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Cyclotriphosphazene, a scaffold for ^{19}F MRI contrast agents

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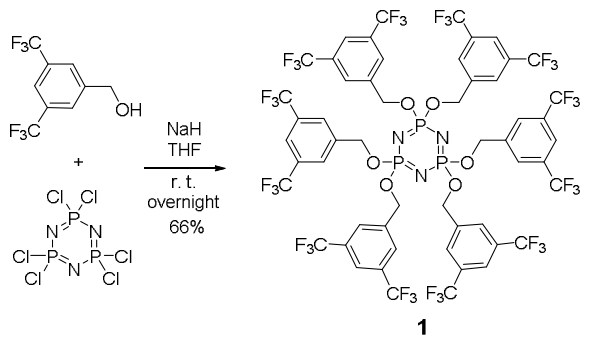
ABSTRACT

A cyclotriphosphazene substituted with six 3,5-bis(trifluoromethyl) benzyloxy units was designed as a novel ^{19}F MRI contrast agent. The resulting molecule has 36 magnetically equivalent fluorine atoms and exhibited suitable MRI properties with high imaging sensitivity, confirming the proof-of-concept as a convenient scaffold for the production of new ^{19}F MRI contrasts agents.

A favourable treatment outcome is dependent on the efficacy of the treatment as well as its early detection, rendering imaging technologies crucial. Magnetic resonance imaging (MRI) is amongst the most widely used imaging techniques, and a number of gadolinium paramagnetic complexes are available as contrast agents.[1] Nonetheless, their side effects and nephrotoxicity have raised considerable concerns.[2] Using ^{19}F atoms as MRI contrast agents offers significant advantages: its 100% natural abundance, its absence in biological media which avoids confusion with other possible signal sources, and its large gyromagnetic ratio which ensures good sensitivity.[3-4]

An important consideration in the design of ^{19}F MRI agents is the need for a single NMR/MRI signal, hence the ^{19}F atoms must be magnetically equivalent. The design of ^{19}F-based contrast agents therefore often includes the use of trifluoromethyl groups. Grafting as many CF_3 groups as possible on a polymeric scaffold, possibly dendrimeric with pseudo-equivalent ^{19}F atoms, is one of the preferred options.[5] Small non-toxic molecules used at higher concentrations, such as perfluoro-18-crown-6-ether[6a] and Perfecta which has 36 equivalent ^{19}F atoms, have also been developed.[6b] The flexibility of the trifluoromethyl group has proved to play a role in ensuring an intense MRI signal.[7] On the other hand, cyclophosphazenes have often been used as dendrimeric cores or as scaffolds for polyfunctionalization, and are biocompatible.[8-9] The convenient insertion of substituents by nucleophilic substitution, usually high-yielding, is another advantage of this molecular basis.

All these parameters have been taken into account to design ^{19}F MRI contrast agents using a cyclotriphosphazene scaffold. A commercially available benzyl alcohol bearing two equivalent trifluoromethyl moieties was selected, relying on the flexibility afforded by the benzylic methylene compared to a phenol that would be, conversely, more reactive. The chlorine nucleophilic substitution reaction performed in tetrahydrofuran using sodium hydride as the base gave **1** in excellent yield (Scheme 1) and chromatographic purification was facile thanks to the difference in polarity of **1** and the benzylic alcohol. Reproduction of the reaction and simply washing the crude precipitate also allowed the isolation of **1** with a satisfactory purity (>90%), which represents a good starting point for future up-scaled synthesis.



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Scheme 1. Preparation of **1**.

