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Stepwise Filtering of the Internal Layers of Dendrimers by Transverse-Relaxation-Edited NMR

Luiz F. Pinto, Ricardo Riguera,* and Eduardo Fernandez-Megia*

Department of Organic Chemistry and Center for Research in Biological Chemistry and Molecular Materials (CIQUS), University of Santiago de Compostela, Jenaro de la Fuente s/n, 15782 Santiago de Compostela, Spain

Supporting Information

ABSTRACT: The characteristic distribution of transverse relaxation times (T_2) within dendrimers (shorter values at the core than the periphery) can be exploited in T_2-edited 1D and 2D NMR experiments for the stepwise filtering of internal nuclei according to their topology within the dendritic structure. The resulting filtered spectra, which can be conceived as corresponding to virtual hollow dendrimers, benefit from reduced signal overlap, thus facilitating signal assignment and characterization. The generality of the method as a powerful tool in structural and end-group analysis has been confirmed with various dendritic families and nuclei (^{1}H, ^{13}C, ^{31}P).

Dendrimers are synthetic treelike macromolecules composed of repetitive layers of branching units that are prepared in a controlled iterative fashion through generations with precise molecular weight and discrete properties. As a function of generation, globular architectures and sizes emerge at the nanoscale that render dendrimers attractive for many applications in the fields of catalysis, nanomaterials, and nanomedicine.[1] NMR spectroscopy is the technique of choice for routine structural characterization of dendrimers.[2] NMR relaxation is also recognized as a versatile way to study their dynamics by measuring longitudinal (T_1) and transverse (T_2) relaxation times and nuclear Overhauser effects (NOEs).[3] In spite of conflicting theoretical models (dense core[4] vs dense shell[5]) that initially obscured the dynamical analysis of dendrimers, a consensus around the dense-core model has recently emerged.[6] We recently reported an NMR relaxation study on the dynamics of dendrimers (^{1}H and ^{13}C T_1 and T_2, NOE) showing that slower internal dynamics are accompanied by a reduction in T_2 in going from the periphery to the core.[7] Herein we report that this characteristic distribution of T_2 can be used for the stepwise filtering of the internal dendritic layers in 1D and 2D NMR spectra as a powerful tool for easier signal assignment and characterization.

In NMR experiments, spin systems in a nonequilibrium condition after a 90° pulse immediately return to equilibrium by longitudinal and transverse relaxation. The intensity of the transverse component of the magnetization, I_{xy}, decays to zero by spin−spin relaxation according to the equation

$$I_{xy} = I_0 \exp\left(-t/T_2\right) \quad (1)$$

where I_0 is the intensity at time $t = 0$.[8] In complex mixtures of species with different T_2 values (e.g.; macromolecules with short T_2 and low-molecular-weight molecules with long T_2), the resonances with the shortest T_2 can be filtered from the NMR spectrum by means of spin-echo pulse sequences, such as the Carr−Purcell−Meiboom−Gill (CPMG) sequence $[90°_x-(\tau-180°_y-\tau)_n$, where 2τ is a fixed echo time, n is the number of echoes, and $2\tau n$ is the total echo duration].[9] CPMG and related sequences[10] keep the magnetization in the transverse plane for a time t equal to $2\tau n$ before acquisition of the free induction decay, allowing the magnetization of each nucleus to decay independently according to its characteristic T_2 value (with larger T_2 leading to slower decay). Eventually, after a certain time t (known as the T_2 or CPMG filter), differences in T_2 can be exploited for the selective suppression of the NMR signals of macromolecular species while enhancing the detection of small molecules. Successful examples of this filtering strategy include the metabolic profiling of cells, tissues, and biological fluids,[11] where T_2 filters at least 5−7 times the T_2 values of the signals to be filtered are implemented to ensure their 99.3−99.9% suppression (eq 1). Application of the same concept to the rich internal distribution of T_2 values in dendrimers was envisaged to be an approach for the stepwise filtering of the internal signals with shorter T_2 values. The resulting filtered spectra, which can be conceived as corresponding to a collection of virtual hollow dendrimers, benefit from reduced signal overlap, which facilitates NMR assignment and characterization.

