Bioorganic & Medicinal Chemistry Letters 20 (2010) 1861–1865

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Ultrasmall particle of iron oxide—RGD peptidomimetic conjugate: synthesis and characterisation

Vincent Rerat [a], Sophie Laurent [b], Carmen Burtéa [b], Benoît Driesschaert [a], Vincent Pourcelle [a], Luce Vander Elst [b], Robert N. Muller [b], Jacqueline Marchand-Brynaert [a,*]

[a] Unité de Chimie Organique et Médicinale, Université Catholique de Louvain, Bâtiment Lavoisier, Place Louis Pasteur 1, B-1348 Louvain-la-Neuve, Belgium
[b] Department of General, Organic and Biomedical Chemistry, NMR and Molecular Imaging Laboratory, University of Mons, Belgium

ARTICLE INFO

Article history:
Received 16 December 2009
Revised 27 January 2010
Accepted 29 January 2010
Available online 4 February 2010

Keywords:
Iron particle
RGD peptidomimetic
Magnetic labeling
Jurkat cell
XPS

ABSTRACT

Ultrasmall particles of iron oxide (USPIOs) coated with 3,3′-bis(phosphonate)propionic acid were covalently coupled to a home-made Arg-Gly-Asp (RGD) peptidomimetic molecule via a short oligoethyleneglycol (OEG) spacer. The conjugation rate was measured by X-ray photoelectron spectroscopy (XPS). The particle size and magnetic characteristics were kept. Our novel conjugate targeted efficiently Jurkat cells (increase of 229% vs the control).

© 2010 Elsevier Ltd. All rights reserved.

Non invasive diagnosis methods and cellular labelling for biomedical researches represent important application domains of medical imaging (MI). Recent developments in this field rely upon the discovery of functional radiotracers and contrast agents targeted at particular cell types, such as tumor cells and migrating vascular endothelial cells.[1] These cells express $\alpha_v\beta_3$ integrin transmembrane receptors[2] that are involved in their migration, invasion, proliferation and survival. The $\alpha_v\beta_3$ integrin recognizes matrix proteins containing the cell-adhesion tripeptide motif Arg-Gly-Asp (RGD)[3] of which the biologically active conformation is known thanks to the cyclic peptide named Cilengitide (*cyclo*-[RGD*f*N(Me)V]-)[4] featuring high selectivity and binding affinity in the nanomolar range.

Accordingly, RGD cyclic peptides have been conjugated to various carriers for selective gene[5] and drug delivery[6] in cancer chemotherapy, but also linked to radioactive labels and contrast agents for selective cell imaging.[7] For instance, conjugation to DOTA (1,4,7,10-tetraazadodecane-*N*,*N*′,*N*″,*N*‴-tertraacetic acid) and radiolabelling with ^{111}In,[8] conjugation to 5-carboxylate-2,2′-bipyridine (BPy)[9] or 6-(2-(2-sulfonatobenzaldehyde)hydrazino)-nicotinyl (HYNIC) and radiolabelling with ^{99m}Tc,[10] have been described to image tumors by positron emission tomography (PET) and single photon emission computed tomography (SPECT), respectively. For magnetic resonance imaging (MRI), cyclic RGD peptides have been coupled to Gd^{3+} complexes[11] as positive contrast agents (effect on T_1, longitudinal relaxation time) and to iron oxide nanoparticles[12] as negative contrast agents (effect on T_2, transverse relaxation time).