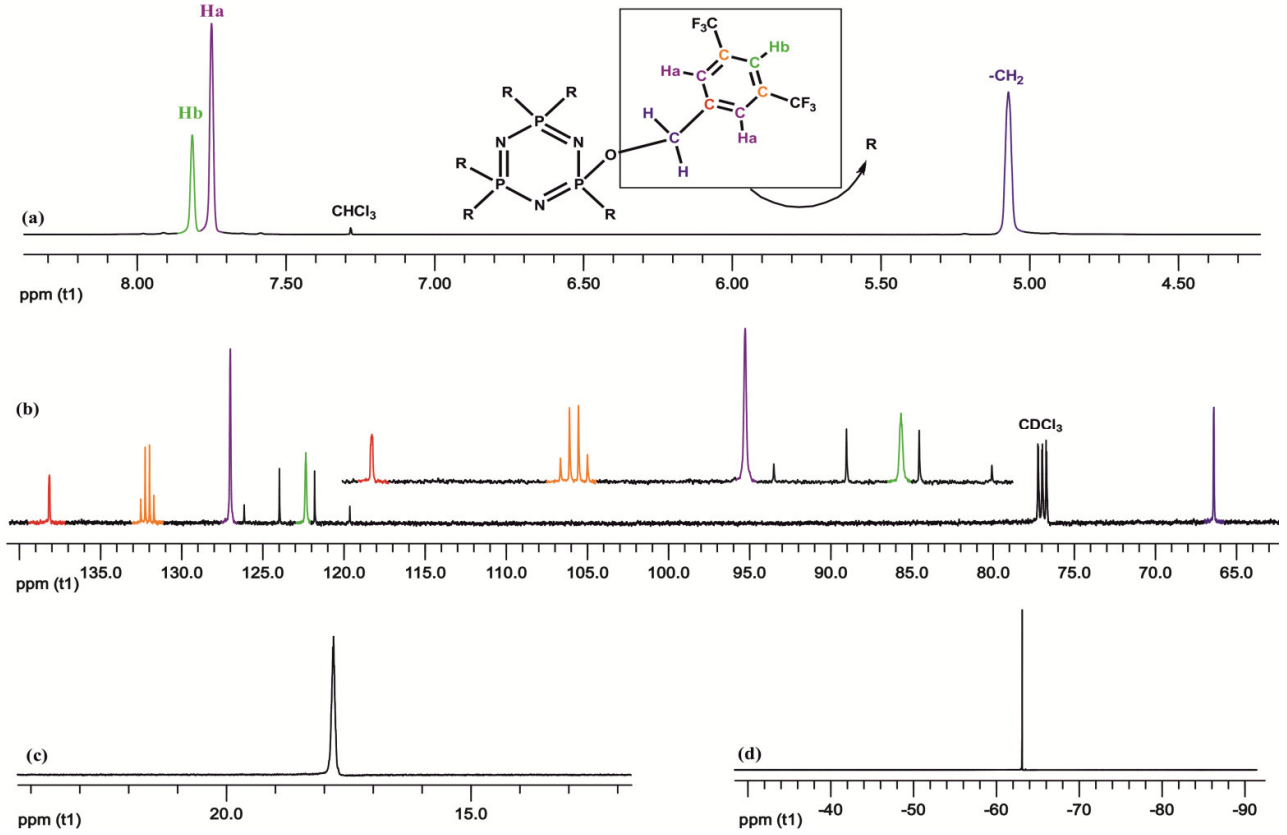

Figure 1. ^{1}H (a), ^{13}C (b), ^{31}P (c) and ^{19}F (d) NMR spectra of **1** in $CDCl_3$.

Exhaustive NMR characterization of **1** was performed (Fig. 1 and ESI). All peaks in the ^{1}H decoupled ^{13}C NMR spectrum were assigned using HSQC experiments (ESI, Fig. S5), which was particularly useful to discriminate *C*Ha and *C*Hb_2 (Fig. 1). The ^{1}J coupling constant between the carbon and fluorine atoms of the CF_3 groups had a value of 272.7 Hz, while the ^{2}J coupling (*C*-C*F*$_3$) had a value of 33.7 Hz. ^{19}F NMR spectroscopy confirmed the magnetic equivalence of the 36 ^{19}F atoms. Similarly, and as can be also expected, all ^{31}P atoms were equivalent.

Single crystals of compound **1** were obtained from the slow diffusion of a CH_2Cl_2/hexane mixture and the solid-state structure was solved and refined using single-crystal X-ray diffraction data (Fig. 2). Compound **1** crystallizes in the orthorhombic system and the non-centrosymmetrical $P2_12_12$ space group (Flack parameter refined to the value 0.11(17)) was retained. Data collection conditions as well as refinement results are presented in the ESI (Table S1). Selected bond lengths and angles are reported in Tables S2 and S3, respectively. The P-N (from 1.574(4) Å to 1.585(4) Å) and P-O (from 1.569(3) Å) to 1.580(3) Å) bond lengths are in good agreement with the previous structure of this type of cyclotriphosphazene, as well as the P–N–P (122.7(3)°) and N–P–N (117.2(2)° and (118.2(3°) angles.[10-11] All lengths for C-O bonds (from 1.450(5) Å to 1.460(6) Å) involving the first C atom of the phenyl ring and the O atom attached to the P atom of the cyclotriphosphazene ring are in the normal ranges. The 3-D network of **1** is built from C-F···F-C interactions between neighboring molecules and lead to planes perpendicular to the [100] direction of the unit-cell (Fig. 2).

