Chapter 4

Multiple Recognition of Barbiturate Guests by "Hamilton" Receptor-Functionalized Dendrimers

Abstract: Four generations (Gx; x = 1, 2, 3, 4) of "Hamilton" receptor-functionalized poly(propyleneamine) dendrimers ("HR-dendrimers") have been synthesized and characterized for the first time using $^1$H NMR, $^{13}$C NMR and MALDI-TOF mass spectrometry. The photophysical properties of the HR-dendrimers have been investigated using UV-Vis and time-resolved fluorescence spectroscopy. The HR-dendrimers are used as multivalent hosts for different barbiturate guests. Barbital (B1) and [Re(Br)(CO)$_3$(barbi-bpy)] (barbi-bpy = 5-[4-(4'-methyl)-2,2'-bipyridyl]methyl-2,4,6-(1H,3H,5H)-pyrimidinetione) (B2) have been synthesized and employed as guests. The stable adducts formed between the dendritic architectures, the hosts, and the barbiturate guests B1 and B2 have been investigated using $^1$H NMR and photophysical methods. The binding constants of the barbiturate guests to G0 were found to be $1.4 \times 10^3$ M$^{-1}$ and $1.5 \times 10^5$ M$^{-1}$ for B1 and B2 respectively in chloroform. The binding of B1 to the dendrimers causes an enhancement of the weak emission of the "Hamilton" receptor, showing also a generation dependency, since it was found to be the most pronounced in case of G0 and the least in case of G4. The unexpected increase in the emission quantum yields could be caused by the rather rigid conformation due to the intramolecular aggregation of the "Hamilton" receptors and steric hindrance at the periphery. The photoinduced energy transfer process occurring from the excited state of the HR-dendrimers to the guest B2 has been used to probe the formation of the host-guest complex Gx-B2 (x = 1, 2, 3, 4). The rate of the energy transfer process was calculated to be $3.6 \times 10^{10}$ s$^{-1}$. Since energy transfer in G0-B2 could only be performed in the presence of a strong base, such as a third generation poly(propyleneamine) dendrimer (DAB-dendr-Am$_{16}$), the presence of the basic amine core in case of the HR-dendrimers proved to be crucial for this photoinduced process. The binding between B2 and the dendrimer is perfectly reversible, since B2 can be exchanged with a competitive guest such as B1 and the emission of the HR-dendrimers is restored.

4.1 Introduction

An important feature in supramolecular chemistry is the assembly of multiple components in a predefined way in order to perform specific functions, such as photoinduced energy or electron transfer processes.\textsuperscript{1-18} Generally, self-assembly and molecular recognition involve the use of a mono- or bifunctionalized host or guest. Multi-binding events in artificial systems within the same molecule are very rare, especially when hydrogen bonds are used to glue the complementary components. Dendrimers have proven to be suitable supramolecular hosts for guest molecules.\textsuperscript{19-40} Due to their monodisperse, highly branched three dimensional structure, a microenvironment is created where guest molecules can be encapsulated based on topological entrapment (hydrophilic, hydrophobic interactions).\textsuperscript{19-34} Such non-bonding interactions are unspecific and even the encapsulation of solvent molecules can be considered as a form of topological entrapment.

Since dendrimers are built up very regularly, it is possible to incorporate receptor sites in the core, in the branches or at the periphery.\textsuperscript{19-24,35-40} These receptors can be based on acid-base, electrostatic or hydrogen-bonding interactions. The organization of binding sites in a specific part of a dendritic structure allows the formation of multiple stable host-guest systems within one molecule.

The creation of large structures, that can bind selectively a certain class of compounds, contributes to the development of artificial binding sites closely resembling those found in proteins.\textsuperscript{41-43} Particularly interesting are host-guest systems in which the binding of a guest can be used to promote new functions such as energy and electron transfer processes. The use of dendrimers containing multiple chromophoric units or receptor sites is extremely appealing, since there is a great need for well characterized systems, in which it is possible to perform simultaneously sensor functions and immobilization of biological substrates.\textsuperscript{44}

