# ELECTRONIC SUPPLEMENTARY INFORMATION

# for

## One-pot synthesis of a piperidine-based rigidified DTPA analogue and its bifunctional chelating agent.

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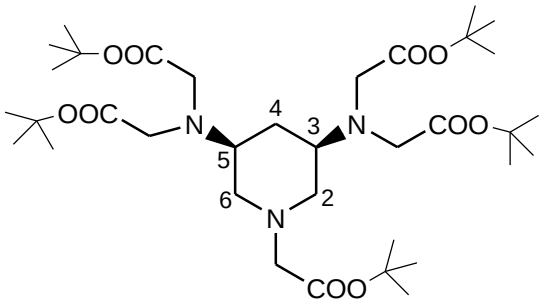
### 1. Materials and Instrumentation

All chemicals were purchased from Sigma-Aldrich Co. or Alfa Aesar Co. and were used without purification unless otherwise stated. "Petroleum ether" (PetEt) refers to petroleum ether with boiling point in the range 40-60 °C. Thin-layer chromatography (TLC) was carried out on silica plates (silica gel 60 $F_{254}$, Merck 5554) and visualized by UV lamp (254 nm) or stained in basic $KMnO_4$ solution. Preparative column chromatography was carried out using silica gel (Merck Silica Gel 60, 230 ± 400 mesh) pre-soaked in the starting eluent. $^{1}H$ and $^{13}C$ NMR spectra were recorded on a JEOL Eclipse Plus 400 and on a Bruker AvanceIII spectrometers operating at 9.4 and 11.7 T, respectively. Chemical shifts are reported relative to TMS and were referenced using the residual proton solvent resonances. Chemical shifts are reported in ppm and coupling constants in Hz. Splitting patterns are described as singlet (s), broad singlet (br s), doublet (d), double doublet (dd), triplet (t), quartet (q), multiplet (m) or broad multiplet (br m). Electrospray ionization mass spectra (ESI MS) were recorded on a SQD 3100 Mass Detector (Waters), operating in positive or negative ion mode, with 1% v/v HCOOH in methanol as the carrier solvent. HPLC analyses were carried out on a 1525 Waters liquid chromatograph equipped with Waters 2489 UV/vis and Waters SQD 3100 MS detectors and using a Waters RPC18 column (150 mm x 4.6 mm, 5µm, Atlantis®); Solvent A: TFA/$H_2O$ 0.1%, Solvent B: TFA/$CH_3OH$ 0.1%; t=0 100% A, t=5 minutes 100% A, t=15 minutes 100% B (linear gradient), t=18min 100% B; flow rate of 1 mL/min. Infrared (IR) spectra were recorded in the range 4000–400 $cm^{-1}$ at 4 $cm^{-1}$ resolution using a Bruker Equinox 55 spectrometer.

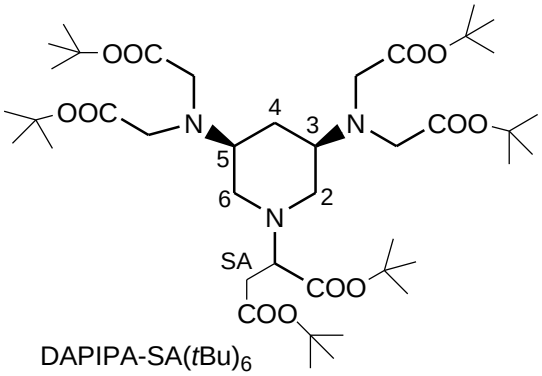
## 2. Experimental Section

*Synthesis of protected ligands via N-alkylation-Stevens rearrangement*

*t*-Butylbromoacetate (1.03 mL, 7 mmol) was slowly added to a suspension of *cis*-3,5-diaminopiperidine (358 mg, 1 mmol), $K_2CO_3$ (2.07 g, 15 mmol) and KI (830 mg, 5 mmol) in $CH_3CN$ (50 mL). The resulting mixture was heated under reflux for 16h. After filtration of the insoluble salts the solvent was removed *in vacuo*. The residue was then dissolved in diethyl ether (30 mL) and washed with 0.1M HCl, $H_2O$ and brine (20 mL each). Column chromatography ($SiO_2$, PetEt/EtOAc 8:2) yielded the two products DAPIPA(*t*Bu)$_5$ (TLC: PetEt/EtOAc 7:3, $R_f$ 0.32; 290 mg, 0.42 mmol, 42% yield) and DAPIPA-SA(*t*Bu)$_6$ (TLC: PetEt/EtOAc 7:3, $R_f$ 0.60; 280 mg, 0.35 mmol, 35% yield).




