Multivalent Supramolecular Dendrimer-Based Drugs

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Supramolecular complexes consisting of a hydrophobic dendrimer host [DAB-dendr-(NHCONH-Ad)$_{64}$] as well as solubilizing and bioactive guest molecules have been synthesized using a noncovalent approach. The guest−host supramolecular assembly is first preassembled in chloroform and transferred via the neat phase to aqueous solution. The bioactive guest molecules can bind to a natural (serotonin 5-HT$_3$) receptor with nanomolar affinity as well as to the synthetic dendrimer receptor in aqueous solution, going toward a dynamic multivalent supramolecular construct capable of adapting itself to a multimeric receptor motif.

Introduction

The generation of particulate systems with a specific shape and size plays a crucial role in the development of modern drug delivery systems. Dendrimers have been explored extensively in this field because their structure is well-defined and can be tailored to specific applications.[1−3] They can act as nanoscale containers capable of hosting drugs in their interior or as nanoscaffolds, where multiple copies of a drug or relevant bioactive ligands are displayed at their surface.[4] The latter approach was successfully applied in our group to inhibit Pneumococcal cell-wall hydrolysis by using various generations of choline functionalized dendrimers.[5] The effectiveness of this approach is based on multivalency, which makes use of two concepts: the statistical effect (higher local concentration of the bioactive ligand, Figure 1A) and multivalent receptor binding, where more than one of the receptors can be accessed by one multivalent construct (Figure 1B).[6]

Moreover, in the field of cellular signal transduction it has been shown that the most effective communication with cell receptors is achieved by allowing the ligands to be mobile on the surface of the synthetic scaffold. Ligands embedded in a lipid bilayer (2D mobility) form different synaptic patterns when binding various immune cell types.[7] Apparently, the pattern into which the mobile ligands can organize matches the cells' receptor motif better than the engineered covalent analogs. Here we apply these concepts by attaching the ligands noncovalently to a dendrimer scaffold (Figure 2A), thus allowing the ligands to be mobile.

The 5-HT$_3$ receptor motif, well studied in our laboratories, shows the pentameric structure characteristic of the ligand-gated ion channels, such as nicotinic acetylcholine, glycine, and type A γ-aminobutyric acid receptors.[8] The ion channel mediates fast depolarizing responses and is apparently selective for the monovalent cations Na^+ and K^+ and for the divalent ones Ca^{2+} and Mg^{2+}.[9] The medicinal chemistry research on the 5-HT$_3$ receptor has produced a large number of potent and selective antagonists based on the arylpiperazine moiety,[10] which have shown an excellent efficiency in the control of the emesis induced by anticancer chemotherapy and few adverse side effects.[11]

In this article we present the first steps in obtaining a bioactive adjustable multivalent supramolecular construct toward effective

Figure 1. Schematic representation of the different interaction modes for a multivalent construct with covalently attached ligands bound to protein or cell surfaces: (A) the construct binds to only one receptor at a time; (B) the construct binds multiple receptors simultaneously.

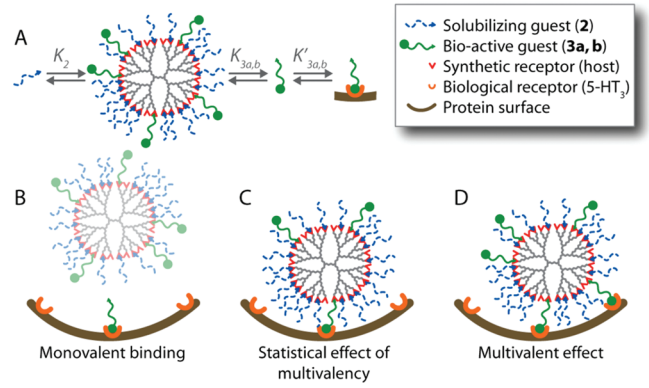

Figure 2. Overview of possible guest−host interactions. (A) Relevant binding equilibria. (B−D) Different interaction modes of the supramolecular construct in binding to the 5-HT$_3$ receptors: (B) monovalent binding, (C) the construct binds to only one biological receptor at a time, and (D) the supramolecular construct binds to multiple biological receptors simultaneously.