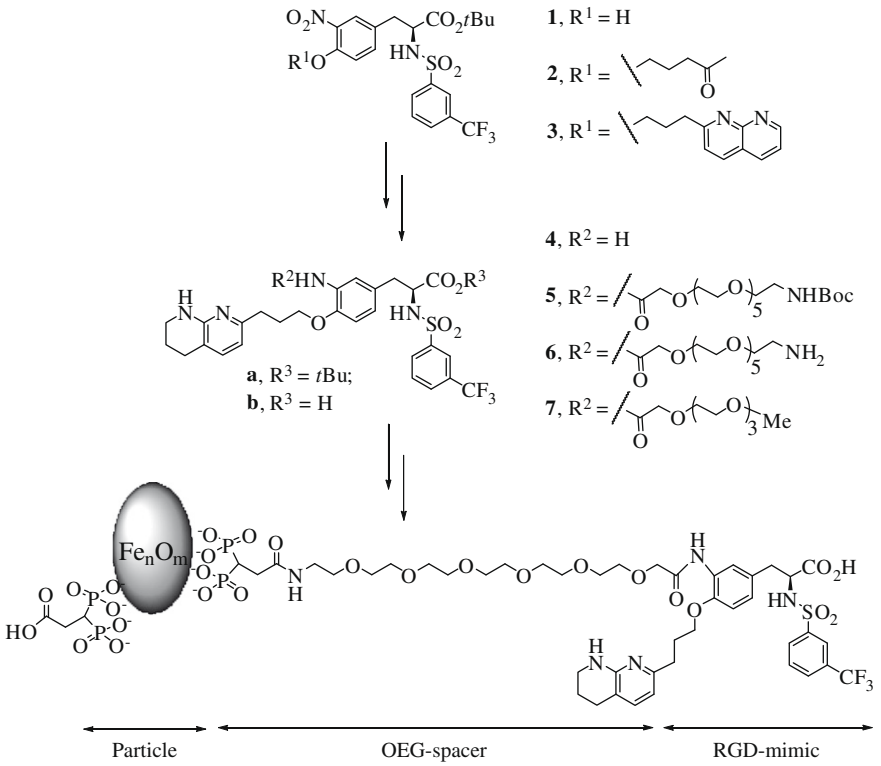
The growing interest in non peptide RGD mimics (peptidomimetics), as orally bioavailable $\alpha_v\beta_3$ antagonists for therapeutical purposes,[13] stimulates the interest to use these molecules in MI applications. RGD peptidomimetics conjugated to ^{111}In-[14] and ^{89}Yb-DOTA complexes,[15] and to Gd-DTPA (Gd-diethylenetriamine pentaacetate) complexe,[16] have been developed to target the $\alpha_v\beta_3$ receptor (see discussion in Supplementary data).

To our knowledge, iron oxide nanoparticles have not been conjugated to RGD peptidomimetics till now. However, small particles of iron oxide possessing superparamagnetic properties are the most commonly used systems for the magnetic labelling of cultured cells and their detection by MRI.[17] In this letter, we describe the grafting of a home-made RGD peptidomimetic on ultrasmall particles of iron oxide (USPIO) coated with a thin layer of 3,3′-bis(phosphonate)propionic acid,[18] and the determination of the grafting rate by X-ray photoelectron spectroscopy (XPS). The RGD peptidomimetic—USPIO conjugate has been characterized by dynamic light scattering (DLS), magnetometric and relaxometric profiles, and its capacity for targeting leukemic cells in vitro.

The structure of our functional magnetic contrast agent that targets $\alpha_v\beta_3$-displaying cells is shown in Scheme 1: the carboxylated iron particle is connected to the RGD peptidomimetic via an OEG

* Corresponding author.
E-mail address: Jacqueline.marchand@uclouvain.be (J. Marchand-Brynaert).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.01.150



Scheme 1. Synthesis of USPIO–petidomimetic conjugate.

(oligoethylene glycol) spacer; amide linkages are used for the assembly of the three building blocks.

The peptidomimetic design stems from previous works of our team[19] dedicated to surface modification of polymer substrates for promoting cellular attachment. The L-tyrosine scaffold **1** was used to introduce the basic motif (Arg mimic) and the OEG spacer respectively at the *para*- and *meta*-positions of the aromatic ring, in agreement with a recent modeling study.[20] In this work, we used the RGD peptidomimetic **6b**, ready for grafting on materials; it features the tetrathydro-naphthyridinyl moiety[21] as the Arg basic mimic and a spacer-arm of six OEG units. The complete synthetic protocols toward **6b** are given as Supplementary data. Briefly, the known precursor **1**[19b] (*t*-butyl-(S)-3-(4′-hydroxy-3′-nitro-phenyl)-2-(3′-trifluoromethyl-1-benzenesulfonylamino)-propionate) was reacted with 3-acetyl-1-propanol under Mitsunobu conditions (DIAD, Ph_3P, THF, 20 °C, 17 h) to give the corresponding phenol ether **2** (57%). Then, a Friedländer condensation reaction (2-amino-3-pyridin-carboxaldehyde, L-proline, EtOH, reflux, 48 h) led to the naphthyridin derivative **3** (55%). Catalytic hydrogenation (Pd/C, H_2 1 atm., EtOH, 20 °C, 17 h) reduced simultaneously the nitro group and the naphthyridin ring to furnish compound **4a** (R^3 = *t*Bu, 95%). *t*-Butyl ester deprotection (TFA, DCM, 20 °C, 2 h), afforded the RGD peptidomimetic **4b** ($R^2 = R^3$ = H, 95%) devoid of spacer-arm, for biological evaluation.

