Supporting Information for:

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

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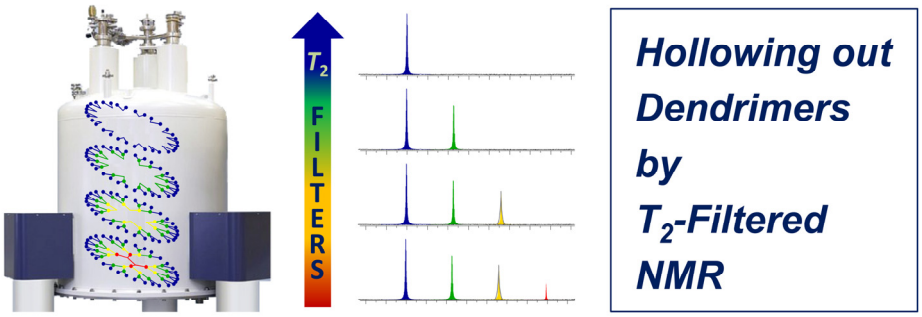

Table of Contents

1. Materials

G4 Poly(amido amine) (PAMAM) with 64 primary amine end groups was purchased as a 10 wt% solution in MeOH from Sigma-Aldrich. G4 poly(propylene imine) (PPI) dendrimer with 32 primary amine end groups was purchase from Symo-Chem. G2 P-dendrimer carrying 24 aldehyde end groups was kindly gifted by Prof. Jean-Pierre Majoral (CNRS, Toulouse, France). G2 and G3 Fréchet-type poly(aryl ether) dendrimers incorporating a 1,1,1-tris(4'-hydroxyphenyl)ethane core were synthesized following a modified procedure of that reported by Yamazaki and co-workers,[1] incorporating extra purification steps by flash chromatography (230-400 mesh silica gel) after chlorination step [CH_2Cl_2:hexane, 3:2 (G2), 3:1 (G3)] and coupling to 3,5-dihydroxybenzyl alcohol [CH_2Cl_2:hexane 25:1 (G2); gradient CH_2Cl_2:hexane 50:1 to 100:1 (G3)]. G4 PPI-TEG was synthesized as previously described by the groups of Ford and Zhu.[2] Partially acetylated G4 PAMAM dendrimer (70% by ^{1}H NMR) was prepared according to previous literature procedures.[3] 2-[2-(2-methoxyethoxy)ethoxy]acetic acid, 1-hydroxybenzotriazole hydrate (HOBt), ibuprofen, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), 1,1,1-tris(4'-hydroxyphenyl)ethane, 3,5-dihydroxybenzyl alcohol, benzyl chloride, tetramethylammonium hydroxide pentahydrate, and acetic anhydride were purchased from Sigma-Aldrich. Thionyl chloride was purchase from Across Organics. CH_2Cl_2 and Et_3N were distilled from CaH_2. 1-Methyl-2-pyrrolidinone, DMF, toluene, and MeOH were dried over molecular sieves. K_2CO_3 was dried under reduced pressure at 65 ˚C overnight. Ultrafiltration was performed on stirred cells with Amicon YM1 membranes.

2. Preparation of G4 PPI-ibuprofen and characterization

Ibuprofen (125 mg, 0.606 mmol, 1.5 eq. relative to NH_2 groups), EDC (116 mg, 0.605 mmol), and HOBt (82 mg, 0.607 mmol) were dissolved in dry CH_2Cl_2 (2 mL) and stirred at rt for 30 min under Ar. Then, G4 PPI (44 mg, 0.401 mmol, 32 terminal NH_2 groups) was dissolved in dry CH_2Cl_2 and added to the reaction mixture. After 72 h of stirring at rt, the solvent was evaporated and the crude product was dissolved in EtOAc and washed with H_2O (3 x 50 mL), 0.5 M HCl (3 x 50 mL), 1 M NaOH (3 x 50 mL), and H_2O (3 x 50 mL). The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure to give a crude product that was purified by ultrafiltration (Amicon YM1, acetone 6 x 30 mL) to give G4 PPI-ibuprofen (92 mg, 77 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.20 (br s, 64H), 7.02 (br s, 64H), 3.60 (br s, 32H), 3.13 (br s, 64H), 2.49-2.24 (m, 178H), 1.80(br s, 34H), 1.44 (br s, 160H), 0.86 (br s, 192H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 175.1, 140.2, 139.2, 129.3, 127.3, 52.2, 51.2, 46.3, 45.1, 37.9, 30.3, 26.8, 22.5, 18.8; MALDI-TOF MS (dithranol, linear mode, *m*/*z*): 9538. Calcd. for $[M+H]^+$, $C_{600}H_{945}N_{62}O_{32}$: 9539.