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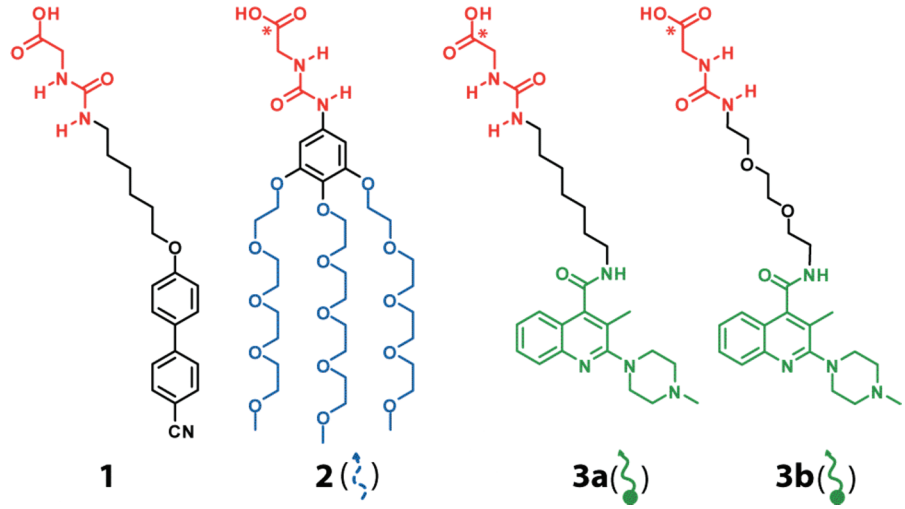

Figure 3. Structure of guest molecules **1**, **2**, and **3a**,**b**: the ureido acetic acid moiety (red) binds to the dendrimer host; the ethylene glycol tails (blue) provide the solubility in both $CHCl_3$ and water; the arylpiperazine moiety (green) binds to the 5-HT_3 receptor. The asterisks mark a ^{13}C label.

targeting of the 5-HT_3 receptor by combining solubilizing (**2**) and bioactive (**3a** and **3b**) guest molecules which interact with an urea adamantyl-functionalized poly(propylene imine) dendrimer [DAB-dendr-$(NHCONH\text{-}Ad)_{64}$] host molecule (Figure 2). In the designed supramolecular system, a dynamic binding equilibrium could be obtained between the solubilizing guest molecules free in solution and bound to the host (Figure 2A), with equilibrium constant K_2, and a similar binding equilibrium of free and bound bioactive guest, with equilibrium constant K_{3a} or K_{3b}. Moreover, the bioactive guest can bind to the 5-HT_3 receptor (with equilibrium constant K'_{3a} or K'_{3b}). Various modes of interaction of the supramolecular construct as a whole with the 5-HT_3 receptors can be envisaged, depending on the different equilibrium constants and on the linker length of the bioactive guest. For instance, the bioactive guest molecule can bind in its molecularly dissolved state to the biological receptor (monovalent binding, Figure 2B). Furthermore, the entire supramolecular construct can bind to only one biological receptor at a time (multivalent binding, due to statistical effect, Figure 2C) or to multiple biological receptors simultaneously (multivalent binding, multivalent effect, Figure 2D).