In this view we discuss in this Chapter poly(propyleneamine) (also called POPAM or poly(propylenimine) or PPI)\textsuperscript{24} dendrimers substituted at the periphery with receptors that can bind barbiturates and its derivatives via six hydrogen bonds. Such a receptor based on multiple hydrogen bonds containing 2,6-diaminopyridine was introduced in 1988 by Hamilton et al. (Figure 4-1).\textsuperscript{45}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{barbiturate_receptor.png}
\caption{The barbiturate receptor published in 1988 by Hamilton et al.\textsuperscript{45}}
\end{figure}
Since then several papers appeared reporting on its ability to subtract barbiturates from serum\textsuperscript{46,47} its use as a model for enzyme catalysis,\textsuperscript{48-50} as a building block in supramolecular materials\textsuperscript{51} and as a receptor in photoactive hydrogen bond-based assemblies.\textsuperscript{11,52,53} From these studies it was clear that the receptor can be a powerful tool to create stable supramolecular assemblies with barbiturates, even in solvents that are competitive for the receptor site.\textsuperscript{54}

In this Chapter the synthesis performed in the group of prof. dr. F. Vögtle at the University of Bonn (Germany), of four generations of poly(propyleneamine) dendrimers substituted at the periphery with barbiturate receptors (Figure 4-2), which will be called from now on "Hamilton" receptors, is briefly discussed.

\textbf{Figure 4-2.} A schematic representation of the HR-dendrimers (G\textsubscript{x}; x = 0, 1, 2, 3, 4) and the structures of G\textsubscript{0} and G\textsubscript{2}.
For the first time the photophysical properties of the "Hamilton" receptor itself will be discussed in more detail as well as the photophysical properties of the "Hamilton" receptor-functionalized dendrimers (HR-dendrimers).

Subsequently, two barbiturate guests have been prepared, namely Barbital\textsuperscript{55} (B1) and [Re(\text{Br})(\text{CO})\textsubscript{3}(barbi-bpy)] (barbi-bpy = 5-[4-(4'-methyl)-2,2'-bipyridyl]methyl-2,4,6-(1\textit{H},3\textit{H},5\textit{H})-pyrimidinetrione)\textsuperscript{53} (B2), which are both able to form a host-guest complex with the HR-dendrimers as depicted in Scheme 4-1. The host-guest complex G\textit{0}·B (B = B1, B2) has been characterized using \textsuperscript{1}H NMR. The binding constant of the barbiturate guests B1 and B2 to the "Hamilton" receptor could be determined using \textsuperscript{1}H NMR and fluorescence spectroscopy respectively.

Finally, it will be shown that also the receptor itself can be used as a chromophore to transfer energy, upon excitation, to the guest across hydrogen bonds. Very suitable for this purpose is B2, which has a triplet excited state, a metal-to-ligand charge transfer state (\textsuperscript{3}MLCT state), at lower energy than the excited state of the "Hamilton" receptor. The energy transfer process has been studied using both steady state and time-resolved fluorescence spectroscopy.

\begin{flushleft}
\textbf{Scheme 4-1.} The formation of the host-guest complex between the HR-dendrimers and the guest molecules B1 and B2.
\end{flushleft}
4.2 Results and Discussion

4.2.1 Synthesis and Characterization of the HR-dendrimers

Four generations of "Hamilton" receptor-functionalized dendrimers (HR-dendrimers) have been synthesized according to Scheme 4-2.\textsuperscript{56}

![Scheme 4-2. A schematic illustration of the synthesis of the HR-dendrimers starting from an amine-functionalized "Hamilton" receptor.](image)

The PTFE ester was used instead of an acid chloride to ensure full substitution of the periphery of the dendrimers, a strategy that was previously reported by Meijer et al.\textsuperscript{57}
4.2.2 Determination of the Association Constants ($K_{\text{ass}}$)

In order to gain more insight in the association constants ($K_{\text{ass}}$) of the host-guest systems, titrations have been performed for $G0$ to determine its association constant with Barbital ($B1$) and $[\text{Re}(\text{Br})(\text{CO})_3(\text{barbi-bpy})]$ ($B2$). The association constant of $G0$-$B1$ has been determined using $^1\text{H}$ NMR spectroscopy (in CDCl$_3$) and could be calculated from the change in chemical shift of selected proton signals of $G0$ upon addition of $B1$ (Figure 4-3), rendering a $K_{\text{ass}}$ of $1.4 \times 10^3$ M$^{-1}$.

![Figure 4-3](image)

**Figure 4-3.** The $^1\text{H}$ NMR spectra of $G0$ and $G0$-$B1$ showing the binding induced changes in chemical shift of proton signals corresponding to $G0$. The $^1\text{H}$ NMR signals of $B1$ are marked with an asterix (*).

