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Preparation of HOPO-containing lariate ethers based on the diaza-18-crown-6 scaffold

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Abstract
Cyclic and acyclic ligands containing the hydroxypyridinone (HOPO) moiety as donor group are known as strong coordinating compounds for a wide variety of metal ions. Based on the diaza-crown[18]ether Kryptofix K22, five different tendentate ligands were prepared using 1,2-HOPO, 1,2,3-HOPO and 2,3-Me-HOPO as additional binding moieties. The diaza-crown ether basic skeleton was furnished with two primary amine functions and subsequently reacted with the respective HOPO acids or the HOPO acid chlorides to obtain the desired HOPO derivatives in two synthesis steps after final deprotection. All compounds were evidenced by NMR and MS analyses.

Keywords HOPO · Multidentate ligand · Diazacrown ether

Introduction

Multidentate complexing compounds containing hydroxypyridinone (HOPO) moieties as binding motif shown exemplarily in Scheme1 have been studied in the past for their ability to coordinate hard metallic cations (Santos 2002). In particular, they have been considered as tools for the complexation of Fe^{3+} for the treatment of iron overload (Turcot et al. 2000; Abergel and Raymond 2006). Sequestering agents bearing the HOPO residue were developed, e.g. for decontamination or decorporation applications due to the electronic properties of actinide cations being similar to Fe^{3+} (Gorden et al. 2003). Furthermore, the stable complexation of Gd^{3+} was proven using HOPO-based chelators associated with an improved relaxometry and sensitivity of Gd-based contrast agents for magnetic resonance imaging (MRI) (Raymond and Pierre 2005; Werner et al. 2008; Datta and Raymond 2009).

In the field of radiopharmacy, HOPO compounds have been also applied as ligands for the stable cation complexation of radionuclides. Examples are known for both isotopes ^{67}Ga (γ emitter) and ^{68}Ga (β^+ emitter) (Clevette et al. 1990; Chaves et al. 2011; Ma et al. 2016) or for the β^+ emitter ^{89}Zr (Deri et al. 2014; Deri et al. 2015; Guérad et al. 2017; Roy et al. 2021). They are in use for nuclear imaging being subjects for a safe radionuclide chelation using HOPO ligands. Even other cations from radionuclides like $^{43/44/47}$Sc (Phipps et al. 2021), $^{149/152/155/161}$Tb (Mishiro et al. 2019), ^{86}Y (Carter et al. 2020) or ^{227}Th (Ramdahl et al. 2016; Hammer et al. 2017, 2020) as therapeutic radionuclide especially for targeted alpha or beta therapies use multidentate HOPO chelators for a stable complexation (Zhou et al. 2021). Interestingly, the majority of multidentate HOPO ligands used for radiopharmaceutical applications is based on open-chain molecule backbones, while only little is known about the combination of aza-crown ethers containing HOPO binding residues. In this paper, we present the synthetic access to five new HOPO-based aza-crown ethers using Kryptofix K22 (1,4,10,13-tetraoxa-7,16-diazacyclooctadecane) as basic chemical scaffold.

Florian Passler and Linda Belke have contributed equally to this work.

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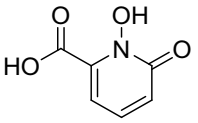
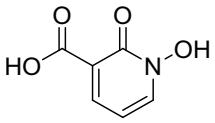
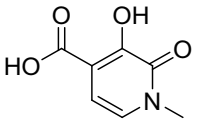
Scheme 1 Known used HOPO-acid scaffolds



1,2-HOPO-COOH



1,2,3-HOPO-COOH



2,3-Me-HOPO-COOH

Experimental

All chemicals were purchased from commercial suppliers and used without further purification unless otherwise specified. Anhydrous THF was purchased from Sigma-Aldrich (Schnelldorf, Germany), and deuterated solvents were purchased from deutero GmbH (Kastellaun, Germany). NMR spectra of all compounds were recorded on an Agilent DD2-400 MHz NMR or an Agilent DD2-600 MHz NMR spectrometer with ProbeOne. Chemical shifts of the ^{1}H and ^{13}C spectra were reported in parts per million (ppm) using TMS as internal standard for ^{1}H and ^{13}C spectra. Mass spectrometric (MS) data were obtained on an Advion Expression CMS by electron spray ionization (ESI). TLC detections were performed using silica gel 60 F_{254} sheets from Merck (Darmstadt, Germany). TLCs were developed by visualization under UV light ($\lambda = 254$ nm). Chromatographic separations were accomplished by using an automated silica gel column chromatography system Biotage Isolera Four and appropriate columns (Biotage, Sfär Silica HC D). A reversed phase HPLC system (Knauer Azura) with Zorbax 300SB-C18 (250×4.6 mm) semi-preparative column and acetonitrile/water (0.1% TFA each) as mobile phase was used for final HPLC purification (10–40% acetonitrile in H_2O within 35 min).