DAPIPA(*t*Bu)$_5$




DAPIPA-SA(*t*Bu)$_6$

**DAPIPA(*t*Bu)$_5$**: $^1$H-NMR ($CDCl_3$) 400 MHz δ = 3.46 (4H, d, *J*=17.6 Hz, C$H_2$CO), 3.37 (4H, d, *J*=17.6 Hz, C$H_2$CO), 3.10 (2H, s, C$H_2$CO), 2.94 (2H, m, $CH_{2(2,6eq)}$), 2.87 (2H, m, NC$H_{(3,5)}$), 2.08 (1H, d, *J*=10.2 Hz, $CH_{2(4eq)}$), 1.96 (2H, t, *J*=10.2 Hz, $CH_{2(2,6ax)}$), 1.40 (45H, s, C$H_3$), 1.11 (1H, dd, *J*=10.2, 2.1 Hz, $CH_{(4ax)}$). $^{13}$C-NMR ($CDCl_3$) 100 MHz δ = 171.5, 169.3 (*C*OO*t*Bu), 81.1, 80.8 (*C*$CH_3$), 59.7 (*C*$H_2$CO), 58.2 (*C*$H_{(3,5)}$), 56.4 (*C*$H_{2(2,6)}$), 53.7 (*C*$H_2$CO), 33.2 (*C*$H_{2(4)}$), 28.1 (*C*$H_3$). IR (KBr disk), cm$^{-1}$: 3389, 3005, 2911, 1747, 1644, 1522, 1356, 1145, 1072. ESI MS (m/z): 686.55 ($M+H^+$) (calc. for $C_{35}H_{63}N_3O_{10}$: 685.89).

**DAPIPA-SA(*t*Bu)$_6$**: $^1$H-NMR ($CDCl_3$) 400 MHz δ = 3.49 (1H, t, *J*=8.0 Hz, NC$H_{(SA)}$), 3.35 (8H, s, C$H_2$CO), 2.82 (2H, d, *J*=10.5, $CH_{2(2,6eq)}$), 2.71 (1H, t, *J*=10.5 Hz, $CH_{(3,5)}$), 2.64 (1H, t, *J*=10.5 Hz, $CH_{(3,5)}$), 2.56 (1H, dd, *J*=14.5, 8.0 Hz, C$H_2$C$H_{(SA)}$), 2.33 (1H, dd, *J*=14.5, 8.0 Hz, C$H_2$C$H_{(SA)}$), 2.30 (1H, t, *J*=10.5 Hz, $CH_{2(6ax)}$), 2.01 (1H, d, *J*=10.5 Hz, $CH_{2(4eq)}$), 1.84 (1H, t, *J*=10.5 Hz, $CH_{2(2ax)}$), 1.33 (54H, s, C$H_3$), 1.02 (1H, q, *J*=10.5 Hz, $CH_{2(4ax)}$). $^{13}$C-NMR ($CDCl_3$) 100 MHz δ = 171.3, 170.4, 169.9 (*C*OO*t*Bu), 81.3, 80.7, 80.3 (*C*$CH_3$), 64.6 (*C*$H_{(SA)}$), 58.8, 58.6 (*C*$H_{(3,5)}$), 56.8 (*C*$H_{2(6)}$), 53.6 (*C*$H_2$CO), 50.3 (*C*$H_{2(2)}$), 36.2 (*C*$H_2$C$H_{(SA)}$), 33.8 (*C*$H_{2(4)}$), 28.3, 28.1, 27.7 (*C*$H_3$). IR (KBr

disk), $cm^{-1}$: 3371, 2975, 2914, 1731, 1639, 1520, 1370, 1149. ESI MS (m/z): 801.18 ($M+H^+$) (calc. for $C_{41}H_{73}N_3O_{12}$: 800.03).

*Synthesis of deprotected ligands*

The protected ligands (0.3 mmol) were dissolved in $CH_2Cl_2$ and trifluoroacetic acid (1:1 v:v, 2 mL) and stirred at RT overnight. The reaction mixture was concentrated *in vacuo* and the product was precipitated with diethyl ether, isolated by centrifugation and washed with diethyl ether obtaining a white solid. Quantitative yield.

**DAPIPA**: HPLC Analysis: RT= 4.95 min. $^1$H-NMR ($D_2O$) 400MHz δ = 3.97 (2H, s, C$H_2$CO), 3.76 (8H, s, C$H_2$CO), 3.74 (2H, m, $CH_{2(2,6eq)}$), 3.50 (2H, m, NC$H_{(3,5)}$), 2.99 (2H, t, *J*=11.7 Hz, $CH_{2(2,6ax)}$), 2.44 (1H, d, *J*=11.7 Hz, $CH_{2(4eq)}$), 1.71 (1H, q, *J*=11.7 Hz, $CH_{2(4ax)}$). $^{13}$C-NMR ($CDCl_3$) 100MHz δ = 173.9, 168.8 (*C*OOH), 57.8 ($CH_{2(2,6)}$), 56.3 ($CH_{(3,5)}$), 53.2 ($CH_2CO$), 52.9 ($CH_2CO$), 27.8 ($CH_{2(4)}$). IR (KBr disk), $cm^{-1}$: 3335, 3015, 2958, 2916, 2849, 1733, 1636, 1400, 1226, 1145, 972, 890, 721. ESI MS (m/z): 404.25 ($M-H^+$) (calc. for $C_{15}H_{23}N_3O_{10}$: 405.36).