The feasibility of the strategy was first demonstrated in ^{1}H NMR analysis of Fréchet-type poly(aryl ether) dendrimers.[12] Figure 1 depicts the structure and ^{1}H NMR spectrum of a third-generation (G3) poly(aryl ether) dendrimer, which shows well-resolved signals for the benzylic (*a*, *bc*, *d*) and aromatic (*A*, *BD*, *C*, *Z*) protons. The potential of T_2 filters for selective suppression of the ^{1}H signals according to their topology within the dendritic structure (different T_2 values) was assessed by plotting the normalized ^{1}H signal intensities (I_{xy}/I_0) as functions of the echo duration. As shown in Figure 1, I_{xy}/I_0 for the different nuclei decreased with the echo duration in a topology-dependent fashion that ensured their stepwise selective suppression from the core to the periphery. Indeed, as indicated by colored arrows in the T_2-filtered spectra, sequential suppression of the aliphatic protons was possible by implementation of increasing T_2 filters from 170 ms (core methyl) to 840 ms (benzylic *d*) to 1.4 s (benzylic *bc*) to 3.4 s (benzylic *a*). Similarly, from the I_{xy}/I_0 plot of the aromatic signals, filters could be selected for their stepwise suppression from the core (*Z* protons) to the periphery, finally

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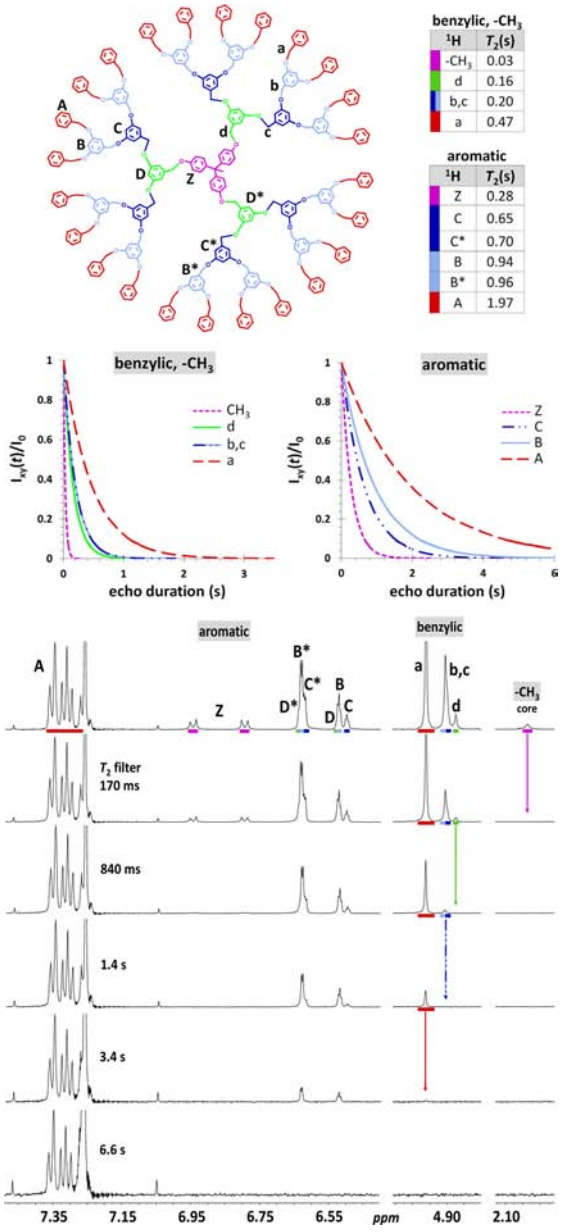

Figure 1. Top: Structure of the G3 poly(aryl ether) dendrimer [1,1,1-tris(4′-hydroxyphenyl)ethane core] and tables of ^{1}H T_2 values. Middle: Normalized ^{1}H intensities (I_{xy}/I_0) as functions of the echo duration. Bottom: Original and T_2-filtered ^{1}H NMR spectra (500 MHz, $CDCl_3$, 298 K).

affording a ^{1}H NMR spectrum showing only the most exposed protons *A* (longest ^{1}H T_2) after a 6.6 s filter (Figure 1).

The generality of the approach for filtering the NMR spectra of dendrimers was demonstrated by applying the method to other classical dendritic families, including Majoral's phosphorus P-dendrimers[13] and Tomalia's poly(amido amine) (PAMAM) dendrimers.[14] In the first case, the similarity of the ^{1}H T_2 values for the internal layers in a G2 P-dendrimer allowed their selective suppression with a T_2 filter of 3 s, which rendered a spectrum showing only the most peripheral aromatic and aldehyde signals (Figure 2). In a second step, filtering of the external aromatic protons was also possible with a longer 8 s filter (both filters were ca. 6 times the longest T_2 signal to be suppressed). The fidelity of the strategy was demonstrated by application of the method to T_2-filtered 2D NMR experiments in which the CPMG sequence

