h; b) pentanoyl chloride, Et_3N, THF, room temp., 16 h; c) 2-ethylhexanoyl chloride, Et_3N, THF, room temp., 16 h.

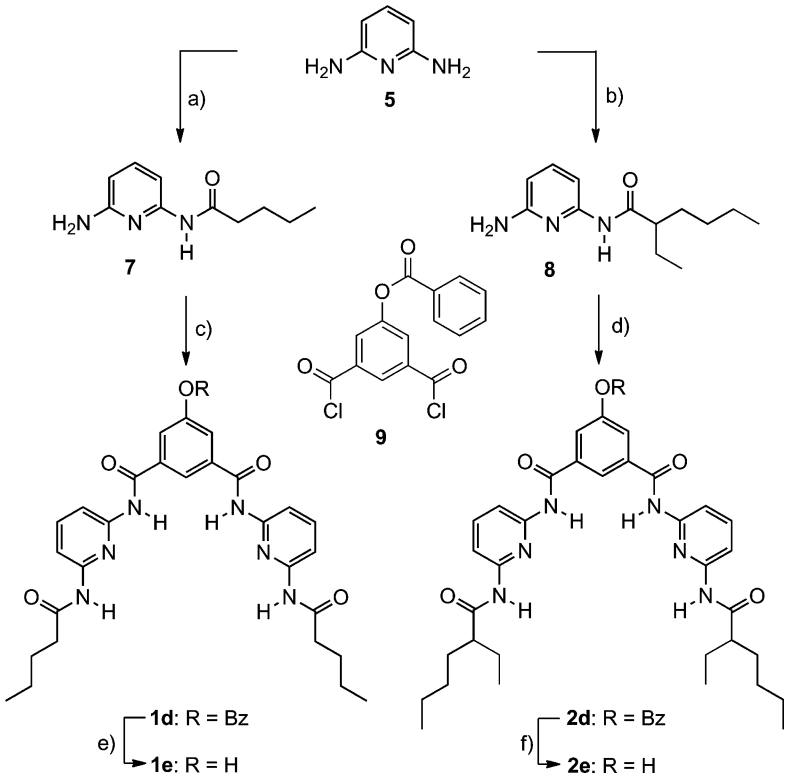
Results and Discussion

Syntheses of the Hamilton Receptors

The syntheses of the Hamilton receptors were carried out by two different reaction pathways. In both cases the parent compounds were isophthalic acid derivatives with functional groups either at their 5- or at their 4- and 6-positions. The next step was the formation of isophthalamides either with 2,6-diaminopyridine (**5**, Scheme 1) or with the already monoacylated diaminopyridines **7** or **8** (Scheme 2, below).

The preparation of the iodine Hamilton receptor **1a** was developed in our group in 2002 in analogy to the synthesis described by Chang and Hamilton.[13] First of all, 5-aminoisophthalic acid was converted into 5-iodoisophthalic acid by a literature procedure, with sodium nitrite, potassium iodide, and a catalytic amount of iodine.[19] 5-Iodoisophthaloyl chloride (**4a**, Scheme 1) could be obtained by treatment with oxalyl dichloride in toluene.[19] 5-Nitroisophthaloyl chloride (**4b**) was synthesized by the same procedure from commercially available 5-nitroisophthalic acid. Furthermore, 4,6-dibromoisophthaloyl chloride (**4c**) was prepared in three steps starting from *m*-xylene, which was first brominated, then oxidized, and finally treated with thionyl chloride.[20–22] These stable isophthaloyl chlorides **4a**–**4c** were each treated with 2,6-diaminopyridine (**5**, excess) to afford the corresponding diamines **6a**–**6c** in a universally applicable precursor. Finally, the different Hamilton receptors **1a**–**c** and **2a** and **2b** with different solubilities were synthesized by use of pentanoyl chloride or 2-ethylhexanoyl chloride. Two factors improve the solubility of the ethylhexanoyl derivatives: the branching itself and the formation of diastereomeric mixtures due to the fact that two chiral centers are introduced.

Since the first description of a synthesis of these isophthalamides, an alternative route to build up the angular receptor has been reported in the literature.[23,24] According to these procedures, we synthesized four additional Hamilton receptors (Scheme 2) by starting from 5-hydroxyisophthalic acid, which was protected with a benzoyl group and afterwards converted into the isophthaloyl chloride **9**.[25] Unlike in the syntheses described above, in this route the side chain was introduced prior to treatment with the acid chloride **9**. 2,6-Diaminopyridine (**5**) was monoacylated with an aliphatic acid chloride such as pentanoyl chloride or 2-ethylhexanoyl chloride. Next, the amidoaminopyridines **7** or **8** were linked to 5-(benzoyloxy)isophthaloyl chloride (**9**) to form the diamides **1d** and **2d**. Crystals were obtained from 5-benzoyloxy-*N*,*N*′-bis(6-pentanoylaminopyrid-2-yl)isophthalamide (**1d**) and the X-ray structure could be solved, although the crystals were non-merohedrally twinned.



Scheme 2. Synthesis of different 5-oxy-substituted Hamilton receptors: a) pentanoyl chloride, NEt_3, THF, 0 °C, 3 h, room temp., 16 h; b) 2-ethylhexanoyl chloride, NEt_3, THF, 0 °C, 3 h, room temp., 16 h; c) and d) 5-(benzoyloxy)isophthaloyl chloride (**9**), NEt_3, THF, room temp., 30 min, reflux, 18 h; e) and f) EtOH, NaOH (1 N), room temp., 3 h, HCl (2 N).

The Hamilton receptor **1d** crystallized in the triclinic space group $P\bar{1}$. The almost planar orientation of the Hamilton receptor binding sites is shown in its X-ray structure (Figure 2). The planarity is comparable to that in another structure published by Vögtle and De Cola.[15,26] Unlike their receptor, which contained tertiary butyl groups, our receptor **1d** has longer unbranched chains; nevertheless, no twist is visible and the binding sites are still in-plane. According to the crystal structure, there is no steric hindrance to binding of a guest.

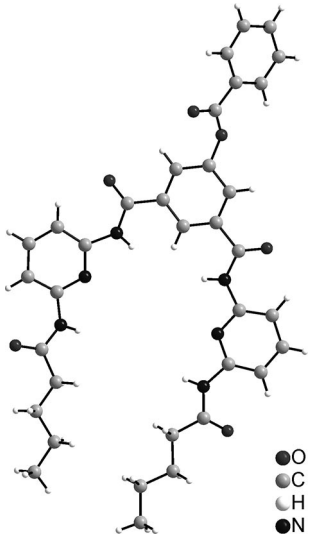


Figure 2. X-ray structure of 5-benzoyloxy-*N*,*N*′-bis(6-pentanoylaminopyrid-2-yl)isophthalamide (**1d**). Only one orientation of the disordered benzoyl protecting group is shown.

The last step in the syntheses of **1e** and **2e** was the deprotection of the hydroxy group. Ester cleavage was achieved in alkaline medium followed by acidification. The solubility of the Hamilton receptor **1e** with unbranched pentanoyl chains was limited, and it dissolved only in DMSO, DMF, and THF. Through the introduction of the branched 2-ethylhexanoyl substituents, the solubility was improved, and less polar and aprotic solvents such as chloroform can be used for further syntheses and measurements. Thanks to these results, the two chloroform-soluble protected Hamilton receptors **1d** and **2d** were used for the determination and comparison of binding constants with barbital (**3**). The hydroxy derivatives **1e** and **2e** were only synthesized to allow incorporation of Hamilton receptors into larger arrays, so investigation of the esters **1d** and **2d**, rather than the corresponding phenols **1e** and **2e**, seemed more relevant.

Investigation of the Host–Guest Systems

The determination of association constants is an essential element when dealing with supramolecular structures or self-assembly. Various methods for the determination of association constants for host–guest complexes are known. In this work, ^{1}H NMR CIS titrations, ^{1}H NMR diffusion experiments, and isothermal titration calorimetry (ITC)

were selected to investigate several Hamilton receptor/barbital systems (see Figure 1). All measurements were carried out in $CDCl_3$ or $CHCl_3$ at 25 °C.

