Supplementary data for

Dual-targeted Gd-based contrast agent for magnetic resonance imaging in tumor diagnosis

Lin Yu, ‡[a] Kaiqi Zhang, ‡[a] Yiyao Zhang,[a] Xun Wang,[b] Peng Dong,[a] Yanming Ge,[a] Guangmao Ni,[a] Zan Liu,*[b] Yanhui Zhang*[a]

[a]*School of Medical Imaging, Weifang Medical University, Weifang, 261053 (China).*

[a]*School of Pharmacy, Weifang Medical University, Weifang, 261053 (China).*

Text S1 MRI experimental parameters

Aqueous MRI experimental parameters

T_1map-RARE: TE=7 ms，TR=200, 400, 800, 1500,3000, 5500 ms, Averages=2, Repetition=1, echo spacing=7.0 ms, Slice=5，Slice Thickness=1.0 mm, Slice Gap=0 mm， MTX=128×128，FOV=15×15 mm^2.

T_2map-MSME: TR=2200 ms, TE=7.5, 15, 22.5, 30, 37.5, 45, 52.5, 60, 67.5, 75, 82.5, 90, 97.5, 105 ms, Averages=1, Repetition=1, echo spacing=7.5 ms, Slice=5，Slice Thickness=1.0 mm，Slice Gap=0 mm， MTX=192×192，FOV=15×15 mm^2.

T_1-RARE: TR=750 ms，TE=6.5 ms，Averages=2, Slice Thickness=1 mm，Slice Gap=0 mm，Slice=5，MTX=256×256，FOV=15×15 mm^2.

T_2-TurboRARE: TR=2500 ms，TE=33 ms，Averages=2, Slice Thickness=1 mm，Slice Gap=0 mm，Slice=5，MTX=256×256，FOV=15×15 mm^2.

***In vitro* cellar MRI experimental parameters**

T_1-RARE:TR=500 ms, TE=5.2 ms, average=4, Slice Thickness=0.8 mm，Slice Gap=0.2 mm, MTX=96×96, FOV=12×12 mm^2.

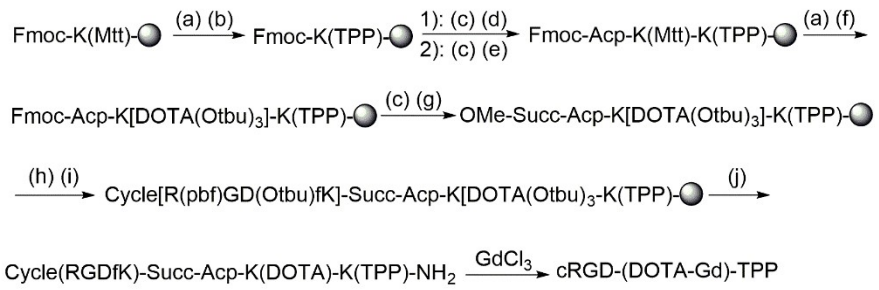
T_1map-RARE: TE=7 ms, TR=40, 70, 100, 180, 300, 500, 750, 1000, 1500, 3000, 5000 ms, average=2, Slice Thickness=0.8 mm，Slice Gap=0.2 mm, MTX=96×96, FOV=12×12 mm^2.

***In vivo* cellar MRI experimental parameters**

Axial T_2-TurboRARE: TR=2500 ms，TE=33 ms，Averages=4, Slice Thickness=1 mm，Slice Gap=0 mm，Slice=18，MTX=128×128，FOV=35×35 mm^2.

Text S2 Synthesis of RGD-(DOTA-Gd)-TPP (RDP)

The synthesis procedure of RGD-(DOTA-Gd)-TPP was illustrated in Scheme S1 and described as following:



(a) 3% TFA in DCM; (b) $TPP(CH_2)_4COOH$, DIC, HOBT; (c) 20% piperidine in DMF; (d) Fmoc-K(Mtt)-OH, TBTU, HOBT, DIPEA; (e) Fmoc-Acp-OH,TBTU, HOBT, DIPEA; (f) $DOTA(Otbu)_3$, HATU, NMM; (g) Succ-OMe,TBTU, HOBT, DIPEA; (h) NaOH, MeOH; (i) Cycle[R(pbf)GD(Otbu)fK]-NH_2, TBTU, HOBT, DIPEA; (j) 95%TFA in DCM.

