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Pyridine-based receptors with high affinity for carbohydrates. Influence of the degree of steric hindrance at pyridine nitrogen on the binding mode

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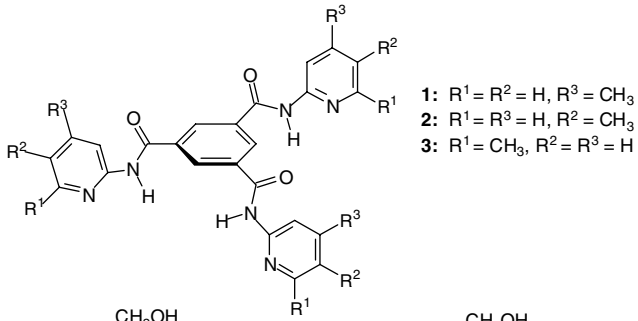
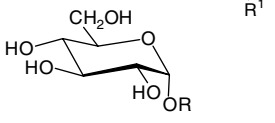
Abstract—Remarkable changes in the binding affinity and selectivity of pyridine-based receptors toward monosaccharides have been observed when the degree of steric hindrance at pyridine nitrogen atom decreases.

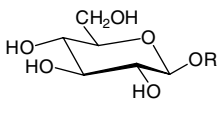
The molecular recognition of carbohydrates by synthetic receptors forms an area of high current interest, arising from the extreme importance of sugar molecules within biological systems. Carbohydrates actively control a whole range of biological processes including cell growth and differentiation. A range of carbohydrate binding proteins mediate the interactions of cells with their environment, primarily via interactions with the carbohydrates on the surfaces of cell.[1] Thus, the carbohydrates play fundamental roles in cell–cell recognition and other interactions processes. The protein–carbohydrate interactions involve the hydrogen bonding, metal coordination, van der Waals forces, and hydrophobic interactions.[1,2] These interactions have inspired the development of a number of different artificial receptor structures for the recognition of carbohydrates.[3] Particularly, many representatives of hydrogen bonding receptors have been obtained and studied.[4,5] However, the selective and effective molecular recognition of carbohydrates by a synthetic receptor is still a challenging goal in artificial receptor chemistry.

In this paper we describe the remarkable changes in the affinity as well as selectivity of pyridine-based receptors when the degree of steric hindrance at pyridine nitrogen atom decreases. The acyclic receptors **1** and **2**,[6] compared to the previously reported receptor **3**,[5b] reflect the change from α,α- to the α,β- or α,γ-disubstituted pyridine groups. The presence of an unsubstituted α-position in the pyridine ring is a typical feature of the receptors **1** and **2**. Consequently, the pyridine units are sterically less hindered at nitrogen and can be involved much easier and effective in the binding interactions. These receptors contain neighboring acceptor/donor groups (pyridine-N/amide-NH), which are able to participate in cooperative hydrogen bonds with the sugar hydroxyls. As shown by our earlier studies, the property of cooperativity is of particular importance in the area of synthetic carbohydrate receptors.[4i,5] Although, the structural variation of the receptor structure is minimal, the binding properties of the new receptors change dramatically.





4: R = C_8H_{17}, **4a:** R = CH_3



5 : R = C_8H_{17}

Keywords: Molecular recognition; Carbohydrate receptors; Hydrogen bonding.

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The evidence for the particularly strong complexation of monosaccharides with the receptors **1** and **2** was obtained by the NMR spectroscopy and extraction experiments. The crystal structures of the receptors **1** and **2** are shown in Figure 1.

All ^{1}H NMR titration experiments were carried out in the concentration range, in which the receptors do not self-aggregate ([**1**] or [**2**] < 0.0015 mol/L). The concentration range was estimated on the basis of a series of dilution experiments. The ^{1}H NMR titrations show that the addition of only 0.5 equiv of α-glucopyranoside **4** leads to practically complete complexation of host **1**. The signal due to amide NH of **1** shifted downfield by 1.10 ppm and the aromatic phenyl proton peak moved upfield by 0.40 ppm. Both the stoichiometry observed by fitting the binding isotherm and the molar ratio plots verify that host **1** forms 2:1 receptor:sugar complexes with α-glucopyranoside. A clear break in the molar ratio plot indicates that the formation of a complex with high stability constant is taking place.[8] Additionally, receptor **1** is able to extract 0.5 equiv of methyl-α-D-glucopyranoside (**4a**) from the solid state into a $CDCl_3$ solution.

