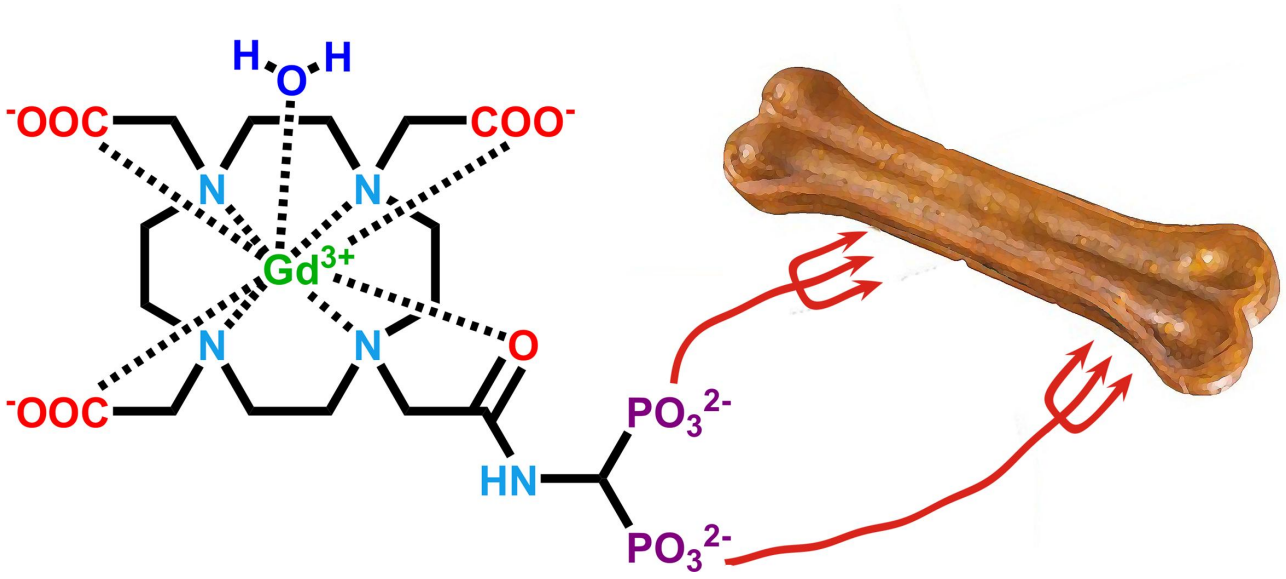
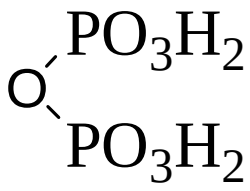
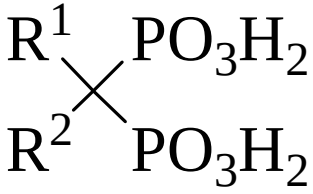
A bis(phosphonate) mono-amide analogue of DOTA: a potential agent for bone-targeting



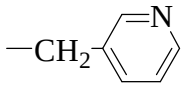
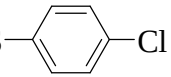
Bis(phosphonates)



