Electronic Supplementary Information

Photoresponsive host-guest chemistry and T_1/T_2* relaxation time of fluorinated cyclodextrin and arylazopyrazole-functionalized DOTA metal complexes

Julian Simke,[a] Till Böckermann,[a] Klaus Bergander,[a] Sina Klabunde,[b] Michael Ryan Hansen[b] and Bart Jan Ravoo*[a]

a) Organic Chemistry Institute and Center for Soft Nanoscience,

Westfälische Wilhelms-Universität Münster, Corrensstrasse 36, D-48149 Münster, Germany

b) Institute of Physical Chemistry,

Westfälische Wilhelms-Universität Münster, Corrensstrasse 28/30, D-48149 Münster, Germany

Email: b.j.ravoo@uni-muenster.de

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1. General information

Chemicals and solvents

Unless stated otherwise, all chemicals were purchased from the following companies and used without further purification: Sigma Aldrich, Acros Organics, Alfa Aesar, abcr GmbH or TCI. Inert conditions were performed by using standard *Schlenk*-technique as well as dry solvents were used for moisture-sensitive reactions.

Milli-Q water

ELGA Purelab$_{TM}$ UHQ II water purification system (*ELGA LabWater*, High Wycombe, Buckinghamshire, UK) was used to produce ultrapure water with an electric resistance of > 18 MΩ. Milli-Q water was used for the preparation of all aqueous solutions.

Freeze drying

For lyophilization *Alpha 1-2 LD plus freeze dryer* (*Martin Christ GmbH*, Osterode, Germany) was used. For preparation, all compounds were dissolved in ddH_2O and frozen under rotation in liquid nitrogen.

Isothermal titration calorimetry (ITC)

For ITC measurements a *TA Instruments Nano ITC Low Volume* (*Ta Instruments Waters Corporation*, Milford, Massachusetts, USA) with *ITC Run Version 2.1.7.0 Firmware Version 1.31* (*TA Instruments Waters Corporation*, Milford, Massachusetts, USA) as software was used. All samples were prepared in ddH_2O and all titrations were carried out by titrating 20 injections (50 µL) of host molecule into an aqueous solution of guest molecule at 25 °C with a stirring rate of 350 rpm. Titration of host molecule into pure ddH_2O served as reference. *NanoAnalyse Data Analysis Version 3.11.0* (*TA Instruments Waters Corporation*, Milford, Massachusetts, USA), *Microsoft Excel Version 1808 as part of Microsoft Office 365 ProPlus 2019* (*Microsoft Corporation*, Redmond, Washington, USA) and *OriginPro 9.7.5.184* (*ORIGINLAB CORPORATION*, Northampton, Massachusetts, USA) were used to analyze all ITC data.

Mass spectrometry

The recording of mass spectra was performed by using different methods and instruments such as electrospray ionization (ESI): MicroTof ESI (*Bruker Daltonics*, Bremen, Germany) or *Orbitrap LTQ XL* (*Thermo-Fisher Scientific*, Bremen, Germany) and matrix-assisted laser

desorption/ionization (MALDI): *Autoflex Speed MALDI-TOF* (*Bruker Daltonics*, Bremen, Germany). All matrices and/or solvents which were used for the sample preparations are stated for each compound in the experimental procedure.

NMR spectroscopy

The recording of NMR spectra was performed by using the following instruments: *AV-300*-spectrometer with 300.1 Hz (^{1}H), 75.5 Hz (^{13}C), 282 Hz (^{19}F) (*Bruker Corporation*, Billerica, Massachusetts, USA); *AV-400* spectrometer with 400.1 Hz (^{1}H), 100.1 Hz (^{13}C) (*Bruker Corporation*, Billerica, Massachusetts, USA); *DD2-600*-spectrometer with 600 Hz (^{1}H), 151 Hz (^{13}C), 564 Hz (^{19}F) (*Agilent Technologies*, Santa Clara, California, USA). ^{1}H NMR chemical shifts are given relative to TMS and are referenced to the solvent signal. Spectra of other nuclei such as ^{19}F, are referenced according to the proton resonance of TMS as the primary reference for the unified chemical shift scale (IUPAC recommendation 2001).[1] Deuterated solvents were used for all measurements and the chemical shifts (δ) are stated in parts per million (ppm). The coupling constants are reported in Hertz (Hz) and the following abbreviations are used for the observed multiplicities of the signals: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) and broad signal (br). *MestReNova* 12.0.4 (*Mestrelab Research* S.L., Santiago de Compostela, Spain) was used to analyze all NMR spectra.

Electron paramagnetic resonance (EPR)

The continuous-wave (CW) EPR experiments were carried out on a BRUKER EMXnano spectrometer operating at X-band (9.6 GHz) frequencies in the range from 100–4900 G. Initial experiments were performed at room temperature and at 140 K in CH_2Cl_2 (no EPR signal detectable) and then subsequently at 140 K on a powdered sample of **Fe-APP** (~0.3 mg in an EPR tube flushed with nitrogen gas to remove oxygen and residual water). The applied microwave power was 0.32 mW. For the acquisition a sweep rate of 80 G s^{-1} and a time constant of 1.28 ms were used. A total of 10k data points were recorded, and 10 scans were performed. The modulation frequency was set to 100 kHz and a modulation amplitude of 4 G was used. The resulting data were processed and simulated using the fitting program SpinFit by *Bruker* and the simulation software Easyspin.[2,3]

Photo-irradiation

For photoswitching experiments two different light sources were used: *UV LED Gen 2 Emitter* with λ = 365 nm (*LED Engin Inc.*, San Jose, California, USA) and *LSC-G HighPower-LED* with λ = 520 nm (*Cree Inc.*, Durham, North Carolina, USA).

Preparative high-performance liquid chromatography (HPLC)

For preparative HPLC a *Knauer* HPLC System with *ChromeGate Client Version 3.3.2.980* (*Wissenschaftliche Gerätebau Dr. Ing. Gerbert Knauer GmbH*, Berlin, Germany) as software was used to purify the final compounds. The HPLC System consists of a *Smartline Manager 5050*, a *Smartline Pump 1050*, a *Smartline UV Detector 2550*, a *Dynamic mixing chamber* with a *VariTide RPC column* (250×21.2 mm, *Varian Inc.*, *Palo Alto*, California, USA) and a *Foxy® R1 fraction collector* (*Teledyne Isco*, Lincoln, Nebraska, USA). HPLC gradient grade solvents were used and purchased from *Carl Roth* (*Carl Roth GmbH & Co. KG*, Karlsruhe, Germany). Degassed ddH_2O (eluent A) and degassed ACN/ddH_2O (80%/20% v/v, eluent B) were used as eluents. The method which was used is shown in Table S1.

Table S1: Gradient for preparative HPLC.

Time (min)	Volume ratio eluent A	Volume ratio eluent B
0	95	5
5	95	5
50	20	80
60	20	80
65	95	5
80	95	5

Preparative silica gel column chromatography

Geduran® Si 60 (*Merck KGaA*, Darmstadt, Germany) with a grain size of 0.060 – 0.200 mm was used for preparative silica gel column chromatography. The different eluents which were used as well as their composition, given in volumetric ratios, are stated for each compound in the experimental procedure.

Preparative size exclusion chromatography

Sephadex® LH20 (*GE Healthcare*, Chalfont St. Giles, Buckinghamshire, UK) with methanol p.a. as eluent was used for preparative size exclusion chromatography.

Relaxation time measurements

Relaxation time measurements were performed on a *Agilent DD2 600* (*Agilent Technologies*, Santa Clara, California, USA) 600 MHz NMR spectrometer. A calibrated 90° pulse was used as the transmitter pulse. The relaxation delay was varied according to the different samples. The transversal relaxation time T_2^* was measured by ^{19}F NMR and calculated from the widths of the signals at half the signal intensity (half-value width $h_{1/2}$).

Thin layer chromatography (TLC)

Silica gel coated aluminium plates (model: *60 F_{254}*, *Merck KGaA*, Darmstadt, Germany) were used for analytical thin layer chromatography. By using a *Dual Wavelength UV Lamp (254 nm and 366 nm*, *CAMAG*, Muttenz, Switzerland) or a basic permanganate solution the spots were visualized.

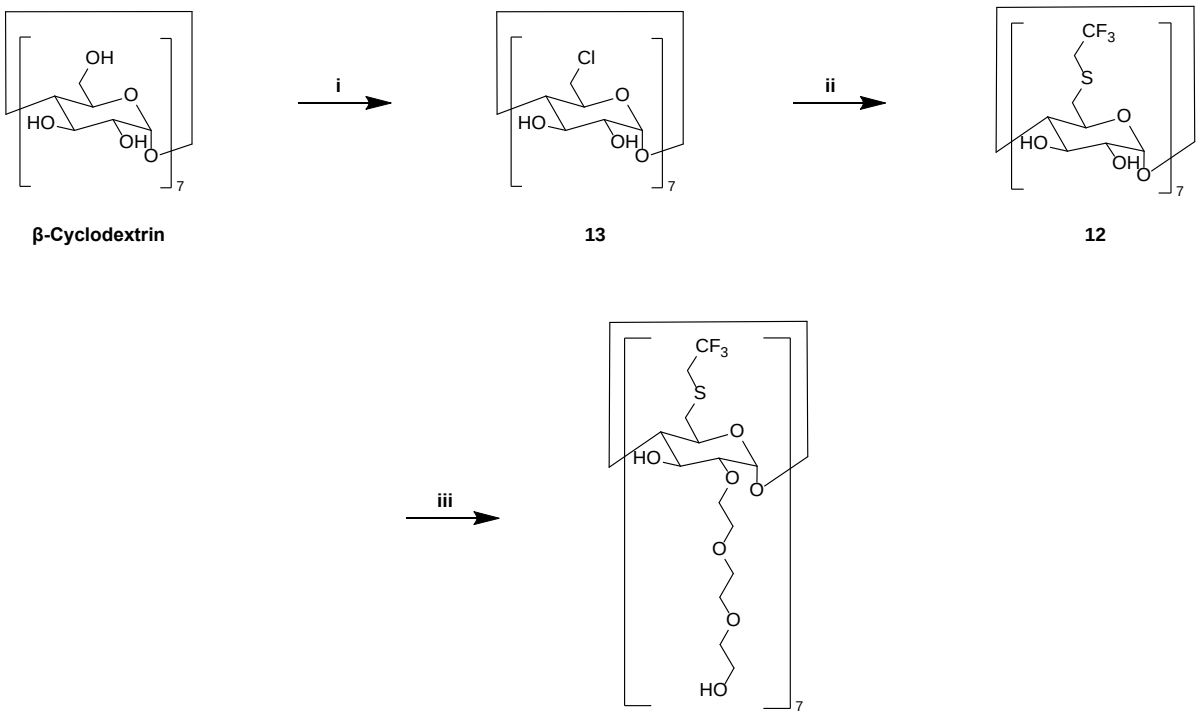
UV/Vis spectroscopy

The recording of UV/Vis spectra was performed with a *JASCO V-650 double-beam spectrophotometer* (*JASCO Germany GmbH*, Gross-Umstadt, Germany). The samples were prepared in 1 mL disposable PMMA cuvettes (*BRAND GmbH & Co. KG*, Wertheim, Germany) in ddH_2O and measured at 25 °C against ddH_2O. *Spectra Manager Version 2.08.04* (*JASCO Germany GmbH*, Gross-Umstadt, Germany) was used as software and *OriginPro 9.7.5.184* (*ORIGINLAB CORPORATION*, Northampton, Massachusetts, USA) was used to analyze the collected data.