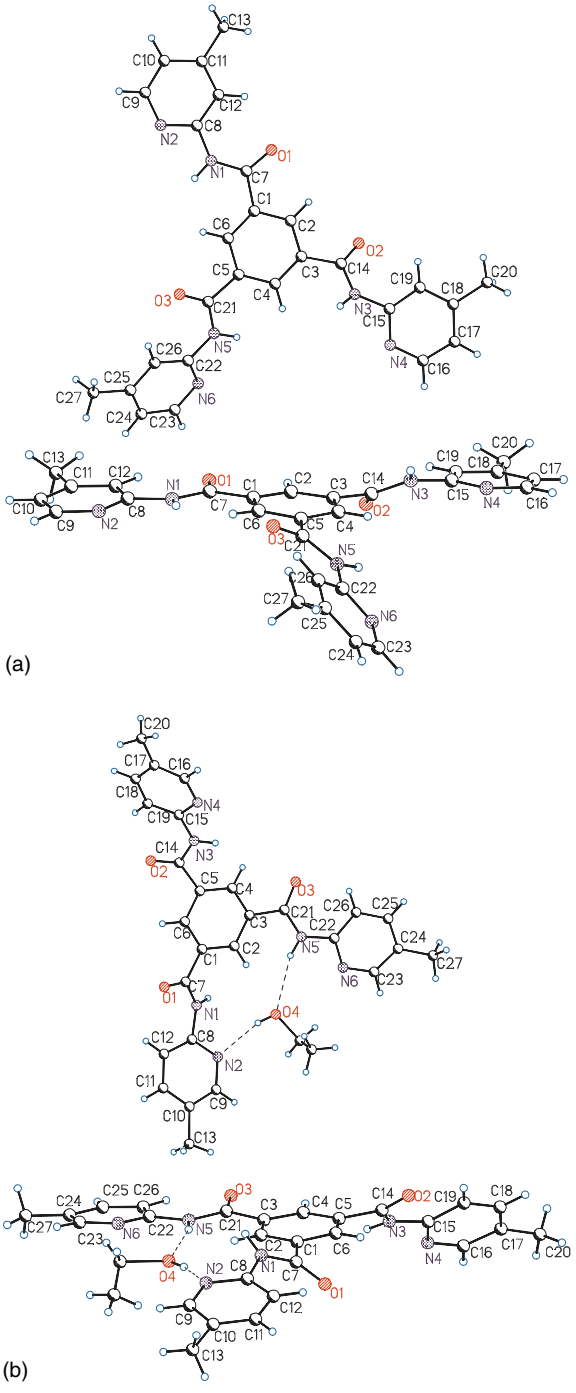

Figure 1. Crystal structure of **1** (a) and **2** (b),[7] top and side views.

The binding affinity toward α-glucopyranosides **4**–**4a** obtained with receptor **1** is comparable to that obtained with the host **2**. The complexation between **4** and receptor **2** is again evidenced by several changes in the ^{1}H NMR spectra, particularly by the significant downfield shifts of the receptor amide protons ($\Delta\delta_{max} = 1.10$ ppm) and upfield shifted resonances for the aromatic phenyl protons of **2** ($\Delta\delta_{max} = 0.37$ ppm), as shown in Figure 2a. After 0.5 equiv of octyl α-D-glucopyranoside (**4**) had been added, almost no change was observed in the NMR spectra.

The plot of the observed and calculated downfield chemical shifts of the NH resonances of **2** as a function of added sugar **4** is illustrated in Figure 3a.[9] The graph rises linearly with increasing the sugar concentration until $\Delta\delta_{max}$ is reached at 1:0.5 receptor:sugar stoichiometry. In the first part of the titration curve the receptor is the excess component and sugar the minor component, thus the 1:1 complex is formed followed by the immediate formation of a strong 2:1 receptor–sugar complex.

