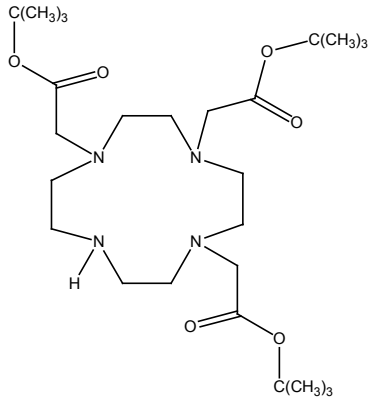
Hybrid nano-sized particles of boehmite with lanthanides complexes as imaging agents

Estefanía Delgado-Pinar,[a] M. Teresa Albelda,[a] Juan C. Frías,[a] Olga Barreiro,[b] Emilio Tejera,[b] Vojtěch Kubíček,[c] Luis J. Jiménez-Borreguero,[b] Francisco Sánchez-Madrid,[b] Éva Tóth,[c] Javier Alarcón*[d] and Enrique García-España*[a]*

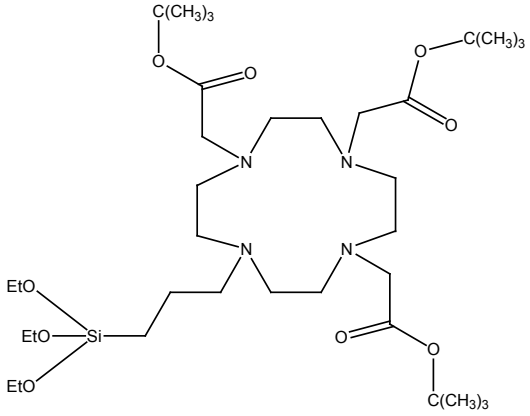
DO3A-tBu



0.750 g (4.21 mmol) of 1,4,7,10-Tetraazacyclododecano (cyclen) and 1.17 g of $NaHCO_3$ (13.90 mmol) were stirred on 25 mL of dried CH_3CN at 0ºC. 2.03 mL (13.90 mmol) of tert-butyl bromoacetate was added dropwise. The reaction was left to react for 30 hours. Protected cyclen was separated by column chromatography (SiO_2 CH_2Cl_2:MeOH). Yield:46 %

NMR: δ_H:1.44(27H, s, *tert*-Bu), 2.87(4H, s, CH_2), 2.90(8H, s, CH_2), 3.08(4H, s, CH_2-amine), 3.27(2H, s, CH_2-CO_2), 3.36(4H, s, CH_2-CO_2), 9.96(1H, s, NH). δ_C 28.5, 47.8, 51.7, 58.5, 82.0,170.0, 170.8; *m/z* (CI) 515(MH^+).

DO3A-tBu-TMSi



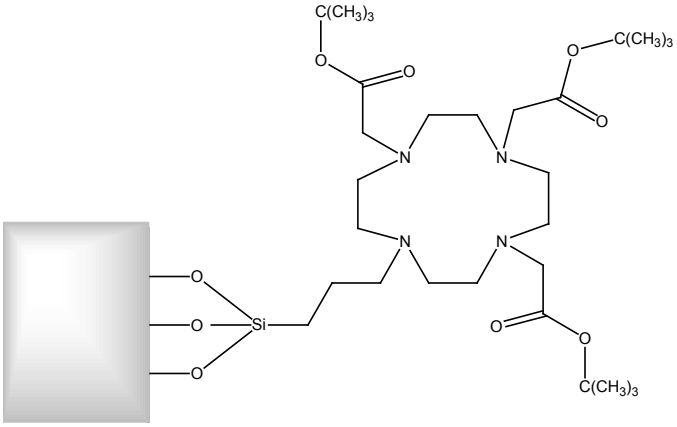
0.100 g (0.94553 mmol) of protected cyclen and 0.26 g (1.94553 mmol) of K_2CO_3 were stirred in dry CH_3CN under reflux. 39.27 µL (0.94553 mmol) of (3-iodopropyl)trimethoxysilane were added and the mixture refluxed for 48 hours. After 48

h se solution was cooled at room temperature and the solvent vacuum removed. The oil obtained is used for the next step without further purification.

