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A bisphosphonate for ^{19}F-magnetic resonance imaging

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ABSTRACT

^{19}F-magnetic resonance imaging (MRI) is a promising technique that may allow us to measure the concentration of exogenous fluorinated imaging probes quantitatively *in vivo*. Here, we describe the synthesis and characterisation of a novel geminal bisphosphonate (^{19}F-BP) that contains chemically-equivalent fluorine atoms that show a single and narrow ^{19}F resonance and a bisphosphonate group that may be used for labelling inorganic materials based in calcium phosphates and metal oxides. The potential of ^{19}F-BP to provide contrast was analysed *in vitro* and *in vivo* using ^{19}F-MRI. *In vitro* studies demonstrated the potential of ^{19}F-BP as an MRI contrast agent in the millimolar concentration range with signal-to-noise ratios (SNR) comparable to previously reported fluorinated probes. The preliminary *in vivo* MRI study reported here allowed us to visualise the biodistribution of ^{19}F-BP, showing uptake in the liver and in the bladder/urinary system areas. However, bone uptake was not observed. In addition, ^{19}F-BP showed undesirable toxicity effects in mice that prevent further studies with this compound at the required concentrations for MRI contrast. This study highlights the importance of developing ^{19}F MRI probes with the highest signal intensity achievable.

1. Introduction

MRI is a medical imaging technique that offers high-resolution images of soft tissues without the need for ionising radiation. In addition, and unlike other techniques such as those based on radionuclides, it does not require the injection of contrast agents in order to obtain meaningful images. However, for some imaging procedures such as angiography or molecular imaging, chemical compounds can be used to enhance the contrast of the specific tissue of interest. In this context, one area that MRI currently lags behind other imaging modalities, particularly positron emission tomography (PET) and single photon emission computed tomography (SPECT), is the quantitative measurement of the signal provided by these contrast agents. This is a key requirement for molecular imaging applications. Current contrast-based MR techniques rely on the detection of imaging agents containing paramagnetic ions such as gadolinium, manganese or iron. However, interpretation of the results is difficult due to the varying underlying signal hyper- and hypo-intensities in MRI. In answer to this ^{19}F-MRI has been implemented. The use of fluorine as the nucleus for magnetic resonance has several advantages over protons. First, the lack of endogenous MR-visible fluorine provides an unambiguous readout of the introduced fluorine-containing compounds location. In addition the ^{19}F MR signal can be quantified, giving a measure of the contrast agent's concentration. This is in contrast to paramagnetic contrast agents used in ^{1}H-MRI and based on Gd, Mn and particularly Fe, where *in vivo* absolute quantification is not achievable.

The main uses of ^{19}F-MRI in biomedical imaging to date has been for cell tracking [1–5] visualisation of inflammation [6–9] and for imaging angiogenesis [10,11] all using ^{19}F nanoparticles. This is an obvious choice due to the capacity of nanoparticles to carry the many fluorine atoms required to obtain sufficient signal. More recently attempts have been made to image smaller compounds by modulating the ^{19}F signal using lanthanide metals [12,13] and used for the detection of gene expression [14]. Despite these early promising results and clear advantages for molecular imaging compared to ^{1}H-MRI, ^{19}F-MRI remains underused in clinical practice. This is due to a major disadvantage, which is low sensitivity [15]. As a consequence most ^{19}F-MRI probes designed to date need to have many fluorine atoms to provide enough signal in the tissues of interest (~20–50 mM ^{19}F). However, the number of fluorine atoms that a molecule can carry is limited for several reasons. First is solubility, as the fluorine content of a molecule increases, the water solubility decreases. The second limitation is the number of ^{19}F signals, the ideal ^{19}F-MRI contrast agent having

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one single narrow resonance to maximise signal and avoid imaging artifacts. To achieve this all the fluorine atoms must be in the same chemical and magnetic environment. Another limitation of ^{19}F-MRI is related to the long longitudinal relaxation times (T_1) of the fluorine nucleus (~1–2 s). This translates into long acquisition times for the MRI procedure due to the 5–10 s required between radiofrequency (RF) pulses, which results in long times or more complex non-standard MRI sequences.

We are interested in developing ^{19}F-MRI contrast agents for molecular imaging that show single and narrow ^{19}F resonances and short T_1 relaxation times. Previously we have shown that 1,1-bisphosphonates (BPs) bind very strongly to metabolically active bone and calcium phosphate materials such as hydroxyapatite using SPECT and PET imaging [16–19]. In addition, we found that BPs also bind very strongly to many nanomaterials based on lanthanide metal oxides of the type M_2O_3 (M = Gd, Er, among others) with known relaxation rate-enhancement properties [19]. We hypothesised that a fluorinated BP molecule could be an useful tool in the development of ^{19}F-MRI probes, that would allow to combine of the amplification properties of nanoparticle-based platforms (high numbers of equivalent fluorine atoms) with the relaxation-enhancement properties of lanthanide-based materials (short acquisition times) without affecting their water solubility. In this way we could potentially achieve ^{19}F-MRI probes with high signal intensity and sensitivity that could be imaged in a short time. In addition, their solution and *in vivo* properties could be easily controlled by surface modification using the same BP chemistry. In this work, we report our first attempts at achieving this aim by synthesizing and characterising a new fluorinated BP (**^{19}F-BP**,Scheme 1) and evaluate for the first time its properties as a single molecule for ^{19}F-MRI *in vitro* and *in vivo.*

