Supplementary Informations

Synthesis of Gd-DO3adamantane (Scheme 1)

***N*-(benzyloxycarbonyl)-2-bromoethylamine (II)**

A solution of 2-bromoethylamine hydrobromide (71.7 g, 0.35 mol) in water (150 ml) and ethanol (150 ml) was brought with NaOH 10 N at 10 ºC to pH 7.1. A solution of benzyl chloroformate (50.0 ml, 0.35 mol) in dimethoxy ethane (100 ml) was added to the mixture dropwise over 2 h at < 20 ºC, maintaining the pH at 7. After 16 h at RT the organic solvent was removed by evaporation and the product was extracted with dichloromethane (3 x 50 ml). The organic layer was washed with water (3 x 30 ml), HCl 0.1 N (3 x 30 ml), water (30 ml), brine (30 ml), dried and evaporated to give a white solid (79.8 g).

^{1}H NMR (300 MHz, CD_3OD): δ 3.30 (t, 2H), 3.61 (t, 2H), 5.12 (s, 2H), 7.38 (m, 5H). ^{13}C NMR(300 MHz, CD_3OD): δ 20.8, 42.7, 66.5, 127.9, 128.2, 128.6, 137.3, 157.8.

1-[*N*-(benzyloxycarbonyl)-2-aminoethyl]-1,4,7,10-tetraazacyclododecane-4,7,10-triacetic(1,1-dimethylethyl ester) (III)

DO3A-tris-*tert*-butylester[1] **I** (37.1 g, 0.072 mol, the free base was obtained by passage of water/ethanol solution of DO3A HBr on Amberlite® IRA 410) was dissolved in acetonitrile (150 ml) and micronized K_2CO_3 (11.9 g, 0.086 mol) was added. The mixture was stirred at RT and a solution of **II** (20.4 g, 0.079 mol) in acetonitrile (150 ml) was slowly dropped over a period of 1 h. The reaction mixture was left stirring for 4 days at 50 ºC. After filtration the solvent was removed by evaporation under reduced pressure and the residue was treated with diethyl ether (100 ml) to give a white solid (DO3A HBr unreacted). After filtration the solution was evaporated in a vacuum and the product was purified by flash chromatography on silica with dichloromethane/methanol gradient. Fractions containing the product were combined and evaporated to give a yellow oil (34 g).

^{1}H NMR (300 MHz, CD_3OD): δ 1.48 (s, 27H), 1.70-3.18 (b m, 26H), 5.18 (s, 2H), 7.38 (m, 5H). MS [M+H$^+$] calcd: 691.9 found: 692.83.

1-(2-aminoethyl)-1,4,7,10-tetraazacyclododecane-4,7,10-triacetic(1,1-dimethylethyl ester) (IV)

Compound **III** (34.0 g, 0.049 mol) was dissolved in methanol (200 ml) and 10% Pd/C (2.0 g) was added. The reaction was carried out under hydrogen atmospheric pressure at RT for 6 h. The mixture was then filtered and the solvent was evaporated (28.6 g).

^{1}H NMR(300 MHz, CD_3OD): δ 1.45 (s, 27H), 1.85-3.24 (b m, 26H). MS [M+H$^+$] calcd: 557.77 found: 558.65.

4-(methyloxycarbonylmethylenoxy)benzenesulfonyl chloride (V)

To a solution of methyl phenoxyacetate (16.6 g, 0.1 mol) in dichloromethane (100 ml) at 0 °C was added dropwise over 1 h a solution of chlorosulfonic acid (16.5 ml, 0.25 mol) in dichloromethane (20 ml). After 16 hours at RT, the solution was poured into chopped ice and the mixture transferred to a separatory funnel with an additional 80 ml of dichloromethane. The organic phase was washed three times with ice water and then evaporated under reduced pressure to give a white solid (24.5 g).

^{1}H NMR (300 MHz, $CDCl_3$): δ 3.67 (s, 3H), 4.97 (s, 2H), 7.23 (d, 2H), 7.65 (d, 2H). ^{13}C NMR (300 MHz, CD_3OD): δ 54.8, 67.5, 117.6, 131.9, 139.5, 165.1, 170.2.