**DAPIPA-SA**: HPLC Analysis: RT= 4.05 min. $^1$H-NMR ($CDCl_3$) 400 MHz δ = 3.98 (1H, m, NC$H_{(SA)}$), 3.94 (8H, s, C$H_2$CO), 3.54 (2H, br m, $CH_{(3,5)}$), 3.31 (2H, m, $CH_{2(2,6eq)}$), 2.89, 2.80 (2H, m, C$H_2$CH$_{(SA)}$), 2.82 (1H, m, $CH_{2(2ax)}$), 2.64 (1H, d, *J*=10.5 Hz, $CH_{2(6ax)}$), 2.46 (1H, br m, $CH_{2(4eq)}$), 1.75 (1H, br m, $CH_{2(4ax)}$). $^{13}$C-NMR ($CDCl_3$) 100 MHz δ = 174.5, 173.3, 172.5 (*C*OOH), 63.8 ($CH_{(SA)}$), 59.6 ($CH_{(3,5)}$), 53.9 ($CH_2CO$), 50.3, 48.1 ($CH_{2(2,6)}$), 33.0 ($CH_2CH_{(SA)}$), 26.7 ($CH_{2(4)}$). IR (KBr disk), $cm^{-1}$: 3335, 3016, 2918, 2849, 1727, 1634, 1399, 1337, 1237, 1199, 967, 900, 721. ESI MS (m/z): 462.24 ($M-H^+$) (calc. for $C_{17}H_{25}N_3O_{12}$: 463.39).

*$^1$H NMR relaxation measurements:*

The water proton longitudinal relaxation rates as a function of the magnetic field strength were measured on a fast field-cycling Stelar SmartTracer relaxometer (Mede, Pv, Italy) and on a Bruker WP80 NMR electromagnet adapted to variable-field measurements (15–80 MHz) Stelar Relaxometer. The exact concentration of gadolinium was determined by measurement of bulk magnetic susceptibility shifts of tBuOH $^1$H NMR signal. The pH dependence was measured in the interval 3-11 by rising the pH from 6.5 to the basic region by slow addition of 0.1 mM NaOH and than lowering it to pH = 4 by slow addition of 0.1 mM HCl.

*Serum stability measurements:*

The water proton longitudinal relaxation rates (20MHz, 25°C) of GdDAPIPA and GdDAPIPA-SA in human serum (Seronorm™ purchased from SERO AS Norway) was measured over a period of

48 h. The lack of a significant change of relaxivity over time gives a good indication of the stability of GdDAPIPA and GdDAPIPA-SA in human serum (Figure S1).

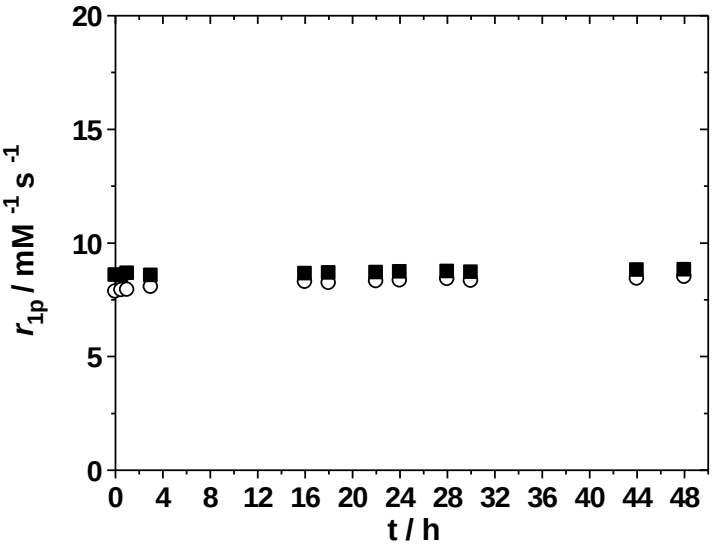



Figure S1. Plot of $^{1}$H relaxivity $r_1$ at 20 MHz and 25 °C for GdDAPIPA$^{2-}$ (○),GdDAPIPA-SA $^{-}$ (■) in seronorm over time.