DTPA-TMSi

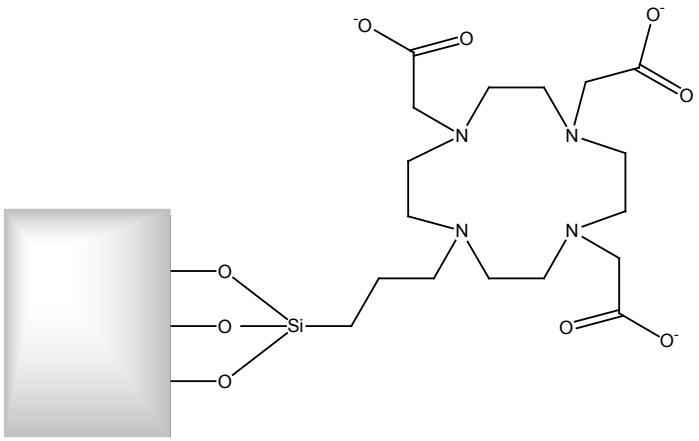
DTPA-TMSi was prepared in a similar fashion as DO3A-tBu-TMSi.

Grafted DO3A-tBu-TMSi



The oil obtained from the previous step was dissolved in toluene. To this solution was added 0.291 mmol of boehmite and the mixture refluxed for 24 hours. The solid is separated by centrifugation at 4000 rpm for 10 minutes and washed 4 times with EtOH. No unreacted (3-iodopropyl)trimethoxysilane or the hydrolyzed form was found in the nanoparticles as measured later on by EDX.

Deprotected DO3A-TMSi



The solid obtained is stirred for 3-4 hours in a mixture of CH_2Cl_2:TFA (3:1). The acid is removed by vacuum evaporation. The traces of acid are removed by washing and centrifuging the solid with CH_2Cl_2, CH_2Cl_2/EtOH and EtOH.

Grafted DTPA-TMSi

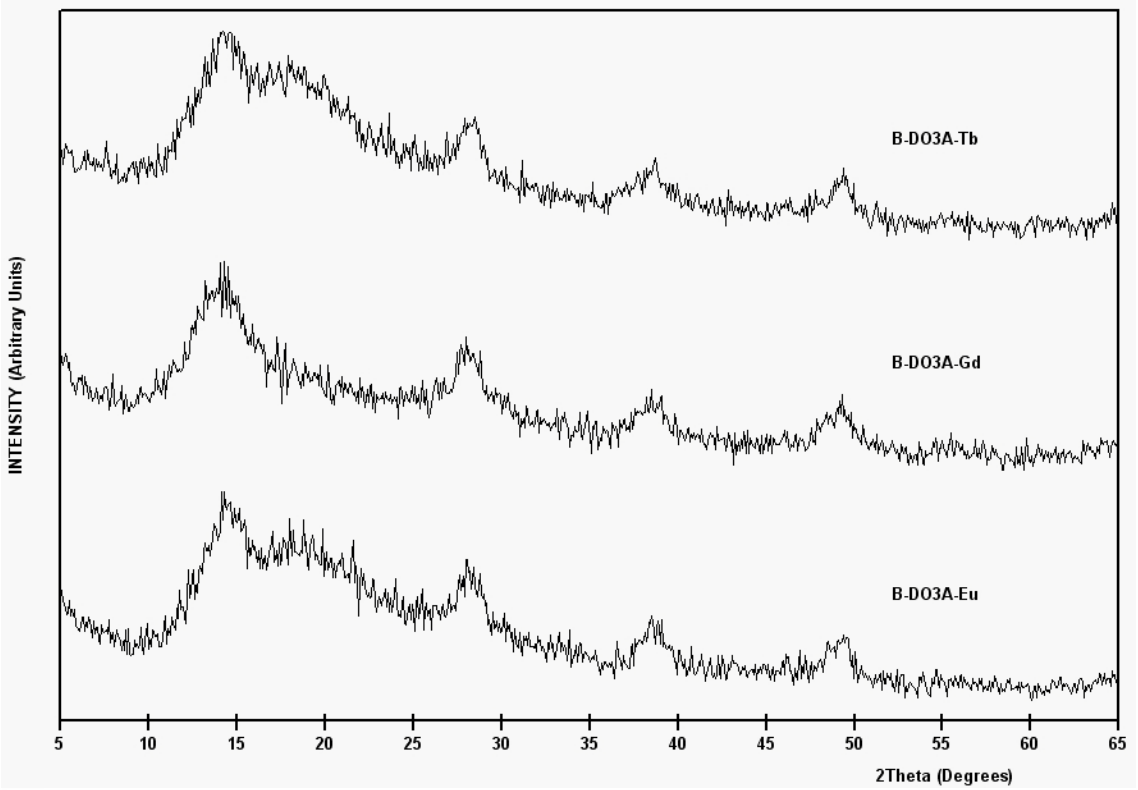
Grafted DTPA-TMSi was supported on boehmite in a similar way as DO3A-tBu-TMSi