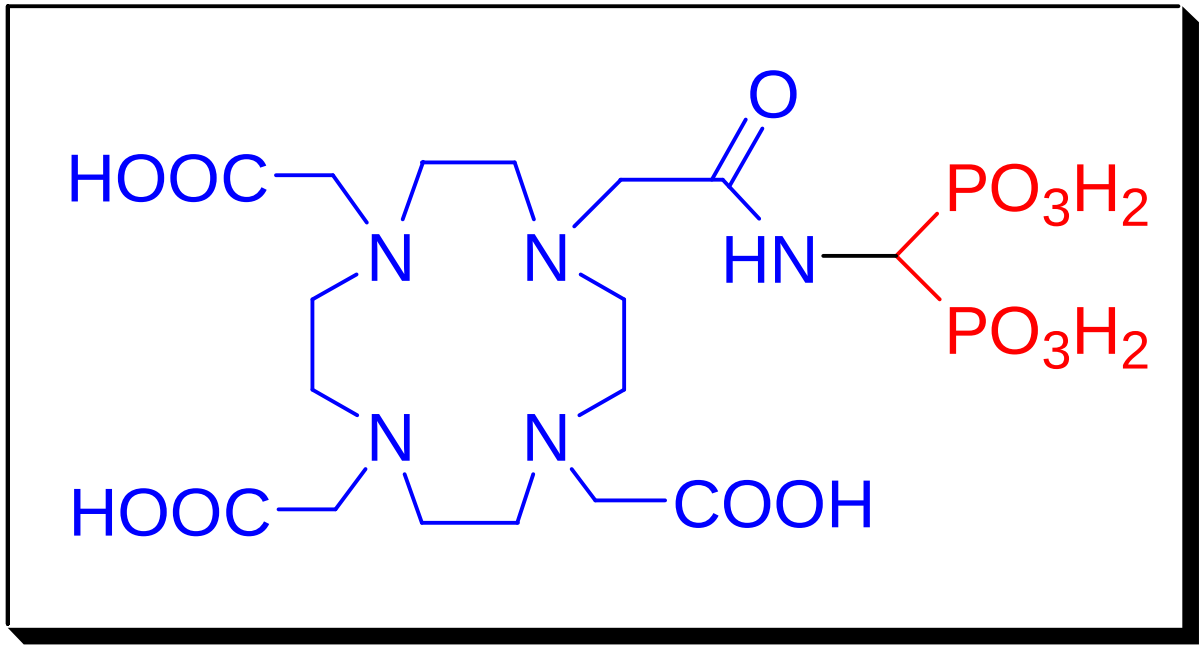
Pyrophosphate



Geminal bis(phosphonate)

Commercial name	R^1	R^2
Aledronate	—OH	—$(CH_3)_3$—NH_2
Clodronate	—Cl	—Cl
Etidronate	—OH	—CH_3
Ibadronate	—OH	—$(CH_3)_2$—N(CH_3)($(CH_2)_4$—CH_3)
Pamidronate	—OH	—$(CH_3)_2$—NH_2
Risedronate	—OH	—CH_2— 
Tiludronate	—H	—S—  —Cl