The determination of binding constants by ^1H NMR titrations, by recording of changes in the chemically induced shifts (CISs), is an easily applicable and well established method.[27] The association constants for host–guest formation between the Hamilton receptors **1a**, **1d**, **2a**, and **2d** as hosts and barbital (**3**) as guest were calculated from the CIS of the N–H signal of barbital (**3**) upon addition of increasing amounts of the Hamilton host to a solution of the guest (see Table 1 and Figure 3 for an example). The reason for the inverse titration was the parallel determination of the diffusion parameters (see below). The results for the receptors **1a**, **1d**, **2a**, and **2d** are compared in Table 1.

Table 1. K_{ass} values of complexes **1·3** and **2·3** determined by ^1H NMR CIS titration and ^1H NMR diffusion experiments in $CDCl_3$ at 298 K with a concentration of **3** of 0.4 mM. All errors were smaller than 20% except for values in parentheses. For exact errors see the Supporting Information.

	K_{ass} [10^3 M^{-1}] (CIS)	K_{ass} [10^3 M^{-1}] (diffusion)
1a	64	59
2a	34	(33)
1d	(76)	85
2d	44	37

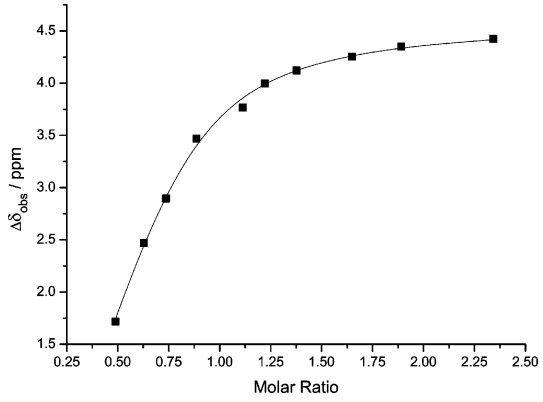


Figure 3. ^1H NMR CIS titration curve of barbital (**3**, 5 mM) with the Hamilton receptor **2d** in $CDCl_3$. The CIS of the NH signals of **3** is plotted against the **2d/3** molar ratio.

All four host–guest systems **1a/2a·3** and **1d/2d·3** show association constants (K_{ass}) of the same order of magnitude. Nevertheless, a difference between the Hamilton receptors with unbranched acyl residues (**1a**, **1d**) and those with the branched chains (**2a**, **2d**) is detectable. The smaller association constants (K_{ass}) for receptors **2a** and **2d** are probably the result of greater steric hindrance during complexation. With regard to the substituents at the isophthalic acid part, there is only a marginal difference in the association constants (K_{ass}).

For the determination of association constants (K_{ass}) through ^1H NMR diffusion experiments, the solutions from the titrations of barbital (**3**) with increasing amounts of the Hamilton receptors **1** or **2** were used. By detection of spin echoes with pulsed gradients the diffusion constant (D) of barbital (**3**) in each mixture was measured, and finally the association constants (K_{ass}) were calculated.[28] The signals of the guest **3** were chosen because of the larger difference in molecular weight, and thus diffusion constant, between barbital (**3**) and the complexes **1·3** or **2·3** (for an example see Figure 4).

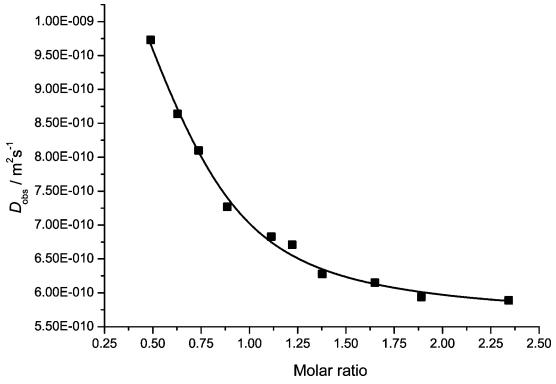


Figure 4. Titration curve of barbital (**3**, 5 mM) with the Hamilton receptor **2d** determined by ^1H NMR diffusion spectroscopy in $CDCl_3$. The signal of the CH_2 groups of barbital (**3**) was used to determine the diffusion constant (D_{obs}), which is plotted against the **2d/3** molar ratio.

Table 1 summarizes the association constants (K_{ass}) determined by the diffusion method and compares them with the constants (K_{ass}) obtained from ^1H NMR CIS titrations. The latter analyze the chemically induced shifts of certain H atoms, preferentially those participating in hydrogen bond formation (see above). The K_{ass} values determined this way thus only register the complexes that are indeed bound by hydrogen bonds. In contrast, the hydrodynamic radius of a guest will always increase when it is coordinated to a host, regardless of whether it is bound by hydrogen bonds or by other intermolecular forces such as van der Waals interactions or π–π stacking. If such other forces play a role, it is obvious that K_{ass} values determined from diffusion experiments may be larger than those determined by CIS titrations. Despite these differences, the binding constants (K_{ass}) for **1·3** or **2·3** determined by diffusion experiments are in the expected range, and they even have a smaller calculation error than the CIS titration data.

The Hamilton receptors **2a** and **2d** with the branched acyl groups show weaker binding of barbital (**3**) than the unbranched **1a** and **1d**. Furthermore, the substituent on the isophthalic acid moiety appears to be irrelevant for the binding; K_{ass} is not influenced. These results are in accordance with the measurements described in the NMR CIS titration section.

In addition to the applied NMR methods, the association constants (K_{ass}) of the receptors **1a**, **2a**, **1d**, and **2d** and three other complexes **1b·3**, **1c·3**, and **2b·3** were determined by isothermal titration calorimetry. ITC is a calorimetric method increasingly used to determine the thermodynamic parameters of supramolecular interactions. The results for the association constants (K_{ass}), the enthalpy changes (ΔH), and also the changes in entropy (ΔS) are summarized in Table 2.

Table 2. Association constants (K_{ass}) and thermodynamic parameters ΔH and ΔS for titration of the Hamilton receptors **1** or **2** (ca. 0.4 mM) with barbital (**3**) in chloroform as determined by ITC. Note that kcal rather than kJ is used.

	K_{ass} [10^3 M^{-1}]	ΔH [kcal mol^{-1}]	ΔS [cal mol^{-1} K^{-1}]
1a	98	–37	–103
1b	100	–36	–99
1c	40	–37	–104
1d	91	–39	–109
2a	59	–40	–115
2b	67	–37	–103
2d	58	–40	–113

The ITC association constants had larger values than those obtained in the NMR CIS experiments. This is not too surprising, because the CIS changes observed during a ^{1}H NMR titration only occur when the corresponding proton becomes part of a hydrogen bond – in contrast to calorimetric measurements, which register any binding regardless of whether this binding is caused by hydrogen bonds or by other interactions.[9b] In this respect, ITC measurements are similar to NMR diffusion experiments. The measurements confirm the expected results: the differences between the association constants (K_{ass}) for the interaction of the Hamilton receptors bearing unbranched (**1a**, **1d**) and branched acyl groups (**2a**, **2d**) with barbital (**3**) became obvious (for an example see Figure 5). Again, the substituents at the central phthaloyl moiety had only a minor influence on the association constants (K_{ass}). In addition to the pairs

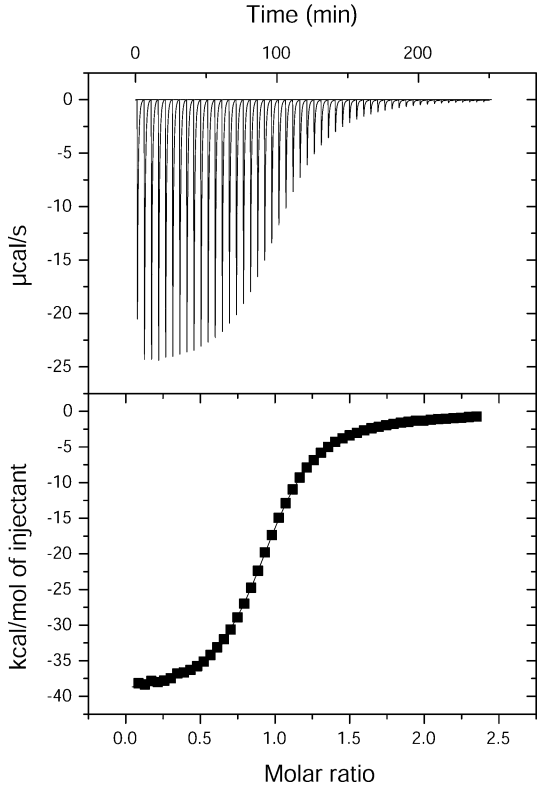


Figure 5. Isothermal titration calorimetry (ITC). Titration of barbital (**3**, 0.5 mM) with the Hamilton receptor **2d** (5 mM) in chloroform (energy in kcal and not in kJ.).

