Supramolecular Chemistry

DOI: 10.1002/anie.201402400

Modular Construction of Dynamic Nucleodendrimers**

*Valentina Abet, Robert Evans, Florian Guibbal, Stefano Caldarelli, and Raphaël Rodriguez**

Dedicated to Professor Max Malacria on the occasion of his 65th birthday

Abstract: *Isoguanosine-containing dendritic small molecules self-assemble into decameric nucleodendrimers as observed by 1D NMR spectroscopy, 2D DOSY, and mass spectrometry. In particular, apolar building blocks readily form pentameric structures in acetonitrile while the presence of alkali metals promotes the formation of stable decameric assemblies with a preference for cesium ions. Remarkably, co-incubation of guanosine and isoguanosine-containing nucleodendrons results in the formation of decameric structures in absence of added salts. Further analysis of the mixture indicated that guanosine derivatives facilitate the formation, but are not involved in decameric structures; a process reminiscent of molecular crowding. This molecular system provides a powerful canvas for the rapid and modular assembly of polyfunctional dendritic macromolecules.*

Dendrimers are globular macromolecules harboring a high density of peripheral functional groups.[1] As a result, dendritic structures exhibit unique physicochemical properties and have found widespread applications spanning from catalysis to molecular sensing.[2] The polymeric nature of these structures makes their syntheses challenging, often resulting in the production of polydisperse mixtures. In pioneering work, Zimmerman et al. have shown that dendritic macromolecules can readily arise from the self-assembly of monomers in organic solvents by means of noncovalent interactions (e.g. hydrogen bonding).[3] Betancourt and Rivera have shown that guanosine (G) residues embedded with an extended aromatic surface form hexadecameric self-organized structures in the presence of potassium ions.[4] Guanosine is known to form square-planar assemblies (e.g. G-quartet), composed of four guanosine residues engaged in a Hoogsteen-type hydrogen-bond network.[5] G-quartets have the ability to pile-up and form multilayered higher-order structures resembling G-quadruplex nucleic acids.[6] While particular guanine-rich oligonucleotides readily form G-quadruplex structures at physiological conditions, self-assembled dendrimers involving multiple building blocks are entropically disfavored and heavily rely on solvent polarity, temperature, and the presence of organic (e.g. aromatic template) and inorganic stabilizers (e.g. metal ions).[1] This dependency provides the opportunity to design tunable supramolecular devices.[7] For example, it has been shown that such assemblies can serve as thermo-, photo-, and metallo-responsive structures, which can be exploited for the purpose of drug delivery.[8]

Herein, we describe the synthesis and self-assembly of isoguanosine-containing dendritic derivatives named "nucleodendrons" (**iG-NDs**). Seminal work from Davis has shown that a low-molecular-weight lipophilic isoguanosine residue could self-assemble as pentamers around alkali metals.[9] This property mainly relies on a network of hydrogen bonds dominated by a larger bond angle compared to the one observed for guanosine residues. Based on this, we reasoned that high-molecular-weight iG dendritic building blocks could self-assemble in a dynamic and controllable manner to form "nucleodendrimers". This work hypothesis was formulated on the ground that specific monomers might confer distinct physicochemical properties to the corresponding assembly based on different shape, polarity, metal ion preferences, and overall size. We anticipated that the hydrophobic nature of the dendritic core would help drive and modulate the stability of the structure in polar solvents, despite higher costs in entropy compared to its G counterpart. Moreover, the central channel being wider for pentameric structures compared to that of G-quartets, the former was expected to be poorly affected by electrostatic repulsion imposed by oxygen lone pairs laying inside the central cavity as is the case for G-quadruplex structures. A representative scheme of putative assemblies is depicted in Figure 1.