Syntheses

N,N′-[(1,4,10,13-Tetraoxa-7,16-diazacyclooctadecane-7,16-diyl)bis(ethane-2,1-diyl)]bis[1-(benzyloxy)-6-oxo-1,6-dihydropyridine-2-carboxamide] (*3a*)

Compound **1a** (161 mg, 0.46 mmol), 1,2-HOPO-acid **2** (283 mg, 1.15 mmol), EDC•HCl (221 mg, 1.15 mmol), and Oxyma (164 mg, 1.15 mmol were dissolved in anhydrous acetonitrile (15 mL) and stirred overnight at 50 °C. After TLC control, the solvent was removed and the crude product mixture dissolved in chloroform (20 mL). The organic phase was washed with saturated hydrogencarbonate solution (3×20 mL) and afterwards dried over Na_2SO_4. After removal of the solvent, purification was done with using automated column chromatography (eluent: ethyl acetate/EtOH $0 \rightarrow 100\%$) to obtain **3a** as yellow oil (76 mg, 20%). R_f: 0.05 (ethyl acetate/EtOH, 2/3); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.43–2.57$ (m, 12H, $NCH_2 + OCH_2$), 3.20–3.29 (m, 16H, $NCH_2 + OCH_2$), 3.30–3.39 (m, 4H, NCH_2), 5.37 (s, 4H, CH_2Ar), 6.25 (d, 2H, $^{3}J = 6.8$ Hz, Ar–H), 6.66 (d, 2H, $^{3}J = 9.2$ Hz, Ar–H), 7.26 (dd, 2H, $^{3}J = 6.8$ Hz, $^{3}J = 9.2$ Hz, Ar–H), 7.32–7.36 (m, 6H, Bn), 7.54–7.58 (m, 4H, Bn), 8.11 (br. s, 2H, NH); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 38.5$ (br. s, CH_2), 53.6 (br. s, CH_2), 54.4 (CH_2), 69.1 (br. s, CH_2), 69.9 (CH_2), 79.3 (CH_2Ar), 105.0, 123.6, 128.6, 129.3, 130.7 ($5 \times CH_{Ar}$), 133.9 (C_{Ar}), 138.1 (CH_{Ar}), 158.8, 160.7 ($2 \times C{=}O$); MS (ESI+): $m/z = 402$ $[M + 2H]^{2+}$. Anal. Calcd. for $C_{42}H_{54}N_6O_{10}$: C, 62.83; H, 6.78; N, 10.47; O, 19.93; Found: C, 62.81; H, 6.75; N, 10.50.

N,N′-[(1,4,10,13-Tetraoxa-7,16-diazacyclooctadecane-7,16-diyl)bis(propane-3,1-diyl)]bis[1-(benzyloxy)-6-oxo-1,6-dihydropyridine-2-carboxamide] (*3b*)

Compound **2** (200 mg, 0.82 mmol) was suspended in anhydrous chloroform (10 mL), oxalyl chloride (120 µL, 1.41 mmol) and a drop of DMF were added. The reaction mixture was stirred at 40 °C for 4 h. Then, the solvent and the remaining oxalyl chloride were removed in vacuum to obtain the acid chloride. Compound **1b** (100 mg, 0.27 mmol) and $NaHCO_3$ (50 mg, 0.60 mmol) were dissolved in anhydrous THF (10 mL) in another flask and cooled to 0 °C. The acid chloride, dissolved in anhydrous THF (2 mL), was added dropwise at 0 °C to the solution containing compound **1b** and the reaction mixture was stirred at rt overnight. Next, the solvent was changed to chloroform (20 mL) and washed with hydrogen carbonate (3×20 mL). The organic phase was dried over Na_2SO_4, the solvent was removed and the crude product was purified via automated column chromatography (eluent: ethyl acetate/methanol $0\% \rightarrow 100\%$) to obtain compound **3b** as yellowish oil (71 mg, 32%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.53–1.63$ (m, 4H, NCH_2), 2.28–2.55 (m, 12H, $NCH_2 + OCH_2$), 3.26 (s, 8H, OCH_2), 3.30–3.48 (m, 16H, $NCH_2 + OCH_2$), 5.34 (s, 4H, CH_2Ar), 6.23 (d, 2H, $^{3}J = 6.9$ Hz, Ar–H), 6.66 (d, 2H, $^{3}J = 9.2$ Hz, Ar–H), 7.27 (dd, 2H, $^{3}J = 6.9$ Hz, $^{3}J = 9.2$ Hz, Ar–H), 7.30–7.38 (m, 6H, Bn), 7.49–7.56 (m, 4H, Bn), 8.02 (br. s, 2H, NH); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 25.6$, 38.9, 52.9, 53.6, 69.2, 70.2 ($6 \times CH_2$), 79.4 (CH_2Ar), 104.7, 123.5, 128.6, 129.4, 130.6 ($5 \times CH_{Ar}$), 133.7 (C_{Ar}), 138.2 (CH_{Ar}), 158.7, 160.8 ($2 \times C{=}O$); MS (ESI+): $m/z = 831$ $[M + H]^{+}$,

853 $[M+Na]^+$. Anal. Calcd. for $C_{44}H_{58}N_6O_{10}$: C, 63.60; H, 7.04; N, 10.11; O, 19.25; Found: C, 63.41; H, 7.05; N, 10.05.

