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Functionalization of Fe_3O_4 magnetic nanoparticles with RGD peptide derivatives

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The ^{1}H NMR spectra were recorded on a Bruker DRX-400 (400 MHz) spectrometer with TMS as internal standard. Melting points were obtained on a SMP3 apparatus (Barloworld Scientific) and are uncorrected. Optical rotations were measured on a Perkin Elmer M341 polarimeter. Elemental analysis was performed using a Perkin Elmer 2400 II analyzer. The FT-IR spectra were recorded on a Nicolet 6700 FT-IR spectrometer (Thermo Electron Corporation) equipped with Smart Orbit with Diamond crystal ATR accessories. Hydrodynamic size and zeta potentials of particles were determined by dynamic light scattering using Malvern Zetasizer Nano ZS (Malvern Instruments Ltd.).

Di-*tert*-butyl (N^{ω}-2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl-N^{α}-glutaryl)-L-arginyl-glycyl-L-aspartate (*N*-Pbf-RGD-$(OBu^t)_2$, 16).

Mp 98–103 °C. $[\alpha]_D$ +1.7 (*c* 1.0, $CHCl_3$).

Found (%): C, 55.34; H, 7.32; N, 9.88; S, 4.04. Calc. for $C_{38}H_{60}N_6O_{12}S$ (%): C, 55.32; H, 7.33; N, 10.19; S, 3.89.

^{1}H NMR (400 MHz, DMSO-d_6, 100 °C) δ: 1.39 (s, 18H, 2 CO_2t-Bu); 1.41 (s, 6H, 2 CH_3 Pbf); 1.42–1.58 (m, 3H, CH_2); 1.68 (m, 1H, CH_2); 1.75 (m, 2H, CH_2); 2.02 (s, 3H, CH_3 Pbf); 2.17–2.26 (m, 4H, 2 CH_2); 2.44 (s, 3H, CH_3 Pbf); 2.50 (s, 3H, CH_3 Pbf); 2.53 (dd, 1H, C^3H_B Asp, *J* 16.1; 6.5); 2.64 (dd, 1H, C^3H_A Asp, *J* 16.1; 6.4); 2.95 (s, 2H, CH_2 Pbf); 3.05 (q, 2H, NCH_2 Arg, *J* 6.5); 3.72 (m, 2H, CH_2 Gly); 4.21 (ddd, 1H, CH Arg, *J* 7.7; 7.7; 5.6); 4.50 (ddd, 1H, CH Asp, *J* 8.0; 6.5; 6.5);

6.36 (s, 2H, NH_2 guanidine); 6.51 (t, 1H, NH guanidine, *J* 5.1);7.59 (d, 1H, NH Arg, *J* 7.4); 7.72 (d, 1H, NH Asp, *J* 7.8); 7.77 (t, 1H, NH Gly, *J* 5.1); 11.3 (br s, CO_2H).

FT-IR, ν (cm^{-1}): 3425, 3319 (NH_2, NH st); 2975, 2933, 2869 (C-H st, CH_2, CH_3); 1727 (C=O st, COOH (intramolecular H-bond), COO*t*-Bu); 1636 (C=O st, amide I); 1541 (NH δ, N-C=O st sy (amide II, H-bond); 1454, 1407, 1393 (C-N st, O-CONH st as, CH_2 δ, CH_3 δ); 1368 (S=O st); 1280, 1244 (CH_3 γ); 1149 (CH_3 γ, $C(CH_3)_3$); 1090 (S=O st; C-O st, COO*t*-Bu).

Dimethyl (N^{ω}-2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl-N^{α}-glutaryl)-L-arginyl-glycyl-L-aspartate (N-Pbf-RGD-$(OMe)_2$, 17).

Product **17** was purified by flash column chromatography (eluent $CHCl_3$-MeOH).

Mp 99–104 °C. $[\alpha]_D$ +4.6 (*c* 1.0, $CHCl_3$).

Found (%): C, 57.91; H, 6.23, N, 9.61; S, 3.43; Cl, 2.20. Calc. for $C_{42}H_{52}N_6O_{11}S \times 0.2\ CHCl_3$ (%): C, 58.07; H, 6.03; N, 9.63; S, 3.67; Cl, 2.44.