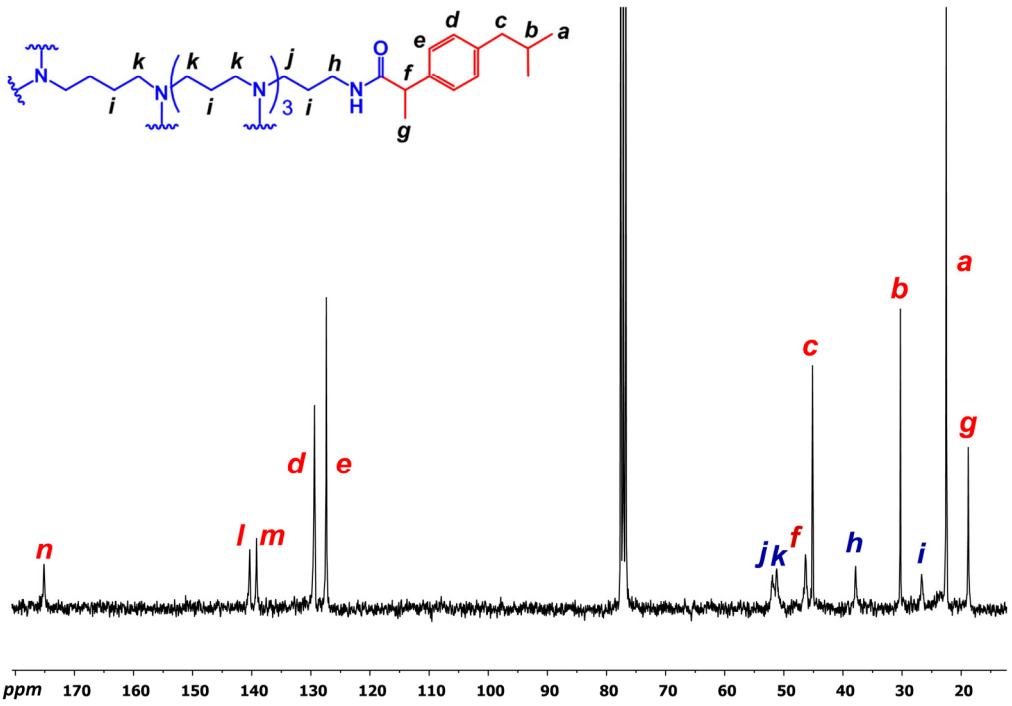

Figure S1. ^{13}C NMR spectrum of G4 PPI-ibuprofen (75 MHz, $CDCl_3$)

