An Octa-Urea [Pd2L4]4+ Cage that Selectively Binds to n-octyl-α-D-Mannoside

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An Octa-Urea $[\text{Pd}_2\text{L}_4]^{4+}$ Cage that Selectively Binds to $n$-octyl-$\alpha$-D-Mannoside


Designing compounds for the selective molecular recognition of carbohydrates is a challenging task for supramolecular chemists. Macrocyclic compounds that incorporate isophthalamide or bisurea spacers linking two aromatic moieties have proven effective for the selective recognition of all-equatorial carbohydrates. Here, we explore the molecular recognition properties of an octa-urea $[\text{Pd}_2\text{L}_4]^{4+}$ cage complex (4). It was found that small anions like NO$_3^-$ and BF$_4^-$ bind inside 4 and inhibit binding of $n$-octyl glycosides. When the large non-coordinating anion ‘BAR’ was used, 4 showed excellent selectivity towards $n$-octyl-$\alpha$-D-Mannoside with binding in the order of $K_s \approx 16 \text{ M}^{-1}$ versus non-measurable affinities for other glycosides including $n$-octyl-$\beta$-D-Glucoside (in CH$_3$CN/H$_2$O 91:9).

1. Introduction

One of the most versatile class of biomolecules are carbohydrates.[9] These natural molecules have been linked to various malignant phenomena such as diabetes, infection, and cancer metastasis.[5] Many healthy biological processes are also mediated by carbohydrate molecules, such as: hormone activities, neuronal development, fertilization, immune surveillance and inflammatory responses.[2] Glycobiology and biomedical research in general thus stand to benefit from studies to understand these processes, with the ultimate goal of unlocking novel medicinal therapies. Strategies to selectively bind carbohydrates can be seen as an essential element of such research efforts. Inspiration can be found in lectins, which are the natural class of molecules that bind carbohydrates. Crystal structures of lectin-carbohydrate complexes reveal a large degree of interaction complementarity, where hydroxyl groups are complemented by hydrogen bonding residues in the lectin and flat CH-surfaces of pyranoses are accommodated by aromatic residues (Phe, Tyr, Trp) for CH···π interactions.[6,7] However, this protein sub-group is hampered, unfortunately, by its non-selective and low affinity binding of the target monosaccharides (typically $K_s \sim 10^{-10} \text{ M}^{-1}$).[6] The interaction complementarity has been mimicked by artificial carbohydrate binding molecules.[6] Two prime examples are macrocycles 1 and 2 shown in Figure 1, which comprise pyrenyl or phenyl surfaces for CH···π interactions and polar isophthalamide or bis-urea spacers for hydrogen bonding. It was found that 1 is selective for GlcNAc-$\beta$-OMe,[6a] while 2 was selective for glucose ($K_s$ of both $\approx 18,000 \text{ M}^{-1}$ in water),[6c] showing better selectivity and affinity than lectins. A major drawback of such covalent structures, however, is that their synthetic route requires one (or more) macrocyclization step(s) with yields rarely exceeding 20%. These drawbacks might be remedied if the cyclization is accomplished by using reversible bonds, so that non-productive oligomerization products can become intermediates towards the desired macrocycle.

Recently, we showed that this could be accomplished by reacting the square planar d$^8$ metal Palladium (in its 2+ oxidation state) with an isophthalamide-linked dipyrild ligand to form 3.[7] Coordination cage 3 is shown in Figure 1 and was found to bind selectively to $n$-octyl-$\beta$-D-glucoside ($S$, with $K_s = \ldots$)
51 M$^{-1}$ versus n-oct-β-D-galactoside (6, with $K_a = 29$ M$^{-1}$) in CD$_2$Cl$_2$/DMSO-d$_6$ (9:1). Given the altered selectivity found for 1 and 2, we wondered what the effect would be of replacing the isophthalamides in 3 to bis-ureas in a structure such as 4 (see Figure 1). The nitrate version of 4 was recently published by Chand et al., where they were mainly interested in studying the effect of utilizing different ligand-isomers.<sup>[8]</sup> Herein, we report that octa-urea cage 4 can host n-octyl glycosides in organic media.