The association constant of $G0$-$B2$ (in CHCl$_3$) could be determined using fluorescence spectroscopy exciting $B2$ at 435 nm. From the decrease in the emission intensity of $B2$ upon addition of $G0$ (Figure 4-4) the association constant was calculated to be $1.5 \times 10^5$ M$^{-1}$.

The association constants found for $B1$ and $B2$ are in good agreement with those found earlier on by Isied et al., who addressed the large difference in binding constants to the ability of the barbiturate to form a keto-enol-enolate equilibrium.$^{58}$ The association constants of the dendritic host-guest systems cannot be determined exactly, because of the high variety of equilibrating species. However, based on their identical behavior in the fluorescence titration experiments, that will be described later on, we assume that the binding constant is similar for each receptor site and resembles that of $G0$. 

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Figure 4-4. The fluorescence spectrum of B2 showing a binding induced decrease in emission upon the addition of GO (CHCl₃; λ_exc = 435 nm).

4.2.3 Photophysical Properties of GO and the HR-Dendrimers (Gₓ; x = 1, 2, 3, 4)

Several studies have focussed on the "Hamilton" receptor and the complexation of barbiturates and its derivatives. In particular the assembly of the two units has been investigated using NMR, X-ray crystallography, UV-Vis and fluorescence spectroscopy.¹¹,⁴⁴-⁵³ However, the photophysical properties of the receptor itself have never been studied in detail. Only Hamilton et al. report an absorption maximum in the UV-Vis spectrum at 303 nm for the "Hamilton" receptor and an emission maximum at 461 nm.⁵² Furthermore, a binding-induced increase in both the absorption and the emission of the receptor is reported.⁵²

As for the single receptor, also the HR-dendrimers have an absorption maximum at 302 nm in CHCl₃ (Figure 4-5a). The molar extinction coefficient is linearly related to the number of "Hamilton" receptors attached to the periphery of the poly(propyleneamine) dendrimers. However, the molar extinction coefficient of the HR-dendrimers (Gₓ; x = 1, 2, 3, 4) was found to be significantly lower as compared to G₀ (Table 4-1). Excitation at 310 nm gives a dual emission, one with a maximum at 440 nm and a shoulder at lower energy centered at about 500 nm for all dendrimers (generations G₁-G₄). In case of G₀ a distinguishable band at 540 nm is observed, although the emission intensity is much lower (Figure 4-5b). All the photophysical data are summarized in Table 4-1.

The emission shows a double exponential decay for all generations in CHCl₃ with one component of 400 ps and a longer one of 1.5 ns (Table 4-1). Remarkably, the quantum yield of emission is larger for the higher generations compared to the smaller ones (Table 4-1). This is most likely related to the aggregation of the "Hamilton" receptors at the periphery of the dendrimer. Increasing dendrimer generation in fact causes a larger steric hindrance, since the periphery becomes more crowded with receptor moieties. The possibility for the receptors to
interact intra- or intermolecularly is therefore higher with increasing generations. Due to the low concentrations used for our experiments, namely $10^{-5}$ M in receptor concentration, we can assess that intramolecular processes are predominant. The aggregation introduces rigidity to the "Hamilton" receptor, which results in a higher quantum yield of emission, since radiationless deactivation is reduced.

![Figure 4-5](image.png)

**Figure 4-5.** Absorption (a) and emission spectra ($\lambda_{\text{exc}} = 310$ nm) (b) of $G_x$ ($x = 0, 1, 2, 3, 4$) measured in CHCl$_3$.

**Table 4-1.** The photophysical properties of $G_x$ ($x = 0, 1, 2, 3, 4$) in CHCl$_3$.

<table>
<thead>
<tr>
<th>$G_x$</th>
<th>$n^{d}$</th>
<th>$\varepsilon$ (M$^{-1}$cm$^{-1}$)$^{b}$</th>
<th>ratio $\varepsilon$</th>
<th>$\Phi_{\text{em}}$</th>
<th>$\tau_1$ (ps)$^{c}$</th>
<th>$\tau_2$ (ns)$^{c}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$G_0$</td>
<td>1</td>
<td>329000</td>
<td>1.33</td>
<td>0.0024</td>
<td>374</td>
<td>1.46</td>
</tr>
<tr>
<td>$G_1$</td>
<td>4</td>
<td>99000</td>
<td>4</td>
<td>0.0103</td>
<td>408</td>
<td>1.51</td>
</tr>
<tr>
<td>$G_2$</td>
<td>8</td>
<td>198000</td>
<td>8</td>
<td>0.0151</td>
<td>408</td>
<td>1.57</td>
</tr>
<tr>
<td>$G_3$</td>
<td>16</td>
<td>377000</td>
<td>15.24</td>
<td>0.0180</td>
<td>369</td>
<td>1.48</td>
</tr>
<tr>
<td>$G_4$</td>
<td>32</td>
<td>785000</td>
<td>31.72</td>
<td>0.0211</td>
<td>429</td>
<td>1.38</td>
</tr>
</tbody>
</table>