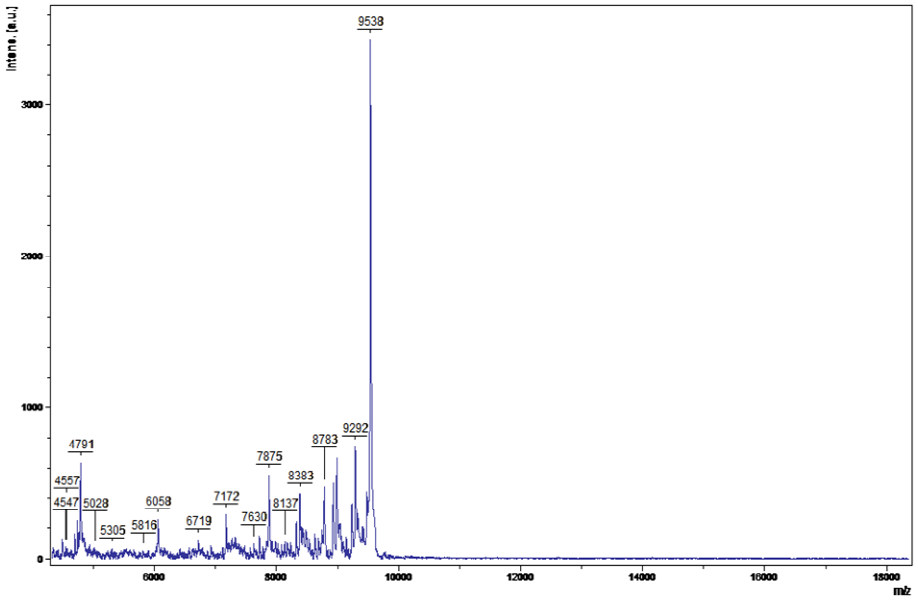

Figure S2. MALDI-TOF MS of G4 PPI-ibuprofen

3. NMR spectroscopy

NMR experiments were recorded at DRX-500 Bruker spectrometer of 11.7 T (^{1}H frequency 500 MHz, ^{31}P frequency 202 MHz) equipped with an inverse detection ^{1}H/X broad-band BBI probe with z gradients and operating under Topspin 1.3 software, and at Agilent Mercury-300 spectrometer of 7.0 T (^{1}H frequency 300 MHz, ^{13}C frequency 75 MHz) equipped with an ATB direct detection X broad-band / ^{1}H dual probe with z gradient and operating under VNMRJ 2.2D software. ^{1}H NMR chemical shifts (δ) are reported in ppm relative to residual $CHCl_3$ at 7.26 ppm or HOD at 4.79 ppm. ^{13}C NMR δ are reported in ppm using $CDCl_3$ as reference at 77.0 ppm. 1D and 2D T_2-edited experiments were performed by replacing the first 90˚ pulse by a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence as previously described[4,5] using a τ value of 0.7 ms. A dendrimer concentration of 0.5 mM was set for all ^{1}H T_2-filtered experiments including COSY (except for PPI-TEG where a 1% wt solution was used for comparison purposes with ref 2). T_2-filtered ^{13}C NMR for G2 poly(aryl ether) dendrimer was done at 2 mM. T_2-filtered ^{1}H-^{13}C HSQC for PPI-ibuprofen was recorded at 8 mM. *MestreNova* software (*Mestrelab Research*) was used for spectra processing and OriginPro7.5 (*Originlab*) to perform the exponential fittings to obtain T_2 relaxation times.

^{1}H T_2 relaxation experiments were acquired at DRX-500 and Mercury-300 spectrometer at 298 K. T_2 values were determined by means of the CPMG pulse sequence using 16 different echo durations (*t*), with a minimum value of 1.4 ms and a maximum of about 6 to 7 times the highest T_2 in the sample. The delay between 180˚ pulses in the CPMG block was 1.4 ms (τ = 0.7 ms), the number of scans was set at 32, and the interscan relaxation delay (d_1) was 18 s. T_2 relaxation times were determined by fitting the absolute signal integrals or intensities (I) at each *t* to the monoexponential eq. S1:

$$I(t)/I_0 = \exp(-t/T_2) \qquad \text{eq. S1}$$

where, I(*t*) and I_0 are the observed signal integrals or intensities at a given value of *t* and at *t* equal to 0, respectively. The *t* dependence of the absolute integrals or intensities for all ^{1}H resonances analyzed showed monoexponential magnetization decays.