1-[*N*-[4-(methyloxycarbonylmethylenoxy)benzenesulfonyl]2-aminoethyl]-1,4,7,10-tetraazacyclododecane-4,7,10-triacetic(1,1-dimethylethyl ester) (VI)

To a solution of **IV** (12.1 g; 0.022 mol) in acetonitrile (40 ml) was added triethylamine (3.6 ml, 0.026 mol) and then a solution of **V** (5.5 g, 0.021 mol) in acetonitrile (30 ml) was dropped. After 3 days of stirring at RT the solvent was evaporated, the oil dissolved in dichloromethane (50 ml) and washed with water (3 x 30 ml) and brine (30 ml). The solvent was evaporated to give a yellow oil, that was purified by flash chromatography on silica with dichloromethane/methanol gradient. Fractions containing the product were combined and evaporated to give a yellow oil (12.8 g).

^{1}H NMR (300 MHz, CD_3OD): δ 1.52 (s, 27H), 1.71-3.25 (b m, 26H), 3.81 (s, 3H), 4.87 (s, 2H), 7.11 (d, 2H), 7.82 (d, 2H). ^{13}C NMR (300 MHz, CD_3OD): δ 27.3, 42.5, 50.4, 52.1, 52.8, 53.7, 53.9, 54.9, 56.8, 57.7, 58.5, 66.2, 83.6, 83.8, 116.4, 130.4, 133.5, 162.8, 170.5, 174.2, 174.6. MS [M+H$^+$] calcd: 785.99 found: 786.30.

1-[*N*-[4-(carboxymethylenoxy)benzenesulfonyl]2-aminoethyl]-1,4,7,10-tetraazacyclododecane-4,7,10-triacetic(1,1-dimethylethyl ester) (VII)

To a solution of **VI** (7.2 g, 0.0092 mol) in methanol (20 ml) and water (40 ml) was dropped NaOH 2N in order to maintain the pH around 12. After 1 h of stirring the pH remained stable: the organic solvent was evaporated and the aqueous solution was brought to pH 4 with HCl 37%. The desired product was extracted with dichloromethane (3 x 20 ml) and the organic phase was dried and evaporated to give a yellow oil (6.7 g).

^{1}H NMR (300 MHz, CD_3OD): δ 1.52 (s, 27H), 1.71-3.25 (b m, 26H), 4.76 (s, 2H), 7.11 (d, 2H), 7.82 (d, 2H). ^{13}C NMR (300 MHz, CD_3OD): δ 27.3, 42.5, 50.4, 52.1, 52.8, 53.7, 53.9, 54.9, 56.8, 57.7, 66.2, 83.6, 83.8, 116.4, 130.4, 133.5, 162.8, 170.5, 174.2, 178.3.

1-[*N*-[4-[(adamantan-1-yl)aminecarbonyloxymethylenoxy]benzenesulfonyl]2-aminoethyl]-1,4,7,10-tetraazacyclododecane-4,7,10-triacetic(1,1-dimethylethyl ester) (VIII)

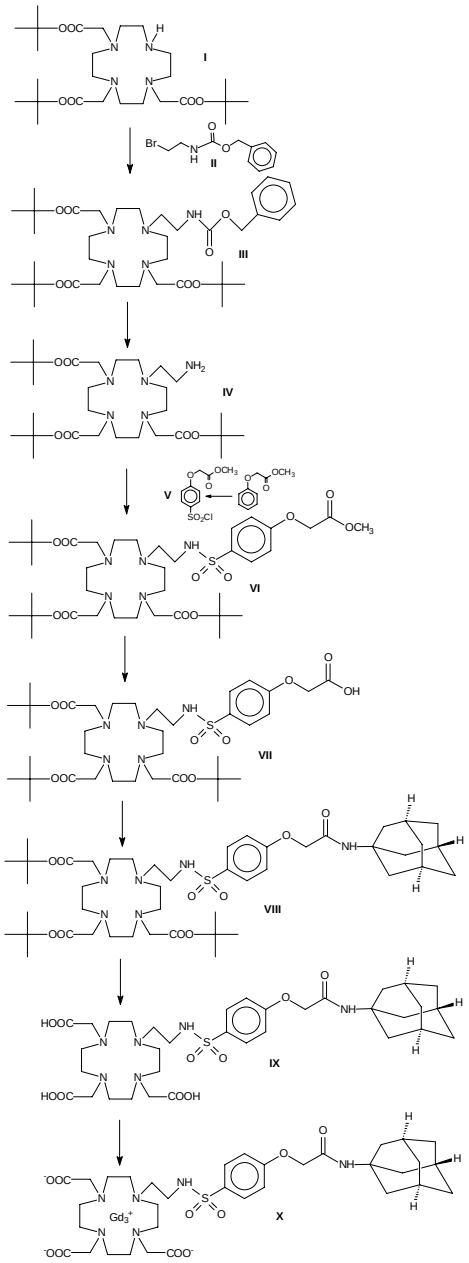
To a solution of **VII** (1.9, 0.0025 mol) in dimethylformamide was first added a solution of 1-adamantamine (0.37 g, 0.0025 mol) in dimethylformamide and then HATU (1.1 g, 0.0029 mol). After 20 h of stirring at RT the solvent was evaporated, the oil was dissolved in dichloromethane (20 ml) and washed with $NaHCO_3$ 5 % (3 x 15 ml), HCl 0.1 N (3 x 15 ml) and brine. The organic phase was dried and evaporated to give a yellow oil (2.5 g) that was purified by flash chromatography on silica with dichloromethane/methanol gradient. Fractions containing the product were combined and evaporated to give an oil (1.69 g).