The spacer-arm was independently prepared according to standard protocols; the use of OEG spacers is a well established strategy for the construction of bifunctional integrin-binding devices.[22] The peptide-like coupling of peptidomimetic **4a** and 2-(2-(2-(2-(2-(2-*t*-butoxycarbonylaminoethoxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-acetic acid made use of Py-Bop (Benzotriazol-1-yl-oxy-tris(dimethylamino)-phosphonium hexafluorophosphate) as activating reagent (iPr_2EtN, DMF, 20 °C, 15 h). Compound **5a** (R^3 = *t*Bu) was isolated in 33% yield after column chromatography, and then fully deprotected (TFA, DCM, 20 °C, 4 h) to quantitatively furnish the target-molecule **6b** (R^3 = H). The reference peptidomimetic, **7b**, was similarly prepared.

The peptidomimetics **4b**, **6b**, and **7b** have been previously evaluated for selectivity and binding affinity towards isolated human $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ receptors by using standard competitive assays.[20c] Results showed that all the tested compounds are $\alpha_v\beta_3$ selective and active in the (sub)nanomolar range with IC_{50} values of 0.1 (**4b**), 0.7 (**6b**), and 0.3 nM (**7b**). The presence of an OEG spacer does not disturb the biological activity of the peptidomimetic core (compare **4b** with **6b**), and the amine end-function of the spacer is not involved in the recognition process (compare **7b** with **6b**). Hence, the graftable RGD peptidomimetic **6b** is a good candidate for conjugation to USPIO.

The iron particles are surface modified with bis-(phosphonate)propionic acid molecules (about 1.6% of organic material vs Fe_nO_m). The phosphonate grips strongly complex the metal core while the carboxyl functions remain available for coupling reactions (see Fig. 1). The USPIO reactivity has been assayed by using a water soluble carbodiimide (WSC) as activating reagent (namely *N*-(3-dimethylamino)propyl *N*′-ethylcarbordiimide hydrochloride) and lysine as molecular probe, in a one-pot grafting protocol.[23] The excess of reagents and self-coupling products were removed by dialysis of the particles. After freeze-drying, the modified particles were analyzed by XPS for determining their surface atomic composition.[24] The native USPIO showed Fe, O, P and C atoms,

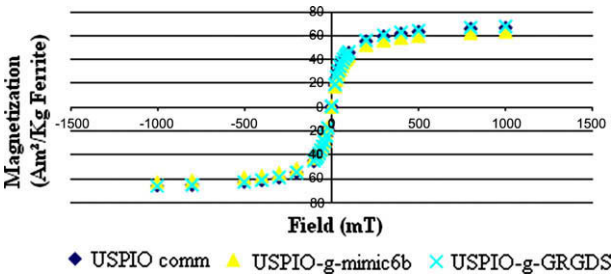


Figure 1. Magnetometric profiles.

Table 1
Surface grafting on USPIO—XPS analysis

Entry	Ligand	Conditions (WSC; dialysis)	N/C × 100 from XPS[a]	Derivatization	
				%[b]	Nbr[c]
1	Lysine	20 °C, 1 h; 48 h	6.35 (0.43)	11.8	6.6
2	Lysine	20 °C, 2 h; 48 h	5.84 (0.36)	10.6	5.9
3	Lysine	20 °C, 4 h; 48 h	5.22 (0.98)	9.3	5.2
4	Lysine	20 °C, 6 h; 48 h	6.23 (0.73)	11.5	6.4
5	Lysine	40 °C, 1 h; 48 h	5.77 (0.42)	10.5	5.9
6	Lysine	40 °C, 2 h; 48 h	6.68 (1.08)	12.5	7.0
7	Mimic **6b**	20 °C, 2 h; 24 h	5.00 (nd)	5.0	2.8
8	GRGDS	20 °C, 2 h; 24 h	7.19 (0.90)	3.2	1.9

[a] Corrected values obtained by subtraction of the blank. Mean of three independent samples with standard deviation into parentheses.
[b] Percentage calculated as described in Ref. 25.
[c] Number of molecules grafted per particle; see Ref. 26.