1a/1d·3 and **2a/2d·3**, measurements of three more Hamilton receptors – **1b**, **1c**, and **2b** – with barbital (**3**) were carried out. The two Hamilton receptors with a nitro group at the 5-position in the isophthalic acid component (**1b**, **2b**) did not differ from other 5-substituted receptors. With regard to the only receptor with substituents in the *ortho* positions (**1c**), there is a decrease in the association constant (K_{ass}) comparable to that when the acyl groups are branched.

Table 2 also shows that the negative Gibbs free enthalpy (ΔG) for the binding results from an enthalpic contribution. Although there is a considerable entropic compensation of ca. 30 kcal mol^{-1} (298 K times ca. 100 cal mol^{-1} K^{-1}), ΔH is ca. 30% larger, resulting in negative Gibbs free enthalpies and corresponding association constants of 40–100×10^3 M^{-1}.

Conclusions

Nine different Hamilton receptors **1** and **2** were synthesized from substituted isophthalic acids, 2,6-diaminopyridine (**5**), and different acyl chlorides. The Hamilton receptors **1** each bear two linear pentanoyl residues, whereas branched substituents were introduced in class **2**, resulting in enhanced solubilities in low-polarity organic solvents such as chloroform. The two amide bonds in the 2- and 6-positions of the pyridine rings in the receptors **1** and **2** could be formed in either sequence. For **1d** it was possible to obtain an X-ray crystal structure showing the planar preorganization of the binding sites. The association constants of the receptors **1** and **2** with barbital (**3**) were determined by monitoring of the chemically induced shifts (CISs) in ^{1}H NMR titrations, by ^{1}H NMR diffusion experiments, and by isothermal titration calorimetry (ITC). The K_{ass} values were found to be between 35×10^3 and 100×10^3 M^{-1} by the three methods, displaying differences of a factor of 3 at most. Slight decreases in the K_{ass} values were detected for Hamilton receptors with branched acyl groups (**2a**, **2b**, and **2d**) or for receptor **1c** with two *ortho* substituents at the phthaloyl unit, possibly due to greater steric hindrance.

Experimental Section

General Remarks: NMR spectra were recorded with Bruker AC 200, DRX 500, or AV 600 instruments. Assignments are supported by COSY, HSQC, and HMBC. Even when obtained by DEPT, the type of ^{13}C signal is always listed as singlet, doublet, etc. All chemical shifts are referenced to TMS or to the residual proton or carbon signal of the solvent. All diffusion and titration measurements were carried out with a Bruker AV 600 spectrometer with a triple-resonance cryo probehead with a maximum *z*-gradient strength of 5.67 G mm^{-1}. As pulse sequence a double stimulated echo with bipolar gradient pulses was chosen. The diffusion time in all these experiments was 100 ms and each gradient pair was applied for 2500 µs. The maximum gradient strength was varied between 5 and 95% of the gradient strength in 16 steps. EI/CI mass spectra were recorded with a Finnigan MAT 8200 or MAT 8230. MALDI mass spectra were recorded with a Bruker–Daltonics Biflex III instrument and Cl-CCA (α-cyano-4-chlorocinnamic acid)

as matrix. ESI mass spectra were recorded with an Applied Biosystems Mariner Spectrometry Workstation. IR spectra were recorded with a Perkin–Elmer Paragon 1000, Perkin–Elmer Spectrum 100 fitted with an MKII Golden GateTM Single Reflection ATR unit. Elemental analyses were carried out with a Euro EA 3000 Elemental Analyzer from Euro Vector. 5-(Benzoyloxy)isophthaloyl chloride (**9**) was synthesized by the literature procedure.[25] 5-Iodoisophthaloyl acid and 5-iodoisophthaloyl dichloride (**4a**) were synthesized by the literature procedure.[19] *N*,*N*′-Bis(6-aminopyrid-2-yl)-5-nitroisophthalamide (**6b**) was synthesized analogously to Hamilton et al.[29]

^{1}H NMR CIS Titrations and Diffusion Experiments: A new sample was prepared for each titration measurement. For each component a stock solution in $CDCl_3$ (dry, as purchased) with a concentration of about 5 mmol L^{-1} was prepared. TMS (ca. 10 μL) was added as internal reference to the stock solution of barbital (**3**). The samples were prepared by injecting the stock solution of barbital (**3**, 50 μL) into a 5 mm NMR tube with a standard microliter syringe. A corresponding volume of a stock solution of a Hamilton receptor (**1a**, **2a**, **1d**, **2d**) in $CDCl_3$ was added and the NMR tube was filled up to 600 μL with $CDCl_3$.

The final concentrations of the Hamilton receptors **1** and **2** in the mixtures were checked by integration of the CH_2 group of barbital (**3**) and the CO–CH or CO–CH_2 signals of **1** or **2**, respectively. The barbital (**3**) concentration was assumed to be constant in all measurements. The same signals were used for the diffusion analysis. In cases of slight signal overlap, biexponential fits with respect to two components were applied to the diffusion data to provide more exact integrals for concentration calculations on the one hand and more accurate diffusion constants on the other.

The diffusion analyses were carried out with the aid of Bruker's Topsin 2.1 software. In the case of biexponential fitting, Origin 7.5 including the ONMR plug-in was used for further analyses and plotting/fitting options.[30]

Statistical errors are usually calculated for curve fittings of NMR titrations but in most cases they do not include all errors (weighing error, temp., how many measurements have been carried out at what ratios, systematic error when always adding the same aliquot of a guest solution to a given host solution, etc.). In our experiments, for every data point in the NMR measurements, new solutions were prepared so that weighing errors etc. of each single experiment should cancel out. In this regard, each titration curve is a set of several independent experiments, and the calculated errors include the other errors. The specific statistical error for each titration can be found in the Supporting Information

Isothermal Titration Calorimetry: Experiments were carried out with a VP-ITC microcalorimeter (MicroCal LLC, GE Healthcare) in anhydrous chloroform. A Hamilton receptor **1** or **2** (ca. 1.4 mL, 0.3–0.5 mM) was placed in the calorimeter cell. The titration syringe was loaded with barbital (**3**) at a 10 times higher concentration than in the cell. The titrations were usually carried out with 50 injections of 6 μL each with time intervals of 5 min. The solution was stirred at 300 rpm as suggested by the manufacturer. Titrations were carried out at a cell temperature of 298 K (shield: 297 K) and with a reference power of 10 μcal s^{-1}. ITC data analyses were carried out in Origin 7 SR 2 (OriginLab Corp.) with the provided microcal ITC routines. Please note that all energies are listed as kcal mol^{-1} rather than kJ mol^{-1} by this routine. Each experiment was carried out at least twice. In cases of larger deviations (due to, for instance, changes in room temperature stability), more titrations were carried out. One representative titration curve for the titrations of each host guest pair is shown in the Supporting Information.