^{1}H NMR (300 MHz, CD_3OD): δ 1.51 (s, 27H), 1.58 (s, 6H) 1.62-3.25 (b m, 26H), 1.71 (s, 6H), 2.05 (s, 3H), 4.87 (s, 2H), 7.11 (d, 2H), 7.82 (d, 2H). MS [M+H$^+$] calcd: 905.22 found: 905.58.

1-[*N*-[4-[(adamantan-1-yl)aminecarbonyloxymethylenoxy]benzenesulfonyl]2-aminoethyl]-1,4,7,10-tetraazacyclododecane-4,7,10-triacetic acid (IX)

To a solution of **VIII** (0.82 g; 0.91 mmol) in dichloromethane (5 ml) was slowly added neat trifluoroacetic acid (0.28 ml, 3.6 mmol) and the solution was stirred at RT for 1 h. The dichloromethane was evaporated and the residue treated with trifluoroacetic acid (1.5 ml, 0.019 mol) and triisopropylsilane (0.2 ml) was added. The solution was stirred for 24 h, then diethyl ether was slowly added (20 ml) to give a slightly yellow solid, which was filtered, washed with diethyl ether and dried (0.76 g).
^{1}H NMR (300 MHz, CD_3OD): 1.57 (m, 6H) 1.62-3.25 (b m, 26H), 1.89 (s, 6H), 2.21 (s, 3H), 4.75 (s, 2H), 7.02 (d, 2H), 7.65 (d, 2H). MS [M+H$^+$] calcd: 736.88 found: 737.32.

1-[*N*-[4-[(adamantan-1-yl)aminecarbonyloxymethylenoxy]benzenesulfonyl]2-aminoethyl]-1,4,7,10-tetraazacyclododecane-4,7,10-triacetic gadolinate (1:1) (X)
The complexation was performed with $GdCl_3$ in aqueous solution at pH 7 by the method of the addition of the ligand.[2] An equimolar amount of $GdCl_3$ (82 mg, 0.22 mmol) solution in water was added to the aqueous solution of ligand (274 mg, 0.22 mmol, purity 60%), maintaining the pH value at 7 with NaOH 0.1 N. The mixture was allowed to stir at RT until the pH remained constant at 7. The solution was then lyophilised to give a white solid (399 mg). The amount of residual free Gd^{3+} ion was assessed by the orange xylenol UV method[3] and the corresponding amount of ligand was added in order to avoid the presence of free Gd^{3+} ions.



Scheme 1

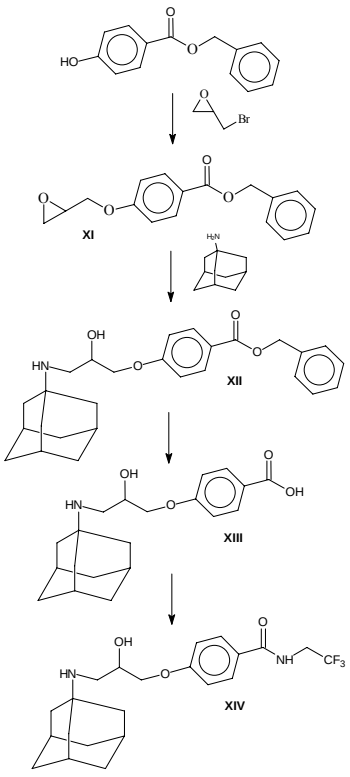
Synthesis of ^{19}F-reporter (Scheme 2)