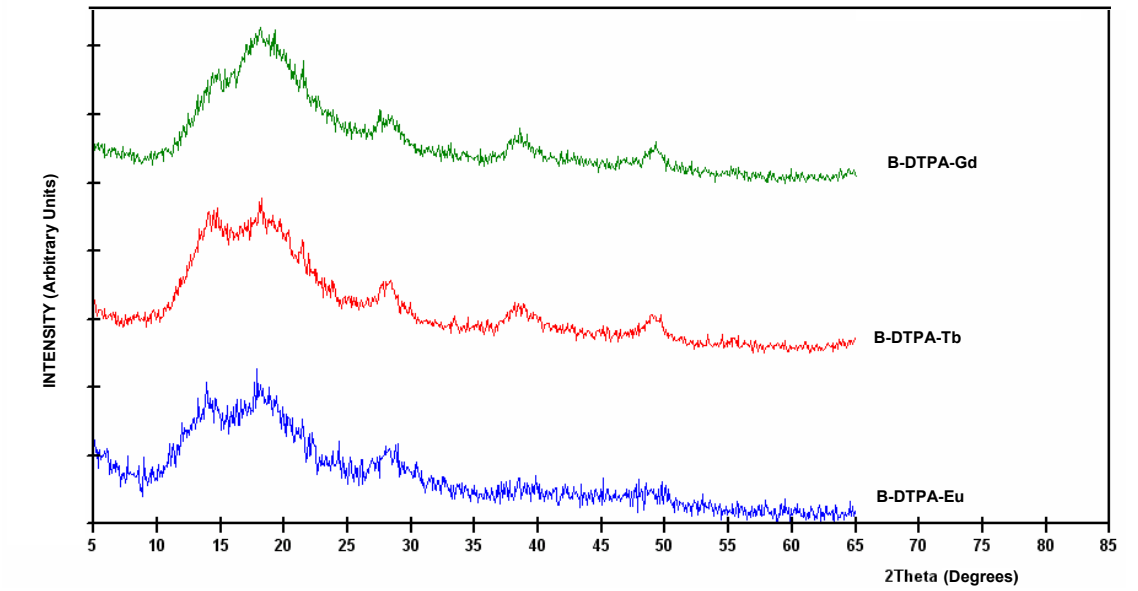
Lanthanide complexes

The solid obtained is dispersed in 25 mL of CH_3CN and an equimolar quantity of lanthanide chloride is added.