4. Figures S3-S6

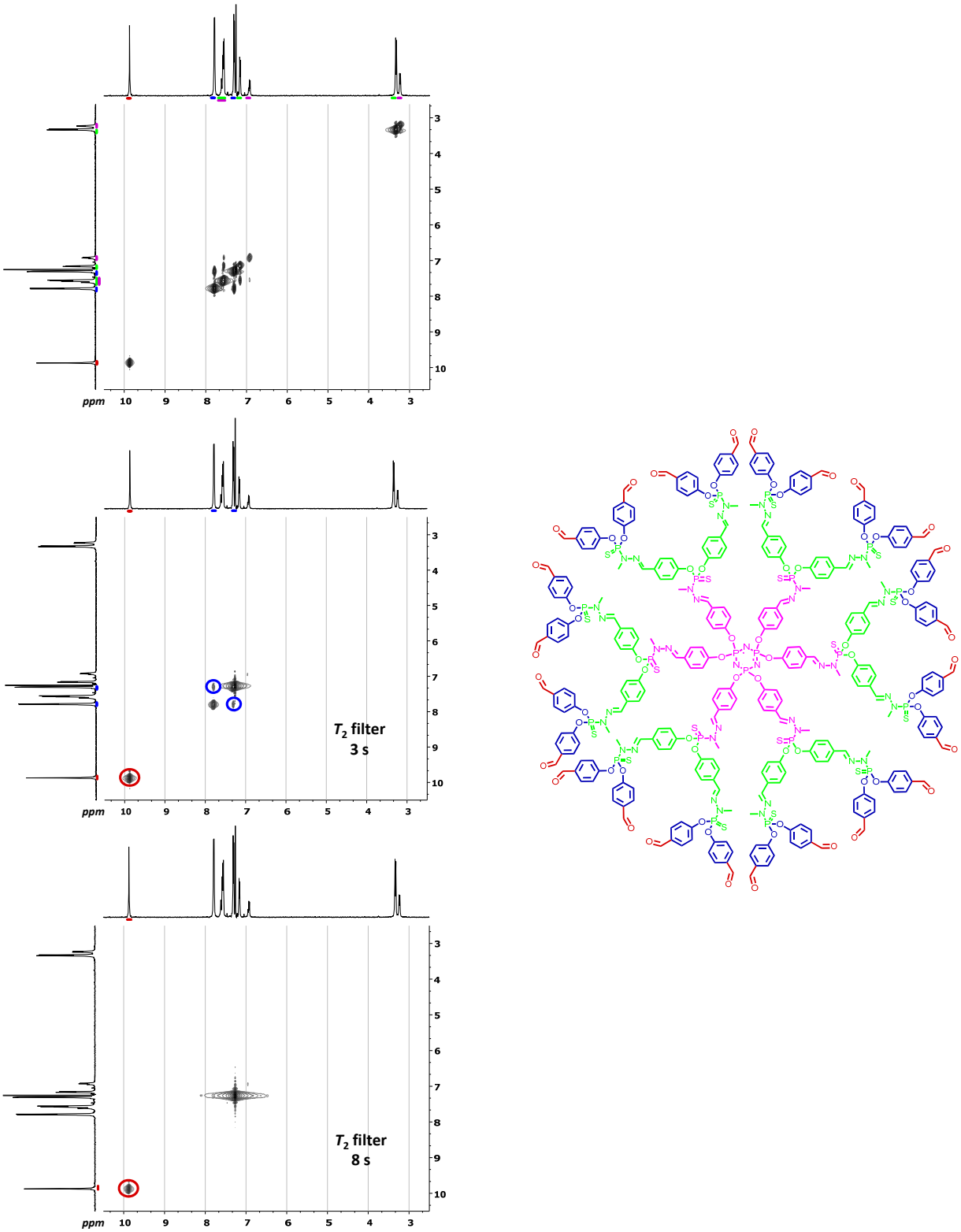

Figure S3. Structure of G2 P-dendrimer and T_2-filtered COSY spectra showing in blue circles the most peripheral aromatic cross-peaks (500 MHz, $CDCl_3$, 298 K).