4[(2,3-epoxy)propyloxy]phenylacetic acid phenyl methyl ester (XI)
To a solution of benzyl 4-hydroxybenzoate (4.0 g, 0.018 mol) in dimethylformamide was added micronized K_2CO_3 (3.3 g, 0.024 mol) and epibromohydrin (3.0 g, 0.022 mol). After 6 h of stirring at 70 °C and 16 h at RT, the suspension was filtered and the salts washed several times with fresh dimethylformamide. The solution was then concentrated, the oil resuspended in ethyl acetate (15 ml) and washed with water (3 x 10 ml) and brine. The organic phase was dried and evaporated to give a yellow solid (5.0 g).
^{1}H NMR (300 MHz, CD_3OD): δ 2.80 (m, 1H), 2.95 (t, 1H), 3.39 (m, 1H), 4.01 (m, 1H), 4.32 (d d, 1H), 5.36 (s, 2H), 6.96 (d, 2H), 7.44 (b m, 5H), 8.06 (d, 2H).

4[*N*(adamantan-1-yl)-3-amine-2-hydroxypropyloxy]phenylacetic acid phenyl methyl ester (XII)
To a solution of 1-adamantamine (1.3 g, 0.008 mol) in acetonitrile (20 ml) was added DIPEA (1.6 ml, 0.0096 mol) and then a solution of **XI** (2.7 g, 0.0096 mol) in acetonitrile (15 ml) was slowly dripped. After 5 days of stirring at 50 °C was observed the formation of a white precipitate that was filtered and washed with cold acetonitrile (2.2 g).
^{1}H NMR (300 MHz, CD_3OD): δ 1.58 (m, 12H), 2.12 (s, 3H), 2.74 (m, 1H), 2.97 (d, 1H), 4.05 (m, 3H), 5.36 (S, 2H), 6.94 (d, 2H), 7.45 (b m, 5H), 8.07 (d, 2H). ^{13}C NMR (300 MHz, CD_3OD): δ 31.8, 38.9, 44.8, 49.1, 53.8, 68.9, 70.5, 73.0, 116.4, 125.0, 130.2, 130.8, 134.1, 138.8, 165.1, 168.2. MS [M+H$^+$] calcd: 435.56 found: 436.17.

4[*N*(adamantan-1-yl)-3-amine-2-hydroxypropyloxy]phenylacetic acid hydrochloride (1:1) (XIII)
Compound **XII** (1.9 g, 0.0044 mol) was dissolved in tetrahydrofurane (30 ml) and 10% Pd/C (0.090 g) was added. The reaction was carried out under hydrogen atmospheric pressure at RT. During the reaction the desired product precipitated and was at the end dissolved in hot slightly acid water (100 ml). The mixture was then filtered and the solvent was evaporated giving a white solid that was dried with NaOH and P_2O_5 (1.56 g).
^{1}H NMR (300 MHz, D_2O): δ 1.51 (d d, 6H), 1.78 (m, 6H), 2.14 (s, 3H), 3.03 (t, 1H), 3.18 (d, 1H), 4.01 (m, 2H), 4.12 (m, 1H), 6.92 (d, 2H), 7.83 (d, 2H). MS [M+H$^+$] calcd: 345.44 found: 346.21.

***N*-(2,2',2''-trifluoroethyl)-4[*N*(adamantan-1-yl)-3-amine-2-hydroxypropyloxy]phenylacetamide (XIV)**
To a solution of **XIII** (0.54 g, 1.35 mmol) in dimethylformamide (10 ml) was added 2,2',2''-trifluoroethylamine (0.61 g, 1.6 mmol) and DIPEA (2.0 ml, 2.97 mmol): the free base immediately precipitated but was dissolved again after that HBTU (0.16 g, 2.0 mmol) was added to the suspension. After 16 h of stirring at RT, the solution was concentrated and the mixture was purified by liquid chromatography on Amberchrom® CG161 resin with a water-acetonitrile gradient. Fractions containing the product were combined and evaporated to give a white solid (0.63 g).
^{1}H NMR (300 MHz, CD_3CN): δ 1.72 (q, 6H), 1.94 (m, 6H), 2.20 (s, 3H), 3.09 (m, 1H), 3.29 (m, 3H), 4.08 (m, 2H), 4.25 (m, 1H), 7.02 (d, 2H), 7.82 (d, 2H). ^{13}C NMR (300 MHz CD_3CN): δ 29.8, 35.6, 38.5, 40.9, 43.7, 59.8, 66.1, 70.6, 114.4, 127.3, 129.9, 160.0, 161.8, 166.6. MS [M+H$^+$] calcd: 426.48 found: 427.21.