5-Iodo-*N*,*N*′-bis[6-(pentanoylamino)pyrid-2-yl]isophthalamide (1a): *N*,*N*′-Bis(6-aminopyrid-2-yl)-5-iodoisophthalamide (**6a**, 4.94 g, 10.4 mmol) and triethylamine (3.0 mL, 22 mmol) were dissolved in anhydrous tetrahydrofuran (80 mL) under nitrogen. A solution of pentanoyl chloride (2.7 mL, 22 mmol) in anhydrous tetrahydrofuran (10 mL) was added dropwise. After the system had been stirred at room temp. for 3 h, the solvent was evaporated in vacuo and the residue was dissolved in chloroform (80 mL) and water (80 mL). The organic layer was washed with sodium hydrogen carbonate (50 mL, 5%) and dried with magnesium sulfate, and the solvent was removed. The residue was purified by column chromatography (silica gel, ethyl acetate/cyclohexane 1:1, R_f = 0.38); yield 5.90 g (88%); m.p. 170–173 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.56 (br. s, 2 H, Ar-CON*H*), 8.33 (s, 2 H, Ar-*H*-4,6), 8.32 (s, 1 H, Ar-*H*-2), 7.98 (br. s, 2 H, H_9C_4CON*H*), 7.95–7.88 (m, 4 H, Pyr-*H*-3,5), 7.70 (t, $^3J_{H,H}$ = 8.1 Hz, 2 H, Py-4-*H*), 2.38 (t, $^3J_{H,H}$ = 7.5 Hz, 4 H, COCH_2), 1.70 (quin, $^3J_{H,H}$ = 7.5 Hz, 4 H, $COCH_2CH_2$), 1.40 (sext, $^3J_{H,H}$ = 7.5 Hz, 4 H, CH_2CH_3), 0.94 (t, $^3J_{H,H}$ = 7.5 Hz, 6 H, CH_2CH_3) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 171.9 (s, *C*OC_4H_9), 163.0 (s, Ar-*C*ONH), 149.8 (s, Py-*C*-2), 149.0 (s, Py-*C*-6), 141.0 (d, Py-*C*-4), 139.7 (d, Ar-*C*-4,6), 136.2 (s, Ar-*C*-1,3), 125.0 (d, Ar-*C*-2), 110.4 (d, Py-*C*-3), 109.7 (d, Py-*C*-5), 94.8 (s, Ar-*C*-5), 37.5 (t, CO*C*H_2), 27.4 (t, $COCH_2CH_2$), 22.3 (t CH_2CH_3), 13.8 (q, CH_3) ppm. IR (KBr): $\tilde{\nu}$ = 3428, 3302 (N–H), 2958, 2930, 2871 (aliph. C–H), 1688, 1586, 1514 (C=O), 1295 cm^{-1}. MS (EI, 70 eV): *m*/*z* (%) = 642 (24) $[M]^{+\cdot}$, 600 (100) $[M - C_3H_6]^{+\cdot}$, 585 (90) $[M - C_4H_9]^+$, 516 (35) $[M - I]^{+\cdot}$. MS (CI, isobutane): *m*/*z* (%) = 643 (26) $[M + H]^+$, 517 (18) $[M - I + H]^+$, 194 (100) $[H_9C_4CONHPyNH + H]^+$. HRMS (EI): calcd. $C_{28}H_{31}IN_6O_4$: 642.14514; found 642.14180, calcd. $C_{27}{}^{13}CH_{31}IN_6O_4$: 643.14850; found 643.14570. $C_{28}H_{31}IN_6O_4$ (642.49): calcd. C 52.34, H 4.86, N 13.08. $C_{28}H_{31}IN_6O_4 \cdot 0.5\,H_2O$ (651.15): calcd. C 51.62, H 4.95, N 12.90; found C 51.60, H 4.96, N 12.90.

5-Nitro-*N*,*N*′-bis[6-(pentanoylamino)pyrid-2-yl]isophthalamide (1b): Compound **1b** was synthesized by the same procedure as used for receptor **1a**, from *N*,*N*′-bis(6-aminopyrid-2-yl)-5-nitroisophthalamide (**6b**, 1.00 g, 2.54 mmol), triethylamine (1.65 mL, 7.50 mmol), and pentanoyl chloride (920 μL, 7.50 mmol) in anhydrous tetrahydrofuran (10 mL). The crude product was purified by column chromatography (silica gel, cyclohexane/ethyl acetate 1:1, R_f = 0.30); yield 1.06 g (74%) as a slightly yellow solid; m.p. 193–195 °C. ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 10.92 (br. s, 2 H, Ar-CON*H*), 10.12 (br. s, 2 H, H_9C_4CON*H*), 8.92 (d, $^4J_{H,H}$ = 1.5 Hz, 2 H, Ar-*H*-4,6), 8.90 (d, $^4J_{H,H}$ = 1.5 Hz, 1 H, Ar-*H*-2), 7.88–7.78 (m, 6 H, Pyr-3,4,5-*H*), 2.41 (t, $^3J_{H,H}$ = 7.5 Hz, 4 H, COCH_2), 1.58 (quin, $^3J_{H,H}$ = 7.5 Hz, 4 H, $COCH_2CH_2$), 1.32 (sext, $^3J_{H,H}$ = 7.5 Hz, 4 H, CH_2CH_3), 0.90 (t, $^3J_{H,H}$ = 7.4 Hz, 6 H, CH_2CH_3) ppm. ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 172.2 (s, *C*OC_4H_9), 163.4 (s, Ar-*C*ONH), 150.6 (s, Py-*C*-2), 149.8 (s, Py-*C*-6), 147.7 (s, Ar-*C*-5), 140.1 (d, Py-*C*-4), 135.8 (s, Ar-*C*-1,3), 133.6 (d, Ar-*C*-2), 125.7 (d, Ar-*C*-4,6), 110.5 (d, Py-*C*-3), 110.3 (d, Py-*C*-5), 35.8 (t, CO*C*H_2), 27.1 (t, $COCH_2CH_2$), 21.7 (t CH_2CH_3), 13.7 (q, CH_3) ppm. IR (ATR): $\tilde{\nu}$ = 3332, 3274 (N–H), 3087 (arom. C–H), 2959, 2931, 2872 (aliph. C–H), 1663 (C=O), 1583, 1443 (arom. C=C), 1511 (C–NO_2) cm^{-1}. MS (EI, 70 eV): *m*/*z* (%) = 504 (5) $[M - C_4H_9]^{+\cdot}$. MS (CI, isobutane): *m*/*z* (%) = 562 (3) $[M + H]^+$. MS (MALDI): *m*/*z* = 562 $[M + H]^+$, 584 $[M + Na]^+$. $C_{28}H_{31}N_7O_6$ (561.59): calcd. C 59.88, H 5.56, N 17.46; found C 59.60, H 5.55, N 17.34.

4,6-Dibromo-*N*,*N*′-bis[6-(pentanoylamino)pyrid-2-yl]isophthalamide (1c): Compound **1c** was synthesized by the same procedure as used

for receptor **1a**, from *N*,*N*′-bis(6-aminopyrid-2-yl)-4,6-dibromoisophthalamide (**6c**, 450 mg, 0.889 mmol), triethylamine (2.00 mL, 9.09 mmol), and pentanoyl chloride (433 μL, 3.56 mmol) in anhydrous tetrahydrofuran (10 mL). The residue was purified by column chromatography (silica gel, ethyl acetate/cyclohexane 1:1, R_f = 0.39); yield 475 mg (70%); m.p. 199–201 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.47 (br. s, 2 H, Ar-CON*H*), 8.05 (br. s, 1 H, Ar-*H*-5), 7.83 (s, 1 H, Ar-*H*-2), 8.20 (br. s, 2 H, H_9C_4CON*H*), 7.93 (d, 2 H, Py-5-*H*), 7.86 (br. d, 4 H, Py-3-*H*), 7.61 (t, $^3J_{H,H}$ = 8.2 Hz, 2 H, Py-4-*H*), 2.38 (t, $^3J_{H,H}$ = 7.5 Hz, 4 H, COCH_2), 1.68 (quin, $^3J_{H,H}$ = 7.5 Hz, 4 H, $COCH_2CH_2$), 1.37 (sext, $^3J_{H,H}$ = 7.4 Hz, 4 H, $CH_2CH_2CH_3$), 0.91 (t, $^3J_{H,H}$ = 7.4 Hz, 6 H, CH_2CH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 172.2 (s, *C*OC_4H_9), 164.0 (s, Ar-*C*ONH), 150.0 (s, Py-*C*-2), 148.5 (s, Py-*C*-6), 141.0 (d, Py-*C*-4), 137.9 (s, Ar-*C*-4,6), 136.2 (s, Ar-*C*-1,3), 130.3 (d, Ar-*C*-5), 122.1 (d, Ar-*C*-2), 110.6 (d, Py-*C*-3), 109.8 (d, Py-*C*-5), 37.6 (t, CO*C*H_2), 27.4 (t, $COCH_2CH_2$), 22.3 (t CH_2CH_3), 13.8 (q, CH_3) ppm. IR (ATR): $\tilde{\nu}$ = 3289 (N–H), 3025 (arom. C–H), 2958, 2930, 2871 (aliph. C–H), 1674 (C=O), 1582, 1505 (arom. C=C), 1445 (CH_2, CH_3) 1052 (arom. C–Br) cm^{-1}. MS (EI, 70 eV): *m*/*z* (%) = 672 (5) [M]$^{+\cdot}$, 615 (8) [M – C_4H_9]$^+$. MS (MALDI): *m*/*z* = 673 [M + H]$^+$, 695 [M + Na]$^+$, 711 [M + K]$^+$. $C_{28}H_{30}Br_2N_6O_4$ (674.38): calcd. C 49.87, H 4.48, N 12.46; found C 49.67, H 4.53, N 12.25.

