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 result in the formation of decameric structures in absence of added salts. Further analysis of the mixture indicated that guanosine derivatives facilitate the formation of, 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 and colleagues have shown that dendritic macromolecules can readily arise from the self-assembly of monomers in organic solvents by means of non-covalent interactions (e.g. hydrogen bonding).[3] Rivera has shown that guanosine (G) residues embedded with an extended aromatic surface and a tree-shaped polyether form hexadecameric self-organized dendrimers in the presence of potassium ions.[4] Guanosine is known to form square planar structures (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).[3b] This dependency provides the opportunity to design tunable supramolecular devices. For example, it was shown that such assemblies can serve as thermo-, photo- and metallo-responsive structures, which can be exploited for the purpose of drug delivery.[7]

Herein, we describe the synthesis and self-assembly of isoguanosine-containing dendritic derivatives named 'nucleodendron' (**iG-ND**). Seminal work from Davis has shown that a low molecular weight lipophilic isoguanosine residue, has the ability to self-assemble as pentamers around alkali metals.[8] 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 functionalized 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 each monomer might confer distinct physicochemical properties to the corresponding assembly based on different shape, polarity, metal ion preferences and overall size. We anticipated that the lipophilic nature of the dendritic core would help drive and modulate the stability of the structure in polar solvents despite a higher cost 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[9] and coupled to iG-azide by means of a copper catalyzed alkyne/azide cycloaddition (see Supporting Information).[10] This short synthetic procedure gave rise to a series of 6 'nucleodendrons' of variable size and polarity, readily available for self-assembly studies (**Figure 1C**).

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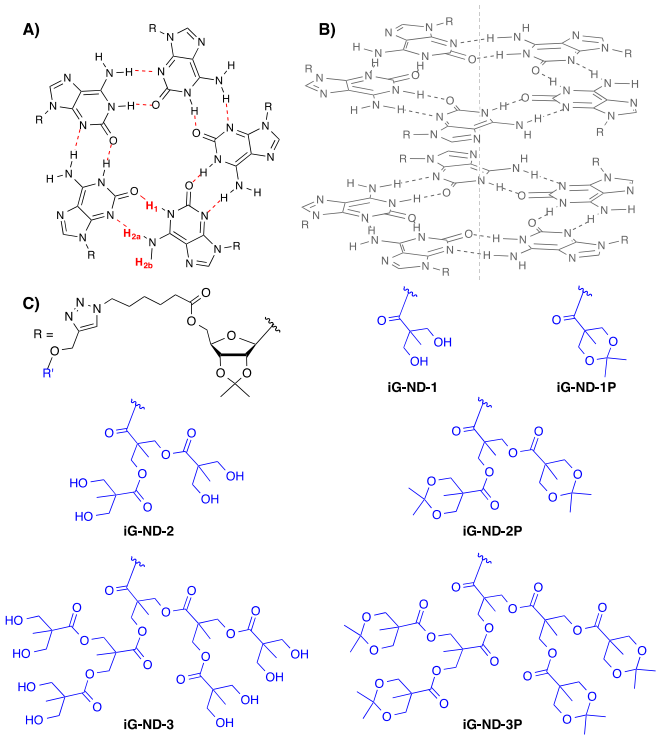

Figure 1. Molecular structures of: **A**) iG-pentamer, **B**) iG-decamer and **C**) iG-derived nucleodendrons (**iG-ND**); **1**, **2** and **3** indicate generation number; **P** indicates acetonide-protected ND.

