Electronic Supporting Information

Multivalent cationic dendrofullerenes for gene transfer: synthesis and DNA complexation

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General. Reagents and solvents were purchased as reagent grade and used without further purification. 1-(chloromethyl)-3,5-bis(prop-2-yn-1-yloxy)benzene (**1a**),[1] 5-

(chloromethyl)-1,2,3-tris(prop-2-yn-1-yloxy)benzene (**1b**)[2] and *tert*-butyl (2-(2-(2-azidoethoxy)ethoxy)ethyl)carbamate (**2**)[3] were prepared according to previously reported procedures. For column chromatography, silica gel 60 (230-400 mesh, 0.040-0.063 mm) was purchased from Scharlab. Thin Layer Chromatography (TLC) was performed on aluminium sheets coated with silica gel 60 F_{254} purchased from E. Merck, visualization by UV light. IR spectra (cm^{-1}) were measured on a Bruker Tensor 27 instrument equipped with an ATR device FTIR instrument and using KBr for water-soluble compounds or dissolved in the proper solvent when possible. NMR spectra were recorded on a Bruker AC 300, AC 500 or AC 700 with solvent peaks as reference. ^{1}H and ^{13}C NMR spectra were obtained for solutions in $CDCl_3$ and DMSO-d^6. All the assignments were confirmed by two-dimensional NMR experiments.

^{1}H NMR (top) and ^{13}C NMR (bottom) spectra of amino compounds.

Compound 3a.

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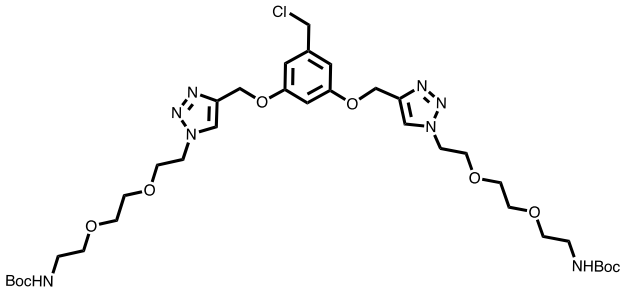
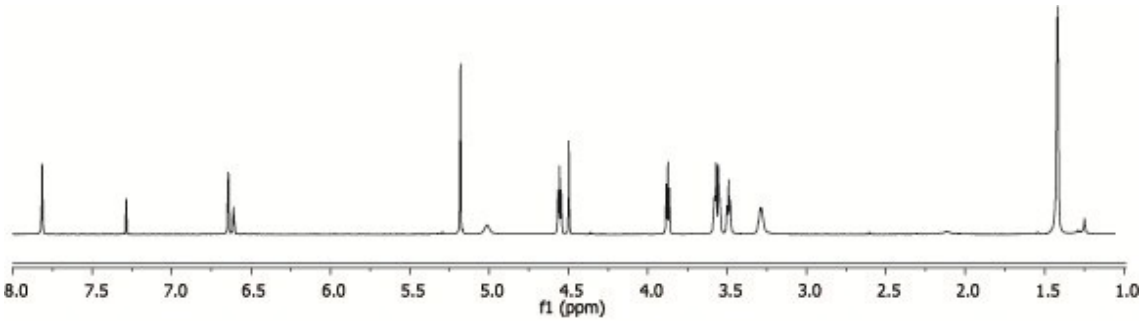
[1] Wu, P.; Feldman, A. K.; Nugent, A. K.; Hawker, C. J.; Scheel, A.; Voit, B.; Pyun, J.; Fréchet, J. M. J.; Sharpless, K. B.; Fokin, V. V. *Angew. Chem. Int. Ed.* **2004***, 43*, 3928-3932.

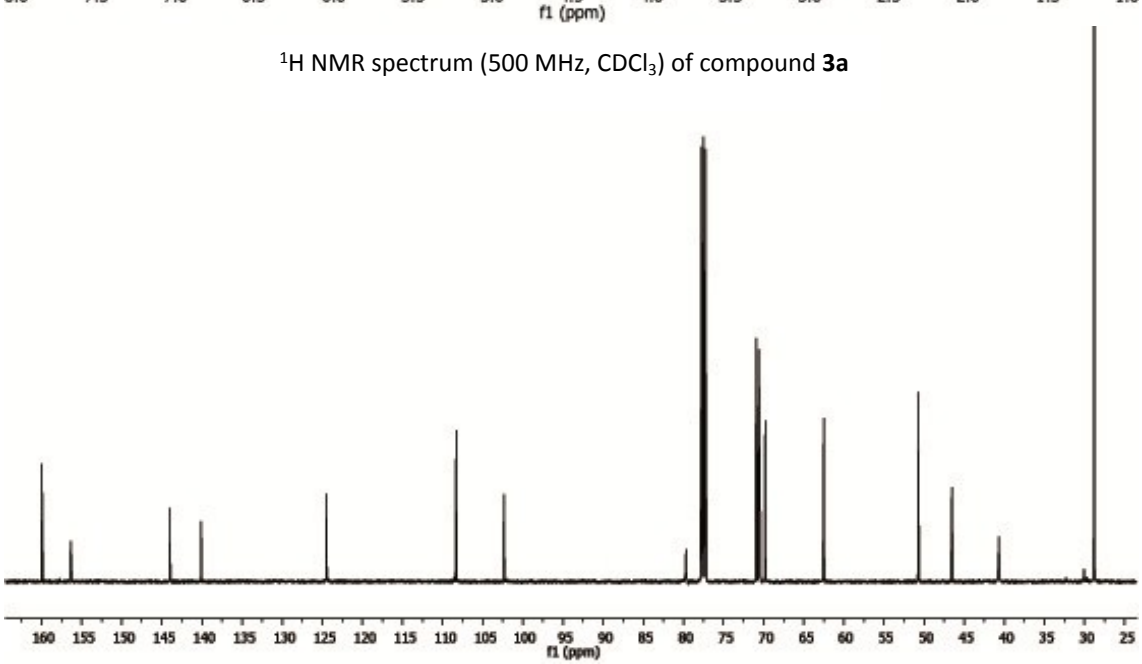
[2] Qin, T.; Li, X.; Chen, J.; Zeng, Y.; Yu, T.; Yang, G.; Li, Y. *Chem. Asian J.* **2014***, 9*, 3641-3649.

[3] Itoh, Y.; Ishikawa, M.; Kitaguchi, R.; Sato, S.; Naito, M.; Hashimoto, Y. *Bioorg. Med. Chem.* **2011***, 19*, 3229-3241.



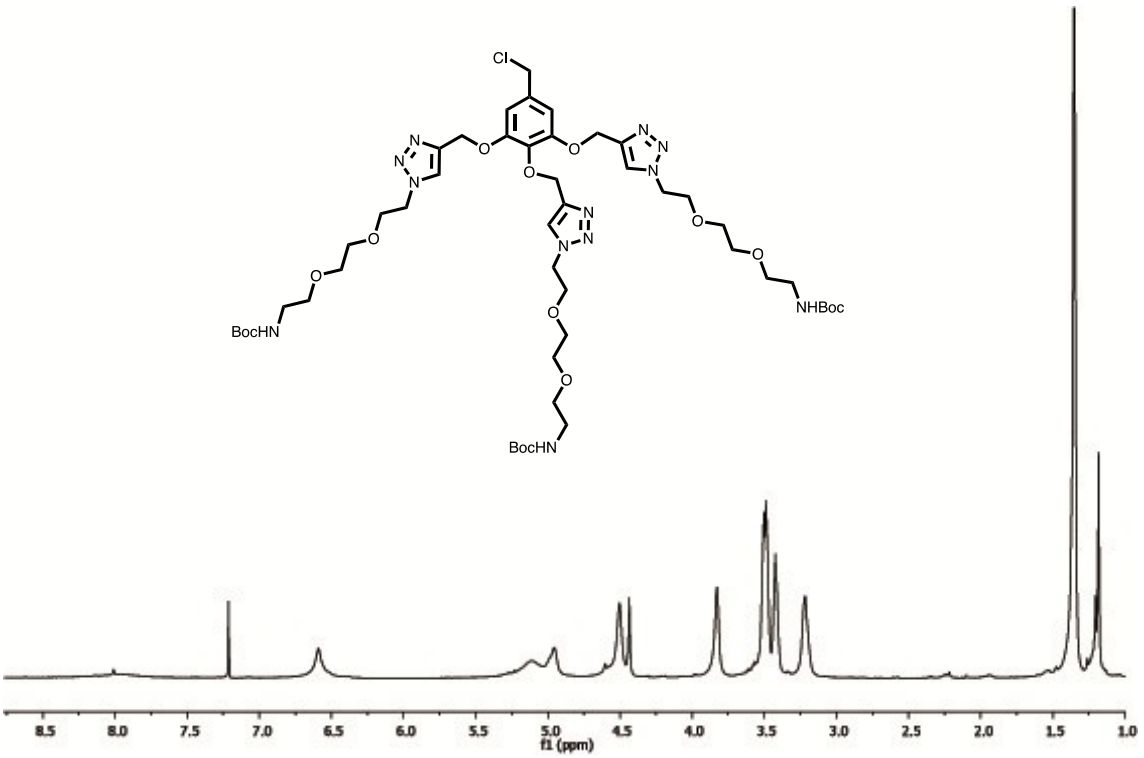


^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **3a**

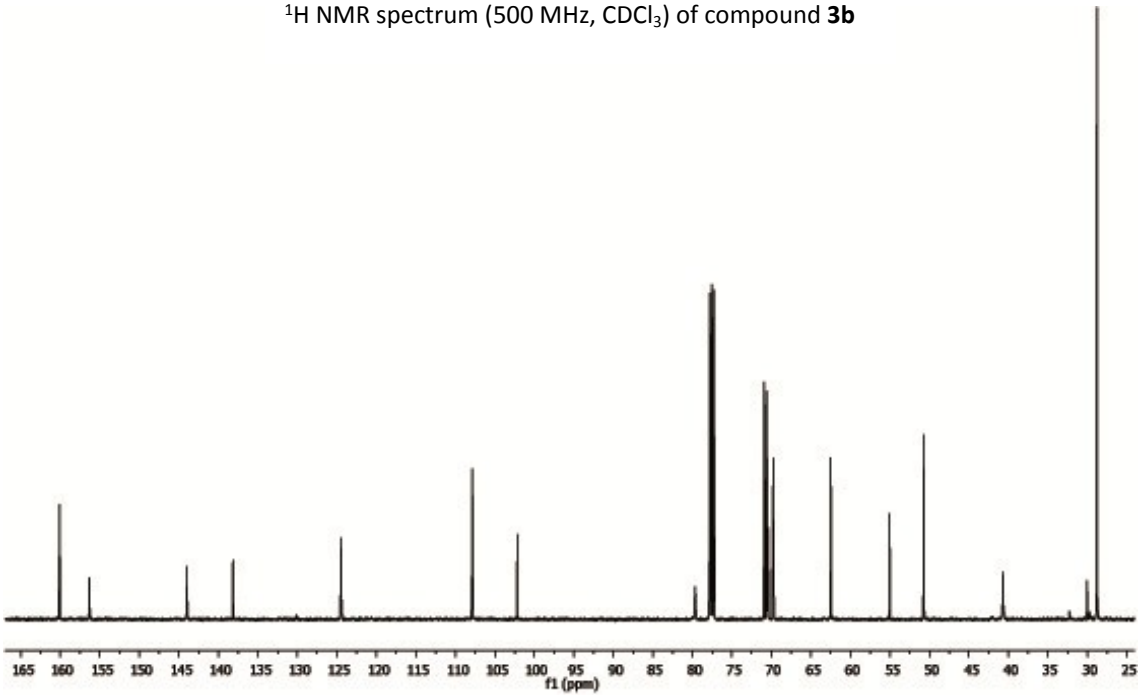


^{13}C NMR spectrum (125 MHz, $CDCl_3$) of compound **3a**

Compound 3b.