## 2. Results and Discussion

As is detailed in the supporting information (section S3a), attempts to utilize the [4][NO$_3$]$_4$ complex for carbohydrate binding studies bore no fruit. The lack of binding was ascribed to firm binding of the nitrate anions within the interior of 4, as was observed in the crystal structure of [4][NO$_3$]$_4$<sup>[8]</sup> Moreover, the poor solubility of the nitrate version of 4 in solvents other than DMSO hampered further studies.

To enable us to the study the binding properties of 4, the BF$_4^-$ and BArF$^-$ versions were prepared by mixing the appropriate Pd$^{2+}$ salt with the dipyridyl ligand (see section S2 for details, BArF$^-$ = tetrakis[3,5-bis(trifluoromethyl)phenyl]borate). As is detailed in section S3b, synthesis of [4][BF$_4$]$_4$ and [4][BArF]$_4$ in pure DMSO-d$_6$ gave complex $^1$H-NMR spectra. These spectra were somewhat resolved at elevated temperatures (80°C) or when adding a glycoside, thus hinting at the capacity of 4 to bind carbohydrates. However, the complexity of the spectra during titration experiments hampered a firm characterization of binding in DMSO-d$_6$.

Changing the solvent from DMSO-d$_6$ to CD$_3$CN with a few percentages of water resulted in $^1$H-NMR spectra with one clear major species for both [4][BF$_4$]$_4$ and [4][BArF]$_4$. This is illustrated in Figure 2, showing assigned $^1$H-NMR spectra of the dipyridyl ligand in pure acetonitrile (a) and in 3% water in acetonitrile (b) to which 0.5 eq. of the appropriate palladium salt was added (either BF$_4^-$ in c, or BArF$^-$ in d).

The large downfield shift of protons such as a (8.19—8.38 or 8.53), c (7.95—8.17 or 8.44), d (8.59—9.00 or 9.12), and e (8.14—8.51 or 9.15) for [4][BF$_4$]$_4$ and [4][BArF]$_4$ respectively, are highly indicative for pyridyl-Pd coordination. DOSY NMR of the BArF$^-$ version of 4 (Figure 2e) revealed that the diffusion constants (D) of the major species is smaller for the ligand (log (D) = −8.82) than for [4][BArF]$_4$ (log(D) = −9.04), which is also in line with complex formation. Moreover, the diffusion constant measured for the BArF$^-$ anion in [4][BArF]$_4$ of log(D) = −8.89 is significantly less than that of 4, implying that these anions are largely dissociated. For the [4][BF$_4$]$_4$ complex on the other hand, a ($^1$H$-^{19}$F)-HOESY spectrum revealed a clear nOe signal between CH proton d and BF$_4^-$, thus showing this anion is bound to the interior of 4 (see Figure S2-11). Lastly, the tetra-cationic 4 was measured by cold-spray ionization mass spectroscopy of [4][BF$_4$]$_4$ and [4][BArF]$_4$ solutions. The measured isotope distribution and highest monoisotopic mass ($m/z = 401.585$) are in agreement with the 2:4 Pd : ligand ratio expected for 4 (see also Figure 2f).

To probe the possible binding properties of 4, various binding studies were conducted with carbohydrates 5–12 listed in Table 1, as well as with the aromatic dimethyl terephthalate (13). We opted for the BArF$^-$ version of 4 because of the previously noted interior binding of BF$_4^-$ evidenced by a ($^1$H$-^{19}$F)-HOESY experiment (see section S2).

As several $^1$H-NMR spectra of [4][BArF]$_4$ between 0.560 to 0.245 mM (Figure S3-3) showed that the resonances of the cage were unperturbed, any significant self-association of 4 could be
the inwards facing NH proton e shifted downfield and the
inwards pointing CH proton d shifted upfield, while the
outwards facing g and h remained nearly stationary.