A series of monomers were prepared from isoguanosine, first protected as an acetonide, then acylated on the 5′-OH using 6-azidohexanoic acid in the presence DCC/DPTS to provide the corresponding iG-azide building block in 79% yield. Three generations of alkyne-containing side chains either protected or harboring free primary alcohols, making up for the core of the assembly, were prepared in solution and coupled to iG-azide by means of a copper catalyzed alkyne/azide cycloaddition (see the Supporting Information).[10,11] This short synthetic procedure gave rise to a series of six nucleodendrons of variable size and polarity, readily available for self-assembly studies (Figure 1 C).

[*] Dr. V. Abet, F. Guibbal, Dr. S. Caldarelli, Dr. R. Rodriguez
Centre de Recherche de Gif
Institut de Chimie des Substance Naturelles du CNRS
Avenue de la Terrasse, 91198 Gif-sur-Yvette (France)
E-mail: raphael.rodriguez@cnrs.fr
Homepage: http://rodchembiolab.com
Dr. R. Evans
Chemical Engineering and Applied Chemistry
Aston University, Birmingham, B4 7ET (UK)

[**] This research was funded by the Centre National de la Recherche Scientifique and the Institut de Chimie des Substances Naturelles. We thank N. Birlirakis for fruitful discussions, N. Elie and O. Gimello for assistance with MS analyses.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201402400.

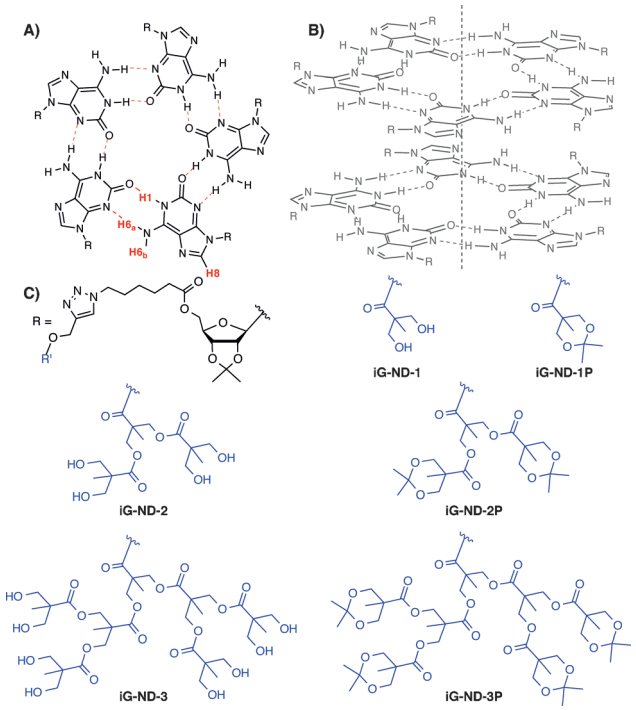

Figure 1. Molecular structures of: A) iG-pentamer, B) iG-decamer, and C) iG-derived nucleodendrons (**iG-ND**); **1**, **2** and **3** indicate generation number; **P** indicates acetonide-protected **ND**.

Nucleodendrons were independently dissolved and subjected to NMR spectroscopy. Our search for a suitable solvent revealed that CD_3CN provided the best results in terms of solubility and spectral resolution of the sample peaks. Because of its low viscosity, diffusion-ordered spectroscopy (DOSY) data were acquired using a convection-compensated, bipolar-paired double-stimulated echo protocol and were processed as previously described.[12] Interestingly, when **iG-ND-1** or **iG-ND-1 P** were dissolved in CD_3CN in the absence of a metal template, weak NH1 and $NH6_a$ signals were observed at 13.7 and 10.7 ppm, respectively (Figure 2 A). These signals, characteristic of a hydrogen-bond network, indicated that iG building blocks interact with one another in solution, to form a symmetrical structure as opposed to random aggregates as previously observed for G derivatives.[5,9,13] In line with this, DOSY data indicated that a range of small-sized species formed, producing NMR spectra with many overlapping peaks that renders simple DOSY analysis intractable.[14] Diffusion coefficients could be measured using isolated signals and suggested that **iG-ND-1** formed an assembly with an experimental diffusion coefficient of $5.3 \times 10^{-10}\ m^2 s^{-1}$. This compares well to a theoretical value of $5.5 \times 10^{-10}\ m^2 s^{-1}$ for a pentameric assembly.[15] DOSY spectra indicated the presence of a significant amount of smaller oligomeric species, reflecting the dynamic and reversible nature of these structures. Addition of CsI to a solution containing **iG-ND-1 P** resulted in significant sharpening of the proton signals associated with a moderate downfield shift of the hydrogen-bonded $NH6_a$ signal (from 10.7 to 11.1 ppm) (Figure 2 A). This data indicated the formation of a tighter assembly, further suggesting that