N,N′-[(1,4,10,13-Tetraoxa-7,16-diazacyclooctadecane-7,16-diyl)bis(ethane-2,1-diyl)]bis(1-hydroxy-6-oxo-1,6-dihydropyridine-2-carboxamide) (*4a*)

Under an argon atmosphere, compound **3a** (208 mg, 0.26 mmol) was dissolved in anhydrous dichloromethane (5 mL) and cooled to 0 °C. Afterwards, BBr_3 (49 µl, 0.54 mmol) was added and the reaction mixture was stirred at ambient temperature overnight. Next, the solvent and remaining BBr_3 were removed. The crude product was then cooled with liquid nitrogen and MeOH was added under stirring. After warming to rt, the solvent was removed and re-dissolved in a minimum amount of MeOH. Ice-cold diethyl ether was added to precipitate the final product. The diethyl ether was decanted, the product was washed with cold diethyl ether and dried to obtain compound **4a** (130 mg, 80%) as yellow–brown oil. Final purification was done using semipreparative HPLC. ^{1}H NMR (400 MHz, D_2O): δ = 3.54–3.65 (m, 12H, NCH_2+OCH_2), 3.74 (s, 8H, OCH_2), 3.82–3.95 (m, 12H, NCH_2+OCH_2), 6.82 (d, 2H, ^3J = 7.1 Hz, Ar–H), 6.89 (d, 2H, ^3J = 9.2 Hz, Ar–H), 7.63 (dd, 2H, ^3J = 7.1 Hz, ^3J = 9.2 Hz, Ar–H); ^{13}C NMR (101 MHz, D_2O): δ = 34.6, 52.5, 53.5, 63.5, 69.7 (5 × CH_2), 109.0, 121.3, 139.1 (3 × CH_{Ar}), 139.4 (C_{Ar}), 159.9, 163.0 (2 × C=O); MS (ESI+): *m/z* = 623 $[M+H]^+$, 645 $[M+Na]^+$; Anal. Calcd. for $C_{32}H_{44}F_6N_6O_{14}$ (as TFA salt): C, 45.18; H, 5.21; N, 9.88; O, 26.33; Found: C, 45.15; H, 5.23; N, 9.90.

N,N′-[(1,4,10,13-Tetraoxa-7,16-diazacyclooctadecane-7,16-diyl)bis(propane-2,1-diyl)]bis(1-hydroxy-6-oxo-1,6-dihydropyridine-2-carboxamide) (*4b*)

Under an argon atmosphere, compound **3b** (67 mg, 0.08 mmol) was dissolved in anhydrous dichloromethane (5 mL) and cooled to 0 °C. Afterwards, BBr_3 (29 µl, 0.32 mmol) was added and the reaction mixture was stirred at ambient temperature overnight. Next, the solvent and remaining BBr_3 were removed. The crude product was then cooled with liquid nitrogen and MeOH was added under stirring. After warming to rt, the solvent was removed and re-dissolved in a minimum amount of MeOH. Ice-cold diethyl ether was added to precipitate the final product. The diethyl ether was decanted, the product was washed with cold diethyl ether and dried to obtain compound **4b** as yellow–brown oil. Final purification was done using semipreparative HPLC (11.8 mg, 52%). ^{1}H NMR (400 MHz, D_2O): δ = 2.05–2.18 (m, 4H, CH_2), 3.33–3.43 (m, 4H, NCH_2), 3.47–3.62 (m, 12H, NCH_2+OCH_2), 3.77 (s, 8H, OCH_2), 3.85–3.97 (m, 8H, NCH_2+OCH_2), 6.47 (d, 2H, ^3J = 6.8 Hz, Ar–H), 6.86 (d, 2H, ^3J = 9.1 Hz, Ar–H), 7.63 (dd, 2H, ^3J = 6.8 Hz, ^3J = 9.1 Hz, Ar–H); ^{13}C NMR (101 MHz, D_2O): δ = 22.3, 36.6, 50.8, 52.8, 63.6, 69.7 (6 × CH_2), 108.1, 120.9, 139.1 (3 × CH_{Ar}), 140.3 (C_{Ar}), 160.1, 162.7 (2 × C=O); MS (ESI+): *m/z* = 651 $[M+H]^+$, 673 $[M+Na]^+$. Anal. Calcd. for $C_{34}H_{48}F_6N_6O_{14}$ (as TFA salt): C, 46.47; H, 5.51; N, 9.56; O, 25.49; Found: C, 46.40; H, 5:66; N, 9.46.