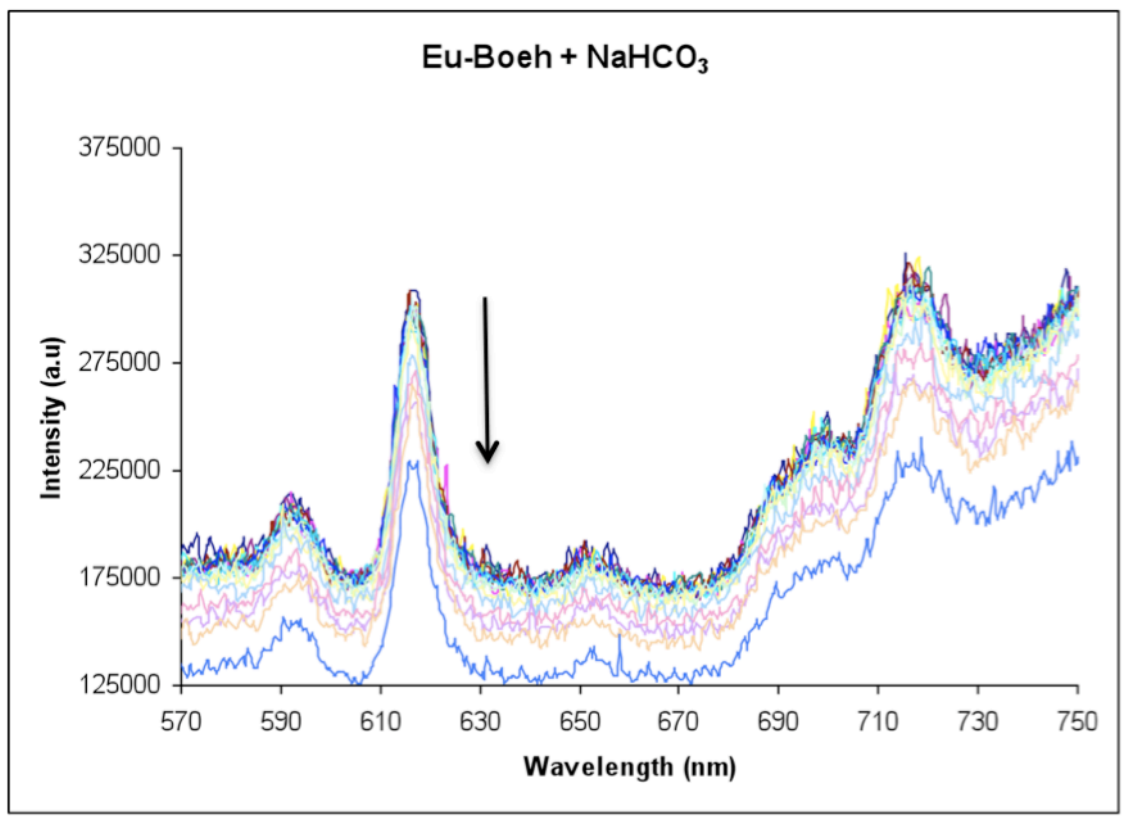
XRD

Supported materials, LnDO3A-boehmite and LnDTPA-boehmite, were checked by conventional solid state techniques. X-Ray powder diffractograms of Eu-, Tb- and GdDO3A-boehmite show the presence of broad peaks at 13.7°, 28.3°, 38.4°, 49.2° and 64.8° 2θ/□which are characteristic of the boehmite structure. This result evidences that the matrix has not been disrupted throughout the grafting step and the width of the peaks confirms the small size measured by electron microscopy.