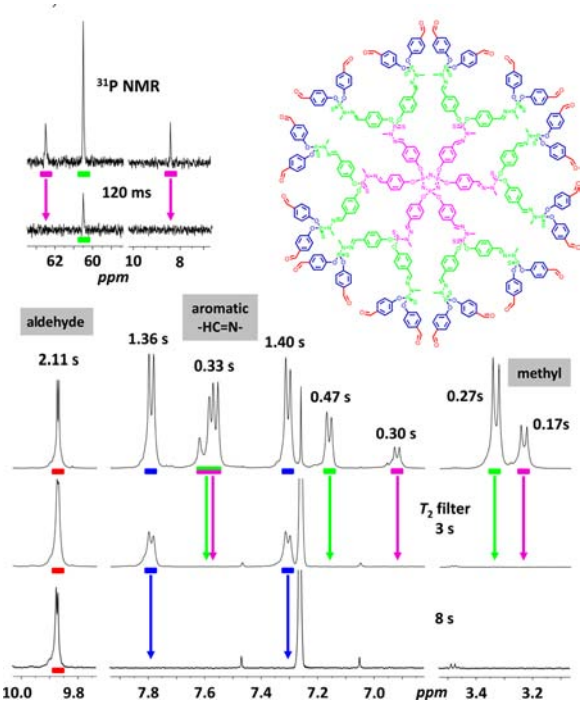

Figure 2. Top right: Structure of a G2 P-dendrimer (cyclotriphosphazene core) carrying 24 peripheral aldehydes. Top left and bottom: ^{31}P and ^{1}H original and T_2-filtered spectra (202 MHz for ^{31}P and 500 MHz for ^{1}H, $CDCl_3$, 298 K). ^{1}H T_2 values are shown above the ^{1}H spectra.

was used as an excitation block, replacing the first excitation pulse.[15] For instance, the use of 3 and 8 s filters in a T_2-filtered correlation spectroscopy (COSY) experiment selectively afforded the desired suppressions for the G2 P-dendrimer [Figure S3 in the Supporting Information (SI)].

PAMAM illustrates a kind of dendrimer with low NMR resolution among nuclei in the different layers that complicates the straightforward identification of the external groups. This characteristic renders PAMAM especially suited to benefit from the selective suppression of broad internal signals by application of T_2 filters. In the absence of a detailed ^{1}H T_2 map for PAMAM, we envisioned the direct implementation of a selection of T_2 filters as the most accelerated filtering strategy. As shown in Figure 3 for G4 PAMAM, the application of four filters between 150 ms and 3 s allowed us to obtain a spectrum using a T_2 filter of 1 s that showed only the signals for the most peripheral protons, which were partially hidden in the original spectrum.

The possibility of filtering internal layers in dendrimers without the necessity of previous knowledge of T_2 was also useful in the characterization and signal assignment of peripherally decorated dendrimers. With this aim, a G4 poly(propylene imine) (PPI) dendrimer[16] was decorated with 32 ibuprofen molecules (see the SI). Implementation of four filters between 150 ms and 3 s again resulted in the complete suppression of the internal PPI signals using a 300 ms filter and the selective visualization of the resonances due to ibuprofen (Figure 4). Remarkably, fruitful characterization of the ibuprofen groups without interference from PPI signals was also possible by application of the very same 300 ms filter to a T_2-filtered COSY spectrum and a heteronuclear ^{1}H−^{13}C T_2-filtered heteronuclear single-quantum coherence (HSQC) spectrum (Figure 4). Since relaxation in ^{1}H heteronuclear 2D NMR experiments (indirect detection) is governed by the ^{1}H nucleus, T_2 filters in HSQC experiments are determined by the ^{1}H T_2 values.

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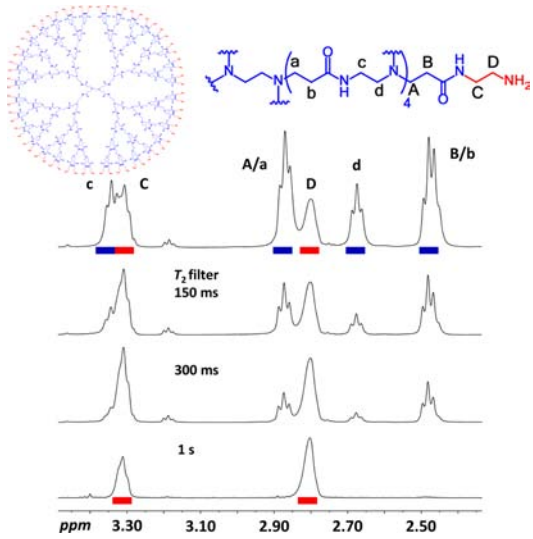

Figure 3. Top: Structure of G4 PAMAM (ethylenediamine core). Bottom: Original and T_2-filtered ^{1}H NMR spectra (500 MHz, $CDCl_3$, 298 K).