2. Syntheses

2.1 Synthesis of FCD

The syntheses of FCD were carried out exactly to the following literature from Becker *et al*.[4]



Scheme S1: i) Methanesulfonyl chloride, DMF, 65 °C, 48 h, 81%; **ii)** 2,2,2-Trifluoroethanethiol, NaH, DMF, 75 °C, 5 d, quant.; **iii)** Triethylene glycol *p*-toluenesulfonate**,** NaH, DMF, rt, 18 h, 67%.

Heptakis(6-chloro-6-deoxy)-β-cyclodextrin (13)[4]

Yield: 4.15 g (3.28 mmol, 81%).

^{1}H-NMR (300 MHz, DMSO-*d*6) δ = 6.32–5.34 (m, 14H), 4.96 (d, *J* = 3.6 Hz, 7H), 4.08 (d, *J* = 10.4 Hz, 7H), 3.99–3.71 (m, 14H), 3.69–3.51 (m, 7H), 3.47–3.25 (m, 14H) ppm.

^{13}C-NMR (75 MHz, DMSO-*d*6) δ = 102.0, 83.6, 72.5, 72.0, 71.2, 45.0 ppm.

MS (m/z) (MALDI, DHB (H_2O/ACN)): Calculated for $[C_{42}H_{63}Cl_7O_{28}Na]^+$: 1283.12; found 1283.12.

Heptakis-6-deoxy-6-((2,2,2-trifluoroethyl)thio)-β-cyclodextrin (12)[4]

Yield: 963 mg (529 µmol, quant.).

^{1}H-NMR (300 MHz, DMSO-*d*6) δ = 5.99 (d, *J* = 6.7 Hz, 7H), 5.87 (d, *J* = 2.2 Hz, 7H), 4.93 (d, *J* = 3.5 Hz, 7H), 3.93–3.73 (m, 7H), 3.69–3.55 (m, 7H), 3.51–3.35 (m, 28H), 3.17 (d, *J* = 13.5 Hz, 7H), 3.00 (dd, *J* = 14.1, 7.0 Hz, 7H,) ppm.

^{13}C-NMR (75 MHz, DMSO-*d*6) δ = 128.2, 102.0, 84.2, 72.4, 72.0, 71.5, 33.9 ppm.

^{19}F-NMR (282 MHz, DMSO-*d*6) δ = -65.68 ppm.

MS (m/z) (MALDI, DHB (EtOAc)): Calculated for $[C_{56}H_{77}F_{21}O_{28}S_7Na]^+$: 1843.22; found 1843.53.

Heptakis-2-oligo-ethylene-6-desoxy-6-((2,2,2-trifluoroethyl)thio)-*β*-cyclo-dextrin (FCD)[4]

Yield: 300 mg (110 µmol, 67%).

^{1}H-NMR: (300 MHz, $CDCl_3$) δ = 5.15 – 4.81 (m, 7H), 4.35 – 3.99 (m, 7H), 4.02 – 3.77 (m, 21H), 3.80 – 3.47 (m, 70H), 3.50 – 3.34 (m, 14H), 3.37 – 3.10 (m, 21H), 3.07 – 2.81 (m, 7H) ppm.

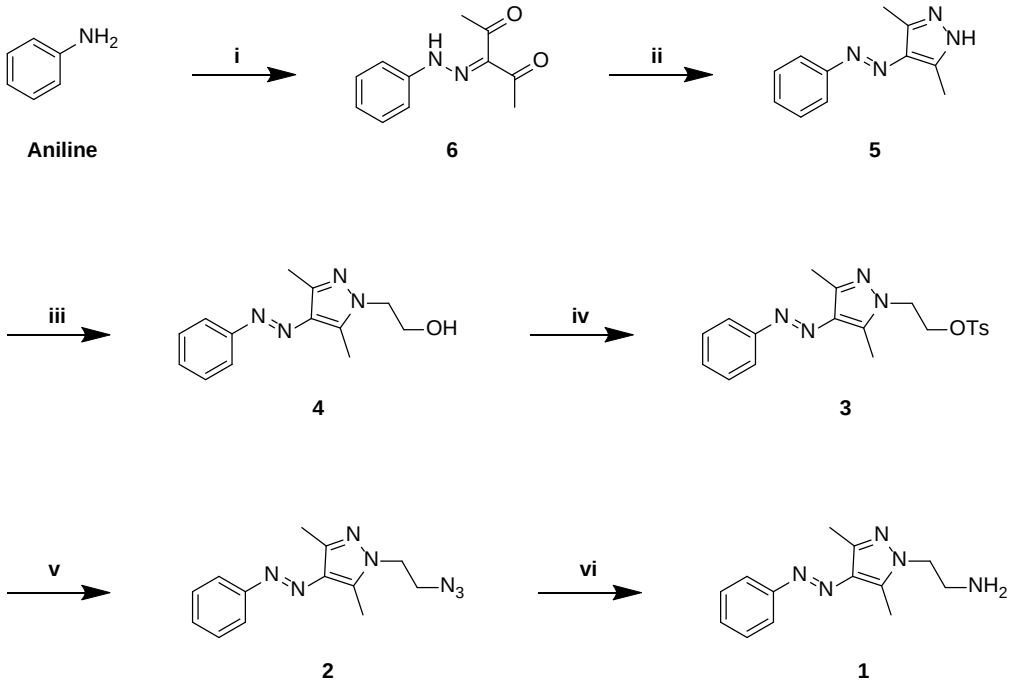
^{13}C-NMR: (75 MHz, $CDCl_3$) δ = 128.1, 101.2, 85.8, 81.0, 73.0 – 70.4, 61.7, 34.1 ppm.

^{19}F-NMR: (282 MHz, $CDCl_3$) δ = -66.65 ppm.

MS (*m/z*): (MALDI, DHB (H_2O/ACN)) Calculated for $[C_{98}H_{161}F_{21}O_{49}S_7Na]^+$: 2767.77; found 2767.34.

2.2 Synthesis of AAP 1

The synthesis of the AAP core **5** was completed in two steps starting from commercially available aniline by following the procedure of Fuchter and co-workers.[5] The functionalization of AAP **5** to yield the amino AAP **1** was carried out over four steps.[6]



Scheme S2: i) $NaNO_2$, acetylacetone, AcOH, HCl, NaOAc, EtOH, H_2O, rt, 30 min, quant.; **ii)** Hydrazine × hydrate, EtOH, 78 °C, 3 h, 99%; **iii)** 2-Bromoethanol, K_2CO_3, ACN, 82 °C, 3 d, 73%; **iv)** Et_3N, TsCl, DMAP, DCM, rt, 18 h, 94%; **v)** NaN_3, DMF, 90 °C, 18 h, 97%; **vi)** PPh_3, THF, H_2O, rt, 36 h, 90%.

3-(2-Phenylhydrazineylidene)pentane-2,4-dione (6)[5]

Yield: 4.79 g (23.5 mmol, quant.).

^{1}H-NMR: (300 MHz, $CDCl_3$) δ = 14.74 (s, 1H), 7.50–7.33 (m, 4H), 7.24–7.16 (m, 1H), 2.61 (s, 3H), 2.49 (s, 3H) ppm.

^{13}C-NMR: (75 MHz, $CDCl_3$) δ = 198.0, 197.2, 141.6, 133.3, 129.7, 126.0, 116.3, 31.8, 26.7 ppm.

MS (*m/z*): (ESI, MeOH) Calculated for $[C_{11}H_{12}N_2O_2Na]^+$: 227.0796; found 227.0797.

(*E*)-3,5-Dimethyl-4-(phenyldiazenyl)-1*H*-pyrazole (5)[5]

Yield: 4.66 g (23.3 mmol, 99%).

^{1}H-NMR: (400 MHz, $CDCl_3$) δ = 8.74 (s, 1H), 7.87–7.75 (m, 2H), 7.52–7.44 (m, 2H), 7.44–7.3 (m, 1H), 2.61 (s, 6H) ppm.

^{13}C-NMR: (101 MHz, $CDCl_3$) δ = 153.5, 141.6, 134.8, 129.7, 129.0, 121.9, 11.7 ppm.

MS (*m*/*z*): (ESI, MeOH) Calculated for $[C_{11}H_{12}N_4H]^+$: 201.1140; found 201.1157.

(*E*)-2-(3,5-Dimethyl-4-(phenyldiazenyl)-1*H*-pyrazol-1-yl)ethan-1-ol (4)[6]

Yield: 415 mg (1.70 mmol, 73%).

^{1}H-NMR: (400 MHz, $CDCl_3$) δ = 7.81–7.75 (m, 2H), 7.49–7.43 (m, 2H) 7.41–7.34 (m, 1H), 4.17-4.12 (m, 2H), 4.07–4.02 (m, 2H), 2.97 (s, 1H) 2.60 (s, 3H), 2.50 (s, 3H) ppm.

^{13}C-NMR: (101 MHz, $CDCl_3$) δ = 153.6, 142.9, 139.6, 135.1, 129.6, 129.0, 121.9, 61.6, 50.3, 14.1, 10.0 ppm.

MS (*m*/*z*): (ESI, MeOH) Calculated for $[C_{13}H_{16}N_4OH]^+$: 245.1402; found 245.1404.

(*E*)-2-(3,5-Dimethyl-4-(phenyldiazenyl)-1*H*-pyrazol-1-yl)ethyl-4-methyl-benzenesulfonate (3)[6]

Yield: 619 mg (1.55 mmol, 94%).

^{1}H-NMR: (300 MHz, $CDCl_3$) δ = 7.83-7.76 (m, 2H), 7.62–7.56 (m, 2H), 7.53–7.44 (m, 2H) 7.43-7.36 (m, 1H), 7.21–7.16 (m, 2H), 4.46–4.40 (m, 2H), 4.30–4.23 (m, 2H), 2.55 (s, 3H), 2.36 (s, 3H), 2.34 (s, 3H) ppm.

^{13}C-NMR: (75 MHz, $CDCl_3$) δ = 153.6, 145.1 143.0, 140.5, 135.0, 132.2, 129.9, 129.7, 129.1, 127.8, 127.2, 121.9, 68.4, 47.7, 42.1, 21.8, 14.3, 14.1, 9.9 ppm.

MS (*m*/*z*): (ESI, MeOH) Calculated for $[C_{20}H_{22}N_4O_3SNa]^+$: 421.1310; found 421.1305.

(*E*)-1-(2-Azidoethyl)-3,5-dimethyl-4-(phenyldiazenyl)-1*H*-pyrazole (2)[6]

Yield: 393 mg (1.46 mmol, 97%).

^{1}H-NMR: (300 MHz, $CDCl_3$) δ = 7.84–7.75 (m, 2H), 7.50–7.42 (m, 2H) 7.41–7.32 (m, 1H), 4.15 (t, J = 6.1, 5.2 Hz, 2H), 3.76 (t, J = 6.2, 5.2 Hz, 2H), 2.62 (s, 3H), 2.52 (s, 3H) ppm.

^{13}C-NMR: (75 MHz, $CDCl_3$) δ = 153.6, 143.3, 140.0, 135.2, 129.5, 129.0, 121.9, 50.8, 47.8, 14.2, 9.9 ppm.

MS (*m/z*): (ESI, MeOH) Calculated for $[C_{13}H_{15}N_7H]^+$: 270.1467; found 270.1459.