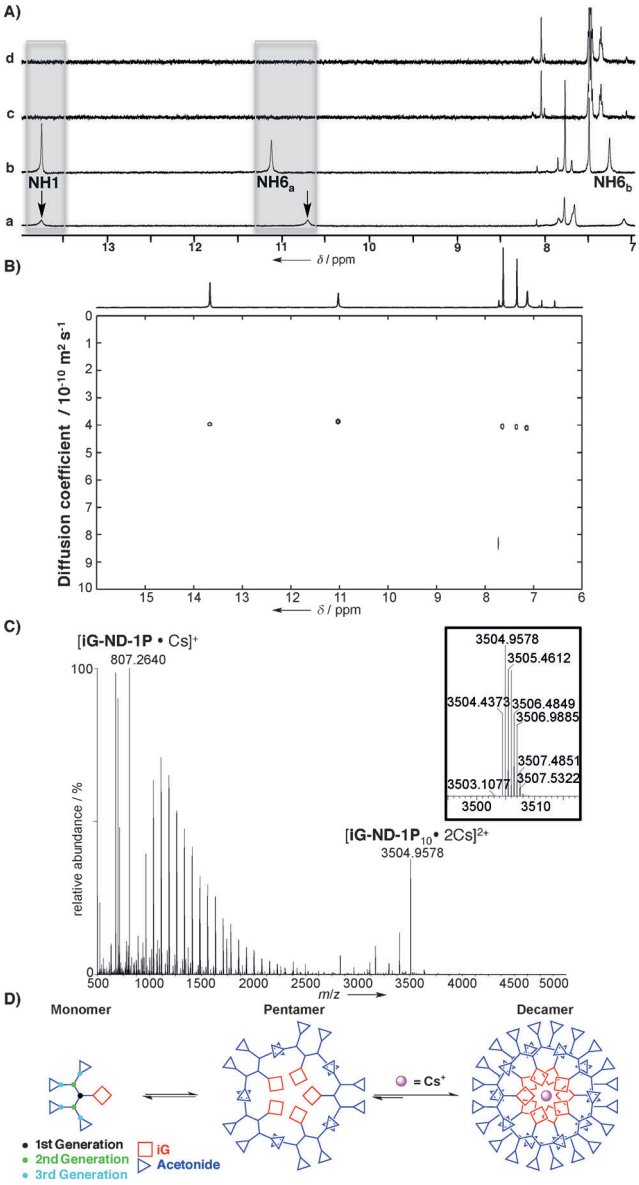

Figure 2. A) ^1H NMR spectra (500 MHz, CD_3CN, 298 K), arrows indicate weak ^1H signals. a) **iG-ND-1 P**; b) **iG-ND-1 P** after addition of 0.2 mol equiv CsI; c) **(iG-ND-1 P)P**; d) **(iG-ND-1 P)P** after addition of 0.2 mol equiv CsI. B) 2D DOSY spectra (600 MHz, CD_3CN, 298 K) of **iG-ND-1 P** with 0.2 mol equiv CsI; C) ESI mass spectra of **iG-ND-1 P** in the presence of CsI; D) Schematic representation of dynamic nucleodendrimer formation.