2. Results and discussion

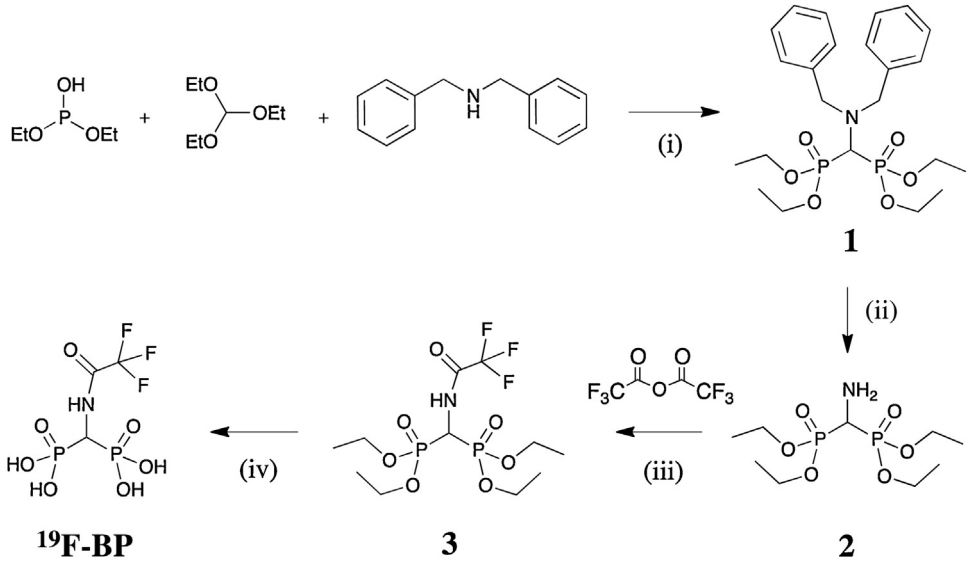
2.1. Synthesis

The reaction scheme for the synthesis of **^{19}F-BP** is shown in Scheme 1. Tetraethyl aminomethyl-bisphosphonate (**2**) was synthesized following published methods [20,21]. Briefly, diethyl phosphite, triethylorthoformate and dibenzylamine were reacted for 29 h at 150–160 °C to yield the benzylated bisphosphonate (**1**). The amino group of **1** was deprotected with H_2 and 10% Pd/C catalyst to yield **2**. After removal of the catalyst, **2** was reacted with 2.9 equivalents of trifluoroacetic anhydride (TFAA) in dry DCM for 3 h. Excess TFAA was used in order to prevent low reaction yields due to potential hydrolysis of the anhydride. After evaporation of the volatiles and work-up, **3** was recrystallised from cold hexanes in good yields (78%). The compound was characterised by NMR, HR-MS and the structure confirmed by X-ray crystallography (Fig. 1 and Fig. SI)

The ethyl-protected bisphosphonate group of **3** was deprotected by reacting with excess bromotrimethylsilane followed by methanolisis at room temperature. The reaction gave quantitative yields of **^{19}F-BP** as assessed by NMR and MS, confirming complete removal of the ethyl protecting groups. ^{19}F-NMR and ^{31}P-NMR also confirmed the stability of the trifluoromethyl and bisphosphonic groups, respectively. The solubility properties of **3** changed from hydrophobic to hydrophilic after deprotection, as expected for bisphosphonic acids, and allowed us to perform our imaging studies in water. One of the main advantages of this compound over most ^{19}F-MRI contrast agents reported to date based on perfluorinated molecules is the chemical equivalence of its F atoms. Non-equivalent F atoms result in broad and/or multiple resonances that have a negative effect on the final ^{19}F-MRI signal. In **^{19}F-BP**, however, having a narrow single ^{19}F resonance (−76.15 ppm, $\omega_{1/2}$ = 4.9 Hz), maximises imaging signal and minimises the appearance of image artefacts.

2.2. In vitro *MR imaging studies*

Phantom MRI studies were performed to evaluate the contrast properties of ^{19}F-BP (Fig. 2). The compound was dissolved in water at pH 7 at several concentrations (27, 54 and 108 mM) and imaged in a preclinical 9.4 T MRI scanner. A clear concentration-dependent increase in signal intensity and signal to noise ratio (SNR) was found, demonstrating that **^{19}F-BP** can be imaged in the high mM concentration range. Stability studies were also performed using these samples. The ^{1}H NMR and ^{19}F-MRI spectra remained stable for 5 h at pH 7 and 37 °C, confirming the stability of **^{19}F-BP** at these



Scheme 1. The synthetic scheme of ^{19}F-BP. (i) 29 h at 150–160 °C; (ii) H_2, 10% Pd/C catalyst in EtOH, room temperature; (iii) 3 h in dry DCM; (iv) (a) 24 h, Me_3SiBr (15 eq) in dry DCM, room temperature (b) 1.5 h MeOH, 1.5 mL, room temperature.