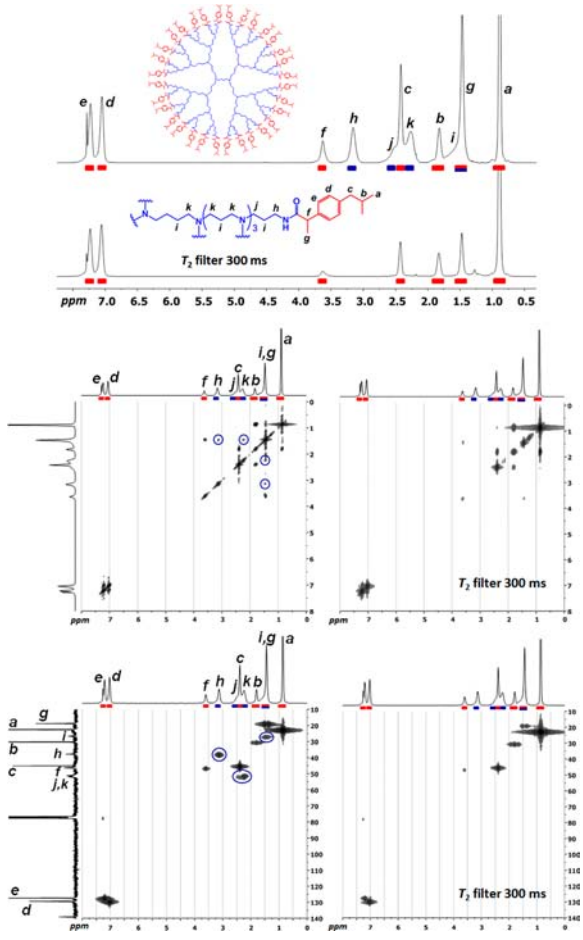

Figure 4. Top: Structure of G4 PPI–ibuprofen (1,4-diaminobutane core) and original and T_2-filtered ^{1}H NMR spectra. Middle and bottom: (left) Original and (right) T_2-filtered COSY and ^{1}H–^{13}C HSQC spectra (500 MHz, $CDCl_3$, 298 K). PPI cross-peaks are circled in blue.

The potential of this filtering tool was further evaluated by application to a dendrimer for which T_2 data were available in the literature, allowing filters to be determined directly as 7 × T_2 to ensure 99.9% signal suppression according to eq 1. We selected a G4 PPI dendrimer decorated with triethylene glycol (TEG) groups that was previously reported by the groups of Ford and Zhu.[17] Figure 5 depicts the structure of PPI–TEG along with the

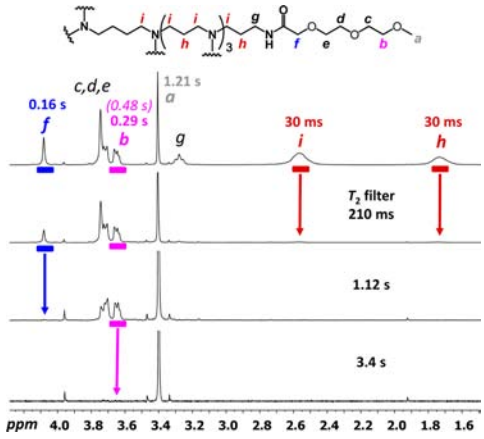

Figure 5. Structure of G4 PPI-TEG (1,4-diaminobutane core) and original and T_2-filtered ^{1}H NMR spectra (300 MHz, 1 wt % in D_2O, 298 K). The ^{1}H T_2 values are shown above the original spectrum.