preformed pentamers operate as suitable receptors for Cs^+ ions. DOSY experiments revealed a diffusion coefficient of $3.9 \times 10^{-10}\ m^2 s^{-1}$ for the largest species of the mixture (Figure 2 B), consistent with the formation of a higher-order decameric structure as depicted in Figure 1 B, with a predicted coefficient of $3.9 \times 10^{-10}\ m^2 s^{-1}$. In agreement with this, mass spectrometry analysis revealed the mass of Cs^+-containing decameric species (Figure 2 C).[16] As a control experiment, a nucleodendron where both the aromatic amine and carbonyl groups were protected (**(iG-ND-1 P)P**, see the Supporting Information), thereby preventing hydrogen-bond formation, was not able to form structured assemblies

as demonstrated by the absence of spectral evolution upon addition of CsI (Figure 2A). Dynamic self-assembly was observed for other monomers of the series, prompting us to propose a general model whereby nucleodendrons exist as multimeric species in dynamic equilibrium with a cyclic pentameric assembly, which upon addition of a template is driven towards the formation of a more stable decameric entity as depicted in Figure 2D. The fact that these structures exhibited a single set of proton signals above 10 ppm supports the notion that the heart of self-assembled nucleodendrimers display a high level of symmetry.

Further analysis of this system revealed a marked dependency on the size and polarity of the nucleodendrons to self-assemble. We found that increasing the generation number significantly impaired the ability of monomers to form stable structures in the unprotected series. For example, a comparative NMR analysis of first and second generations showed that while the first generation formed a decameric structure in the presence of CsI, the proportion of structured species was considerably decreased for its second-generation counterpart (Figure 3A). Given that a low-molecular-weight lipophilic

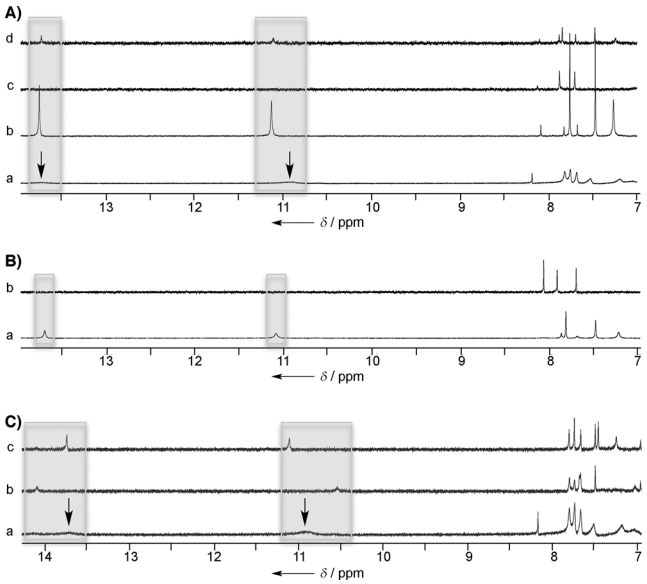

Figure 3. ^{1}H NMR spectra (500 MHz, CD_3CN, 298 K). A) a) **iG-ND-1**; b) **iG-ND-1** after addition of 0.2 mol equiv CsI; c) **iG-ND-2**; d) **iG-ND-2** after addition of 0.2 mol equiv CsI; B) a) **iG-ND-3P** after addition of 0.2 mol equiv CsI; b) **iG-ND-3** after addition of 0.2 mol equiv CsI; C) a) **iG-ND-1**; b) **iG-ND-1** after addition of 0.1 mol equiv KI; c) **iG-ND-1** after addition of 0.1 mol equiv KI followed by addition of 0.1 mol equiv CsI.