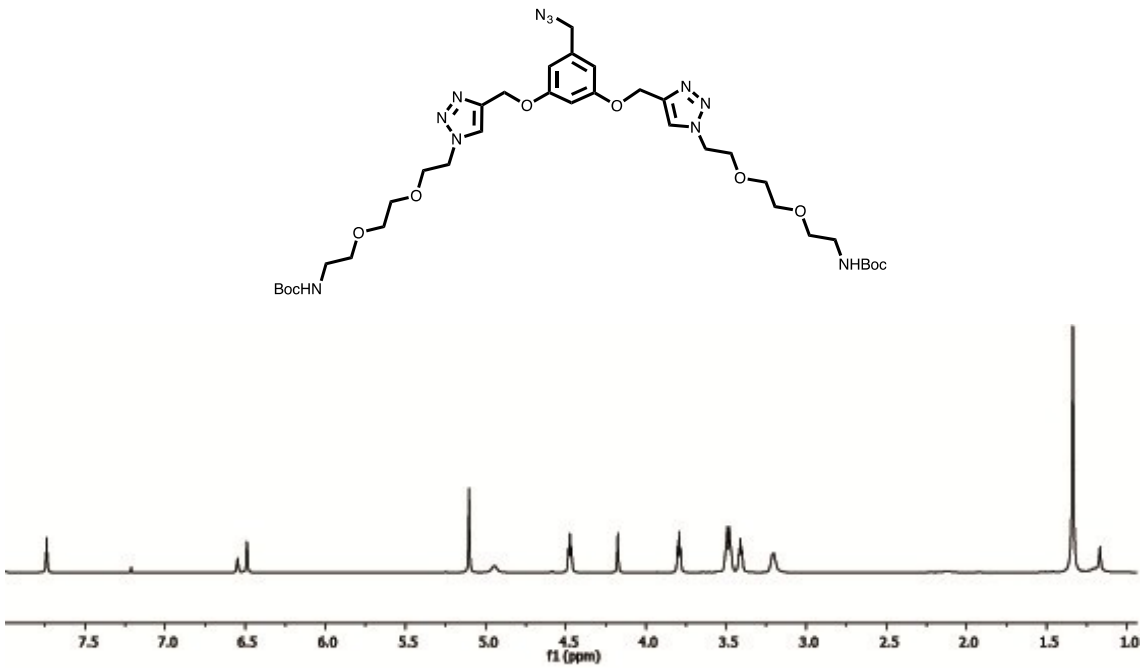


^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **3b**

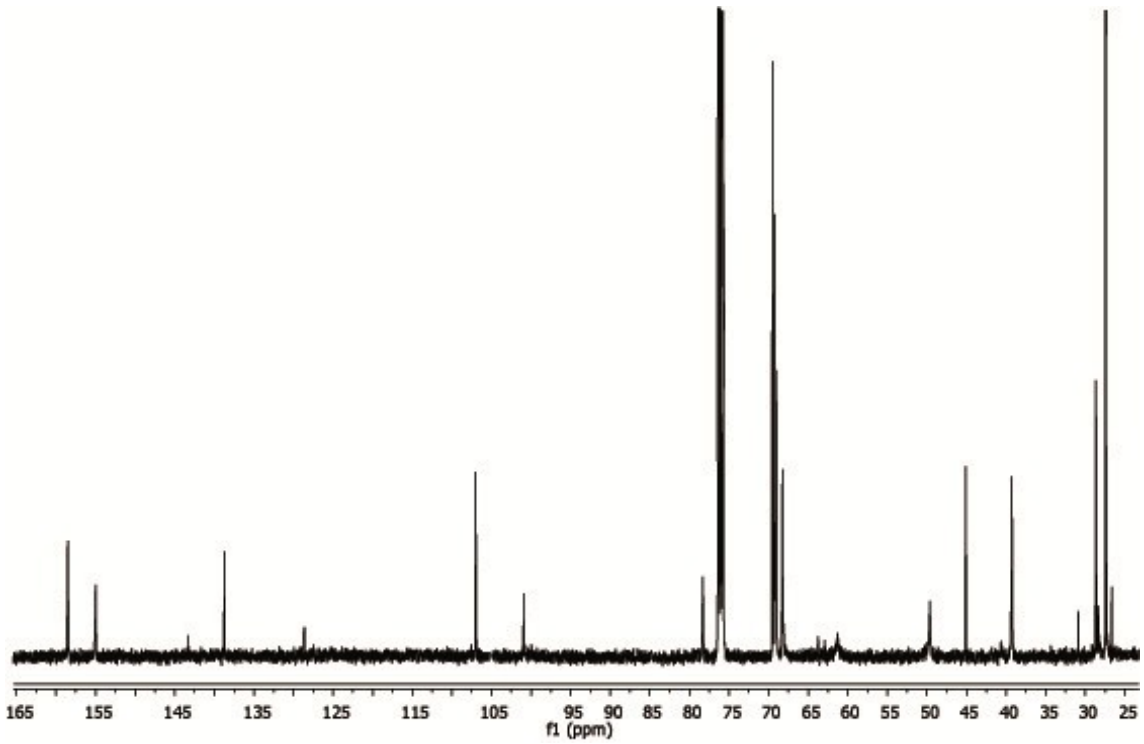


^{13}C NMR spectrum (125 MHz, $CDCl_3$) of compound **3b**

Compound 4a.

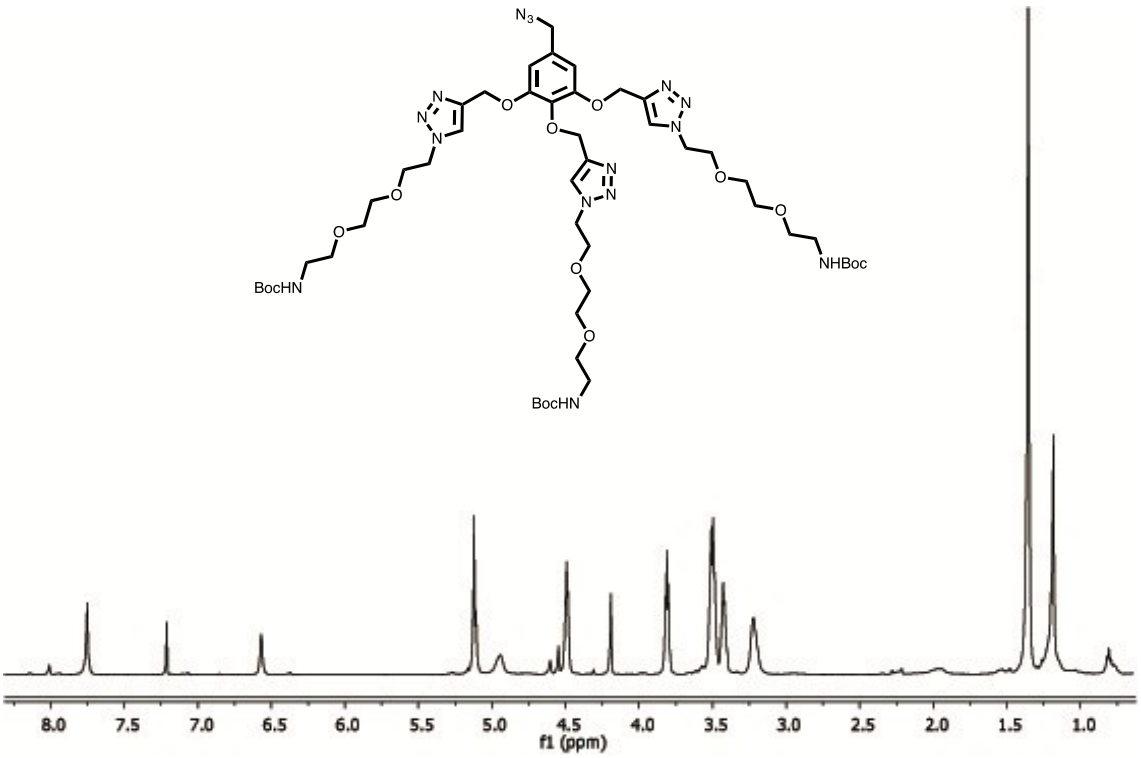


^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **4a**

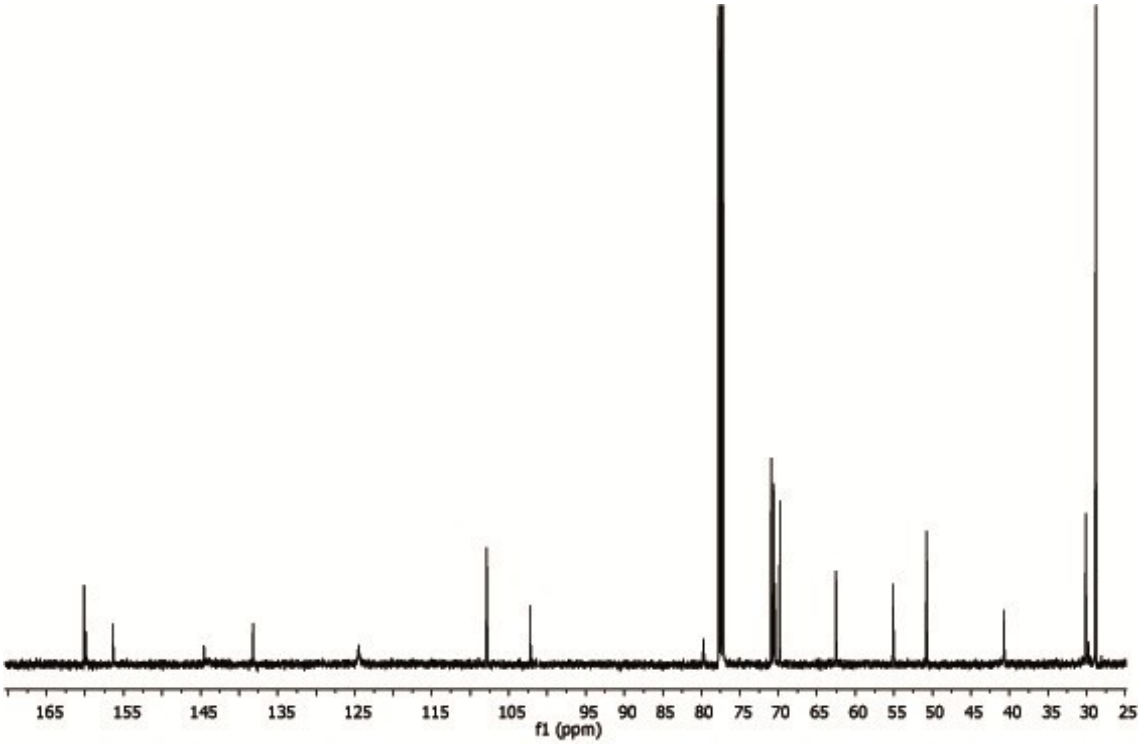


^{13}C NMR spectrum (125 MHz, $CDCl_3$) of compound **4a**

Compound 4b.