N,N′-[(1,4,10,13-Tetraoxa-7,16-diazacyclooctadecane-7,16-diyl)bis(ethane-2,1-diyl)]bis[1-(benzyloxy)-2-oxo-1,2-dihydropyridine-3-carboxamide] (*7a*)

Compound **5** (114.6 mg, 0.47 mmol) was suspended in anhydrous toluene (10 mL), oxalyl chloride (40 µL, 0.47 mmol) and a drop of DMF were added. The reaction mixture was stirred at 40 °C for 4 h. Then, the solvent and the remaining oxalyl chloride were removed in vacuum to obtain **6**. Compound **1a** (74 mg, 0.21 mmol) and triethylamine (73 µL, 52 mmol) were dissolved in anhydrous THF (10 mL) in another flask and cooled to 0 °C. Compound **6**, dissolved in anhydrous THF (2 mL), was added dropwise at 0 °C to the solution containing compound **1a** and the reaction mixture was stirred at rt overnight. Next, the solvent was changed to chloroform (20 mL) and washed with hydrogen carbonate (3 × 20 mL). The organic phase was dried over Na_2SO_4, the solvent was removed and the crude product was purified via automated column chromatography (eluent: ethyl acetate/ethanol 0% → 100%) to obtain compound **7a** as yellowish oil (69 mg, 40%).^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.78 (t, 4H, ^3J = 6.6 Hz, CH_2N), 2.88 (t, 8H, ^3J = 5.8 Hz, CH_2N), 3.48–3.55 (m, 4H, CH_2N), 3.60 (s, 8H, CH_2O), 3.64 (t, 8H, ^3J = 5.8 Hz, CH_2O), 5.27 (s, 4H, CH_2Ar), 6.12 (t, 2H, ^3J = 7.2 Hz, Ar–H), 7.29 (dd, 2H, ^3J = 6.9 Hz, ^4J = 2.2 Hz, Ar–H), 7.33–7.39 (m, 10H, Bn), 8.40 (dd, 2H, ^3J = 7.3 Hz, ^4J = 2.2 Hz, Ar–H), 9.64 (t, 2H, ^3J = 5.4 Hz, NH); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 37.9 (br. s, CH_2), 54.2 (CH_2), 54.6 (br. s, CH_2), 70.2 (br. s, CH_2), 70.8 (CH_2), 79.0 (CH_2Ar), 104.6 (CH_{Ar}), 123.7 (C_{Ar}), 129.0 (Bn), 129.8 (Bn), 130.2 (Bn), 133.3 (CH_{Ar}), 139.6 (C_{Ar}), 142.4 (CH_{Ar}), 158.8 (CH_{Ar}), 163.5 (C=O); MS (ESI+): *m/z* = 402 $[M+2H]^{2+}$. Anal. Calcd. for $C_{42}H_{54}N_6O_{10}$: C, 62.83; H, 6.78; N, 10.47; O, 19.93; Found: C, 62.79; H, 6.81; N, 10.49.

N,N′-[(1,4,10,13-Tetraoxa-7,16-diazacyclooctadecane-7,16-diyl)bis(propane-3,1-diyl)]bis[1-(benzyloxy)-2-oxo-1,2-dihydropyridine-3-carboxamide] (*7b*)

Compound **5** (200 mg, 0.82 mmol) was suspended in anhydrous chloroform (10 mL), oxalyl chloride (120 µL, 1.41 mmol) and a drop of DMF were added. The reaction mixture was stirred at 40 °C for 4 h. Then, the solvent and the remaining oxalyl chloride were removed in vacuum to obtain the acid chloride. Compound **1b** (100 mg, 0.27 mmol) and $NaHCO_3$ (50 mg, 0.60 mmol) were dissolved in anhydrous