Scheme 2

Determination of Binding parameters between the Gd-complex and Poly-β-Cyclodextrin

The affinity binding of the adamantane functionalized Gd-complex towards Poly-β-CD substrate has been assessed through the Proton Relaxation Enhancement (PRE) method. This is a non-separative technique in which the binding parameters can be obtained by exploiting the differencies in water proton relaxation rates between the bound and unbound species. Since the relaxation rate can be markedly increased when a paramagnetic substrate interacts with a macromolecular system, this method is perfectly taylored to investigate the binding of a paramagnetic metal chelate. Besides providing informations about the association constant (K_A) this technique also allows the assessment of the relaxivity of the paramagnetic complex bound to the macromolecular substrate (r_{1p}^b).

In the case of the following binding equilibrium between a Gd-complex (GdL) and a macromolecular host (H):

$$GdL + H \leftrightarrow GdL - H \qquad (1)$$

The affinity constant K_A is given by the equation:

$$K_A = \frac{[GdL - H]}{[GdL][nH]} \qquad (2)$$

in which [nH] indicates the concentration of the equivalent and independent binding sites on the host.

The measured longitudinal proton relaxation rate (R_1^{obs}) is given by the sum of the contribution arising from the unbound and the bound species as well as the diamagnetic contribution of the host (R_{1H}) :

$$R_{1obs} = \left(r_1[GdL] + r_1^b[GdL - H]\right)1000 + R_{1H} \qquad (3)$$

Where r_1 and r_1^b are the millimolar relaxivity of the unbound and bound GdL respectively.

Combination of the equations 2 and 3, allow to correlate the measured R_{1obs} to the binding parameters K_A and n, as follows:

$$R_{1obs} = \frac{(K_A GdL_T + nK_A H_T + 1) - \sqrt{(K_A GdL_T + nK_A H_T + 1)^2 - 4K_A^2 GdL_T nH_T}}{2K_A}$$
$$-(r_1^b - r_1 + r_1 GdL_T)1000 + R_{1H} \qquad (4)$$

Where GdL_T and H_T are the total molar concentrations of the GdL and the host respectively.

The experimental procedure consists of carrying out a titration in which a fixed concentration of Gd-complex (0.1 mM) is titrated with increasing concentrations of macromolecular host; the relative observed relaxation rates increase according to the K_A and r_{1p}^b values (Fig. 1). In this case a K_A of 1300 M^{-1} and a r_{1p}^b of 17.7 $mM^{-1}s^{-1}$ have been determined.

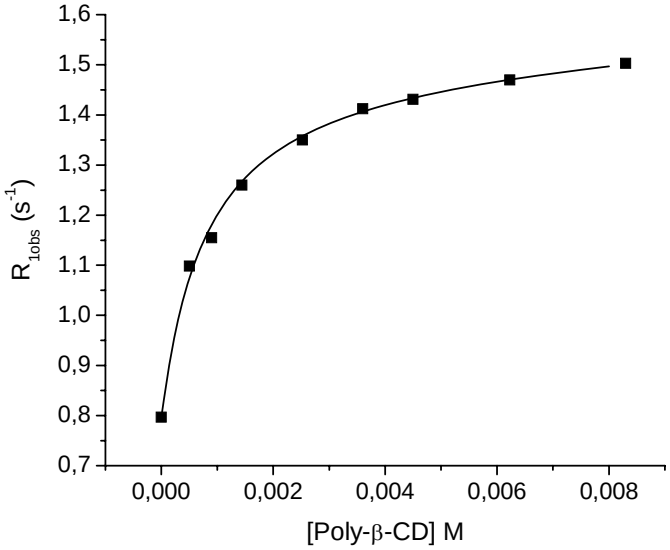


Figure 1

Determination of Binding association constant between the ^{19}F-reporter and Poly-β-Cyclodextrin

In the case of the diamagnetic ^{19}F-reporter, the affinity constant toward Poly-β-CD has been determined by measuring the chemical shift changes of the ^{1}H-NMR signals of the molecule as a consequence of the addition of increasing amounts of Poly-β-CD. The experimental data have been fitted with equations analogous to those reported in the previous section but accounting for a chemical shift variation instead of a relaxation rate one. In Fig. 2 the experimental data relative to three signals of the adamantane moiety of a 4.3 mM solution of ^{19}F-reporter titrated with increasing amounts of Poly-CD (600 MHz, 298K) are reported. Ter-But 5mM was used as a reference. Fitting of the three set of experimental data invariantly gave a K_A value of 14000 M^{-1}.