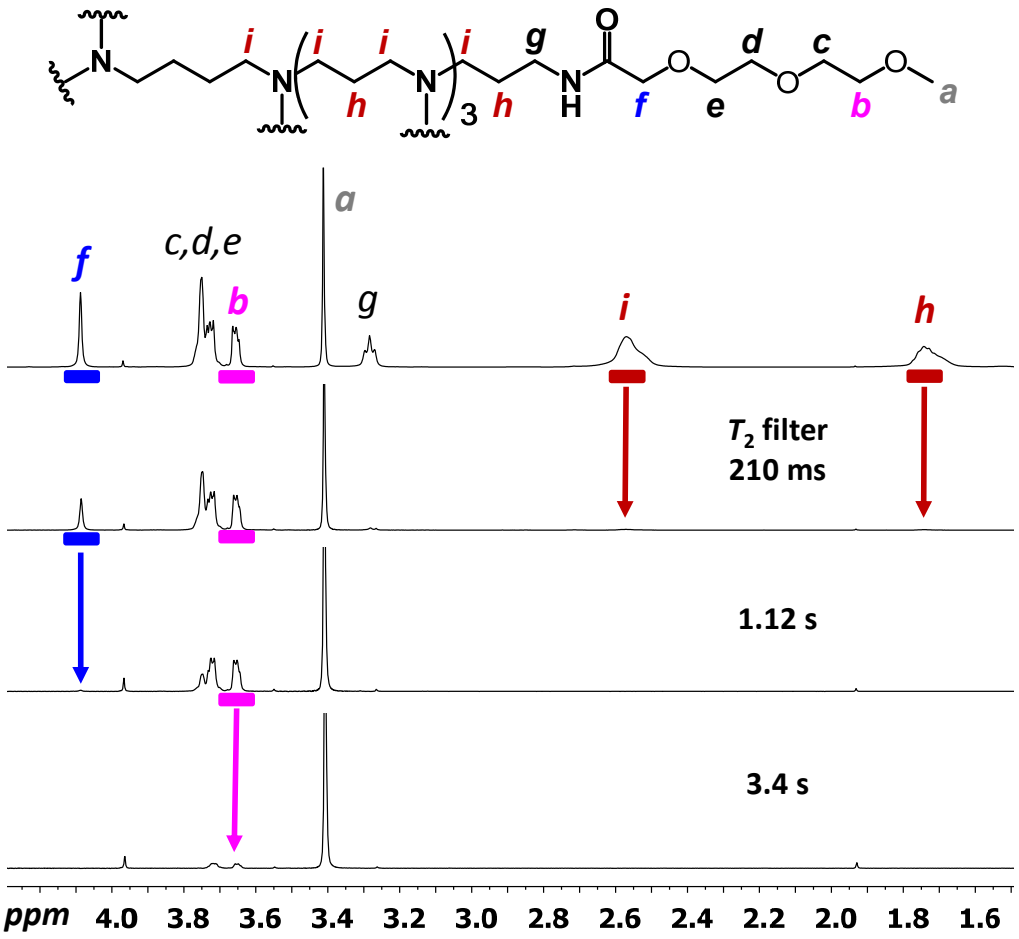

Figure S4. Structure of G4 PPI-TEG and ^{1}H T_2-filtered NMR spectra (500 MHz, 1 wt.% in D_2O, 298 K).

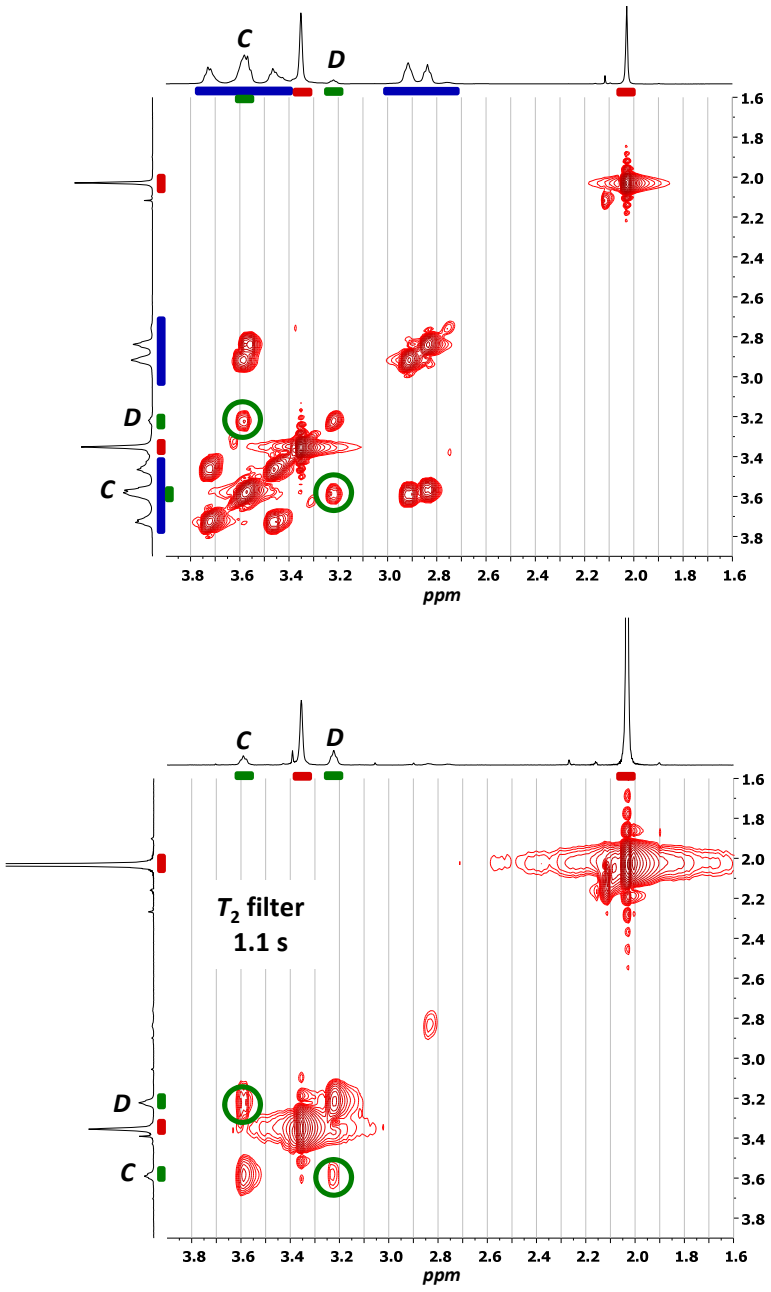

Figure S5. T_2-filtered COSY of partially acetylated G4 PAMAM (500 MHz, D_2O, pD 3.8, 298 K).