while the lysine-coupled USPIO revealed the presence of supplementary N atoms, in larger amounts as compared to the blank samples. The grafting rates calculated from the experimental N/C atomic ratios[25] are collected in Table 1 (entries 1–6). The percentages of derivatization remained in the range of 9–12%, corresponding to 5–7 molecules of lysine per particle,[26] whatever the coupling conditions had been (20 °C for 1 h, 2 h, 4 h, 6 h and 40 °C for 1 h, 2 h). This means that the coupling occurs quite rapidly and is well reproducible. The conjugation protocol validated with lysine (20 °C, 2 h) was further applied for the coupling of the peptidomimetic molecule **6b** (Fig. 1) and the commercial pentapeptide Gly-Arg-Gly-Asp-Ser (GRGDS) considered as the reference ligand of $\alpha_v\beta_3$ receptor.[27] The derivatization rates recorded by XPS analysis were quite lower, about 3–5% corresponding to 2–3 molecules of $\alpha_v\beta_3$ ligand per particle (Table 1, entries 7 and 8).[23,25,26] Interestingly, by using the experimental F/C atomic ratio we calculated a derivatization rate for USPIO-g-Mimic**6b** of 4.9% corresponding to an average of 2.7 molecules of RGD peptidomimetic per particle, in good agreement with the result of Table 1 (entry 7).

Physico-chemical properties of the novel USPIO conjugates, namely USPIO-*g*-Mimic**6b** and USPIO-*g*-GRGDS, were measured to verify the keeping of particle size and magnetic characteristics[28] (Table 2): hydrodynamic mean diameter was determined by dynamic light scattering (DLS, see Supplementary data); from magnetometric (Fig. 1) and relaxometric profiles (Fig. 2), the magnetization at saturation (Ms) and Fe microcrystal radius were obtained (see Supplementary data). As already reported,[29] the magnetometric mean diameter is lower than the relaxometric one (Table 2) because of the distribution of the crystal sizes which influences the mean size obtained by various methods. This size dispersion is also responsible for the lower relaxometric specific magnetization as compared to the magnetometric one.

The physicochemical properties of the USPIO-*g*-Mimic **6b** included a r_1 of 35.0 $mM^{-1}\,s^{-1}$ and a r_2 of 72.1 $mM^{-1}\,s^{-1}$ at 20 MHz and a r_1 of 17.5 $mM^{-1}\,s^{-1}$ and a r_2 of 73.4 $mM^{-1}\,s^{-1}$ at 60 MHz (37 °C). For the USPIO-*g*-GRGDS, r_1 was of 37.2 $mM^{-1}\,s^{-1}$ and r_2 of 72.3 $mM^{-1}\,s^{-1}$ at 20 MHz and r_1 of 19.0 $mM^{-1}\,s^{-1}$ and r_2 of 71.8 $mM^{-1}\,s^{-1}$ at 60 MHz (37 °C). The grafting of GRGDS peptide or of peptidomimetic **6b** on the nanoparticle surface does not change significantly the relaxometric properties.

Table 2
Characteristics of USPIO conjugates

Entry	Sample	D^a (nm)	R^b (nm) Method A/B	Ms (A m^2/kg)[c] Method A/B
1	Native USPIO	24	4.71/5.20	66.12/58.4
2	USPIO-*g*-Mimic **6b**	37	4.78/5.51	62.51/54.0
3	USPIO-*g*-GRGDS	21	4.67/5.16	66.7/56.9

[a] Apparent hydrodynamic diameter measured by DLS.
[b] Microcrystal radius determined from magnetometric profile (method A) or from NMRD profile (method B).
[c] Magnetization at saturation.

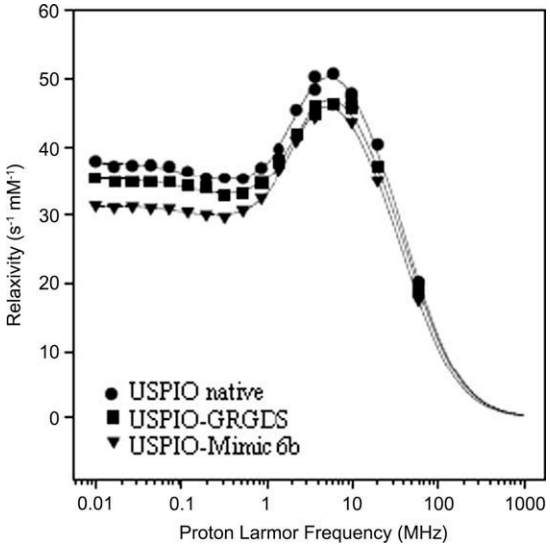


Figure 2. Relaxometric profiles.