THF (10 mL) in another flask and cooled to 0 °C. The acid chloride, dissolved in anhydrous THF (2 mL), was added dropwise at 0 °C to the solution containing compound **1b** and the reaction mixture was stirred at rt overnight. Next, the solvent was changed to chloroform (20 mL) and washed with hydrogen carbonate (3 × 20 mL). The organic phase was dried over Na_2SO_4, the solvent was removed and the crude product was purified via automated column chromatography (eluent: ethyl acetate/methanol 0% → 100%) to obtain compound **3b** as yellowish oil (66 mg, 30%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.71–1.81 (m, 4H, NCH_2), 2.60 (t, ^3J = 7.1 Hz, 4H, NCH_2), 2.78 (t, ^3J = 5.8 Hz, 8H, OCH_2), 3.26 (s, 8H, OCH_2), 3.39–3.66 (m, 20H, $NCH_2 + OCH_2$), 5.27 (s, 4H, CH_2Ar), 6.13 (t, 2H, ^3J = 7.0 Hz, Ar–H), 7.29 (dd, 2H, ^4J = 2.0 Hz, ^3J = 7.0 Hz, 2H, Ar–H), 7.27 (dd, 2H, ^3J = 6.9 Hz, ^3J = 9.2 Hz, 2H, Ar–H), 7.32–7.42 (m, 10H, Bn), 8.41 (dd, 2H, ^4J = 2.0 Hz, ^3J = 7.5 Hz, 2H, Ar–H), 9.58 (br. s, 2H, NH); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 27.4, 37.9, 53.4, 54.0, 70.0, 70.8 (6 × CH_2), 79.0 (CH_2Ar), 104.7, 123.7, 129.0, 129.8, 130.1 (5 × CH_{Ar}), 133.2 (C_{Ar}), 139.4 (C_{Ar}), 142.4 (CH_{Ar}), 158.8, 163.3 (2 × C=O); MS (ESI+): *m/z* = 831 $[M + H]^+$, 853 $[M + Na]^+$. Anal. Calcd. for $C_{44}H_{58}N_6O_{10}$: C, 63.60; H, 7.04; N, 10.11; O, 19.25; Found: C, 63.55; H, 6.99; N, 10.14.

N,N'-[(1,4,10,13-Tetraoxa-7,16-diazacyclooctadecane-7,16-diyl)bis(ethane-2,1-diyl)]bis(1-hydroxy-2-oxo-1,2-dihydropyridine-3-carboxamide) (*8a*)

Under an argon atmosphere, compound **7a** (128 mg, 0.16 mmol) was dissolved in anhydrous dichloromethane (5 mL) and cooled to 0 °C. Afterwards, BBr_3 (49 µL, 0.54 mmol) was added and the reaction mixture was stirred at ambient temperature overnight. Next, the solvent and remaining BBr_3 were removed. The crude product was then cooled with liquid nitrogen and MeOH was added under stirring. After warming to rt, the solvent was removed and redissolved in a minimum amount of MeOH. Ice-cold diethyl ether was added to precipitate the final product. The diethyl ether was decanted, the product was washed with cold diethyl ether and dried to obtain compound **8a** (98 mg, 99%) as yellow–brown oil. Final purification was done using semipreparative HPLC. ^{1}H NMR (400 MHz, D_2O): δ = 3.51–3.71 (m, 20H, $NCH_2 + OCH_2$), 3.81–3.94 (m, 12H, $NCH_2 + OCH_2$), 6.68 (t, 2H, ^3J = 7.1 Hz, Ar–H), 8.20 (d, 2H, ^3J = 9.2 Hz, Ar–H), 8.36 (dd, 2H, ^3J = 7.1 Hz, ^3J = 9.2 Hz, Ar–H), 10.03 (t, 2H, ^3J = 5.6 Hz, NH); ^{13}C NMR (101 MHz, D_2O): δ = 34.5, 53.6, 53.7, 63.6, 69.7 (5 × CH_2), 106.7 (CH_{Ar}), 119.1 (C_{Ar}), 140.4, 142.1 (2 × CH_{Ar}), 158.9, 166.8 (2 × C=O); MS (ESI+): *m/z* = 623 $[M + H]^+$, 645 $[M + Na]^+$. Anal. Calcd. for $C_{32}H_{44}F_6N_6O_{14}$ (as TFA salt): C, 45.18; H, 5.21; N, 9.88; O, 26.33; Found: C, 45.13; H, 5.20; N, 9.87.

N,N'-[(1,4,10,13-Tetraoxa-7,16-diazacyclooctadecane-7,16-diyl)bis(ethane-2,1-diyl)]bis(1-hydroxy-2-oxo-1,2-dihydropyridine-3-carboxamide) (*8b*)

Under an argon atmosphere, compound **7b** (66 mg, 0.08 mmol) was dissolved in anhydrous dichloromethane (5 mL) and cooled to 0 °C. Afterwards, BBr_3 (49 µL, 0.54 mmol) was added and the reaction mixture was stirred at ambient temperature overnight. Next, the solvent and remaining BBr_3 were removed. The crude product was then cooled with liquid nitrogen and MeOH was added under stirring. After warming to rt, the solvent was removed and redissolved in a minimum amount of MeOH. Ice-cold diethyl ether was added to precipitate the final product. The diethyl ether was decanted, the product was washed with cold diethyl ether and dried to obtain compound **8b** (71 mg, > 99%) as yellow–brown oil. Final purification was done using semipreparative HPLC. ^{1}H NMR (400 MHz, D_2O): δ = 2.04–2.16 (m, 4H, CH_2), 3.30–3.39 (m, 4H, NCH_2), 3.42–3.60 (m, 12H, $NCH_2 + OCH_2$), 3.68 (s, 8H, OCH_2), 3.79–3.92 (m, 8H, $NCH_2 + OCH_2$), 6.66 (t, 2H, ^3J = 7.2 Hz, Ar–H), 8.16 (d, 2H, ^3J = 6.5 Hz, Ar–H), 8.36 (d, 2H, ^3J = 7.2 Hz, Ar–H), 9.89 (t, 2H, ^3J = 4.9 Hz, NH); ^{13}C NMR (101 MHz, D_2O): δ = 22.7, 36.2, 50.5, 52.7, 63.5, 69.6 (6 × CH_2), 106.8 (CH_{Ar}), 119.5 (C_{Ar}), 139.9, 141.7 (2 × CH_{Ar}), 158.9, 165.9 (2 × C=O); MS (ESI+): *m/z* = 651 $[M + H]^+$, 673 $[M + Na]^+$, 689 $[M + K]^+$. Anal. Calcd. for $C_{34}H_{48}F_6N_6O_{14}$ (as TFA salt): C, 46.47; H, 5.51; N, 9.56; O, 25.49; Found: C, 46.55; H, 5:59; N, 9.67.