Scheme S1 Synthesis procedure of RGD-(DOTA-Gd)-TPP (RDP)

The targeting moiety Cycle[R(pdf)GD(Otbu)fK]-NH_2 and $TPP(CH_2)_4COOH$ and MRI moiety $DOTA(OtBu)_3$ used in this work were coupled to a scaffold of a peptide backbone by a standard solid phase N-9-Fluorenylmethoxycarbonyl
(Fmoc) peptide synthesis strategy according to the designed peptide sequence from C to N terminal. Fmoc-K(Mtt) -PEG Resin (0.5 mmol/g) as the solid support was swelled well in DMF for 2 h. Mtt was deprotected with adding 3% TFA in DCM for 30 min and washing thoroughly six times with DMF. Then the $TPP(CH_2)_4COOH$ was conjugated onto the side chain of lysine with the molar ration of resine: $TPP(CH_2)_4COOH$: DIC: HOBT = 1: 3: 3: 3. And Fmoc was deprotected with adding 20% piperidine in DMF for 30 min following by washing six times with DMF. Then Fmoc-K(Mtt)-OH and Fmoc-Acp-OH were coupled to the scaffold in sequence respectively with the resine: peptide: TBTU: HOBT: DIPEA = 1: 2: 2: 2: 4. After successful conjugation, Mtt was deprotected and $DOTA(Otbu)_3$ was coupled to the side chain of lysine with a molar ration of $DOTA(Otbu)_3$:HATU:NMM = 2: 2: 4. After that, the Fmoc was deprotected and a linker of Succ-OMe was conjugated to the scaffold with a molar ration of Succ-OMe: TBTU: HOBT: DIPEA =2: 2: 2: 4. Then the OMe was deprotected with NaOH in MeOH solution following by coupling Cycle[R(pdf)GD(Otbu)fK]-NH_2 onto the scaffold. The condensation efficiency was determined by measuring free residual amine group with the quantitative ninhydrin assay. After that, the the crude product was cleaved off the resin accompanied by removing the protective-groups by treatment with 95% TFA for 2 h at room temperature and precipitated with ether for three times to obtain white solid. Then the crude product was purified with semi-preparative HPLC with a gradient of 0.1% TFA CH_3CN from 20% to 40% in 20 min using an elution speed of 10 mL/min and UV detection at 220 nm to get the product with a purity of more than 95%. Subsequently, the product was chelated with Gd^{3+} to get crude product of RGD-(DOTA-Gd)-TPP (RDP) and purified with 500 D dialysis bag.