Previously, we have shown a dendrimer-based guest−host system using urea adamantyl-functionalized poly(propylene imine) dendrimers (i.e., the host), which is able to bind multiple ureido-acetic acid guest molecules (**1**) by means of hydrogen bonding and electrostatic interactions (Figure 3).[12] In one of the recent systems, the ureido-acetic moiety of guest **2** is linked to oligo(ethylene glycol) tails to allow the production of nanometer scale guest (**2**)−host assemblies, which are stable in water.[13] In short, oligo ethylene glycol-based solubilizing guest molecules (**2**) are complexed to the host in chloroform, and the whole guest−host complex is transferred to water via the neat state (chloroform is thoroughly evaporated). By choosing the right conditions, a guest−host complex consisting of a single dendrimer host and multiple guest **2** molecules can be obtained.[13c]

Results and Discussion

Interaction of Bioactive Guest Molecules 3a,b with the Natural 5-HT_3 Receptor. Bivalent ligands **3a**,**b** were synthesized as described in Supporting Information and their affinity for the serotonin 5-HT_3 receptor located in the central nervous system was measured by means of displacement studies performed with radiolabeled granisetron specifically bound to the 5-HT_3 receptor in rat cortical membranes (see Supporting Information for details).[14] These experiments show that both compounds **3a**,**b** are capable of interacting with 5-HT_3 binding site exhibiting inhibition constants in the nanomolar range. In particular, compound **3a** with a heptamethylene spacer (K'_{3a} = 26 ± 1.0 nM) is almost 1 order of magnitude more potent than **3b** (K'_{3b} = 141 ± 6.9 nM), containing a oxygenated spacer (Figure 4).

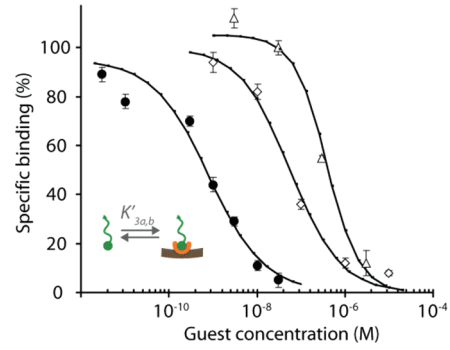

Figure 4. Concentration-dependent displacement of specific [^3H]-granisetron binding to rat cerebral membranes by granisetron (●), **3a** (◇), and **3b** (△).

Interaction of Bioactive Guest Molecules 3a,b with the Synthetic Dendrimer Receptor. The guest−host interactions of **3a**,**b** to the host were first studied in $CDCl_3$ by means of NMR spectroscopy experiments (^1H, ^{13}C, and DOSY), because the supramolecular guest−host complex (Figure 1A) is first preassembled in chloroform solution before being transferred to an aqueous solution. Moreover, the absence of hydrophobic effects between the host molecules simplifies the analysis. The guest interaction of **3a**,**b** with the host in aqueous solution will be discussed below.

The solubility of **3a** in chloroform is very limited, probably owing to the intermolecular interaction between the carboxyl group and the protonated terminal piperazine nitrogen atom. This is reflected in the ^1H NMR chemical shift of the methyl group linked to the terminal piperazine nitrogen, which is shifted downfield when comparing **3a** in deuterated methanol (2.81