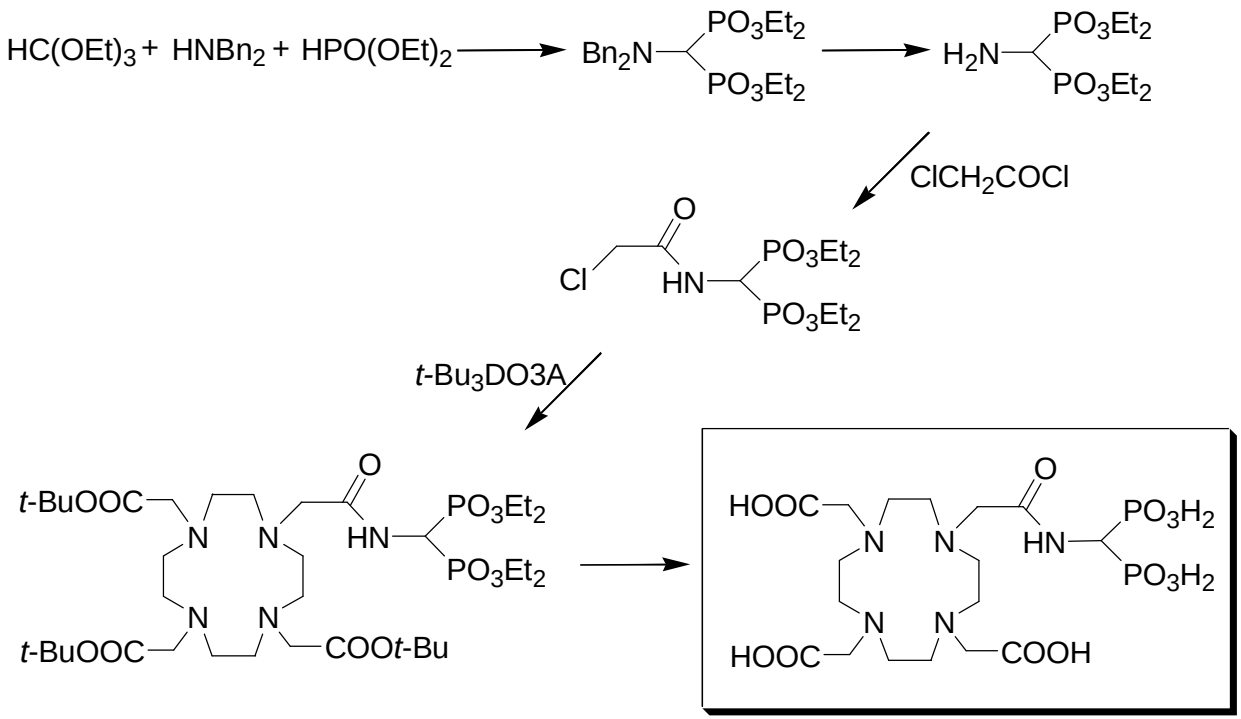
Ligand BPAMD



Bis(phosphonic acid) group – bone targeting group

DOTA monoamide – chelating group for lanthanide(III) ions

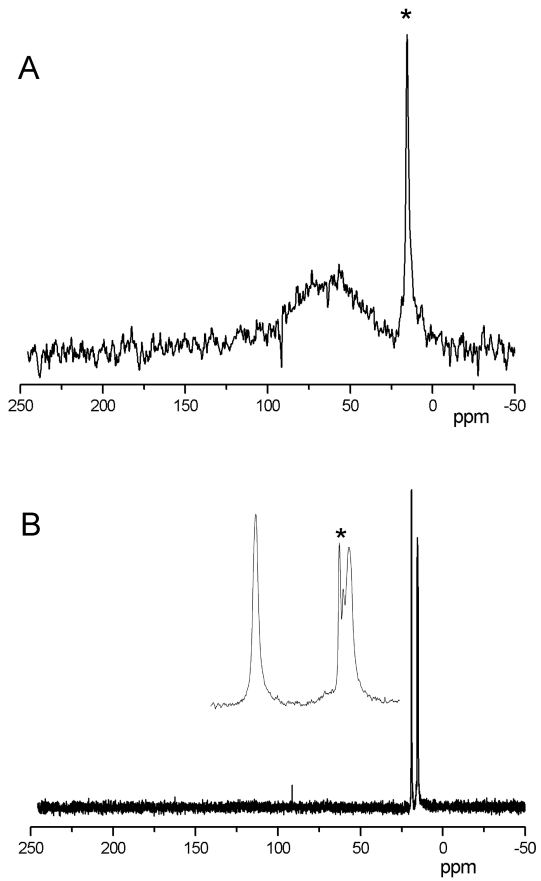
Synthesis


HC(OEt)3 + HNBn2 + HPO(OEt)2
Bn2N
PO3Et2
PO3Et2
H2N
PO3Et2
PO3Et2
ClCH2COCl
O
Cl
HN
PO3Et2
PO3Et2
t-Bu3DO3A
t-BuOOC
t-BuOOC
N
N
N
N
O
HN
PO3Et2
PO3Et2
COOt-Bu
HOOC
HOOC
N
N
N
N
O
HN
PO3H2
PO3H2
COOH

Complexation of Ln(III) ions

3 step process:

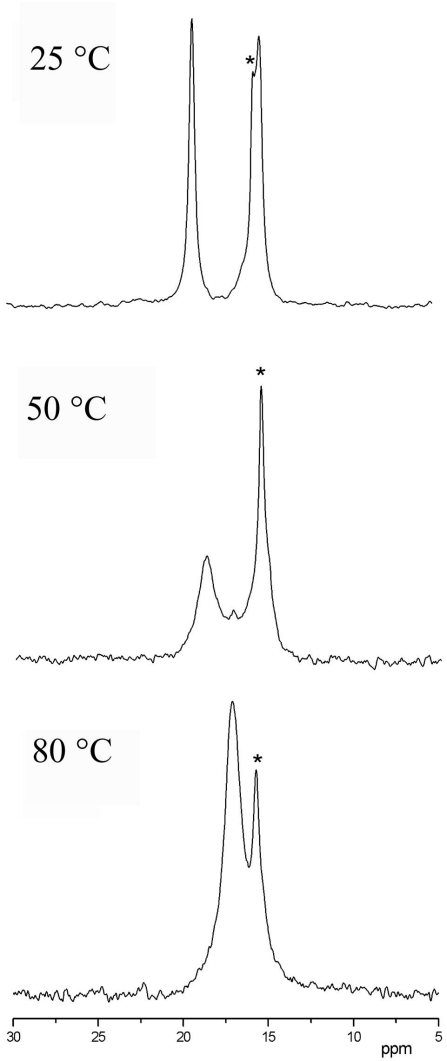
- pH 2–4: only phosphonates are coordinated
- pH 7–9: “out of cage” complex (A)
- After heating: DOTA-like complex (B)



The signals of excess of the free ligand are marked with asterisks

Ln-BPAMD complexes

- All pendants coordinated
- One molecule of water in the inner coordination sphere
- Bis(phosphonic acid) group is not coordinated
- Phosphorus atoms are nonequivalent



The signals of excess of the free ligand are marked with asterisks

Relaxometry of Gb-BPAMD

- Slow water exchange
- High value of relaxivity
- Expected second hydration sphere

Ligand	Δ^2 [$s^{-2}/10^{19}$]	τ_v [ps]	τ_r [ps]	τ_M [μs]	r_1 (20 MHz) [s^{-1} mM^{-1}]
BPAMD	3.7 ± 0.2	17 ± 1	88 ± 3	1.18 ± 0.6	5.3
DOTA[a]	1.6	11	77	0.244	4.8
DOTA-bis(methylphosphonic) monoamide[b]	1.8	21	97	1.6	6.2

[a] Powell, D. H.; Dhubhghaill, O. M. N.; Pubanz, D.; Helm, L.; Lebedev, Y. S.; Schlaepfer, W.; Merbach, A. E. *J. Am. Chem. Soc.* **1996**, *118*, 9333–9346
[b] Aime, S.; Botta, M.; Garino, E.; Geninatti Crich, S.; Giovenzana, G.; Pagliarin, R.; Palmisano, G.; Sisti, M. *Chem. Eur. J.* **2000**, *6*, 2609–2617