In order to quantify the observed shifts in terms of a
binding constant for 5 and 7, the shifts were initially fitted to a
simple 1:1 binding model. This model did not fit very well, in
particular at the beginning of the titrations, at low concentra-
tions of carbohydrate. This was ascribed to small near-linear
shifts, often in opposite direction of the main shifts, which were
also present in many titrations with the other substrates. This
phenomenon cannot be cage aggregation, as the dilution study
did not reveal such shifts (see Figure S3-3). One might speculate
that carbohydrates can also be very loosely associated with the
cage’s exterior, leading to higher stoichiometries with small
shifts. Alternatively, the small initial shifts might result from
changes in the equilibrium composition of the cage’s con-
formers and/or coordination oligomers. This phenomenon
notwithstanding, the data could be fitted with reasonable
accuracy as is shown in Figures 3c and d ($R^2 > 0.99$ over all
55 data points). In these fits, the initial small shifts were taken
into account by using a 1:2 model and fixing the ‘first’ event to
6 M$^{-1}$. This gave the reported values of an assumed 1:1 binding
with $K_b = 9 \text{ M}^{-1}$ for 5 (in 3% $\text{H}_2\text{O}$ in CD$_3$CN) and $16 \text{ M}^{-1}$ for 7 (in
9% $\text{H}_2\text{O}$ in CD$_3$CN, see also Table 1). The order of magnitude
of these values is consistent with the saturation observed with the
concentration of guest used (up to 140 mM). When the titration
with glucoside 5 was repeated in acetonitrile with 9% water, no
significant peak shifting was observed (entry 2 in Table 1, see
also Figure S3-5). This implies a clear preference of 4 for
mannoside 7 over glucoside 5.

To verify if the observed shifts were indeed caused by
binding of glycosides 5 and 7, a series of selective 1D nOe
spectra were measured of the final titration solutions. As is
exemplified in Figure 4 for both glycosides, when proton d was
irradiated, large signals were observed in the carbohydrate
region (3.0–4.5 p.p.m.). In contrast, irradiation of the outward
pointing pyridyl proton c, or phenyl proton h did not result in
such large nOe signals in the carbohydrate region. These nOe
data thus provide evidence that binding occurred and that 5
and 7 reside within the cage’s interior. Lastly, the final titration
solution of [4][BAR$_7^{13}$] with glucosides 5 was investigated with
cryospray ionization high resolution mass spectrometry (CSI-
HRMS). As is shown in the top-left inset figure of Figure 4, a
species was observed with a mass and isotope distribution
consistent with a 1:1 stoichiometry of a [4–5]$^{14}$+ (see also
Table S3-2).

While the exact molecular geometry of 4 bound to glyco-
sides 5 and 7 could not be measured, molecular modeling was
used to obtain likely approximate geometries. Details of the
approach can be found in the supporting information, section S4.
The energy minimum conformer of unbound 4 was approximated by
the model shown in Figure 5a. This model indicates that the two urea moieties of each dipyrrol ligand
establish an intramolecular hydrogen bond (also shown as red
dashed line in the schematic representation). Moreover, the
interior of this model has the indicated estimated dimensions,
which are generally congruent with those of a carbohydrate (see Figure S4-2).

Starting from this presumed energy minimum conformer, models of 4 bound to n-octyl glycosides 5–8 were generated by conformational searches and DFT geometry optimizations as detailed in section S4b. As an example, the energy minimum found for [4]\(^{\text{+}}\) is shown in Figure 5b. Interestingly, in this structure (as well as the others) the carbohydrate pyranose ring plane is not coplanar with the N\(\text{4}\)Pd\(\text{2}^+\) planes. In the case of [4]\(^{\text{+}}\) these angles are about 40°. As can be seen in the right-hand side of Figure 5b, the mannoside in the model is held in place by a total of nine traditional hydrogen bonding interactions involving the cage’s urea groups. Six of these hydrogen bonding distance can be seen as typical for charge neutral hydrogen bonds (H···O = 1.5–2.2 Å) while three can be seen as weak (H···O > 2.2 Å).