(*E*)-2-(3,5-Dimethyl-4-(phenyldiazenyl)-1*H*-pyrazol-1-yl)ethan-1-amine (1)[6]

Yield: 202 mg (830 µmol, 90%).

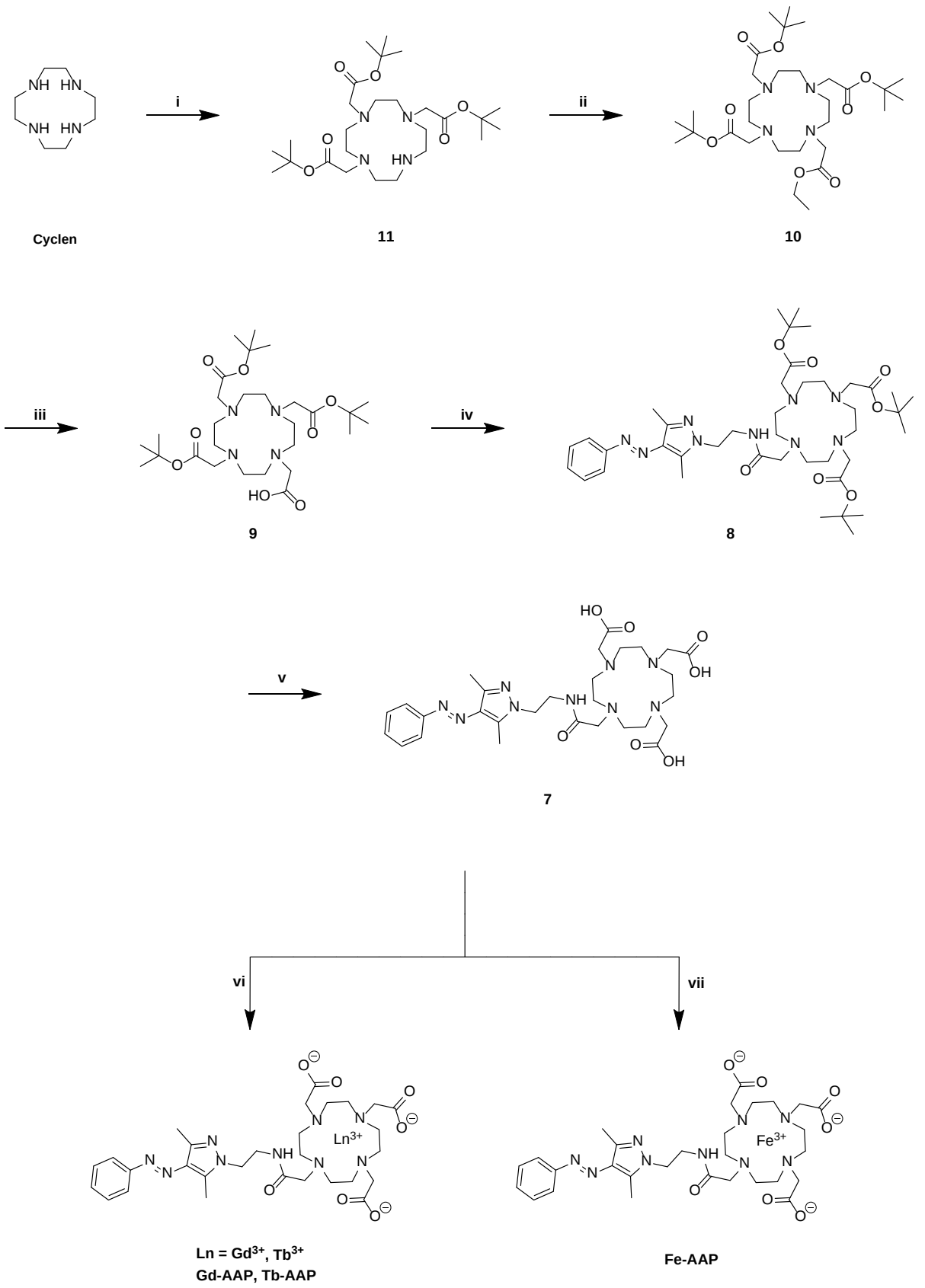
^{1}H-NMR: (400 MHz, $CDCl_3$) δ = 7.81–7.74 (m, 2H), 7.49–7.41 (m, 2H) 7.40–7.32 (m, 1H), 4.09 (t, *J* = 5.9 Hz, 2H), 3.17 (t, *J* = 5.9 Hz, 2H), 2.61 (s, 3H), 2.50 (s, 3H), 1.63 (s, 2H) ppm.

^{13}C-NMR: (101 MHz, $CDCl_3$) δ = 153.7, 142.8, 139.4, 135.2, 129.5, 129.0, 121.9, 120.4, 51.6, 41.8, 14.2, 10.1 ppm.

MS (*m/z*): (ESI, MeOH) Calculated for $[C_{13}H_{17}N_5H]^+$: 244.1562; found 244.1557.

2.3 Syntheses of Gd-AAP, Tb-AAP and Fe-AAP

The syntheses of **Gd-AAP**, **Tb-AAP** and **Fe-AAP** were carried out as follows: Starting from commercially available cyclen, firstly,three of four amines were substituted with *tert*-butyl bromoacetate. The remaning one was substituted with ethyl bromoacetate and subsequently hydrolyzed to obtain the carboxylic acid. Afterwards, amino AAP **1** was attached to the DOTA core via peptide coupling. After deprotection and complexation with $GdCl_3$, $TbCl_3$ and $FeOTf_2$, all three complexes **Gd-AAP**,**Tb-AAP** and **Fe-AAP** were obtained.



Scheme S3: i) *tert*-Butyl bromoacetate, $NaHCO_3$, ACN, rt, 24 h, 57%; **ii)** Ethyl bromoacetate, K_2CO_3, ACN, 82 °C, 3 d, 92%; **iii)** NaOH, MeOH, rt, 2 d, quant.; **iv)** AAP **1**, HOBt, EDCl, NMM, DMF, rt, 18 h, 78%; **v)** TFA, DCM, rt, 3 d, quant.; **vi)** $Ln(III)Cl_3 \times 6H_2O$ (Ln = Gd, Tb), H_2O, 60 °C, 18 h, 37% (Gd), 25% (Tb); **vii)** $Fe(II)OTf_2 \times 6H_2O$, MeOH, ACN, 65 °C, 19 h, 39%.

1,4,7-Tris(*tert*-butoxycarbonyl-methyl)-1,4,7,10-tetraazacyclododecane (11)

Cyclen (1.03 g, 5.97 mmol, 1.0 eq.) was dissolved in ACN (20 mL) and $NaHCO_3$ (2.56 g, 30.5 mmol, 5.1 eq.) was added. The reaction mixture was stirred at rt for 15 min. After that, *tert*-Butyl bromoacetate (2.73 mL, 18.5 mmol, 3.1 eq.) was added dropwise at 0 °C and the reaction mixture was stirred overnight at rt. $NaHCO_3$ was filtered off and the solvent was removed under reduced pressure. Purification of the crude product *via* column chromatography (SiO_2, DCM/MeOH/NH_4OH 10:1:0.1, R_f = 0.3) yielded **11** as a colourless solid.

Yield: 1.74 g (3.38 mmol, 57%).

^{1}H-NMR: (400 MHz, $CDCl_3$) δ = 10.07 (s, 1H), 3.77–2.42 (br, 22H,), 1.45 (d, J = 2.9 Hz, 27H) ppm.

^{13}C-NMR: (101 MHz, $CDCl_3$) δ = 173.0, 170.6, 169.7, 82.6, 82.4, 82.0, 81.9, 81.8, 58.3, 55.7, 51.4, 49.3, 47.6, 28.3, 28.3, 28.1, 28.0 ppm.

MS (*m/z*): (ESI, MeOH) Calculated for $[C_{26}H_{50}N_4O_6H]^+$: 515.3809; found 515.3803.

1-(Ethyl-acetate)-4,7,10-tris(*tert*-butoxycarbonyl-methyl)-1,4,7,10-tetra-azacyclododecane (10)[7]

K_2CO_3 (282 mg, 2.04 mmol, 2.0 eq.) and ethyl bromoacetate (113 µL, 1.02 mmol, 1.0 eq.) in ACN (5 mL) were added to a solution of **11** (525 mg, 1.02 mmol, 1.0 eq.) in ACN (40 mL). The reaction mixture was refluxed for 3 d. K_2CO_3 was filtered off, the solvent was removed under reduced pressure and the crude product was purified by column chromatography (SiO_2, DCM/MeOH 10:1, R_f = 0.3) resulting in a pale yellow solid.

Yield: 564 mg (939 µmol, 92%).

^{1}H-NMR: (400 MHz, $CDCl_3$) δ = 4.13 (q, J = 9.0 Hz, 2H), 3.78–1.65 (br, 24H), 1.44 (s, 27H), 1.25 (t, J = 7.1 Hz, 3H) ppm.

^{13}C-NMR: (101 MHz, $CDCl_3$) δ = 173.7, 173.2, 173.1, 82.0, 81.0, 61.3, 55.8, 55.8, 55.0, 28.0, 14.3 ppm.

MS (*m/z*): (ESI, MeOH) Calculated for $[C_{30}H_{56}N_4O_8Na]^+$: 623.3996; found 623.3990.

1-(Ethyl-acetate)-4,7,10-tris(*tert*-butoxycarbonyl-methyl)-1,4,7,10-tetra-azacyclododecane (9)[8]

To a stirred solution of **10** (554 mg, 923 µmol, 1.0 eq.) in MeOH (4 mL) at rt sodium hydroxide (60.0 mg, 1.50 mmol, 1.6 eq.) in H_2O (2 mL) was added slowly. The reaction mixture was stirred at rt for 2 d. Afterwards *Dowex-H*$^+$ resin (250 mg) was added and the mixture was stirred until the pH was neutral. The resin was filtered off and washed several times with MeOH. After the solvent was removed under reduced pressure **9** was obtained as a pale yellow film without further purification.

Yield: 530 mg (925 µmol, quant.).

^{1}H-NMR: (300 MHz, CD_3OD) δ = 3.89–1.87 (br, 24H), 1.46 (s, 27H) ppm.

^{13}C-NMR: (75 MHz, CD_3OD) δ = 177.4, 172.7, 81.3, 57.9, 55.9, 55.3, 27.1, 27.0, 27.0 ppm.

MS (*m/z*): (ESI, MeOH) Calculated for $[C_{28}H_{52}N_4O_8H]^+$: 573.3863; found 573.3858.

Tri-*tert*-butyl-2,2',2''-(10-(2-((2-(3,5-dimethyl-4-(phenyldiazenyl)-1*H*-pyrazol-1-yl)ethyl-amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)*(E)*-triacetate (8)

HOBt×H_2O (84.2 mg, 623 µmol, 1.2 eq.) and EDCl (120 mg, 623 µmol, 1.2 eq.) were added to a stirred solution of **9** (297 mg, 519 µmol, 1.0 eq.) in dry DMF (11 mL). After 30 min **1** (152 mg, 623 µmol, 1.2 eq.) in dry DMF (6 mL) was added dropwise, followed by NMM (70.0 µL, 623 µmol, 1.2 eq.). The reaction mixture was stirred at rt for 18 h. The solvent was removed under reduced pressure and the residue was dissolved in EtOAc. The organic layer was washed with H_2O (30 mL), NaCl (sat., 30 mL), dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified *via* column chromatography (SiO_2, DCM/MeOH/NH_4OH 10:1:0.1, R_f = 0.2) yielding **8** as an orange solid.