5-Benzoyloxy-*N*,*N*′-bis[6-(pentanoylamino)pyrid-2-yl]isophthalamide (1d): *N*-(6-Aminopyrid-2-yl)pentanamide (**7**, 1.12 g, 6.18 mmol) and anhydrous triethylamine (1.12 mL, 8.03 mmol) were dissolved in anhydrous tetrahydrofuran (60 mL) under nitrogen. A solution of 5-(benzoyloxy)isophthaloyl chloride (**9**, 1.00 g, 2.37 mmol) in anhydrous tetrahydrofuran (15 mL) was then added dropwise. After the system had been stirred at room temp. for 30 min it was heated to reflux for 18 h. The resulting dispersion was filtered and the solvent was evaporated in vacuo. The residue was purified by column chromatography (silica gel, ethyl acetate/cyclohexane 3:2, R_f = 0.8); yield 911 mg (60%); m.p. 115 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.22 (s, 1 H, Ar-*H*-2), 8.21 (d, $^3J_{H,H}$ = 8.1 Hz, 2 H, Py-*H*-5), 8.05–7.94 (m, 6 H, Ar-*H*-4,6, Bz-*H*-2,6, Py-*H*-3), 7.81–7.74 (m, 2 H, Bz-*H*-3,5), 7.70 (t, $^3J_{H,H}$ = 7.5 Hz, 1 H, Bz-*H*-4), 7.56 (t, $^3J_{H,H}$ = 8.1 Hz, 2 H, Py-*H*-4), 2.38 (t, $^3J_{H,H}$ = 7.5 Hz, 4 H, COCH_2), 1.73 (quint, $^3J_{H,H}$ = 7.5 Hz, 4 H, $COCH_2CH_2$), 1.42 (sext, $^3J_{H,H}$ = 7.5 Hz, 4 H, $COCH_2CH_2CH_2$), 0.96 (t, $^3J_{H,H}$ = 7.3 Hz, 6 H, CH_3), ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 172.1 (s, NH*C*O), 165.1 (s, NH*C*O), 163.7 (s, O*C*O), 151.5 (s, Ar-*C*-5), 150.0 (s, Py-*C*-2), 149.1 (s, Py-*C*-6), 140.9 (d, Py-*C*-4), 136.1 (s, Ar-*C*-1,3), 134.3 (d, Bz-*C*-4), 130.3 (s, Bz-*C*-1), 128.8 (d, Bz-*C*-2,6), 128.3 (d, Bz-*C*-3,5), 125.0 (d, Ar-*C*-4,6), 122.9 (d, Ar-*C*-2), 110.3 (d, Py-*C*-3), 109.4 (d, Py-*C*-5), 37.4 (t, CH_2), 27.4 (t, CH_2), 22.4 (t, CH_2), 13.8 (q, CH_3) ppm. IR (KBr): $\tilde{\nu}$ = 3408 (N–H), 2962 (aliph. C–H), 1686 (C=O), 1584, 1523 (arom.), 1260 (N–H) cm^{-1}. MS (ESI): *m*/*z* (%) = 637 [M + H]$^+$, 659 [M + Na]$^+$. $C_{35}H_{36}N_6O_6$ (636.7): calcd. C 66.02, H 5.70, N 13.20. $C_{41}H_{48}N_6O_6 \cdot 2.5\,H_2O$: calcd. C 61.66, H 6.06, N 12.33; found C 61.81, H 6.04, N 12.23.

5-Hydroxy-*N*,*N*′-bis[6-(pentanoylamino)pyrid-2-yl]isophthalamide (1e): 5-Benzoyloxy-*N*,*N*′-bis[6-(pentanoylamino)pyrid-2-yl]isophthalamide (**1d**, 570 mg, 896 μmol) was dissolved in ethanol (5 mL). After addition of sodium hydroxide (1 N, 10 mL), the solution was stirred for 3 h and then acidified with hydrochloric acid (2 N). The mixture was filtered, and the solid was washed with tetrahydrofuran. The organic filtrate was dried with sodium sulfate and the solvent was evaporated in vacuo; yield 449 mg (94%); m.p. 172 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 10.4 (br. s, 2 H, CON*H*), 10.2 (br. s, 2 H, CON*H*), 8.00 (s, 1 H, Ar-*H*-2), 7.52 (s, 2 H, Ar-*H*-4,6), 7.86–7.75 (m, 6 H, Py-*H*-3,4,5), 2.41 (t, $^3J_{H,H}$ = 7.4 Hz, 2 H, COCH_2), 1.58 (quint, $^3J_{H,H}$ = 7.4 Hz, 4 H, $COCH_2CH_2$), 1.32 (sext, $^3J_{H,H}$ = 7.5 Hz, 4 H, $COCH_2CH_2CH_2$), 0.86 (t, $^3J_{H,H}$ = 7.3 Hz, 6 H, CH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 172.1 (s, $CH_3CH_2CH_2CH_2CO$), 166.1 (s, NH*C*O), 150.5 (s, Ar-*C*-5), 150.3 (s, Py-*C*-2), 139.9 (Py-*C*-6, Py-*C*-4), 135.1 (s, Ar-*C*-1,3), 119.5 (Ar-*C*-4,6, Ar-*C*-2), 110.1 (d, Py-*C*-3), 109.4 (d, Py-C-5), 35.8 (t, COCH_2), 27.1 (t, $COCH_2CH_2$), 21.7 (t, CH_2CH_3), 13.7 (q, CH_3) ppm. IR (KBr): $\tilde{\nu}$ = 3376 (O–H, N–H), 1654 (C=O), 1585, 1530 (arom.), 1293 (N–H) cm^{-1}. MS (ESI): *m*/*z* (%) = 533 [M + H]$^+$, 555 [M + Na]$^+$. $C_{28}H_{32}N_6O_5$ (532.6): calcd. C 63.14, H 6.06, N 15.78. $C_{28}H_{32}N_6O_5 \cdot 0.5\,H_2O$: calcd. C 62.05, H 6.14, N 15.52; found C 61.85, H 6.15, N 15.59.