The magnetic labelling of cells expressing the $\alpha_v\beta_3$ integrin was performed with Jurkat T lymphocytes. For preliminary validation, Jurkat cells are usually considered as good cell model of $\alpha_v\beta_3$ integrin expression,[30] but not as a model of certain pathology although such cells are cancerous cells (acute T cell leukemia). In previous studies, this cell model was routinely employed to validate various $\alpha_v\beta_3$ integrin-targeted imaging probes.[16,31] Jurkat cells are known to express the $\alpha_v\beta_3$ receptors under stimulation with phorbol 12-myristate 13-acetate (PMA, 50 ng/mL, 3 h, 37 °C, 5% CO_2). Cells (1.5×10^6 cells/mL), stimulated or not (negative control), were incubated in USPIO-conjugate solutions (0.5 mM) during 2 h at 25 °C. After washing out the excess of particles, cells were seeded in a gelatin matrix for measuring T_2 (CPMG pulse sequence, Bruker Minispec Mq-60, 60 MHz, 37 °C). The efficiency of USPIO capture by cells is determined by the R_2^{norm} values (where $R_2 = 1/T_2$, while R_2^{norm} is the normalized R_2 which is calculated by subtracting the R_2 of cells free of USPIO from R_2 of the cells incubated with iron oxide nanoparticles), the highest values corresponding to the best cell targeting. Results of Figure 3 showed a clear difference between activated cells (PMA) and non activated ones (CONtrol). The native particles gave some unspecific cell adhesion, independently of their activation state, which is justified by the absence of any stealth coating. After conjugation to the RGD peptidomimetic **6b**, the particles were more efficiently trapped by the activated cells (increase of 229% as compared to CON cells; $p < 0.05$). This targeting effect was also visible in the case of particles conjugated to the reference peptide GRGDS, but the peptide appeared less active than the peptidomimetic probably because the reaction of conjugation to USPIO could have altered the affinity of the reference peptide for the target. With its nanomolar binding affinity, the new RGD peptidomimetic **6b** represents an interesting candidate ligand for contrast agent vectorization. For sensitive MRI applications (i.e., detection of low biomolecule concentration) iron oxide superparamagnetic agents[28] are very promising after rendering them enough furtive to circumvent the inevitable capture by the phagocytic cells of the reticuloendothelial system. The novel USPIO-RGD peptidomimetic **6b** could thus assist the robust tumor cell detection by MRI,[32] possibly taking advantage of the $\alpha_v\beta_3$ receptor-mediated endocytosis phenomenon for particle uptake.[33] Optimiz-

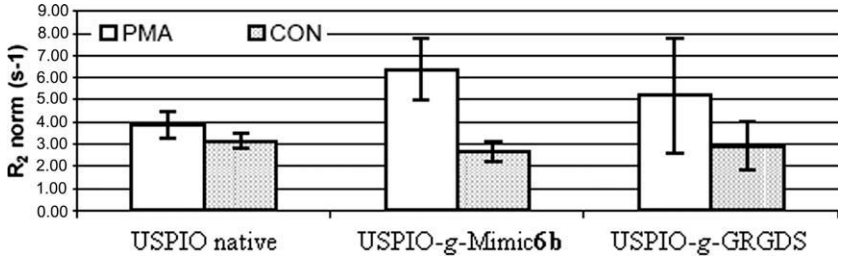


Figure 3. Targeting Jurkat cells with USPIO conjugates. PMA for activated cells and CON for control cells.

ing the conjugation yield on USPIO coated with an adequate stealth layer (i.e., pegylation) is currently under investigation.

Acknowledgements

This work was supported by the F.R.S.-FNRS and the ARC Program 05/10-335 of the French Community of Belgium. The support and sponsorship accorded by COST Action D38, EMIL NoE of the FP6 of the EC, and Encite (Contract No. 201842) are kindly acknowledged. We thank Ir. Michel Genêt for XPS facilities (UCL-CIFA) and advices. V.R. was F.R.I.A. (Belgium) fellow.