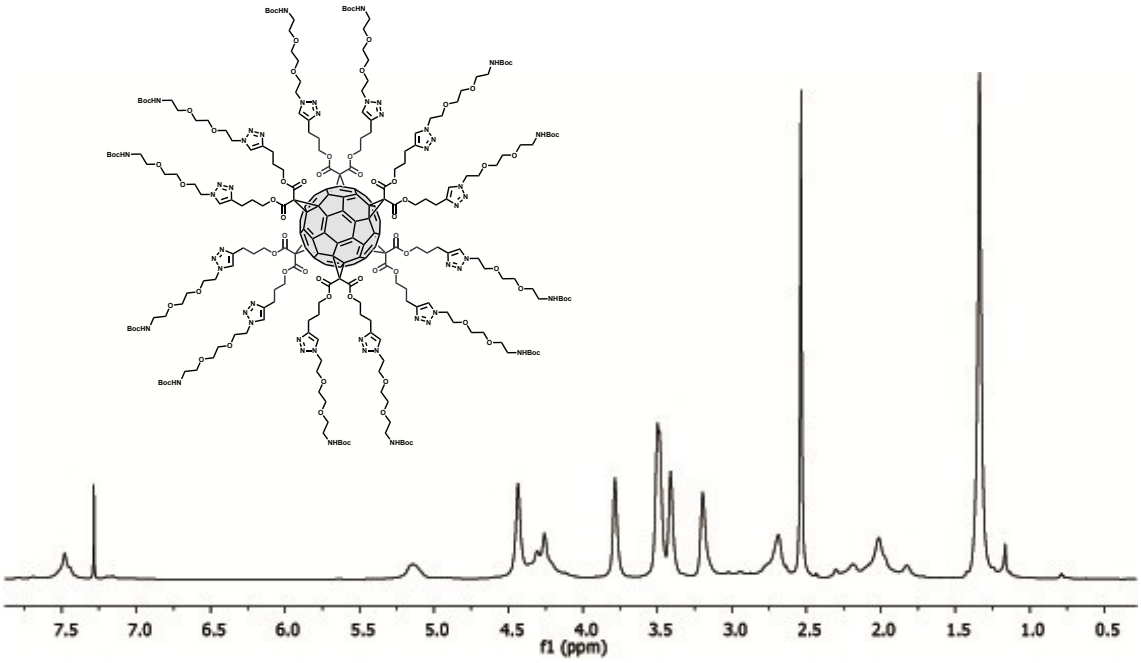


^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **4b**

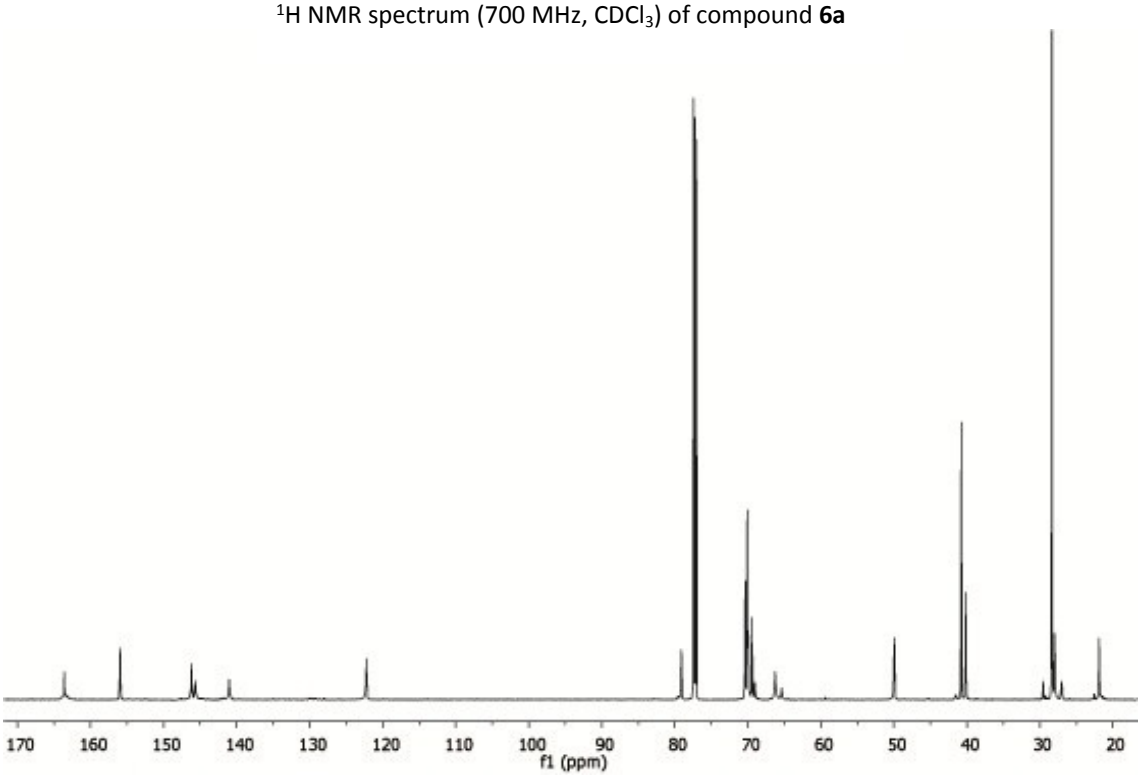


^{13}C NMR spectrum (125 MHz, $CDCl_3$) of compound **4b**

Compound 6a.