^{1}H NMR (400 MHz, DMSO-d_6, 24 °C) *δ*: 1.41 (s, 6H, 2 CH_3 Pbf); 1.32–1.52 (m, 3H, γ-CH_2, β-CH_B Arg); 1.62 (m, 1H, β-CH_A Arg); 1.70 (tt, 2H, $\underline{CH_2}CH_2CO_2H$, *J* 7.4); 2.01 (s, 3H, CH_3 Pbf); 2.18 (dt, 2H, CH_2CO_2H, *J* 16.3; 7.5); 2.22 (dt, 2H, $C\underline{H_2}CONH$, *J* 16.6; 7.5); 2.42 (s, 3H, CH_3 Pbf); 2.48 (s, 3H, CH_3 Pbf); 2.71 (dd, 1H, C^3H_B Asp, *J* 16.6; 6.8); 2.80 (dd, 1H, C^3H_A Asp, *J* 16.6; 6.2); 2.97 (s, 2H, CH_2 Pbf); 3.02 (m, 2H, δ-CH_2 Arg); 3.60 (s, 3H, CO_2Me); 3.62 (s, 3H, CO_2Me); 3.67 (dd, 1H, CH_B Gly, *J* 16.8; 5.7); 3.74 (dd, 1H, CH_A Gly, *J* 16.8; 5.8); 4.16 (dt, 1H, α-CH Arg, *J* 7.3; 6.0); 4.67 (dt, 1H, C^2H Asp, *J* 7.6; 6.6); 6.3–7.0 (m, 3H, NH Arg); 8.04 (d, 1H, NH Arg, *J* 7.3); 8.22 (d, 1H, NH Gly, *J* 6.0); 8.26 (d, 1 H, NH Asp, *J* 8.1); 12.1 (br s, CO_2H).

FT-IR, ν ($см^{-1}$): 3428, 3313 (NH_2, NH st); 2962, 2931, 2869 (C-H st, CH_2, CH_3); 1737 (C=O st, COOMe); 1650, 1620 (C=O st, COOH (intramolecular H-bond) and amide I); 1541 (NH δ, N-C=O st sy (amide II, H-bond); 1438, 1407, 1370 (C-N st, O-CONH st as, CH_2 δ, CH_3 δ); 1370 (S=O st); 1258, 1229 (CH_3 γ); 1087, 1014 (S=O st; C-O st, COOMe).

MNPs (19) modified with 3-aminopropylsilane.

FT-IR, ν (cm^{-1}): 3395 (NH st); 2958, 2921, 2864 (C-H st, CH_2); 1627 (NH δ); 1008 (Si-O st,); 619,

542, 432 (Fe-O st).

MNPs (20) modified with N^{ω}-Pbf-L-Arg-Gly-L-Asp(OBu^{t})$_2$ tripeptide.

FT-IR, ν (cm^{-1}): 3263 (NH st); 2967, 2926, 2863 (C-H st, CH_2, CH_3); 1729 (C=O st, COO*t*-Bu); 1622 (C=O st, amide I); 1538 (NH δ, N-C=O st sy (amide II); 1455, 1369 (C-N st, O-CONH st as); 1369 and 1091 (S=O st); 1253 (CH_3 γ); 1091 (C-O st, COO*t*-Bu); 995 (Si-O st,); 619, 542, 432 (Fe-O st).

MNPs (21) modified with N^{ω}-Pbf-L-Arg-Gly-L-Asp(OMe)$_2$ tripeptide.

FT-IR, ν (cm^{-1}): 3307 (NH st); 2932 (C-H st, CH_2, CH_3); 1730 (C=O st, COOMe); 1629 (C=O st, amide I); 1538 (NH δ, N-C=O st sy (amide II); 1436, 1389 (C-N st, O-CO-NH st as); 1369 and 1090 (S=O st); 1259 (CH_3 γ); 1190 (C-O st, COOMe); 996 (Si-O st,); 619, 534, 433 (Fe-O st).

MNPs (22) modified with L-Arg-Gly-L-Asp(OH)$_2$ tripeptide.

FT-IR, ν (cm^{-1}): 3255 (NH_2, NH st); 2923 (C-H st, CH_2); 1667 (shoulder), 1629 (broad band, C=O st, COOH (intramolecular H-bond) and C=O st, amide I); 1541 (NH δ, N-C=O st sy (amide II); 1405, 1382 (C-N st, O-CO-NH st as); 994 (Si-O st); 618, 536, 434 (Fe-O st).