Supplementary data

Materials and methods, general synthesis of RGD peptidomimetics (**2**, **3**, **4a**, **5a**, **6b**), NMR spectra, USPIO derivatization rates and comments on $\alpha_v\beta_3$ receptor are provided. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.01.150.

References and notes

1. (a) Temming, K.; Schiffelers, R. M.; Molema, G.; Kok, R. J. *Drug Resistance Updates* **2005**, *8*, 381; (b) Meyer, A.; Auernheimer, J.; Modlinger, A.; Kessler, H. *Curr. Pharm. Des.* **2006**, *12*, 2723. and references cited therein.
2. Hynes, R. O. *Cell* **2002**, *110*, 673. and references cited therein.
3. Ruoslahti, E. *Matrix Biol.* **2003**, *22*, 459. and references cited therein.
4. (a) Dechantstreiter, M. A.; Planker, E.; Mathae, B.; Lohof, E.; Hoelzemann, G.; Jonczyk, A.; Goodman, S. L.; Kessler, H. *J. Med. Chem.* **1999**, *42*, 3033; (b) Xiong, J.-P.; Stehle, T.; Zhang, R.; Joachimiak, A.; Frech, M.; Goodman, S. L.; Arnaout, M. *Science* **2002**, *296*, 151.
5. Oba, M.; Fukushima, S.; Kanayama, N.; Aoyagi, K.; Nishiyama, N.; Koyama, H.; Kataoka, K. *Bioconjugate Chem.* **2007**, *18*, 1415.
6. Chen, X.; Plasencia, C.; Hou, Y.; Neamati, N. *J. Med. Chem.* **2005**, *48*, 1098.
7. Thumshirn, G.; Hersel, U.; Goodman, S. L.; Kessler, H. *Chem. Eur. J.* **2003**, *9*, 2717.
8. Dijkgraaf, I.; Kruijtzer, J. A. W.; Frielink, C.; Soede, A. C.; Hilbers, H. W.; Oyen, W. J. G.; Corstens, F. H. M.; Liskamp, R. M. J.; Boerman, O. C. *Nucl. Med. Biol.* **2006**, *33*, 953.
9. Zhang, X.; Chen, X. *Appl. Radiat. Isotopes* **2007**, *65*, 70.
10. Shi, J.; Wang, L.; Kim, Y.-S.; Zhai, S.; Liu, Z.; Chen, X.; Liu, S. *J. Med. Chem.* **2008**, *51*, 7980.
11. Weibo Cai, S. S. G.; Chen, X. *Biotechniques* **2005**, *39*, S6.
12. Nasongkla, N.; Bey, E.; Ren, J.; Ai, H.; Khemtong, C.; Setti Guthi, J.; Chin, S.-F.; Sherry, A. D.; Boothman, D. A.; Gao, J. *Nano Lett.* **2006**, *6*, 2427.
13. (a) Miller, W. H.; Keenan, R. M.; Willette, R. N.; Lark, M. W. *Drug Discovery Today* **2000**, *5*, 397; (b) Cacciari, B.; Spalluto, G. *Curr. Med. Chem.* **2005**, *12*, 51; (c) Dayam, R.; Aiello, F.; Deng, J.; Wu, Y.; Garofalo, A.; Chen, X.; Neamati, N. *J. Med. Chem.* **2006**, *49*, 4526.
14. Jang, B.-S.; Lim, E.; Hee Park, S.; Soo Shin, I.; Danthi, S. N.; Hwang, I. S.; Le, N.; Yu, S.; Xie, J.; Li, K. C. P.; Carrasquillo, J. A.; Paik, C. H. *Nucl. Med. Biol.* **2007**, *34*, 363.
15. Harris, T. D.; Kalogeropoulos, S.; Nguyen, T.; Liu, S.; Bartis, J.; Ellars, C.; Edwards, S.; Onthank, D.; Silva, P.; Yalamanchili, P.; Robinson, S.; Lazewatsky, J.; Barrett, J.; Bozarth, J. *Cancer Radiother. Radiopharm.* **2003**, *18*, 627.
16. Burtea, C.; Laurent, S.; Murariu, O.; Rattat, D.; Toubeau, G.; Verbruggen, A.; Vansthertem, D.; Vander Elst, L.; Muller, R. N. *Cardiovascular Res.* **2008**, *78*, 148.
17. (a) Boutry, S.; Brunin, S.; Mahieu, I.; Laurent, S.; Vander Elst, L.; Muller, R. N. *Contrast Media Mol. Imaging* **2008**, *3*, 223; (b) Radermacher, K. A.; Beghein, N.; Boutry, S.; Laurent, S.; Vander Elst, L.; Muller, R. N.; Jordan, B.; Gallez, B. *Invest. Radiol.* **2009**, *44*, 398.
18. Port, M.; Corot, C.; Raynal, I.; Rousseaux, O. US Patent 2004, 0253181 A1.
19. (a) Biltresse, S.; Attolini, M.; Marchand-Brynaert, J. *Biomaterials* **2005**, *26*, 4576; (b) Biltresse, S.; Attolini, M.; Dive, G.; Cordi, A.; Tucker, G. C.; Marchand-Brynaert, J. *Bioorg. Med. Chem.* **2004**, *12*, 5379.
20. (a) Rerat, V. Ph.D. Thesis, Université catholique de Louvain (Louvain-la-Neuve), 2008 (http://hdl.handle.net/2078.1/20874).; (b) Marchand-Brynaert, J. *Cardiovascular Pathol.* **2008**, *17*, 276; (c) Rerat, V.; Dive, G.; Tucker, G. C.; Cordi, A.; Bareille, R.; Amédée, J.; Bordenave, L.; Marchand-Brynaert, J. *J. Med. Chem.* **2009**, *53*, 7029.
21. Kinney, W. E.; Teleha, C. A.; Thompson, A. S.; Newport, M.; Hansen, R.; Ballentine, S.; Ghosh, S.; Mahan, A.; Grasa, G.; Zanotti-Gerosa, A.; Dingenen, J.; Schubert, C.; Zhou, Y.; Leo, G. C.; McComsey, D. F.; Santulli, R. J.; Maryanoff, B. E. *J. Org. Chem.* **2008**, *73*, 2302.