Relaxometry of Gb-BPAMD

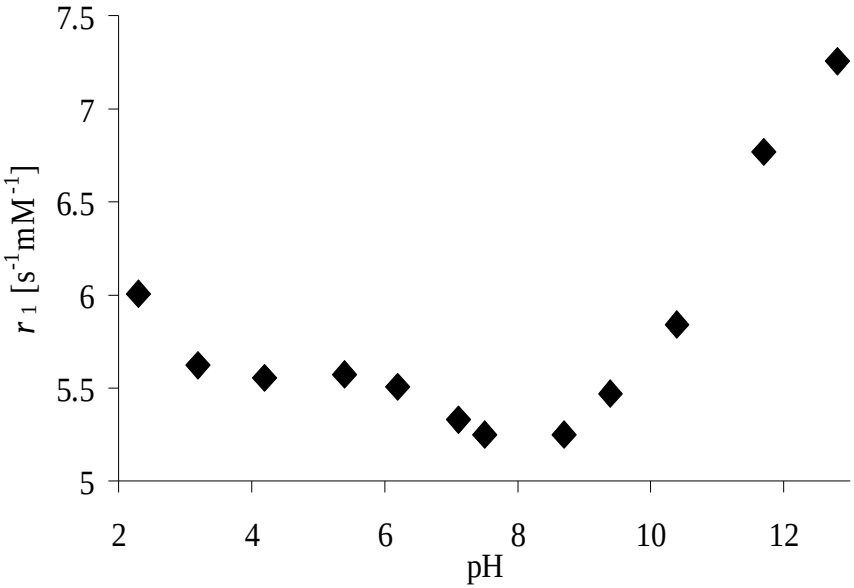
pH dependence of relaxivity

- Two steps in relaxivity: pH = 2–3 and 6–8

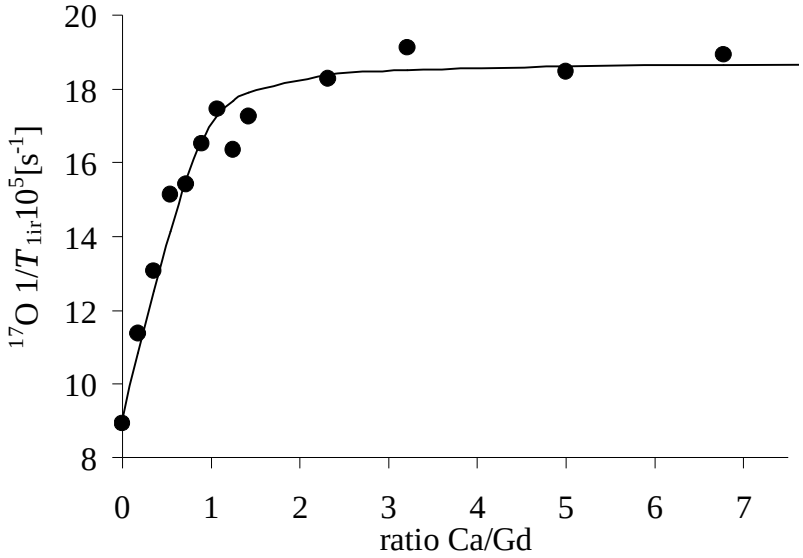
  ➡ protonation of bis(phosphonic) acid group

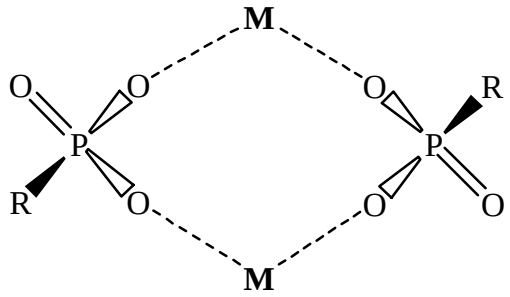
- Increase in basic region:

  ➡ prototropic exchange



Interaction of Gd-BPAMD with Ca(II) ions

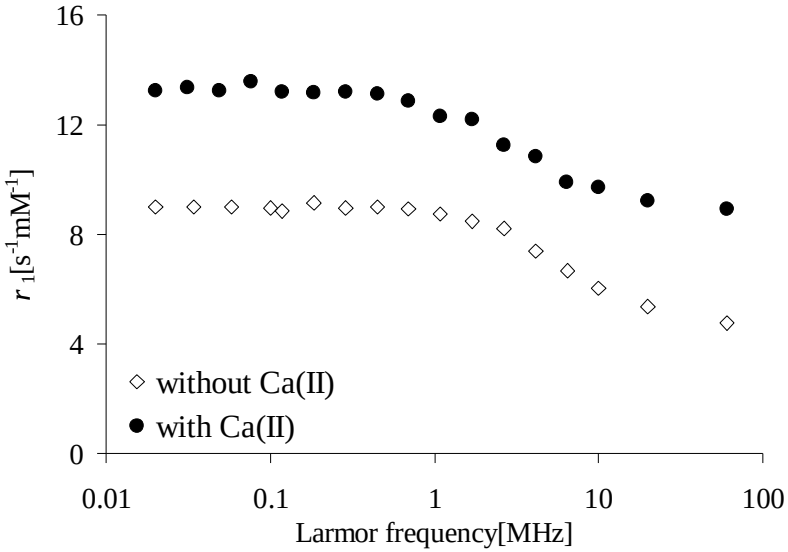


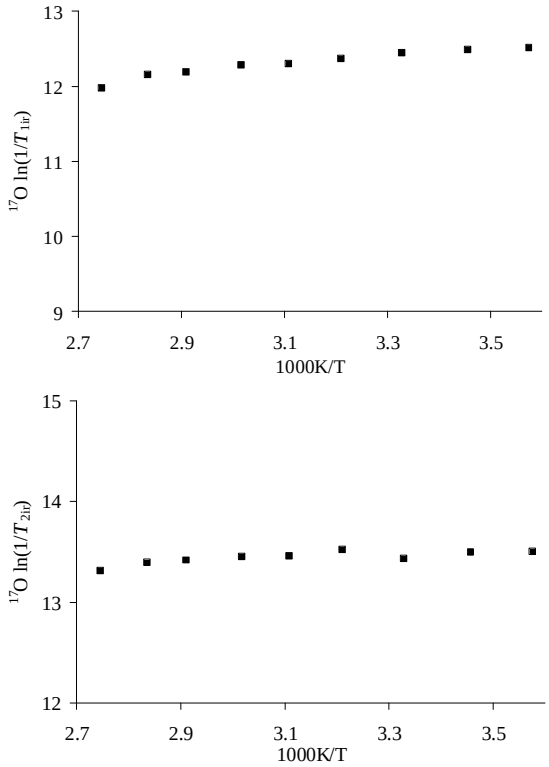


- 1:1 complex formation – log*b* = 2.2
- 2 times shorter ^{17}O T_{1ir} ➡ increase of rotation correlation time
- Formation of 2+2 complexes – 8 member ring typical for phosphonate complexes

Interaction of Gd-BPAMD with Ca(II) ions

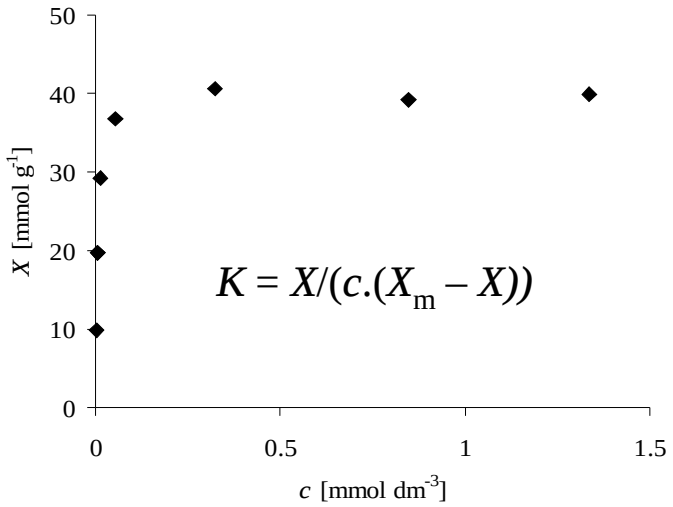
- Increase of relaxivity upon interaction with Ca(II) ions
- Unusually flat ^{17}O temperature relaxation profiles
- Different complexing mode at higher temperature

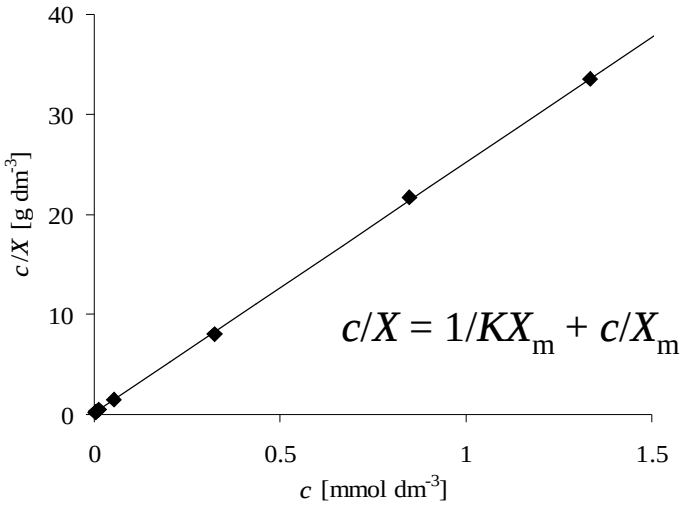




Sorption of Tb-BPAMD complex on the hydroxyapatite (HA)

- Fast and fully reversible sorption
- Maximum adsorption capacity $X_m = 4.0\times10^{-5}$ mol g^{-1} (specific surface of HA 53 m^{2}g^{-1}) indicates monomolecular layer formation
- Affinity constant $K = 2.1\times10^{5}$ dm^{3}mol^{-1}





