Journal of Fluorine Chemistry 153 (2013) 172–177

Contents lists available at SciVerse ScienceDirect

Journal of Fluorine Chemistry

journal homepage: www.elsevier.com/locate/fluor

Short communication

Easily available, low cost ^{19}F MRI agents: Poly(ethylene-glycol)-functionalized fluorinated ethers

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ARTICLE INFO

Article history:
Received 26 November 2012
Received in revised form 28 April 2013
Accepted 30 April 2013
Available online 22 May 2013

Keywords:
Fluorinated polymer
^{19}F NMR
Poly(ethylene-glycol)
Magnetic resonance imaging
Perfluoro compounds

ABSTRACT

A simple derivatisation of commercially available poly(ethylene-glycols) of different molecular weight followed by the reaction with the selected fluorinated organic molecule, perfluoro-*tert*butanol, allowed an easy synthesis of several novel polymers of different fluorine content. The properties of the new materials as MRI agents were preliminarily investigated. In all cases a single ^{19}F signal at NMR in deuterated chloroform and D_2O was registered; for a few fluorinated polymeric candidates a good solubility in water was observed and MR imaging of the fluorinated polymer successfully provided images. Most interesting results were obtained with the sample of 1436 MW (24% fluorine content) that not only gave a spectrum with a single resonance line, but, most importantly, was tolerated in low doses when *in vivo* experiments on animals (mice) were conducted.

1. Introduction

Modified poly(ethylene-glycol)s (PEGs) are very popular soluble supports. They are inexpensive, readily functionalized with different spacers and linkers, and, provided that their MW is greater than 2000 Da, are insoluble in nonpolar solvents (hexanes, diethyl ether, *tert*-butyl methyl ether) and simply purified by precipitation. As a consequence, the synthesis and the immobilization of several organic molecules on PEG supports have been reported over the last few years [1]. Indeed, PEGs allow to run the functionalization reaction under homogeneous (and likely best performing) conditions and to isolate and recover the PEG-supported organic residue as if it were bound to an insoluble polymer. Therefore they profit from both the advantageous features of homogeneous solution chemistry (high reactivity, lack of diffusion phenomena, analytical simplicity) and of solid-phase methods (ready isolation and easy purification of the products) [2].

Significantly poly(ethylene-glycol) is also a non-toxic, biocompatible, water soluble polymer; the method of grafting hydrophilic residues onto water insoluble reactants relies on a simple concept (increased solubility = increased reactivity), that has found extensive applications for instance in medicinal chemistry where poly(ethylene-glycols) (PEGs) chains have been widely employed to increase bioavailability of certain drugs [3]. We thought to take advantage of the positive features of this widely employed polymer in medicinal chemistry in the design of novel nanomedicine systems [4]; in this context our project would like to explore the development of novel macromolecular materials bearing a fluorinated residue suitable for ^{19}F MRI, which may be further implemented with other functions, for example a diagnostic one or, in the future, even a therapeutic one. Because of the lack of any ^{19}F background in the body, observed signals originating from injected ^{19}F containing agent exhibit an excellent degree of specificity and merging of recorded ^{19}F images on ^{1}H images enables an exact anatomic localization of fluorinated substances as "hot spots" [5].

Although ^{19}F MRI is only four years younger than ^{1}H MRI, it is not in clinical use, testifying for the difficulty in developing suitable imaging agents. In this area different polymeric (nano)-carriers have been investigated, including linear polymers, hyperbranched polymers, and dendrimers.[6] Only recently a very promising, dendritic ^{19}F imaging tracer has been developed. Structurally, such MRI agent is an amphiphilic dendrimer, where the two dissimilar dendrons are connected by a metabolically stable amide bond [7]. Our approach aims to develop a synthesis of the new MRI agents that should be highly flexible, easily tunable, relatively simple and not requiring many purification procedures, as it happens for example for dendrimers, that are macromolecules

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http://dx.doi.org/10.1016/j.jfluchem.2013.04.010

whose synthesis is not flexible and needs purifications for each step, with relatively low final yields [8].

2. Results and discussion

In a preliminary approach, in order to verify our hypothesis, several commercially available poly(ethylene-glycols) (PEGs) of different molecular weight were properly modified to act as carrier of the fluorinated residue. (Scheme 1) Poly(ethylene-glycol) was converted into its bis-mesylate derivative in quantitative yields and then reacted with the sodium salt of perfluoro-*tert*butanol in DMF.[9] Three different commercially available PEGs were employed, having MW of 400 Da, 1000 Da and 2000 Da to afford three novel fluorinated polymers, **A**, **B** and **C** of 836 Da, 1436 Da and 2436 respectively, with yields ranging from 47% to 71%.

All the materials showed a single ^{19}F signal at NMR in deuterated chloroform and D_2O and they were all indeed soluble in water (Fig. 1). It must be noted that all the fluorine atoms in the materials are equivalent, so that the quality of imaging signals is not significatively affected by the distribution of molecular weights; this approach should avoid the intrinsic problems involved in the dendrimer synthesis, that, being based on the repetition of the same synthetic scheme for each growth generation, requires many purification steps and it is often affected by low final yield.

The stability of the fluorinated polymers was checked, both in chloroform and in deuterated water, by performing NMR analysis; after one week no appreciable signs of degradation were observed.