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. Due to its low viscosity, DOSY experiments were acquired using a convection-compensated, bipolar-paired double-stimulated echo protocol and data were processed as previously described.[11] Interestingly, when **iG-ND-1** or **iG-ND-1P** were dissolved in acetonitrile in absence of metal template, weak NH_1 and NH_{2a} signals were observed at 13.7 and 10.7 ppm, respectively (**Figure 2A**). 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. In line with this, 2D DOSY NMR spectroscopy indicated that a range of small-sized species formed, producing NMR spectra with many overlapping peaks that renders simple DOSY analysis poorly tractable.[12] 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 x 10^{-10} m^2 s^{-1}. This compares well to a theoretical value of 5.5 x 10^{-10} m^2 s^{-1} for a pentameric assembly.[13] It is noteworthy that DOSY signals 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-1P** resulted in significant sharpening of the proton signals associated with a moderate downfield shift of the hydrogen-bonded NH_{2a} signal (10.7 to 11.1 ppm), consistent with the formation of a tighter assembly, further suggesting that preformed pentamers operate as suitable receptors for Cs^+ (**Figure 2A**). An experimental diffusion coefficients of 4.0 x 10^{-10} m^2 s^{-1} compared to a theoretical value of 3.9 x 10^{-10} m^2 s^{-1} was consistent with the formation of a higher-order decameric structure as depicted in **Figure 1B**. In agreement with this, mass spectrometry analysis revealed the mass of Cs^+ containing decameric species (**Figure 2C**).[14] As a control experiment, a dendron where both amine and carbonyl groups were protected (**PiG-ND-1P**, see Supporting Information), thereby preventing hydrogen bonding, was not able to form structured assemblies based on the absence of signals above 10 ppm for NH_1 (**Figure 2A**). This general behavior was also observed for other monomers of the series (see Supporting Information), prompting us to propose a general model whereby nucleodendron 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 hearts of self-assembled nucleodendrimers display a high level of symmetry.

