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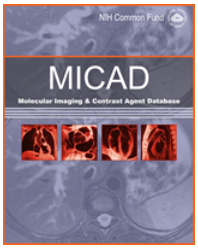

(1-(2-(β-Galactopyranosyloxy)propyl)-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane) gadolinium(III)

EGadMe

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Chemical name:	(1-(2-(β-Galactopyranosyloxy)propyl)-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane) gadolinium(III)	
Abbreviated name:	EGadMe	
Synonym:		
Agent category:	Small molecule	
Target:	β-Galactosidase	
Target category:	Enzyme	
Method of detection:	Magnetic resonance imaging (MRI)	
Source of signal/contrast:	Gadolinium	
Activation:	Yes	
Studies:	• *In vitro*	No structure is available in PubChem.

Background

[PubMed]

β-Galactosidase (β-Gal) derived from *Escherichia coli* (*E. coli*) is the gene product of the bacterial *lacZ* operon (1). This 465-kDa tetrameric enzyme is responsible for hydrolysis of lactose and other β-galactosides to monosaccharides (2). β-Gal exhibits fairly strict specificity for sugars in galactosyl positions in a wide variety of aglycones (3). Several chromogenic sugar substrates have been synthetically modified so that their hydrolyzed products are easily detectable by a conventional colorimeter (2, 4). For example, β-Gal hydrolyzes 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal) to produce a 5-bromo-4-chloro-indigo in blue color (5). The development of substrates like X-Gal makes β-Gal a common enzymatic reporter protein to assay clonal insertions, transcriptional activations, protein expressions, and protein interactions *in vitro* and *in vivo* (2, 6). The increase in β-Gal activity in particular has been used as an indirect measure of the enhanced transcription of the *lacZ* reporter gene (6). A major advantage of using β-Gal as a reporter protein is its high sensitivity originating from efficiently enzymatic turnover (4).

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1-(2-(β-Galactopyranosyloxy)propyl)-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane) gadolinium(III) (EGadMe) is a modified sugar substrate containing a gadolinium (Gd) chelate (7), which is used to enhance the imaging contrast of β-Gal in magnetic resonance imaging (MRI). EGadMe consists of a high-affinity chelator that occupies eight of the nine coordination sites on Gd^{3+} and a galactopyranose residue that is positioned to block the remaining site on Gd^{3+} from water access. An α-methyl group is attached to the ethylenic carbon on the sugar linkage arm of the tetraazacarboxylic macrocycle to restrict the rotation of the galactopyranose blocking group. As a result, it not only enhances the rigidity of the chelator but also limits the bulk water access to Gd^{3+}. The T_1 relaxivity of Gd chelates contains the contributions from water molecules in two environments: the water molecules that are directly bonded to the metal ions (the inner sphere water) and the water molecules that are not directly bonded to the metal ions (the outer sphere water) (8, 9). For Gd with one structural water (q = 1) in the fast reorientation motion region (small free Gd chelates), the inner sphere water contributes 50–70% to the T_1 relaxivity depending on the magnetic field strength (8). In the absence of β-Gal, EGadMe is in a water-inaccessible conformation (uncleaved); its T_1 relaxivity is low because the primary contribution is only from the outer sphere water. The presence of β-Gal enzymatically cleaves the galactopyranose from EGadMe (cleaved), freeing one coordination site (q ~ 1) and causing a substantial increase in the T_1 relaxivity. Such a substantial change in T_1 relaxivity allows for measuring the activity of β-Gal by MRI.

Xenopus embryos comprise an important cell system used for biological studies of cell signaling, cell lineage, and cell movement (10). The embryos are typically 1 mm in diameter, which is sufficiently large for easy visualization and manipulation at low magnification. Cell division as early as the stage of the first six divisions generates easily identifiable rows and tiers of cells (blastomeres) (11). A fate map of these blastomeres has been established for identification of the descendents during embryo development (11). The β-Gal gene can be introduced into a *Xenopus* embryo *via* transfection of messenger ribonucleic acid (mRNA) of β-Gal or plasmid deoxyribonucleic acid (DNA) carrying the *lacZ* gene at the two-cell stage (7). At the same time, EGadMe as a MRI lineage tracer is microinjected into both cells, and it remains within the originally labeled cell and its progeny (the descendents) during the growth of embryo. As a result, the transfected cell is easily distinguished from the nontransfected one by MRI and their descendents as well.

Synthesis

[PubMed]

Louie et al. reported the details of synthesis of EGadMe in several steps (7). 1-Bromopropan-2-ol was reacted with aceto-1-bromogalactose in the presence of silver trifluoromethanesulfonate (AgOTf) in CH_2Cl_2 to yield α- and β-anomers of 2,3,4,6-aceto-1-ethylbromo-galactose. The β-anomer was isolated by flash chromatography and reacted with cyclen to form monosubstituted cyclen. The substituted cyclen was treated with four equivalents of bromoacetic acid to produce the ligand, followed by complexation with $GdCl_3$ to yield EGadMe.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The water relaxation properties of EGadMe were examined on an 11.7-T magnetic resonance spectrometer (7). The T_1 relaxivity was found to be 2.72 $mM^{-1}•s^{-1}$ for its cleaved conformation and 0.903 $mM^{-1}•s^{-1}$ for its uncleaved conformation. The kinetic properties of EGadMe as a substrate of β-Gal were examined in solutions (7). The Michaelis constant (K_m) of 0.0182 mM and maximum initial velocity (V_m) of 2.4×10^{-3} nmol $U^{-1}•s^{-1}$ were extracted from Lineweaver-Burke plots.

The *in vivo* study of magnetic resonance contrast enhancement was conducted in living *X. laevis* embryos on an 11.7-T imager (7). Both cells in embryos at the two-cell stage were microinjected with 4 nl of 400 M EGadMe. Then, one of the two cells was transfected with either 4 nl of 0.5-μg/μl mRNA of β-Gal or 4–16 nl of 20-ng/μl

linearized plasmid DNA carrying the *lacZ* gene; the other cell was microinjected with water as a control. Consequently, the transfection led to the generation of ~13,000 copies of the 3,000 bp sequence per cell or ~88–352 copies of the 8.9 kb plasmid per cell in the animal at the 100,000-cell stage. To confirm the MRI labeling, 0.025 μg/μl mRNA of nuclear-localized green fluorescent protein (GFP) was also microinjected into the transfected cells to generate GFP reporter. Both cells contained EGadME, but one existed as the enzymatically processed contrast agent and the other as the unprocessed one. After multiple cell division stages, the two cells developed into the left and right sides of animals. The descendents of the labeled cells remained only on one side as predicted by the embryo development fate map for (11). As a result, the images of the embryo injected with both β-Gal mRNA and EGadMe (cleaved) demonstrated an expected increase in signal intensity compared to that of the embryo injected with EGadMe alone (uncleaved). A significant signal intensity enhancement of MRI (57%) was found on the side that expressed β-Gal compared to the equivalent somites on the other side that did not express β-Gal. This result was further confirmed by light microscopy of the embryo fixation with X-Gal staining and nuclear-localized GFP fluorescence measurement. In the embryo transfected with plasmid DNA of the *lacZ* gene, the magnetic resonance image correlated well with the image of the same embryo after fixation and staining with X-Gal.

Animal Studies

Rodents

[PubMed]

No publication is currently available.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

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