***N*,*N*′-Bis[6-(2-ethylhexanoylamino)pyrid-2-yl]-5-iodoisophthalamide (2a):** Compound **2a** was synthesized by the same procedure as used for receptor **1a**, from *N*,*N*′-bis(6-aminopyrid-2-yl)-5-iodoisophthalamide (**6a**, 1.23 g, 2.60 mmol), triethylamine (750 μL, 5.50 mmol), and 2-ethylhexanoyl chloride (950 μL, 5.50 mmol). Column chromatography (silica gel, ethyl acetate/cyclohexane 1:1, R_f = 0.59); yield 1.51 g (80%); m.p. 130–132 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.40 (br. s, 2 H, Ar-CON*H*), 8.40–8.35 (m, 3 H, Ar-*H*-2,4,6), 8.03, 7.97 (2 × d, $^3J_{H,H}$ = 8.0 Hz, 4 H, Pyr-3,5-*H*), 7.90 (br. s, 2 H, $H_{15}C_7CON$*H*), 7.73 (t, $^3J_{H,H}$ = 8.0 Hz, 2 H, Py-4-*H*), 2.19 (m_c, 2 H, COC*H*), 1.80–1.50 (m, 8 H, $COCHCH_2$), 1.32 (m_c, 8 H, $CH_2CH_2CH_3$), 0.97 (t, $^3J_{H,H}$ = 7.4 Hz, 6 H, $CHCH_2CH_3$) 0.88 (m_c, 6 H, $CHCH_2CH_2CH_2CH_3$) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 174.9 (s, *C*OC_7H_{15}), 162.8 (s, Ar-*C*ONH), 149.8 (s, Py-*C*-2), 148.9 (s, Py-*C*-6), 141.0 (d, Py-*C*-4), 139.6 (d, Ar-*C*-2), 136.3 (s, Ar-*C*-1,3), 125.0 (d, Ar-*C*-4,6), 110.5 (d, Py-*C*-3), 109.7 (d, Py-*C*-5), 94.8 (s, Ar-*C*-5), 50.7 (d, CO*C*H), 32.4 (t, $CH_2CH_2CH_2CH_3$), 29.8 (t, $CH_2CH_2CH_2CH_3$), 26.1 (t, $CHCH_2CH_3$), 22.8 (t, $CH_2CH_2CH_2CH_3$), 14.0 (q, $CH_2CH_2CH_2CH_3$), 12.0 (q, $CHCH_2CH_3$) ppm. IR (ATR): $\tilde{\nu}$ = 3279 (N–H), 3071 (arom. C–H), 2958, 2929, 2859 (aliph. C–H), 1668 (C=O), 1580, 1504 (arom. C=C), 1441 (alkyl C–H) cm^{-1}. MS (EI, 70 eV): *m*/*z* (%) = 726 (15) [M]$^{+\cdot}$, 670 (72) [M – C_4H_8]$^{+\cdot}$, 627 (100) [M – C_7H_{15}]$^+$, 600 (5) [M – I]$^{+\cdot}$. MS (CI, isobutane): *m*/*z* (%) = 727 (100) [M + H]$^+$, 601 (18) [M – I + H]$^+$. MS (ESI): *m*/*z* (%) = 727 [M + H]$^+$. MS (MALDI): *m*/*z* (%) = 727 [M + H]$^+$, 749 [M + Na]$^+$, 765 [M + K]$^+$. $C_{34}H_{43}IN_6O_4$ (726.24): calcd. C 56.20, H 5.96, N 11.57; found C 56.20, H 6.13, N 11.60.

***N*,*N*′-Bis[6-(2-ethylhexanoylamino)pyrid-2-yl]-5-nitroisophthalamide (2b):** Compound **2b** was synthesized by the same procedure as used for receptor **1a**, from *N*,*N*′-bis(6-aminopyrid-2-yl)-5-nitroisophthalamide (**6b**, 661 mg, 1.68 mmol), triethylamine (785 μL, 3.56 mmol), and 2-ethylhexanoyl chloride (615 μL, 3.56 mmol). The crude product was purified by column chromatography (silica gel, cyclohexane/ethyl acetate 1:1, R_f = 0.60); yield 500 mg (46%) as a slightly yellow solid; m.p. 119–121 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.87 (d, $^4J_{H,H}$ = 1.5 Hz, 2 H, Ar-*H*-4,6), 8.83 (t, $^4J_{H,H}$ = 1.5 Hz, 1 H, Ar-*H*-2), 8.76 (br. s, 2 H, Ar-CON*H*), 8.02 (d, $^3J_{H,H}$ = 8.0 Hz, 2 H, Pyr-5-H), 7.99 (br. s, 2 H, H_9C_4CON*H*), 7.95 (d, $^3J_{H,H}$ = 8.0 Hz, 2 H, Pyr-3-*H*), 7.74 (t, $^3J_{H,H}$ = 8.0 Hz, 2 H, Py-4-*H*), 2.21 (m_c, 2 H, COC*H*), 1.80–1.50 (m, 8 H, $COCHCH_2$), 1.32 (m_c, 8 H, $CH_2CH_2CH_3$), 0.97 (t, $^3J_{H,H}$ = 7.5 Hz, 6 H, $CHCH_2CH_3$), 0.88 (t, $^3J_{H,H}$ = 6.9 Hz, 6 H, $CHCH_2CH_2CH_2CH_3$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 175.1 (s, *C*OC_7H_{15}), 162.1 (s, Ar-*C*ONH), 149.9 (s, Py-*C*-2), 148.8 (s, Py-*C*-6), 148.7 (s, Ar-*C*-5), 141.0 (d, Py-*C*-4), 141.0 (d, Ar-*C*-2), 136.5 (s, Ar-*C*-1,3), 125.3 (d, Ar-*C*-4,6), 110.8 (d, Py-*C*-3), 109.8 (d, Py-*C*-5), 50.7 (d, CO*C*H), 32.4 (t, $CH_2CH_2CH_2CH_3$), 29.8 (t, $CH_2CH_2CH_2CH_3$), 26.1 (t, $CHCH_2CH_3$), 22.8 (t, $CH_2CH_2CH_2CH_3$), 14.0 (q, $CH_2CH_2CH_2CH_3$), 12.0 (q, $CHCH_2CH_3$) ppm. IR (ATR): $\tilde{\nu}$ = 3283 (N–H), 3050 (arom. C–H), 2959, 2931, 2872 (aliph. C–H), 1669 (C=O), 1583, 1505 (arom. C=C), 1441 (alkyl C–H) cm^{-1}. MS

(EI, 70 eV): *m/z* (%) = 645 (4) $[M]^{+\cdot}$, 616 (10) $[M - C_2H_5]^{+\cdot}$, 546 (100) $[M - C_4H_9]^+$. MS (CI, isobutane): *m/z* (%) = 646 (6) $[M + H]^+$. MS (MALDI): *m/z* = 646 $[M + H]^+$, 668 $[M + Na]^+$, 684 $[M + K]^+$. $C_{34}H_{43}N_7O_6$ (645.749): calcd. C 63.24, H 6.71, N 15.18. $C_{34}H_{43}N_7O_6 \cdot 0.1\,CH_3COOCH_2CH_3$ (663.37): calcd. C 63.12, H 6.74, N 14.98; found C 63.28, H 6.94, N 14.82.

5-Benzoyloxy-*N*,*N*′-bis[6-(2-ethylhexanoyl)aminopyrid-2-yl]isophthalamide (2d): *N*-(6-Aminopyrid-2-yl)-2-ethylhexanamide (**8**, 1.24 g, 5.27 mmol) and anhydrous triethylamine (1.24 mL, 8.96 mmol) were dissolved in anhydrous tetrahydrofuran (60 mL) under nitrogen. A solution of 5-(benzoyloxy)isophthaloyl chloride (**9**, 1.10 g, 3.40 mmol) in anhydrous tetrahydrofuran (15 mL) was then added dropwise. After the system had been stirred at room temp. for 30 min it was heated to reflux for 18 h. The resulting dispersion was filtered, and the solvent was evaporated in vacuo. The residue was purified by column chromatography (silica gel, ethyl acetate/cyclohexane 3:1, R_f = 0.38); yield 850 mg (35%); m.p. 111 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.52 (br. s, 2 H, N*H*), 8.31 (br. s, 2 H, N*H*), 8.25 (s, 1 H, Ar-*H*-2), 8.08 (d, $^3J_{H,H}$ = 7.3 Hz, 2 H, Py-*H*-5), 7.98–7.87 (m, 6 H, Ar-*H*-4,6, Bz-*H*-2,6, Py-*H*-3), 7.65–7.59 (m, 3 H, Bz-*H*-3,4,5), 7.46 (t, $^3J_{H,H}$ = 7.8 Hz, 2 H, Py-*H*-4), 2.23–2.17 (m, 2 H, C*H*), 1.75–1.68 (m, 4 H, $CHCH_2CH_3$), 1.60–1.48 (m, 4 H, $CHCH_2$), 1.29–1.25 (m, 8 H, $CHCH_2CH_2CH_2$), 0.94 (t, $^3J_{H,H}$ = 7.4 Hz, 6 H, $CHCH_2CH_3$), 0.86–0.83 (m, 6 H, CH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 175.2 (s, *C*O), 164.9 (s, O*C*O), 163.5 (s, NH*C*O), 151.5 (s, Ar-*C*-5), 149.9 (s, Py-*C*-2), 149.0 (s, Py-*C*-6), 140.8 (d, Py-*C*-4), 136.1 (s, Ar-*C*-1,3), 134.3 (d, Bz-*C*-4), 130.3 (s, Bz-*C*-1), 128.7 (d, Bz-*C*-2,6), 128.3 (d, Bz-*C*-3,5), 124.7 (d, Ar-*C*-4,6), 122.9 (d, Ar-*C*-2), 110.5 (d, Py-*C*-3), 109.7 (d, Py-*C*-5), 50.6 (d, CO*C*H), 32.4 (t, $COCHCH_2$), 29.8 (t, $COCHCH_2$), 26.9 (t, $COCHCH_2CH_2$), 22.8 (t, $COCHCH_2CH_2CH_2$), 13.9 (q, $COCHCH_2CH_2CH_2CH_3$), 12.1 (q, $CHCH_2CH_3$) ppm. IR (ATR): $\tilde{\nu}$ = 3292 (N–H), 2959 (aliph. C–H), 1670 (C=O), 1582, 1507 (arom.) cm^{-1}. MS (ESI): *m/z* = 721 $[M + H]^+$, 743 $[M + Na]^+$. $C_{41}H_{48}N_6O_6$ (720.8): calcd. C 68.31, H 6.71, N 11.66. $C_{41}H_{48}N_6O_6 \cdot 0.5\,H_2O$: calcd. C 67.47, H 6.77, N 11.51; found C 67.85, H 6.75, N 11.65.