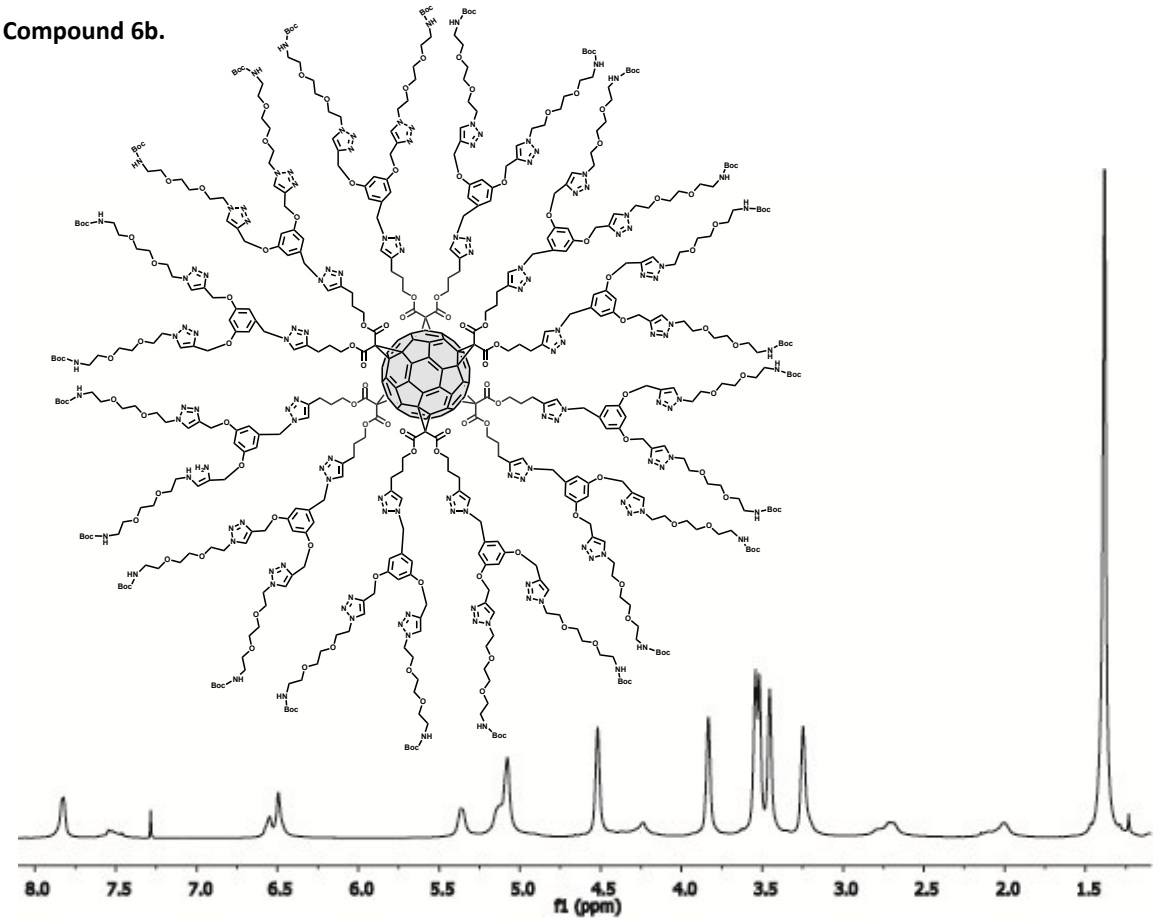


^{1}H NMR spectrum (700 MHz, $CDCl_3$) of compound **6a**

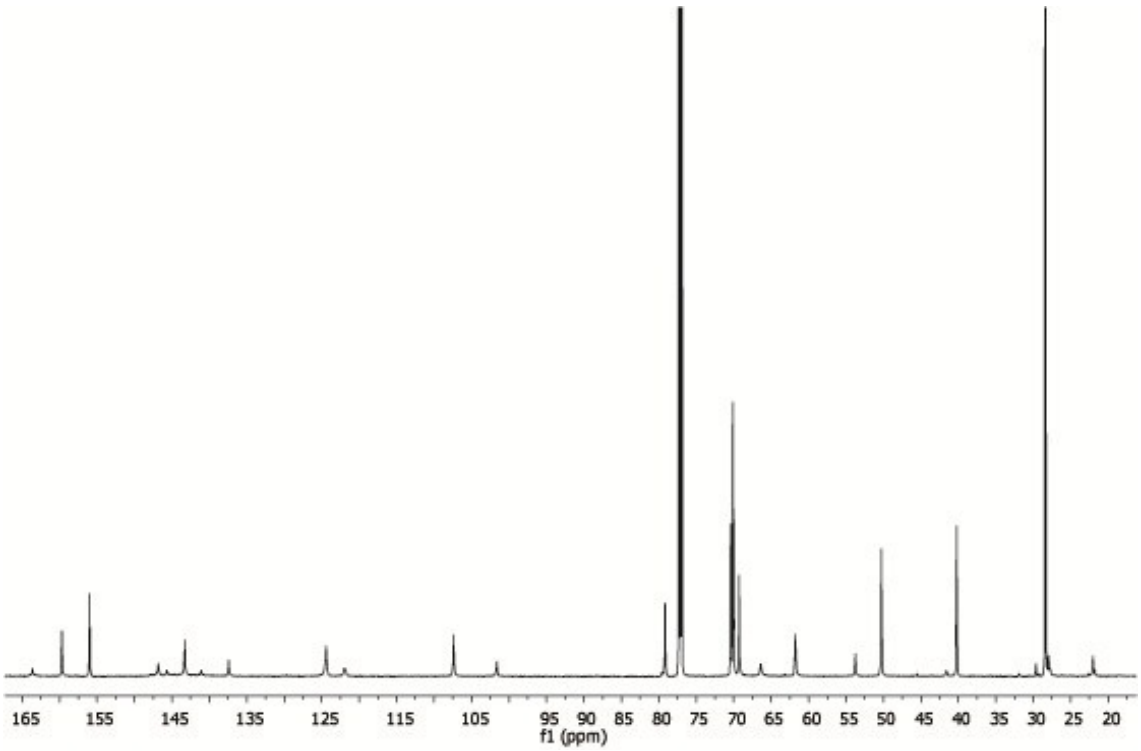


^{13}C NMR spectrum (175 MHz, $CDCl_3$) of compound **6a**

Compound 6b.

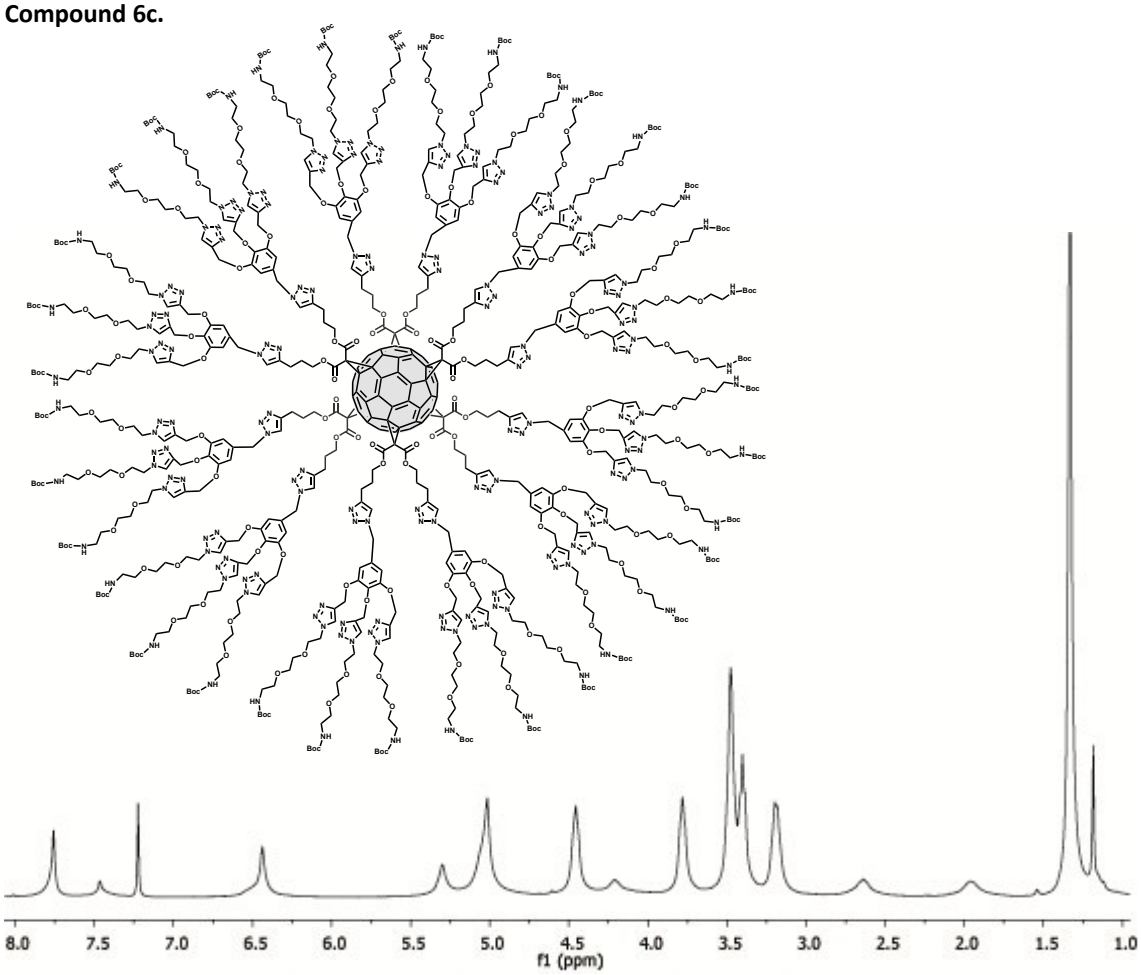


^{1}H NMR spectrum (700 MHz, $CDCl_3$) of compound **6b**

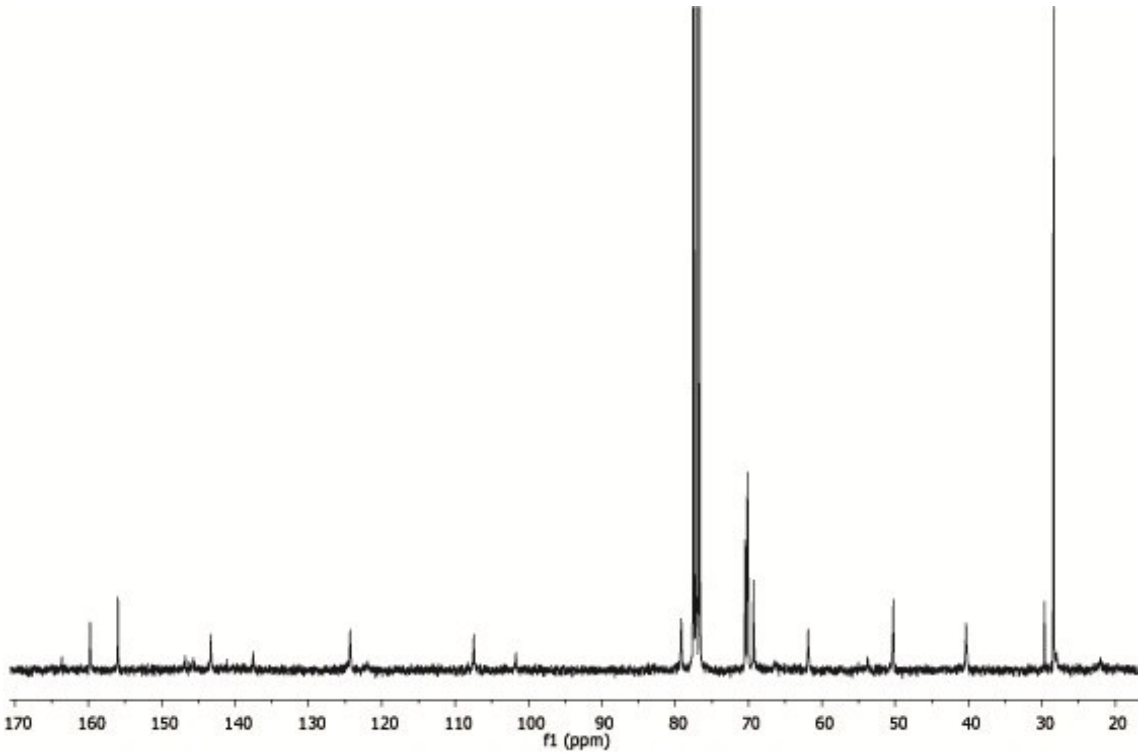


^{13}C NMR spectrum (175 MHz, $CDCl_3$) of compound **6b**

Compound 6c.