MNPs (23) modified with L-Arg-Gly-L-Asp(OMe)$_2$ tripeptide.

FT-IR, ν (cm^{-1}): 3285 (NH_2, NH st); 2953, 2916, 2847 (C-H st, CH_2); 1719 (C=O st, COOMe (intramolecular H-bond)); 1633 (broad band, C=O st (amide I)); 1529 (NH δ, N-C=O st sy (amide II)); 1453, 1375 (C-N st, O-CO-NH st as); 1116, 1067 (C-O st, COOMe); 990 (Si-O st); 619, 542, 434 (Fe-O st).

Calculation of the amounts of RGD peptide derivatives immobilized on MNPs

Amount of immobilized RGD peptide was calculated by subtracting the carbon mass fraction (calculated from the elemental analysis data for the initial APS-modified MNPs ($\omega 1$)) from the carbon mass fraction of MNPs modified with RGD peptide ($\omega 2$). Based on the obtained value of the carbon mass fraction in the sample, the amount RGD peptide was calculated according to formula (1):

$$c = \frac{\omega 2 - \omega 1}{\omega 3 * M * 1g} \quad (1),$$

where *c* is the amount of RGD peptide on the surface of nanoparticles, mol/1 g MNPs;

ω1 is the carbon mass fraction of MNPs modified with RGD peptide according to the elemental analysis data;

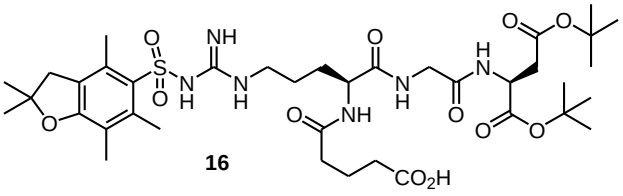
ω2 is the carbon mass fraction of the initial APS-modified MNPs according to the elemental analysis data;

ω3 is the carbon mass fraction in the corresponding derivative of RGD peptide according the elemental analysis data;

M is the molecular weight of the corresponding derivative of RGD peptide.

Table S1 Elemental analysis data for MNPs **19-23**.

	APS-modified MNPs **(19)**	N-Pbf-RGD-(O*t*-Bu)$_2$-modified MNPs **(20)**	N-Pbf-RGD(OMe)$_2$ -modified MNPs **(21)**	RGD-modified MNPs **(22)**	RGD(OMe)$_2$-modified MNPs **(23)**
C, %	2.64	5.66	6.30	3.86	4.69
ω1	0.0264				
ω2		0.0566	0.0630	0.0386	0.0469
ω3		0.5532 (N-Pbf-RGD-(O*t*-Bu)$_2$, **16**)	0.5188 (N-Pbf-RGD-(OMe)$_2$, **17**)	0.4435 (RGD, **24**)	0.4672 (RGD(OMe)$_2$, **25**)
M		824.98 (N-Pbf-RGD-(O*t*-Bu)$_2$, **16**)	740.82 (N-Pbf-RGD-(OMe)$_2$, **17**)	460.44 (RGD, **24**)	488.49 (RGD(OMe)$_2$, **25**)
c, mmol / 1g MNPs		0.067	0.095	0.060	0.090



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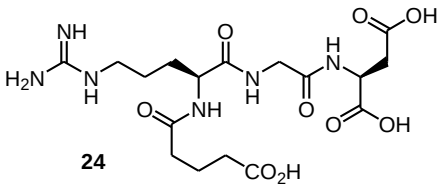

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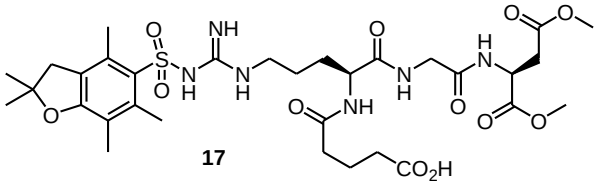

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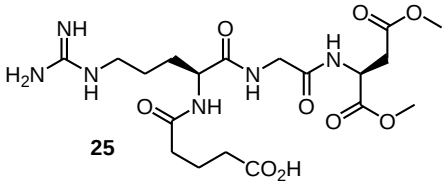