Yield: 324 mg (406 µmol, 78%).

^{1}H-NMR: (400 MHz, $CDCl_3$) δ = 9.26 (t, *J* = 5.9 Hz, 1H), 7.77–7.72 (m, 2H), 7.50–7.41 (m, 2H) 7.38–7.31 (m, 1H), 4.43–1.89 (br, 28H), 2.71 (s, 3H), 2.47 (s, 3H), 1.44 (d, 27H) ppm.

^{13}C-NMR: (101 MHz, $CDCl_3$) δ = 172.3, 171.8, 153.8, 142.3, 135.1, 129.2, 129.0, 121.8, 82.2, 56.4, 55.7, 48.5, 39.1, 29.8, 28.2, 28.1, 14.5, 10.5 ppm.

MS (*m/z*): (ESI, MeOH) Calculated for $[C_{41}H_{67}N_9O_7Na]^+$: 820.5061; found 820.5068.

(*E*)-2,2',2''-(10-(2-((2-(3,5-dimethyl-4-(phenyldiazenyl)-1*H*-pyrazol-1-yl)ethyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)-triacetic acid (7)[9]

8 (285 mg, 357 µmol, 1.0 eq.) was dissolved in DCM/TFA (1:1, 2 mL) and stirred at rt for 3 d under argon atmosphere. DCM (10 mL) was added and removed under reduced pressure (3×). After lyophilisation, the product was isolated as an orange solid without further purification.

Yield: 368 mg (584 µmol, quant.).

^{1}H-NMR: (300 MHz, $(CD_3)_2SO$) δ = 8.69 (t, 1H) 7.96–7.64 (m, 2H), 7.58–7.48 (m, 2H) 7.49–7.40 (m, 1H), 4.38–2.77 (br, 28H), 2.58 (s, 3H), 2.38 (s, 3H) ppm.

^{13}C-NMR: (75 MHz, $(CD_3)_2SO$) δ = 159.2, 158.7, 158.3, 157.9, 153.1, 140.9, 134.6, 129.7, 129.3, 122.9, 121.5, 118.9, 115.0, 69.9, 52.8, 50.4, 48.7, 47.5, 29.1, 14.0, 9.5 ppm.

MS (*m/z*): (ESI, H_2O/ACN) Calculated for $[C_{29}H_{43}N_9O_7H]^+$: 630.3364; found 630.3352.

General procedure I: Synthesis of (*E*)-2,2',2''-(10-(2-((2-(3,5-dimethyl-4-(phenyldiazenyl)-1*H*-pyrazol-1-yl)ethyl)amino)-2-oxoethyl)-1,4,7,10-tetra-azacyclododecane-1,4,7-triyl)triacetic acid lanthanide complexes (Gd-AAp, Tb-AAP)[9]

7 (1.0 eq.) was dissolved in H_2O (33.1 µL/µmol) and Ln(III)Cl_3×$6H_2O$ (1.1 eq.) was added. The pH value was adjusted to pH 5.5 – 7 by with a KOH solution (0.1 M). The reaction mixture was stirred at 60 °C for 18 h. Lyophilization and prep. HPLC led to the desired compound.

Gd(III)-AAP: Yield: 43.7 mg (55.7 µmol, 37%).

MS (*m/z*): (ESI, MeOH) Calculated for $[C_{29}H_{40}GdN_9O_7H]^+$: 785.2370; found 785.2374.

Tb(III)-AAP: Yield: 32.4 mg (41.3 µmol, 25%).

MS (*m/z*): (ESI, MeOH) Calculated for $[C_{29}H_{40}N_9O_7TbNa]^+$: 808.2202; found 808.2194.

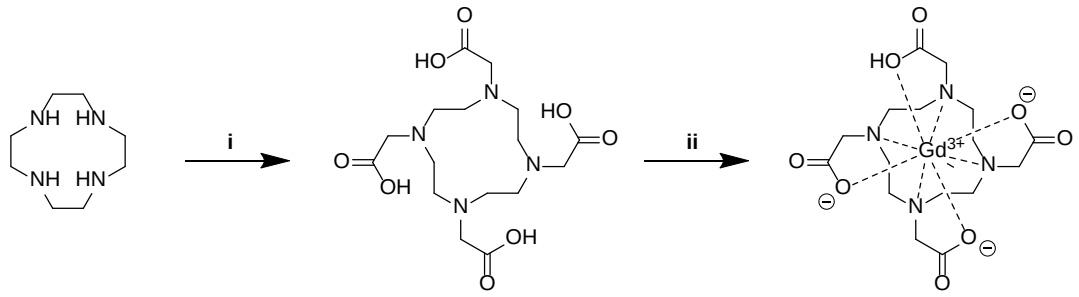
Synthesis of (*E*)-2,2',2''-(10-(2-((2-(3,5-dimethyl-4-(phenyldiazenyl)-1*H*-pyrazol-1-yl)ethyl)amino)-2-oxoethyl)-1,4,7,10-tetra-azacyclododecane-1,4,7-triyl)triacetic acid iron (III) complex (Fe-AAP)[10]

7 (1.0 eq.) was dissolved in a mixture of dry MeOH (4.85 µL/µmol) and dry ACN (19.4 µL/µmol) and Fe(II)OTf_2×$6H_2O$ (1.1 eq.) was added under argon atmosphere. The reaction mixture was stirred overnight at 65 °C. After lyophilization and prep. HPLC the yielded solid was again freeze-dried affording the desired compound.

Fe(III)-AAP (5): Yield: 54.4 mg (79.7 µmol, 39%).

MS (*m/z*): (ESI, MeOH) Calculated for $[C_{29}H_{40}FeN_9O_7]^-$: 682.2400; found 682.2394.

2.4 Synthesis of Gd-DOTA



Scheme S4: i) Chloroacetic acid, H_2O, 100 °C, 22 h, 76%; **ii)** $GdCl_3 \times 6H_2O$, H_2O, 60 °C, 24 h, quant.

<u>1,4,7,10-tetraazacyclododecane-tetraacetic acid</u> (14)[11]

To a stirred solution of cyclen (488 mg, 2.83 mmol, 1.0 eq.) in H_2O (4 mL) a certain amount of a HCl solution (1 M) was added to adjust the pH value to 8.5. Afterwards, chloroacetic acid (1.18 g, 12.5 mmol, 4.4 eq.) was added and the reaction mixture was stirred at 75 °C while the pH value was kept at 9-10 by adding a few drops of a KOH solution (6 M) from time to time. The reaction mixture was refluxed overnight. Then, the pH value was adjusted to 1 with a HCl solution (6 M) whereby a solid precipitate was formed. After filtration, the crude product was purified by recrystallization from HCl (6 M).

Yield: 865 mg (2.14 mmol, 76%).

^{1}H-NMR: (300 MHz, $(CD_3)_2SO$) δ = 3.86 (s, 8H), 3.55–3.03 (m, 16H) ppm.

^{13}C-NMR: (75 MHz, $(CD_3)_2SO$) δ = 170.3, 53.3, 49.5 ppm.

MS (*m/z*): (ESI, ACN/H_2O) Calculated for $[C_{16}H_{28}N_4O_8K]^+$: 443.1544; found 443.1538.

<u>Gd-Dota</u>[9]

$GdCl_3 \times 6H_2O$ (50.5 mg, 136 µmol, 1.1 eq.) was added to a stirred solution of **14** (50.0 mg, 124 µmol, 1.0 eq.) in H_2O (5 mL) and the pH value was adjusted to 5.5 – 7 with a KOH solution (1 M). The reaction mixture was stirred at 60 °C for 24 h. Lyophilisation yielded the desired compound as a white flaked solid which was used without further purification.

Yield: 143 mg (256 mmol, quant.).

MS (*m/z*): (ESI, ACN/H_2O) Calculated for $[C_{16}H_{24}N_4O_8Gd]^-$: 558.0841; found 558.0838.

3. NMR and mass spectra

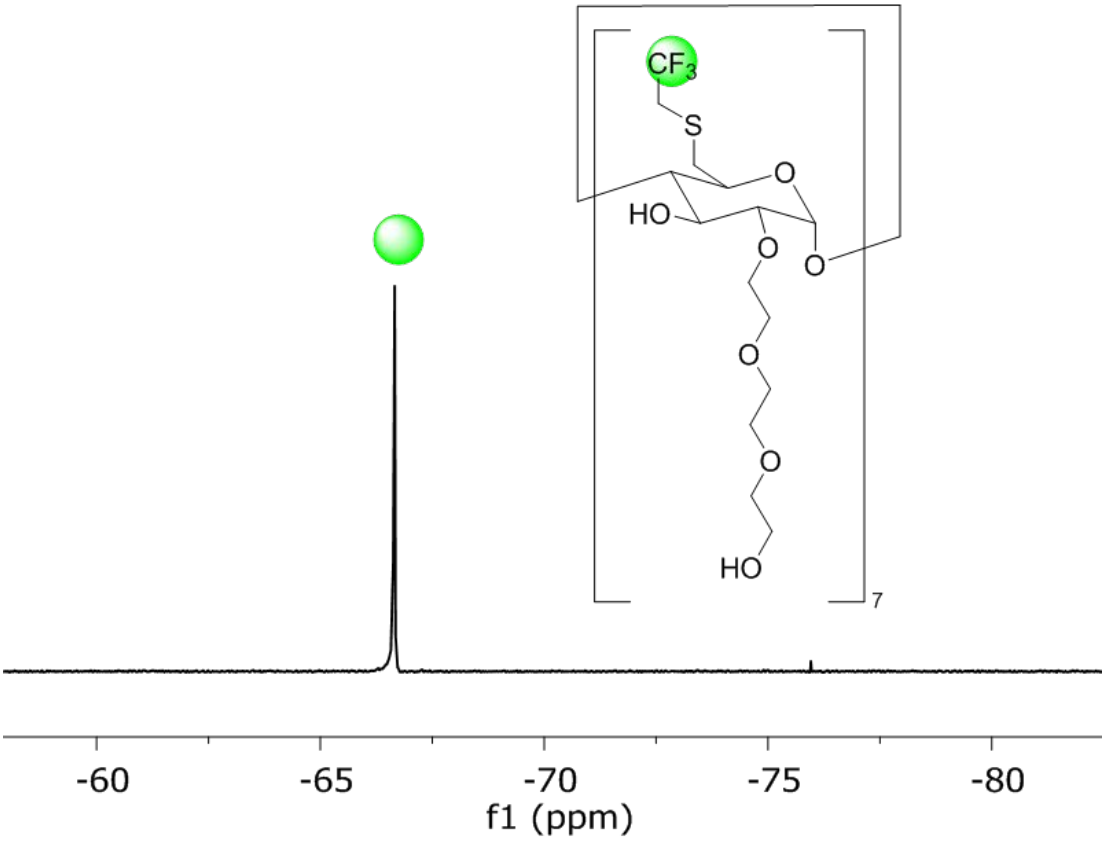


Figure S1: Structure and ^{19}F NMR spectrum of **FCD**.