$^{d}$ $n =$ number of HR; $^{b}$ calculated at 302 nm; $^{c} \lambda_{\text{exc}} = 356$ nm and $\lambda_{\text{probe}} = 480$ nm

In order to prove this concept and due to the lack of conformational evidence to support these findings, an alternative strategy to rigidify the structure of the "Hamilton" receptor comprises the binding of an "innocent" guest molecule. The binding of a barbiturate guest, like B1, "fixes" the "Hamilton" receptor in a certain conformation and reduces in this way the degrees of freedom of the chromophore. Since B1 is an "innocent" guest (i.e. no absorption and emission from B1 at the excitation wavelength used), all changes in the emission of the receptors as a result of the addition of B1 can be attributed to conformational changes of the receptor upon binding the guest. As can
be seen in Figure 4-6a, the quantum yield of emission increases dramatically for G0 upon addition of B1 and the maximum shifts to lower energy (450 nm). Such a shift can be explained considering that the insertion of B1 into G0 causes a planarization of the receptor and a larger delocalization within the system. As a result of the binding of B1 (a large excess of 100 eq. is used), the long-lived component of 1.5 ns of the excited state lifetime of G0 disappears, while only the 400 ps component remains (Figure 4-7).

Figure 4-6. (a) Titration of G0 with B1 followed using fluorescence spectroscopy (in CHCl₃; \( \lambda_{\text{exc}} = 330 \text{ nm} \)), showing a binding-induced increase in the emission of G0 and (b) the binding-induced increase in emission (in CHCl₃; \( \lambda_{\text{exc}} = 330 \text{ nm} \)) as a result of the addition of B1 plotted relative to the original emission (E₀) for all generations HR-dendrimers (Gₓ; \( x = 0, 1, 2, 3, 4 \)).

Figure 4-7. The emission decay probed at 480 nm measured for G0 in the absence and in the presence of a large excess of B1 (exc.: 100 eq.) (CH₂Cl₂; \( \lambda_{\text{exc}} = 324 \text{ nm} \)).
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This indicates that the two different lifetimes refer to two different conformations of the receptor; one in which the receptor arms are in a *trans* configuration (long lifetime), and the other one in which the receptor arms are in a *cis* configuration (short lifetime). Upon binding of a barbiturate guest, the receptor will always be forced into the *cis* configuration.

Interestingly, also for the HR-dendrimers (G1-G4) an increase in emission and a slight shift of the emission maximum to lower energy is observed. This effect becomes smaller going to higher generations (Figure 4-6b). This can only be the case when the "Hamilton" receptors already have restricted conformational freedom in the higher generations due to the aggregation or steric hindrance at the periphery of the dendrimer.

4.2.4 Energy Transfer in the Gx-B2 (x = 0, 1, 2, 3, 4) Complex

Metal complexes, like [Ru(bpy)$_3$] and [Os(bpy)$_3$], but also [Re(X)(CO)$_3$(bpy)] (X = Cl, Br, I), are often used as energy or electron donor or acceptor in photoinduced processes.$^{59-65}$ Their use is often dictated by their photophysical and redox properties, that are suitable to study energy and electron transfer processes. In particular for [Re(Br)(CO)$_3$(bpy)] the lowest excited state is a luminescent $^3$MLCT state. In Chapter 3 we showed that [Re(Br)(CO)$_3$(barbi-bpy)] (B2) forms a stable host-guest complex with the "Hamilton" receptor.$^{53}$ Comparison of the energy levels of the HR-dendrimers (G1-G4) and the rhenium guest (Figure 4-8) shows that the $^3$MLCT state of B2 is at lower energy (17730 cm$^{-1}$ (564 nm)), than the lowest excited state of the HR-dendrimers (27397 cm$^{-1}$ (365 nm)). Excitation at 330 nm will bring predominantly the "Hamilton" receptor in its excited state. It is interesting to notice that due to the different absorption properties of the separate components (Figure 4-8), it is possible to selectively excite the HR-dendrimers. At the selected excitation wavelength ($\lambda_{exc} = 330$ nm) the contribution of the B2 absorption is $\sim 15\%$ in an equimolar solution.