Additionally (highlighted in pink), this axial OH-2 is involved in two charge assisted [C-H]^+···O interactions involving two of the inwards pointing pyridyl CH’s (d) that are trans-coordinated relative to each other. The H–O distances of 2.42 Å and 2.20 Å can be seen as weak and moderate respectively. The possibility to establish three relatively strong hydrogen bonding interactions with the axial hydroxyl of 7 provides a rationalization to the observed selectivity for this carbohydrate over glucoside 5 (where only seven hydrogen bonds were found, none involving the pyridyl CH’s).

Figure 3. Partial \(^1\)H-NMR spectra and HypNMR curve fitting analysis of a) [4][BARF]\(^2_4\) titrated with glucoside 5 in acetonitrile with 3% water and b) titrated with mannoside 7 in acetonitrile with 9% water. c + d) Fitting on protons a and e-f gave a binding constant of 9 M\(^{-1}\) for 5 (c, in 3 % H\(\text{2}O\) in CD\(\text{3}CN\)) and 16 M\(^{-1}\) for 7 (d, in 9 % H\(\text{2}O\) in CD\(\text{3}CN\)) with the indicated goodness of fit (r\(^2\)) calculated over all 55 data points (see note b of Table 1 and main text for details). See also Figure S3-4 and S3-7.
3. Conclusions

The nitrate version of 4 reported by Chand et al. that was only soluble in DMSO-d$_6$ could be modified to the BF$_4^-$ and BArF$_4^-$ analogues which were also soluble in wet (3% or 9% water) CD$_3$CN. Binding studies of 4[BArF$_4^-$]$_4$ with 5–13 showed that binding could only be quantified for glucoside 5 in 3% water in acetonitrile, and for mannoside 7 in 9% water in acetonitrile. In both cases a 1:2 stoichiometry had to be assumed for a proper fit due to initial shifting of some peaks that we consider to be an artifact. The 1:1 binding constants we found are 9 M$^{-1}$ for 5 and 16 M$^{-1}$ for 7 and binding to the interior of 4 could be verified in both cases by selective 1D nOe studies. As no affinity of 4 for 5 was observed in 9% water in acetonitrile, these data indicate a clear preference of 4 for the diaxial n-octyl-α-mannoside 7. This selectivity could be rationalized based on molecular modeling of [4⊂7]$^{4+}$ where several clear hydrogen bonds were found involving the axial hydroxyl of 7, including charge assisted [C–H]$^+$→O interactions that were absent in a model of [4⊂5]$^{4+}$. While the affinities found are low, these studies do show that carbohydrate binding with 4 is possible in very competitive media such as wet acetonitrile. Moreover, the first reported covalently-assembled cage (a biphenyl analogue of 1) for carbohydrate binding in a competitive medium has an affinity of merely 4.6 M$^{-1}$ for D-glucose in water. As such, one can actually consider the affinities in the order of 9–16 M$^{-1}$ observed with 4 as a significant first step. We foresee that installation of a solubility group on the phenyl moiety in the dipyridyl ligand will open up further explorations of the binding potential of the octa-urea 4 in other solvents.

Figure 4. Partial $^1$H-NMR spectrum of [4⊂5][BArF$_4^-$]$_4$ and [4⊂7][BArF$_4^-$]$_4$ and selective 1D nOe’s with $t_{meas} = 500$ ms. The top left inset figure displays the CSI HRMS isotope distribution of a [4⊂5] species as measured (blue, top) and simulated (green, bottom) with m/z = 984.225 for [4⊂5][Cl$_2$]$_2^+$; the chloride anion must originate from the eluent used in the spectrometer.

Figure 5. DFT optimized (ωB97X-D/6-31G*) molecular models of: a) the empty [4]$^{4+}$ cage represented in space filling mode. In each bis-pyridyl ligand, the urea’s form an intramolecular hydrogen bond as shown. Some dimensions are given as well. b) [4⊂7]$^{4+}$ with a magnification of the mannoside and the H-bonds found in the model (yellow dashed lines). See section S4 of the supporting information for details.
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Conflict of Interest

The authors declare no conflict of interest.

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