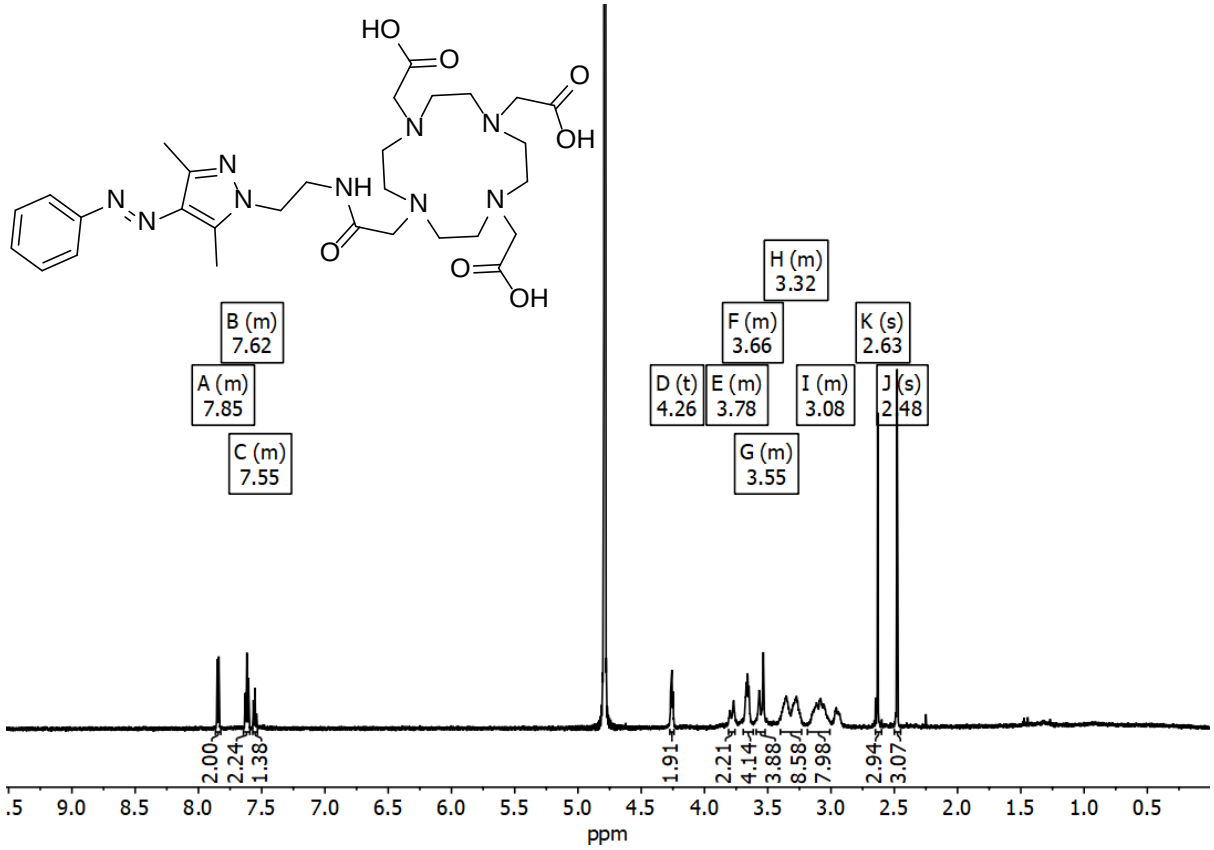


Figure S2: ^{1}H NMR of compound **7**.

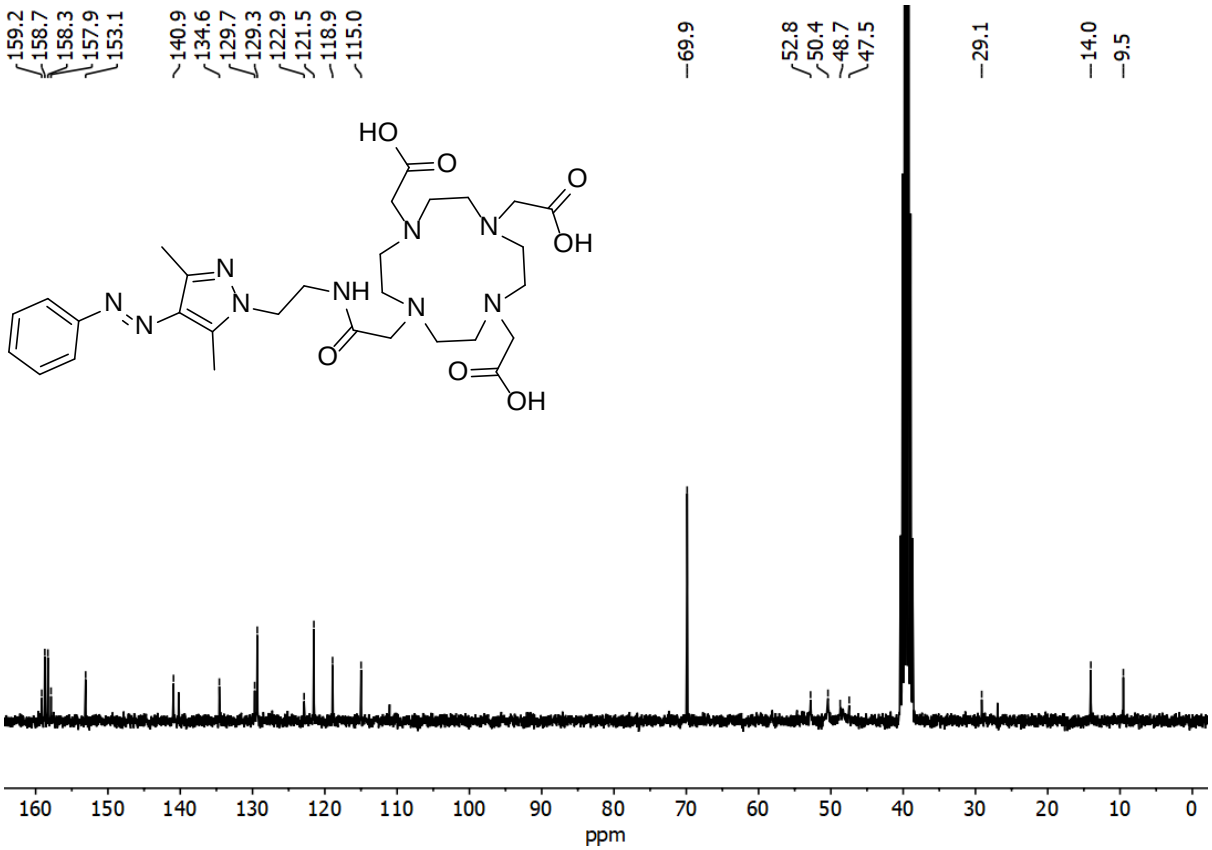


Figure S3: ^{13}C NMR of compound **7**.

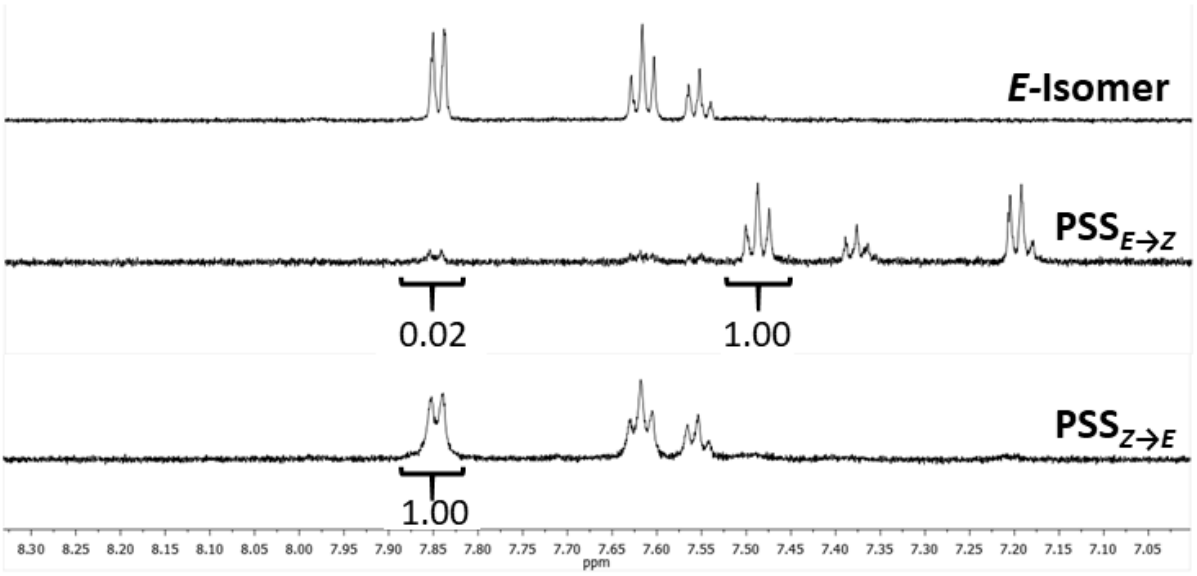


Figure S4: Aromatic region of the ^{1}H-NMR spectra of **7** (*c* = 500 µmol/L in D_2O)..

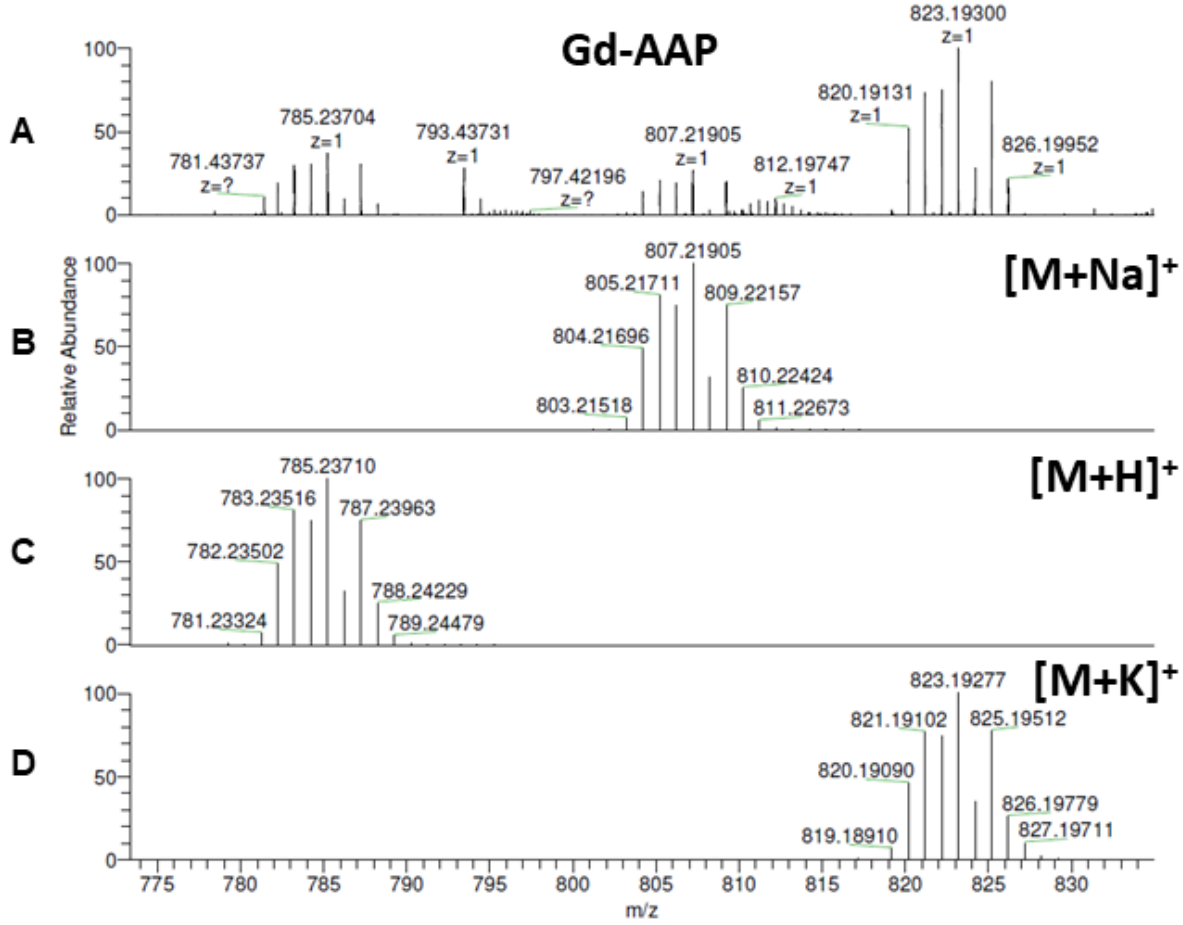


Figure S5: MS of complex Gd-AAP.