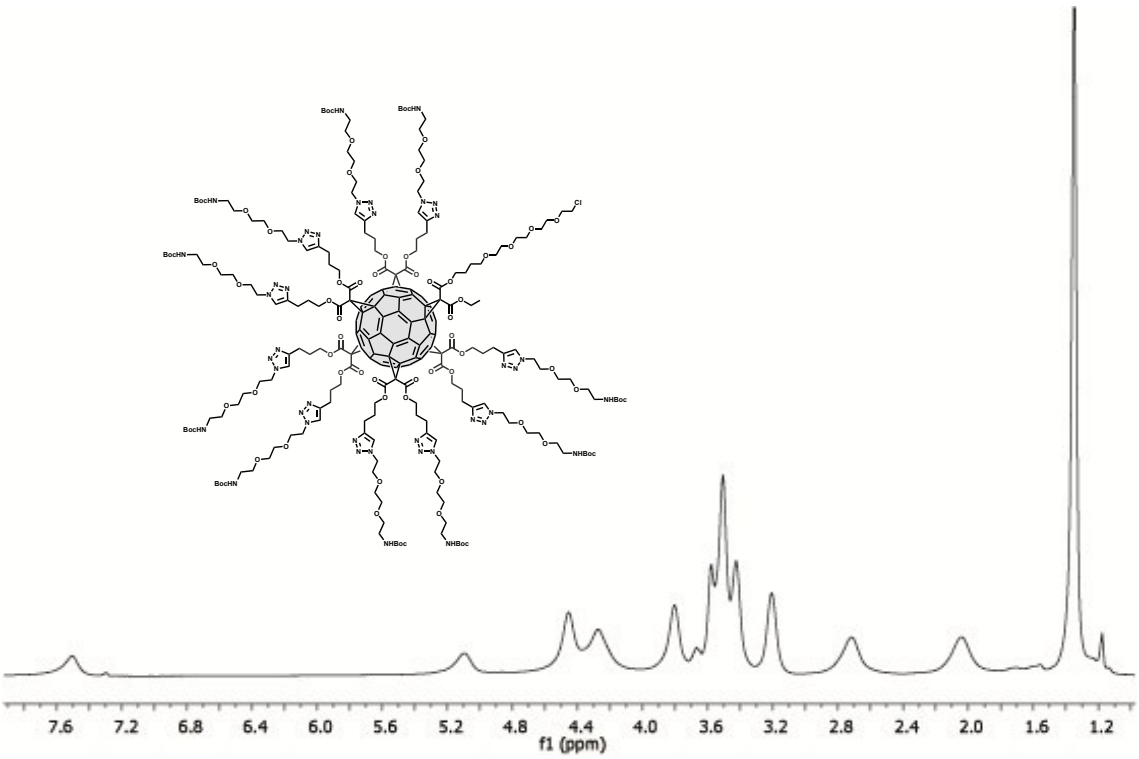


^{1}H NMR spectrum (700 MHz, $CDCl_3$) of compound **6c**

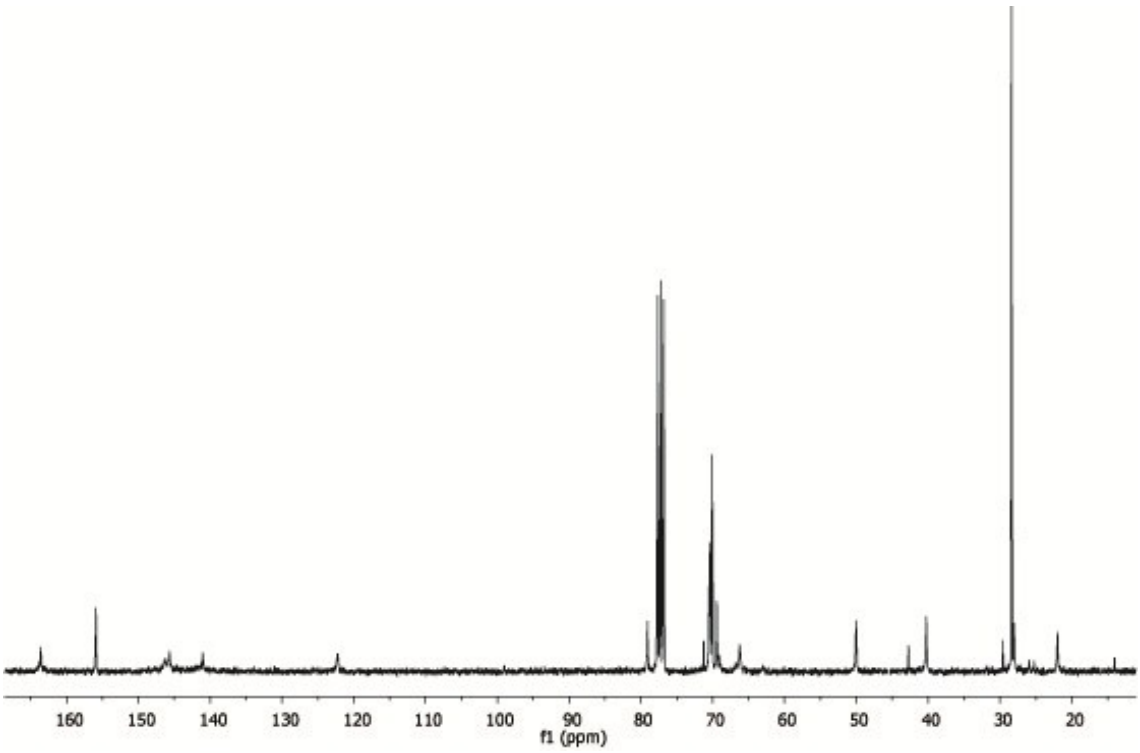


^{13}C NMR spectrum (175 MHz, $CDCl_3$) of compound **6c**

Compound 9.

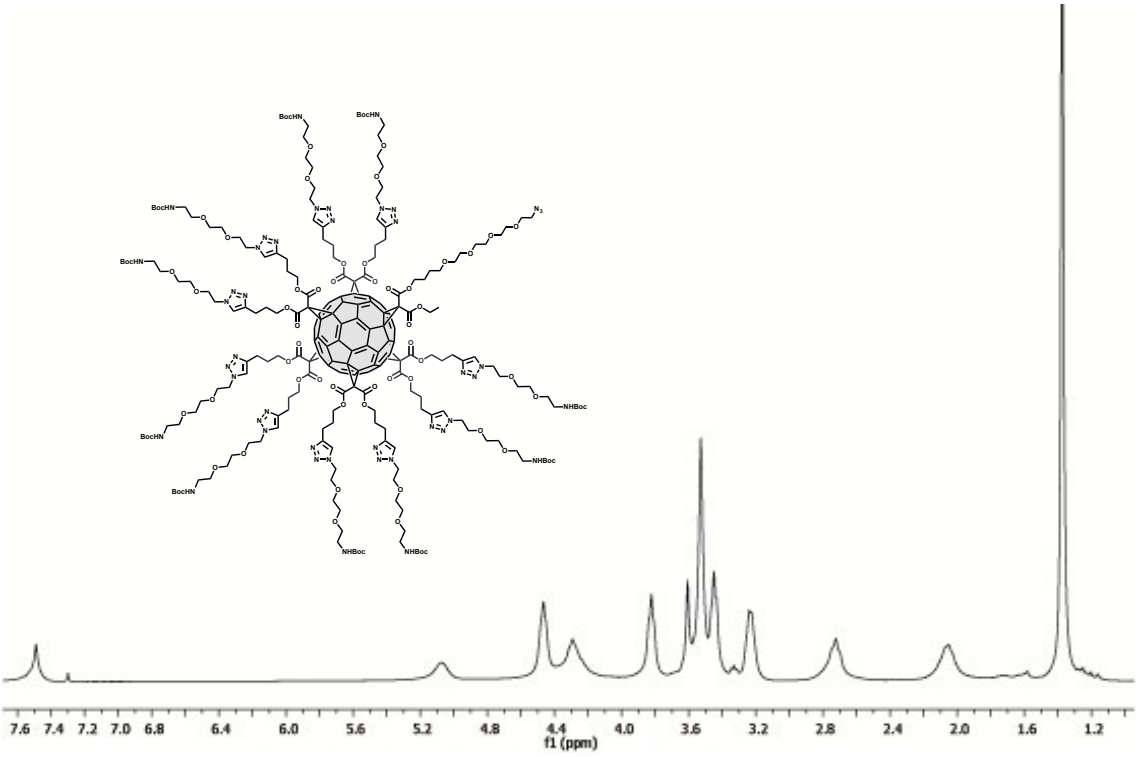


^{1}H NMR spectrum (700 MHz, $CDCl_3$) of compound **9**

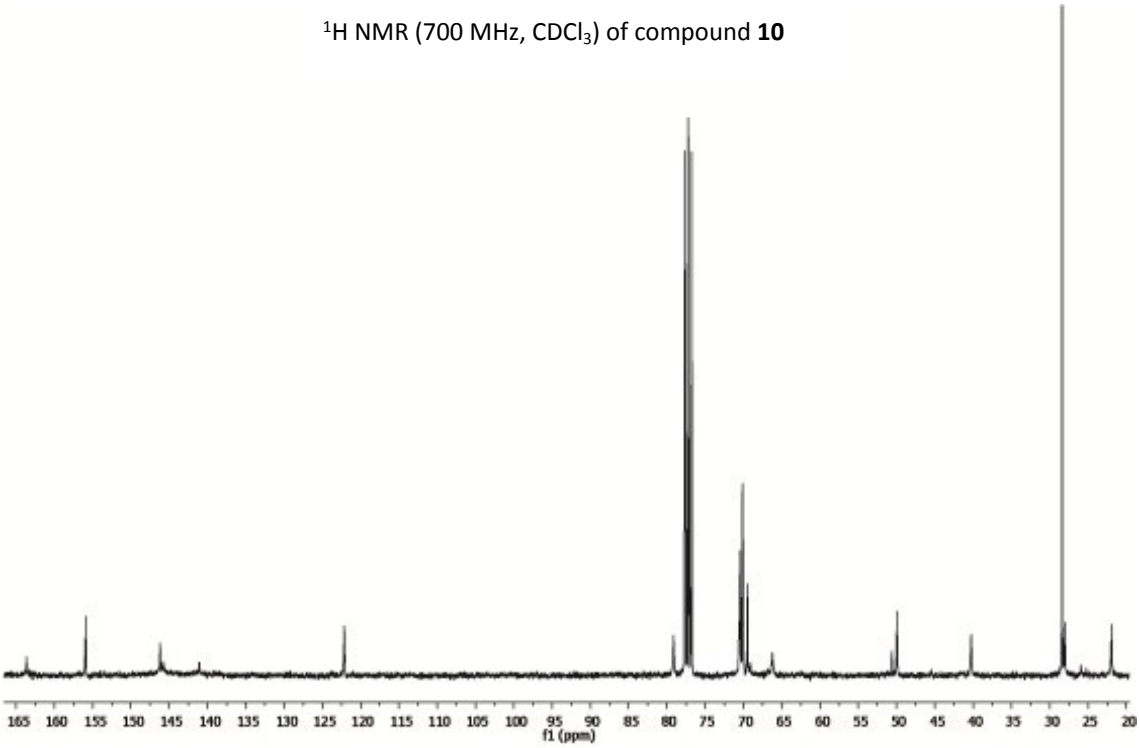


^{13}C NMR spectrum (175 MHz, $CDCl_3$) of compound **9**

Compound 10.