N,N'-[(1,4,10,13-Tetraoxa-7,16-diazacyclooctadecane-7,16-diyl)bis(ethane-2,1-diyl)]bis(3-methoxy-1-methyl-2-oxo-1,2-dihydropyridine-4-carboxamide) (*11*)

Compound **9** (249.4 mg, 1.36 mmol) was suspended in anhydrous toluene (10 mL), oxalyl chloride (1.1 mL, 15.1 mmol), and a drop of DMF were added. The reaction mixture was stirred at 40 °C for 4 h. Then, the solvent and the remaining oxalyl chloride were removed in vacuum to obtain **10**. Compound **1** (200 mg, 0.57 mmol) and triethylamine (174 mg, 1.72 mmol) were dissolved in anhydrous dichloromethane (10 mL) in another flask and cooled to 0 °C. Compound **10**, dissolved in anhydrous dichloromethane (2 mL), was added dropwise at 0 °C to the solution containing compound **1** and the reaction mixture was stirred at rt overnight. Next, the solvent was changed to chloroform (30 mL) and washed with hydrogen carbonate (3 × 30 mL). The organic phase was dried over Na_2SO_4, the solvent was removed and the crude product was purified via automated column chromatography (eluent: ethyl acetate/methanol 50% → 100%) to obtain compound **11** as yellowish oil (125 mg, 0.18 mmol, 32%).^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.74 (t, 4H, ^3J = 6.0 Hz, CH_2N), 2.84 (t, 8H, ^3J = 5.8 Hz, CH_2N), 3.44–3.50 (m, 4H, CH_2N), 3.53–3.57 (m, 14H, $CH_2O + CH_3$), 3.59 (t,

8H, $^{3}J = 5.8$ Hz, CH_2O), 4.06 (s, 6H, CH_3), 6.76 (d, 2H, $^{3}J = 7.2$ Hz, Ar–H), 7.08 (d, 2H, $^{3}J = 7.2$ Hz, Ar–H), 8.43 (t, 2H, $^{3}J = 4.5$ Hz, NH); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 37.7 (CH_3), 37.8, 53.8, 54.0 (3 × CH_2), 60.2 (CH_3), 70.1, 70.8 (2 × CH_2), 104.9 (CH_{Ar}), 130.5 (C_{Ar}), 132.1 (CH_{Ar}), 147.8 (C_{Ar}), 159.7, 163.3 (2 × C=O); MS (ESI +): *m/z* = 340 $[M + 2H]^{2+}$, 679 $[M + H]^{+}$. Anal. Calcd. for $C_{32}H_{50}N_6O_{10}$: C, 56.62; H, 7.43; N, 12.38; O, 23.57; Found: C, 56.67; H, 7.41; N, 12.35.

N,N′-((1,4,10,13-tetraoxa-7,16-diazacyclooctadecane-7,16-diyl)bis(ethane-2,1-diyl))bis(3-hydroxy-1-methyl-2-oxo-1,2-dihydropyridine-4-carboxamide) (*12*)