The data from titrations of **1** or **2** versus α-glucopyranoside **4** suggested a very large value of the overall formation constants and were impossible to follow for precisely binding constant calculations. In order to determine the binding constants, additional inverse titrations were carried out in which the concentration of glycoside **4** was held constant and that of receptor **1** or **2** varied. In these titrations, the motion of the signal due to anomeric CH proton of **4**, which is shifted significantly downfield, was monitored. The typical titration curves are shown in Figure 3c and d. In the first part of the titration curves, the sugar is now the excess component and the receptor the minor component. The anomeric CH proton exhibits chemical shift changes of 0.55 and 0.50 ppm ($\Delta\delta_{max}$) during complexation with **1** and **2** (Fig. 2c), respectively. These shifts are significantly larger in comparison to those reported usually for the anomeric CH in the literature (mostly $\Delta\delta_{max} = 0.05$–0.15 ppm), indicating an important contribution of this unit to the stability of the complexes **4·1** and **4·2**. On the basis of the inverse titrations the binding constants of **4** and receptor **1** were found to be 3640 (K_{a1}) and 82,450 M^{-1} (K_{a2}), while the binding constants for **4·2** amount to 2100 (K_{a1}) and 47,600 M^{-1} (K_{a2}).[9] The stronger binding of **1** in comparison to **2** can be attributed both to the different basicity of the pyridine recognition units (the increase in pK_a is somewhat greater for γ- than for β-methyl substituted pyridine[10]) and to

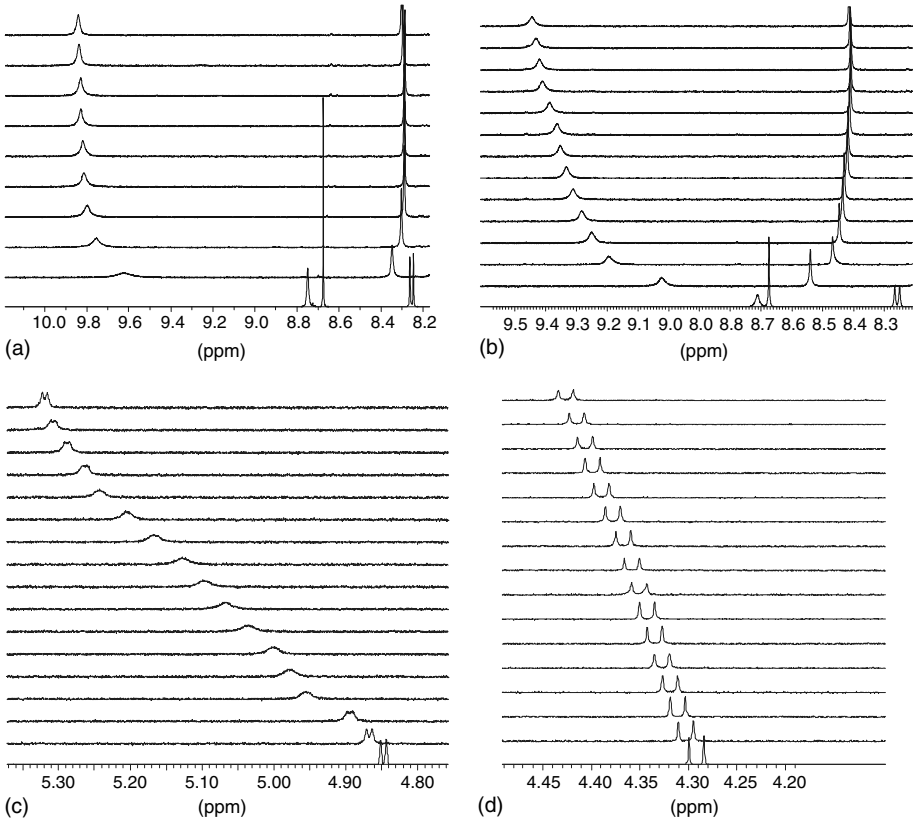

Figure 2. (a) ^{1}H NMR spectra ($CDCl_3$, 25 °C) of receptor **2** (the shifts of the NH and CH_{Ph} resonances are shown) after addition of (from bottom to top) 0.00, 0.39, 0.59, 0.80, 0.99, 1.19, 1.40, 1.59, 1.99, and 2.39 equiv of α-glucopyranoside **4** ([**2**]=1.33 mM). (b) ^{1}H NMR spectra of receptor **2** (the shifts of the NH and CH_{Ph} resonances are shown) after addition of 0.00, 0.25, 0.50, 0.63, 0.75, 0.88, 1.00, 1.13, 1.26, 1.51, 1.76, 2.00, 2.26, and 2.52 equiv of **5** ([**2**]=1.03 mM). (c) The downfield chemical shifts of the anomeric CH resonances of α-anomer **4** during the titration with receptor **2** ([**4**]=0.61 mM, [**2**]=0.10–2.50 mM). (d) The downfield chemical shifts of the anomeric CH resonances of β-anomer **5** during the titration with receptor **2** ([**5**]=0.61 mM, [**2**]=0.10–2.50 mM).