To test the ^{19}F MRI properties of compound **1**, ^{19}F MRI measurements of solutions at varying concentrations of **1** in $CHCl_3$ were conducted at a field strength of 9.4 T. As illustrated in Figure 3a, ^{1}H RARE (rapid acquisition with relaxation enhancement) images are displayed (top) to illustrate the location of the NMR tubes within the resonator. All of the samples with ^{19}F concentrations ranging from 3 to 100 mM could be detected successfully by ^{19}F MRI as shown in the ^{1}H MRI, indicating the

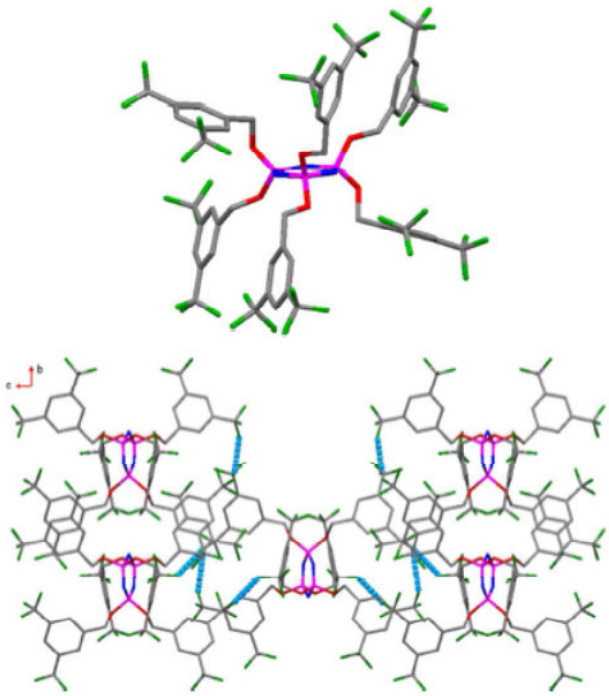

Figure 2. Top: Crystal structure of **1**. Hydrogen atoms have been omitted for clarity. Bottom: Packing views of compound **1** along the (b,c)-plane. F···F interaction are represented with light-blue dashed lines.

high sensitivity of compound **1** as a ^{19}F MRI contrast agent. Moreover, the ^{19}F MRI signal-noise-ratio (SNR) was calculated for illustrating the quantitative nature of the ^{19}F MRI experiments. As shown in Figure 3b, a good linear relationship of ^{19}F MRI SNR to the concentration of sample **1** can be observed (R^2 = 0.982). These observations all indicate that compound **1** is a promising quantitative ^{19}F MRI contrast agent with high imaging sensitivity due to the numerous chemically equivalent fluorine atoms in one molecule (Scheme 1). In the meantime, the ^{19}F NMR T_1 and T_2 relaxation times of **1** were monitored at these imaging conditions (Table S4). It is noteworthy that the T_2/T_1 ratio is above 0.9 for compound **1** at all testing conditions, highlighting the good ^{19}F MR imaging sensitivity.[12] The ^{19}F T_1 of **1** is significantly shorter than the majority of fluorinated small compounds, such as trifluoroethanol (TFE), under same conditions (~1500 vs. ~2400 ms).[13] From a sensitivity point of view, a shorter T_1 allows for a greater number of scans to be obtained in an equivalent time frame and thus better sensitivity.

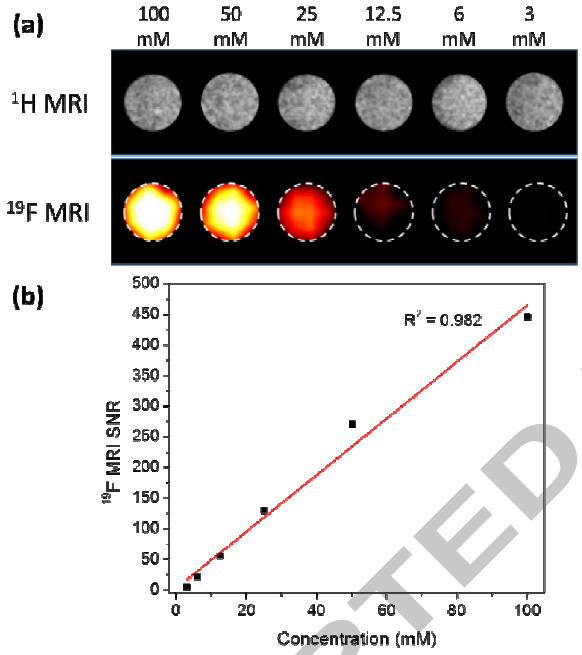

Figure 3. (a) ^{19}F MRI images of solutions of **1** ($CHCl_3$) at various concentrations. (b) Signal-to-noise ratio of **1** increases linearly with respect to concentration of **1**. Field strength : 9.4 T.