Under an argon atmosphere, compound **11** (115 mg, 0.17 mmol) was dissolved in anhydrous dichloromethane (5 mL) and cooled to 0 °C. Afterwards, BBr_3 (100 µL, 292 mmol) was added and the reaction mixture was stirred at ambient temperature overnight. Next, the solvent and remaining BBr_3 were removed. The crude product was then cooled with liquid nitrogen and MeOH was added under stirring. After warming to rt, the solvent was removed and redissolved in a minimum amount of MeOH. Ice-cold diethyl ether was added to precipitate the final product. The diethyl ether was decanted, the product was washed with cold diethyl ether and dried to obtain compound **8b** (108 mg, 98%) as yellow–brown oil. Final purification was done using semipreparative HPLC. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.29–3.88 (m, 38H, $CH_2N + CH_2O + CH_3$), 6.50 (d, 2H, $^{3}J = 7.4$ Hz, Ar–H), 7.21 (d, 2H, $^{3}J = 7.4$ Hz, Ar–H), 8.64 (t, 2H, $^{3}J = 5.3$ Hz, NH), 9.50 (br. s, 2H, OH); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 36.9 (CH_3), 34.4, 52.4, 52.8, 64.4, 69.4 (4 × CH_2), 60.2 (CH_3), 70.1, 70.8 (2 × CH_2), 103.1 (CH_{Ar}), 117.4 (C_{Ar}), 127.9 (CH_{Ar}), 146.8 (C_{Ar}), 158.2, 165.6 (2 × C=O); MS (ESI +): *m/z* = 651 $[M + H]^{+}$, 673 $[M + Na]^{+}$. Anal. Calcd. for $C_{34}H_{48}F_6N_6O_{14}$ (as TFA salt): C, 46.47; H, 5.51; N, 9.56; O, 25.49; Found: C, 46.65; H, 5:76; N, 9.85.

Results and discussion

Diaza-crown ethers are subjected to function as basic skeleton to prepare multidentate cyclic chelators. To introduce the respective HOPO functions, two primary diazacrown ethers **1a,b** were prepared according to the literature in two steps starting from diaza-18-crown-6 ether, which was treated with *N*-(2-bromoethyl)phthalimide (Lukyanenko et al. 2004) or *N*-(3-bromopropyl)phthalimide (Quici et al. 1999), respectively, according to published procedures. The second step comprises the removal of the phthalimide moiety with hydrazine to obtain *N,N′*-bis(aminoethylene) compound **1a** and *N,N′*-bis(aminopropylene) compound **1b**, both containing two free primary amino functions to introduce the HOPO groups. The reaction path is shown in the Supporting Information.

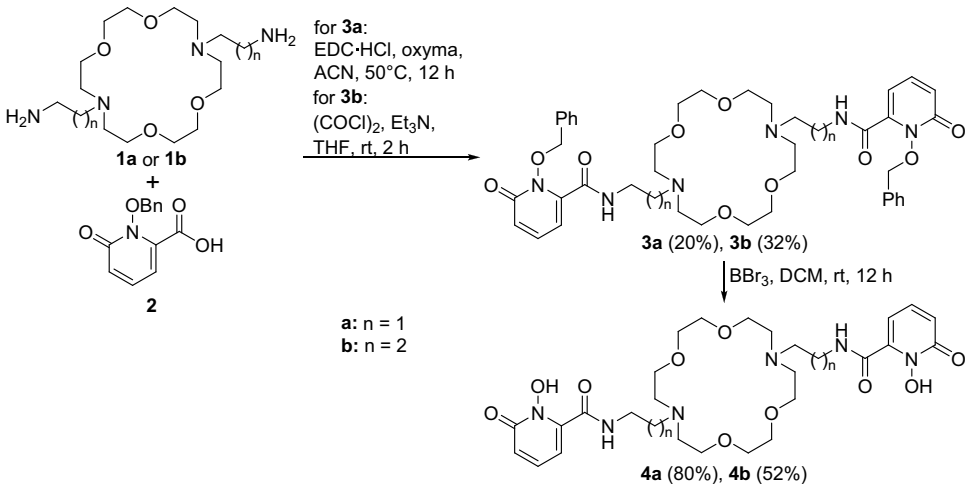
Synthesis of the HOPO-functionalized crown ethers 4a,b, 8a,b, and 12

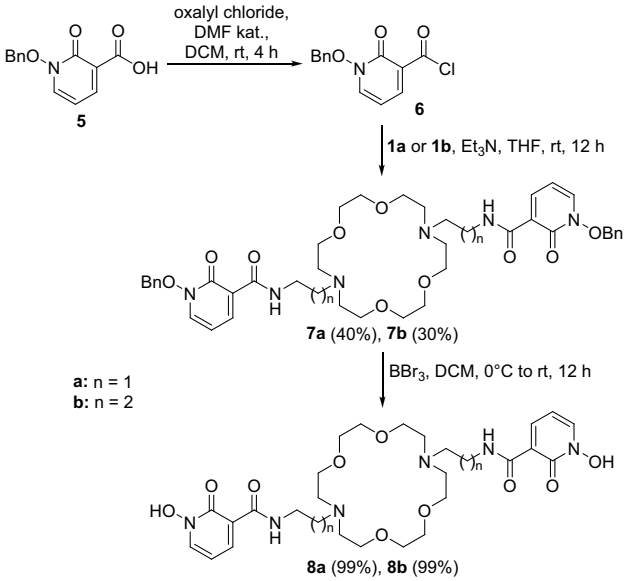
To avoid side reactions, the 1,2-HOPO-core is introduced into multidentate amine skeletons in its *O*-benzyl-protected form as activated ester (succinimidyl ester, see: Huang et al. 2019; TFP ester, see: Workman et al. 2020), using peptide coupling conditions (Daumann et al. 2016) or as acid chloride (see e.g. Phipps et al. 2023). In our case, the *O*-benzyl protected 1,2-HOPO-acid **2** was used as well, which was prepared from the respective acid and benzyl bromide (Deri et al. 2014). Bn-1,2-HOPO-acid **2** was reacted with the basic macrocycle **1a** using EDC•HCl (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) and Oxyma (ethyl cyanohydroxyiminoacetate) to yield the *O*-benzyl-HOPO-functionalized diazacrown ether **3a** (20% yield). In contrast, **3b** was prepared from **2** in 32% yield, which was converted into the acid chloride with oxalyl chloride beforehand and then reacted with **1b**. Finally, the benzyl protecting groups of **3a,b** were cleaved with BBr_3 under anhydrous conditions to obtain the final HOPO-ligands **4a,b** in 80 and 52% yield, respectively. The synthesis procedure to HOPO derivative **4a,b** is shown in Scheme 2.