the different sterical effects. The 2:1 receptor:sugar binding mode is also supported by molecular modeling. A possible structure of the 2:1 and 1:1 complexes of **2** and **4** is shown in Figure 4.

In contrast to α-anomer **4**, the β-anomer **5** showed lower affinity to **1** and **2**. The ^{1}H NMR titration of **1** or **2** versus **5** also produced several spectral changes (Fig. 2b). The largest complexation-induced shifts were observed for the NH-signal (downfield shift of 0.85 and 0.80 ppm for **1** and **2**, respectively,) and for the phenyl CH-signal of the receptors (upfield shift of 0.35 ppm for **1** and 0.30 ppm for **2**). Comparison of Figure 3a and b clearly shows that the process of complexation is not reflected by these chemical shifts upon binding with α- or β-anomer in the same way. Whereas after the addition of 0.5 equiv of α-anomer almost no change was observed in the chemical shift of receptor signals, with the β-anomer chemical shift changes continue to higher receptor:sugar ratios. Nevertheless, for all systems the stoichiometry observed by fitting the binding isotherm is in agreement with that determined by the ratio method, which indicates the formation of 1:2 sugar:receptor complexes. The best fit of the titration data was obtained again with the 'mixed' 1:1 and 2:1 receptor:sugar binding models. The association constants of 660 (K_{a1}) and 24,200 M^{-1} (K_{a2}) were determined for **5**·**1** (Table 1), whereas the binding constants for **5**·**2** amount to 440 (K_{a1}) and 22,600 M^{-1} (K_{a2}). The association constants obtained from these experiments (typical titration curve is shown in Fig. 3e) are identical within the limits of uncertainty to those determined from titrations where the role of host and guest **5** was reversed. As shown in Figure 2d, the peak for the anomeric CH proton of **5** is affected significant weakly by complexation (0.18 and 0.15 ppm by complexation with **1** and **2**, respectively) than that of **4** (Fig. 2c), indicating a weaker binding again.

Notably, the observed α/β-anomer selectivities of **1** and **2** differ from those observed for the hydrogen-bonding host molecules described so far, which usually show higher affinity toward β-anomer. This preference has been ascribed to the hydrogen-bonding abilities of sugar hydroxyl groups. As discussed in Refs. 4f and 11 the axial 1-alkoxy group in α-anomer can form intramolecular hydrogen bonds with 2-OH group more easily than equatorial 1-alkoxy substituent in β-anomer can do. In this context, the 2-OH in β-glucopyranoside **5** is relatively free from intramolecular hydrogen bonding and can interact with receptor molecules more strongly. The strong binding of **4** with **1** or **2** indicates that the intermolecular host–guest H-bonding compete effectively with the intramolecular H-bonding network in