isoguanosine requires the presence of cations to self-assemble,[13] our data support the idea that the hydrophobic nature of the dendritic side chain constitute a driving force for the assembly of nucleodendrimers in acetonitrile, whereas steric hindrance impacts on the ability of higher generations to self-assemble. Consistent with this, we observed that protected nucleodendrons had a stronger propensity to self-assemble compared to their non-functionalized analogues, strengthening the notion that the polarity of individual dendrons affects their capacity to self-assemble (Figure 3B). Overall, we observed the general trends of: **iG-ND-1P** > **iG-ND-2P** > **iG-ND-3P** and **iG-ND-1** > **iG-ND-2** ≫ **iG-ND-3** in the presence of CsI.[17] In particular, **iG-ND-3** was resistant to self-assembly in the presence of equimolar amounts of CsI whereas its protected analogue formed structures in the absence of CsI, reaching the size of small proteins almost quantitatively in the presence of 1 mol equiv CsI (ca. 15 kDa).

The size of the cavity within isoguanosine assemblies is suitable to accommodate Cs^+ and K^+ ions.[13] Therefore, we next evaluated the ability of both ions to induce the formation of nucleodendrimers and to compete each other out after assembly. To do so, **iG-ND-1** was first incubated with KI. Interestingly, we observed a new set of NH signals at 14.1 and 10.5 ppm, distinct from those obtained in the presence of CsI, but still representative of a hydrogen-bonded supramolecular assembly. Remarkably, addition of a substoichiometric amount of CsI shifted the mixture composition towards the decamer containing Cs^+, for which the chemical shifts were identical to the ones observed when **iG-ND-1** was directly incubated with CsI (Figure 3C). In contrast, when **iG-ND-1** was first incubated with CsI prior to addition of an equimolar amount of KI, no evolution of the mixture composition was observed. This data demonstrated that isoguanosine-based nucleodendrimers show a preference for Cs^+ ions reflecting a cavity size that is more suitable for its larger ionic radii, leading to a more stable structure as compared to potassium-containing assemblies.[13] Most importantly, our data indicate that monomers or preformed pentamers are in dynamic equilibrium allowing template exchange, leading to distinct nucleodendrimers.

We then explored the capacity of **G-** and **iG-NDs** to form hybrids species based on a previous finding showing that oligonucleotides containing either G or iG have the ability to form heterodimeric assemblies.[18] To do so, **G-NDs** were synthesized, and sequentially incubated either with CsI or KI. Consistent with previous reports on guanosine containing oligonucleotides, **G-NDs** were resistant to CsI and did not form any well-defined assembly (Figure 4A). In contrast, addition of potassium resulted in the appearance of a series of poorly resolved imino signals near 12 ppm indicating the formation of hydrogen-bonded structures devoid of a predominant species exhibiting a clear symmetry. Strikingly, mixing **iG-ND-3P** with **G-ND-1** resulted in the appearance of a signal at 11.1 ppm at the expense of the one at 10.7 ppm in absence of salts, suggesting the formation of the homodecameric iG-containing nucleodendrimer (Figure 4B). This structure did not significantly evolve upon addition of excess CsI, whereas signals corresponding to the K^+ containing structure emerged as a result of KI addition (Figure 4B). These results support the idea that nucleodendrimers comprising exclusively iG were predominent. The absence of an extra set of imino proton corresponding to G residues further corroborates the fact that **G-ND-1** was not involved in a putative hybrid species per se. Additionally, we failed to identify proton signals of either free or structured **G-ND-1** previously identified (Figure 4A). Together, these results demonstrate that G-derived nucleodendrons facilitate the formation of a decameric iG-containing nucleodendrimer in absence of salts, without forming hybrid G/iG nucleodentritic