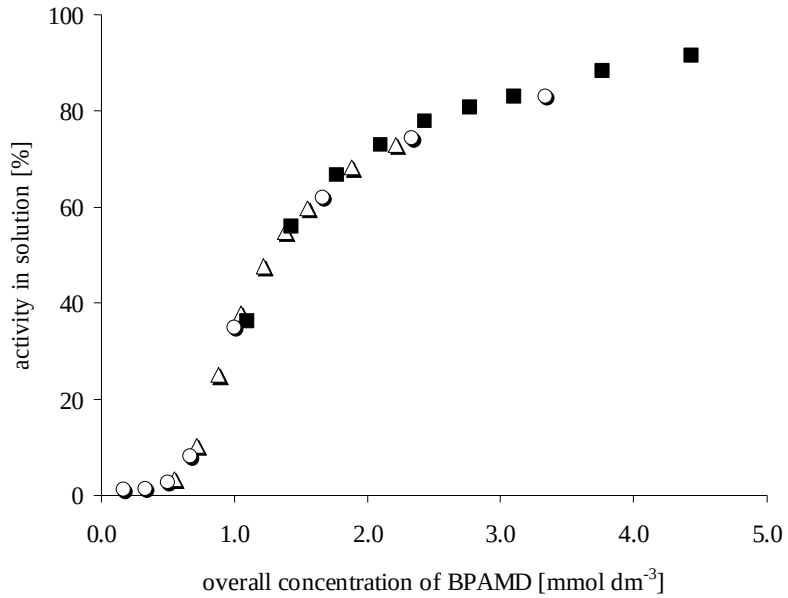
c – equilibrium concentration; X – specific adsorbed amount; X_m – maximum adsorption capacity; K – affinity constant

Sorption of Tb-BPAMD complex on the hydroxyapatite (HA)

- Very strong interaction of Tb-BPAMD complex with HA surface
- Identical sorption abilities of free ligand BPAMD and its Tb(III) complex
- α-amido-bis(phosphonic acid) group – responsible for exceptional properties

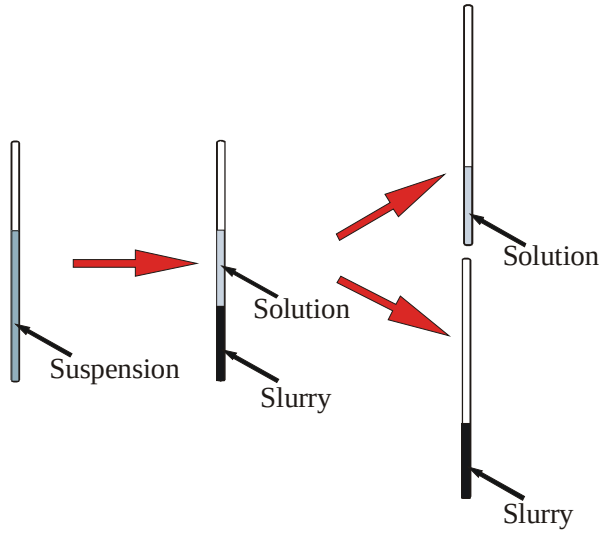
bis(phosphonate)	affinity constant $K \times 10^3$ [$mol^{-1}dm^{-3}$]
HEDP[a,b]	3.3
MDP[a,b]	0.7
Tb-BPAMD	**210**