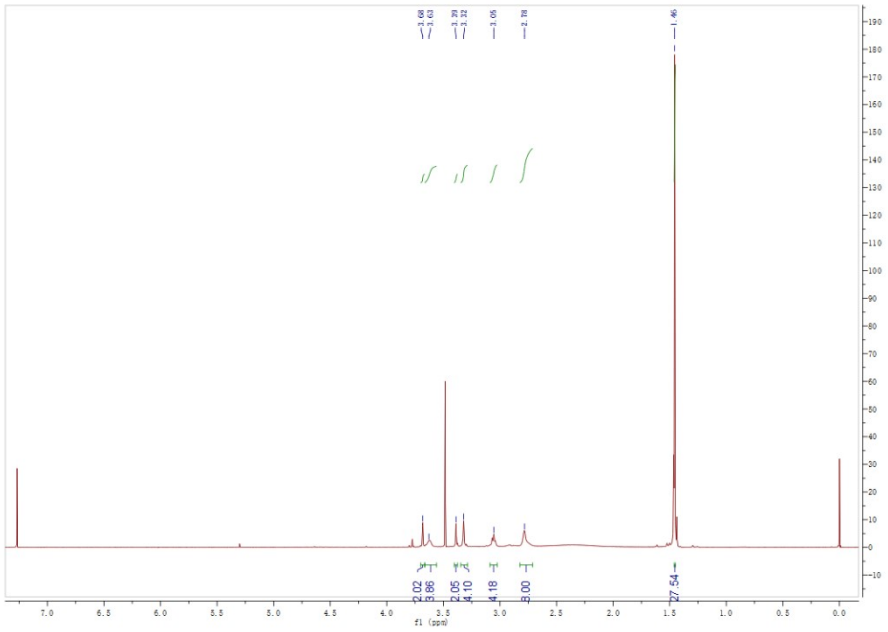


Fig. S1 ^{1}H NMR of DOTA(Otbu)$_3$

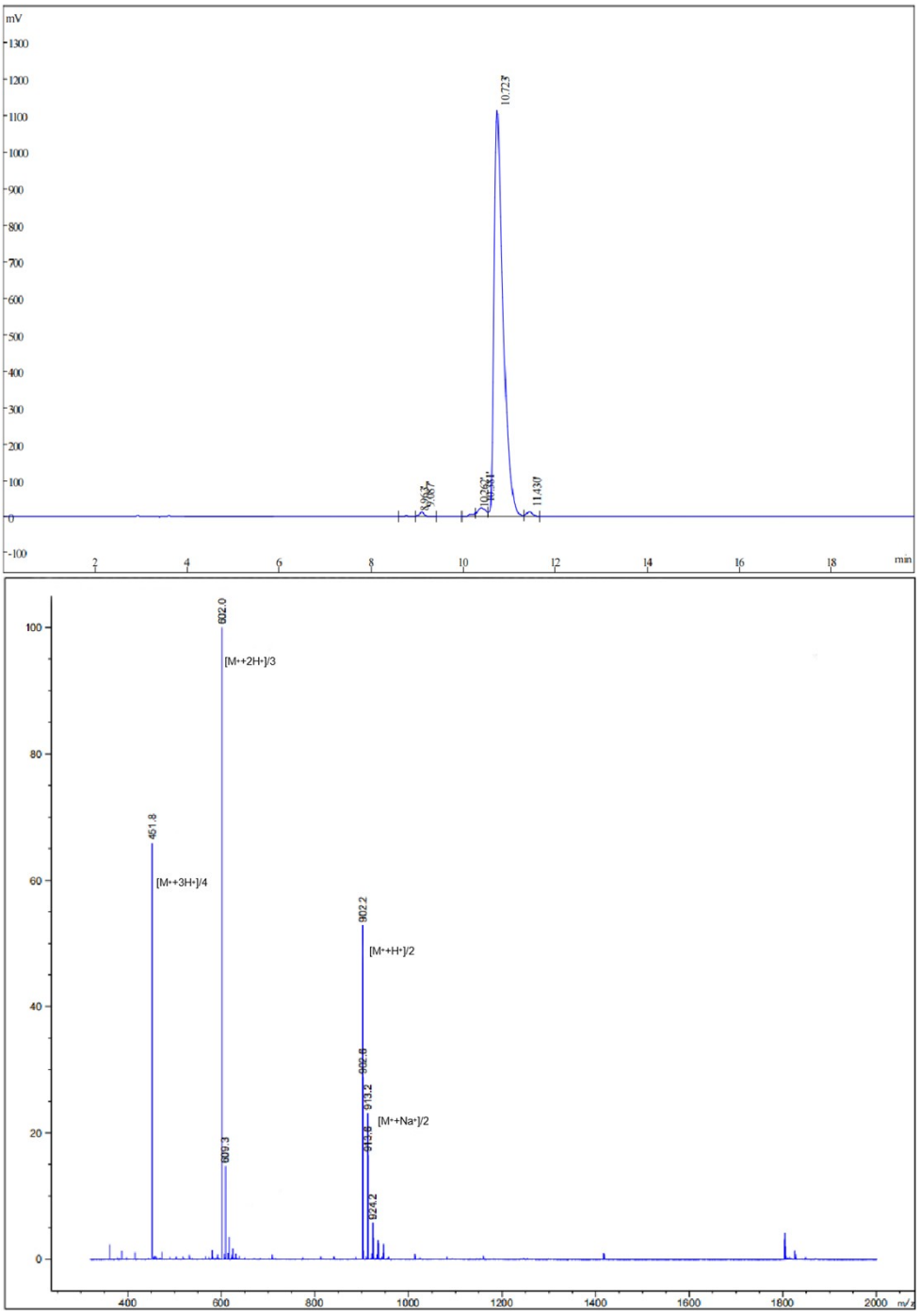


Fig. S2 LC-MS of RGD-DOTA-TPP

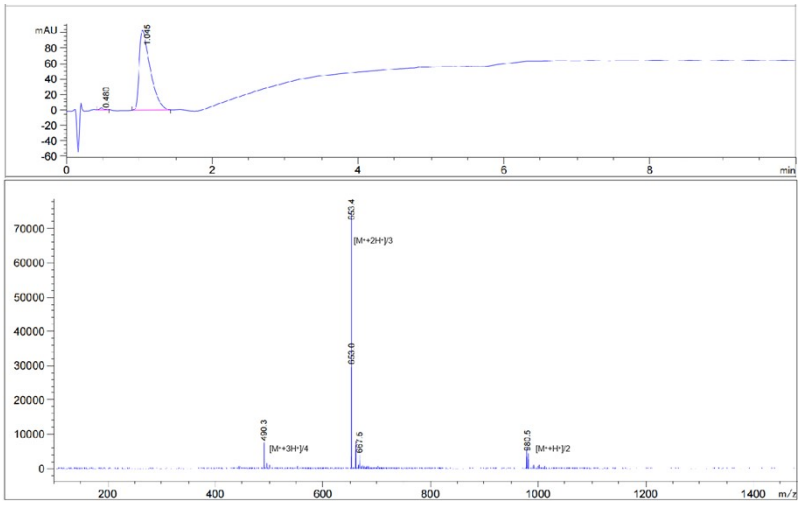


Fig. S3 LC-MS of RGD-(DOTA-Gd)-TPP (RDP)