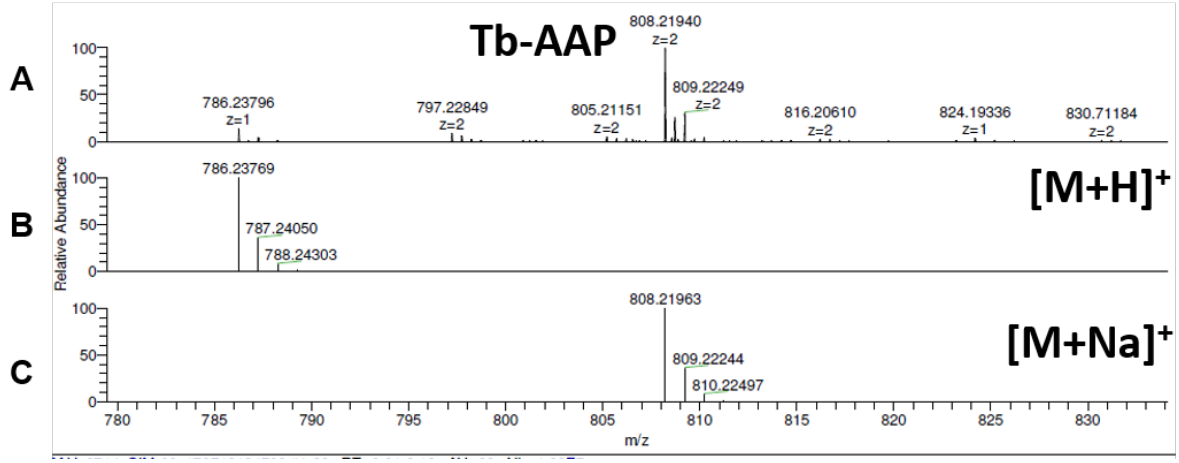


Figure S6: MS of complex Tb-AAP.

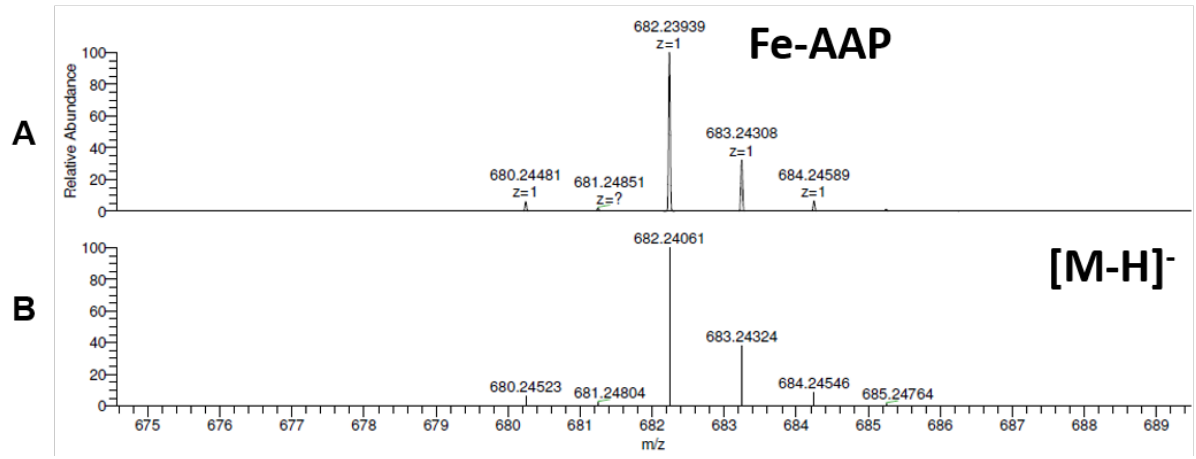


Figure S7: MS of complex Fe-AAP.

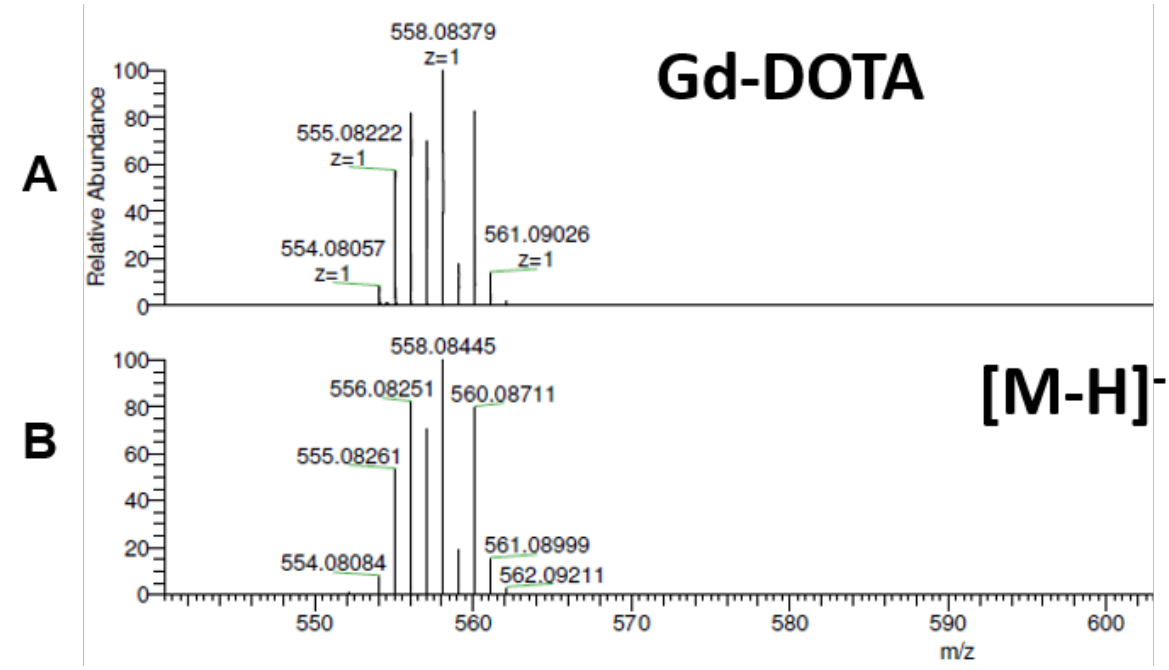


Figure S8: MS of complex Gd-DOTA.

4. UV/Vis spectroscopy

4.1 Gd-AAP

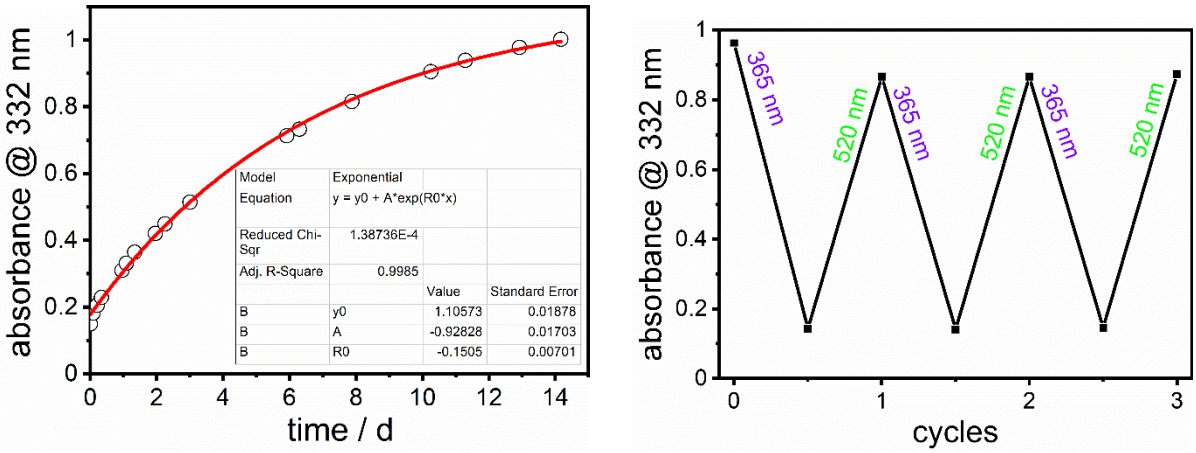


Figure S9: Time dependent absorbance for the determination of the half-life time of Z-isomer Gd-AAP (*c* = 55 µmol in ddH_2O) and absorbance at 332 nm for three switching cycles.

4.2 Tb-AAP

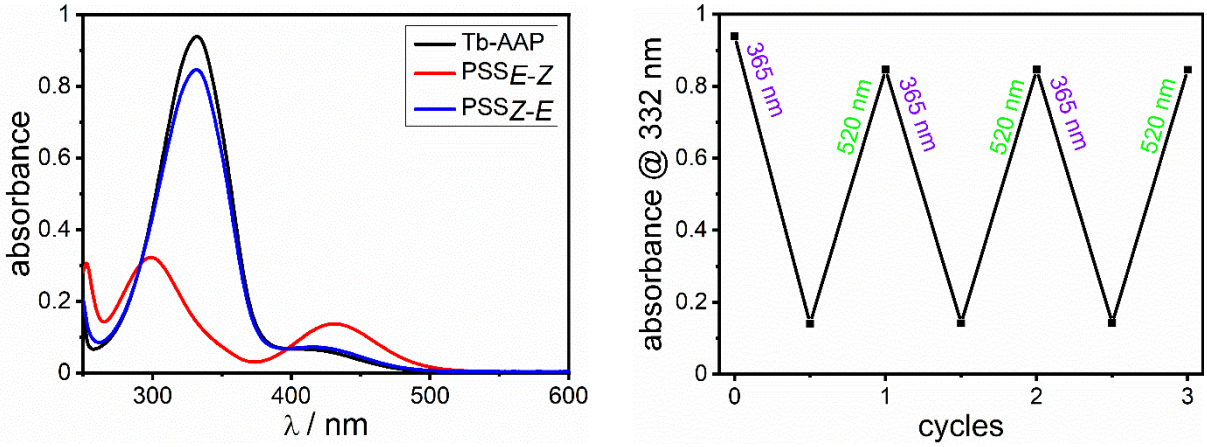


Figure S10: UV/vis spectrum of the photoisomerization of Tb-AAP (*c* = 70 µmol in ddH_2O) and absorbance at 332 nm for three switching cycles.