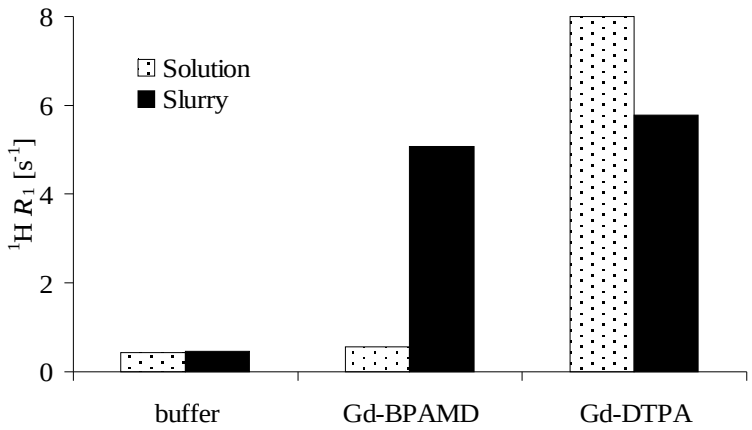
[a] HEDP - 1-hydroxy-ethane-1,1-bis(phosphonic acid)
MDP – methanebis(phosphonic acid)
[b] Claessens, R. A. M. J.; Kolar, I. Z. *Langmuir* **2000**, *16*, 1360–1367



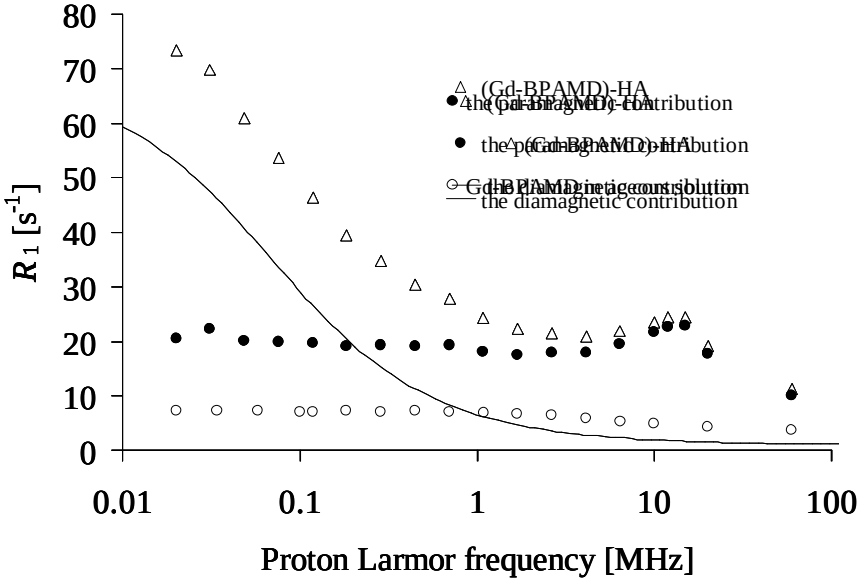
Relaxometry of the hydroxyapatite slurry

- Gd-BPAMD complex is fully adsorbed in the slurry
- Value of the relaxivity of adsorbed Gd-BPAMD comparable with that obtained for the slurry containing Gd-DTPA complex
- Same concentration of Gd-DTPA in the solution and in the slurry
  ✗ different relaxivities due to physicochemical conditions





Relaxometry of the hydroxyapatite slurry



• Superposition of a paramagnetic contribution from the complex and a diamagnetic contribution coming from HA

• Paramagnetic contribution shows a maximum at 10 – 20 MHz typical for Gd(III) complexes with slow rotation of the molecule

• 3-5 times higher milimolar relaxivity than the Gd-BPAMD complex in the solution as result of the hindered rotation

Conclusion

- new bis(phosphonate) containing DOTA monoamide
- three step lanthanide complexation
- DOTA-like Ln(III) complexes
- interaction with Ca(II) ions
- fast and strong adsorption of Ln(III)complexes on hydroxyapatite
- increase of rotation corelation time and relaxivity upon binding on hydroxyapatite

Acknowledgement

Prague

Ivan Lukeš

Petr Hermann

Jakub Rudovský

Jan Kotek

Delft

Joop A. Peters

Hubert Th. Wolterbeek

Zvonimir I. Kolar

Kristina Djanashili

Mons

Robert N. Muller

Luce Vander Elst

Sophie Laurent

Financial support

- Marie Curie training site host fellowship (QLK5-CT-2000-60062)
- Grant Agency of the Czech Republic (No. 203/03/0168)
- COST D18 Action
- EU Network of Excellence (NoE) “European Molecular Imaging Laboratory” (EMIL).