![Figure 4-8. The absorption spectra of G2 (scaled to $\varepsilon$ per HR) and B2 in CHCl$_3$.](image-url)
The association of the two components is anticipated to lead to a very exergonic photoinduced energy transfer process ($\Delta G = -1.20 \text{ eV}$) from the selectively excited ($\lambda_{\text{exc}} = 330 \text{ nm}$) "Hamilton" receptor to the B2-component (Figure 4-9b).

**Figure 4-9.** (a) The titration of G2 with B2 followed by fluorescence spectroscopy (a) (in CHCl3; $\lambda_{\text{exc}} = 330 \text{ nm}$) showing an energy transfer from the excited state of G2 to B2 and energy level diagram (b) representing the energy transfer process between the HR-dendrimers (Gx) and B2.

Indeed, upon addition of the guest a quenching of the excited state ($\lambda_{\text{max}} = 450 \text{ nm}$) of the "Hamilton" receptor is observed as well as a sensitization of the rhenium emission ($\lambda_{\text{max}} = 564 \text{ nm}$) as shown in Figure 4-9a for G2. No isosbestic point is observed, indicating that two different processes occur simultaneously. The first process is the quenching of the excited state of the "Hamilton" receptor by B2 via energy transfer.

**Scheme 4-3.** A schematic representation of the energy transfer between the HR-dendrimers and B2.
The second process, as will be discussed in detail later on, is the deprotonation of the barbituric acid moiety attached to the bipyridine ligand of B\textsubscript{2}, which causes a blue shift (from 610 nm to 564 nm) and an increase in the emission of B\textsubscript{2}.

Time-resolved fluorescence spectroscopy revealed that the emission lifetime of 400 ps, corresponding to the excited state of the "Hamilton" receptor, was reduced to 30 ps. A long-lived component due to the population, via energy transfer, of the \textsuperscript{3}MLCT state (\(\tau = 109\) ns) of B\textsubscript{2} is also present. From this quenched and the unquenched lifetimes of the HR-dendrimers the rate of energy transfer from the "Hamilton" receptors at the periphery of the dendrimer to B\textsubscript{2} was calculated to be \(3.6 \times 10^{10}\) s\(^{-1}\). The same rate, as expected, of such photoinduced process was found for all generations (G\textsubscript{1}-G\textsubscript{4}). A schematic overview of the photophysical processes occurring in G\textsubscript{x}B\textsubscript{2} \((x = 1, 2, 3, 4)\) is given in Scheme 4-3.

It is interesting to notice that upon complexation of B\textsubscript{2} to the HR-dendrimers (G\textsubscript{1}-G\textsubscript{4}) the B\textsubscript{2}-based emission is blue shifted from 610 nm to 564 nm as depicted in Figure 4-10 for G\textsubscript{4}. The shift in the emission of B\textsubscript{2} is attributed to the deprotonation of the barbituric acid by the poly(propyleneamine) core of the HR-dendrimers, forming its enolate form, which is negatively charged (Scheme 4-4).

\textbf{Figure 4-10.} The fluorescence spectrum of B\textsubscript{2}, showing an increase and a blue shift upon addition of G\textsubscript{4} due to the deprotonation of the barbituric acid moiety by the basic poly(propyleneamine) core (CHCl\textsubscript{3}; \(\lambda_{\text{exc}} = 435\) nm).

Since the bipyridine ligand is involved in the MLCT as an electron acceptor, the presence of the electron rich barbiturate will cause the MLCT state to rise to higher energy. In fact, the addition of third generation poly(propyleneamine) dendrimer (DAB-\textit{dendr}-Am\textsubscript{16}), which can only act as a base, to a solution of B\textsubscript{2} in CHCl\textsubscript{3} causes the same shift in emission. Furthermore, an increase of the emission of B\textsubscript{2} was observed upon deprotonation. This is in accordance with the "Energy Gap Law", stating that if the energy difference between the lowest excited state and the ground state increases, non-radiative decay to the ground state decreases. Therefore, in our case the increase in
the energy gap between the ground state and the lowest excited state (\(^3\)MLCT state of B2) causes an increase in the emission from B2. Another possible explanation of the increased quantum yield of emission of B2 lies in the change in conformation of B2 upon deprotonation. The barbiturate ring will become in plane with the linker to the bipyridinine ligand due to a change in hybridization of the deprotonated carbon from \(sp^3\) to \(sp^2\), improving the conjugation between the binding moiety and the rhenium complex.