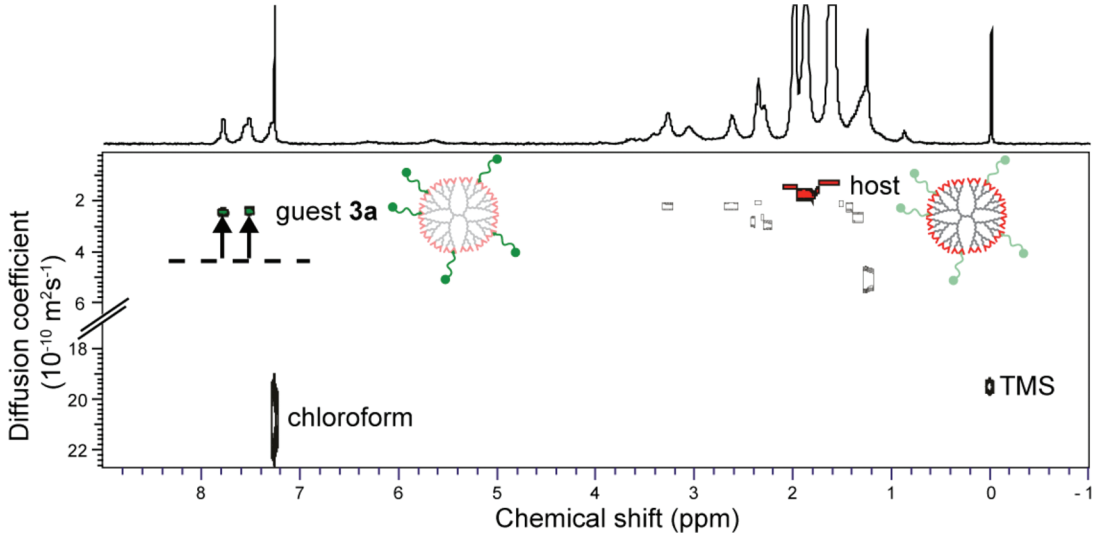

Figure 5. [1]H-DOSY NMR spectrum of the soluble complex consisting of the host (red) and **3a** (green). The dotted horizontal line at 4.4×10^{-10} m^2 s^{-1} represents the diffusion the guest molecule would have if it were molecularly dissolved.

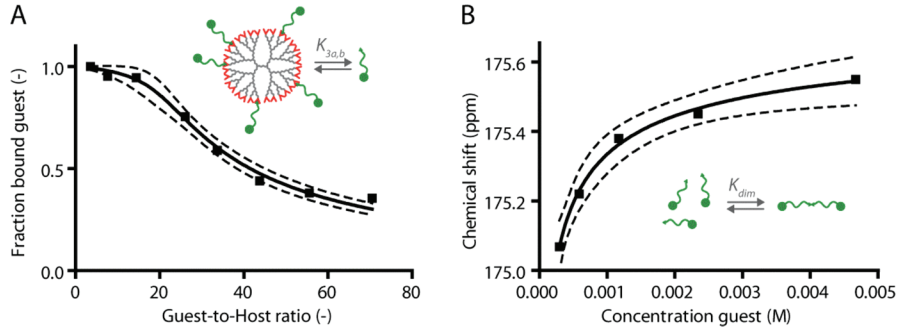

Figure 6. Binding interactions studied by NMR (with 95% confidence intervals). (A) Guest−host titration curve (guest concentration is constant at 7.5×10^{-3} M in $CHCl_3$) fitted with a noncooperative binding model. (B) Guest−guest (**3b**) dimerization determined by ^{13}C NMR.

ppm) to benzyl ester **6a** (2.32 ppm). Despite the guest's poor solubility, the host can pull **3a** into solution upon sonication, yielding a soluble guest (**3a**)−host complex. The changes observed in the ^1H NMR spectrum of this soluble complex (Figure S1 in the Supporting Information) are in agreement with the results previously obtained with guest **1** interacting with the host; however, the maximal guest−host ratio was found to be 24 instead of 32.[12] The ^1H NMR spectrum of the complex shows a significant downfield shift of both urea protons of the host (Figure S1, signals a and b) as well as of the methylene protons adjacent to the tertiary amine of the host outmost shell (Figure S1, signal c). In the case of peaks a and b, the downfield shift indicates the formation of hydrogen bonds between the urea groups of guest and host, while for peak c, it indicates the protonation of the host tertiary amine.