reported ^{1}H T_2 values in D_2O (1 wt %, 300 MHz) for protons *a* (1.21 s), *b* (0.29 s), *f* (0.16 s), *h* (30 ms), and *i* (30 ms). According to these data, the stepwise suppression of *h*/*i*, *f*, and *b* was achieved with T_2 filters of 210 ms, 1.12 s, and 2.03 s, respectively. As expected, the first two filters proceeded very efficiently for the selective suppression of *h*/*i* and *f*. The third filter, however, failed in the goal of completely suppressing proton *b*, suggesting a much longer ^{1}H T_2 for this proton than the reported value of 0.29 s. Confirmation of this point was obtained by determining a ^{1}H T_2 of 0.48 s. Indeed, application of a 3.4 s filter (equivalent to 7 × 0.48 s) achieved the pursued filtration, affording a spectrum showing only the signals for the outermost methyl protons *a*. The relatively small dependence of T_2 on the magnetic field[18] encouraged us to test the robustness of the method at different fields. To this end, the above T_2 filters determined at 300 MHz were implemented in a 500 MHz spectrometer. Gratifyingly, a clean stepwise suppression was revealed, demonstrating the utility of the tool with spectrometers operating at fields different from that used for the T_2 determination (Figure S4).

A practical application of the T_2 filters involves the analysis of partially/incompletely functionalized dendrimers. For instance, it is known that the inherent toxicity of cationic amino-dendrimers can be modulated by partial acetylation, which also results in increased solubility and reduced nonspecific targeting. Such a strategy has been thoroughly studied for PAMAM dendrimers with different degrees of acetylation.[19] A partially (70%) acetylated G4 PAMAM dendrimer was prepared following these procedures. Analysis of the ^{1}H NMR spectrum of this sample showed extensive overlap between the resonances of the peripheral nonacetylated end groups and those of internal nuclei that complicated the characterization (Figure 6). However, suppression of all resonances from internal layers was possible with a T_2 filter of 1.1 s, after which the presence of nonacetylated groups was unambiguously confirmed both by ^{1}H NMR and T_2-filtered COSY experiments (Figures 6 and S5). Application of the same concept is expected to aid the analysis of end-group purity during dendrimer growth and postfunctionalization.

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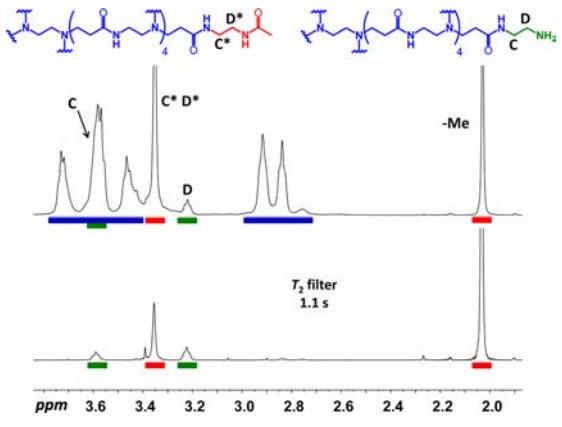

Figure 6. Structure of partially acetylated G4 PAMAM and original and T_2-filtered ^{1}H NMR spectra (500 MHz, D_2O, pD 3.8, 298 K).

Finally, considering the relevance of NMR spectroscopy using nuclei other than ^{1}H in the characterization of dendrimers, we decided to test the viability of this T_2-filtering strategy in ^{13}C and ^{31}P NMR analysis of dendrimers. ^{13}C was especially interesting because of its higher resolution relative to ^{1}H, while ^{31}P was selected to illustrate the relevance of alternative nuclei in the analysis of heteroatom-containing dendrimers (e.g., P-dendrimers). With this aim, a G2 poly(aryl ether) and a G2 P-dendrimer carrying 24 peripheral groups were submitted to T_2-filtered ^{13}C and ^{31}P NMR experiments, respectively. Selective suppression of the resonances due to the internal layers in a stepwise fashion according to their characteristic T_2 values was achieved (Figures 2 and S6), greatly facilitating the signal assignment of both nuclei.

In conclusion, the characteristic distribution of T_2 values within dendrimers (shorter values at the core than the periphery) can be exploited in T_2-edited NMR experiments for the stepwise filtering of nuclei in the internal layers. The resulting spectra corresponding to virtual hollow dendrimers benefit from reduced signal overlap, facilitating signal assignment and characterization. The generality of the method was confirmed through applications to various dendritic families, nuclei (^{1}H, ^{13}C, ^{31}P), and 2D experiments (COSY and HSQC). For cases where no previous knowledge of T_2 is available, an accelerated strategy was developed by implementing selected filters (four filters between 150 ms and 3 s in the case of ^{1}H). The application of T_2 filters is envisaged to aid structural and end-group analysis in related dendritic structures, including block, dendronized, and hyperbranched polymers.

■ ASSOCIATED CONTENT

Supporting Information

Methods and additional data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

ricardo.riguera@usc.es; ef.megia@usc.es

Notes

The authors declare no competing financial interest.

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