Scheme 4-4. The keto-enol-enolate equilibrium of barbiturates bearing an acidic proton and its influence on the binding affinity of the barbiturate for the "Hamilton" receptor.\(^{58}\)

The deprotonation of the barbituric acid moiety of B2 (Scheme 4-4) proved to be essential for the energy transfer from the excited state of the receptor to the excited state of B2. No energy transfer was in fact observed within the host-guest complex G0-B2, but only an increase in emission from G0 due to guest binding. Upon addition of a strong base, such as DAB-dendr-Am\(_{16}\), the receptor emission is quenched and a strong emission from B2 is observed. This proves that the presence of the basic poly(propyleneamine) core is absolutely necessary for the energy transfer to occur, even though the energy gap between the lowest excited state of the receptor and the lowest excited state of B2 does not increase, but even decreases, reducing the driving force for the energy transfer process (vide supra). So, the reason for the efficient energy transfer to occur, must be an improvement in electronic coupling between the receptor and B2. The excited state of the receptor is very short-lived (only 400 ps), so that a strong electronic coupling between the energy donor and the energy acceptor is crucial for the energy transfer to occur.
4.2.5 Competition Experiment between B2 and B1

To show that binding of B2 to the "Hamilton" receptor is causing a photoinduced energy transfer and that the exchange of guest molecules is a clean and reversible process, a competition experiment between B2 and B1 has been performed. Indeed, upon addition of a large excess of B1 to a solution containing Gx-B2 (x = 1, 2, 3, 4; ratio HR/B2 is 1:1), the emission at 450 nm of the "Hamilton" receptors is restored, while the emission from B2 at 564 nm subsequently decreases (at the excitation wavelength almost no direct excitation of the rhenium complex was possible). This shows clearly that B2 has been replaced by B1 (Figure 4-11).

The exchange of guests is a clean and reversible process, which is demonstrated by the isosbestic point at 512 nm. Since the emission of B2 does not shift back to 610 nm once exchanged by B1, apparently B2 remains deprotonated by the dendrimer.

![Figure 4-11. Titration of G3·16B2 with B1 in CHCl₃ followed using fluorescence spectroscopy (λ<sub>exc</sub> = 330 nm). The exchange of B2 with B1 causes the recover of the emission from G3 and the disappearance of the emission from B2.](image)

4.3 Conclusions

In this Chapter it has been demonstrated that both the "Hamilton" receptor itself and the "Hamilton" receptor-functionalized dendrimers can be used as emitting sensors to probe the presence of barbiturates. The emission from the "Hamilton" receptor is strongly related to the rigidity of the chromophoric system. The emission quantum yield was found to be a real dendritic effect. The emission intensity increases due to the aggregation of receptors and steric hindrance at the periphery of the dendrimers. Also the binding of an "innocent" guest, such as Barbital (B1), forces the receptor in a "fixed" conformation, which results in an increase in the emission of the receptor. Binding of a barbiturate guest, which has an excited state at lower energy, e. g.
[Re(Br)(CO)₃(barbi-bpy)] (B2), to the HR-dendrimers results in an energy transfer from the "Hamilton" receptors to B2 with a rate of 3.6 x 10¹⁰ s⁻¹. The emission of B2 shifts towards higher energy upon binding to the HR-dendrimers as a result of deprotonation of the barbituric acid attached to the bipyridine ligand. Deprotonation to obtain a good electronic coupling between the receptor and B2 is necessary, favoring an efficient energy transfer to occur. Furthermore, exchanging B2 with B1 results in the recovery of the receptor emission and a decrease in the emission of B2.

By using the "Hamilton" receptor as a binding motif at the periphery of the poly(propyleneamine) dendrimers, stable supramolecular host-guest complexes can be formed in which photophysical processes, such as energy transfer, can be observed. In the binding studies the emission of the "Hamilton" receptor was successfully used to probe to binding of guest molecules and can be regarded as a sensor for barbiturates. Finally, a smart design of the interior of the dendrimer could allow the assembly of different guests in desired parts of a dendritic structure. This would lead to the possibility of intra-dendritic processes and a more complicated function such as the release of one of the guests induced by light excitation.