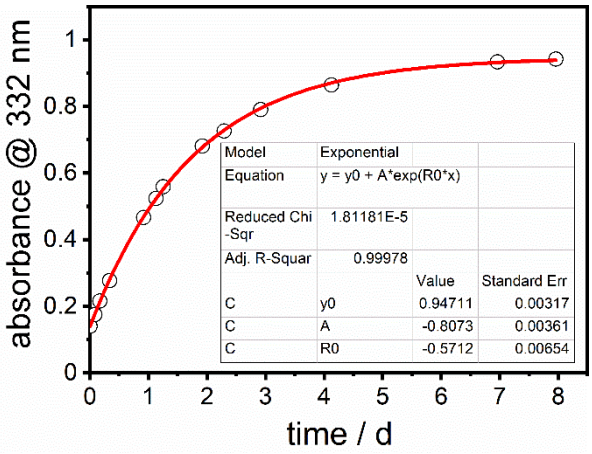


Figure S11: Time dependent absorbance for the determination of the half-life time of Z-isomer Tb-AAP (*c* = 70 µmol in ddH_2O).

4.3 Fe-AAP

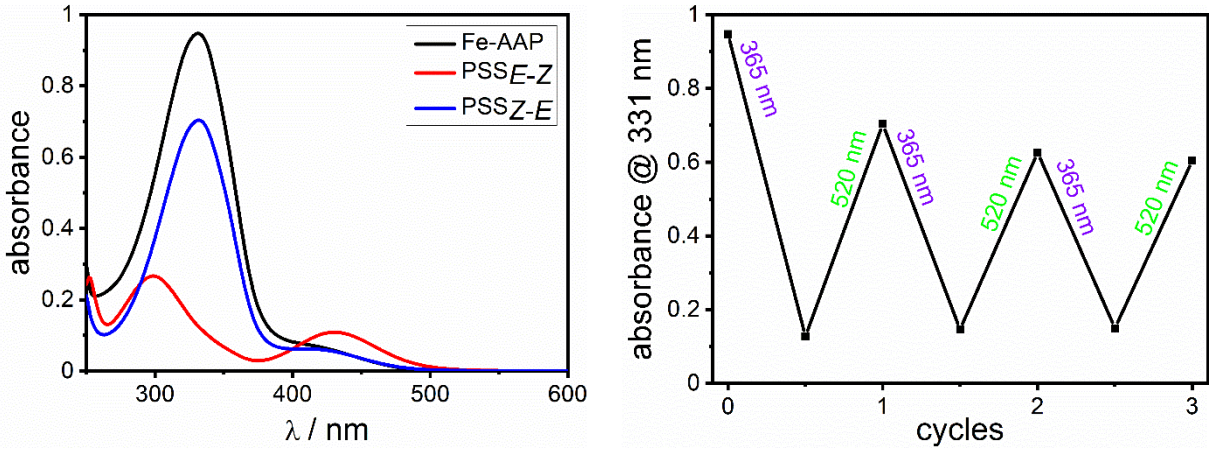


Figure S12: UV/vis spectrum of the photoisomerization of Fe-AAP (*c* = 40 µmol in ddH_2O) and absorbance at 331 nm for three switching cycles.

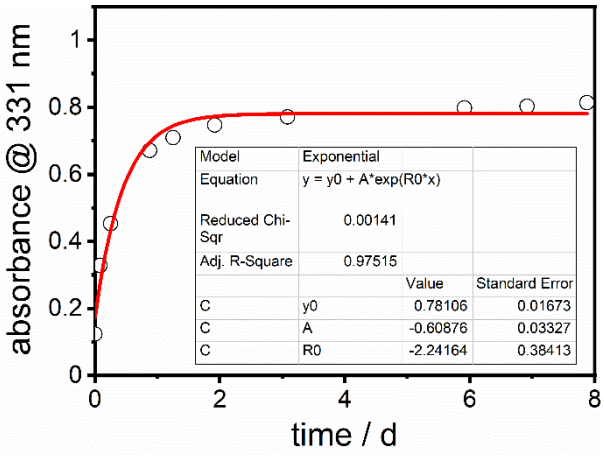


Figure S13: Time dependent absorbance for the determination of the half-life time of Z-isomer Fe-AAP (*c* = 40 µmol in ddH_2O).

5. Isothermal titration calorimetry

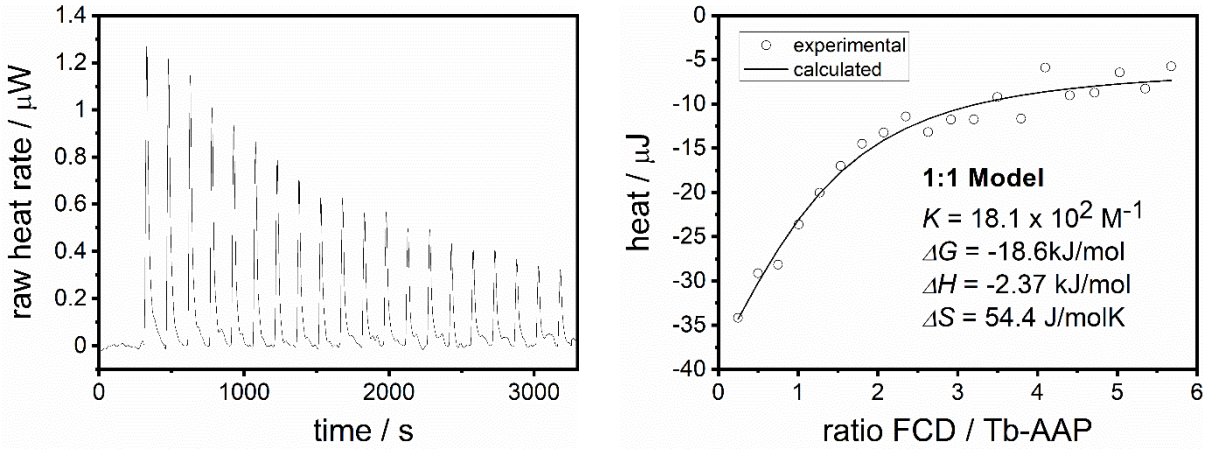


Figure S14: ITC of FCD with terbium complex with Tb-AAP.

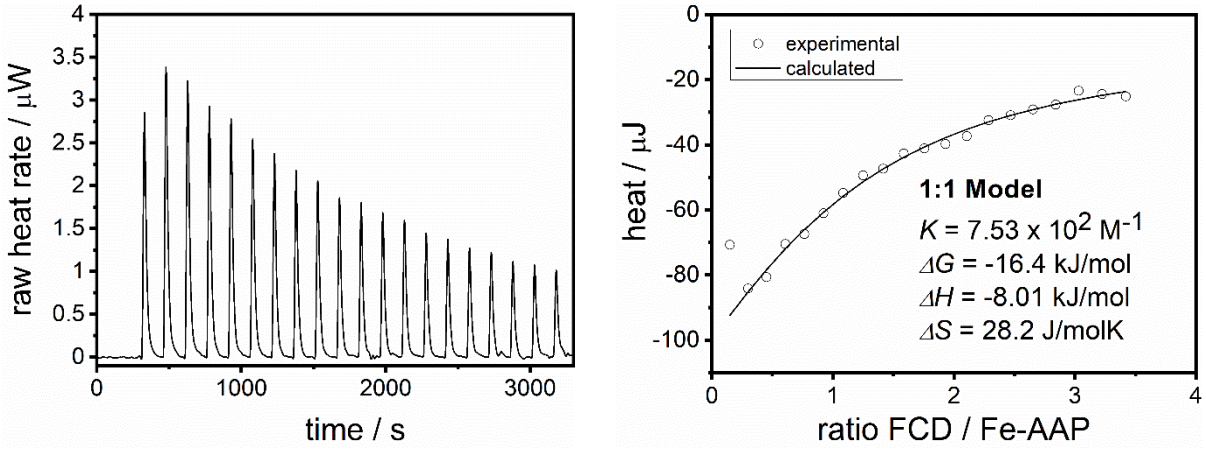


Figure S15: ITC of FCD with iron complex with Fe-AAP.

6. T_1 relaxation time measurement

6.1 Inversion recovery experiments

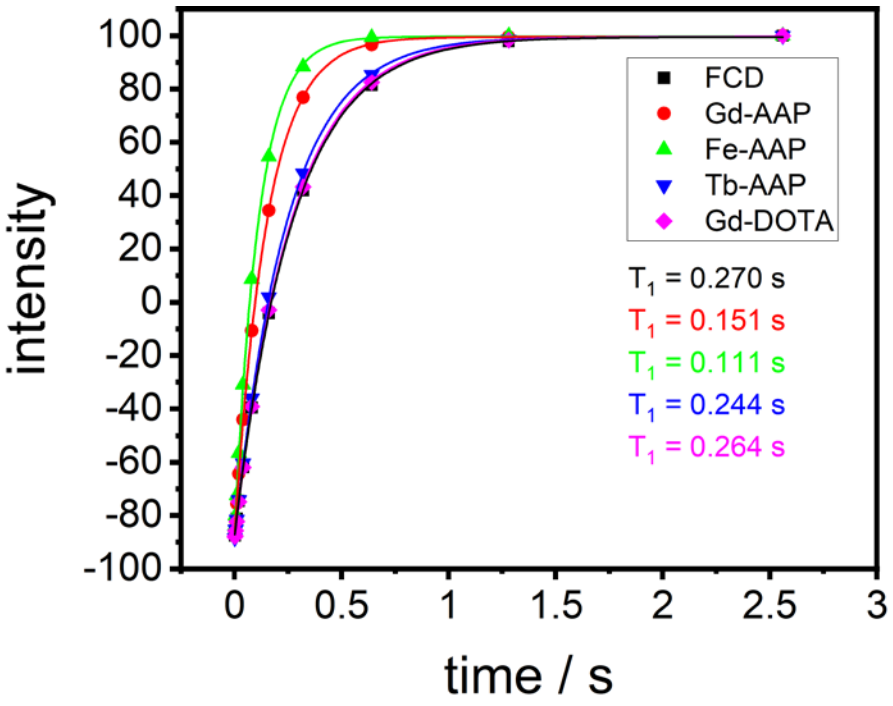


Figure S16: Experimental normalized longitudinal relaxation time (T_1) measurements of FCD (2.5 mM) with AAP-complexes and Gd-DOTA (2.5 mM): Exponential fitting of the signal intensity for the FCD and the equimolar mixtures with Gd-AAP, Fe-AAP, Tb-AAP and Gd-DOTA.

