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Towards potential nanoparticle contrast agents:  
Synthesis of new functionalized PEG bisphosphonates

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and Marc Lecouvey*¹

Abstract  
The use of nanotechnologies for biomedical applications took a real development during these last years. To allow an effective targeting for biomedical imaging applications, the adsorption of plasmatic proteins on the surface of nanoparticles must be prevented to reduce the hepatic capture and increase the plasmatic time life. In biologic media, metal oxide nanoparticles are not stable and must be coated by biocompatible organic ligands. The use of phosphonate ligands to modify the nanoparticle surface drew a lot of attention in the last years for the design of highly functional hybrid materials. Here, we report a methodology to synthesize bisphosphonates having functionalized PEG side chains with different lengths. The key step is a procedure developed in our laboratory to introduce the bisphosphonate from acyl chloride and tris(trimethylsilyl)phosphite in one step.

Introduction  
Numerous researchers are interested in the development of superparamagnetic iron oxide nanoparticles (SPIONPs) because of their biocompatibility which allows there in vivo use both for diagnosis in magnetic resonance imaging and in therapy [1,2]. Most often, it is necessary to modify the surface of SPIONPs to increase the metabolic stability.

To overcome this main drawback, the NP surface could be derivatized by various functional groups. These ligands have to possess certain chemical and biological properties as the flexibility, the hydrophilicity and an absence of in vivo toxicity. In addition, the nanoparticulate systems so obtained must be stable in the various biological compartments and they must be stealthy to avoid the elimination by macrophages.

For this purpose, appropriate coatings have already been reported [3,4]. Some of which consist in the NP surface modification using hydrophilic polymers (dextran, PEG) or
bifunctional molecules substituted by amines, thiols, carboxylates, sulfonates, phosphonates or bisphosphonates [5-7]. Particularly, a strong interaction between the NPs and the phosphonic moiety was observed and more interestingly the best results were obtained with bisphosphonate products [8,9]. For the past years, our group has focused its interest in the synthesis of various functionalized hydroxymethylene bisphosphonates (HMBPs) [10] and their applications in health science, especially in antitumor therapy [11-13]. Herein, we described the synthesis of novel bifunctional PEG-HMBP compounds in order to employ them as anchoring agents for SPIONPs (Figure 1).

The first method, used in the industry, allows accessing the desired products in one step under rather harsh conditions [14]. 1-Hydroxyalkylidenebisphosphonic acids have also been obtained in good yields. However, this widely used method seems not to be compatible with breakable and delicate functionalized substrates. In contrast, our lab has developed a new synthetic strategy starting from an acid chloride and tris(trimethylsilyl) phosphite, followed by a methanolysis step [15].

This one-pot procedure allows the synthesis of various aliphatic and aromatic bisphosphonic acids under mild conditions. Moreover, reactions were very fast and pure products were obtained after evaporation of the volatile fraction. The scope of this reaction was successfully widened in aliphatic and aromatic anhydride [15-21]. The introduction of the HMBP moiety in presence of the PEG tether seems to be critical due to its high sensitivity under harsh conditions. Wherefore, our methodology, which exhibits a high tolerance to various functionalized groups, appears to be an adequate way to introduce the HMBP chain in presence of the PEG moiety.

To obtain the PEG-HMBP 1 compound family, the synthetic strategy consists in mono-protecting and/or mono-functionalizing commercially available PEGs followed by the lab-made HMBP methodology introduced previously (Scheme 2).

Starting materials, the free alcohol PEG and monomethyl ether PEGs (compounds 3a, b) with various chain lengths (n = 4, 7 and 12) were commercially available. Firstly, the free alcohol PEG was selectively monoprotected with a benzyl group (Scheme 3).

Only one alcohol function was indeed deprotonated with one equivalent of sodium hydride at −78 °C in THF after the solution was stirred for 12 hours at room temperature. The alcoholate intermediate reacted smoothly with benzyl bromide at room temperature to afford the monobenzylated PEG 2 in 77% yield (Table 1, entry 1). Afterwards, the alcohols 2 and 3a, b have to be oxidized to the corresponding carboxylic acids 6 and 7a, b. First of all, the direct oxidation reported in the literature in one step has been performed. Thus, tested oxidants were the Jones