To conclude, these proof-of-concept experiments demonstrate for the first time that cyclophosphazene is an excellent scaffold for the construction of ^{19}F MRI contrast agents. The next steps will be formulation studies, chosen to minimize the issues of entrapment and compartmentalisation, for *in vitro* then *in vivo* investigations. Improvement of the relaxation time by conjugation to metal complexes, which was previously proved[14] to considerably optimize the relaxation rate, will also be explored.

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A. Supplementary Data

Supplementary data associated with this article (synthetic and characterization details, spectra, crystallographic data and MRI methods) can be found online at doi…..

References and notes

1. Zhang, L.; Liu, R.; Peng, H.; Li, P.; Xu Z.; Whittaker, A. K. *Nanoscale,* **2016**, *8*, 10491-10510.
2. Rogosnitzky, M.; Branch, S. *Biometals*, **2016**, *29*, 365-376.
3. Zhang, C.; Moonshi, S. S.; Han, Y.; Puttick, S.; Peng, H.; Magoling, B. J. A.; Reid, J. C.; Bernardi, S.; Searles, D. J.; Král P.; Whittaker, A. K. *Macromolecules* **2017**, *50*, 5953-5963.
4. Zhao, W.; Ta, H. T.; Zhang C.; Whittaker, A. K. *Biomacromolecules* **2017,** *18*, 1145-1156.
5. Yu, W.; Yang, Y.; Bo, S.; Li, Y.; Chen, S.; Yang, Z.; Zheng, X.; Jiang Z.-X.; Zhou, X. *J. Org. Chem.* **2015**, *80*, 4443-4449.
6. a) Lin, W.-H.; Bailey, Jr., W. I.; Lagow, R. J. *J. Chem. Soc., Chem. Commun*., 1985, 1350-1352 ; b) Tirotta, I.; Mastropietro, A.; Cordiglieri, C.; Gazzera, L.; Baggi, F.; Baselli, G.; Bruzzone, M. G.; Zucca, I.; Cavallo, G.; Terraneo, G.; Baldelli Bombelli, F.; Metrangolo P.; Resnati, G. *J. Am. Chem. Soc.* **2014**, *136*, 8524-8527.
7. a) Tirotta, I.; Dichiarante, V.; Pigliacelli, C.; Cavallo, G.; Terraneo, G.; Baldelli Bombelli, F.; Metrangolo, P.; Resnati G. *Chem. Rev.* **2015**, *115*, 1106–1129; b) Flogel, U.; Ahrens, E. *Fluorine Magnetic Resonance Imaging*, **2016**, Pan Stanford; c) Fu, C.; Herbst, S.; Zhang C.; Whittaker, A. K. *Polym. Chem*., **2017**, *8*, 4585-4595.
8. Andrianov, A. K. *Polyphosphazenes for Biomedical Applications.* John Wiley & Sons, **2009**.
9. Caminade, A.-M. *Chem. Commun.,* **2017**, *53*, 9830-9838.
10. Patil, B. R.; Machakanur, S. S.; Badiger, D. S.; Hunoor, R. S.; Gudasi, K. B.; Nethaji M.; Bligh, S. W. A. *J. Mol. Struct.* **2011**, *1003*, 52-61.
11. Fidan, I.; Önal, E.; Yerli, Y.; Luneau, D.; Ahsen, V.; Hirel, C. *Inorg. Chem.* **2016**, *55*, 11447-11453.
12. Srivastava, K.; Weitz, E. A.; Peterson, K. L.; Marjańska M.; Pierre, V. C. *Inorg. Chem.* **2017,** *56*, 1546-1557.
13. Bo, S.; Song, C.; Li, Y.; Yu, W.; Chen, S.; Zhou, X.; Yang, Z.; Zheng X.; Jiang, Z.-X. *J. Org. Chem.* **2015**, *80*, 6360-6366.
14. a) Chalmers, K. H.; De Luca, E.; Hogg, N. H. M.; Kenwright, A. M.; Kuprov, I.; Parker, D.; Botta, M.; Wilson, J. I.; Blamire, A. M. *Chem. Eur. J.* **2010**, *16*, 134-148; b) Blahut, J.; Bernašek, K.; Galisová, A.; Herynek, V.; Císarová, I.; Kotek, J.; Lang, J.; Matejková, S.; Hermann P. *Inorg. Chem.* **2017**, 10.1021/acs.inorgchem.7b02119

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- Suitable MRI properties with high imaging sensitivity have been demonstrated

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