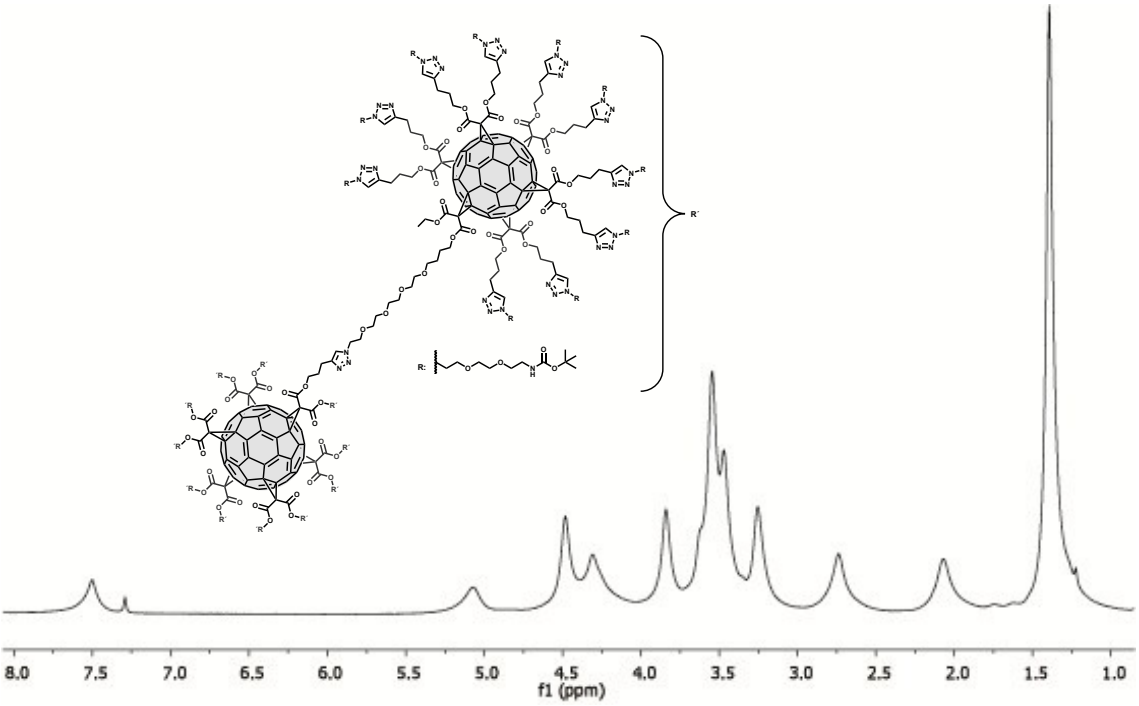


^{1}H NMR (700 MHz, $CDCl_3$) of compound **10**

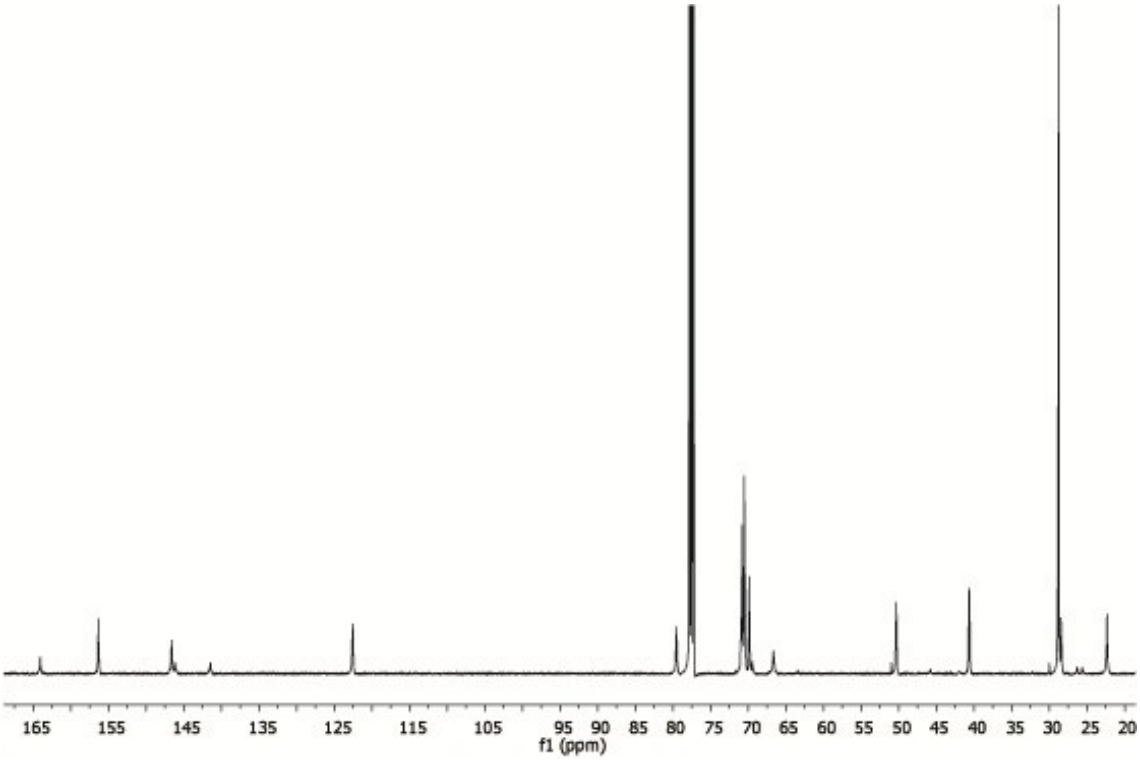


^{13}C NMR (175 MHz, $CDCl_3$) of compound **10**

Compound 11.



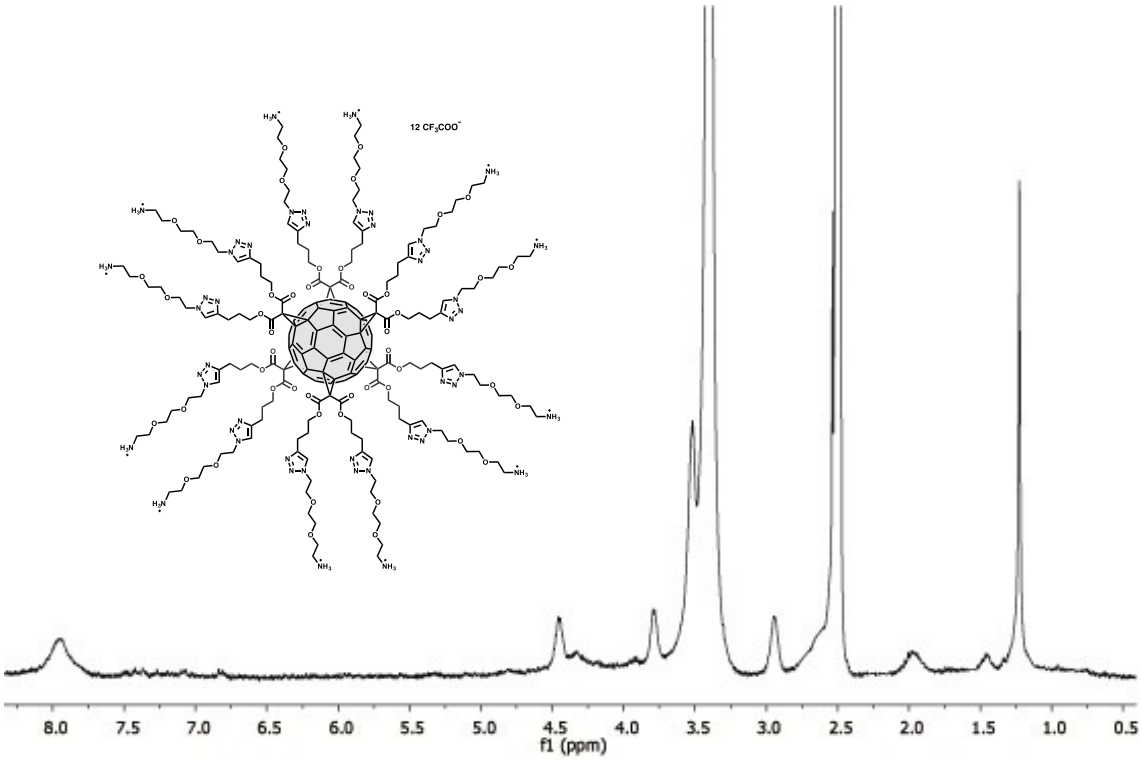
^{1}H NMR (700 MHz, $CDCl_3$) of compound **11**



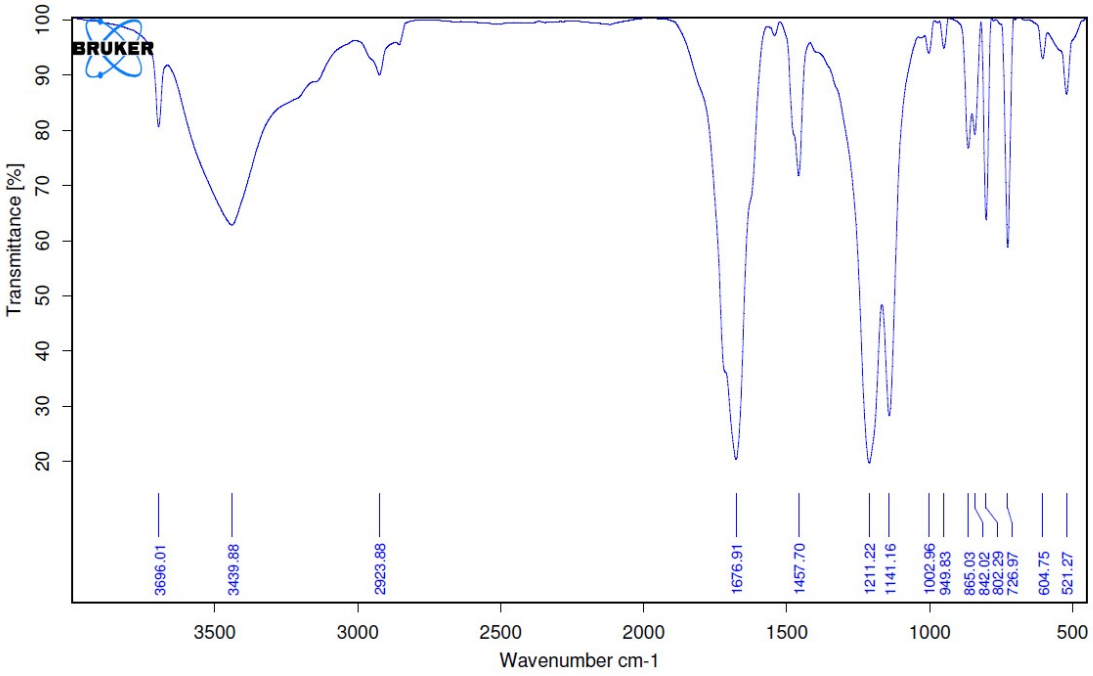
^{13}C NMR (175 MHz, $CDCl_3$) of compound **11**

^{1}H NMR (top) and FTIR (bottom) spectra of compounds 7a-c and 12.

Compound 7a.