4.4 Experimental

4.4.1 Synthesis

"Hamilton" Receptor-Functionalized Dendrimers (HR-dendrimers). The HR-dendrimers were synthesized in the group of prof. dr. F. Vögtle at the University of Bonn (Germany). Details on the synthetic procedures are given in the literature.⁵⁶

Guest Molecules. Barbital⁵⁵ (B1) and [Re(Br)(CO)₃(barbi-bpy)] (barbi-bpy = 5-[4-(4'-methyl)-2,2'-bipyridyl|methyl-2,4,6-(1H,3H,5H)-pyrimidinetrione)⁵³ (B2) have been synthesized according to literature.

4.4.2 Instrumentation

The ¹H NMR binding study was performed on a Varian Inova500 spectrometer at 499.86 MHz. UV-Vis absorption spectra were recorded on a diode-array HP8453 spectrophotometer at 293 K. Fluorescence spectra were recorded on a SPECTAR fluorometer. The lifetime of the emission of the HR dendrimers as well as the lifetime of G0 in the presence of B1, were determined with single photon counting using a picosecond laser. Details on the experimental set-ups used to study the photophysical processes presented in this Chapter are given in the Appendix of this Thesis.

4.4.3 Determination of the Quantum Yields of Emission

The quantum yields of emission in CHCl₃ of the HR-dendrimers have been determined using quinine sulfate in 0.05 M H₂SO₄ (aq) as a reference. The solutions are optically diluted, i. e. having an absorption between 0.05 and 0.15 at the wavelength of excitation (λₑₓₗ = 310 nm).
4.4.4 Determination of $K_{ass}$ of B1 to G0 Using $^1$H NMR Spectroscopy

To 1 mL of a solution of 2.5 mM of G0 in CDCl$_3$ a solution of 28 mM of B1 in CDCl$_3$ is added in aliquots of 10 µL (10 µL contains 0.2 equivalents of B1). From the change in chemical shift of selected proton signals belonging to G0 upon addition of B1 the binding constant could be calculated using a Scatchard-plot.

4.4.5 Determination of $K_{ass}$ of B2 to G0 Using Fluorescence Spectroscopy

To 3 mL of a solution of $1 \times 10^{-5}$ M B2 in CHCl$_3$ a solution of 1.2 mM of G0 in CHCl$_3$ is added in aliquots of 5-50 µL (5 µL contains 0.2 equivalents of G0). The fluorescence intensity at the maximum of the emission ($\lambda_{\text{max}} = 618$ nm) of B2 is probed, exciting at 435 nm. From the decrease in emission intensity the binding constant could be calculated using a Scatchard-plot.

4.4.6 Energy Transfer Study of Gx (x = 1, 2, 3, 4) with B2

A solution of $1 \times 10^{-5}$ M in HR of Gx (x = 1, 2, 3, 4) in CHCl$_3$ and a solution of $1 \times 10^{-5}$ M in HR of Gx (x = 1, 2, 3, 4) containing $2 \times 10^{-5}$ M B2 in CHCl$_3$ are mixed in different ratios. In this way solutions are obtained containing $1 \times 10^{-5}$ M in HR of Gx (x = 1, 2, 3, 4) and increasing amounts of B2 per HR. Fluorescence spectra were recorded exciting at 330 nm. A correction was performed taking into account the direct excitation of B2.

4.4.7 Competition Experiment between B1 and B2

To 3 mL of a solution of $1 \times 10^{-5}$ M in HR of Gx (x = 1, 2, 3, 4) containing 1 equivalent B2 per HR in CHCl$_3$ a solution of 15 mM B1 in CHCl$_3$ is added in aliquots of 5-10 µL (10 µL contains 5 equivalents of B1). Fluorescence spectra were recorded exciting at 330 nm.

4.4.8 Deprotonation of B2 by the Poly(propyleneamine) Core

To a solution of $1 \times 10^{-5}$ M B2 in CHCl$_3$ are added in aliquots of 10-50 µL HR-dendrimer solution in CHCl$_3$ ($6 \times 10^{-4}$ M in HR). Fluorescence spectra were recorded exciting at 435 nm.
4.5 References

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