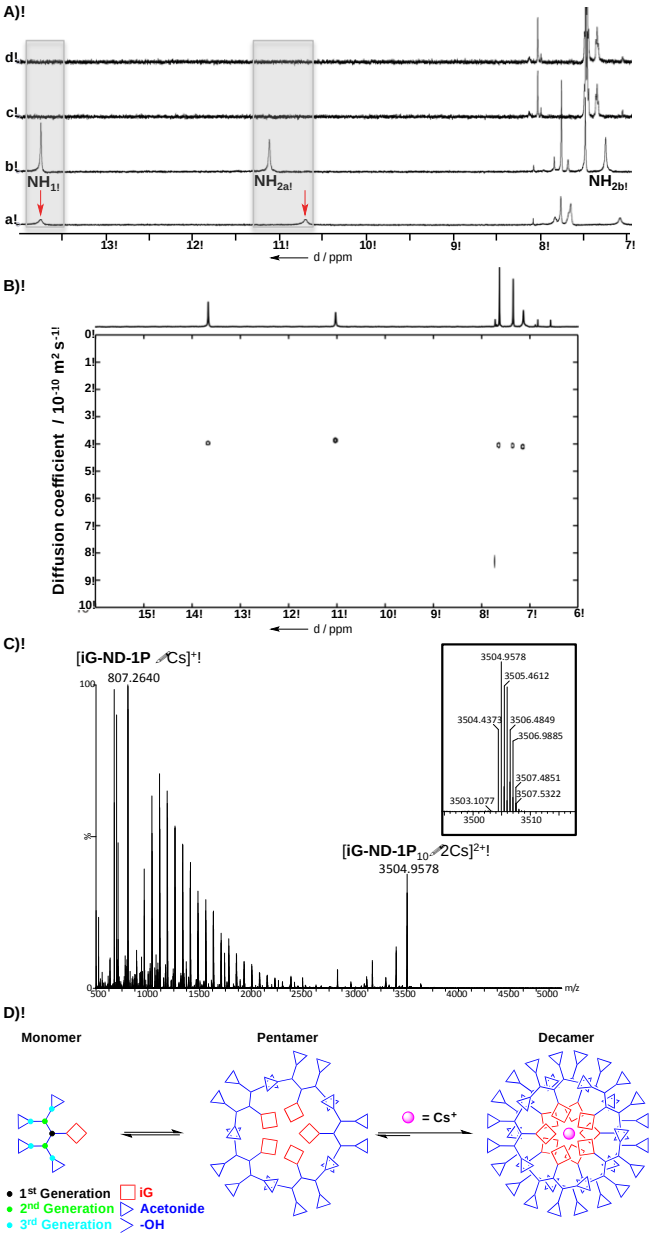

Figure 2. **A**) ^1H NMR spectra (500MHz, CD_3CN, 298K), red arrows indicate weak ^1H signals. a) **iG-ND-1P**; b) **a** after addition of 0.2 mol equiv. CsI; c) **PiG-ND-1P**; d) **c** after addition of 0.2 mol equiv. CsI. **B**) 2D DOSY spectra (600MHz, CD_3CN, 298K) of **iG-ND-1P** with 0.2 mol equiv.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

Further analysis of this system revealed a marked dependency on nucleodendrons polarity and size 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 isoguanosine requires the presence of cations to self-assemble[15], our data support the idea that the lipophilic nature of the dendritic side chain constitute a driving force for the assembly of nucleodendrimers in polar solvents, 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 unfunctionalized analogues, strengthening the notion that deprotected monomers being more soluble in acetonitrile exhibit a weaker capacity to self-assemble (**Figure 3B**). Overall, we observed the general trend of **iG-ND-1P ≥ iG-ND-2P ≥ iG-ND-3P > iG-ND-1 > iG-ND-2 >> iG-ND-3** in the presence of Cs^+ based on the relative intensities of NH_1 and NH_{2a} signals. In particular, **iG-ND-3** was resistant to self-assembly even in the presence of large excess CsI whereas its protected analogue formed structures in absence of CsI, reaching the size of small proteins in the presence of CsI (15kDa).

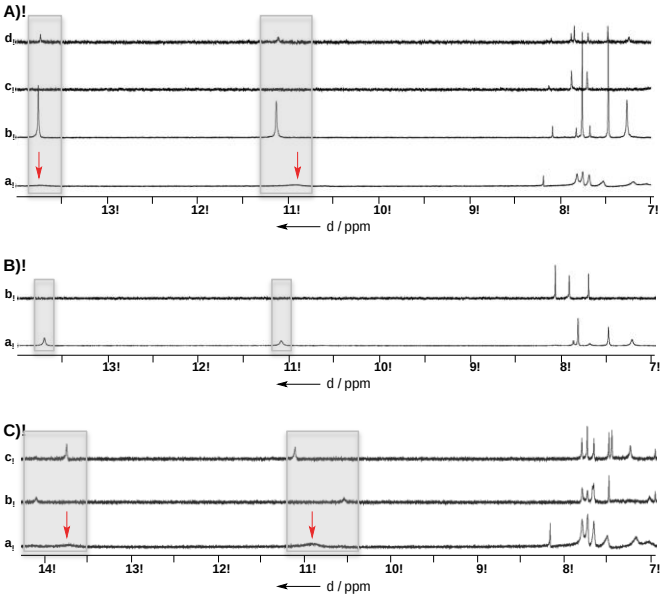

Figure 3. ^{1}H NMR spectra (500MHz, CD_3CN, 298K). **A)** a) **iG-ND-1**; b) **a** after addition of 0.2 mol equiv. CsI; c) **iG-ND-2**; d) **c** after addition of 0.2 mol equiv. CsI; **B)** a) **iG-ND-3P** after addition of 0.2 mol equiv. CsI; c) **iG-ND-3** after addition of 0.2 mol equiv. CsI; **C)**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