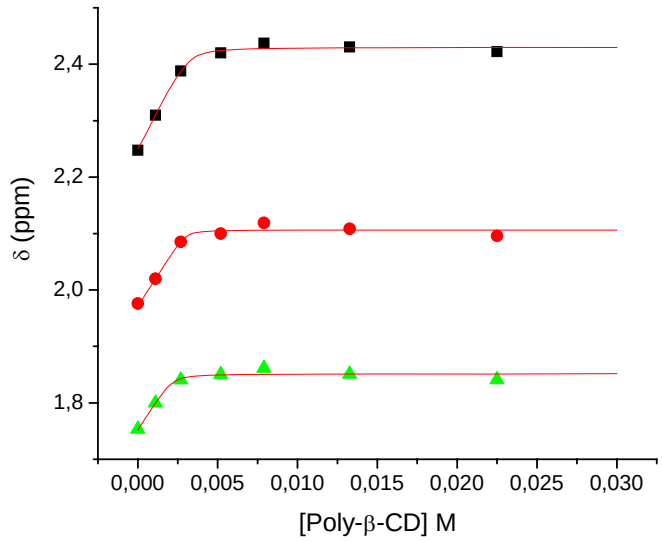


Figure 2

Calibration lines for the determination of Gd(III) concentration

The unknown concentration of Gd-probe in the different capillaries have been calculated by the use of two different calibration lines following the linear correlation between either ^{19}F-R_{1obs} and ^{19}F-signal intensities and the Gd-complex concentration. Both the calibration lines have been acquired at 7.1 T and 298K. For ^{19}F signal intensities measures a reference containing $NaPF_6$ 25 mM was used.

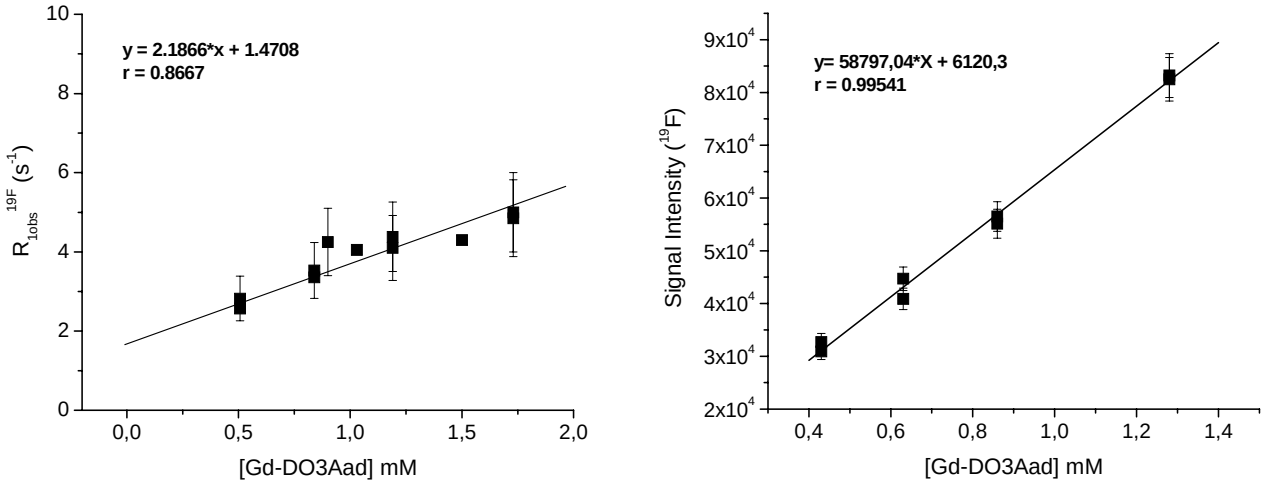


Figure 3

References

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3 A. Barge et al. *Contrast Med. Mol. Imaging,* 2006, **1**, 184.