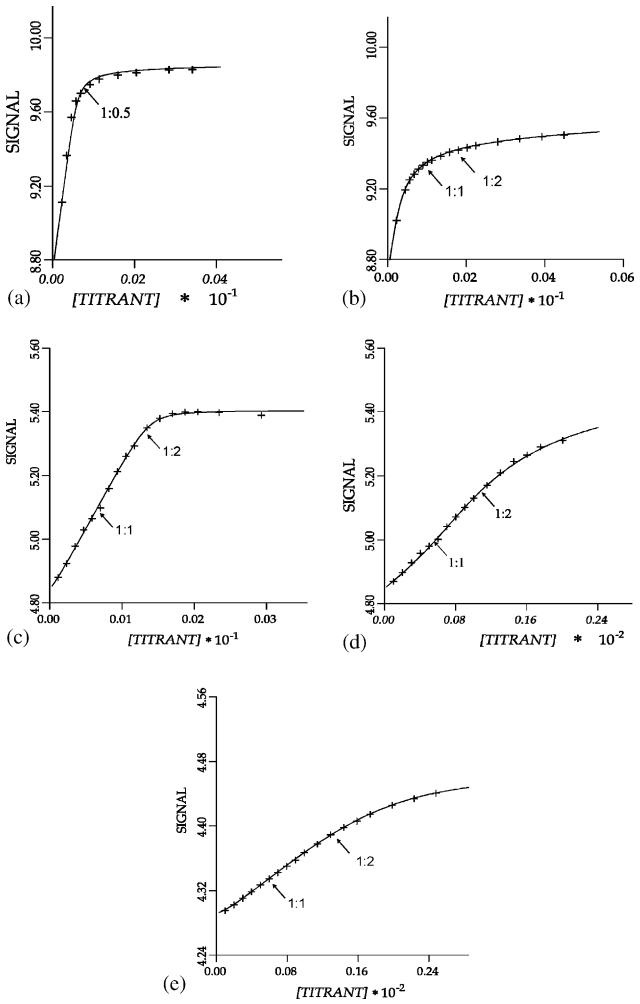

Figure 3. Plot of the observed (×) and calculated (—) downfield chemical shifts of the NH resonances of **2** as a function of added α-glucopyranoside **4** (a) or β-glucopyranoside **5** (b). The [receptor]/[sugar] ratio is marked (a–b). Plot of the downfield chemical shifts of the anomeric CH resonances of sugar **4** as a function of added **1** (c) or **2** (d), respectively. Plot of the downfield chemical shifts of the anomeric CH resonances of sugar **5** as a function of added **2** (e). The [sugar]/[receptor] ratio is marked (c–e).

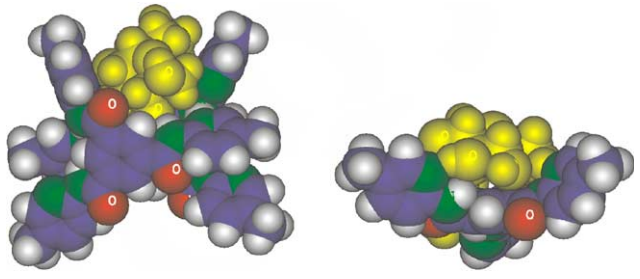

Figure 4. Energy-minimized structure of the 2:1 (left) and 1:1 (right) complexes formed between receptor **2** and glucopyranoside **4** (MacroModel V.6.5, Amber* force field, Monte Carlo conformational searches, 50,000 steps).

carbohydrates. The intramolecular bond is replaced by enough intermolecular H-bonds during the binding process. Molecular modeling studies suggest the existence of hydrogen bonds between OHs of **4** and the amide-NH/pyridine-N of **1** or **2**, including cooperative hydrogen bonds (OH···N-pyr, HO···HN-amide), in which the hydroxyl group acts simultaneously as a hydrogen bond donor and acceptor. The hydrogen bonds are supplemented by interactions of sugar CH moieties with the phenyl and pyridine groups of the receptors. Both sides of the pyranoside ring (α- and β-face) are involved in the stacking interactions with aromatic residues of the two receptor molecules in 2:1 receptor:sugar complexes. Furthermore the complex of **4** is also stabilized by weak CH···N-pyr and CH···O=C hydrogen bonds. These intermolecular interactions were also evidenced by the observed changes of chemical shifts in the NMR spectra of the complexes. In comparison with **4**, the CHs of sugar **5** participate in less intermolecular interactions (as indicated by molecular modeling, **5** is less favorable positioned in the cavity between the two receptors). The 2:1 receptor:sugar complexes display also hydrogen bonding between the two receptor molecules (two amide-NH···N-pyr hydrogen bonds).

Table 1. Association constants K_a[a] (M^{-1}) and corresponding free energy changes $\Delta G°$ (kJ mol^{-1}) for receptors **1**–**2** and glucopyranosides **4**–**5**

Host–guest complex	K_{a1} ($\Delta G°$)	K_{a2}[b] ($\Delta G°$)	$\Delta\delta_{max}$ (ppm)
1·4	3640 (−20.3)	82,450 (−28.0)	0.55[c]/1.10[d]
2·4	2100 (−18.9)	47,600 (−26.7)	0.50[c]/1.10[d]
1·5	660 (−16.1)	24,200 (−25.0)	0.18[c]/0.85[d]
2·5	440 (−15.1)	22,600 (−24.8)	0.15[c]/0.80[d]

[a] Average K_a values from multiple titrations ($CDCl_3$, stored over activated molecular sieves and deacidified with Al_2O_3), values provided by HOSTEST.[9] The reproducibility of the K_a values was ±10–15%. Uncertainty in a single K_a estimation was ±2–10%. Dilution experiments show that receptors do not self-aggregate in the used concentration range.