Little is known about ligand formed by the 1,2,3-HOPO-acid moiety. For the preparation of the 1,2,3-HOPO-ligands **8a,b**, it is also necessary to protect the hydroxy function of the starting HOPO derivative. Thus, the *O*-benzyl protected 1,2,3-HOPO-acid **5** is used, which was prepared from the HOPO acid by *O*-alkylation with benzyl bromide (Workman et al. 2020). They used activated esters based on TFP or mercaptothiazoline for the connection of the 1,2,3-HOPO moiety to the amine. In our case, carbodiimides such as EDC were used to directly react HOPO derivative **5** with the macrocycles **1a,b** without using an activated ester. Notably, both HOPO-functionalized macrocycles **7a,b** were not obtained. Thus, Bn-1,2,3-HOPO-acid **5** was converted into the corresponding acid chloride **6** using oxalyl chloride (Workman et al. 2020) and then subsequently reacted with crown ethers **1a,b** under mild conditions using triethyl amine as base to obtain **7a** in 40% yield and **7b** in 30% yield. Finally, the benzyl groups were quantitatively cleaved with BBr_3 obtaining the final derivatives **8a** and **8b** (see Scheme 3).

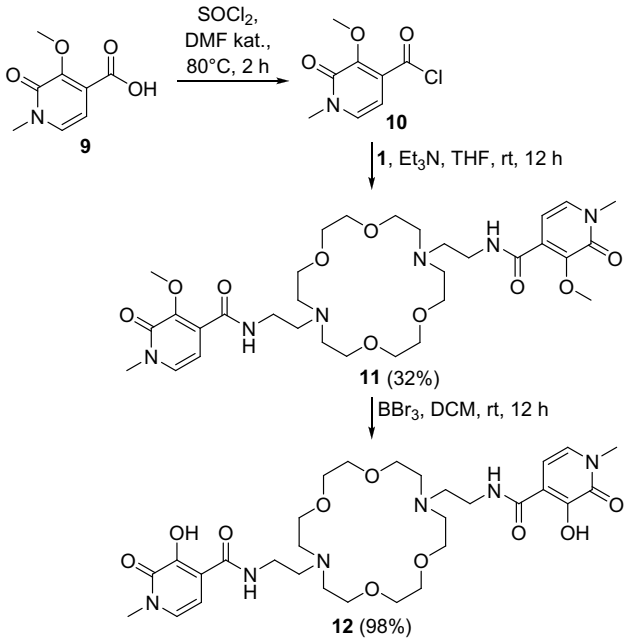
The 2,3-Me-HOPO-acid was mainly used as intermediate in the synthesis of pharmacologically active compounds (Sweeney et al. 2008). In this case, the synthesis route starts from the di-*N,O*-methyl protected 2,3-HOPO acid ethyl ester. The saponification under basic conditions delivered the free acid **9** (Sweeney et al. 2008), which was converted into its respective acid chloride **10**. Compound **10** was then subsequently reacted with **1a** to obtain the desired

Scheme 2 Synthesis of 1,2-HOPO-based lariat ethers **4a** and **4b**





Scheme 3 Synthesis of 1,2,3-HOPO-based lariat ethers **8a** and **8b**



Scheme 4 Synthesis of the 2,3-Me-HOPO-based lariat ether **12**

dimethyl-protected derivative **11** in 32% yield. Finally, the methyl groups were cleaved with BBr_3. An excess of BBr_3 combined with a longer reaction time is necessary. Otherwise the partly deprotected compound will be obtained (data not shown). The whole reaction path is shown in Scheme 4.

Conclusions

The combination of macrocyclic compounds with HOPO-functions delivers new multidentate ligands for a stable complexation of metal ions. For this purpose, five new HOPO-functionalized diazacrown ethers were prepared using a convenient synthesis procedure. Their structures were confirmed by NMR and ESI MS.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11696-024-03376-8.

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