Scheme 3: Synthesis of PEG-HMBPs 1 and 1'.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>R</th>
<th>n</th>
<th>Yield (%)</th>
<th>31P δ (ppm)</th>
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<tr>
<td>1</td>
<td>2</td>
<td>Bn</td>
<td>4</td>
<td>77</td>
<td>–</td>
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<td>4</td>
<td>89</td>
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<tr>
<td>3</td>
<td>5a</td>
<td>Me</td>
<td>7</td>
<td>79</td>
<td>–</td>
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<tr>
<td>4</td>
<td>5b</td>
<td>Me</td>
<td>12</td>
<td>80</td>
<td>–</td>
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<tr>
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<td>6</td>
<td>Bn</td>
<td>4</td>
<td>72</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>7a</td>
<td>Me</td>
<td>7</td>
<td>82</td>
<td>–</td>
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<tr>
<td>7</td>
<td>7b</td>
<td>Me</td>
<td>12</td>
<td>72</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>Bn</td>
<td>4</td>
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<td>–</td>
</tr>
<tr>
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<td>9a</td>
<td>Me</td>
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<td>quant.</td>
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<tr>
<td>11</td>
<td>1</td>
<td>Bn</td>
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<tr>
<td>12</td>
<td>1’a</td>
<td>Me</td>
<td>7</td>
<td>47</td>
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<tr>
<td>13</td>
<td>1 ’b</td>
<td>Me</td>
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<td>10</td>
<td>H</td>
<td>4</td>
<td>72</td>
<td>16.2</td>
</tr>
</tbody>
</table>

aIsolated yield. bproton decoupling 31P NMR experiment.

The carboxylic acids 6 and 7a,b reacted quantitatively with oxalyl chloride to give acyl chlorides 8 and 9a,b at room temperature after 24 hours (Table 1, entries 8–10). The completion of the reaction was monitored by infrared spectroscopy with the disappearance of the hydroxy absorption band and the shifting of the carbonyl vibration band to about 1800 cm⁻¹. The addition of two equivalents of tris(trimethylsilyl) phosphite to the acyl chloride derivatives 8 and 9a,b yielded the corresponding silylated acid derivatives 11–13. The treatment of 1 with dihydrogen and palladium on charcoal in water allowed cleavage of the benzyl moiety and led to the HO-PEG-HMBP 10 in 72% yield (Table 1, entry 14). The ligand 10 permitted to obtain new gadolinium phosphate nanocrystals with luminescent properties [26].
products will allow us to perform click chemistry to introduce various functionalities. Moreover, the amino derivaties will be easily obtained by reducing the azido group. As previously mentioned, the first step was a selective mono-activation of PEG using para-toluenesulfonyl chloride in the presence of sodium hydroxide in a water/THF mixture (Scheme 4).

The tosylated products 11a, b were obtained after three hours at 0 °C with a 95% yield. Next, the monoactivated compounds 11a,b were substituted by sodium azide in DMF at 60 °C within five hours. The azido compounds 12a,b were obtained in 83% and 81% yield, respectively. The alcohols 12a,b were subsequently firstly deprotonated with NaH in DMF and the generated alcoholates were stirred 16 hours with ethyl bromoacetate giving the expected esters 13a,b in moderate yields.

The saponification reactions of the esters 13a,b were carried out with sodium hydroxide in methanol. The completion of the reactions was controlled by TLC. After neutralization with a cationic exchange Dowex® 50WX2 resin, the corresponding carboxylic acids 14a,b were isolated in quantitative yields. The HMBP moiety was subsequently incorporated into the lab-made methodology previously described. The carboxylic acids 14a,b were converted into the expected HMBP-PEG-N₃ 16a,b via the corresponding acyl chloride 15a,b. The reactions were monitored by ³¹P NMR, compounds 16a and 16b were obtained after 12 and 18 hours, respectively.

Thus, HMBP-PEG-N₃ 16a,b were obtained after purification in 72% and 74% yield and characterized by a singlet in ³¹P [27] NMR at about 17 ppm. Finally, the reduction of the azido compounds 16a,b in the presence of palladium on charcoal and dihydrogen led to the targeted amino-PEG-HMBPs 17a and 17b, respectively in moderate 62% and 68% yields.

In order to access available PEG-HMBPs functionalized with a primary amine or a carboxylic acid group usable in peptidic coupling with various molecules for example, the HMBP-PEG-COOH 23 was synthesized (Scheme 5). This compound was obtained in six steps starting from a free alcohol four-unit PEG. It
reacted with ethyl bromoacetate after mono-deprotonation using sodium hydride in THF at –78 °C for 12 hours at room temperature giving PEG 18. HMBP-PEG 22 was next synthesized in four steps with satisfying yields in a similar strategy previously described for compounds 1 and 1’a,b. The last step was the saponification of the ethyl ester group. Different usual conditions were tested, leading to partial degradation of the HMBP. The use of a diluted aqueous solution of potassium hydroxide and sodium hydroxide as well as copies of their supplementary/1860-5397-12-130-S1.pdf [http://www.beilstein-journals.org/bjoc/content/ supplementary/1860-5397-12-130-S1.pdf]

Supporting Information
Supporting Information File 1
Experimental and analytical data of all new compounds as well as copies of their 1H, 31P and 13C NMR spectra.
[http://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-12-130-S1.pdf]

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References
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