^{13}C NMR experiments showed that ^{13}C-labeled carboxyl moiety of the guest (**3a**) was observed only in the presence of adequate concentrations of the host, and the decreasing of the G/H ratio (i.e., the increase in host concentration) resulted only in marginal variations in the chemical shift. This indicates that only the complexed **3a** is responsible for the chemical shift of the peak and a negligible concentration of unbound guest is present in the $CDCl_3$ solution. The formation of a complex is further supported by ^1H-DOSY NMR experiments (Figure 5), which show the diffusion coefficient of the guest **3a** ($2.3 \pm 0.4 \times 10^{-10}$ m^2 s^{-1}) to be quite close to that of the host ($1.7 \pm 0.3 \times 10^{-10}$ m^2 s^{-1}). The broadness of the aromatic proton signals of the guest in the NMR spectrum hampers an accurate fitting of the diffusion coefficient using the Stejskal-Tanner equation, which could explain the small difference in diffusion coefficients between **3a** and host in this case. By employing the Stokes−Einstein equation, the diffusion coefficient can be translated to a hydrodynamic radius, which is 2.4 ± 0.4 nm for the host (almost identical to the previously reported 2.2 nm for this dendrimer) and 1.7 ± 0.3 nm for the guest.[13b] Due to its limited solubility the apparent association constant of **3a** is very high.

Ureido-acetic acid guest **3b** is soluble in chloroform (solubility ca. 14 mM). Similarly to the analysis of **3a**, the signals attributable to the host urea protons and the methylene adjacent to the tertiary amines of the host are shifted downfield as a result of guest−host interaction (Figure S2, signals a−c in the Supporting Information). Moreover, the amide and urea protons of the guest have shifted as well (Figure S2).

The higher solubility of **3b** allowed for guest−host titration experiments measured by ^1H-DOSY NMR. Here, a dynamic guest−host binding equilibrium is established and binding is not governed by poor solubility (as for **3a**). To determine the dissociation constant of guest **3b** to the host, guest−host titration experiments at constant guest concentration were performed and analyzed using ^1H-DOSY NMR (Figure 6A).

From the ^1H-DOSY spectra the fraction of bound guest can be directly determined because the diffusion coefficient of the unbound guest and that of the host are known.[13b] Using a noncooperative binding model, the dissociation constant K_{3b} in $CHCl_3$ is found to be 330 μM. However, guest−guest interactions are prone to obscure the true dissociation constant. Figure 6B clearly shows a dependence of the chemical shift in a solution containing only **3b** in chloroform. The guest−guest

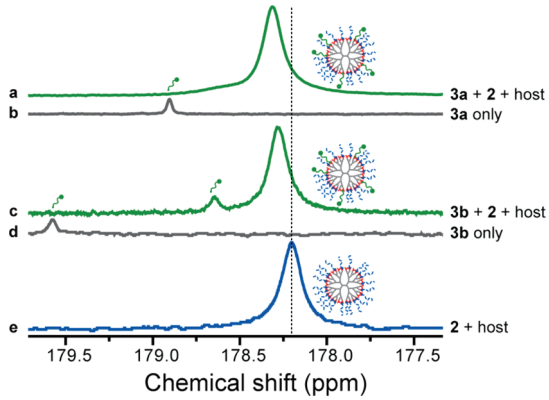

Figure 7. Supramolecular complexes studied by ^{13}C NMR in D_2O (carboxy region): (a) guest **3a** (6.3×10^{-3}M), guest **2** (5×10^{-2} M), and host (7.7×10^{-4}M); (b) guest **3a** (6.3×10^{-3}M); (c) guest **3b** (5.4×10^{-3} M), guest **2** (5×10^{-2} M), and host (7.7×10^{-4}M); (d) guest **3b** (5.4×10^{-3} M); (e) guest **2** (5×10^{-2} M) and host (7.7×10^{-4}M).

interaction can be fitted using a dimerization model (best fit $K_{dim} = 6000\ M^{-1}$). When K_{3b} of **3b** is readjusted, taking into account dimerization, it decreases to 67 μM. The best fit is obtained with 23 binding sites on every host. This is remarkably close to the maximal G/H ratio obtained for **3a** (i.e., 24). The K_{dim} has a large error margin because no plateau can be reached in the chemical shift (Figure 6B) on either side of the curve: at the high end of the concentration the analysis is hampered by the moderate solubility of **3b** and at the low end the sensitivity of NMR measurements is the limiting factor. The measurements presented so far using **3a**,**b** in chloroform show that both guest molecules are indeed capable of binding to the host.