22. (a) Owen, R. M.; Carlson, C. B.; Xu, J.; Mowery, P.; Fasella, E.; Kiessling, L. L. *ChemBioChem* **2007**, *8*, 68; (b) Pilkington-Miksa, M. A.; Sarkar, S.; Writer, M. J.; Barker, S. E.; Shamlou, P. A.; Hart, S. L.; Hailes, H. C.; Tabor, A. B. *Eur. J. Org. Chem.* **2008**, 2900.
23. *Coupling protocol*: USPIO material was received from Guerbet laboratories (Aulnay-sous-Bois, France). The particles feature carboxylated groups on their surface according to Ref. 18. They are suspended in water in order to obtain the [Fe] concentration of 0.407 M. USPIO (1 mL, 0.407 mmol) was introduced in a flask previously washed with 37% HCl, milliQ water, acetone, and ether, and gently stirred with a magnetic bar. Lysine (8.13×10^{-2} mL of a solution of 4.5 mg Lys·HCl in 25 mL milliQ water; 0.0814 mmol) and water soluble carbodiimide (WSC, 54 mg, 0.28 mmol) were successively added, very slowly. The mixture was left under stirring at 20 °C or 40 °C, for 1 h to 6 h (see Table 1). After dialysis against milliQ water (membrane of 12–14,000) during 48 h, the suspension was freeze-dried, and the resulting powder analyzed by XPS (see Supplementary data for the table of results). The so-called blank samples were similarly prepared, but with omitting the carbodiimide. Control samples were similarly prepared but with omitting the lysine. These samples were also analyzed by XPS (see Supplementary data). For the coupling of peptidomimetic **6b**, we used USPIO (0.5 mL, 0.203 mmol), a 10^{-3} M solution of **6b** in water–DMSO, 95:5 (4.06×10^{-2} mL) and WSC (27 mg, 0.14 mmol); the mixture was reacted for 2 h at 20 °C and dialyzed during 24 h. For the coupling of GRGDS peptide, we applied the above protocol with a 10^{-3} M solution of peptide in water.
24. (a) Biltresse, S.; Descamps, D.; Henneuse-Boxus, C.; Marchand-Brynaert, J. *J. Polym. Sci., Part A.: Polym. Chem.* **2002**, *40*, 770; (b) Pourcelle, V.; Devouge, S.; Garinot, M.; Préat, V.; Marchand-Brynaert, J. *Biomacromolecules* **2007**, *8*, 3977; (c) Momtaz, M.; Rerat, V.; Gharbi, S.; Gérard, E.; Pourcelle, V.; Marchand-Brynaert, J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1084.
25. *Calculation of derivatization rate (%) from XPS data*: The simplified atomic formula of USPIO surface derivatized with lysine is: $xC_3 + yC_9N_2$, considering the native surface (C_3) and the modified fraction (C_9N_2); accordingly $x + y = 100$. Thus the atomic ratio $N/C = 2y/(3x + 9y)$. For instance, 10% of grafting ($y = 10$; $x = 90$) gives the calculated N/C atomic ratio of: $N/C = (10 \times 2)/[(90 \times 3) + (10 \times 9)] = 20/(270 + 90) = 0.0555$. The percentages of derivatization are deduced from the N/C experimental values. The formula considered for USPIO grafted with **6b** and GRGDS are respectively: $xC_3 + yC_{44}N_5F_3$ and $xC_3 + yC_{20}N_8$. Accordingly, for **6b**, $N/C = 5y/(3x + 44y)$ and $F/C = 3y/(3x + 44y)$ and for GRGDS, $N/C = 8y/(3\times + 20y)$.
26. *Calculation of the number (Nbr) of grafted molecules per particle*: Magnetite is considered as a crystalline system with a unit cell volume of 0.592 nm^3. The surface of a USPIO particle (sphere with $r = 10$ nm) and of a magnetite unit cell being respectively 1256 nm^2 and 0.71 nm^2, the number of unit cell faces displayed on one particle is 1,256/0.71 = 1769. Assuming the presence of 2 iron atoms per unit cell face, we calculate that one particle surface features about 3538 Fe atoms. With an organic covering of 1.6%, we can determine the average number of 56 bis-(phosphonate)propionic acid molecules per particle. The number of grafted molecules per particle is thus given by the percentage of surface derivatization (% from XPS) × 0.56.
27. Chollet, C.; Chanseau, C.; Remy, M.; Guignandon, A.; Bareille, R.; Labruyère, C.; Bordenave, L.; Durrieu, M.-C. *Biomaterials* **2009**, *30*, 711.
28. (a) Laurent, S.; Forge, D.; Port, M.; Roch, A.; Robic, C.; Vander Elst, L.; Muller, R. N. *Chem. Rev.* **2008**, *108*, 2064; (b) Aimé, S.; Fasano, M.; Terreno, E.; Botta, M. In *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*; Merbach, Toth, Eds.; Wiley, 2001; pp 193–241.
29. Ouakssim, A.; Fastrez, S.; Roch, A.; Laurent, S.; Gossuin, Y.; Pierart, C.; Vander Elst, L.; Muller, R. N. *J. Magn. Magn. Mater.* **2004**, *272*(276), E1711–E1713.