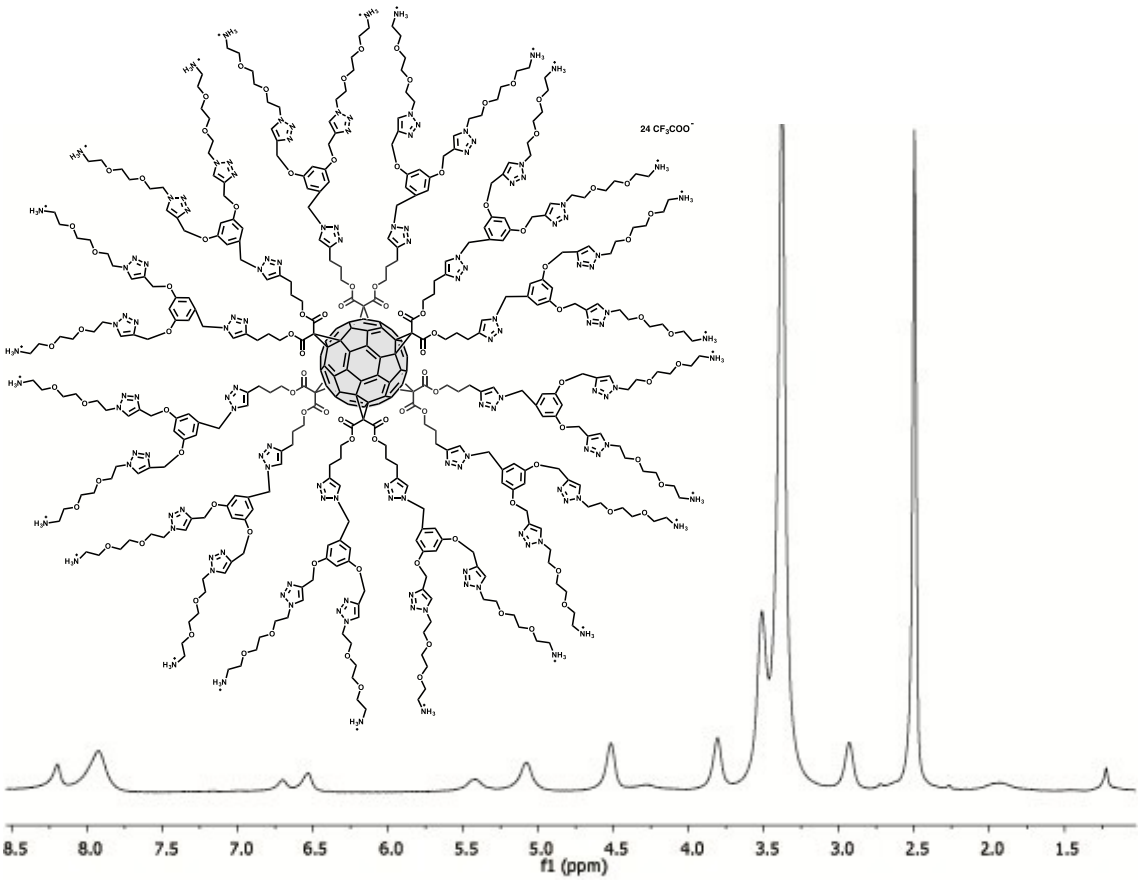


^{1}H NMR (300 MHz, DMSO-d^6) of compound **7a**

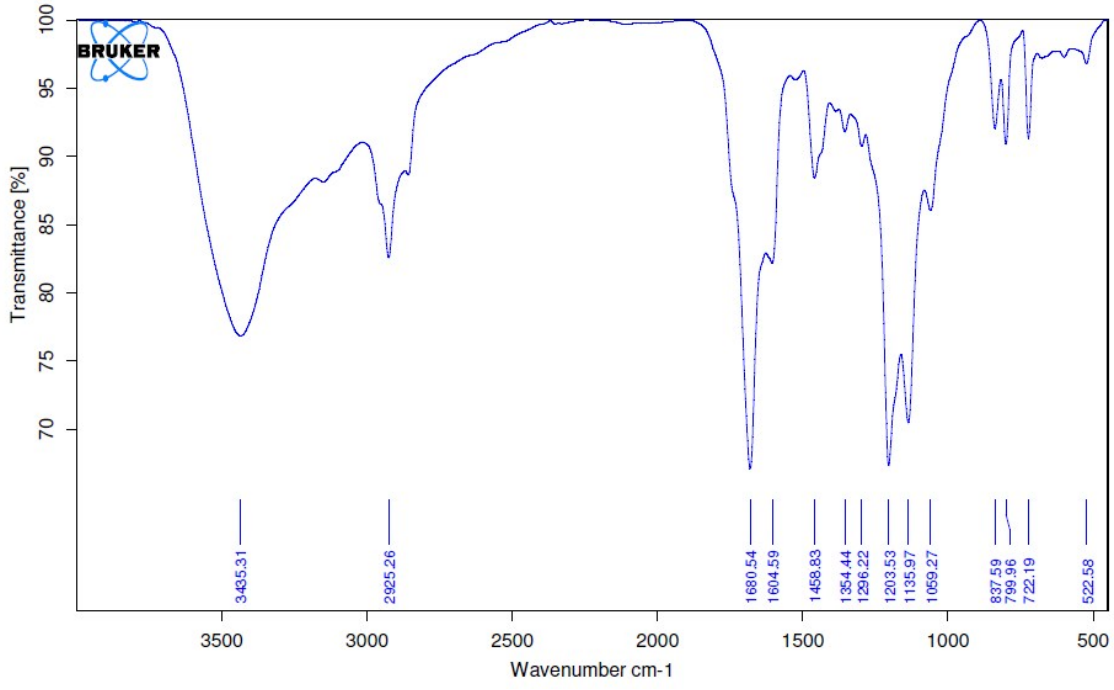


FTIR spectrum (KBr) of compound **7a**

Compound 7b.

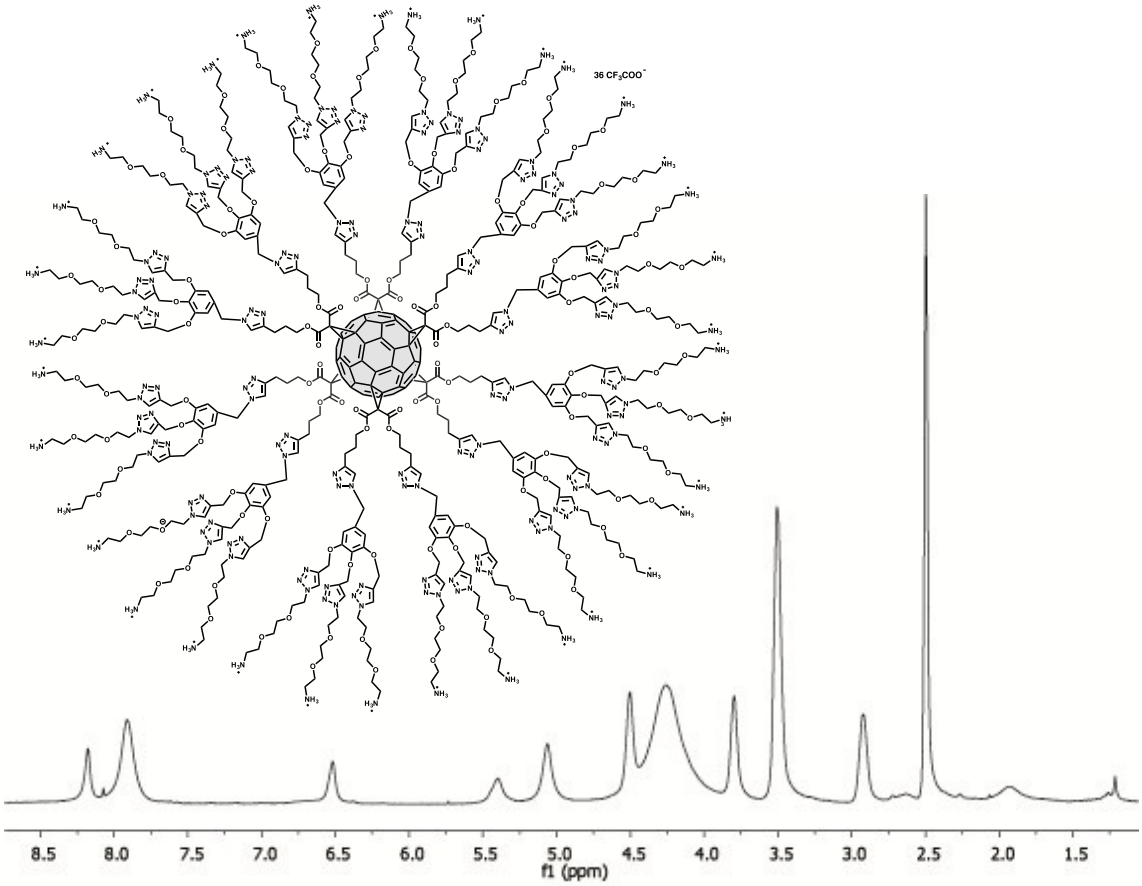


^{1}H RMN (300 MHz, DMSO-d^{6}) of compound **7b**

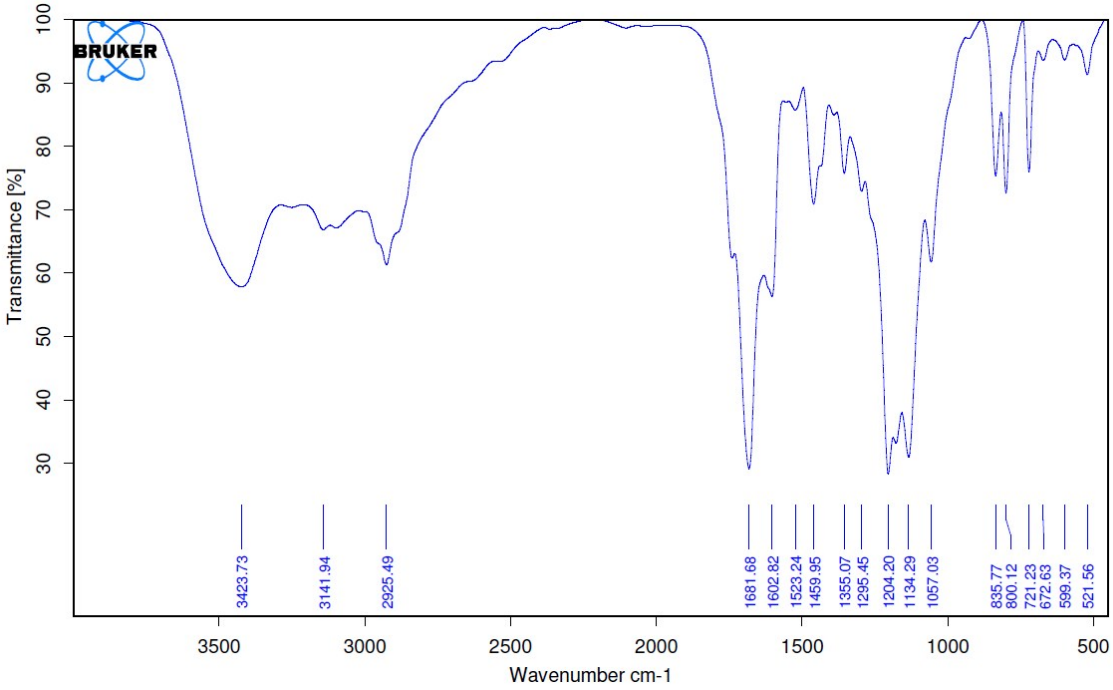


FTIR spectrum (KBr) of compound **7b**

Compound 7c.