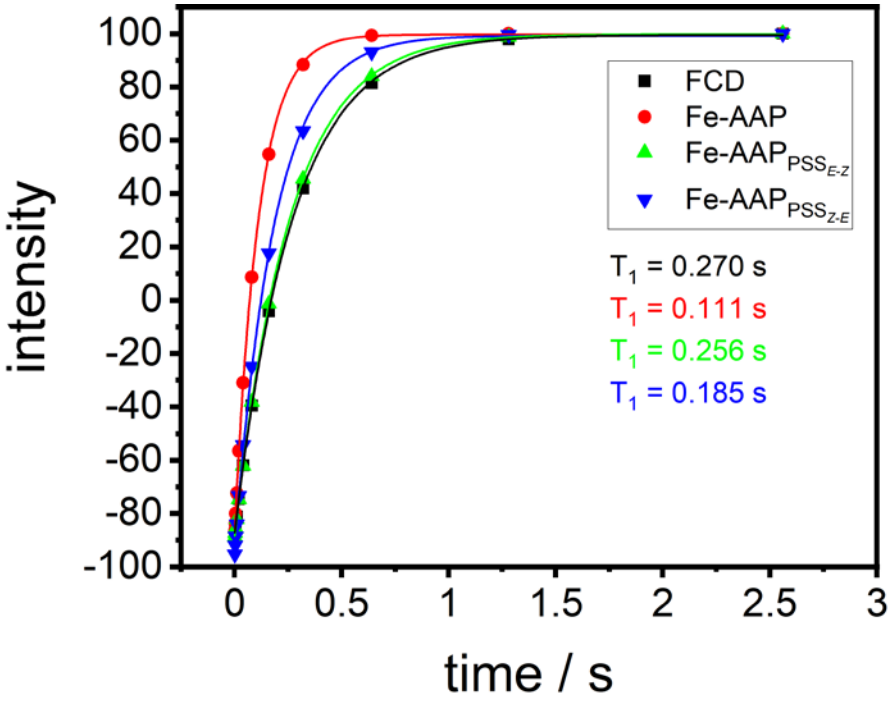


Figure S17: Experimental normalized longitudinal relaxation time (T_1) measurements of FCD (2.5 mM) with Fe-AAP (2.5 mM): Exponential fitting of the signal intensity for the FCD and the equimolar mixtures with Fe-AAP, Fe-AAP(PSS_{E-Z}), Fe-AAP (PSS_{Z-E}).

6.2 Concentration series

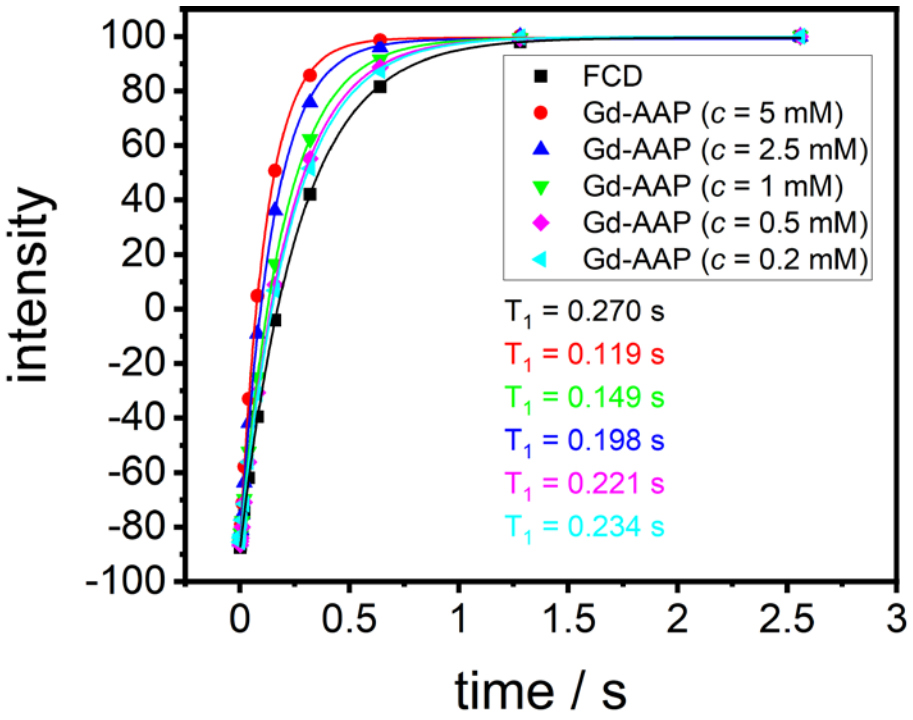
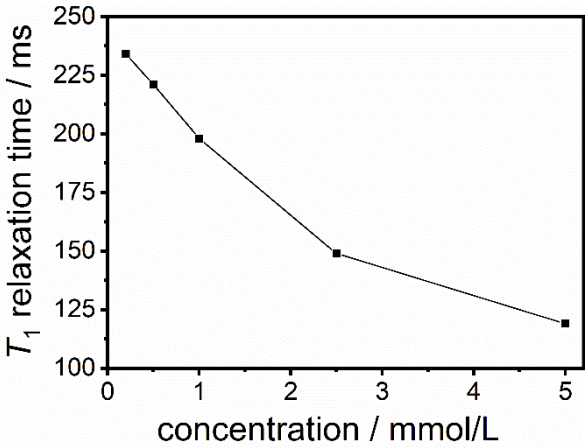


Figure S18: Experimental normalized longitudinal relaxation time (T_1) measurements of FCD (2.5 mM) with Gd-AAP (5 mM to 0.2 mM): Exponential fitting of the signal intensity for the FCD and mixtures of Gd-AAP with different concentrations.

Table S2: Concentration-dependent T_1 relaxation times of FCD/Gd-AAP.



c FCD/Gd-AAP (mmol/L)	T_1 relaxation time (ms)
5	119
2.5	149
1	198
0.5	221
0.2	234

Figure S19: Concentration-T_1 relaxation time-diagram of FCD/Gd-AAP.

7. High performance liquid chromatography (HPLC)

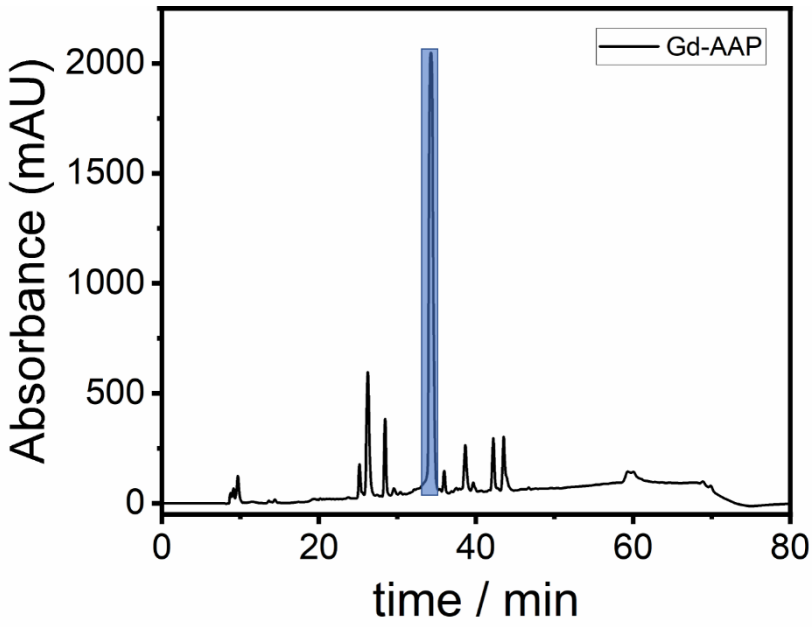


Figure S20: HPLC chromatogram of Gd-AAP during purification after synthesis. The area marked in blue shows the collected peak.

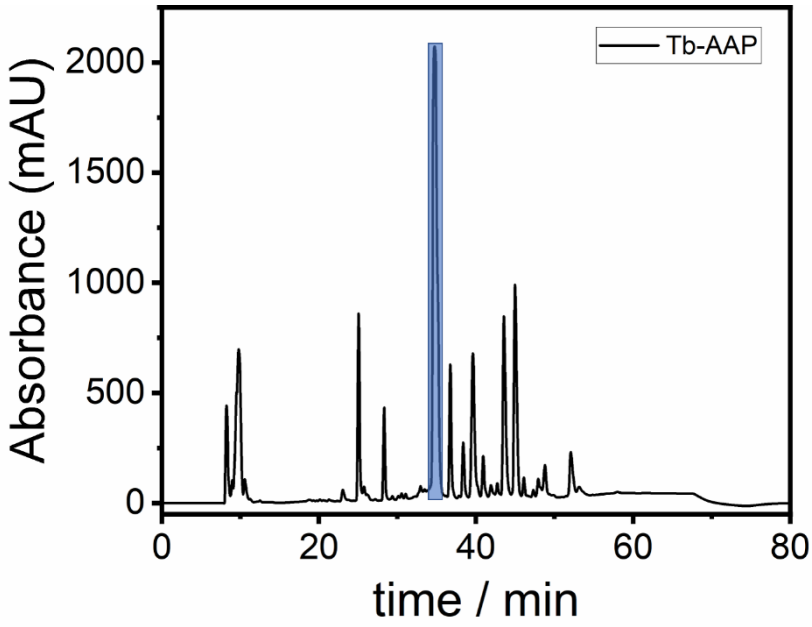


Figure S21: HPLC chromatogram of Tb-AAP during purification after synthesis. The area marked in blue shows the collected peak.

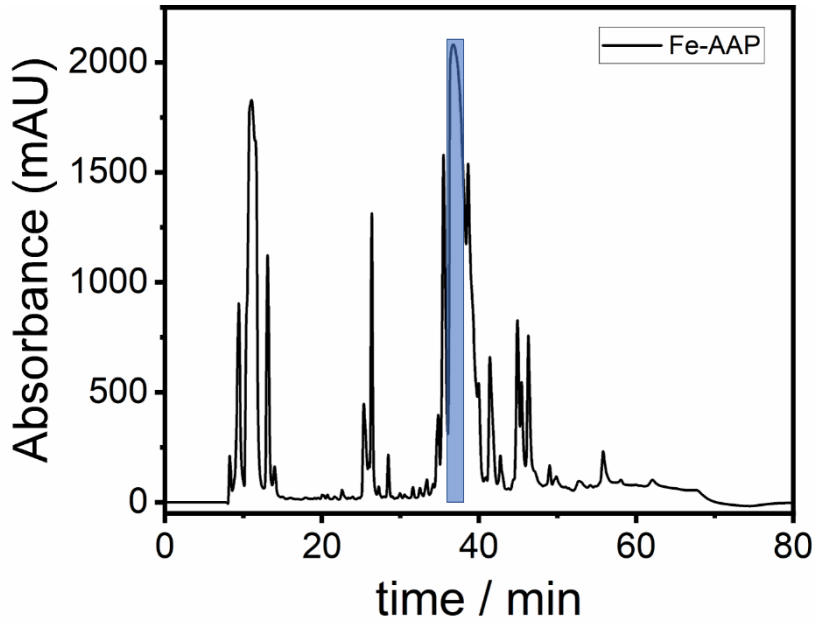


Figure S22: HPLC chromatogram of Fe-AAP during purification after synthesis. The area marked in blue shows the collected peak.

8. Electron paramagnetic resonance (EPR)

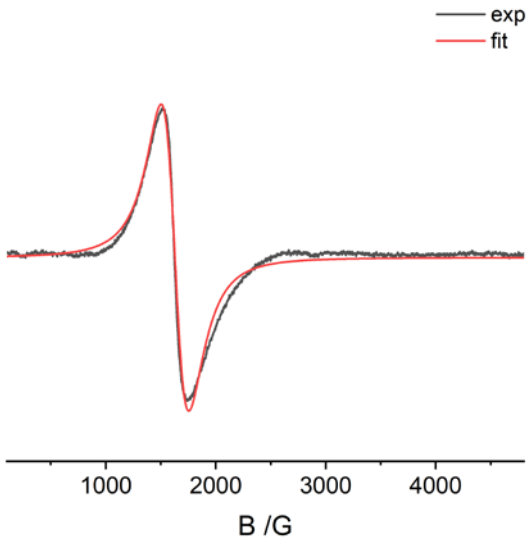


Figure S23. Solid-state CW EPR spectrum of **Fe-AAP** at 140 K recorded at X-band. The black and red curves represent the experimental EPR spectrum and the best fit simulation using Easyspin,[2,3] respectively.

9. Literature

1) *MATLAB Release 2018b*. (2018). MathWorks, Inc.
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