

# MRI of C6 glioma cells tagged with an opioid functionalized DTPA-Gd contrast agent

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## Introduction

The development of new Gadolinium containing contrast agents for sites specific molecular imaging has received increasing interest recently. Opioid based MRI-contrast agents which have not yet been described in the literature allow to image cells expressing opioid receptors. Here, we present first images of cells labelled with a Bis-normorphine-Gd-DTPA complex.

## Methods

**Synthesis:** DTPA-bis-anhydride and the twofold excess of normorphine were allowed to react in DMF at 80° C for 6h. The ligand was obtained as an amorphous solid after solvent removal, washed with chloroform and dried for 6h. Complexation was done with equimolar amounts of ligand and GdCl<sub>3</sub> in aqueous solution by titration with sodium hydroxide to pH=7. The reaction mixture was desalted and lyophilized to obtain an amorphous solid. The chemical structure of Bis-normorphine-DTPA was characterized and confirmed by Electrospray Ionisation-Massspectrometry and NMR. Fig.1 shows the molecular structure of opiate complex.

## Characterization of MR-properties:

T<sub>1</sub>- and T<sub>2</sub>-relaxation rates of the Gd-complex were determined in aqueous solutions of 1mM Gd-complex at 4,7T at 20°C using inversion recovery and Carr-Purcell-Meiboom-Gill sequences, respectively. Complex stability, i.e. the release of Gd from the complex, was estimated with a 1mM solution of the opiate Gd-DTPA-complex in medium at 37°C by longitudinal T<sub>1</sub>-measurements over 28 hours.

**Cell Experiments:** C6-Cells were cultured in DMEM medium with 5% FCS and incubated with 0 μM, 5μM, 15μM, 25μM, 35μM Bis-normorphine-DTPA-Gd-complex for 24 hours. Cells were washed twice with DMEM and immobilized within an agarose gel phantom (1,6% agarose, 0,9% NaCl).

**MR Imaging:** Labelled C6 cells were imaged in a Biospec 4,7/40 MR scanner with a T<sub>1</sub>-weighted 3D-gradient echo sequence (TE/TR = 5/75ms, pulse width =90deg) at an isotropic resolution of 117μm in all 3 dimensions.

## Results and Discussion:

This new Gd-complex can be used to label successfully C6 cells. As shown in fig. 2, only cells incubated with the opioid-DTPA-Gd complex are visible in the T<sub>1</sub>-weighted image. Clearly, the image intensity is dominated by the increased longitudinal relaxation proportional to the complex concentration in the incubation medium. The image intensity is still dominated by T<sub>1</sub> at short echo times of about 5ms, although the paramagnetic complex also increases the T<sub>2</sub>-relaxation. This newly synthesized Gd-Complex shows a similar T<sub>1</sub>-relaxation rate as other DTPA-bisamide complexes (Tab.1). The reduction of R<sub>1</sub>-relaxivity to 80% of its starting value (fig. 3) suggests that Gd might be released from the DTPA ligand.

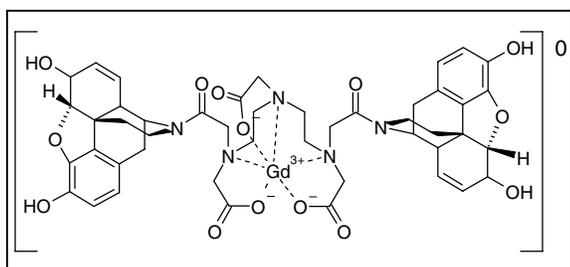


Fig.1 Synthesis of Bis-normorphine-DTPA-Gd-complex

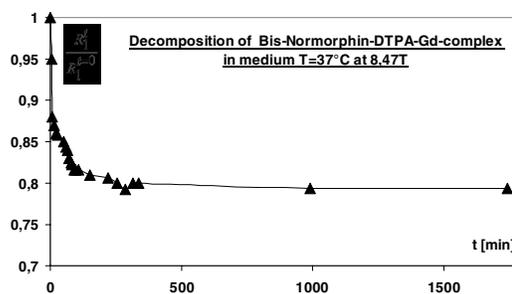


Fig.3 Kinetical decomposition curve of Bis-normorphine-DTPA-Gd

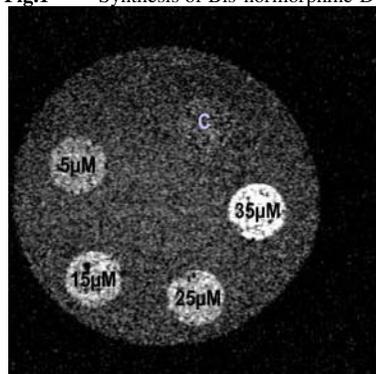


Fig.2 MR-Image of C6-cells tagged with an opiate Gd-complex

Tab.1: Molar T<sub>1</sub>- and T<sub>2</sub>-times of the opioid Gd-complex in water and DMEM at 4,7T and several Bis-amide-DTPA-Gd-complexes

	Medium	Water	
		T <sub>1</sub> [mmol s]	T <sub>2</sub> [mmol s]
Bis-normorphine-DTPA Gd (B <sub>0</sub> =4,70T)	0,179	0,049	0,209
Bis-N-ethylaminomorpholine-DTPA-Gd (B <sub>0</sub> =0,86T) <sup>[2]</sup>	-	-	0,277
Bis-benzylamido-DTPA-Gd (B <sub>0</sub> =0,47T) <sup>[3]</sup>	-	-	0,208
Gd-DTPA-BMA (B <sub>0</sub> =0,47T) <sup>[1]</sup>	-	-	0,244
Water (B <sub>0</sub> =4,70T)	-	-	2,272

## Conclusion

This new Gd-complex tags rather well to opiate receptors, which are involved in many processes and pathways. Opioid-T<sub>1</sub>-relaxation agents opens a new perspective to image their spatial distribution at very high resolution on a cellular level.

## References:

[1] Fosshem S (et al.) JMRI 1997, 7, 251 [2] Geraldes CFGC (et al.) MRM, 13 (3), 401 [3] Laurent S (et al.), Helv. Chim. Acta, 2000, 83, 394