***N*,*N*′-Bis[6-(2-ethylhexanoyl)aminopyrid-2-yl]-5-hydroxyisophthalamide (2e):** 5-Benzoyloxy-*N*,*N*′-bis[6-(2-ethylhexanoyl)aminopyrid-2-yl]isophthalamide (**2d**, 570 mg, 791 µmol) was dissolved in ethanol (5 mL). After addition of aqueous sodium hydroxide (1 N, 10 mL), the solution was stirred for 3 h and then acidified to pH 3 with hydrochloric acid (2 N). The mixture was filtered, and the solid was washed with tetrahydrofuran. The organic filtrate was dried with sodium sulfate, and the solvent was evaporated in vacuo; yield 410 mg (84%); m.p. 134 °C. ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 10.36 (br. s, 2 H, CON*H*), 10.14 (br. s, 2 H, CON*H*), 8.01 (s, 1 H, Ar-*H*-2), 7.86–7.79 (m, 6 H, Py-*H*-3,4,5), 7.53 (s, 2 H, Ar-*H*-4,6), 2.51–2.50 (m, 2 H, C*H*), 1.57–1.55 (m, 4 H, $CHCH_2CH_3$), 1.46–1.36 (m, 4 H, $CHCH_2$), 1.27–1.23 (m, 8 H, $CHCH_2CH_2CH_2$), 0.87–0.83 (m, 12 H, CH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 175.1 (s, *C*O), 165.1 (s, NH*C*O), 157.7 (s, Ar-*C*-5), 150.4 (s, Py-*C*-2), 150.1 (s, Py-*C*-6), 140.1 (d, Py-*C*-4), 135.6 (s, Ar-*C*-1,3), 118.1 (d, Ar-*C*-4,6), 117.6 (d, Ar-*C*-2), 110.3 (d, Py-*C*-3), 110.0 (d, Py-*C*-5), 47.6 (d, CO*C*H), 31.9 (t, $COCHCH_2CH_3$), 29.2 (t, $COCHCH_2$), 26.3 (t, $COCHCH_2CH_2$), 22.2 (t, $COCHCH_2CH_2CH_2$), 13.9 (q, $CH_2CH_2CH_2CH_3$), 11.7 (q, $CHCH_2CH_3$) ppm. IR (ATR): $\tilde{\nu}$ = 3270 (O–H, N–H), 1668 (C=O), 1582, 1506 (arom.) cm^{-1}. MS (ESI): *m/z* = 617 $[M + H]^+$, 639 $[M + Na]^+$. $C_{34}H_{44}N_6O_5$ (616.7): calcd. C 66.21, H 7.19, N 13.63. $C_{41}H_{48}N_6O_6 \cdot 0.5\,H_2O$: calcd. C 65.26, H 7.25, N 13.43; found C 65.13, H 7.19, N 13.26.

***N*,*N*′-Bis(6-aminopyrid-2-yl)-5-iodoisophthalamide (6a):** 5-Iodoisophthaloyl dichloride (**4a**, 2.52 g, 7.69 mmol) in tetrahydrofuran (50 mL) was added dropwise over a period of 3 h to a solution of 2,6-diaminopyridine (**5**, 8.39 g, 76.9 mmol) and triethylamine (2.13 mL, 15.4 mmol) in anhydrous tetrahydrofuran (150 mL). After the system had been stirred for 2 h, the solvent was removed in vacuo and the residue was washed with water (1 L). Purification by column chromatography (neutral alumina, tetrahydrofuran/dichloromethane 3:1, R_f = 0.52) yielded a light yellow solid; yield 1.54 g (79%); m.p. 248 °C. ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 10.35 (br. s, 2 H, N*H*), 8.49 (s, 1 H, Ar-2-*H*), 8.38 (d, $^4J_{H,H}$ = 1.3 Hz, 2 H, Ar-4,6-*H*), 7.45 (t, $^3J_{H,H}$ = 7.8 Hz, 2 H, Pyr-4-*H*), 7.36 (dd, $^3J_{H,H}$ = 7.7, $^4J_{H,H}$ = 0.9 Hz, 2 H, Pyr-3-*H*), 6.28 (dd, $^3J_{H,H}$ = 7.7, $^4J_{H,H}$ = 0.9 Hz, 2 H, Pyr-5-*H*), 5.84 (br. s, 4 H, NH_2) ppm. ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 163.4 (s, Ar-*C*ONH), 158.6 (s, Py-*C*-2), 150.1 (s, Py-*C*-6), 139.3 (d, Py-*C*-4), 139.0 (d, Ar-*C*-4,6), 136.2 (s, Ar-*C*-1,3), 126.3 (d, Ar-*C*-2), 104.3 (d, Py-*C*-3), 101.9 (d, Py-*C*-5), 94.6 (s, Ar-*C*-5) ppm. IR (ATR): $\tilde{\nu}$ = 3326 (CON*H*), 1673, 1612 (*C*ONH) cm^{-1}. MS (EI, 70 eV): *m/z* (%) = 474 (100) $[M]^{+\cdot}$, 366 (9) $[M - NHPyrNH_2]^{+\cdot}$. MS (CI, isobutane): *m/z* (%) = 475 (100) $[M + H]^+$, 349 (18) $[M - I + H]^+$. HRMS (EI): $C_{18}H_{15}IN_6O_2$ calcd. 474.03012; found 474.02408, $C_{17}{}^{13}CH_{15}IN_6O_2$ calcd. 475.03348; found 475.02854.

***N*,*N*′-Bis(6-aminopyrid-2-yl)-4,6-dibromoisophthalamide (6c):** 2,6-Diaminopyridine (**5**, 986 mg, 9 mmol) was dissolved in anhydrous tetrahydrofuran (100 mL), the mixture was stirred for 3 h at room temp., the solution was filtered, and triethylamine (2 mL) was added. The solution was cooled in ice and 4,6-dibromoisophthaloyl chloride (**4c**, 1.08 g, 3.00 mmol) in tetrahydrofuran (10 mL) was added under nitrogen. The reaction mixture was stirred at room temp. for 10 h, concentrated by evaporation, and poured into boiling water (200 mL) to remove excess 2,6-diaminopyridine and triethylamine hydrochloride. The crude product was dried and purified by basic alumina filtration (tetrahydrofuran/methanol 25:1). Recrystallization from tetrahydrofuran/*n*-hexane afforded **6a** as white crystals (1.15 g, 76%); m.p. 250–255 °C (decomp.). ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 10.33 (br. s, 2 H, Ar-CON*H*), 8.03 (s, 1 H, Ar-*H*-5), 7.68 (s, 1 H, Ar-*H*-2), 7.42 (t, $^3J_{H,H}$ = 7.8 Hz, 2 H, Py-4-*H*), 7.31 (br. d, $^3J_{H,H}$ = 7.8 Hz, 2 H, Py-3-*H*), 6.26 (dd, $^3J_{H,H}$ = 7.8, $^4J_{H,H}$ = 1.0 Hz, 2 H, Py-5-*H*), 5.80 (br. s, 4 H, NH_2) ppm. ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 164.51 (s, *C*ONH), 158.6 (s, Py-2-*C*), 149.9 (s, Py-6-*C*), 138.92 (d, Py-4-*C*), 137.0 (s, Ar-1,3-*C*), 136.0 (d, Ar-5-*C*), 129.3 (d, Ar-2-*C*), 120.9 (s, Ar-*C*-4,6), 104.2 (d, Py-3-*C*), 101.4 (d, Py-5-*C*) ppm. IR (ATR): $\tilde{\nu}$ = 3463, 3300, 3197 (N–H), 3056 (arom. C–H), 1668 (C=O), 1620, 1532 1448 (arom. C=C), 1052 (arom. C–Br) cm^{-1}. MS (EI, 70 eV): *m/z* (%) = 504 (48) $[M]^{+\cdot}$, 425 (100) $[M - Br]^+$, 345 $[M - Br_2 + H]^+$. MS (CI, isobutane): *m/z* (%) = 505 (13) $[M + H]^+$. MS (MALDI): *m/z* = 505 $[M + H]^+$, 527 $[M + Na]^+$.