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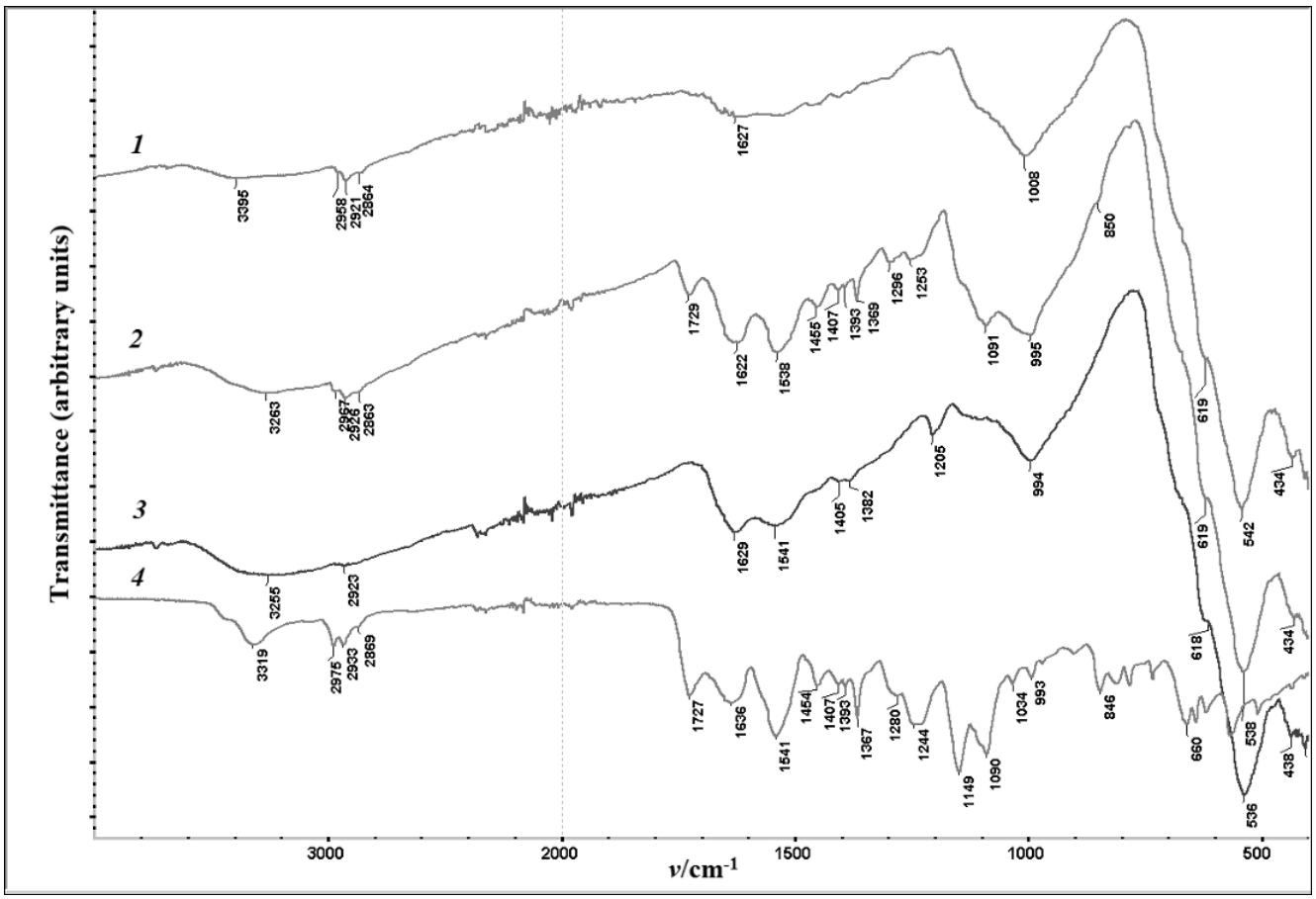

Figure S1 FT-IR spectra of (*1*) APS-modified MNPs **19**, (*2*) MNPs **20** modified with N^{ω}-Pbf-L-Arg-Gly-L-Asp(OBu^{t})$_2$ tripeptide, (*3*) RGD-modified MNPs **22**, and (*4*) tripeptide **16**.