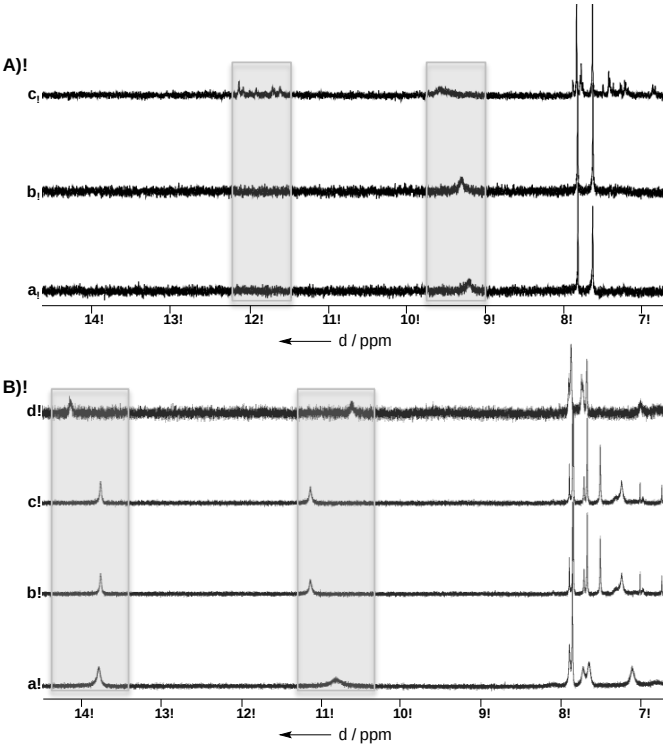

Figure 4. ^{1}H NMR spectra (500MHz, CD_3CN, 298K). **A)** a) **G-ND-1**; b) **a** after addition of excess CsI; c) **a** after addition of excess KI; **B)** a) **iG-ND-3P**; b) **b** with 1 mol equiv. of **G-ND-1**; c) **b** after addition of 0.2 mol equiv. CsI; d) **b** after addition of 0.2 mol equiv.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

It was previously shown that the cavity-size within pentameric isoguanosine could accommodate Cs^+ and K^+.[15] 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 an equimolar 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. In contrast, when **iG-ND-1** was incubated with CsI first, addition of an equimolar amount of KI did not displace the composition of the mixture (**Figure 3C**). This data demonstrated that isoguanosine-based nucleodendrimers exhibit a preference for Cs^+ reflecting a cavity-size that is more suitable for its larger ionic radii, leading to a more stable structure. 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.[16] 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. 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 (**Figure 4A**). 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 was 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 species evocative a molecular crowding situation previously reported for G-quadruplex nucleic acids. 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 *via* hydrogen bonding as previously shown for picrate ions,[5] thereby driving the equilibrium towards a homodecameric iG-nucleodendrimer.[17]

We have described a rapid and versatile access to self-assembled dendritic structures from isoguanosine-containing small molecules using a combination of non-covalent interactions. Our system is modular in that nucleodendritic structures 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 elaborate strategies to desymmetrize isoguanosine-derived structures in a controllable manner to produce monodisperse multifunctional dendritic molecules with tunable physicochemical properties.[18]

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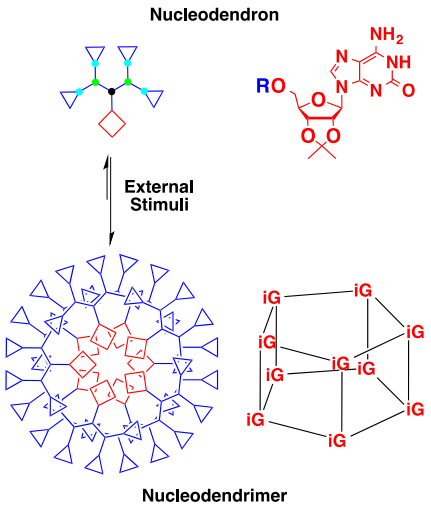

Isoguanosine-containing dendritic small molecules self-assemble into nucleodendrimers. Building blocks readily form pentameric structures alone while the presence of alkali metals promotes the formation of stable decamers. Remarkably, guanosine building blocks favor the formation of isoguanosine-containing decameric structures in absence of salts in a process reminiscent of molecular crowding. This system provides a powerful canvas for the rapid and modular assembly of polyfunctional dendritic macromolecules.