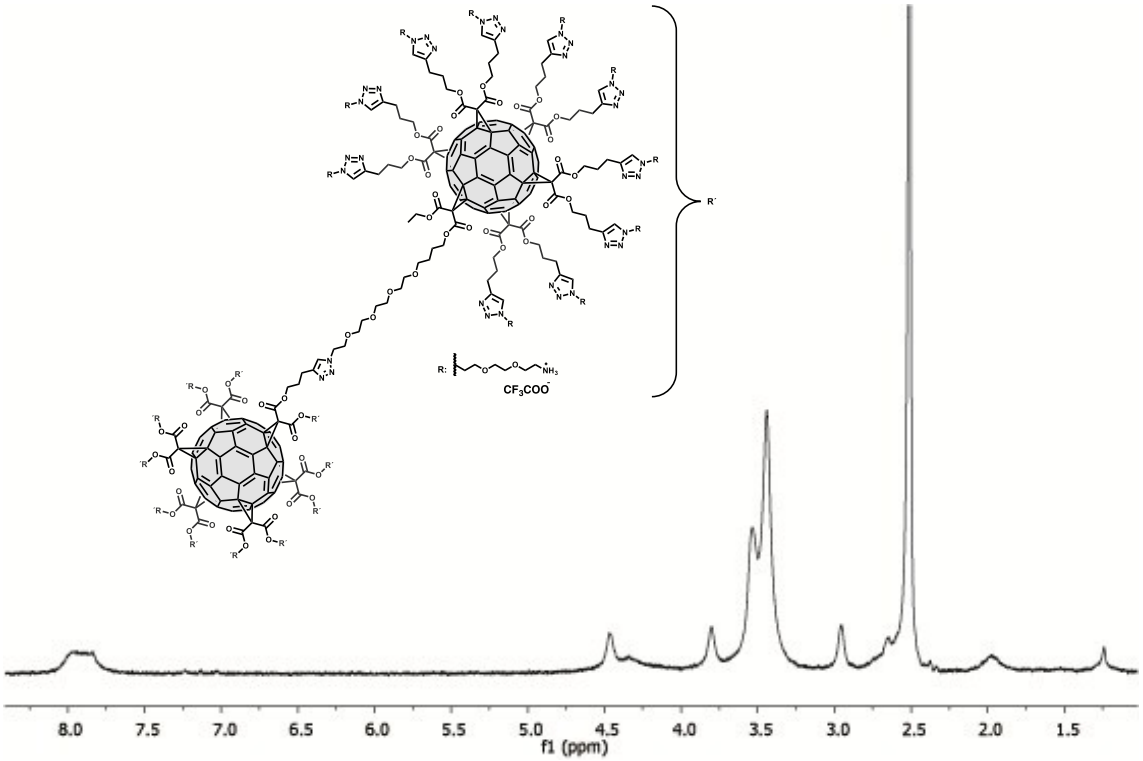


^{1}H NMR spectrum (300 MHz, DMSO-d^6) of compound **7c**

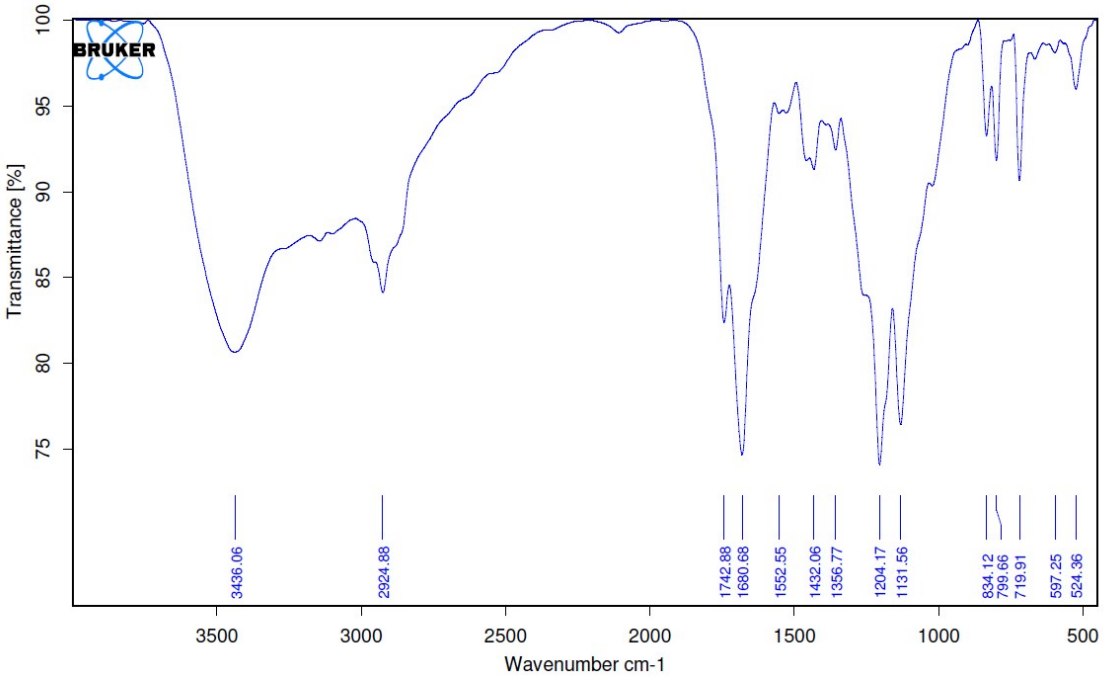


FTIR spectrum (KBr) of compound **7c**

Compound 12.



^{1}H NMR (300 MHz, DMSO-d^{6}) of compound **12**



FTIR spectrum (KBr) of compound **12**

Determination of the N/P ratios

An average molecular mass of 618 g/mol was considered for each A:T or G:C pair. The EGFP-C1 plasmid has 4700 base pairs and a molecular mass of 2904600 g/mol. Each phosphate group provides one negative charge. That means that each plasmid molecule has 9400 negative charges. In the assays we add 1 µg of plasmid, which in equivalent to $3.44 \cdot 10^{-13}$ mol. Considering the Avogadro number we can calculate 2.074 10^{11} plasmid units per assay. In summary, for 1 µg of added plasmid we have 1.95 10^{15} total negative charges. Considering the molecular masses of the fullerene compounds and the amount added, we could obtain the following N/P ratios:

Table S1. N/P Ratios calculated for the concentrations employed in the transfection experiments

	40 µM	***8 µM***	***1.6 µM***	***0.32 µM***	***0.064 µM***
7a	7.4	1.5	0.3	0.06	0.012
7b	15	3	0.6	0.12	0.025
7c	22.25	4.4	0.9	0.18	0.037
7d	74	15	3	0.6	0.12

Cytotoxicity assay

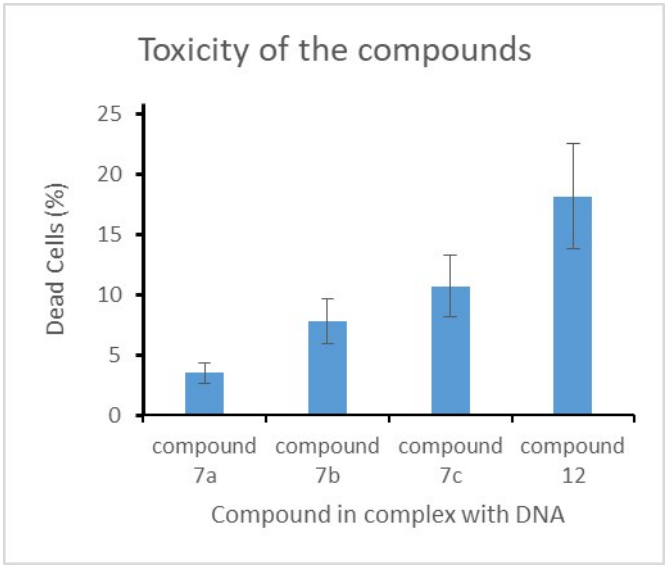


Figure S1. The cytotoxicity exerted by the various fullerene compounds when complexed to plasmidic DNA was determined 12 hours post-addition of the complexes. The amount of dead cells was counted in a field of approximately 1000 cells in total.

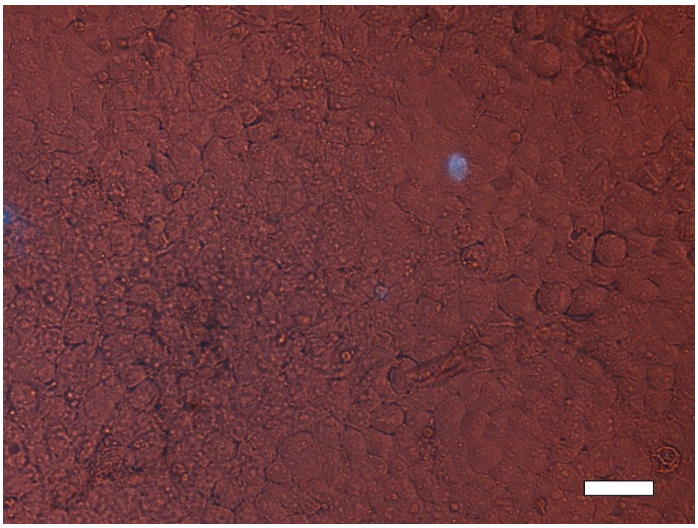


Figure S2. The figure shows an overlay of bright field and 405 nm excitation of confluent HEK293 cells incubated with compound **7a** complexed with plamidic DNA. Emission of blue fluorescence correspond to dead cells with fragmented membranes and the Hoechst dye labelling the cell nuclei. Bar, 50 µM.

DLS experiments

For compounds **7a-c** and **12**, Dynamic Light Scattering measurements were carried out at 25°C on an ALV GSC08 correlator working in a cross correlation mode with an Ar+ laser operating at λ = 514.5 nm. The output signals were obtained with backscatter detection at an angle of 90º and processed with a digital correlator that computed intensity-intensity autocorrelation of the scattered light. Measurements were made in a 1-cm path-length round quartz cell maintained at 298 K. Solution samples of 10^{-4} M in PBS buffer were filtered through nylon Acrodisc syringe filters (Pall Life Sciences) with 0.2-µm pore size.

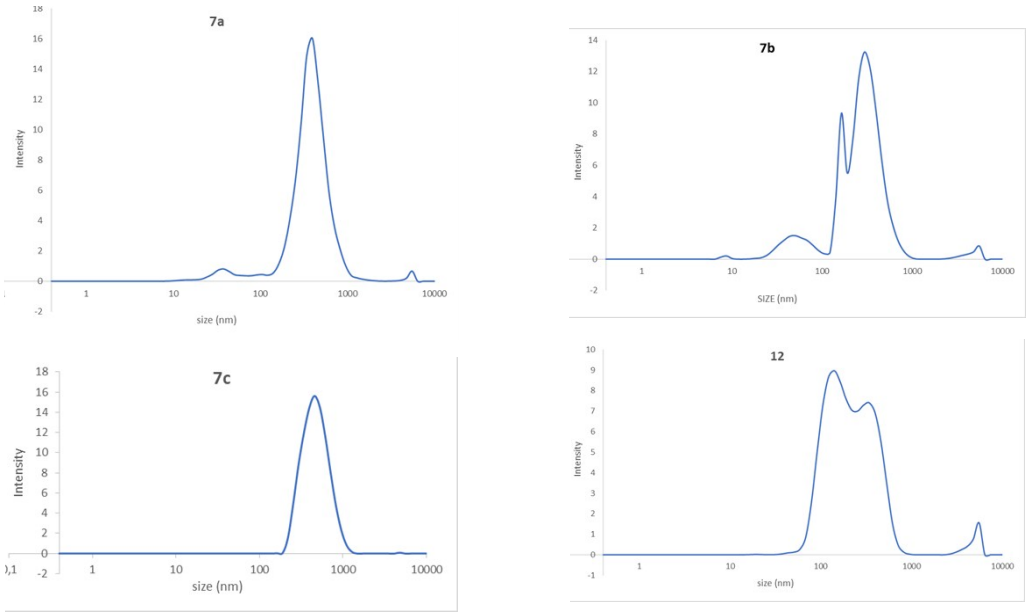


Figure S3. Representative DLS for **7a-c** and **12**, intensity vs. particle size distribution.