30. (a) Huang, S.; Endo, R. I.; Nemerow, G. R. *J. Virol.* **1995**, *69*, 2257; (b) Chen, Z.; Ahonen, M.; Hämäläinen, H.; Bergelson, J. M.; Kähäri, V. M.; Lahesmaa, R. *J. Immunol. Methods* **2002**, *260*, 79.
31. (a) Burtea, C.; Laurent, S.; Roch, A.; Vander Elst, L.; Muller, R. N. *J. Inorg. Biochem.* **2005**, *99*, 1135; (b) Burtea, C.; Laurent, S.; Port, M.; Lancelot, E.; Ballet, S.; Rousseaux, O.; Toubeau, G.; Vander Elst, L.; Corot, C.; Muller, R. N. *J. Med. Chem.* **2009**, *52*, 4725.
32. Lim, E. H.; Danthi, N.; Bednarski, M.; Li, K. C. P. *Nanomedicine* **2005**, *1*, 110.
33. (a) Xiong, X.-B.; Huang, Y.; Lu, W.-L.; Zhang, X.; Zhang, H.; Nagai, T.; Zhang, Q. *J. Control. Release* **2005**, *107*, 262; (b) Alam, Md. R.; Dixit, V.; Kang, H.; Li, Z.-B.; Chen, X.; Trejo, J.; Fisher, M.; Juliano, R. L. *Nucleic Acids Res.* **2008**, *36*, 2764; (c) Kiessling, F.; Huppert, J.; Zhang, C.; Jayapaul, J.; Zwick, S.; Woenne, E. C.; Mueller, M. M.; Zentgraf, H.; Eisenhut, M.; Addadi, Y.; Neeman, M.; Semmler, W. *Radiology* **2009**, *253*, 462.