***N*-(6-Aminopyrid-2-yl)pentanamide (7):** 2,6-Diaminopyridine (**5**, 3.25 g, 29.8 mmol) and anhydrous triethylamine (4.35 mL, 29.8 mmol) were dissolved in anhydrous tetrahydrofuran (50 mL). A solution of pentanoyl chloride (3.80 mL, 31.3 mmol) in anhydrous tetrahydrofuran (6 mL) was added dropwise at 0 °C over 30 min. Afterwards, the mixture was stirred at 0 °C for 3 h and then for 16 h at room temp. After filtration, the solvent was evaporated in vacuo and the residue was purified by column chromatography (silica gel, ethyl acetate/cyclohexane 3:2, R_f = 0.5); yield 3.44 g (60%); m.p. 66 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.65 (br. s, 1 H, N–*H*), 7.55 (d, $^3J_{H,H}$ = 7.9 Hz, 1 H, Py-*H*-3), 7.45 (t, $^3J_{H,H}$ = 7.9 Hz, 1 H, Py-*H*-4), 6.24 (d, $^3J_{H,H}$ = 7.9 Hz, 1 H, Py-*H*-5), 4.29 (br. s, 2 H, NH_2), 2.35 (t, $^3J_{H,H}$ = 7.6 Hz, 2 H, $COCH_2$), 1.70 (quint, $^3J_{H,H}$ = 7.6 Hz, 2 H, $COCH_2CH_2$), 1.40 (sext, $^3J_{H,H}$ = 7.6 Hz, 2 H, $COCH_2CH_2CH_2$), 0.94 (t, $^3J_{H,H}$ = 7.4 Hz, 3 H, CH_3) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 171.9 (s, *C*O), 156.9

(s, Py-*C*-6), 149.8 (s, Py-*C*-2), 140.3 (d, Py-*C*-4), 104.1 (d, Py-*C*-5), 103.2 (d, Py-*C*-3), 37.3 (t, $CO\mathit{C}H_2$), 27.4 (t, $COCH_2\mathit{C}H_2$), 22.2 (t, $COCH_2CH_2\mathit{C}H_2$), 13.7 (q, $\mathit{C}H_3$) ppm. IR (KBr): $\tilde{\nu}$ = 3410 (N–H), 2961 (aliph. C–H), 1673 (C=O), 1617, 1542 (arom.), 1299 (N–H) cm^{-1}. MS (ESI): *m*/*z* = 194 $[M + H]^+$, 216 $[M + Na]^+$. $C_{10}H_{15}N_3O$ (193.2): calcd. C 62.15, H 7.82, N 21.74; found C 62.09, H 7.97, N 21.62.

***N*-(6-Aminopyrid-2-yl)-2-ethylhexanamide (8):** 2,6-Diaminopyridine (**5**, 3.25 g, 29.8 mmol) and anhydrous triethylamine (4.35 mL, 29.8 mmol) were dissolved in anhydrous tetrahydrofuran (50 mL). A solution of 2-ethylhexanoyl chloride (5.36 mL, 31.3 mmol) in anhydrous tetrahydrofuran (6 mL) was added dropwise over 30 min. Afterwards, the mixture was stirred at 0 °C for 3 h and then for 16 h at room temp. After filtration, the solvent was evaporated in vacuo and the residue was purified by column chromatography (silica gel, ethyl acetate/cyclohexane 1:2, R_f = 0.37); yield 3.77 g (54%); m.p. 102 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.61 (br. d, $^3J_{H,H}$ = 7.8 Hz, 2 H, Py-*H*-3, N–*H*), 7.44 (t, $^3J_{H,H}$ = 7.9 Hz, 1 H, Py-*H*-4), 6.24 (d, $^3J_{H,H}$ = 7.9 Hz, 1 H, Py-*H*-5), 4.30 (br. s, 2 H, NH_2), 2.10–2.06 (m, 1 H, C*H*), 1.83–1.65 (m, 2 H, $CHC\mathit{H}_2$), 1.56–1.47 (m, 2 H, $C\mathit{H}_2$), 1.32–1.26 (m, 4 H, $C\mathit{H}_2C\mathit{H}_2$), 0.93 (t, $^3J_{H,H}$ = 7.4 Hz, 3 H, $CHCH_2C\mathit{H}_3$), 0.87 (t, $^3J_{H,H}$ = 6.9 Hz, 3 H, $C\mathit{H}_3$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 174.5 (s, *C*=O), 157.0 (s, Py-*C*-6), 149.8 (s, Py-*C*-2), 140.2 (d, Py-*C*-4), 104.2 (d, Py-*C*-5), 103.3 (d, Py-*C*-3), 50.9 (d, CO*C*H), 32.5 (t, $COCH\mathit{C}H_2CH_3$), 29.8 (t, $COCH\mathit{C}H_2$), 26.1 (t, $COCHCH_2\mathit{C}H_2$), 22.8 (t, $COCHCH_2CH_2\mathit{C}H_2$), 13.9 (q, $CH_2CH_2\mathit{C}H_3$) 12.06 (q, $CHCH_2\mathit{C}H_3$) ppm. IR (ATR): $\tilde{\nu}$ = 3201 (N–H), 2922 (aliph. C–H), 1638 (C=O) cm^{-1}. MS (EI, 70 eV): *m*/*z* (%) = 235 (22) $[M]^+$, 109 (100) $[M - C_8H_{15}O]^+$. MS (MALDI): *m*/*z* = 236 $[M + H]^+$, 276 $[M + Na]^+$. $C_{13}H_{21}N_3O$ (235.3): calcd. C 66.35, H 8.99, N 17.86; found C 66.13, H 9.05, N 17.80.

X-ray Crystal Structure Determination of 1d: Suitable crystals were grown by allowing the test tubes to stand overnight after column chromatography. Empirical formula $C_{35}H_{36}N_6O_6 \cdot 3H_2O$, *Fw* = 690.75 g mol^{-1}, *a* = 11.6929(9) Å, *b* = 13.1904(10) Å, *c* = 13.2977(9) Å, α = 108.951(8)°, β = 96.455(9)°, γ = 109.105(9)°, *V* = 1777.2(2) Å^3, *T* = 170(2) K, $p_{calcd.}$ = 1.291 Mg m^{-3}, μ = 0.094 mm^{-1}, triclinic, space group $P\bar{1}$, *Z* = 2, STOE Imaging Plate Diffraction System (IPDS-1), Mo-K_α (λ = 0.71073 Å), 7076 measured reflections in the $2.2° < 2\theta < 26.0°$ range, 3762 independent reflections used for refinement. R_{int} = 0.0850. The structure was solved with SHELXS-97. Structure refinement was performed against F^2 with use of SHELXL-97; 514 parameters, R_1 = 0.0704 for 3205 data with $F_o > 4\sigma(F_o)$, R^1 = 0.0784 and wR_2 = 0.1978 for all 3762 data, GoF = 1.104, residual electron density 0.225/–0.283 (e Å^{-3}). All non-hydrogen atoms were refined anisotropically. The hydrogen atoms were positioned with idealized geometry and were refined by use of a riding model. The O–H hydrogen atoms were located in the difference map, their bond lengths were set to ideal values and finally they were refined by use of a riding model. The terminal six-membered ring is disordered in two orientations and was refined by use of a split model. All crystals investigated were non-merohedrally twinned. The two individual forms were therefore indexed and integrated separately and overlapping reflections were omitted.

CCDC-803919 (for **1d**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Supporting Information (see footnote on the first page of this article): NMR spectra and titration curves.

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