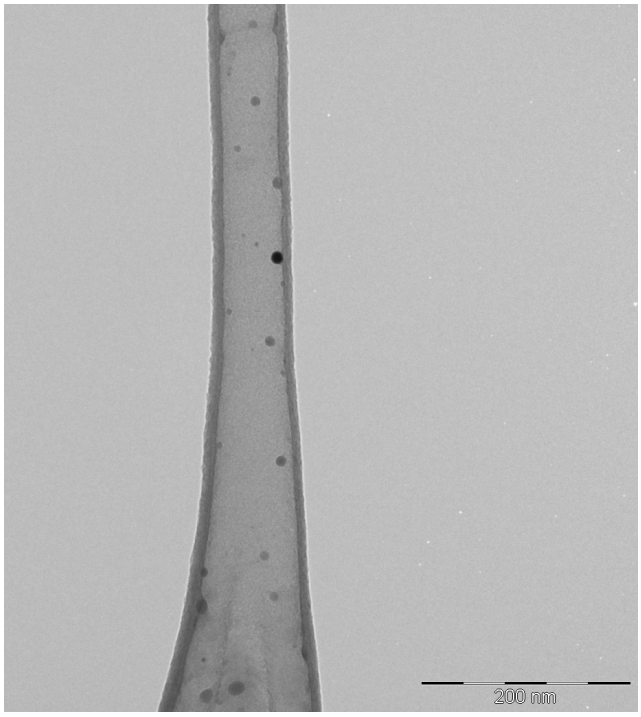


XRD reflection patterns for Tb-boehmite, Gd-boehmite and Eu-boehmite chelated by DO3A or DTPA.



Eu-DO3A-boehmite titration at pH 7.4 showing no changes in the $\Delta J = 2/\Delta J = 1$ ratio.

TEM



TEM micrograph of Eu-boemite-DTPA.

Laser confocal microscopy

Human HeLa cells were seeded on glass coverslips and grown in DMEM (Sigma Chemical Co.; St Louis, MO) supplemented with 10% fetal bovine serum (Linus, Spain), 4.5 g/L glucose, L-glutamine, 500 U/mL penicillin/streptomycin and 20 mM Hepes (Biowhitaker, Walkersville, MD). When reached confluence, monolayers were incubated with a suspension of fresh medium containing 100 μM of europium or terbium particles for 4 hours. Then, coverslips were extensively washed and fixed with 4% paraformaldehyde in PBS. Immunofluorescence staining was performed as previously described.[3] Anti-CD44 (HP2/9) (a cell-surface glycoprotein involved in cell-cell interactions, cell adhesion and migration), generated in our laboratory at CNIC, was used as primary antibody. Goat anti-mouse Alexa 647 (Molecular Probes, Invitrogen, Carlsbad, CA) was employed as secondary antibody. Next, samples were mounted using Prolong (Molecular Probes). Series of optical sections were obtained with a Leica TCS-SP5 confocal laser scanning unit (Leica Microsystems, Heidelberg, Germany), using a 63x oil immersion objective (NA 1.4), a 405 nm diode for the nanoparticles excitation and a 633 nm laser line for Alexa 647 visualization. Images were analyzed using Leica Confocal Software.

Flow cytometry analysis

Human HeLa cells were cultured and incubated with lantanides as described above. Then, cells were trypsinized and washed in PBS, followed by incubation with annexin-V Alexa 647 (Molecular Probes) for 15 min at room temperature. Finally propidium iodide was added to a final concentration of 0.001% and samples were analyzed in a flow cytometer (FACSCantoTM II, BD Biosciencies). Cells were subdivided in 4 categories: negative for both stainings (viable), positive for propidium iodide (early apoptotic), double positive (intermediate apoptotic) and only positive for annexin V (necrotic).

References

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(2) Ek, S.; Iiskola, E. I.; Niinistö, L.; Vaittinen, J.; Pakkanen, T. T.; Root, A. *J. Phys. Chem. B*, **2004**, *108*, 11454.

(3) Barreiro, O.; Yañez-Mo, M.; Sala-Valdes, M.; Gutierrez-Lopez, M. D.; Ovalle, S.; Higginbottom, A.; Monk, P. N.; Cabañas, C.; Sanchez-Madrid, F. *Blood* **2005**, *105*, 2852.