Supporting Information

A Supramolecular Approach to Multivalent Target-Specific MRI Contrast Agents for Angiogenesis

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Solvents and Starting Materials. Unless stated otherwise, all reagents and solvents were purchased from commercial sources and used without any further purification.

Instrumentation. Reversed phase high pressure liquid chromatography (RP HPLC) was performed on a Varian Pro Star HPLC system coupled to an UV-Vis detector probing at 214 nm using a VydacTM protein & peptide C18 column. Electrospray ionization mass spectrometry (ESI-MS) was performed on a Perkin Elmer PE SCIEX Turbo Ionspray. The longitudinal ionic relaxivity ($r_1$) was determined by a concentration dependent measurement of the longitudinal relaxation time ($T_1$) via an inversion recovery pulse sequence at 1.5 T and 20 °C on a Philips Gyroscan S15/ACS. The gadolinium content was determined by means of inductively coupled plasma atomic emission spectroscopy (ICP-AES) on a Leeman Labs Echelle spectrometer.

Synthesis:

AcC(SAcm)NGRC(SAcm)GGMPAL (1). The peptide containing the target-specific NGR sequence with a thioester at its C-terminal was synthesized as previously reported.  

C-Biotin (2). C-Biotin was synthesized according to a literature procedure.  

AcC(SAcm)NGRC(SAcm)GGC-Biotin (3). 59.4 mg (0.057 mmol) of 1 and 1.1 equivalent (24.6 mg, 0.066 mmol) of 2 were dissolved in 1 mL of 6 M Guanidine in 0.07 M Tris (aq). To this solution 20 µL (2 v-%) of thiophenol and 20 µL (2 v-%) of benzylmercaptan were added. The pH was adjusted to pH ~7 by the addition of small aliquots of 0.5 M NaOH (aq). The reaction was continued for 2 hours at 37 °C. The reaction mixture was filtered and the product was purified employing preparative RP HPLC over a C18 column (gradient: 7–27% MeCN in H2O, 0.1% TFA in 90 minutes). Freeze drying rendered 57.6 mg (0.047 mmol, 83%) of 3 as a fluffy white powder: ESI-MS calcd. for C45H76N18O14S4 ([M+H]+): 1221.5, found 1221.3.

malDTPA (4). The synthesis of 4 will be published elsewhere.
AcC(SAcm)NGRC(SAcm)GGC(S-DTPA)-Biotin (5). 45.0 mg (0.0369 mmol) of 3 was dissolved in 1 mL 0.1 M Tris (aq, pH 6.9). The solution was added to 24.5 mg (0.0369 mmol) of 4. The pH of the solution was adjusted to pH 6.5 by the addition of small aliquots of 0.5 M NaOH (aq) and the reaction was continued for 2 hours at room temperature. The reaction was monitored employing analytical RP HPLC over a C18 column (gradient: 0–67% MeCN in H2O, 0.1% TFA in 30 minutes) and showed that the reaction went to completion. The reaction mixture was used for the next reaction step without any purification. ESI-MS calcd. for C74H113N23O27S4 ([M+H]+): 1884.7, found 1885.0.

Ac-cNGR-GGC(S-DTPA)-Biotin (6). The reaction mixture was diluted ~ 30 times by adding 40.5 mL of 0.1 M Tris (aq, pH 6.9) and 4.5 mL (i.e. 10 v-%) of acetic acid. Subsequently, 870 µL of a 0.075 M solution of I2 in MeOH (0.064 mmol of I2, 1.75 equivalents) were added and the reaction was continued for 1 hour at room temperature. The product was purified employing preparative RP HPLC over a C18 column (gradient: 10–30% MeCN in H2O, 0.1% TFA in 90 minutes). Freeze drying rendered 17.2 mg (9.9 µmol, 27%) of 6 as a fluffy white powder. ESI-MS calcd. for C68H101N21O25S4 ([M+H]+): 1740.6, found 1740.9.

HPLC traces of 4, 5, and 6 (analytical RP HPLC; gradient: 0–33.5% MeCN in H2O, 0.1% TFA in 15 minutes):
Ac-eNGR-GGC(S-Gd(III)DTPA)-Biotin (7). 14.6 mg (8.4 µmol) of 6 was dissolved in 5 mL of H₂O. The pH was adjusted to pH 7 by adding small aliquots of 0.5% NH₄OH (aq). To this solution was added 0.5 mL of a 16.8 mM solution GdCl₃ in H₂O (8.4 µmol). This was done in a stepwise manner and followed with ESI-MS to ensure full complexation, avoiding the addition of an excess of GdCl₃. Freeze drying rendered 7 in quantitative yield (> 99%). ESI-MS calcd. for C₆₈H₉₈GdN₂₁O₂₅S₄ ([M-H]⁻): 1893.5, found 1893.8. ICP-AES gadolinium content: 81%.

ESI-MS spectrum of 7 (negative mode):
Longitudinal relaxivity measurement ($r_1$) of 7. For the relaxivity measurements a dilution series of 7 was prepared in PBS buffer (pH 7.4): 1.6 mM, 1.2 mM, 0.8 mM, 0.4 mM, 0.08 mM. Each sample had a volume of 0.5 mL. For each concentration the longitudinal relaxation time $T_1$ was determined, giving a good linear fit ($R^2 > 0.999$) to the equation $(1/T_1)_{\text{observed}} = (1/T_1)_{\text{diamagnetic}} + r_1[\text{Gd(III)}]$. The $r_1$ was calculated in terms of the actual Gd(III) content as determined with ICP-AES.
Longitudinal relaxivity (\(r_1\)) measurement of 7:

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\frac{1}{T_1}/\text{s}^{-1} = 8.1 \text{ mM}^{-1}\text{s}^{-1}
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Binding studies of 7 with avidin:

**HABA assay.**\(^3\) 166 \(\mu\)L of a 30 \(\mu\)M solution of avidin (from egg-white) in PBS buffer (pH 7.4), 20 \(\mu\)L of a 5 mM solution of 4’-hydroxyazobenzene-2-carboxylic acid (HABA), and 314 \(\mu\)L of PBS buffer (pH 7.4) were mixed in a quartz cuvette (\(l = 1\) cm). To this solution was added in aliquots of 10 \(\mu\)L a 0.26 mM solution of 7. The absorption was probed at 500 nm, which is in the absorption maximum of HABA bound to avidin.

**Longitudinal relaxivity (\(r_1\)) measurements: effects of the binding to avidin.** To 0.5 mL of a 0.08 mM solution of 7 in PBS buffer (pH 7.4) was added stepwise in aliquots of 25 \(\mu\)L a 0.12 mM solution of avidin (from egg-white) in PBS buffer (pH 7.4). The \(T_1\) was measured after each addition.