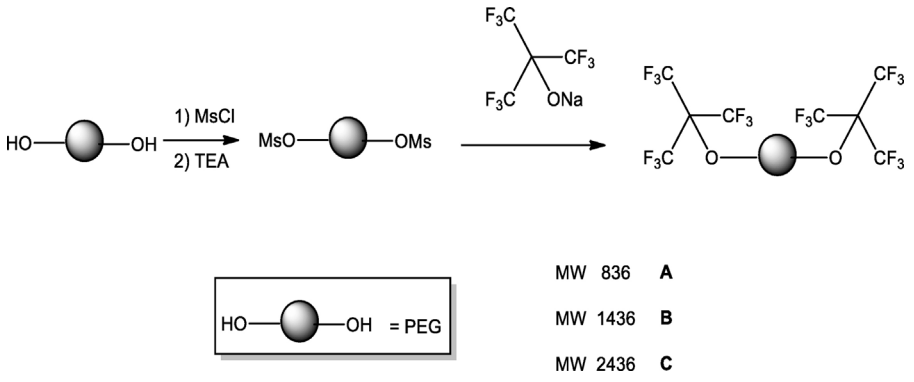
Size exclusion chromatography (SEC) analyses were performed in anhydrous CH_2Cl_2 at room temperature using a six Ultrasyragel column set (10^5, $2*10^4$, $2*10^3$ and 500 Å) with a isocratic Waters 1515 HPLC pump running at 1 mL/min and a Waters 2487 UV detector operating both at 244 nm and 230 nm. According to well established procedures, molecular weights were determined using a calibration curve determined by using polystyrene (PS) monodisperse standards. Curves obtained at wavelength of 244 nm are shown in Fig. 2. The analyses were performed by dissolving approximately 20 mg of sample in 1 mL of solvent (injection volume is 50 μl); 1,2-dichlorobenzene was used as internal reference. Out of clarity, curves were normalized according to the main peak (Fig. 2). SEC analyses were used to investigate the presence of species having different molecular weights in the newly synthesized PEG derivatives. Since in the case of low molecular weight polymers derivatization involves a high increase of the molecular weight of the material and therefore the modification of the commercially available starting material can be easily observed, SEC curves of standard PEG400 and of its fluorinated derivative are here reported as the most significant example. The SEC curve of commercial PEG400 itself shows a distribution of molecular weights around the main peak and the presence of a second smaller peak of species having higher hydrodynamic volume; PS calibration indicates a Mp (Molecular weight of the peak) on the main peak of 943 Da (PS equivalents). The perfluoro-*tert*butanol curve (not shown here) has a Mp of 535 Da (PS equivalents) and a polydispersity of 1.02.

SEC curve of the fluorinated polymer shows a distribution of molecular weights and a bimodal curve at lower elution times; the main peak has a Mp of 2451 Da (PS equivalents) and the smaller one has Mp of 1854 Da; the difference between them is very similar to the weight of the fluorinated alcohol alone (around 590–600 Da); therefore the two peaks can be assigned to the completely fluorinated molecule (the peak at lower elution times, around 2980 s) and to the monofunctionalized PEG400 (the peak at around 3070 s), respectively. The difference between pure PEG400 and the monofluorinated residue is higher (around 900 Da) because the presence of a residue having a steric hindrance much higher than the one of –OH group forces the polymer to change its hydrodynamic volume that becomes different from the one of a pure linear PEG chain. Also some residual PEG is observed, but only the higher molecular weight peak of the original PEG400 seems to remain unchanged. This might imply that commercial sample contains some non-reactive fractions (see Fig. 2). The product, that contains small impurities (already present in the commercial poly(ethylene glycol), can be considered a mixture of mono and bis fluorinated PEG400. The analysis does not show the presence of any other contaminant and/or polymer.

When experiments of *in vitro* MR imaging were conducted [10] a clear imaging was obtained both with polymers **A** and **B**, featuring 41% and 24% of fluorine content in weight respectively (see Fig. 3) [11]. All MRI experiments were performed with a Bruker Biospec 70/30 using a volume transmit/receive $^1H/^{19}F$ coil with a 3 cm inner diameter [12]. All the samples have been dissolved in phosphate buffer saline in order to obtain a 250 mM solution [7]. Afterwards ^{19}F NMR spectrum and ^{19}F MRI image have been acquired. ^{19}F NMR spectrum gave a spectrum with a single (or very dominant) resonance line [13]. In Fig. 3 a comparison between samples **B** and **C** is reported and it clearly shows that the material of 2436 MW, with 14% of fluorine in weight was not able to give a clear imaging. The fluorine content of the sample is a crucial point in the design of a successful candidate for MRI imaging, that should have a fluorine content in weight higher than 25–30% over the whole molecular weight of the sample.

After having successfully synthesized water soluble fluorinated polymers able to guarantee a clear imaging, their toxicity was preliminarily investigated.

Male C57Bl/6 mice (10-week old, 20–25 g, Harlan Laboratories, Italy) were used [14]. The polymer **B** was administered intraperitoneally in a volume of 150 μl (23 mM solution concentration). The formulation to be administered was as bland



Scheme 1. Synthesis of fluorinated poly(ethylen glycol) derivatives.