[b] 2:1 Receptor:sugar complex.

[c] Complexation-induced shifts observed for the anomeric CH of sugar (titrations of sugar vs receptor, inverse titrations).

[d] Complexation-induced shifts observed for the NH of receptor (titrations of receptor vs sugar).

In contrast to receptors **1** and **2**, receptor **3**, in which both pyridine α-positions are blocked, shows significantly lower affinity to both anomers and the formation of 1:1 complexes. The binding constant for α-anomer **4** and receptor **3** was found to be 4000 M^{-1}, the one for β-anomer **5** and host **3** amounts to 8700 M^{-1}.[5] Thus, the binding properties of the new receptors **1** and **2** apparently indicate that the steric interactions involving the pyridine substituents significantly affect the binding properties. These results reflect clearly the great importance of hydrogen-bonding interactions with the pyridine nitrogen atom for the binding affinity of the receptors. According to molecular modeling the steric effects from the α-position of the pyridine rings of **3** hinder the formation of intermolecular interactions, which are typical for 2:1 receptor:sugar complexes between **1/2** and glucopyranosides. The decreased degree of steric hindrance at the pyridine nitrogen allows for

the development of much stronger host:guest hydrogen-bonding interactions in the complexes. This knowledge may also enable the design of further hydrogen-bonding receptors, which may lead to the development of new chemosensors. Structures of type **1** or **2** can be incorporated into more sophisticated architectures to create a range of receptors, which should be able to form energetically favorable 1:1 complexes with sugar derivatives. Studies to synthesis of new receptors of this type are in progress.

Acknowledgements

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6. Compounds **1** and **2** were synthesized from benzene-1,3,5-tricarbonyl chloride and 2-amino-4-methyl-pyridine or 2-amino-5-methyl-pyridine, respectively (CH_2Cl_2 or THF, NEt_3, room temperature). *N*,*N*′,*N*″-Tris-(4-methylpyridin-2-yl)benzene-1,3,5-tricarbonamide (**1**): ^{1}H NMR ($CDCl_3$): $\delta = 2.40$ (s, 9H, $3\times CH_3$), 6.93 (d, $3H_{pyr}$, $J = 5.2$ Hz), 8.17 (d, $3H_{pyr}$, $J = 5.2$ Hz), 8.20 (s, $3H_{pyr}$), 8.68 (s, $3H_{Ph}$), 8.82 (s, 3H, $3\times NH$). ^{13}C NMR ($CDCl_3$): $\delta = 21.35$, 114.98, 121.51, 129.38, 135.70, 147.58, 150.07, 151.27, 163.94. HR-MS, calcd for $C_{27}H_{24}N_6O_3$: 480.1910. Found: 480.1903. *N*,*N*′,*N*″-Tris-(5-methyl-pyridin-2-yl)benzene-1,3,5-tricarbon-amide (**2**): ^{1}H NMR (200, $CDCl_3$): $\delta = 2.32$ (s, 9H, $3\times CH_3$), 7.58 (dd, $3H_{pyr}$, $J = 8.6/2.20$ Hz), 8.13 (d, $3H_{pyr}$, $J = 2.2$ Hz), 8.25 (d, $3H_{pyr}$, $J = 8.6$ Hz), 8.67 (s, $3H_{Ph}$), 8.73 (s, 3H, $3\times NH$). ^{13}C NMR ($CDCl_3$): $\delta = 17.87$, 113.84, 129.17, 129.83, 135.73, 139.07, 147.96, 148.97, 163.38. HR-MS, calcd for $C_{27}H_{24}N_6O_3$: 480.1910. Found: 480.1907.
7. Crystals of **1** were obtained from THF/heptane solution, crystals of **2** from ethanol solution (one hydrogen-bonded ethanol molecule is shown). Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no CCDC-229363 (**1**) and 229364 (**2**).
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