Noncovalent Synthesis of the Supramolecular Complex. As described in the Introduction, the supramolecular complex (Figure 2A) is first preassembled in chloroform and transferred to aqueous solution via the neat state. Because of the interesting properties shown by oligo- and polyethylene glycol in the interaction with different microenvironments in the human body, **2** would potentially confer solubility in the physiological environment and prevent the interaction with blood protein and cells.[15] Similarly to chloroform, ^{13}C NMR and ^{1}H-DOSY NMR measurements were used to study the binding of **3a**,**b** to the host (solubilized by **2**) in aqueous solution.

In ^{13}C NMR a shift is observed in the carboxyl moiety of the ureido acetic acid group of both guest molecules (minor peaks in Figure 7). The major peak in the spectra in Figure 7 corresponds to the ^{13}C-labeled carboxyl moiety of the solubilizing guest (**2**), which is present in a guest (**2**)−host complex (Figure 7e). The shift of **3a**,**b** in the presence of the host indicates the formation of an additional acid−base interaction upon addition of the dendrimer host, which is probably caused by the tertiary amine functionalities of the host, as we have shown before. Also the chemical shift of the solubilizing guest **2** shifts downfield (Figure 7a,c), indicating interaction with the bioactive guest (**3a**,**b**).

^{1}H-DOSY NMR shows that the diffusion coefficient of guest **3a** or **3b** is lowered significantly, from $3.5 \pm 0.5 \times 10^{-10}$ m^2 s^{-1} for the molecularly dissolved guest molecule to $2 \pm 0.5 \times 10^{-10}$ m^2 s^{-1} when the supramolecular complex is present, which indicates binding (not shown). No increase in the diffusion of the host is observed, which indicates that the size of the bioactive complexes is identical to that of the complex consisting of just **2** and host; no aggregation of the complexes is thus induced by either guest **3a** or **3b**. The presence of only one signal for the ^{13}C-labeled carboxyl moiety and a diffusion coefficient, which resides between that of the molecularly dissolved guest and that of the host, indicates that the exchange rates are fast on the NMR time scale (<2.4 ms).

Conclusions

In summary, dynamic multivalent complexes (as depicted in Figure 2A) composed of a hydrophobic urea adamantyl-functionalized poly(propylene imine) dendrimer [DAB-dendr-(NHCONH-Ad)$_{64}$, host], a solubilizing oligo(ethylene glycol)-based guest molecule (**2**), and arylpiperazine bivalent guest molecules (**3a**,**b**) have been synthesized by means of a noncovalent approach. The solubilizing guests (**2**) as well as the bioactive guests (**3a**,**b**) are in dynamic equilibrium on the NMR time scale. Bivalent ligands **3a**,**b** were found capable of binding to the natural 5-HT$_3$ receptor ($K'_{3a} = 26$ and $K'_{3b} = 141$ nM) as well as to the synthetic dendrimer receptor.

Preliminary results using the supramolecular complex in buffered solutions show that the stability of the bioactive supramolecular complexes has to be increased further to allow for in vivo experiments using the construct (Figure 2A). We are currently investigating new guest molecules (containing multiple ureido acetic acid moieties) to increase the interaction strength with the host and to produce supramolecular constructs able to bind multiple biological receptors simultaneously (multivalent binding, Figure 2D). Notwithstanding the lack of in vivo stability, the results presented in this paper open a new way to fabricate dynamic and adjustable noncovalent complexes mimicking the biological tissue they have to communicate with.

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Supporting Information Available. Full experimental details for the synthesis and the characterization of compounds **3a**,**b** and their intermediates (chemistry, NMR, MS). This material is available free of charge via the Internet at http://pubs.acs.org.

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