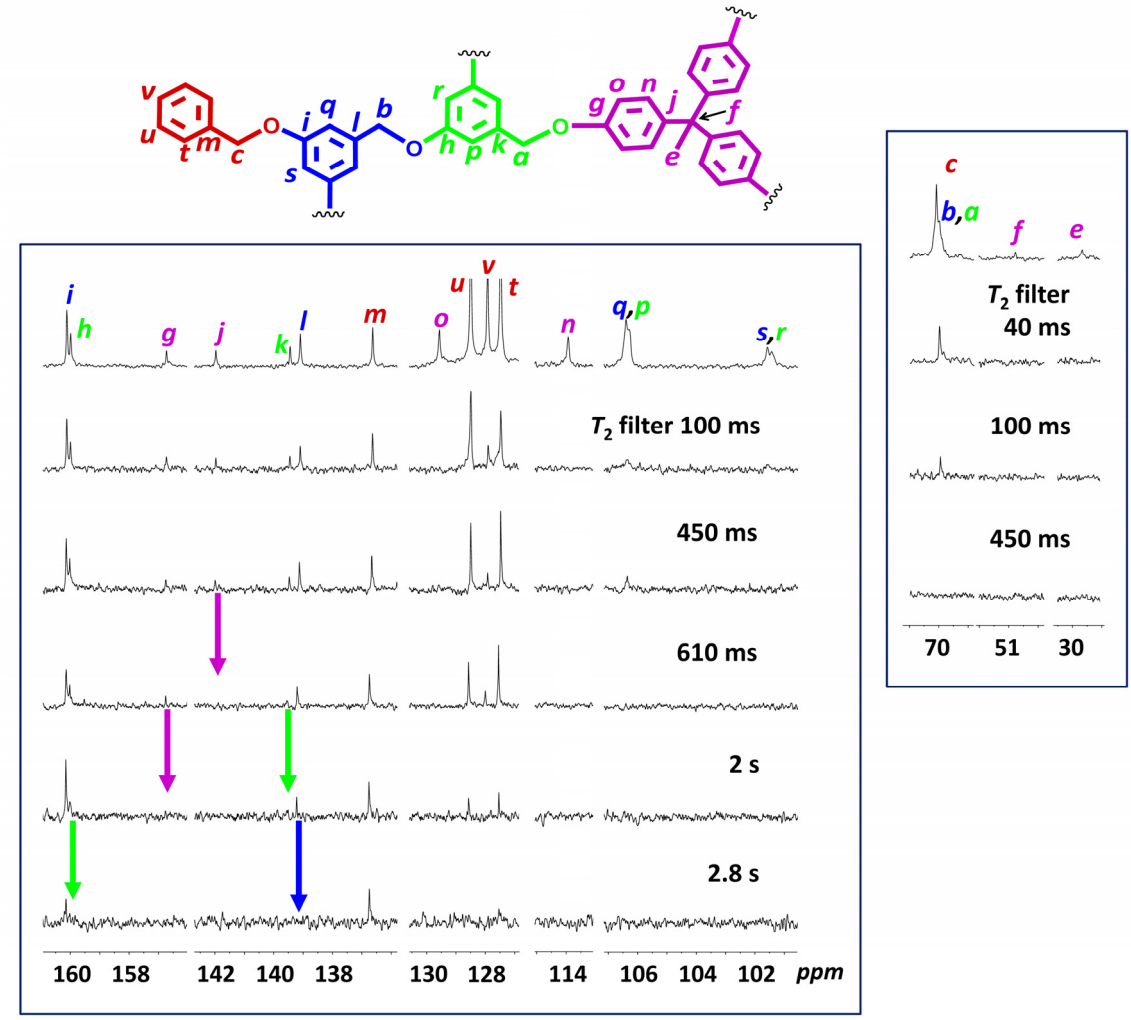

Figure S6. ^{13}C T_2-filtered NMR spectra of aromatic and aliphatic carbons in G2 poly(aryl ether) dendrimer (75 MHz, $CDCl_3$, 298 K). The stepwise suppression of the ^{13}C resonances is illustrated with arrows for the quaternary aromatic signals.

5. References

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5 Williams, P. G.; Saunders, J. K.; Dyne, M.; Mountford, C. E.; Holmes, K. T. *Magn. Reson. Med.* **1988**, *7*, 463.