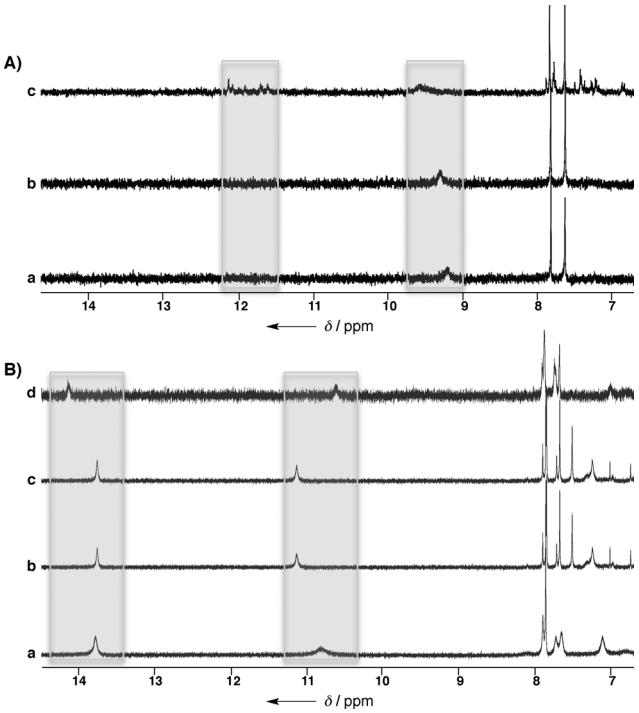

Figure 4. ^{1}H NMR spectra (500 MHz, CD_3CN, 298 K). A) a) **G-ND-1**; b) **G-ND-1** after addition of excess CsI; c) **G-ND-1** after addition of excess KI; B) a) **iG-ND-3 P**; b) **iG-ND-3 P** with 1 molar equiv of **G-ND-1**; c) **iG-ND-3 P** with 1 molar equiv of **G-ND-1** followed by addition of 0.2 mol equiv CsI; d) **iG-ND-3 P** with 1 molar equiv of **G-ND-1** after addition of excess KI.

species, evocative of a molecular crowding situation previously reported for G-quadruplex nucleic acids.[19] It is tempting to speculate that **G-ND-1** being unable to self-assemble interacts instead at the surface of iG-pentamers by means of π-π interactions or in the groove of iG-decamers by hydrogen bonding as previously shown for picrate ions,[5] thereby driving the equilibrium towards a homodecameric iG-nucleodendrimer.

We have described a rapid and versatile access to self-assembled dendritic structures from isoguanosine-containing small molecules using a combination of noncovalent interactions. Our system is modular in that dendrons can respond to organic and inorganic stimuli to form nucleodendritic structures that can vary in size, polarity, stability, geometry and peripheral functionalities. This dynamic system could in principle be used to produce differentially functionalized heterodendrimers by strategically mixing **iG-NDs**. Because of its high degree of symmetry, a challenging endeavour will be to desymmetrize *meso* isoguanosine-derived structures in a controllable manner to produce multifunctional dendritic molecules with tunable physicochemical properties.[20]

Received: February 13, 2014
Published online: ■■ ■■, ■■■■

Keywords: cesium · dendrimers · isoguanosine · NMR spectroscopy · self-assembly

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Communications

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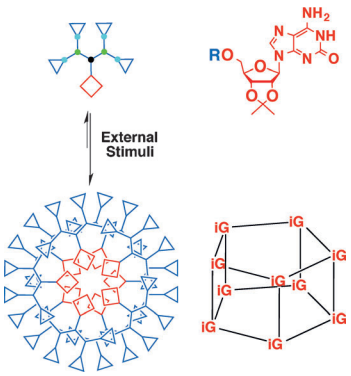
Modular Construction of Dynamic Nucleodendrimers



Dendritic macromolecules: Isoguanosine-containing dendritic small molecules self-assemble into isoguanosine-containing dendrimers named nucleodendrimers (see picture). The building blocks alone form pentameric structures while the presence of alkali metals promotes the formation of stable decamers. This system provides a powerful canvas for the rapid and modular assembly of polyfunctional dendritic macromolecules.